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**Diagnosis, treatment and immunopathogenesis of
the HIV-associated tuberculosis immune
reconstitution inflammatory syndrome**

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**Thesis Presented for the Degree of
DOCTOR OF PHILOSOPHY
in the Department of Medicine
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Professor Robert J. Wilkinson
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DECLARATION

I, Graeme Ayton Meintjes, do hereby declare that this thesis includes five journal manuscripts. Four of these manuscripts (Chapter 3-6) have been published and the fifth (Chapter 7) has been submitted for publication in an international journal. The contents of each of these manuscripts remains unchanged from that which has been published or submitted for publication. The manuscripts are listed below, with a description of my contribution and the contribution of each author to the study.

CHAPTER 3

Meintjes G, Lawn SD, Scano F, Maartens G, French MA, Worodria W, Elliott JH, Murdoch D, Wilkinson RJ, Seyler C, John L, van der Loeff MS, Reiss P, Lynen L, Janoff EN, Gilks C, Colebunders R; International Network for the Study of HIV-associated IRIS. Tuberculosis-associated immune reconstitution inflammatory syndrome: case definitions for use in resource-limited settings. Lancet Infect Dis. 2008;8(8):516-23.

This manuscript arose from the founding meeting of the International Network for the Study of HIV-associated IRIS (INSHI) held in Kampala, Uganda, in 2006. Over 100 researchers attended. One of the aims of the meeting was to develop consensus case definitions for TB-IRIS to allow for standardization of research reports on TB-IRIS. Several groups presented the case definitions they were using at the meeting. Our group was conducting a clinical trial of TB-IRIS treatment at the time. We therefore had developed detailed clinical case definitions for TB-IRIS and had experience of using them in a prospective clinical study. These were presented at the meeting by Graeme Meintjes and formed the basic framework for the consensus case definitions that were developed at the meeting. Graeme Meintjes was given the role of heading the writing committee for the consensus case definitions and over the next year he co-ordinated via email the write-up of the manuscript that presents these case definitions. This involved drafting a manuscript, several rounds of comments from all co-authors and inclusion of these comments into revised versions by Graeme Meintjes who finalized the manuscript for submission.

He was assisted in particular by Stephen D. Lawn, Robert Colebunders, Gary Maartens and Robert J. Wilkinson. He was the first author on the final manuscript.

CHAPTER 4

Meintjes G, Rangaka MX, Maartens G, Rebe K, Morroni C, Pepper DJ, Wilkinson KA, Wilkinson RJ. Novel relationship between tuberculosis immune reconstitution inflammatory syndrome and antitubercular drug resistance. Clin Infect Dis. 2009;48(5):667-76.

Graeme Meintjes was the lead investigator on this clinical study. He designed the study. He was involved in patient assessment and collected clinical data. He was responsible together with Robert J. Wilkinson for data entry, management and analysis. He wrote the final manuscript which was reviewed by all authors critically.

Dominique J. Pepper, Kevin Rebe and Molebogeng X. Rangaka worked on the study at the clinical site and were involved in patient assessment and collection of clinical data.

Gary Maartens and Robert J. Wilkinson were involved in study design and supervised the study and writing of the manuscript. Robert J. Wilkinson also designed the study database.

Chelsea Morroni and Molebogeng X. Rangaka assisted with statistical analysis.

Katalin Wilkinson performed and supervised the performance of the ELISpot assays in the IIDMM laboratory and was involved in analysis of this data.

CHAPTER 5

Meintjes G, Wilkinson KA, Rangaka MX, Skolimowska K, van Veen K, Abrahams M, Seldon R, Pepper DJ, Rebe K, Mouton P, van Cutsem G, Nicol MP, Maartens G, Wilkinson RJ. Type 1 helper T cells and FoxP3-positive T cells in HIV-tuberculosis-

associated immune reconstitution inflammatory syndrome. Am J Respir Crit Care Med. 2008;178(10):1083-9.

Graeme Meintjes was responsible for recruitment, clinical assessment, clinical data collection, entry and analysis at the GF Jooste site. He was involved in study design and was also involved in supervision of the patient recruitment at the Ubuntu TB-HIV Clinic, Site B Khayelitsha. He assisted with performance of the ELISpot assays in the laboratory. He was involved in laboratory data analysis and write up of the manuscript.

Robert J. Wilkinson conceived and designed the study. He was responsible for overall supervision of the two clinical sites and the laboratory work. He designed the study database and assisted with data entry and was involved in study data analysis.

Katalin Wilkinson supervised the laboratory assays (ELISpot and flow cytometry) that were performed by Kerryn Matthews (née van Veen), Ronette Seldon, Keira Skolimowska and Graeme Meintjes.

The following were also involved in patient assessment and recording of clinical data: Dominique J. Pepper, Kevin Rebe, Musaed Abrahams, Gilles van Cutsem, Priscilla Mouton and Molebogeng X. Rangaka.

Gary Maartens and Marc Nicol were involved in study design and review of the manuscript.

CHAPTER 6

Meintjes G, Wilkinson RJ, Morrioni C, Pepper DJ, Rebe K, Rangaka MX, Oni T, Maartens G. Randomized placebo-controlled trial of prednisone for paradoxical tuberculosis-associated immune reconstitution inflammatory syndrome. AIDS. 2010;24(15):2381-90.

Graeme Meintjes was the lead investigator on this clinical trial. He was involved in study design and responsible for on-site co-ordination of the study. He was involved in patient

recruitment, assessment, follow-up and collected clinical data. He clinically assessed and provided consultant input on all 110 patients enrolled in the trial. He was responsible together with the study statistician (Chelsea Morroni) for data management and analysis. He wrote the final manuscript which was reviewed by all authors critically. Graeme Meintjes was also a co-author on the successful grant application to the Medical Research Council (MRC) of South Africa that funded the study and he played a key role in writing this grant.

Robert J. Wilkinson was involved in study design and co-ordination. Gary Maartens was the study principal investigator and involved in study design and co-ordination. Both supervised the study and writing of the manuscript.

Dominique J. Pepper, Kevin Rebe, Molebogeng X. Rangaka and Tolu Oni worked on the study at the clinical site and were involved in patient recruitment, follow-up and collection of clinical data.

CHAPTER 7

Meintjes G, Skolimowska KH, Wilkinson KA , Conesa Botella A, Matthews K, Tadokera R, Rangaka MX, Rebe K, Pepper DJ, Morroni C, Colebunders R, Maartens G, Wilkinson RJ. Corticosteroid modulated immune activation in HIV / tuberculosis-associated immune reconstitution inflammatory syndrome. Submitted for publication.

This immunology study was conducted on samples collected from patients during the randomized controlled trial of prednisone for TB-IRIS. The role of Graeme Meintjes and others in the clinical aspects of this trial have been described above.

The immunology study was designed and supervised by Robert J. Wilkinson. The laboratory work for this study was supervised by Katalin Wilkinson and Robert J. Wilkinson. The assays (ELISpot, PCR and Luminex multiplex) were performed by Keira Skolimowska, Katalin Wilkinson, Graeme Meintjes, Kerry Matthews, Rebecca Tadokera and Anali Conesa Botella.

Graeme Meintjes was involved in study design and was responsible for laboratory data analysis, taking the raw laboratory data, synthesizing it with the clinical data, data cleaning and statistical analysis. He wrote the first draft of the manuscript, which was critically reviewed by all co-authors prior to submission. Keira Skolimowska and Anali Conesa Botella assisted with data analysis.

I confirm that no part of this thesis has been submitted in the past, or is being, or is to be submitted for a degree at this or any other university. I hereby grant the University of Cape Town free license to reproduce this thesis in whole or part for the purposes of research or teaching.

This thesis is presented for examination in fulfillment of the requirements for the degree of Doctor of Philosophy in Medicine.

Signed,

Signed by candidate

Signature Removed

Graeme Ayton Meintjes

1 August 2011

ABSTRACT

PhD candidate: Graeme Ayton Meintjes

Title: Diagnosis, treatment and immunopathogenesis of the HIV-associated tuberculosis immune reconstitution inflammatory syndrome

Date: 8 August 2011

Background

The paradoxical tuberculosis-associated immune reconstitution inflammatory syndrome (TB-IRIS) is a frequent complication of antiretroviral therapy (ART) among patients who start ART while on treatment for tuberculosis (TB). The condition manifests with new, worsening or recurrent inflammatory features of TB due to immunopathology attendant on rapidly recovering immune function.

Methods

We screened consecutive patients referred with suspected paradoxical TB-IRIS using standard TB-IRIS case definitions and excluded alternative causes for clinical deterioration. A randomized placebo-controlled clinical trial of prednisone for the treatment of paradoxical TB-IRIS was conducted. Participants received prednisone (n = 55) or placebo (n = 55) for 4 weeks. Immunology studies were conducted, exploring the role of mycobacterial-specific IFN γ -producing T cells and regulatory T cells in pathogenesis and the effect of prednisone on the immune response in TB-IRIS. The TB-IRIS case definitions we developed formed the framework for consensus case definitions developed by the International Network for the Study of HIV-associated IRIS.

Results

Among patients presenting with suspected paradoxical TB-IRIS, 10.1% (95% confidence interval = 3.9 – 16.4%) were found to have undiagnosed rifampicin-resistant TB once those with known rifampicin resistance and alternative diagnoses were excluded. In the clinical trial, the combined primary endpoint of days hospitalised and outpatient therapeutic procedures (counted as an additional day) was more frequent in the placebo than the prednisone arm (median 3 days versus 0 days, p=0.04). Prednisone also resulted in more

rapid improvements in symptoms, quality of life and chest radiology. In serum, significant decreases in IL-6, IL-10, IL-12p40, TNF α , IFN γ and IP-10 concentrations during prednisone, but not placebo, treatment were observed. Paradoxical TB-IRIS cases had higher median ELISpot responses to several mycobacterial antigens in cross-sectional analysis compared with non-IRIS controls, but responses were heterogenous and dynamic in both cases and controls in longitudinal analysis. No association with regulatory T cell numbers was demonstrated.

Conclusions

Drug resistant TB should be excluded in all patients presenting with suspected paradoxical TB-IRIS. Prednisone reduced morbidity in paradoxical TB-IRIS and resulted in more rapid symptom and radiographic improvement. This appears to be mediated through reductions in concentrations of pro-inflammatory cytokines. Although T cell responses likely contribute to TB-IRIS pathogenesis they do not appear to be causal.

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I thank my supervisors, Professor Robert J. Wilkinson and Professor Gary Maartens, for stimulating conversations, detailed review and critique of manuscripts as they were developed towards publication, and wise advice and guidance all the way. I also wish to thank Katalin Wilkinson who patiently taught me the ropes in the laboratory.

I thank the patients who participated in these studies and the staff at the facilities where they were conducted (GF Jooste Hospital and Ubuntu TB-HIV Clinic in Khayelitsha). I thank Priscilla Mouton and Rene Goliath, the study nurses, whose dedication and care for patients contributed to the success of these studies. I thank all colleagues who participated in the clinical studies (Kevin Rebe, Dominique Pepper, Molebogeng Rangaka, Tolu Oni, Musaed Abrahams and Gilles van Cutsem), the laboratory studies (Keira Skolimowska, Rebecca Tadokera, Kerryn Matthews, Anali Conesa Botella and Ronett Seldon) and Chelsea Morrioni (the study statistician). I also wish to thank Kathryn Wood and Reyhana Solomon for administrative support.

Finally, I thank my wife, Cecile, for constant support and encouragement and many sacrificed hours. And to my parents, Mike and Irene, thank you for respecting independent thought from a young age, and always being there to encourage me.

Graeme Meintjes

1 August 2011

CHAPTER 1

Introduction

Context

Antiretroviral therapy (ART) for HIV infection has dramatically altered the natural history of the disease. As a consequence of immune restoration on ART, HIV-infected patients are protected from opportunistic infections and experience improved quality of life and prolonged survival. However, ART is accompanied by a number of potential complications. The immune reconstitution inflammatory syndrome (IRIS) is an important and frequent early complication of ART, particularly in patients who start ART with advanced immunosuppression and co-existent opportunistic infections. IRIS occurs during initial rapid immune recovery on ART and manifests with clinical deterioration accompanied by features of an inflammatory process, which is the consequence of an immunopathological reaction to the antigens of an opportunistic infection. Tuberculosis-associated IRIS (TB-IRIS) is the most common form of IRIS worldwide.

Over the last two decades South Africa has experienced an upsurge of the dual epidemics of HIV and TB. The HIV epidemic has undermined the TB control programme and fuelled the incidence of TB in the country, and TB is the major cause of morbidity and mortality among HIV-infected Africans (1). In 2006, South Africa reported the fourth highest number of TB cases globally (2). TB case notification rates have risen nearly four-fold from 1986 to 2006 (from 163 per 100 000 population in 1986 to 628 per 100 000 population in 2006) (2). The country is the worst affected in the world by the co-epidemic of HIV and TB. While it is estimated that 0.7% of the world's population lives in South Africa, approximately one-quarter of all global cases of HIV-associated TB occur in the country (3). Since 2004 there has been a massive scale-up of ART in public sector clinics in South Africa. It is estimated that approximately 1.4 million peoples have accessed ART in the South African public sector to date (4).

Many patients learn their HIV status when they are diagnosed with active TB and require the initiation of ART soon after starting TB treatment because of a low CD4+ T-lymphocyte count. In ART clinics in Cape Town up to 42% of patients starting ART are concurrently on TB treatment (5), reflecting that the diagnosis of TB is frequently the entry point into HIV care in this setting (6). Patients starting ART following a recent diagnosis of active TB are at

risk for paradoxical TB-IRIS, which typically occurs in the first weeks of ART and manifests with new, recurrent or worsening symptoms, signs and/or radiographic manifestations of TB.

Whilst many cases are self-limiting, paradoxical TB-IRIS results in considerable morbidity and places considerable burden on the health services. Patients frequently require hospitalization, diagnostic and therapeutic procedures. In South Africa, given the large numbers of HIV-TB co-infected patients with advanced immunosuppression and the rapidly expanding availability of ART in the public sector to effectively treat these patients, paradoxical TB-IRIS has become a frequent reason for presentation at primary care and higher referral levels of the health care system. There is no confirmatory diagnostic test and the diagnosis is made on the basis of characteristic clinical features after excluding alternative diagnoses that could otherwise explain the symptomatic deterioration. This condition poses considerable challenges for clinicians. A major reason for this is that our evidence base regarding the diagnosis, treatment and pathogenesis of this condition is limited.

The studies reported in this thesis aimed to establish an evidence-based approach to the diagnostic work-up of patients presenting with paradoxical TB-IRIS, define the role of prednisone in the treatment of paradoxical TB-IRIS in a randomized-controlled clinical trial and investigate the immunopathogenesis of the condition. A better understanding of the immunopathogenesis of TB-IRIS may allow for predictive and diagnostic markers to be discovered and more targeted and rational therapies for the condition.

Key research questions

The key research questions addressed in this thesis are developed to address gaps in knowledge that were identified when the research work was initiated. They are:

- 1) When assessing patients with suspected paradoxical TB-IRIS what are the important alternative diagnoses to exclude?
- 2) What are the common clinical features in patients with paradoxical TB-IRIS?
- 3) What is the immunopathogenesis of TB-IRIS, with a focus on the role of mycobacterial-specific interferon- γ -producing T cells and regulatory T cells?

- 4) Do the clinical benefits of prednisone used to treat paradoxical TB-IRIS outweigh potential risks in these patients who have advanced HIV?
- 5) Given that prednisone did provide clinical benefit, what is its effect on the mediators of inflammation in paradoxical TB-IRIS?

Setting

These studies were conducted in Cape Town in the Western Cape Province of South Africa. The randomised controlled trial was conducted at GF Jooste Hospital. This is a public sector referral hospital located in Manenberg on the Cape Flats, which during the period of the studies served as the adult referral hospital for a population of approximately 1.3 million people. The communities served by this hospital included Khayelitsha, Gugulethu, Nyanga and Mitchells Plain. The antenatal HIV seroprevalence in Khayelitsha was 33% and TB annual notification rate 1612 / 100 000 during the study. There were 16 public sector TB clinics and 10 ART clinics in the referral area of the hospital during the study. All patients attending these clinics who require specialist level investigations or opinion or hospital admission are referred to the hospital. During the clinical trial primary care clinicians were also educated about the study and asked to refer all suspected paradoxical TB-IRIS cases to GF Jooste Hospital for assessment by the study team even if they did not fulfill the usual referral criteria. GF Jooste Hospital runs an Infectious Diseases Referral Unit that supports these TB and ART clinics by providing a service to which patients experiencing complications in primary care or with problems that cannot be adequately investigated or managed in primary care can be referred and rapidly assessed. Until 2008, a primary care ART clinic was also run within the outpatient department of GF Jooste Hospital. By June 2009, there were 16 828 adults receiving ART at the primary care ART clinics in the referral area of the hospital.

The largest of these clinics is the Ubuntu TB-HIV clinic in Site B Khayelitsha. The longitudinal study described in Chapter 5 was conducted at this facility. A pilot ART clinic was started at this facility in 2001, a collaboration between Medecins sans Frontieres and the Provincial Government of the Western Cape prior to the national public sector ART roll-out

(7). Currently over 5000 adults receive ART at this facility. The HIV and TB clinics have been integrated at this facility.

The laboratory studies described in Chapter 5 and Chapter 7 were conducted in the laboratory of Professor Robert J. Wilkinson at the Institute of Infectious Diseases and Molecular Medicine at the Faculty of Health Sciences of the University of Cape Town

Chronology of studies

The randomised controlled trial was designed in 2004 and a funding application to the Medical Research Council was successful that year. The trial recruited patients from June 2005 until December 2007. During the course of this trial 287 patients with suspected paradoxical TB-IRIS were screened and 110 participants were enrolled in the clinical trial. Pre-defined case definitions for TB-IRIS were used to screen patients for the clinical trial and during the work-up for the trial patients were investigated for drug resistant TB and alternative diagnoses. The first 100 patients screened in this way formed the basis for the study reported in Chapter 4 which reports the important differential diagnoses and clinical features of patients with paradoxical TB-IRIS with the most important finding that 10% of patients were found to have had previously undiagnosed rifampicin-resistant TB during work-up.

A further prospective cohort study enrolled 63 patients starting ART at Ubuntu TB-HIV clinic between January 2006 and September 2007. The main purpose of this study was to obtain blood samples for the longitudinal immunology study described in Chapter 5. Furthermore, blood samples were taken from patients seen at GF Jooste Hospital with TB-IRIS prior to enrollment in the clinical trial (included in the cross-sectional study described in Chapter 5) and during the clinical trial (included in the study described in Chapter 7 that assesses the effect of prednisone on mediators of inflammation).

In 2004-5, prior to commencing the clinical trial, we developed clinical case definitions for paradoxical TB-IRIS. These case definitions incorporated aspects of previous case definitions (8, 9), but were designed to be useful in a busy clinical setting utilizing no more than routine

laboratory tests. In November 2006, a meeting of IRIS researchers from around the world was held in Kampala, Uganda, and at this meeting the International Network for the Study of HIV-associated IRIS (INSHI) was formed. The major output of this meeting was the development of consensus case definitions for TB-IRIS (both the unmasking and paradoxical forms). The paradoxical TB-IRIS case definitions drew heavily from our case definitions and our experience of using these in our studies, and also incorporated aspects of other groups' case definitions. I was asked to be the head of the writing committee and lead author responsible for consolidating the consensus case definitions and preparing a paper for publication. The consensus case definitions were published in 2008 (10) and are described in Chapter 3.

Outline of the thesis

In **Chapter 2** (Background and literature review) background regarding the dual epidemic of HIV and TB in South Africa, antiretroviral therapy and its effect on the CD4 T cell recovery, the ART programme in South Africa and the Western Cape Province, issues related to the co-treatment of HIV and TB and the timing of ART in HIV-TB patients is presented. Thereafter the literature on three topics is reviewed in detail: clinical studies of TB-IRIS, immunological studies of TB-IRIS and studies that report the use of corticosteroids in the treatment of TB.

In **Chapter 3** the consensus case definitions developed at the 2006 INSHI meeting are presented. Consensus case definitions for the following conditions were developed: paradoxical TB-associated IRIS, ART-associated TB and unmasking TB-associated IRIS. The process of the development of consensus around these case definitions is discussed.

In **Chapter 4** the prospective clinical study in which clinical case definitions for paradoxical TB-IRIS were assessed among 100 patients with suspected paradoxical TB-IRIS referred to the Infectious Diseases Referral Unit or medical wards at GF Jooste Hospital is presented. The aim of this study was to ascertain the major differential diagnoses for paradoxical TB-IRIS in HIV-TB patients presenting with deteriorating TB after starting ART. The clinical features of the cases of paradoxical TB-IRIS are also described.

Chapter 5 reports two related studies that investigated the immunopathogenesis of paradoxical TB-IRIS: one a cross-sectional study of patients who presented with paradoxical TB-IRIS at GF Jooste Hospital and the other a longitudinal study of patients with HIV-associated TB starting ART at Ubuntu TB-HIV Clinic in Khayelitsha. In the cross-sectional study samples of 35 patients at the time of presentation with TB-IRIS were compared with two groups of controls. The first control group (n=29) was patients on TB treatment and ART who did not develop TB-IRIS (sampled at 2 weeks on ART) and the second control group (n=31) was HIV-associated TB patients prior to starting treatment for TB or ART. In the longitudinal study, samples were taken at 0, 2 and 4 weeks on ART and the serial samples of 10 cases who developed paradoxical TB-IRIS were compared with 29 controls who did not.

The hypothesis of these studies was that paradoxical TB-IRIS was associated with expansions of Th1 lymphocytes that had distinct *Mycobacterium tuberculosis* antigen specificity and that TB-IRIS was associated with greater expansions of Th1 lymphocytes, but reduced expansion of T-regulatory cells (CD4+ expressing FoxP3) in response to *Mycobacterium tuberculosis* antigen stimulation *in vitro*.

Experiments that we performed in these two studies to test these hypotheses were: 1) Interferon-gamma enzyme-linked immunospot (ELISpot) assays using the following antigens: PPD, ESAT-6, α -crystallin 1 and 2 and 38kDa cell wall antigen; and 2) flow cytometry on culture supernatants following stimulation with heat-killed MTB, staining for CD4, FoxP3 and the T cell activation markers HLA-DR and CD71.

Chapter 6 reports the randomized placebo-controlled clinical trial of prednisone for the treatment of paradoxical TB-IRIS. This was the first clinical trial of treatment for TB-IRIS. Prior to this there were anecdotal reports of response to corticosteroid therapy (11, 12). However, corticosteroids are potentially harmful in patients with advanced HIV, having been associated with herpes virus infection reactivations and Kaposi's sarcoma development and exacerbation (13-15). Given this equipoise, a clinical trial of corticosteroids to treat paradoxical TB-IRIS was warranted. The central hypothesis of this clinical trial was that a 4-week course of prednisone would reduce morbidity without an excess of corticosteroid side effects or infections in patients presenting with non-life threatening paradoxical TB-IRIS.

In **Chapter 7** an immunological study that arose out of the clinical trial is presented. In this study the effects of prednisone on mediators of inflammation in TB-IRIS was explored. The hypothesis of this study was that the clinically beneficial effect of corticosteroid treatment in TB-IRIS is mediated through effects on specific immune mediators. Our aim in this study was to define the effects that prednisone has on RNA expression, concentrations of specific cytokines and chemokines, and T cell expansions in vitro in response to mycobacterial antigens. In addition unstimulated serum was assayed for cytokine/chemokine concentrations. During the clinical trial samples were collected at 0, 2 and 4 weeks of the trial from 31 prednisone-treated patients and 27 placebo-treated patients. We performed three sets of experiments to explore the role of prednisone (with patients who were treated with placebo the controls in each experiment). The following experiments were performed: 1) ELISpot assays using the following antigenic stimuli: ESAT 6, α -crystallin 1 and 2, heat-killed H37Rv, 37kDa antigen and PPD; 2) real-time PCR on culture supernatants of samples cultured with heat-killed MTB to determine the RNA expression levels of the genes of 18 cytokines/chemokines longitudinally; and 3) assay of the protein levels of 17 of these cytokines/chemokines in the same tissue culture supernatants using multiplex cytokine fluorescent bead-based immunoassay as well as 12 cytokines/chemokines in unstimulated serum.

In **Chapter 8** the findings of the studies are summarized and conclusions from across all the studies are presented. The implications of the research for the field in general are discussed and priorities for future TB-IRIS research are identified.

Coherence of the thesis

The coherence of this body of work is underpinned by four common themes. Firstly, I (Graeme Meintjes) was the first author on all five of the papers included and was the lead investigator on all the studies. Secondly, all these studies have been undertaken under the joint supervision of Professors Robert J. Wilkinson and Gary Maartens (my PhD supervisors) while I have been based at the Institute of Infectious Diseases and Molecular Medicine at UCT and GF Jooste Hospital over the past 6 years. Thirdly, all of the research investigates

the same clinical condition (paradoxical TB-IRIS) within the same geographical and clinical setting. Finally, the work presented represents an evolution of investigation into this condition from clinical diagnosis, to immunopathogenesis, to treatment and finally an investigation of the immune mechanisms of effective treatment.

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CHAPTER 2

Background and literature review

The dual epidemic of HIV and tuberculosis in South Africa

South Africa is the country most severely affected by the global HIV epidemic. It is estimated that 5.5 million people live with HIV infection in SA (10.9% of the population over 2 years of age are HIV-infected according to the 2008 Human Sciences Research Council (HSRC) household survey (1)). While the South African population accounts for 0.7% of the world's population it accounts for 17% of all HIV infections worldwide (2).

The first case of AIDS was documented in South Africa in 1982. Through the 1980's the epidemic was largely concentrated among men who have sex with men and haemophiliacs who had received infected blood transfusions. The circulating virus was subtype B, which is the dominant strain in high-income countries (3, 4). There was little evidence of HIV spread in the general population. The early 1990's represented the initiation of a generalized subtype C epidemic (2, 3). Subtype C is the dominant strain in sub-Saharan Africa. The predominant modes of transmission shifted to heterosexual and mother to child transmission. The doubling time of the HIV epidemic was just over one year during the early 1990's (3). Through the late 1990's there was continued rapid spread through the general population. Data from government antenatal clinics demonstrate an increase in HIV seroprevalence among pregnant women from 0.8% in 1990 to 20.5% by 2000 (2). After 2000 the incidence of AIDS in the general population rose sharply with increasing numbers of people dying. By 2006 it was estimated that there were 345 640 deaths annually due to HIV (2). Life expectancy has fallen by almost 20 years and mean life expectancy is 48.4 years for men and 51.6 years for women according to 2008 estimates from Statistics South Africa (2). The most severely affected sector of the population is young women with an HIV prevalence of 32.7% among women aged 25-29 years documented in the 2008 HSRC survey. In men HIV prevalence peaks in the 30-34 year age group at 25.8% (Figure 1) (1). The response of the South African government until 2008 was characterized by denial, lack of political will and inefficiencies in implementation of policies (2). The HIV epidemic has profoundly impacted the economy of South Africa both at a macro-economic level and at the level of households from loss

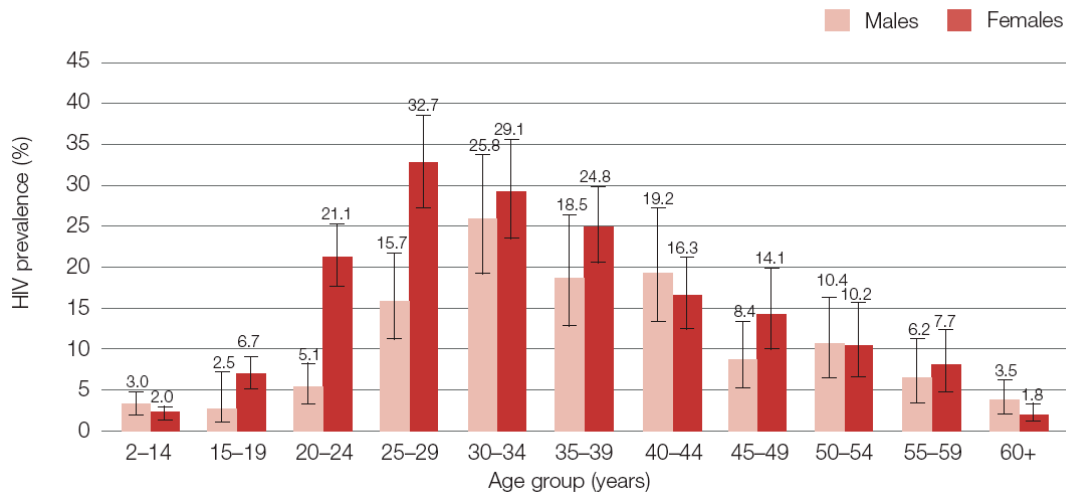


Figure 1: HIV prevalence, by sex and age, South Africa 2008.

From the Human Sciences Research Council South African National HIV Prevalence, Incidence, Behaviour and Communication Survey 2008 (reproduced from reference (1))

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of breadwinners and growing numbers of HIV orphans (5).

HIV impairs cell-mediated immunity that plays a critical role in containment of tuberculosis (TB). Patients with HIV, and particularly those with advanced HIV, are thus at heightened risk of reactivation of latent TB and progression from infection or re-infection to active disease. In South African individuals with advanced HIV the annual risk of active TB is as high as 30% (6). However, even in the first year of HIV infection the risk of active TB doubled compared with those who remained HIV negative in a study done on the South African gold mines (7). Thereafter, during the chronic phase of HIV infection, TB incidence steadily increased and by 11 years nearly half these men had developed active TB (8). In patients with advanced HIV and CD4 counts < 200 cells/mm³ the risk of active TB increases exponentially (6, 9). The HIV epidemic is undermining TB control efforts in many regions of the world (10, 11). Sub-Saharan Africa is now most severely impacted by the dual epidemic of HIV and TB, with TB incidence rates increasing by up to five-fold in many countries in the region over the last two decades (10). In South Africa annual TB case notification rose from 163 per 100 000 population in 1986 to 628 per 100 000 population in 2006 (2). The actual incidence of TB is greater given that not all cases are detected. In 2009, according to World Health Organization (WHO) estimates, 74% of cases were detected giving an estimated incidence of TB of 971 / 100 000 population for that year (2). In 2009, there were a total of 405 982 cases of TB notified in South Africa and 58% of those patients tested were HIV seropositive (2). However, other estimates suggest that up to 70% of TB cases are HIV co-infected (2). Certain communities are more severely affected, such as Khayelitsha in Cape Town where the TB case notification rate was 1612 / 100 000 in 2005 (City of Cape Town data (12)). The South African population is 0.7% of the world's population, but the country accounted for 28% of the global burden of HIV-associated TB in 2006 (8).

TB patients with HIV co-infection have a substantially higher case-fatality rate and TB is the leading cause of death in HIV-infected patients in developing world countries (13, 14). The WHO estimates that in 2007 there were 456 000 HIV-associated TB deaths globally accounting for a quarter of the estimated 2 million

HIV-related deaths that year (8). It is estimated that there were 105 000 TB deaths in South Africa in 2006 (2), the majority among HIV co-infected individuals.

The HIV epidemic has also coincided with an increase in the prevalence of drug-resistant TB in South Africa (15). The HIV epidemic has overwhelmed TB services undermining programme performance and has probably indirectly led to the emergence of drug resistant TB in this way. In addition, in congregate settings such as hospital wards and outpatient clinics transmission of drug resistant TB to patients with advanced HIV may result in outbreaks. HIV infection was associated with institutional outbreaks of multi-drug resistant (MDR) TB in the United States, Italy, Spain and Argentina in the 1980's and 1990's (15). These outbreaks mainly occurred in hospital settings where infection control practices were poor and patients with infectious TB and advanced HIV were in close proximity. The mortality rate was high (generally > 70%) with delay to the diagnosis of MDR a major factor contributing to this (15). The outbreak of extremely drug resistant (XDR) TB in Tugela Ferry in Kwazulu-Natal from 2005 onward could also be traced largely to hospital acquisition (16) and was initially associated with 98% mortality and all patients tested were HIV seropositive (17). The strain causing this outbreak was identified as F15/LAM4/KZN (18). Despite these associations with drug resistant TB outbreaks, HIV does not appear to be an independent risk factor for drug resistant TB in a community setting: people with HIV infection are at heightened and similar risk of both drug susceptible and resistant TB (15). In a cross-sectional survey among adult TB clinic attendees conducted in Khayelitsha in 2008 MDR was diagnosed among 3.3% of new TB cases and 7.7% of retreatment cases. There was not a significant association between rifampicin resistance and HIV infection in multivariate analysis (19). In South Africa as a whole, the number of MDR cases confirmed in the laboratory annually tripled between 2005 and 2007 from 2000 to 7350 and 5.6% of MDR cases were confirmed to have XDR between 2004-7 (20).

Antiretroviral therapy and immune recovery

Antiretroviral therapy (ART) first became available with the discovery that the nucleoside reverse transcriptase inhibitor zidovudine (AZT) had activity against HIV. However, it soon became apparent that the benefits of AZT were short lived due to

the development of drug resistance (21). This was followed by an era of dual therapy with nucleoside reverse transcriptase inhibitors, but with similar disappointing long-term results (22, 23). The discovery of the protease inhibitor class of drugs, which, when used with dual nucleoside reverse transcriptase inhibitors, made sustained suppression of HIV replication possible with durable long-term clinical benefit (24, 25). Since the mid-1990's combined antiretroviral therapy, usually with a combination of 3 drugs, has made it possible to achieve sustained suppression of HIV viral load in plasma to below 50 copies/ml. This allows for both quantitative and qualitative reversal of the immune suppression caused by HIV. After initiation of ART the majority of patients achieve a CD4 count greater 200 cells/mm³ (26). However, about a third of patients fail to achieve a CD4 count greater than 500 cells/mm³ despite viral suppression after 5 years. In the Swiss HIV Cohort 36% did not achieve a CD4 count above 500 cells/mm³ after 5 years despite continuous suppression of viral load below 1000 copies/ml (27). However, in a select group of patients on continuous ART for 10 years without a detectable viral load at any time point, CD4 counts continue to rise and reach normal levels regardless of the nadir CD4 count (28). Full restoration of the CD4 T cell compartment is thus possible on ART provided HIV replication remains suppressed and immune activation is efficiently controlled for a prolonged period (29).

This recovery of immune function has resulted in dramatic declines in AIDS-related morbidity and mortality (30, 31). Several studies have now demonstrated that with currently available ART options decades of life expectancy can be added for an HIV-infected person (32-34). There has been a dramatic and sustained reduction in the incidence of AIDS-related opportunistic infections in developed world countries since the mid 1990's when triple drug ART became available (35). Across studies performed in high and low TB burden settings ART use is associated with a 67% (95% confidence interval = 61-73%) reduction in TB incidence rates among HIV-infected people (36).

Autran and colleagues (37) demonstrated soon after the introduction of triple drug ART that there are three phases of T cell reconstitution. There is an early rise of memory CD4 T cells. This is thought to reflect recirculation of cells previously recruited into productively infected tissues once viral replication is suppressed

representing recovery from inflammatory responses to the HIV infection. There is little de novo production of immune cells in the first 2-3 months of ART (29). The second phase is characterized by a reduction in T cell activation with improved CD4+ T cell reactivity to recall antigens. There is a later rise of naïve CD4 T cells while CD8 T cells decline (37). The regeneration of naïve CD4 T cells is accompanied by restoration in the diversity of the CD4 T cell receptor repertoire (38).

Reconstitution of mycobacterial-specific CD4 T cell immune responses was explored using flow cytometry after PPD stimulation of peripheral blood mononuclear cells (PBMC) in a cohort of 19 HIV-infected patients with latent TB starting ART in South Africa (39). The first cells to proportionally expand were central memory CD4+ T cells by 12 weeks, representing the strongest correlate of ART mediated immunity. This was followed by expansion of naïve CD4 T cells by 36 weeks. Terminally differentiated effector cells proportionately decreased by 12 weeks, however the absolute number of PPD-specific IFN- γ producing cells determined by ELISpot increased during this period.

CD4+ CCR5+ T-lymphocytes are almost completely depleted in the gut-associated lymphoid tissue during the first weeks of acute HIV infection and this depletion is sustained through chronic HIV infection. Despite reconstitution of systemic CD4 T cells, CD4 T cell reconstitution in the small intestine mucosa has been demonstrated to be poor and occurs much more slowly than in peripheral blood. It is hypothesized that this is related to ongoing low level HIV replication in the gut mucosa and fibrotic damage to the Peyer's patches, which impairs their ability to support CD4+ T cell reconstitution in the gut (40).

Predictors of poor reconstitution of systemic CD4 T cell counts on ART include: older age (27, 41, 42), longer duration of HIV infection prior to ART (27), lower CD4 T cell count at ART initiation (43, 44), lower number of naïve CD4 T cells (45), higher levels of immune activation (46) and intermittent viral replication during ART (47). Even if there is adequate CD4 T cell count recovery on ART, restoration of functional immunity on ART may be incomplete as reflected in studies that have assessed immune responses to vaccination. One study showed that despite reconstitution of CD4 T cell numbers on ART memory B and T cell responses to

tetanus and diphtheria toxoid were impaired in patients with low nadir CD4 T cell counts and not related to current CD4 T cell count on ART (48).

ART programme in South Africa and the Western Cape Province

ART was available in the South African private sector from the 1990's initially as dual therapy for cost reasons. Because of the prohibitive costs of the ART drugs at the time and the skepticism towards ART from the national government of the time, no ART was available in the public sector in South Africa until 2004. However, through partnerships between non-governmental organizations and the Provincial Government of the Western Cape, ART was made available to public sector patients at three clinics in Khayelitsha and one in Gugulethu in Cape Town from 2001 (49, 50). In the programme initiated at the 3 Khayelitsha clinics by Medecins sans Frontieres the annual enrolment in the ART programme increased from 80 patients in 2001 to 2087 by 2006 (51). Data on patients starting and maintained on ART in the Western Cape province is prospectively captured into facility-based registers from which monthly cross-sectional activity and quarterly cohort reports are aggregated (52). Data from this reporting system show that by the end of February 2011, 88 100 adults and 6432 children were receiving ART in the Western Cape province public-sector programme and it is estimated that 1.4 million people have accessed ART nationally.

The South African public sector ART programme has followed a public health approach with standardized criteria for initiating, monitoring and switching ART. The first line and second line regimens are also standardized with substitutions allowed in the event of drug toxicities. The initial first line regimen used in the national programme was stavudine (D4T), lamivudine (3TC) and a non-nucleoside reverse transcriptase inhibitor (nevirapine or efavirenz). Efavirenz is advised in patients who are on rifampicin-based TB treatment when starting ART. In April 2010, tenofovir was substituted for stavudine in the first line regimen due to the toxicity issues related to stavudine (53-55).

Five-year outcome data from the Khayelitsha clinics (n=7323) shows that 9.8% of people were lost to follow-up for at least 6 months of whom one-third had died. Corrected mortality at 5 years was 20.9%, but mortality fell over time as patients

accessed ART with higher CD4 counts (the median CD4 count in patients starting ART was 43 cells/mm³ in 2001 and 131 cells/mm³ in 2006). By 5 years 14% of patients had failed first line ART virologically (51).

Co-treatment of HIV and TB

In patients who are diagnosed with active TB and require the initiation of ART there are a number of complexities to co-treatment of both conditions. These include shared drug toxicities, drug interactions, high pill burden (which potentially impacts adherence) and the paradoxical TB-associated immune reconstitution inflammatory syndrome (TB-IRIS).

Shared drug toxicities include drug-induced liver injury (which may be caused by rifampicin, isoniazid, pyrazinamide and the non-nucleoside reverse transcriptase inhibitors (NNRTI's) and protease inhibitors (PI's)), drug rashes (which may be caused by many of the TB drugs and the NNRTI's), peripheral neuropathy (which may be caused by stavudine, didanosine and isoniazid), renal impairment (related to tenofovir or aminoglycosides used to treat TB) and gastro-intestinal intolerance (related to many of the TB and ART drugs) (56). When severe drug toxicity occurs in a patient on treatment for both TB and HIV, treatment often needs to be interrupted and a complex rechallenge process follows. Such interruptions may contribute to morbidity and mortality due to inadequate therapy and the emergence of drug resistance.

Pharmacokinetic drug interactions are mainly related to rifampicin being a potent inducer of cytochrome P450 enzymes, particularly isoenzyme 3A4/5, and the drug transporter p-glycoprotein. The effect of rifampicin is to moderately reduce the levels of efavirenz (although standard doses are sufficient), but more significantly reduce nevirapine concentrations (with an effect on virological outcomes) (57-59).

Rifampicin causes very substantial reduction in the concentrations of all PI's and if used with PI's such as lopinavir and saquinavir then additional boosting with ritonavir is required (56).

Because of these clinical challenges the optimal timing of ART in HIV-infected TB patients has been an important research issue internationally.

The timing of ART in TB patients

The timing of ART in HIV-infected TB patients with low CD4 counts needs to balance the potential complexities of co-treatment with the high risk of HIV disease progression with associated mortality in such patients. ART has been shown to decrease mortality in HIV-infected TB patients by 64-95% (60). To answer the question of when the optimal time to start ART in such patients is, a number of randomized strategy trials have compared different timing of ART initiation during TB treatment.

The CAMELIA study (61) conducted in Cambodia among patients with smear positive TB and $CD4 \leq 200$ cells/mm³ compared starting ART 2 weeks versus 8 weeks after starting TB treatment. Patients in this trial had very advanced HIV with median CD4 count of 25 cells/mm³ and body mass index of 17. There was a 34% reduction in mortality in those who started at 2 weeks. The ACTG 5221 STRIDE study (62) was a multi-country study that enrolled patients with confirmed or suspected TB that had a CD count < 250 cells/mm³. ART was started 2 weeks after TB treatment in the one arm and between 8-12 weeks in the other. There was no difference in the combined endpoint of AIDS progression and death between the two arms. However, in a sub-analysis of only those with CD4 counts ≤ 50 cells/mm³ AIDS progression and death was reduced by 42% among those who started at 2 weeks.

Similar findings were demonstrated in the SAPIt study (63) conducted in Durban. This study enrolled patients with smear-positive PTB and CD4 counts < 500 cells/mm³. The most recent report from this study, presented at the 18th Conference on Retroviruses and Opportunistic Infections (64), compared outcomes in the two integrated arms of the study: one arm started ART within 4 weeks of starting TB treatment and the other within 4 weeks of the completion of intensive phase of TB treatment. There was no difference in the combined endpoint of AIDS progression or

death comparing the two arms, but again in a sub-analysis of those with CD4 < 50 cells/mm³, earlier ART (at a median of 8 days) reduced AIDS progression or death by 68% (marginally significant, p=0.06). In all three of these studies, the incidence of paradoxical TB-IRIS was approximately 2 to 3 fold higher among those starting ART in the earlier arm.

Finally, in a study of ART timing in patients with TB meningitis conducted in Vietnam (65) there was no difference in survival among patients starting ART immediately or deferring 2 months. Mortality at 9 months was around 60% in both arms. Patients in this study were treated with adjunctive high dose dexamethasone for the first 6-8 weeks of TB treatment. Grade 4 adverse events occurred more frequently in patients who started immediately.

In summary, these studies demonstrate that TB patients with a CD4 < 50 cells/mm³ benefit from starting ART within 2 weeks of starting TB treatment with one study showing this reduced mortality and two demonstrating a reduction in a combined endpoint of AIDS progression and death among these patients, despite a higher incidence of paradoxical TB-IRIS. Among those with higher CD4 counts deferring ART up to 2 months may reduce the risk of TB-IRIS without compromising outcome. In TB meningitis mortality is extremely high and unaffected by the exact timing of ART within the first 2 months of TB treatment and deferring ART a few weeks may reduce risk of severe adverse events.

Immune reconstitution inflammatory syndrome: history and spectrum

The immune reconstitution inflammatory syndrome (IRIS) was first described among Australian patients in the early 1990's. After commencing AZT monotherapy a localized form of *Mycobacterium avium intracellulare* (MAI) infection occurred concurrently with restoration of cutaneous delayed-type hypersensitivity responses to mycobacterial antigens. This was characterized by lymphadenitis and fevers 1-2 weeks after commencing AZT, and differed in clinical presentation to the MAI infections that had been previously encountered in patients with advanced HIV which typically presented with wasting, fevers, gastro-intestinal symptoms and cytopaenias.

The atypical presentations were thought to result from the restoration of cellular immunity (66).

Since then a wide range of IRIS conditions has been described in association with viral infections (eg. cytomegalovirus immune recovery uveitis), fungi (eg. cryptococcal IRIS), other mycobacteria (eg. TB-IRIS), parasites (eg. schistosomiasis IRIS), tumours (eg. Kaposi's sarcoma IRIS), auto-immune conditions (eg. Grave's disease of the thyroid) and other inflammatory conditions such as sarcoidosis (67). IRIS may occur in the context of treated or untreated infections and has been most frequently described in association with mycobacterial, fungal and herpesvirus infections (68). IRIS associated with HIV itself has also been described in patients with HIV encephalopathy who deteriorate neurologically after starting ART due to encephalitis which has been shown on brain histology to be related to CD8+ T-lymphocyte infiltration (69). Synonymous terms used to describe IRIS that have been used in the literature are immune restoration disease (IRD) and immune reconstitution syndrome (IRS).

IRIS has also been described in HIV-negative patients recovering from iatrogenic immunosuppression (70, 71). The most common situations in which this is described is with recovery of neutrophil counts following engraftment of bone marrow transplants and in patients where iatrogenic immunosuppression is withdrawn. An example of the latter is patients on tumour necrosis factor α (TNF α) receptor inhibitors who are diagnosed with TB, started on TB treatment and thereafter the TNF α receptor inhibitor is withdrawn. A life threatening paradoxical reaction involving the lungs has been described in such a patient (72).

Tuberculosis-associated immune reconstitution inflammatory syndrome (TB-IRIS) and unmasking of tuberculosis by antiretroviral therapy

It was well recognized prior to the HIV era that the immune response to TB contributes to pathology as well as protection. Paradoxical TB reactions (discussed below) and the basal exudate complicated by endarteritis that may develop in TB meningitis are examples of this immunopathology. During the early period of rapid immune recovery on ART, immunopathology in the form of IRIS has emerged as an

important clinical entity altering the clinical presentation and course of TB infection. TB-IRIS is thought to result from dysregulated recovering immune responses driving exaggerated inflammation directed towards the antigens of *Mycobacterium tuberculosis* resulting in clinical deterioration in patients experiencing immune recovery during early ART (67, 73).

TB-IRIS is a frequent early complication of ART, especially in countries where TB is prevalent. Two forms of TB-IRIS are recognized: paradoxical and unmasking. Paradoxical TB-IRIS manifests with new or recurrent TB symptoms or signs in patients being treated for TB during early ART and unmasking TB-IRIS is characterized by an exaggerated, unusually inflammatory initial presentation of TB following initiation of ART (74).

The diagnosis of TB is a common reason for HIV testing and entry into HIV care in sub-Saharan Africa. ART dramatically improves survival in HIV-infected people (75) and reduces TB risk by 70-90% (76-79), but very high TB incidence rates have been noted in the first 3 months of ART in developing countries (80-82). A substantial proportion of patients starting ART in sub-Saharan Africa are on treatment for active TB (up to 42% (51)) or have undiagnosed active TB (81), and are therefore at risk of TB-IRIS.

Paradoxical TB reactions in patients not on ART

Paradoxical reactions during TB treatment (new or recurrent TB symptoms or signs occurring after initial response to treatment) are recognized to occur in HIV-uninfected patients and HIV-infected patients not on ART. Up to 25% of patients with TB lymphadenitis will experience a paradoxical reaction usually manifesting as enlargement of the lymph nodes (83, 84). Other manifestations include recurrent fevers, worsening pulmonary infiltrates, enlarging pleural effusions, the development of tuberculous meningitis (TBM), new or enlarging tuberculomas or tuberculous lesions developing at other anatomical sites (85-87). In a South African case series, reported prior to widespread ART availability, 23% of TBM in HIV-infected patients was diagnosed in patients already on TB treatment, so-called “breakthrough” TBM (88).

These paradoxical reactions are thought to reflect an immunologically-mediated deterioration rather than TB treatment failure. The pathogenesis has variably been attributed to a combination of the following factors: persistence of lipid rich insoluble cell wall antigen in infected tissue (84), exposure and release of new antigen targets during mycobacterial killing (89), hypersensitivity to such antigens (89) and exaggerated immune restoration (following TB-induced immunosuppression) occurring on TB treatment (86). The development of paradoxical reactions in patients not infected with HIV is associated with greater increases in total lymphocyte count on TB treatment (86).

Paradoxical TB-IRIS: clinical aspects

Paradoxical TB-IRIS is a form of paradoxical TB reaction that occurs during early ART-mediated immune recovery and which is often more severe and more frequently involves multiple organ systems than paradoxical reactions seen in patients not on ART. Paradoxical reactions are also far more frequent in the period after ART initiation than in HIV negative patients and HIV-infected patients not on ART (36% vs 7% vs 2% respectively in one study (90)).

Paradoxical TB-IRIS occurs in 8-46% of patients starting ART while on TB treatment (Table 1). In a meta-analysis of studies reporting the incidence of paradoxical TB-IRIS the pooled cumulative incidence was 15.7% (95% credibility interval calculated using Bayesian methods = 9.7 – 24.5%) (91). In the same meta-analysis the mortality risk associated with the development of TB-IRIS was reported as 3.2% (95% credibility interval = 0.7 – 9.2%). Death due to paradoxical TB-IRIS is infrequent apart from when the central nervous system is affected (discussed below) and certain of the deaths occurring in TB-IRIS patients are not due to TB-IRIS but due to other infections (92). In cohort studies the mortality rate in patients who develop paradoxical TB-IRIS has been no different to TB patients starting ART who do not develop paradoxical TB-IRIS (93, 94). In contrast, the mortality risk associated with cryptococcal IRIS is much higher: the estimate was 20.8% (95% credibility interval = 5.0 - 52.7%) in the same meta-analysis (91).

TABLE 1: Cohort studies reporting the incidence, treatment and outcome of paradoxical tuberculosis-associated immune reconstitution inflammatory syndrome (TB-IRIS) in adult patients ^a

First author (Country and year of publication)	Incidence of paradoxical TB-IRIS	Treatment of IRIS episode	Duration symptom onset to resolution of IRIS episode	Number of deaths (% of IRIS cases)
Narita (90) (United States, 1998)	12/33 (36%)	Corticosteroids (2) ART interruption (1)	NR	NR
Breen (95) (UK, 2004)	8/28 (29%)	Prednisone (8 of 14) ^b	NR	NR
Breton (96) (France, 2004)	16/37 (43%)	Corticosteroids (6) NSAIDs (2) ART interruption (7) Surgery (1)	NR	NR
Kumarasamy (97) (India, 2004)	11/144 (8%)	Corticosteroids (6) NSAIDs (5) Aspiration (1)	NR	0 (0%)
Michailidis (98) (UK, 2005)	9/28 (32%)	Corticosteroids (11) IL-2 and GM-CSF (1) Aspiration (10) ^c	Median 2.5 months (range 0.5-15 months) ^c	NR
Manosuthi (94) (Thailand, 2006)	21/167 (13%)	Corticosteroids (11)	NR	2 (10%)
Lawn (93) (South Africa, 2007)	19/160 (12%)	Corticosteroids (2) Laparotomy (1)	NR	2 (11%)
Burman (99) (United States, 2007)	19/109 (17%)	Corticosteroids (4) Aspirations (11) Surgical drainage (6) ^d	Median 60 days (range 11-442 days) ^d	1 (5%)
Tansuphasawadikul (100) (Thailand, 2007)	15/101 (15%)	Corticosteroids (6)	NR	0 (0%)
Serra (101) (Brazil, 2007)	10/84 (12%) ^c	Prednisone (8) NSAIDs (2)	Mean 91 days +/- 30 days	0 (0%)
Baalwa (102) (Uganda, 2008)	13/45 (29%)	NR	NR	NR
Manosuthi (103) (Thailand, 2009)	22/126 (18%)	NR	NR	0 (0%)
Elliott (104) (Cambodia, 2009)	15/75 (20%)	NR	NR	0 (0%)

Bourgarit (105) (France, 2009)	11/24 (46%)	NR	NR	NR
Sharma (106) (India, 2010)	18/237 (8%)	Corticosteroids (2) Surgical drainage (1) NSAIDs or paracetamol (15)	NR	0 (0%)
Eshun-Wilson (107) (South Africa, 2010)	35/333 (11%)	Corticosteroids (13)	NR	1 (2%)
Worodria (92) (Uganda, 2011)	61/258 (24%)	NR	NR	6 (10%)
Haddow (108) (South Africa, 2011)	14/102 (14%)	NR	NR	NR

Legend for Table 1

^a This table is restricted to cohort studies that assessed the incidence and outcomes of paradoxical TB-IRIS in adult patients starting ART while on TB treatment and where 8 or more cases of TB-IRIS were documented. Cohorts in which all-cause IRIS was assessed are not included.

^b This study reported 14 paradoxical reactions in HIV-infected patients, 8 of them in patients who started ART after TB treatment (paradoxical TB-IRIS). Among all 14, 8 were treated with corticosteroids.

^c This study reported 14 cases of paradoxical reaction, 9 of which were paradoxical TB-IRIS. Data regarding treatment and duration relate to all 14 patients.

^d Burman et al reported 25 cases of paradoxical reaction in HIV-infected patients, 19 of which were in patients starting ART after TB treatment. Data regarding treatment and duration are for all 25 cases.

^e Of these 10 patients who had paradoxical reactions, 9 were on ART when the paradoxical reaction occurred and 1 was not.

NR = Not reported

NSAIDs = Non-steroidal anti-inflammatory drugs

The median interval from ART initiation to onset of paradoxical TB IRIS is typically 2-4 weeks (90, 93-96, 101), but cases may occur within a few days and rarely onset may be months after ART is started (109). The median duration of symptoms is reported to be 2-3 months (98, 99, 109). A minority of cases have manifestations that last for more than a year (99, 109, 110) and recurrent episodes are described up to 4 years after ART initiation (111).

The most frequent clinical features of TB-IRIS are recurrent symptoms, fever, enlarging inflammatory lymph nodes (Figure 1) and new or enlarging serous effusions. In addition, worsening of radiographic pulmonary infiltrates (Figure 2) is seen in up to 45% of TB patients starting ART and patterns observed include consolidation, cavitation, miliary infiltrates and cystic changes (112, 113). Subcutaneous and deep tissue abscesses may form (99, 110). Common manifestations are listed in Table 2. There are case reports of other rare manifestations such as hypercalcaemia (attributed to TB-IRIS granulomas increasing production 1,25 dihydroxy-Vitamin D3) (114, 115), choroidal granuloma enlargement and retinal detachment (116) and chylothorax (117).

Abdominal manifestations of TB-IRIS are frequent, but not widely reported. Hepatic and splenic involvement may occur together with intestinal lesions, peritonitis, ascites, enlargement of intra-abdominal lymphadenopathy (Figure 3) and formation of abscesses including psoas abscesses (118, 119). Abdominal symptoms reported include abdominal pain, nausea, vomiting and diarrhea (94, 118). Hepatic involvement, which occurs in between 21-56% of TB-IRIS cases, can be difficult to differentiate from drug-induced hepatitis (118, 120). Hepatic TB-IRIS manifests with tender liver enlargement, cholestatic liver function derangement with or without jaundice and frequently there is evidence of TB-IRIS at other sites. A granulomatous hepatitis is found on liver biopsy (120). Such patients may have had subclinical abdominal involvement at the time of TB diagnosis and only manifest with abdominal features at the time of IRIS.

Table 2: Common manifestations of paradoxical TB-IRIS

Worsening or recurrence of TB symptoms

Weight loss

Fever

Systemic inflammatory syndrome

Lymph node enlargement

Cold abscess formation

Respiratory manifestations

Progressive pulmonary infiltrates on chest radiography (several patterns described, including miliary infiltrates and alveolitis)

Pleural effusions

Lymph node compression of airways

Abdominal manifestations

Hepatic enlargement and abscesses

Splenic enlargement, rupture and abscesses

Intestinal involvement, eg. ileo-caecal perforation

Peritonitis

Ascites

Psoas and intra-abdominal abscesses

Central nervous system manifestations

Tuberculoma enlargement

Meningitis

Myeloradiculitis

Genito-urinary tract manifestations

Renal involvement with acute renal failure

Ureteric compression

Epididymo-orchitis

Other system involvement

Arthritis and osteitis

Pericardial effusion

Bone marrow involvement

(Originally produced for reference (121))

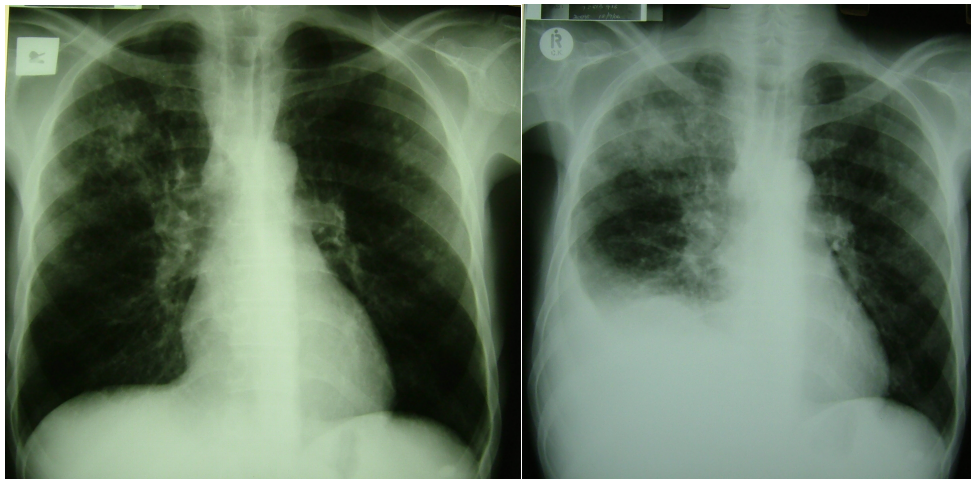
Figure 1: Supraclavicular lymphadenitis due to TB-IRIS

This 23 year-old HIV-infected woman with a CD4 count of 143 cells/mm³ was diagnosed with pulmonary TB on the basis of a sputum culture that grew *Mycobacterium tuberculosis* susceptible to isoniazid and rifampicin. At the time of TB diagnosis she had small cervical lymph nodes. She reported initial symptom response on TB treatment and started ART 3 weeks later. Three weeks after starting ART she developed painful right supraclavicular lymphadenitis that enlarged to a nodal mass measuring 15 x 8 cm with overlying erythema and tenderness characteristic of TB-IRIS lymphadenitis.



Figure 2: Worsening pulmonary infiltrate and new pleural effusion

A 49-year old HIV-infected man with CD4 of 29 cells/mm³ was diagnosed with drug-susceptible pulmonary TB (chest radiograph A). He started ART 2 weeks after TB treatment. Two weeks later he developed recurrent TB symptoms, worsening of pulmonary infiltrate and new pleural effusion due to paradoxical TB-IRIS (chest radiograph B). The TB sputum culture and pleural aspirate TB culture were negative at the time of TB-IRIS.



A

B

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Figure 3: Abdominal lymphadenitis due to TB-IRIS

A 21-year old HIV-infected woman with a CD4 count of 77 cells/mm³ was diagnosed with pulmonary TB (*Mycobacterium tuberculosis* isolated from sputum was susceptible to rifampicin and isoniazid). She commenced ART (stavudine, lamivudine and efavirenz) five weeks after starting 5-drug antitubercular therapy that she was taking as an inpatient. After two weeks on ART she developed progressively worsening abdominal pain and severe vomiting. The pain required opiate analgesia. A contrasted CT scan of her abdomen revealed mesenteric and retroperitoneal rim-enhancing, coalescent lymph nodes with necrotic centres (arrowed), that were thought to be the cause of her symptoms and the consequence of paradoxical TB-IRIS. A percutaneous aspirate of one of these nodes grew drug-susceptible *Mycobacterium tuberculosis*.

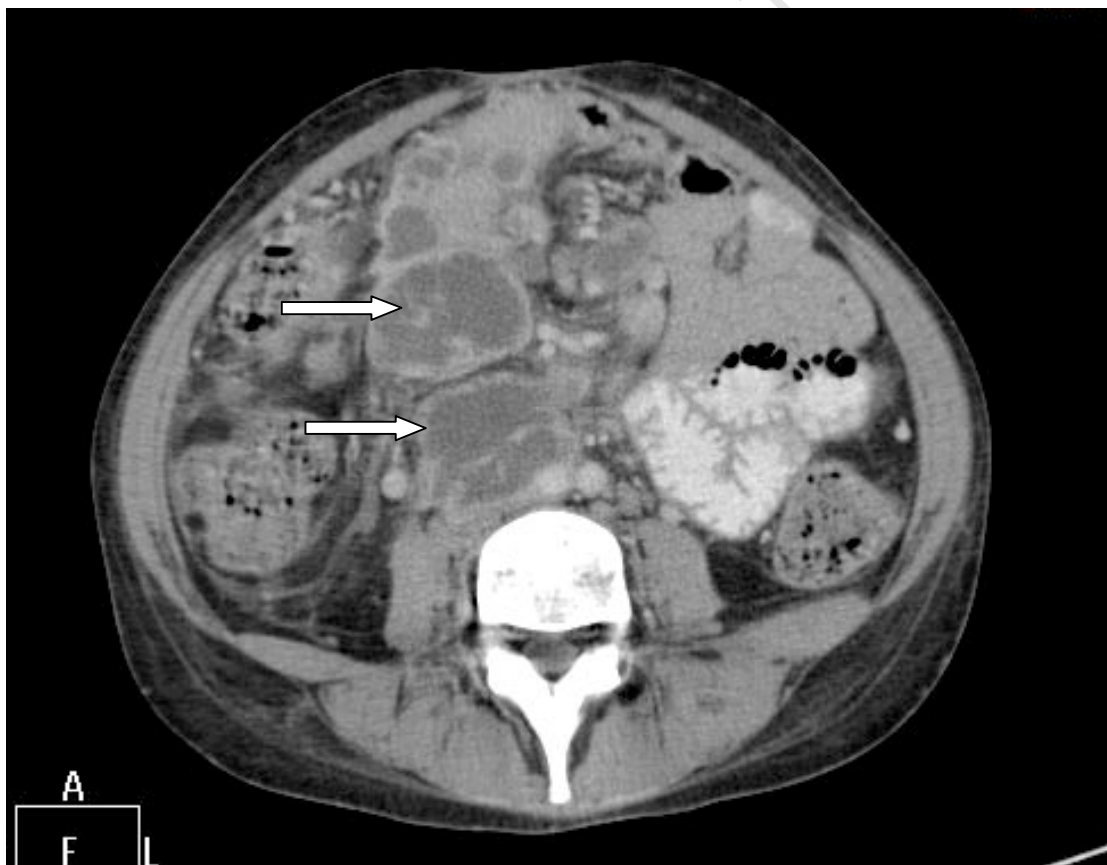


Figure 4: Fatal enlargement of intracerebral tuberculoma due to TB-IRIS

A 34-year old HIV-infected woman with a CD4 count of 26 cells/mm³ was diagnosed with TB meningitis after presenting with headaches and neck stiffness. Lumbar puncture demonstrated lymphocytic meningitis, CSF cryptococcal antigen test was negative and chest radiograph was compatible with pulmonary TB. She started TB treatment and prednisone and improved significantly. She was able to self-care after discharge and started ART 6 weeks after TB treatment. Two weeks after starting ART she was admitted following several generalized convulsions and had a depressed level of consciousness and right hemiparesis. CT scan showed a large left fronto-parietal ring-enhancing lesion with extensive surrounding oedema and mid-line shift. ART was stopped, she was commenced on high dose dexamethasone and anitconvulsants, but deteriorated and died 2 days later.



Neurological deterioration has been reported as 12% of paradoxical TB-IRIS cases (122). Manifestations include new or recurrent meningitis, enlarging tuberculomas (Figure 4) and radiculomyelopathy. In the case series (n=23) of neurologic TB-IRIS reported by Pepper et al (122), only 70% of patients were known to be alive at 6 months; and of the survivors 6 of 16 had long term neurologic disability. Other life threatening manifestations of paradoxical TB-IRIS include pericardial tamponade (123), acute renal failure (124), splenic rupture (96, 125), intestinal perforation (126), airway compromise due to compression by enlarging nodes (96) and respiratory failure. As stated above overall reported deaths from paradoxical TB-IRIS are rare (Table 1), although this may represent publication bias. There is one study that has reported a high mortality rate among paradoxical TB-IRIS cases from Ethiopia (5 of 11 (45%) of cases died), but the causes of these deaths was not reported (127). Whilst many cases are self-limiting, paradoxical TB-IRIS results in considerable morbidity and places considerable burden on the health services. Patients frequently require hospitalization, diagnostic and therapeutic procedures.

The risk factors for paradoxical TB-IRIS most consistently identified are: disseminated TB, low CD4 count prior to ART and shorter interval from TB treatment to ART (93-96, 98, 99, 128). Should ART therefore be delayed in order to reduce the risk of TB-IRIS? This consideration needs to be counterbalanced by the risk that delaying ART confers in terms of increased HIV disease progression and the additional opportunistic infections and mortality associated with this. A further complication is that patients at highest risk for TB-IRIS are those with low CD4 counts who are most at risk of HIV complications if ART is delayed. In addition, delaying ART until after 2 months of TB treatment does not necessarily prevent paradoxical TB-IRIS. In a Ugandan study, 22% of patients starting ART within 2 months developed TB-IRIS whereas 31% of those starting after 2 months developed TB-IRIS (102). As discussed above, several randomized clinical trials have now demonstrated that mortality (or the combined endpoint of mortality and AIDS progression) is reduced if ART is started within 2 weeks of having started TB treatment in patients with low CD4 counts ($CD4 < 50 \text{ cells/mm}^3$). This survival benefit of early ART in such patients clearly takes precedence over concerns of a higher incidence of paradoxical TB-IRIS.

Paradoxical TB-IRIS is diagnosed on the basis of a characteristic clinical presentation, temporal relationship to the initiation of ART and exclusion of alternative explanations for clinical deterioration. It is important to investigate for other opportunistic infections and malignancies, TB treatment failure (due to non-adherence, TB drug resistance or malabsorption of TB drugs) or drug reaction. In addition, paradoxical TB-IRIS may develop in patients with undiagnosed rifampicin resistance, clinically indistinguishable from TB-IRIS that occurs in patients with drug-susceptible disease (data in this regard is presented in Chapter 4)(118). Where possible drug susceptibility testing, preferably using a rapid diagnostic assay, should be performed in all patients presenting with paradoxical TB-IRIS. Other investigations in a particular case will depend on the nature of the clinical presentation. Consensus case definitions for use in resource limited settings have been developed (presented in Chapter 3)(110).

Mild cases require reassurance and symptomatic treatment. In cases with more significant symptoms non-steroidal anti-inflammatory drugs and corticosteroids have been used. Corticosteroid therapy will exacerbate other untreated infections or drug-resistant TB, so should only be considered for paradoxical TB-IRIS when alternative diagnoses have been excluded. Breen reported 8 HIV-infected patients with paradoxical reactions or paradoxical TB-IRIS who all responded to prednisone at a range of doses (10-80mg/day)(95). Our randomized placebo-controlled trial of prednisone for the treatment of paradoxical-TB IRIS (presented in Chapter 6) demonstrated a significant reduction in a combined endpoint of days hospitalized plus outpatient therapeutic procedures in prednisone-treated cases. Significant benefit was also demonstrated for symptom improvement. Patients received prednisone or placebo at a dose of 1.5mg/kg for 2 weeks followed by 0.75mg/kg for 2 weeks. Cases with immediately life-threatening manifestations were excluded from this trial (129). Corticosteroid use is associated with risks such as reactivations of herpes virus infections, Kaposi's sarcoma and metabolic side effects (130-132). Some patients require corticosteroids for several months (133) where side effects are likely to be greater. There is a case report of successful treatment with the TNF α receptor antagonist infliximab of a TB paradoxical reaction involving the central nervous system (134) and the role of these agents in life-threatening TB-IRIS warrants further

investigation. Needle aspiration to provide symptom relief and for aesthetic reasons for suppurative lymphadenitis or cold abscesses may be required (99). ART interruption may rarely be considered in life-threatening cases, for example those with depressed level of consciousness due to neurological involvement.

Immunopathogenesis of paradoxical TB-IRIS

IRIS occurs during the initial weeks to months of effective ART during a period of rapid recovery in immune function. While IRIS is characterized by exaggerated inflammatory responses clinically, driven by this rapid recovery in immunity, the precise pathogenic mechanisms are incompletely understood. Inflammatory features are heterogenous: mycobacterial and fungal IRIS are characterized by delayed type hypersensitivity responses, granuloma formation and in some cases suppuration whereas in viral conditions CD8⁺ T-lymphocyte responses predominate (67, 119, 135).

The pathogenesis of TB-IRIS is related to recovery of MTB-specific immunity but the relative contribution of various components and cells of the immune system is a field of intense and ongoing study (Figure 5). The first report to suggest that mycobacterial IRIS coincided with recovery of mycobacterial-specific immune responses was in patients who developed a localized form of *Mycobacterium avium* complex (MAC) disease, such as lymphadenitis, after starting AZT monotherapy in the early 1990's. Reversion of tuberculin or MAC purified protein derivative (PPD) skin tests from anergic to positive was demonstrated (66). This was later also reported in patients with anergic PPD skin tests who developed paradoxical TB-IRIS who developed strongly positive skin tests around the time of IRIS (90). These studies implicate recovery of delayed type hypersensitivity responses in pathogenesis. Certain patients with TB-IRIS in the latter study (90) had not had a CD4 count rise at the time of IRIS, suggesting that at a tissue level IRIS may occur prior to CD4 count recovery in peripheral blood. This has also been observed in patients with MAC IRIS (136).

The association between increases in mycobacterial-specific T cells in peripheral blood and paradoxical TB-IRIS has been investigated using enzyme-linked immunospot (ELISpot) assays, whole blood interferon-gamma release assays and

flow cytometry (104, 105, 137-140). Bourgarit et al (137) showed that TB-IRIS was associated with large expansions of PPD-specific interferon- γ producing T cells. Expansions were not seen in the TB-IRIS patients when CMV antigens or ESAT-6 were used as antigen stimuli. Similar findings have been reproduced in other studies (104, 139). Bourgarit et al (105) subsequently reported that these PPD-specific T cells are multifunctional (IFN- γ + TNF- α + IL2-), of the CD4+ effector memory phenotype and highly activated. In contrast, our study presented in Chapter 5 showed that while TB-IRIS patients had greater median IFN- γ -producing T cell expansions in response to a number of mycobacterial antigens in cross-sectional analysis, the responses in both cases and controls were heterogenous, and in longitudinal analysis responses were highly variable and dynamic (with expansions and contractions occurring in both cases and controls). That these expansions are the cause of TB-IRIS is questioned (138). In addition, a Thai study found no significant differences in cytokine assays for T helper 1 cytokines (IL-2, IL-12, and IFN- γ) following PPD and RD1 antigen stimulation between patients who developed TB-IRIS and controls prior to ART or at time of IRIS (140).

Bourgarit et al (105) also observed that TB-IRIS patients had high levels of $\gamma\delta$ T cells not expressing KIR (killer Ig-related receptor) at baseline and at TB-IRIS, suggesting a role for these cells in the pathogenesis of TB-IRIS and that they may be deficiently regulated. $\gamma\delta$ T cells respond to mycobacterial phospho-antigens and produce large amounts of IFN- γ .

It has been suggested that paradoxical TB-IRIS is precipitated by a “cytokine storm” (141). TB-IRIS was accompanied by elevations in a range of Th1 and inflammatory cytokines and chemokines, but not Th2 cytokines in one study (137). In a South African study (142) cytokine concentrations were studied in patients who developed TB-IRIS and compared to controls (patients with HIV-associated TB who did not develop TB-IRIS on ART) at 2 weeks on ART. A broad range of cytokines and chemokines were elevated after in vitro re-stimulation but the most consistently elevated cytokines and those significantly elevated in unstimulated serum after correction for multiple comparisons were TNF- α , IFN- γ and IL-6. Oliver et al (143) demonstrated that TB-IRIS was associated with higher concentrations of CXCL10 and IL-18 and lower levels of CCL2 in unstimulated samples and suggest therefore

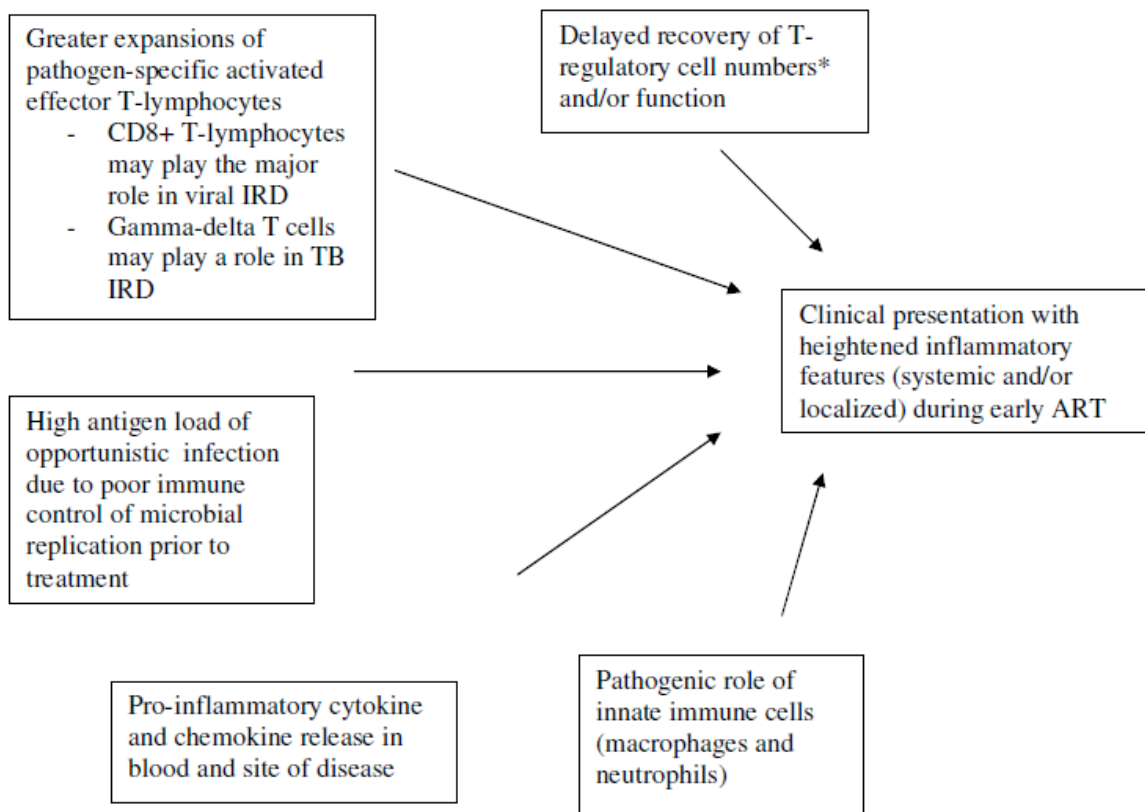
that perturbations of the innate immune responses may contribute to pathogenesis. CXCL10 (also known as IFN- γ inducible protein-10 (IP-10)) is chemotactic for effector T cells. IL-18 is a macrophage-derived inducer of IFN- γ that contributes to protective responses to TB. The results were interpreted to indicate that production of IL-18 and CXCL10 augment effector T cell responses to MTB antigens in TB-IRIS (144). Another study that analysed IFN- γ and IL-5 concentrations in whole blood mitogen-stimulated cultures demonstrated no differences in concentrations when comparing TB-IRIS cases and controls and the levels of these two cytokines correlated with each other in both patient groups. The interpretation of this data was that TB-IRIS is not caused by an imbalance of Th1- and Th2-polarised T cells (145).

In a nested case-control study conducted in Durban, South Africa, 9 patients with paradoxical TB-IRIS were compared with 12 controls with TB who started ART and developed non-IRIS clinical events. At the time of the clinical event, which was similar in time from ART initiation in cases and controls, TB-IRIS patients had lower IL-10 and MCP-1 (monocyte chemotactic protein-1 also known as CCL2) concentrations, higher C-reactive protein:IL-10 ratio when compared with controls and undetectable IL-12p70 and GM-CSF. It was concluded that this reflected lower monocyte and regulatory T cell activity and higher pro-inflammatory activity (108).

It has been hypothesized that paradoxical TB-IRIS may reflect a relative delay in recovery of regulatory T cell numbers and function compared with pro-inflammatory responses (141, 146). Two studies (one presented in Chapter 5) have demonstrated no deficiency of regulatory T cells in TB-IRIS patients (138, 139). Seddiki et al (147) demonstrated expansions of CD127^{lo} Foxp3⁺ CD25⁺ T regulatory cells and a higher ratio of T regulatory to effector/memory subsets in TB-IRIS patients. In this same study (147) in vitro suppression assays demonstrated reduced functional capacity of and reduced IL-10 secretion from suppressor cells in TB-IRIS patients, suggesting that while T regulatory cells numbers are increased their ability to downregulate aberrant immune responses is impaired. Another study of two patients with mycobacterial IRIS demonstrated impaired IL-10 and increased IFN- γ production. The authors suggested that an imbalance of regulatory and effector cytokine responses may be the cause of IRIS (146). As discussed above, low pre-ART levels of inhibitory NK receptors (CD94/NKG2, CD158a and CD158b) on mycobacterial-specific V δ 2

TCR $\gamma\delta$ T cells have been shown to predict paradoxical TB-IRIS (105). Thus while no deficiency in T-regulatory cell numbers in mycobacterial IRIS has been shown, data suggest there may be functional impairment of regulatory components of the immune system that contributes to dysregulated inflammation.

Figure 5: Immunopathogenesis of IRIS: mechanisms that have been hypothesized and studied



*No study has demonstrated deficient numbers of T-regulatory cells in TB-IRIS patients although studies have suggested impaired function

(Originally produced for reference (68))

What of the role of the innate immune system? It has been suggested that the recovery of mycobacterial-specific T cell numbers and function is not sufficient to explain the occurrence of TB-IRIS and that recovery of innate immune function may play an important role in pathogenesis (143, 144). Our study (reported in Chapter 5) demonstrated that some patients who developed TB-IRIS showed minimal or no ELISpot responses to mycobacterial antigens whereas some patients who had high numbers of mycobacterial-specific T cells on ELISpot did not develop TB-IRIS (138). It has been hypothesized that macrophages have an important role in the pathogenesis of IRIS (148). A fatal case of unmasking pulmonary TB-IRIS was found to have bronchiolitis obliterans organizing pneumonia at autopsy with an infiltrate consisting predominantly of macrophages (149). Neutrophils are likely involved given that suppurative lymphadenitis and abscess formations are frequent features of TB-IRIS. In a mouse model of MAC-IRIS marked alterations in blood and tissue CD11b⁺ myeloid cells were observed (150). The finding of higher levels of CXCL10 and IL-18 in TB-IRIS patients by Oliver and colleagues discussed above was also suggested to be evidence of a role for the innate immune system (143). A study of 5 cases of TB-IRIS and 9 matched non-IRIS controls suggested a role for Toll-like receptor 2 (TLR-2) induced pro-inflammatory cytokines produced by monocytes and dendritic cells in TB-IRIS pathogenesis. At 24 weeks on ART, TLR-2 expression on monocytes and lipomannan-induced TNF α production was significantly higher in TB-IRIS cases. Lipomannan is a TLR-2 ligand. Lipomannan-induced TNF α and IL-12p40 responses paralleled TB-IRIS in certain patients with high TLR-2 expression monocytes and myeloid dendritic cells, without a parallel increase in IL-10 production (151).

A genetic predisposition to IRIS has been suggested from genetic polymorphism studies conducted in Australia. Although these studies were small, an association of alleles of certain cytokine genes (TNFA-308*2 and IL-6-174*G) with mycobacterial IRIS, and not IRIS associated with herpes virus infections, was shown (152).

The finding that lower CD4 T cell counts (93, 98), lower concentrations of CCL2 (143) and lower plasma levels of antibodies to PGL-Tb1 (153) prior to ART are associated with development of paradoxical TB-IRIS is evidence that immunodeficiency prior to ART may be an important factor in the development of TB-IRIS. It is possible that this immunodeficiency plays a role by allowing more

dissemination of TB bacilli prior to ART, thereby providing a greater antigen stimulus (of *Mycobacterium tuberculosis* antigen) when ART is commenced precipitating TB-IRIS.

Unmasking TB-IRIS and unmasking of TB by ART

High TB incidence rates (5.6 - 23 TB cases per 100 person years) in the first 3 months of ART have reported from developing country ART programmes (80-82). It is likely that a number of factors account for this. Patients may seek medical attention and enter HIV care because of the symptoms of TB. Many such patients have TB diagnosed before ART, but because of the insensitivity of sputum smear (154) and chest radiography(155) in patients with advanced immunosuppression the diagnosis may be missed before ART initiation. Other patients may have subclinical TB at the time they start ART that becomes clinically apparent on ART. Other patients may reactivate latent TB or be infected or re-infected with TB around the time of ART initiation. Increased clinical surveillance in patients attending for clinical care likely also plays a role.

There is a spectrum of clinical presentations among such TB cases occurring during early ART that is postulated to be influenced by the complex interplay between the infectious load of mycobacterial organism and immune recovery (which tends to result in TB becoming more clinically overt)(156). The dynamics of this interplay may result in TB remaining subclinical, a typical clinical presentation or an exaggerated inflammatory presentation. The effect of ART-mediated immune restoration, particularly in the presence of a high mycobacterial load, may be three fold: the timing of onset of TB symptoms may be brought forward, there may be more rapid symptom onset and it may result in heightened inflammatory clinical manifestations (156). Lawn et al propose that only the latter category should be regarded as IRIS, and be termed unmasking TB-IRIS (156).

Some authors have regarded any presentation of TB diagnosed while patients are on ART as TB-IRIS provided there was a CD4 rise and viral load reduction (157), however there is increasing consensus that IRIS plays a role in presentation in only a subset of patients presenting with TB on ART (110, 156). A nomenclature has been

proposed (156). All TB diagnosed while on ART should be termed “ART-associated TB”. TB that presents soon after ART initiation due to restoration of TB antigen-specific immune responses should be termed “unmasking TB”. As stated above, a subset of these cases presenting with heightened intensity of clinical manifestations, particularly when there is evidence of a marked inflammatory component, during the first 3 months of ART should be termed “unmasking TB-IRIS”.

“Unmasking” TB-IRIS is less well characterized than paradoxical TB-IRIS with fewer cases reported. Cases described include patients presenting with rapid onset of severe respiratory presentations (110, 149, 158, 159), one of whom required mechanical ventilation for adult respiratory distress syndrome associated with miliary TB (158). A fatal case of unmasking TB-IRIS presenting after 6 weeks on ART was shown at post-mortem to have extensive infiltrate of the upper lobe of the right lung with histological appearance compatible with bronchiolitis obliterans organizing pneumonia (149). Complicated neurological involvement in unmasking TB-IRIS cases has been described (160, 161), as has pyomyositis (162).

Breen et al reported 13 patients diagnosed with active TB in the first 3 months of ART. These patients more frequently developed paradoxical reactions (62%) than patients who were diagnosed with TB later on ART, none of whom had paradoxical reactions. They conclude that an “inflammatory phenotype” associated with early ART may have resulted in these paradoxical reactions, perhaps another manifestation of IRIS (163). Lawn et al noted in a South African cohort that during the first 4 months of ART the adjusted TB incidence rate for those with a CD4 cell count < 200 cells/ μ L was 1.7-fold higher than the incidence rate for patients with an updated CD4 cell count < 200 cells/ μ L during long term ART. This excess adjusted risk was attributed to “unmasking” of active TB by recovering immunity (164). The proportion of these cases whose presentation was suggestive of unmasking TB-IRIS was not quantified. In Haiti, a three-fold increase in mortality in those diagnosed with TB in the first 3 months on ART (27% mortality) compared to other patients with AIDS and TB (8% mortality) was noted (165). This higher mortality may in part be attributable to unmasking TB-IRIS.

Clinicians should screen for TB symptoms prior to ART, investigate those with symptoms, be aware that a subset of patients with advanced HIV may have subclinical TB and be vigilant for the development of unmasking TB and TB-IRIS during early ART. It has been reported that around 50-70% of patients diagnosed with TB in the first 3 months of ART had TB symptoms at the time of ART initiation (163, 165). A simple screening tool for active TB in patients entering ART programmes or on ART in resource-poor settings has been proposed (166). The diagnosis of TB may be difficult to prove in patients with advanced HIV given the insensitivity of sputum smears and empiric treatment based on strong clinical suspicion and compatible radiography should be considered particularly in patients whose condition is rapidly deteriorating (167).

Further research to characterize the clinical manifestations and immunological mechanisms of unmasking TB-IRIS will help in refining the proposed clinical case definition for this condition in the future (110).

Corticosteroids for the treatment of tuberculosis

Corticosteroids are known to exert anti-inflammatory effects on most types of immune cells through direct effects on transcription of inflammatory mediators via the Glucocorticoid Responsive Element, indirect genomic effects via interference with other transcriptional factors such as NF- κ B and AP-1, and non-genomic effects on anti-inflammatory proteins (168, 169). These effects result in increased transcription of a number of anti-inflammatory mediators and decreased transcription of pro-inflammatory cytokines, chemokines, enzymes, receptors and adhesion molecules (170, 171). In addition corticosteroids have been shown to reduce T cell survival by enhancing apoptosis (171).

Corticosteroids have been used as adjunctive treatment in TB for about six decades (106, 172). Because the host immune response plays an important part in the pathology caused by TB, corticosteroids have been used in both pulmonary and many forms of extrapulmonary forms of TB with the intention of improving outcomes and reducing complications such as pericardial constriction, hydrocephalus, focal

neurological deficits, pleural adhesions and intestinal strictures. However, evidence of benefit from controlled clinical trials exists only for TB meningitis and pericardial TB. In other forms of TB the clinical benefit is anecdotal, minor or not present (106).

Thwaites and colleagues (173) conducted a randomized, double-blind, placebo-controlled trial of dexamethasone for the treatment of TB meningitis in Vietnam among patients older than 14 years of age (n=545). The initial dose of intravenous dexamethasone used was 0.4mg/kg/d for patients with Grade 2 and 3 TBM disease and 0.3mg/kg/d in patients with Grade 1 disease. The total duration of dexamethasone (initially intravenous followed by oral) was 8 weeks in those with Grade 2 and 3 disease and 6 weeks in those with Grade 1 disease. At 9 months follow-up, dexamethasone was associated with a reduced risk of death (relative risk = 0.69, 95% CI= 0.52 – 0.92), but no reduction in the proportion of patients with severe disability. Eighteen percent of participants were HIV seropositive and in a pre-specified subgroup analysis of these patients dexamethasone was associated with a non-significant trend towards reduced mortality (relative risk = 0.78, 95%CI = 0.59 -1.04). Significantly fewer severe adverse events occurred in patients who received dexamethasone. In particular, 8 cases of severe hepatitis (one was fatal) occurred in the placebo group and none in the dexamethasone group.

A Cochrane systematic review of corticosteroids as an adjunct to TB treatment in TB meningitis published in 2008 included seven trials and a total of 1140 participants (with 411 deaths). Dexamethasone or prednisolone was the corticosteroid used in all studies. Corticosteroids reduced the risk of death (relative risk = 0.78, 95% CI = 0.67 - 0.91). The survival benefit occurred irrespective of the severity of TB meningitis. In three trials in which this was assessed, corticosteroids reduced the risk of death or disabling residual neurological deficit as a combined endpoint significantly. Adverse events that occurred across studies included gastro-intestinal bleeding, bacterial and fungal infections and hyperglycaemia, but were mild and treatable (174).

A randomized, double blind, placebo-controlled trial of prednisolone (for 11 weeks) for the treatment of TB pericardial effusion was conducted in Transkei, South Africa, in the 1980's prior to the HIV epidemic by Strang and colleagues. Patients in this study were also randomized to open pericardial biopsy and complete drainage of

pericardial fluid on admission or percutaneous aspiration when required. Among patients who did not have open drainage on admission, 3% given prednisolone compared with 14% given placebo died of pericarditis ($p < 0.05$) (175). Patients who received prednisolone required repeat pericardiocentesis less frequently. A Cochrane review (176) of controlled trials evaluating the role of adjuvant corticosteroids for TB pericarditis included 2 trials from the pre-HIV era with a total of 383 participants (the 2 trials included were the study published by Strang and colleagues in 1988 (175) and a prior trial in patients with TB constrictive pericarditis conducted by the same group (177)). There was a non-significant trend towards reduced death in the intervention group (relative risk = 0.65, 95% CI = 0.36–1.16). There was a significant reduction in the combined endpoint of death or disability at 2 years although there was substantial heterogeneity in the trials. In a small randomised controlled trial of prednisolone for TB pericarditis in HIV-infected patients conducted in Zimbabwe ($n=58$) (178) there were 10 deaths among those who received placebo compared with 5 deaths among those who received prednisone. This represented significantly lower mortality in the prednisolone group using the log-rank test to compare Kaplan Meier survival curves (178). However, when cumulative mortality was compared the difference was not significant (RR=0.50, 95% CI = 0.19 - 1.28) (176). The Cochrane review concluded that corticosteroids could have clinical benefit, but the trials published to date are too small to demonstrate an effect (176). In particular the efficacy and safety of corticosteroids in HIV-infected patients needs to be evaluated in a larger clinical trial. Adjuvant prednisolone for TB pericarditis is being evaluated in the IMPI Pilot trial, a multicenter collaboration involving sites in South Africa, Zimbabwe, Nigeria and India, currently recruiting both patients who are HIV-infected or uninfected (<http://clinicaltrials.gov/ct2/show/NCT00810849>).

Tuberculomas involving the brain parenchyma or spinal cord may develop despite effective TB treatment resulting in focal neurological deficits or seizures. The host immune response is thought to play an important role in these paradoxical TB reactions. Corticosteroids have been used with anecdotal reports of symptomatic benefit (85). However, no clinical trials have been conducted. In TB pleural effusions a Cochrane systematic review found that corticosteroids had no significant effect on mortality, respiratory function, resolution of pleural effusion at 8 weeks, or development of pleural adhesions. However risk of pleural thickening was reduced,

the significance of which is unclear (179). In a Ugandan trial that evaluated prednisolone in HIV-infected patients with TB pleural effusions, prednisolone was associated with more rapid clinical and radiological improvement, but an excess of Kaposi's sarcoma (131). In peritoneal TB a small trial (n=47) conducted in India demonstrated a non-significant reduction in the late fibrotic complication of intestinal obstruction in patients who received corticosteroids (180). In miliary and pulmonary TB initiation of TB treatment may be complicated by the development of adult respiratory distress syndrome with acute respiratory failure (181). Corticosteroids are frequently used in this situation, but efficacy has not been determined in an adequately powered clinical trial. One study of 55 patients with miliary TB showed a non-significant trend towards improved survival with corticosteroids (182).

Smego and Ahmed (183) have conducted a systematic review of studies that have evaluated corticosteroids in patients with pulmonary TB. Ten of the 11 studies identified were conducted in the pre-HIV era. Two of the studies involved patients receiving rifampicin-based regimens. In the larger of these two studies (n=600) corticosteroids had no significant effect on radiological or bacteriological outcomes (184). In 9 of the 11 trials corticosteroids were associated with more rapid radiographic resolution of pulmonary infiltrates and in 6 more rapid closure of cavities. In many of the studies patients receiving corticosteroids experienced moderate clinical benefit in terms of more rapid defervescence and weight gain. On the basis of the review it was concluded that corticosteroids confer little, if any, significant effect on long-term pulmonary function in patients with TB. In the majority of studies corticosteroids did not have any appreciable effect on the speed or rate of sputum conversion. Importantly in only one of these 11 studies did corticosteroids delay sputum conversion suggesting that bacteriological outcome is not adversely affected by receipt of corticosteroids while on effective TB treatment. No serious side effects or increase in rate of bacteriological relapse was noted. Thus in pulmonary TB, corticosteroids result in modest benefits in terms of more rapid clinical and radiological improvement, but do not impact on mortality or the development of late complications such as chronic restrictive lung disease (172).

A review of the literature of corticosteroids used for all forms of TB also concluded that corticosteroids do not diminish the efficacy of TB treatment (172). In contrast in

patients with latent TB corticosteroids increase the risk of reactivation and active TB disease (185). Risk of TB is increased approximately two-fold in rheumatological patients receiving corticosteroids in North American clinics (186).

One study has been conducted in Uganda specifically in HIV-infected patients with pulmonary TB. In a phase 2 trial of short-term prednisolone in HIV-infected TB patients with CD4 count ≥ 200 cells/mm³, prednisolone was associated with more rapid clearance of MTB from sputum, reduced immune activation and resulted in a non-significant increase in CD4 count, but caused a transient increase in HIV viral load and worsened underlying hypertension and caused fluid retention and hyperglycaemia. On the basis of these findings the investigators advised against the use of prednisolone in these patients because risks outweigh benefits (132).

There are special considerations when considering the use of corticosteroids for TB in HIV-infected patients. Some investigators have documented no serious adverse effects of corticosteroid therapy in HIV-infected patients (178, 187, 188), whereas other studies have raised concerns related to reactivation of herpes infections and the development of Kaposi's sarcoma (130, 131). Another important factor to be considered in the use of corticosteroids with TB treatment is the drug-drug interaction with rifampicin, a potent inducer of cytochrome P450 enzymes. Rifampicin increases the clearance of prednisolone by 45% resulting in reduction in the area under the plasma concentration time curve of prednisolone by 66% (189).

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CHAPTER 3

Tuberculosis-associated immune reconstitution inflammatory syndrome: case definitions for use in resource-limited settings

Tuberculosis-associated immune reconstitution inflammatory syndrome: case definitions for use in resource-limited settings

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The immune reconstitution inflammatory syndrome (IRIS) has emerged as an important early complication of antiretroviral therapy (ART) in resource-limited settings, especially in patients with tuberculosis. However, there are no consensus case definitions for IRIS or tuberculosis-associated IRIS. Moreover, previously proposed case definitions are not readily applicable in settings where laboratory resources are limited. As a result, existing studies on tuberculosis-associated IRIS have used a variety of non-standardised general case definitions. To rectify this problem, around 100 researchers, including microbiologists, immunologists, clinicians, epidemiologists, clinical trialists, and public-health specialists from 16 countries met in Kampala, Uganda, in November, 2006. At this meeting, consensus case definitions for paradoxical tuberculosis-associated IRIS, ART-associated tuberculosis, and unmasking tuberculosis-associated IRIS were derived, which can be used in high-income and resource-limited settings. It is envisaged that these definitions could be used by clinicians and researchers in a variety of settings to promote standardisation and comparability of data.

Introduction

The immune reconstitution inflammatory syndrome (IRIS; also known as immune reconstitution disease, immune reconstitution syndrome, or immune restoration disease) is a widely recognised phenomenon that can complicate antiretroviral therapy (ART).^{1,2} The condition results from rapid restoration of pathogen-specific immune responses to opportunistic infections, causing either the deterioration of a treated infection or the new presentation of a previously subclinical infection. IRIS typically occurs during the initial months of ART and is associated with a wide spectrum of pathogens, most commonly mycobacteria, herpesviruses, and deep fungal infections such as cryptococcal meningitis.^{1–3}

In recent years, access to ART has increased rapidly in resource-limited settings, reaching over 2 million people by December, 2006, with an estimated 1340 000 of these individuals living in sub-Saharan Africa.⁴ Since the burden of HIV/tuberculosis co-infection is very high in many low-income and middle-income countries,⁵ many of the patients who enter ART programmes in these settings have a current diagnosis of tuberculosis, or later develop tuberculosis following initiation of ART. For example, one South African study reported that 238 (25%) of 944 patients attending a community-based ART programme were receiving tuberculosis treatment at ART initiation and in the first year of ART the incidence of tuberculosis was 13.4 cases per 100 person-years (95% CI 10.4–16.9).⁶ Up to one-third of patients with HIV/tuberculosis co-infection who begin ART in such settings could be at risk of developing tuberculosis-associated IRIS (also known as TB-IRIS),³ and this condition is emerging as an important clinical challenge in resource-limited settings.^{7–10}

Since there is no diagnostic test for IRIS, confirmation of the disease relies heavily upon case definitions incorporating clinical and laboratory data. However, clinical management and research on IRIS are hindered by the lack of consensus case definitions and definitions that are specific to particular opportunistic infections. To address this shortcoming, an international meeting of researchers working in this field was convened in Kampala, Uganda, in November, 2006, and the International Network for the Study of HIV-associated IRIS (INSHI) was formed. The specific aim of the meeting was to develop consensus case definitions for tuberculosis-associated IRIS that are appropriate for low-income settings where laboratory capacity is often limited, and that can be used by researchers working in different settings to permit comparability of results. We present these consensus case definitions in this paper.

Participants and consensus methods

The need for a public-health definition for tuberculosis-associated IRIS was first proposed at the WHO consultation on tuberculosis and HIV research priorities in resource-limited settings in February, 2005.¹¹ The organisers of the meeting in Kampala contacted individuals involved in research related to tuberculosis-associated IRIS, particularly those working in resource-limited settings or collaborating with researchers in these settings. Contacting these individuals was dependent on whether they had published or presented data about tuberculosis-associated IRIS at international conferences, whether they were involved in ongoing research projects about the disease, or whether they had clinical experience of the disease. 97 researchers from 16 countries on six continents attended the meeting. Among the delegates were

microbiologists, immunologists, clinicians, epidemiologists, clinical trialists, public-health specialists, and representatives from WHO.

At the meeting a subgroup was assembled to develop the case definitions. Two participants presented published IRIS case definitions (panel 1)^{2,7,12–14} as well as eight different tuberculosis-associated IRIS case

definitions currently being used by researchers in ongoing cohort and intervention studies. The common features among these case definitions were highlighted, and their practical use in resource-limited settings was discussed. Tuberculosis-associated IRIS case definitions were agreed and taken back to a plenary session for further discussion and consensus building. Thereafter,

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Panel 1: Existing case definitions for IRIS and tuberculosis-associated IRIS that have been most widely used

General IRIS case definition 1 (French et al, 2004)²

Diagnosis requires two major criteria (A+B) or major criterion (A) plus two minor criteria to be fulfilled:

Major criteria

(A) Atypical presentation of opportunistic infections or tumours in patients responding to ART

- Localised disease
- Exaggerated inflammatory reaction
- Atypical inflammatory response in affected tissues
- Progressive organ dysfunction or enlargement of pre-existing lesions after definite clinical improvement with pathogen-specific therapy before the initiation of ART and exclusion of treatment toxicity and new alternative diagnoses

(B) Decrease in plasma HIV RNA concentration by more than 1 log₁₀ copies per mL

Minor criteria

- Increase in blood CD4 T-cell count after starting ART
- Increase in an immune response specific to the relevant pathogen—eg, delayed-type hypersensitivity skin test response to mycobacterial antigens
- Spontaneous resolution of disease without specific antimicrobial therapy or tumour chemotherapy with continuation of ART

General IRIS case definition 2 (Shelburne et al, 2006)¹³

Criteria for IRIS diagnosis include:

- HIV-infected patient
- Receiving effective ART as evidenced by a decrease in HIV-1 RNA concentration from baseline or an increase in CD4+ T cells from baseline (may lag behind HIV-1 RNA decrease)
- Clinical symptoms consistent with inflammatory process
- Clinical course not consistent with expected course of previously diagnosed opportunistic infection, expected course of newly diagnosed opportunistic infection, or drug toxicity

Case definition specific for tuberculosis-associated IRIS (Colebunders et al, 2006)⁷

For patients receiving treatment for tuberculosis and starting ART:

Suspected tuberculosis-associated IRIS case

Cases must meet the following three criteria:

- An initial clinical response to tuberculosis treatment, based on a combination of some of the following factors: cessation of fever, relief of pulmonary symptoms, decrease in lymph node size, termination of signs of meningeal irritation (depending on presenting symptoms)
- New persistent fevers without another identifiable cause and/or one or more of the following: worsening or emergence of dyspnoea, stridor, an increase in lymph node size, development of abscesses, development of abdominal pain with ultrasound evidence of abdominal adenopathies, unexplained CNS symptoms
- Adequate adherence to ART and tuberculosis treatment

Confirmed tuberculosis-associated IRIS case

Cases must meet the following three criteria:

- Radiological examinations showing worsening or emergence of intrathoracic lymphadenopathy, pulmonary infiltrates, pleural effusions, abdominal lymph nodes, hepatosplenomegaly
- A good virological response and/or increase in CD4+ lymphocyte count, and/or conversion of tuberculin skin test from negative to positive, and/or adequate adherence to ART and tuberculosis treatment
- A clear exclusion of other conditions that could explain the clinical manifestations of the patient, such as tuberculosis treatment failure or other concomitant infections, tumours, or allergic reactions

ART=antiretroviral therapy. IRIS=immune reconstitution inflammatory syndrome.

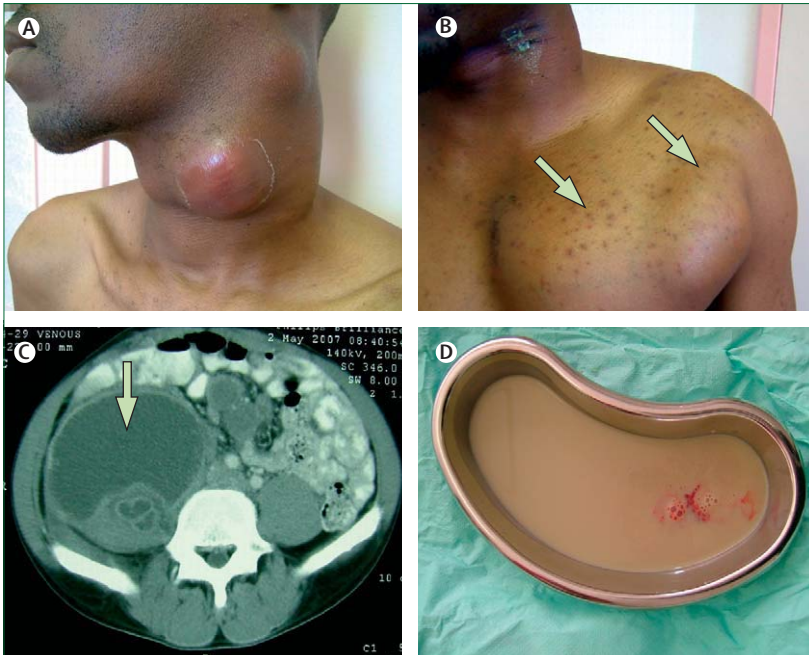


Figure 1: Illustrative case of paradoxical tuberculosis-associated IRIS

A 36-year-old HIV-infected man was diagnosed with culture-positive pulmonary tuberculosis (sensitive to rifampicin and isoniazid) without evidence of extrapulmonary involvement. His CD4 count was 39 cells per μL and HIV-1 viral load 1 300 000 copies per mL. He commenced antiretroviral therapy (ART; stavudine, lamivudine, and efavirenz) 7 weeks after initiating antituberculous therapy. 1 week later he presented with a recurrence of tuberculosis symptoms and cervical node enlargement. Paradoxical tuberculosis-associated IRIS was diagnosed. Over the next 18 months he presented with several tuberculosis-associated IRIS manifestations that sequentially emerged, despite corticosteroid therapy, then resolved. Photographs show development of massive cervical lymphadenitis (A), a chest wall cold abscess (B, arrows), and a massive right psoas abscess shown here on CT scan (C, arrow) from which over 2 L of pus was aspirated (D). Repeated mycobacterial cultures of aspirates from these collections have been negative. After 6 months on ART his CD4 count was 181 cells per μL and viral load undetectable. After 12 months his CD4 count was 448 cells per μL and viral load 35 copies per mL. This was an unusually prolonged course for paradoxical tuberculosis-associated IRIS given that the median duration of symptoms is reported to be 2 months (see text).

a writing committee of 17 members was appointed to finalise the consensus case definitions.

Changes to existing case definitions

General case definitions for IRIS have previously been published (panel 1).^{2,12–14} These case definitions include the following criteria: confirmed HIV diagnosis, temporal association with initiation of ART, demonstration of response to ART (ie, plasma viral load reduction, blood CD4 cell count increase, or another marker of immune recovery such as conversion of tuberculin skin test from negative to positive for mycobacterial IRIS), clinical deterioration with an inflammatory process, and exclusion of other causes that could explain deterioration (such as antimicrobial drug resistance, drug hypersensitivity reaction, or another opportunistic infection). However, since manifestations of IRIS are infection-specific, it has been recognised that particular definitions applicable to individual diseases such as tuberculosis would be useful.⁷ The case definitions presented in this manuscript focus specifically on the clinical manifestations of tuberculosis-associated IRIS.

Case definitions should be readily applicable in resource-limited settings where the vast majority of patients requiring ART live and yet where facilities for diagnosis and management of the complications of ART are least well developed. In this respect, the requirement within existing definitions for documentation of changes in CD4 cell count and plasma viral load is not achievable in these settings. Viral load testing has limited availability and is very costly. In the South African public sector a viral load test costs US\$39, more than the cost of 1 month's supply of first-line ART. Even where CD4 and viral load testing are available (such as in South Africa), use of these tests under programmatic conditions is usually permitted for monitoring of ART at 6-monthly intervals only and not for individual patient diagnostic work-up.

We believe that omission of these laboratory parameters would not substantially compromise case definitions for tuberculosis-associated IRIS. First, within the initial months of ART—when most cases of tuberculosis-associated IRIS arise—most ART-naïve patients adhering to treatment have substantial viral load reductions;^{15–17} thus, inclusion of viral load changes in definitions is largely redundant in the context of a patient who adheres to therapy. Second, tuberculosis-associated IRIS frequently develops shortly after initiation of ART and before any measurable increase in peripheral blood CD4 cell count. In a series of 51 patients presenting with non-tuberculous mycobacterial IRIS, six (12%) of 51 IRIS events occurred without a substantial increase in CD4 cell count (four patients had a CD4 increase from baseline to time of IRIS diagnosis of less than 25 cells per μL and in two patients the CD4 cell count had actually fallen at the time of presentation).¹⁸ The number of CD4 T cells measured in peripheral blood does not necessarily reflect function nor how many cells are actually present at the site of an opportunistic infection. Moreover, it is very likely that CD4 T cells are not the only cellular mediators of IRIS.^{19,20} For these reasons we, like others,¹² propose that a rise in peripheral blood CD4 cell count should not be a necessary marker for the diagnosis of tuberculosis-associated IRIS.

A further important modification to existing definitions is the inclusion of a timeframe of the first 3 months of ART. Such a timeframe is not present in the widely used case definitions to date (panel 1). Onset of the clinical manifestations of tuberculosis-associated IRIS should occur within this timeframe for a diagnosis of tuberculosis-associated IRIS to be made, since this represents the period when rapid immune recovery usually occurs.^{3,21}

Categories of tuberculosis-associated IRIS

Tuberculosis-associated IRIS can present as one of two main syndromes: (1) a paradoxical reaction after the start of ART in patients receiving tuberculosis treatment (here termed paradoxical tuberculosis-associated IRIS), or (2) a new presentation of tuberculosis that is “unmasked” in the weeks following initiation of ART with an exaggerated

	Country	Number of patients on tuberculosis treatment starting ART	Number of patients with paradoxical tuberculosis-associated IRIS	Interval from initiation of ART to IRIS presentation	Risk factors for tuberculosis-associated IRIS
Narita et al (1998) ²²	USA	33	12 (36%)	Mean 15 days (SD 11 days)	Purified protein derivative conversion
Breen et al (2004) ²³	UK	28	8 (29%)	Median 11 days (range 8–18 days)	Starting ART within 6 weeks of tuberculosis diagnosis
Breton et al (2004) ²⁴	France	37	16 (43%)	Median 12 days (range 2–114 days)	Greater increase in CD4 percentage and CD4/CD8 ratio; disseminated tuberculosis
Kumarasamy et al (2004) ⁸	India	144	11 (8%)	Median 42 days (range 10–89 days)	..
Shelburne et al (2005) ²¹	USA	86	26 (30%)	Median 46 days (range 3–658 days)	Shorter interval to starting ART; more rapid initial fall in viral load
Michailidis et al (2005) ²⁵	UK	28	9 (32%)	Median 0.6 months (IQR 0.1–9.1 months)	Lower baseline CD4 cell count; disseminated tuberculosis; greater CD4 rise on ART
Manosuthi et al (2006) ⁹	Thailand	167	21 (13%)	Median 32 days (IQR 14–115 days)	Extrapulmonary tuberculosis
Lawn et al (2007) ¹⁰	South Africa	160	19 (12%)	Median 2 weeks (IQR 1.5–3.5 weeks)	Lower baseline CD4 cell count; shorter interval to starting ART
Burman et al (2007) ²⁶	USA	109	19 (17%)	Median 34 days (IQR 8–97 days)	Black ethnic origin; shorter interval to starting ART; extrapulmonary tuberculosis

..=not reported. ART=antiretroviral therapy. IRIS=immune reconstitution inflammatory syndrome. *Only studies where more than eight patients with paradoxical tuberculosis-associated IRIS were reported are included. This table is an updated version of a previously published table.²⁷ Studies are presented in chronological order. †The authors reported 57 cases of tuberculosis, *Mycobacterium avium* complex, and cryptococcal IRIS (26 of 57 were tuberculosis-associated IRIS). Five of these 57 patients started ART before the opportunistic infection was diagnosed, and were thus not paradoxical IRIS cases. The data shown regarding risk factors and median interval relate to all 57 patients. ‡14 tuberculosis-associated IRIS cases were reported. Nine of these were paradoxical tuberculosis-associated IRIS cases. Data shown regarding risk factors relate to all 14 cases.

Table: Paradoxical tuberculosis-associated IRIS: cohort studies reported in the literature*

inflammatory clinical presentation or complicated by a paradoxical reaction (here termed unmasking tuberculosis-associated IRIS).

Paradoxical tuberculosis-associated IRIS

In paradoxical tuberculosis-associated IRIS, patients have been diagnosed with active tuberculosis before initiation of ART, and have typically been responding to antituberculosis treatment. Following initiation of ART, IRIS presents as the development of recurrent, new, or worsening symptoms or signs of tuberculosis, such as fever, return of cough, or lymph node enlargement, or recurrent, new, or deteriorating radiological manifestations (figure 1). These symptoms typically occur within the first few weeks and up to 3 months after ART is initiated, restarted, or changed because of treatment failure.

Reports of the frequency of paradoxical tuberculosis-associated IRIS using a variety of existing case definitions range from 8% to 43% (table).^{8–10,21–28} Paradoxical tuberculosis-associated IRIS has been linked with large expansions of purified protein derivative-specific T cells in peripheral blood and increased pro-inflammatory cytokine levels.²⁹ Risk factors for the disease are shown in table 1 and include more advanced HIV disease with lower CD4 cell count, disseminated and extrapulmonary tuberculosis, a shorter delay between the start of tuberculosis treatment and initiation of ART, and a more vigorous immunological and virological response to ART. Most cases of paradoxical tuberculosis-associated IRIS are

self-limiting. The median duration of symptoms reported in the literature is 2 months,^{26,28} but this ranges from mild cases where symptoms resolve after a few days to isolated prolonged cases that have still been symptomatic after more than a year (figure 1).²⁸ Mortality from tuberculosis-

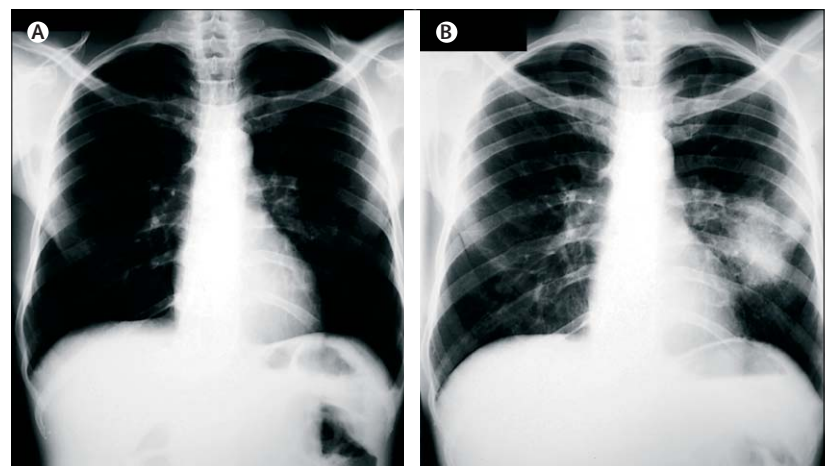


Figure 2: Illustrative case of unmasking tuberculosis-associated IRIS
 A 48-year-old HIV-infected man with a CD4 count of 10 cells per μL presented with low-grade fevers, retrosternal chest pain, and a dry cough. Examination was non-contributory. He could not produce sputum and his chest radiograph showed no features of active tuberculosis (A). No other investigations for tuberculosis were available in this resource-limited setting (Uganda). Antiretroviral therapy (ART) was started (zidovudine, lamivudine, and efavirenz). 10 days later he returned acutely unwell with a productive cough. His temperature was 38.7°C and he was in respiratory distress. Chest radiograph now showed left mid-zone consolidation (B) and his sputum was positive for acid-fast bacilli. The unusual rapidity and clinical severity of his tuberculosis presentation was attributed to unmasking tuberculosis-associated IRIS. He responded well to continued ART and tuberculosis treatment.

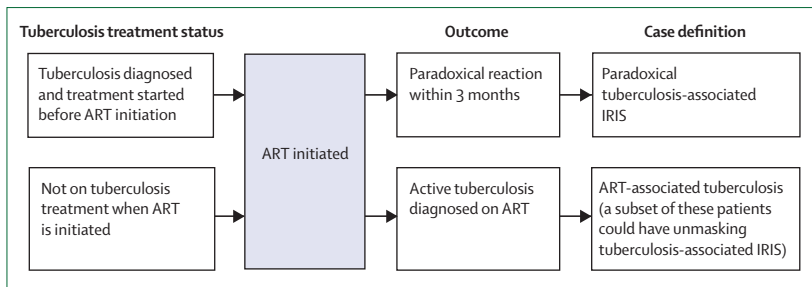


Figure 3: Schematic representation showing the different forms of tuberculosis-associated IRIS and ART-associated tuberculosis
ART=antiretroviral therapy.

associated IRIS has been reported infrequently in the literature,^{3,9,10,26} but morbidity and the need for hospital admission and therapeutic procedures can be substantial.²⁶ Rates of morbidity and mortality attributable to paradoxical

tuberculosis-associated IRIS may be higher in resource-limited settings where diagnostic and treatment options are restricted. Neurological tuberculosis-associated IRIS in particular can be associated with poor outcome.

Tuberculosis paradoxical reactions, such as enlargement of lymph nodes or cerebral tuberculomas, can also occur in HIV-uninfected individuals and HIV-infected individuals who are receiving appropriate tuberculosis treatment but who are not receiving ART;^{30–32} however, the frequency of paradoxical reactions is much lower in these groups compared with patients receiving ART.^{22,23} In one study, paradoxical reactions following tuberculosis treatment occurred in one (2%) of 55 HIV-seronegative patients, two (7%) of 28 HIV-infected patients not on ART, and 12 (36%) of 33 HIV-infected patients on tuberculosis treatment and ART.²² The timing of the paradoxical reaction in the latter group was more closely related to the initiation of ART than it was to the initiation

Panel 2: Case definition for paradoxical tuberculosis-associated IRIS

There are three components to this case definition:

(A) Antecedent requirements

Both of the two following requirements must be met:

- Diagnosis of tuberculosis: the tuberculosis diagnosis was made before starting ART and this should fulfil WHO criteria for diagnosis of smear-positive pulmonary tuberculosis, smear-negative pulmonary tuberculosis, or extrapulmonary tuberculosis⁴⁴
- Initial response to tuberculosis treatment: the patient’s condition should have stabilised or improved on appropriate tuberculosis treatment before ART initiation—eg, cessation of night sweats, fevers, cough, weight loss. (Note: this does not apply to patients starting ART within 2 weeks of starting tuberculosis treatment since insufficient time may have elapsed for a clinical response to be reported)

(B) Clinical criteria

The onset of tuberculosis-associated IRIS manifestations should be within 3 months of ART initiation, reinitiation, or regimen change because of treatment failure.

Of the following, at least one major criterion or two minor clinical criteria are required:

Major criteria

- New or enlarging lymph nodes, cold abscesses, or other focal tissue involvement—eg, tuberculous arthritis
- New or worsening radiological features of tuberculosis (found by chest radiography, abdominal ultrasonography, CT, or MRI)
- New or worsening CNS tuberculosis (meningitis or focal neurological deficit—eg, caused by tuberculoma)
- New or worsening serositis (pleural effusion, ascites, or pericardial effusion)

Minor criteria

- New or worsening constitutional symptoms such as fever, night sweats, or weight loss
- New or worsening respiratory symptoms such as cough, dyspnoea, or stridor
- New or worsening abdominal pain accompanied by peritonitis, hepatomegaly, splenomegaly, or abdominal adenopathy

(C) Alternative explanations for clinical deterioration must be excluded if possible*

- Failure of tuberculosis treatment because of tuberculosis drug resistance
- Poor adherence to tuberculosis treatment
- Another opportunistic infection or neoplasm (it is particularly important to exclude an alternative diagnosis in patients with smear-negative pulmonary tuberculosis and extrapulmonary tuberculosis where the initial tuberculosis diagnosis has not been microbiologically confirmed)
- Drug toxicity or reaction

ART=antiretroviral therapy. IRIS=immune reconstitution inflammatory syndrome. *It might be difficult or impossible in resource-poor settings to confirm tuberculosis drug resistance and to exclude certain other infections or neoplasia. Cases where alternative diagnoses cannot be fully excluded because of limited diagnostic capacity should be regarded as “probable paradoxical tuberculosis-associated IRIS”. In these probable cases, should resolution of clinical or radiological findings of the suspected IRIS episode occur without a change in tuberculosis treatment or ART having been made, they could then be reclassified as “paradoxical tuberculosis-associated IRIS” cases.

of tuberculosis treatment. Thus, the greatly increased frequency of paradoxical reactions in patients receiving ART suggests that ART-related immunological changes have an important role in their aetiology. Additionally, our clinical experience is that paradoxical tuberculosis-associated IRIS is more severe and more frequently a multisystemic condition in ART patients than paradoxical reactions in patients not receiving ART.

ART-associated tuberculosis and unmasking tuberculosis-associated IRIS

Compared with paradoxical tuberculosis-associated IRIS, there is much less clarity surrounding the second major category of tuberculosis-associated-IRIS. High rates of tuberculosis have been diagnosed during ART, especially in the initial months of treatment in ART programmes in resource-limited settings.^{6,33–36} The mechanisms underlying the presentation of tuberculosis after the initiation of ART are likely to be heterogeneous.³⁷ Since ART-induced immune recovery is a time-dependent process and some patients initially fail to show an increased circulating CD4 T-cell count,^{38,39} a proportion of cases might present as a result of persisting immunodeficiency. Diagnoses of active tuberculosis before ART initiation might be missed because of the inherent insensitivity of tuberculosis diagnostics in patients with advanced immunodeficiency and only confirmed later during ART. Other patients might have active subclinical disease at the time of ART initiation and presentation of symptomatic disease might result from ART-induced restoration of an immune response against *Mycobacterium tuberculosis* antigens that causes inflammation. Some patients with a missed tuberculosis diagnosis or active subclinical tuberculosis at the time of ART initiation may later present with exuberant inflammatory clinical features that are consistent with a diagnosis of unmasking tuberculosis-associated IRIS (figure 2).

Paradoxical reactions in patients started on tuberculosis treatment while receiving ART have also been described,^{26,40} and one study reported that paradoxical reactions are more frequent in patients who are diagnosed with tuberculosis in the first 3 months of ART than in patients who start ART after tuberculosis treatment (eight [62%] of 13 patients vs nine [30%] of 30 patients, respectively, $p=0.05$).⁴⁰ This finding suggests that ART-related immunological changes have a role in the development of paradoxical reactions in patients who present with tuberculosis while receiving ART and that these reactions are a form of tuberculosis-associated IRIS.

Only a few cases of unmasking tuberculosis-associated IRIS have been described in the literature to date.^{40–43} In the absence of a diagnostic test, it is currently difficult to differentiate the varied mechanisms underlying most cases of tuberculosis that present during early ART, especially in resource-limited settings where rates of infection are high. We therefore propose that, as elsewhere,³⁷ the term ART-associated tuberculosis is used

Panel 3: Case definition for ART-associated tuberculosis and provisional case definition for unmasking tuberculosis-associated IRIS

ART-associated tuberculosis

We propose that ART-associated tuberculosis (all cases of tuberculosis that are diagnosed during ART) should be defined as follows:

- Patient is not receiving treatment for tuberculosis when ART is initiated
- Active tuberculosis is diagnosed after initiation of ART
- The diagnosis of tuberculosis should fulfil WHO criteria for smear-positive pulmonary tuberculosis, smear-negative pulmonary tuberculosis, or extrapulmonary tuberculosis⁴⁴

Unmasking tuberculosis-associated IRIS (provisional)

We propose that the following could suggest a diagnosis of unmasking tuberculosis-associated IRIS:

- Patient is not receiving treatment for tuberculosis when ART is initiated and then presents with active tuberculosis within 3 months of starting ART
- AND one of the following criteria must be met:
- Heightened intensity of clinical manifestations, particularly if there is evidence of a marked inflammatory component to the presentation. Examples include tuberculosis lymphadenitis or tuberculosis abscesses with prominent acute inflammatory features, presentation with pulmonary tuberculosis that is complicated by respiratory failure due to adult respiratory distress syndrome, and those who present with a marked systemic inflammatory syndrome related to tuberculosis. See example in figure 2
 - Once established on tuberculosis treatment, a clinical course that is complicated by a paradoxical reaction

ART=antiretroviral therapy. IRIS=immune reconstitution inflammatory syndrome. Researchers in the field are encouraged not to regard all patients with ART-associated tuberculosis as having tuberculosis-associated IRIS, but only those that fit this provisional unmasking tuberculosis-associated-IRIS case definition. We suggest that the clinical manifestations of all patients developing ART-associated tuberculosis should be well characterised and reported in studies, which will assist with refinement of this case definition in the future. Studies of the immunological processes underlying the presentation of these cases are also likely to assist with refining this case definition.

to refer to all patients who present with active tuberculosis while receiving ART (figure 3). We also suggest a provisional case definition for unmasking tuberculosis-associated IRIS and clinical scenarios where the diagnosis could be considered.

Further research into the clinical characteristics and immunological mechanisms underlying cases of ART-associated tuberculosis will permit a more refined case definition for unmasking tuberculosis-associated IRIS in the future. However, in view of the heterogeneity in the natural history and clinical manifestations of tuberculosis it is unlikely that a clinical case definition that robustly separates patients with unmasking tuberculosis-associated IRIS from others with ART-associated tuberculosis will be derived.³⁷

Case definitions

With the rationale described above, we have developed case definitions for “paradoxical tuberculosis-associated IRIS” (panel 2), “ART-associated tuberculosis” (panel 3), and “unmasking tuberculosis-associated-IRIS” (panel 3). The case definitions are presented schematically in figure 3. These case definitions have been designed for use in resource-limited settings and are consensus case definitions that need validation in clinical practice.

Search strategy and selection criteria

Data for this Personal View were obtained by searching Medline for articles published from 1990 to 2008. Search terms included “immune reconstitution”, “immune restoration”, “immune recovery”, “IRIS”, “antiretroviral”, “tuberculosis”, and “paradoxical reaction”. Only English language papers were reviewed. Additionally, unpublished data and tuberculosis-associated IRIS case definitions presented by researchers at the INSHI meeting were used.

Conclusions

The use of standardised case definitions in different populations will help to provide greater insight into the incidence, clinical manifestations, risk factors, and impact of tuberculosis-associated IRIS, ultimately leading to better prevention and management strategies for this condition. Further clinical and immunological research on patients with ART-associated tuberculosis is needed to better differentiate the subset of cases that have unmasking tuberculosis-associated IRIS and to further refine this case definition. It is hoped that open research networks such as INSHI will provide opportunities for researchers to engage in collaborative research into tuberculosis-associated IRIS using these case definitions.

Conflicts of interest

We declare that we have no conflicts of interest.

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CHAPTER 4

Novel relationship between tuberculosis immune reconstitution inflammatory syndrome and antitubercular drug resistance

Novel Relationship between Tuberculosis Immune Reconstitution Inflammatory Syndrome and Antitubercular Drug Resistance

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(See the editorial commentary by Friedland on pages 677–9)

Background. Tuberculosis (TB) immune reconstitution inflammatory syndrome (IRIS) is emerging as an important early complication of combination antiretroviral therapy in patients with TB in developing countries. The differential diagnosis of TB IRIS includes deterioration caused by other human immunodeficiency virus-related morbidities and drug-resistant TB.

Methods. We prospectively evaluated consecutive patients with suspected TB IRIS from February 2005 through July 2006 at a community-based secondary hospital in Cape Town, South Africa, by means of clinical case definitions for TB IRIS. Specimens were sent for TB culture and susceptibility testing, and a rapid test (FASTplaque-Response) was performed to expedite determination of rifampin susceptibility.

Results. One hundred patients with suspected TB IRIS were evaluated, 26 of whom were being retreated for TB. IRIS symptoms developed a median of 14 days (interquartile range, 7–25 days) after the initiation of combination antiretroviral therapy. In 7 patients, an alternative opportunistic disease was diagnosed. Rifampin-resistant TB was present in 13 patients, 9 of whom received a diagnosis after study entry (7 of 9 had multidrug-resistant TB). Undiagnosed rifampin-resistant TB was thus present in 10.1% of patients (95% confidence interval, 3.9%–16.4%) who presented with TB IRIS, once those with alternative diagnoses and TB with known rifampin resistance were excluded. In the remaining 80 patients, TB IRIS without rifampin resistance was the final diagnosis.

Conclusions. TB IRIS that is clinically indistinguishable from TB IRIS that occurs in the context of drug-susceptible disease may occur in patients with undiagnosed multidrug-resistant TB. Antitubercular drug resistance should be excluded in all cases of suspected TB IRIS, and corticosteroids should be used with caution for patients with presumed TB IRIS until the result of drug-susceptibility testing is known.

The scale-up of combination antiretroviral therapy (cART) in the developing world is progressing rapidly, improving survival among HIV-infected persons [1, 2].

An emerging complication of cART in countries with high rates of tuberculosis (TB) is TB immune reconstitution inflammatory syndrome (IRIS). “Paradoxical” TB IRIS manifests with new, worsening, or recurrent symptoms, signs, and/or radiological manifestations of TB after cART is initiated in patients receiving treatment for TB [3]. It occurs in 8%–43% of patients who initiate cART while receiving TB treatment [4–11] and is associated with exuberant antimycobacterial immune responses [12]. There is no diagnostic test for TB IRIS, and the differential diagnosis is wide, including failure of TB treatment attributable to antimicrobial resistance, or suboptimal antitubercular drug concentrations [13], drug reactions, or an alternative opportunistic condition. Published case definitions require that these be

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Table 1. Case definitions for tuberculosis (TB) immune reconstitution inflammatory syndrome (IRIS).

Criteria that must be met for the diagnosis of TB IRIS before the initiation of cART
Microbiologic, histologic, or very strong clinical evidence of TB
Initial improvement of ≥ 1 of the following during multidrug TB treatment: symptoms, Karnofsky score, weight, fever, clinical signs, or radiographic findings
The infecting strain of <i>Mycobacterium tuberculosis</i> is susceptible to rifampin (if this result is available)
The patient was receiving antitubercular therapy when cART was initiated
Criteria that must be met for the diagnosis of TB IRIS within 3 months after the initiation of cART
New or recurrent TB-related symptoms and/or
New or worsening TB manifestations, such as ≥ 1 of the following:
New or expanding lymph nodes
New or expanding tuberculous cold abscesses
New or expanding intracranial tuberculomas
New or expanding pulmonary infiltrates (radiographically confirmed)
New or recurrent tuberculous meningitis (after exclusion of bacteria and fungi)
New or enlarging serous effusions (pericardial, pleural, or ascitic; radiographically confirmed)
New or worsening granulomatous hepatitis
New or worsening granulomatous infiltration of bone marrow
Other new or worsening tuberculous lesions
No other opportunistic disease to explain the new or recurrent symptoms and/or new or worsening TB manifestations

excluded before a diagnosis of TB IRIS is made [14, 15]. TB IRIS can be severe and life-threatening, and there are anecdotal reports that suggest the use of adjunctive corticosteroid therapy [16, 17].

Concurrent with the increase in the prevalence of TB IRIS, the emergence of multidrug-resistant (MDR) and extensively drug resistant TB in settings in Southern Africa where HIV infection is prevalent has recently been highlighted [18, 19]. Determining the cause of deterioration in patients with TB during cART in resource-limited settings is important, because adjunctive corticosteroid therapy may worsen an already immunosuppressed patient's condition if used in the presence of incompletely efficacious TB treatment or other opportunistic infections. This study prospectively evaluated clinical case definitions of TB IRIS among 100 patients who were considered to have likely cases of TB IRIS. We found a high prevalence of unsuspected drug-resistant TB in this cohort, which has important implications for the diagnosis and management of this condition, as well as wider policy implications.

PATIENTS AND METHODS

Study site and participants. A prospective observational study was conducted under program conditions that involved 100 consecutive patients who were referred to GF Jooste Hospital (Cape Town, South Africa) with likely cases of TB IRIS. The TB incidence rate in the Western Cape province in 2006 was 1031 cases per 100,000 population [20], and the prevalence of antenatal HIV infection was as high as 33% [21]. More than 10,000 people have initiated cART within the catchment area of GF Jooste Hospital (M. Osler, Provincial Government of the

Western Cape, personal communication). The national TB program treats new TB cases with 6 months of therapy (rifampin, isoniazid, pyrazinamide, and ethambutol for 2 months, followed by rifampin and isoniazid for 4 months). The retreatment regimen includes the addition of streptomycin, as follows: 2 months of rifampin, isoniazid, pyrazinamide, ethambutol, and streptomycin; 1 month of rifampin, isoniazid, pyrazinamide, and ethambutol; and 5 months of rifampin, isoniazid, and ethambutol. Routine TB drug susceptibility testing (DST) is not performed for new TB cases. Patients receiving retreatment and patients not responding to TB treatment may have DST performed. DST was only performed for rifampin, isoniazid, and ethambutol during the study, in accordance with national guidelines.

First-line cART in South Africa is stavudine, lamivudine, and either nevirapine or efavirenz. Efavirenz is preferred for patients receiving rifampin-based TB treatment. Patients with a CD4 cell count < 200 cells/ μ L and/or World Health Organization stage 4 disease are eligible to commence cART.

Clinical case definitions of TB IRIS (table 1) were prepared, circulated, and discussed with participating primary care physicians. The definitions were in accordance with other published case definitions for IRIS [14, 15], in that they excluded patients with drug-resistant TB. Our case definitions, however, specifically used known resistance to rifampin as an exclusion criterion. We obtained information regarding initial TB diagnosis from the referral letter and by search of records from the regional laboratory, which processes all TB microbiologic specimens in our referral area. All patients aged ≥ 13 years who were referred to the hospital with suspected TB IRIS were in-

cluded. TB treatment adherence was assessed by self-report and on the basis of the patient's TB clinic card, on which each daily dose taken was documented with a tick. Patients who had <80% adherence to TB treatment reported were not included as patients with suspected TB IRIS. The Research Ethics Committee of the University of Cape Town approved this study (REC 337/2004).

Clinical assessment sought to exclude differential diagnoses based on clinical presentation. For example, for patients with respiratory symptoms, bacterial and pneumocystis pneumonia were investigated. Clinical specimens were sent for TB microscopic examination, culture, and DST at the time of presentation with suspected TB IRIS. DST for rifampin and isoniazid was performed at 2 nationally accredited laboratories. Both laboratories used the indirect proportion method: one laboratory used liquid media with the MGIT system, and the other used solid culture medium (Middlebrook 7H11 agar). For 36 patients, a rapid rifampin resistance assay was performed (FASTplaque-Response; Biotec Laboratories) [22]. The result of this assay was confirmed by culture-based DST, and the latter result is reported unless stated otherwise. A purified protein derivative enzyme-linked immunospot assay was performed, as described elsewhere [23]. Patients were classified as having TB IRIS if 2 clinicians agreed that, at initial assessment and during the follow-up period, the patient fulfilled at least 1 of the case definitions.

Statistical methods. Fisher's exact test was used to compare proportions, and the Mann Whitney *U* test was used to analyze differences between medians. The unpaired Student's *t* test with Welch's correction was used to compare enzyme-linked immunospot assay results.

RESULTS

One hundred patients (66 female and 34 male patients) with suspected TB IRIS were evaluated from February 2005 through July 2006. The median age was 31 years (interquartile range [IQR], 26–35 years), and the median baseline CD4 cell count was 50 cells/ μ L (IQR, 26–94 cells/ μ L). Twenty-six patients had received ≥ 1 course of prior TB treatment. Patients developed symptoms prompting referral with suspected TB IRIS a median of 14 days (IQR, 7–25 days) after starting cART. These patients were assessed during screening for a randomized placebo-controlled trial of prednisone for mild and moderate TB IRIS (ISRCTN 21322548). Thirty-eight patients were enrolled in that study, and 25 received corticosteroid treatment for TB IRIS outside that study, usually for severe TB IRIS. Final diagnoses are shown in figure 1.

Follow-up CD4 cell counts during the first year of cART were available for 77 patients. In 73 patients (95%), the follow-up CD4 cell count increased (median increase, 139 cells/ μ L; IQR, 64–241 cells/ μ L) from the pre-cART value. In 4 patients,

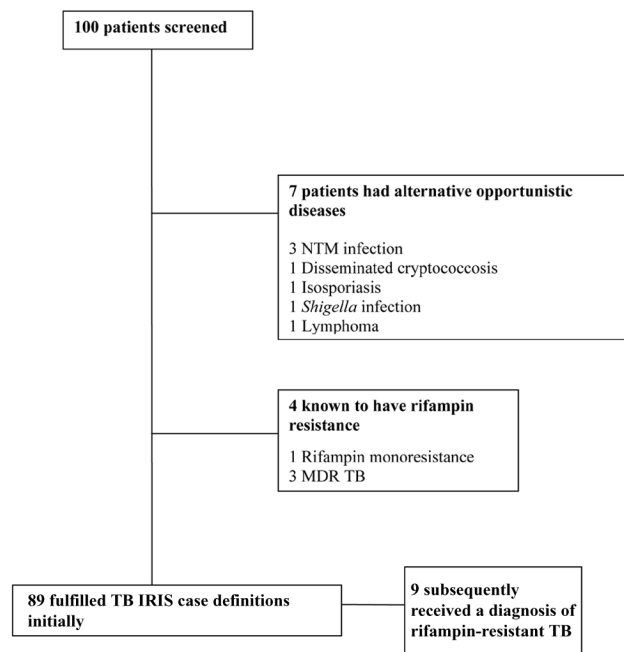


Figure 1. Final diagnosis for 100 patients with suspected cases of tuberculosis (TB) immune reconstitution inflammatory syndrome (IRIS). MDR, multidrug resistant; NTM, nontuberculous mycobacteria.

the CD4 cell count decreased by 1–50 cells/ μ L during the first year of cART. Follow-up viral loads, usually measured after 6 months of cART, were available for 74 patients. The viral load was <400 copies/mL in 65 patients, 400–1000 copies/mL in 5, and >1000 copies/mL in 4. In all 4 of these patients, the CD4 cell count increased by 77–365 cells/ μ L during cART.

Among the whole cohort, the initial TB diagnosis was made on the basis of culture of *Mycobacterium tuberculosis* in a clinical specimen for 41 patients and positive smear microscopy results for 31 patients. For 25 patients, a diagnosis of smear-negative or extrapulmonary TB was made on the basis of clinico-radiological data [24–26]. Although the other 3 patients were receiving TB treatment at presentation, the initial diagnosis of TB was incorrect, and they had nontuberculous mycobacterial infection.

Seven of the 25 patients with a clinico-radiological diagnosis had microbiological confirmation when they presented with suspected TB IRIS (4 were smear positive, and 3 were culture positive). For the other 18 patients, the diagnosis of TB was not microbiologically proven. These patients had TB symptoms and lymphadenopathy on abdominal ultrasound (5 patients), on chest radiograph (2), or peripherally (1); miliary infiltrates on chest radiograph (4); other radiographic pulmonary infiltrates (2); pericardial effusion (2); or pleural effusion (2). One of the patients who received a diagnosis on the basis of symptoms and abdominal nodes on ultrasound was subsequently found to have lymphoma and probably did not have TB.

Table 2. Cases of rifampin-resistant tuberculosis (TB) diagnosed after presentation with suspected TB immune reconstitution inflammatory syndrome (IRIS).

Patient	Age, years	Sex	Nadir CD4 cell count, cells/ μ L	Previous TB	Initial TB diagnosis	Course of events	TB IRIS manifestations
1	24	F	94	No	Pleural and pulmonary; not confirmed microbiologically	Symptoms improved and pleural effusion decreased while receiving Rif, INH, Pza, and Eth; started cART 8 weeks later; suspected TB IRIS onset 14 days later; sputum culture negative for TB twice at IRIS onset; sputum and pleural aspirate samples from 8–10 weeks after IRIS onset grew MDR <i>M. tuberculosis</i>	Recurrent constitutional, respiratory, and abdominal symptoms; fever and weight loss; cervical node enlargement, hepatomegaly; CXR showed progressively worsening nodular infiltrates and enlarging pleural effusion
2	29	F	16	No	Disseminated (pulmonary infiltrates on CXR and ascites on ultrasound); not confirmed microbiologically	Started on Rif, INH, Pza, and Eth; symptoms improved; started cART 13 weeks later; suspected TB IRIS onset 39 days later; bronchoscopy performed 8 weeks later (bronchial brushings grew MDR TB); thereafter, ongoing IRIS manifestations despite MDR TB treatment	High fevers, weight loss, and abdominal and constitutional symptoms; right hilar node enlargement on CXR with bronchial compression (on bronchoscopy); new right middle lobe infiltrate on CXR; enlarging cervical nodes
3	26	F	44	Yes	Disseminated; lymphadenopathy and hepatosplenomegaly on abdominal ultrasound; urine sample culture positive for <i>Mycobacterium tuberculosis</i> ; DST not requested at the time	Started Rif, INH, Pza, Eth, and Stm; reported 2-kg weight gain, and CRP level decreased (202 to 69 mg/L); started cART 1 month later; 5 days later, developed suspected TB IRIS; DST requested on initial urine isolate revealed Rif monoresistance	Abdominal pain, constitutional symptoms, and weight loss; progressively enlarging hepatomegaly, cervical node enlargement, para-umbilical cold abscess, peritonism, and marked tachycardia
4	51	F	35	No	Pulmonary; sputum sample cultured for <i>M. tuberculosis</i> ; DST not performed at the time	Symptomatic improvement while receiving Rif, INH, Pza, and Eth (cough and night sweats resolved, and patient felt stronger); started cART 2 months later; 7 days later, developed suspected TB IRIS; lymph node aspirate culture positive for MDR <i>M. tuberculosis</i>	Constitutional symptoms, marked weight loss, and respiratory symptoms; cervical node enlargement; CXR showed pulmonary infiltrates and enlarging thoracic nodes
5	31	F	3	No	Disseminated (CXR revealed pulmonary infiltrates and cervical adenitis); lymph node aspirate smear positive and sputum culture positive for <i>M. tuberculosis</i> ; no DST performed at the time	Initially improved while receiving Rif, INH, Pza, and Eth (night sweats resolved and general condition improved); then had chronic diarrhea followed by recurrent TB symptoms prior to cART; started cART 4 months after TB treatment; 3 days later, began deteriorating more rapidly, requiring admission; sputum culture positive for MDR <i>M. tuberculosis</i>	Recurrent cough; constitutional and abdominal symptoms; new pulmonary infiltrates with cavitation on CXR

(continued)

Table 2. (Continued.)

Patient	Age, years	Sex	Nadir CD4 cell count, cells/ μ L	Previous TB	Initial TB diagnosis	Course of events	TB IRIS manifestations
6	24	M	47	No	Disseminated; CXR revealed pulmonary infiltrates; abdominal ultrasound showed lymphadenopathy and splenic microabscesses; urine sample culture positive for <i>M. tuberculosis</i> ; no DST was performed at the time	Reported symptomatic improvement while receiving Rif, INH, Pza, and Eth; started cART 6 months after TB treatment; 29 days later, developed suspected TB IRIS; node aspirate grew <i>M. tuberculosis</i> and NTM; thus, formal DST could not be done; FASTplaque demonstrated Rif resistance	Constitutional symptoms, fever, new axillary lymphadenopathy, and tender hepatomegaly with jaundice
7	28	F	187	Yes ^a	Pulmonary (smear positive)	Started receiving Rif, INH, Pza, Eth, and Stm with some adherence lapses, but symptomatically better when started cART 5 months later; 7 days after starting cART, developed suspected TB IRIS; sputum culture positive for MDR <i>M. tuberculosis</i>	Fever and constitutional symptoms; pulmonary infiltrates on CXR
8	28	F	103	No	Disseminated; abdominal ultrasound showed lymphadenopathy and splenic microabscesses; sputum culture positive for <i>M. tuberculosis</i> ; susceptible to Rif and INH	Symptomatically improved while receiving Rif, INH, Pza, and Eth, with reported weight gain; 6 weeks after TB treatment, started cART; developed suspected TB IRIS 13 days later; then sputum culture positive for MDR <i>M. tuberculosis</i>	Respiratory, abdominal, and constitutional symptoms; weight loss, fever, and tachycardia; peritonism on examination; cervical node enlargement; CXR showed progressive pulmonary infiltrates and enlarging thoracic nodes
9	31	M	12	Yes ^a	Disseminated; CXR revealed pulmonary infiltrates and CSF smear positive for acid-fast bacilli; patient also received a diagnosis of cryptococcal meningitis before initiation of cART	Symptomatically improved while receiving treatment for TB and cryptococcal meningitis prior to cART; 48 days after starting cART, developed suspected TB IRIS; sputum culture positive for MDR <i>M. tuberculosis</i>	Respiratory and constitutional symptoms, weight loss, and headaches; hepatomegaly; CXR showed infiltrates with cavitation; CSF showed worsened lymphocytic meningitis that could have been due to TB or cryptococcal IRIS

NOTE. cART, combined antiretroviral therapy; CRP, C-reactive protein; CXR, chest radiograph; DST, drug susceptibility testing; Eth, ethambutol; INH, isoniazid; MDR, multidrug resistant; NTM, nontuberculous mycobacteria; Pza, pyrazinamide; Rif, rifampin; Stm, streptomycin.

^a Previously defaulted TB treatment.

Seven patients with suspected TB IRIS had clear evidence of an alternative opportunistic condition (figure 1). In 3 patients, this was a nontuberculous mycobacterial infection. These patients had experienced some symptomatic improvement while receiving TB treatment before initiation of cART and were then referred with suspected TB IRIS after commencing cART. Review of the initial sputum culture results revealed nontuberculous mycobacterial infection. In 2 of the 3 patients, nontuberculous mycobacteria were also cultured from blood samples at the time of assessment for suspected TB IRIS.

Twenty-five patients had DST performed at initial TB diagnosis. For 19 of these patients, the isolate was susceptible to

rifampin and isoniazid; 2 had isolates monoresistant to isoniazid, 1 had an isolate monoresistant to rifampin, and 3 had MDR TB. In an additional patient (patient 3), DST of the isolate at TB diagnosis was performed retrospectively after presentation with suspected TB IRIS, and the isolate demonstrated monoresistance to rifampin (table 2). For this patient, the culture result at the time of presentation with TB IRIS was negative. All 4 patients who presented with suspected TB IRIS with known rifampin resistance were receiving appropriate therapy for MDR TB and reported at least partial clinical improvement before initiation of cART. These patients then presented with clinical deterioration 7–45 days after starting cART. Their clin-

Table 3. Characteristics of the cohort.

Characteristic	Patients			
	Received a diagnosis of TB IRIS without Rif resistance (n = 80)	Presented with suspected TB IRIS and then received a diagnosis of Rif-resistant TB (n = 9)	Known to have Rif-resistant TB at presentation with suspected TB IRIS (n = 4)	Presented with suspected TB IRIS and then received a diagnosis of an alternative OD (n = 7)
Female sex	52 (65)	7 (78)	1 (25)	6 (86)
Age, years	31 (27–36)	29 (26–31)	31 (28–35)	30 (24–34)
Previous TB	18 (23)	3 (33)	2/3 (67)	3 (43)
Prior cART ^a	4 (5)	1 (11)	1 (25)	0 (0)
WHO clinical stage ^b				
3	28 (35)	2 (22)	0 (0)	2 (29)
4	52 (65)	7 (78)	4 (100)	5 (71)
cART regimen at assessment				
d4T, 3TC, and EFV	64 (80)	8 (89)	4 (100)	6 (86)
Other	16 (20)	1 (11)	0(0)	1 (14)
TB disease form ^c				
Pulmonary	36 (45)	3 (33)	2 (50)	3 (43)
Extrapulmonary	44 (55)	6 (67)	2 (50)	4 (57)
Time from start of TB treatment to start of cART, days	68 (35–93)	84 (47–115)	199 (121–447)	69 (21–125)
Time from start of cART to TB IRIS onset, days	14 (8–23)	13 (7–29)	35 (17–45)	35 (7–52)
Baseline CD4 cell count, cells/ μ L	50(26–94) ^d	44(16–94)	84 (55–184)	72 (13–85)
Baseline viral load measured	61 (76)	5 (56)	0 (0)	5 (71)
Baseline viral load, value $\times 10^5$ copies/mL	2.1 (9.8– 5.0)	1.7 (1.4–5.6)	...	2.5 (1.1–5.0)

NOTE. Data are no. (%) of patients or median value (interquartile range). cART, combination antiretroviral therapy; d4T, stavudine; EFV, efavirenz; IRIS, immune reconstitution inflammatory syndrome; OD, opportunistic disease; Rif, rifampin; TB, tuberculosis; 3TC, lamivudine; WHO, World Health Organization.

^a Excludes patients who received single-dose nevirapine for prevention of mother-to-child transmission.

^b WHO staging includes current TB diagnosis.

^c The pulmonary TB category includes patients who had pulmonary TB only, and the extrapulmonary TB category includes patients with extrapulmonary TB only and those with both pulmonary TB and extrapulmonary TB.

^d Seventy-nine of these 80 patients had a baseline CD4 cell count available.

ical manifestations included fever or night sweats (2 patients), marked weight loss (2), new peripheral nodes (1), new pulmonary infiltrates on chest radiograph (2), and other recurrent TB symptoms. Although these features are typical of TB IRIS, the case definitions that we used excluded this diagnosis.

Eighty-five patients had at least 1 culture for *M. tuberculosis* performed during assessment for TB IRIS. Cultures for *M. tuberculosis* were performed on sputum (for 55% of patients), lymph node or abscess aspirate (18%), pleural fluid (8%), CSF (8%), or other clinical specimens (11%). Culture yielded *M. tuberculosis* for 17 patients (20%; 8 had MDR TB, 1 had isoniazid-monoresistant TB, and 7 had TB that was susceptible to rifampin and isoniazid). Drug susceptibility was undetermined for 1 patient (patient 6) (table 2), because the culture of the patient's sample yielded both nontuberculous mycobacteria and *M. tuberculosis*. This patient had a FASTplaque assay indicative of rifampin resistance. Including the patient whose initial isolate was rifampin resistant (patient 3) (table 2) and the patient with a FASTplaque result indicating rifampin resistance (patient 6) (table 2), 10 patients were found to have rifampin-resistant TB at the time of TB IRIS presentation. One of these patients was previously known to have MDR TB; thus, 9 (10.1%; 95% CI, 3.9%–16.4%) of 89 patients with suspected

TB IRIS had rifampin-resistant TB that was previously unsuspected (table 2). All 9 of these patients reported symptomatic improvement after commencing standard TB treatment. For 1 of these patients (patient 8) (table 2), culture and DST at initial TB diagnosis revealed susceptibility to rifampin and isoniazid, but repeat DST performed for suspected TB IRIS revealed MDR TB.

Table 3 compares the baseline characteristics of the 4 diagnostic groups. No statistically significant differences existed in univariate analysis, with the exception of shorter duration from initiation of TB treatment to initiation of cART in the group of patients who received diagnoses of TB IRIS with no rifampin resistance, compared with those who were known to have rifampin-resistant disease when they presented with suspected TB IRIS (median, 68 days vs. 199 days; $P = .02$).

Table 4 shows clinical, radiological, and laboratory features of the TB IRIS episode for the 80 patients who received a final diagnosis of TB IRIS without rifampin resistance. The TB IRIS case definitions that were fulfilled are also shown. Many patients fulfilled >1 case definition, and frequently, TB IRIS involved >1 organ system. The most frequent TB IRIS symptoms among these 80 patients were constitutional (68 patients; 85%), including night sweats, malaise, anorexia, and weight loss; re-

Table 4. Clinical, radiographic, and laboratory features of tuberculosis (TB) immune reconstitution inflammatory syndrome (IRIS) in 80 patients who received a diagnosis of TB IRIS without rifampin resistance.

Variable	Patients
TB IRIS case definitions fulfilled	
New, recurrent, or worsening symptoms	80 (100)
New or worsening TB manifestations	59 (74) ^a
New or expanding lymph nodes	35 (44)
New or expanding cold abscesses	3 (4)
New or expanding intracranial tuberculomas	1 (1)
New or expanding pulmonary infiltrates	22 (28)
New or recurrent TB meningitis ^b	6 (8)
New or enlarging serous effusions	13 (16)
New or worsening granulomatous hepatitis	2 (3)
New or worsening granulomatous bone marrow infiltrate	5 (6)
Other new or worsening TB lesion ^c	3 (4)
Other physical signs	
Fever (temperature, >37.4°C)	31 (39)
Hepatomegaly	45 (56)
Splenomegaly	7 (9)
Peritonism	4 (5)
New neurological signs	7 (9)
Radiographic features	
On chest radiograph	
Pulmonary infiltrates	52/80 (65)
Hilar/mediastinal nodes	30/80 (38)
Pleural effusion	17/80 (21)
On ultrasound	
Abdominal nodes	27/35 (77)
Ascites	10/35 (29)
Pericardial effusion	10/35 (29)
Focal splenic lesions	7/35 (20)
On CT: head lesions	3/8 (38) ^d
Laboratory features	
Total bilirubin level, $\mu\text{mol/L}$ ($n = 13$) ^e	54 (16–103) [0–21]
Alkaline phosphatase level, IU/L ($n = 56$)	181 (103–326) [40–120]
Alanine aminotransferase level, IU/L ($n = 62$)	41.5 (28–59.8) [5–40]
γ -Glutamyl transferase level, IU/L ($n = 15$)	217 (152–482) [0–35]
Hemoglobin level, g/dL ($n = 73$)	9.1 (7.8–10) [M: 13.0–17.0; F: 12.0–15.0]
WBC count, value $\times 10^9$ cells/L ($n = 71$)	5.4 (3.7–8.7) [4.0–10.0]
Platelet count, value $\times 10^9$ cells/L ($n = 65$)	362 (268–462) [178–400]
C-reactive protein level, mg/L ($n = 72$)	96 (70–152) [0–10]
C-reactive protein level >10 mg/L ($n = 72$)	71 (99)

NOTE. Data are no. (%) of patients or median value (interquartile range) [reference range].

^a The other 21 patients fulfilled only the case definition relating to new, worsening, or recurrent symptoms.

^b TB IRIS meningitis was defined as ≥ 5 lymphocytes and/or polymorphs in a CSF sample from a patient with new or recurrent meningism or other new neurological symptoms after exclusion of bacterial and fungal meningitis.

^c The other manifestations were arthritis (in 2 patients) and bursitis (in 1 patient).

^d Eight patients had CT performed: tuberculomas were shown for 1 patient and a new infarct (in 1 patient) and hydrocephalus (in 1 patient) were shown for the others.

^e Bilirubin level measurement was performed mainly for those patients in whom jaundice was clinically apparent. Nine of the 13 patients who had their bilirubin level measured had a total bilirubin level >21 $\mu\text{mol/L}$.

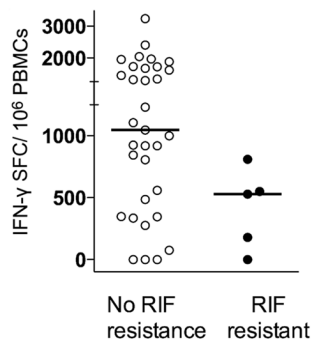


Figure 2. Comparison of purified protein derivative enzyme-linked immunospot assay results for 33 patients with tuberculosis (TB) immune reconstitution inflammatory syndrome (IRIS) with no rifampin (RIF) resistance (median, 1047 spot-forming cells [SFC] \times 10⁶ PBMCs; interquartile range, 417–1700 SFC \times 10⁶ PBMCs) with those for 5 patients who received a diagnosis of RIF-resistant TB after presenting with TB IRIS (median, 529 SFC \times 10⁶ PBMCs; interquartile range, 89–680 SFC \times 10⁶ PBMCs; $P = .04$).

spiratory (48; 60%); and abdominal (47; 59%), including abdominal pain, nausea, vomiting, and diarrhea. The symptoms, signs, and radiological findings for the 9 patients with rifampin-resistant TB diagnosed after presentation with suspected TB IRIS did not differ significantly from those of the patients who had TB IRIS without rifampin-resistant TB, with the exception of more frequent presence of lymphadenopathy on chest radiograph (7 of 9 patients vs. 30 of 80 patients; $P = .03$). Blood investigations were also similar between the 2 groups, although for the 9 patients with rifampin-resistant TB, the median C-reactive protein level was higher (179 mg/L [IQR, 100–212 mg/L] vs. 96 mg/L [IQR, 70–152 mg/L]; $P = .05$). Purified protein derivative enzyme-linked immunospot assay demonstrated evidence of immune activation in both groups, although the degree was less in the patients with rifampin-resistant TB (figure 2).

DISCUSSION

In Cape Town, the triple coincidence of very high TB case notification rates, an expanding epidemic of HIV infection, and the large-scale roll-out of cART has led to a large increase in the number of cases of TB IRIS (to date, there are hundreds of cases) presenting at our health care service. We prospectively evaluated clinical case definitions for TB IRIS for 100 cases; this was, to our knowledge, the largest TB IRIS case series reported to date. Once patients with alternative conditions (7 patients) and known rifampin-resistant TB (4 patients) were excluded, 9 (10%) of the remaining 89 patients were found to have rifampin-resistant TB only after presenting with suspected TB IRIS. This has important implications for the diagnosis and treatment of HIV-infected patients with TB whose conditions deteriorate after the introduction of cART.

In the 9 patients with rifampin-resistant TB, presentation was suggestive of TB IRIS, with improvement while receiving TB treatment before initiation of cART and then deterioration during the weeks after the initiation of cART. The obvious question is whether the condition of the patients with drug-resistant TB deteriorated because of suboptimally treated TB, TB IRIS, or both? Our case definitions, which are similar to those used by other researchers [14, 15], classified known rifampin resistance as excluding TB IRIS. However, in light of our observations, we propose that antitubercular drug resistance and TB IRIS are not mutually exclusive and may overlap in the same person. First, given that TB IRIS immunopathology is attributable to restored antigen-specific immunity to *M. tuberculosis* antigens [12], it is reasonable to conclude that TB IRIS may occur in response to drug-susceptible or drug-resistant strains, whether the latter are treated or untreated. The antigen stimulus for TB IRIS is unlikely to differ in these scenarios.

Second, in the 4 patients with known rifampin-resistant TB, all improved while receiving treatment for MDR TB, and then their conditions deteriorated after cART initiation. The deterioration of their conditions was most likely attributable to TB IRIS, given the timing. These patients may be at higher risk of TB IRIS than patients with rifampin-susceptible TB. *M. tuberculosis* bacillary load has been suggested as a risk factor for the condition [7], and even those patients who are effectively treated for MDR TB are likely to have slow bacillary clearance. This may partially account for our observation that these 4 patients developed TB IRIS despite a long interval between initiation of TB therapy and initiation of cART (table 3).

Third, 9 patients had undiagnosed rifampin-resistant TB when they presented with suspected TB IRIS. These patients all reported at least partial symptomatic response to standard TB treatment before the initiation of cART. It is possible for patients with MDR TB to initially respond to first-line TB treatment [27–29], either because the organism is sensitive to ethambutol and pyrazinamide or because the patient is dually infected with MDR TB and a susceptible strain [30]. In addition, patients may improve while receiving treatment for drug-susceptible TB and then be reinfected with drug-resistant TB, or the infecting organism may develop rifampin resistance during treatment. Either of these scenarios could have occurred for patient 8 (table 2). For these 9 patients who received a diagnosis of rifampin-resistant TB after presenting with suspected TB IRIS, symptomatic deterioration occurred 3–48 days after the initiation of cART—the characteristic timing of TB IRIS. The clinical and radiological features of these patients when they presented with suspected TB IRIS, with the exception of more frequent presence of lymphadenopathy on chest radiograph, were not significantly different from those of patients with TB IRIS with no drug resistance. We propose that TB IRIS

exacerbated undiagnosed drug-resistant TB. Support for the idea that TB IRIS was present in this group comes from the enzyme-linked immunospot assay data, which showed expansions of purified protein derivative-specific IFN- γ -producing T cells (which are a reported characteristic of TB IRIS [12]), irrespective of drug susceptibility. The overlap of IRIS and drug resistance with respect to cryptococcal infection has been highlighted elsewhere [31].

Seven patients with suspected TB IRIS had alternative opportunistic diseases that explained their clinical deterioration. Patients with advanced immunosuppression may have multiple opportunistic conditions. A thorough examination for alternative infections and malignancies before diagnosis of TB IRIS is essential. Three patients receiving TB treatment at their local clinic actually had nontuberculous mycobacterial infection when the result of the original culture was followed up; this underscored the importance of reviewing the original TB diagnosis during the assessment of these patients. The performance of these case definitions may not be the same in settings with lower TB incidence rates, where patients might more frequently present with other reasons for deterioration.

Frequently, patients had signs, symptoms, and radiological manifestations suggestive of multiple organ system involvement with TB IRIS. This may be explained by profound immunosuppression (median nadir CD4 cell count, 50 cells/ μ L), predisposing to disseminated TB. The most frequent organ systems involved were the respiratory system (respiratory symptoms and chest radiograph infiltrates) and, hitherto little appreciated, the abdominal organs. Of the 80 patients with TB IRIS with no rifampin resistance, 59% had abdominal symptoms; 56% had hepatomegaly, 9% had splenomegaly, and 5% had peritonism. Abdominal nodes were present in 77% of those who underwent ultrasonography. Liver function derangement, particularly a cholestatic pattern, was common. These findings suggest that, if patients develop abdominal symptoms or liver function derangement after commencing cART, TB IRIS should be considered in the differential diagnosis in addition to drug-related adverse effects, such as pancreatitis, lactic acidosis, and drug-induced hepatitis.

There were several limitations to our study. For most patients, the initial TB diagnosis had been made in primary care according to program guidelines, and only 41% of the cases were confirmed by culture. Most patients also initiated cART at the same facilities; thus, examination and radiographic data were incomplete. All patients had a chest radiograph performed to investigate TB IRIS, but few were performed at the time of cART initiation. In addition, patients who are smear positive do not routinely undergo chest radiography at TB diagnosis. Therefore, for many patients with symptoms of TB IRIS who had radiographic pulmonary infiltrates (65% had infiltrates), it was impossible to determine whether these were new or

expanding, which potentially underestimated the number of cases fulfilling the pulmonary infiltrate case definition and, similarly, the serous effusion case definition. Many patients had hepatomegaly (56% of patients) with cholestatic liver derangement, which suggested granulomatous hepatitis [32], but this was only confirmed in 2 patients, because severity otherwise was insufficient to warrant a biopsy. The number of cases fulfilling the granulomatous hepatitis case definition might, for this reason, also be underestimated. It is for these reasons that the diagnosis of TB IRIS was made exclusively on the basis of a clear history of symptom improvement before the initiation of cART, followed by new, worsening, or recurrent TB symptoms after the initiation of cART in many patients (26% of patients). Follow-up CD4 cell count and HIV viral load measurements during cART were unavailable for one-quarter of the patients. These tests are performed every 6 months during cART under program conditions; however, many patients were transferred to other facilities, and some were lost to follow-up or died by 6 months. Determination of viral load or CD4 cell count at the time of TB IRIS diagnosis did not, however, form part of our case definitions. For patient 6 (table 2), the FASTplaque assay demonstrated rifampin resistance. This could not be confirmed by culture-based DST. The presence of nontuberculous mycobacterial coinfection in the patient's specimen may have affected the FASTplaque result, but it is worth noting that the specimen was culture positive for *M. tuberculosis* after 7 months of TB treatment; this supported a diagnosis of rifampin-resistant TB.

Corticosteroids have been proposed as a treatment for TB IRIS, although the only evidence currently available is anecdotal [17]. There are potential risks of corticosteroid treatment for HIV-infected patients with TB, including herpes virus reactivation and Kaposi sarcoma [33, 34]. There is no present clinical trials evidence supporting the use of corticosteroids for the treatment of TB IRIS, other than for TB meningitis and, perhaps, pericardial disease (neither with evidence in the context of cART [35, 36]). Assessment of suspected TB IRIS should trigger re-evaluation of the TB diagnosis and the adequacy of antimicrobial therapy. Such assessment should occur before the instigation of corticosteroid therapy; otherwise, there is a risk that patients who remain deeply immunosuppressed (despite IRIS) may receive steroid therapy without adequate antimicrobial coverage. Our study highlights the pressing need to develop and implement rapid techniques to diagnose drug resistance that are appropriate to resource-limited conditions. The use of the FASTplaque assay in the present study was an attempt to overcome this problem, and there are encouraging data emerging from other studies of rapid tests [37]. Overall, our data also support the use of routine DST for HIV-infected patients with TB.

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CHAPTER 5

Type 1 helper T cells and FoxP3-positive T cells in HIV-tuberculosis-associated immune reconstitution inflammatory syndrome

Type 1 Helper T Cells and FoxP3-positive T Cells in HIV–Tuberculosis-associated Immune Reconstitution Inflammatory Syndrome

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Rationale: Tuberculosis-associated immune reconstitution inflammatory syndrome (TB–IRIS) induced by combination antiretroviral therapy (cART) has been attributed to dysregulated expansion of tuberculin PPD–specific IFN- γ –secreting CD4⁺ T cells.

Objectives: To investigate the role of type 1 helper T cell expansions and regulatory T cells in HIV–TB IRIS.

Methods: Longitudinal and cross-sectional studies of *Mycobacterium tuberculosis*–specific IFN- γ enzyme-linked immunospot responses and flow cytometric analysis of blood cells from a total of 129 adults with HIV-1–associated tuberculosis, 98 of whom were prescribed cART.

Measurements and Main Results: In cross-sectional analysis the frequency of IFN- γ –secreting T cells recognizing early secretory antigenic target (ESAT)-6, α -crystallins 1 and 2, and PPD of *M. tuberculosis* was higher in patients with TB–IRIS than in similar patients treated for both HIV-1 and tuberculosis who did not develop IRIS (non-IRIS; $P \leq 0.03$). The biggest difference was in the recognition of α -crystallin molecules: peptide mapping indicated a polyclonal response. Flow cytometric analysis indicated equal proportions of CD4⁺ and CD8⁺ cells positive for activation markers HLA-DR and CD71 in both patients with TB–IRIS and non-IRIS patients. The percentage of CD4⁺ cells positive for FoxP3 (Forkhead box P3) was low in both groups (TB–IRIS, 5.3 ± 4.5 ; non-IRIS, 2.46 ± 2.46 ; $P = 0.13$). Eight weeks of longitudinal analysis of patients with tuberculosis who were starting cART showed dynamic changes in antigen-specific IFN- γ –secreting T cells in both the TB–IRIS and non-IRIS groups: the only significant trend was an increased response to PPD in the TB–IRIS group ($P = 0.041$).

Conclusions: There is an association between helper T-cell type 1 expansions and TB–IRIS, but the occurrence of similar expansions in non-IRIS brings into question whether these are causal. The defect in immune regulation responsible for TB–IRIS remains to be fully elucidated.

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AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Little is known of the pathogenesis of the HIV–TB immune reconstitution inflammatory syndrome (TB–IRIS), although preliminary analyses have attributed it to dysregulated helper T-cell type 1 (Th1) expansion.

What This Study Adds to the Field

Dynamic *Mycobacterium tuberculosis*–specific Th1 responses associate with TB–IRIS, although the presence of such responses in patients who did not develop the syndrome suggests that other regulatory factors are involved.

Keywords: tuberculosis; HIV; immune reconstitution inflammatory syndrome; FoxP3; type 1 helper T cells

HIV-1–associated tuberculosis is a major cause of mortality in sub-Saharan Africa (1). The widespread introduction of combination antiretroviral therapy (cART) has, however, been associated with a decrease of between 50 and 80% in the annual risk of tuberculosis in HIV-1–infected persons (2–4). cART partially and beneficially restores pathogen-specific immunity (5), but is also associated under some circumstances with paradoxical deterioration of existing, or unmasking of, unapparent infection (6, 7). This phenomenon is presumed to reflect the simultaneous restoration of immunopathological as well as protective immunity during antitubercular and cART. Deterioration of mycobacterial disease was recognized early as one of the forms of aberrant immune restoration or immune reconstitution inflammatory syndrome (IRIS) (8, 9). A number of case series of tuberculosis–IRIS (TB–IRIS) have been reported (reviewed by Dhasmana and coworkers [10]) with recognized risk factors being disseminated tuberculosis, a low nadir CD4⁺ cell count, high viral load, and short interval between starting antituberculosis chemotherapy and cART. Initially reported from specialized centers (11, 12), TB–IRIS is now a frequent diagnosis in areas of the world where tuberculosis and HIV frequently coexist (13, 14).

The diverse clinical presentations of TB–IRIS have been characterized (10), but little is known of its immunopathogenesis. It has been related to expansion of tuberculin purified protein derivative (PPD)–specific interferon- γ –secreting CD4⁺ T cells during cART (8, 9, 15). In the most comprehensive analysis to date, expansion of PPD-specific T cells was sug-

gested as the cause of the syndrome: the maximal median value in 7 patients who developed TB–IRIS being higher than the maximal median values obtained from 12 similar patients who did not experience IRIS (16). In addition, T-cell reactivity to the species-specific and immunodominant early secretory antigenic target (ESAT)-6 protein was reported as low in patients with IRIS, suggesting that TB–IRIS may be associated with specific recognition of components of PPD. These findings in turn have led to the suggestion that regulatory T cells that secrete transforming growth factor- β or IL-10 may not expand at an equal rate during cART, thereby setting up conditions favorable to immunopathology (17, 18). This study was therefore performed to investigate the role of helper T-cell type 1 (Th1) expansions and regulatory cells in much larger numbers of patients than has hitherto been possible. We chose a range of previously well-characterized antigens of *Mycobacterium tuberculosis* including the species-specific, secreted and immunodominant antigen ESAT-6, the 38-kD cell wall antigen, and two cytoplasmic heat shock proteins, α -crystallin-1 (Acr1) and Acr2, to address the possibility that the immune response underlying TB–IRIS is antigen specific. Some of the results of these studies have been previously reported in the form of an abstract (19, 20).

METHODS

Subjects

The University of Cape Town (Cape Town, South Africa) Research Ethics Committee approved the study (REC references 337/2004 and 173/2005). In a cross-sectional analysis 35 cases of paradoxical TB–IRIS (see Table E1 in the online supplement for the case definition and also Meintjes and coworkers [21] for the consensus definition) were compared with 29 persons treated for HIV-associated tuberculosis with similar baseline CD4⁺ cell count and duration of combination antiretroviral treatment (cART) who did not develop IRIS (non-IRIS). A third group consisted of 31 HIV-infected persons with tuberculosis sampled before antitubercular treatment and who were not receiving cART (untreated HIV+TB). Tuberculosis was diagnosed primarily on the basis of smear or culture positivity. Where this was negative or unavailable, diagnosis was in line with guidelines for smear-negative or extrapulmonary tuberculosis in HIV-infected persons (22, 23). TB–IRIS was diagnosed according to case definitions shown in Table E1. New TB cases were treated with 6 months duration of first-line therapy (isoniazid, rifampin, pyrazinamide, and ethambutol [HRZE] for 2 months followed by HR for 4 months [2HRZE/4HR]). The retreatment regimen added streptomycin (S) as follows: 2HRZES/1HRZE/5HRE. All non-IRIS patients and 28 of 35 patients with TB–IRIS were prescribed the cART combination of stavudine (d4T), lamivudine (3TC), and efavirenz (EFZ). Six patients with TB–IRIS were prescribed zidovudine (AZT), 3TC, and EFZ and one was prescribed AZT, 3TC, and nevirapine (NVP). No patient had received prior cART.

Longitudinal analysis was of a prospective cohort of 63 adults who started cART in primary care while undergoing TB treatment between January 2006 and September 2007. Participants were monitored four times for a period of 2 months after initiation of cART. The main outcome was TB–IRIS, with suspected cases being referred to secondary care for investigation and further management.

Immunological Analysis

Blood was drawn for IFN- γ enzyme-linked immunospot (ELISpot) analysis and peptide mapping as previously described (24, 25). For flow cytometric analysis peripheral blood mononuclear cells (PBMCs) were separated by standard protocols, and plated at 10⁶/ml per well in 24-well plates in RPMI–10% fetal calf serum (FCS). Heat-killed *M. tuberculosis* H37Rv was added to the wells at a multiplicity of infection of 1:15 (hkH37Rv:PBMC) and the plates were incubated overnight at 37°C in 5% CO₂. Cells were then surface stained for 20 minutes on ice, using the following fluorescent antibodies (all from BD Biosciences, San Jose, CA): CD3–allophycocyanin (APC), CD4–peridinin chloro-

phyll protein (PerCP), CD8–PerCP, HLA-DR–fluorescein isothiocyanate (FITC), and CD71–phycoerythrin (PE). After washing, stained cells were fixed in phosphate-buffered saline (PBS)–2% FCS containing 1.6% paraformaldehyde and acquired with a FACSCalibur flow cytometer (BD Biosciences). Intracellular staining for FoxP3 (Forkhead box P3) was performed with the PE-conjugated anti-human FoxP3 staining set from eBioscience (San Diego, CA). Cells were stimulated as described previously for 2 hours, after which brefeldin A was added at a concentration of 5 μ g/ml (an unstimulated control well was also included). Cells were first surface stained with anti-CD3–APC and anti-CD4–PerCP, followed by incubation for 45 minutes on ice in eBioscience Fix/Perm buffer. After washing in permeabilization buffer, cells were incubated for 15 minutes on ice in 2% normal rat serum, followed by incubation for 30 minutes on ice in 10 μ l of anti-human FoxP3 (clone PCH101) antibody. The cells were then washed again, fixed in PBS–2% FCS containing 1.6% paraformaldehyde, and acquired. Analysis was performed with FlowJo software (Tree Star, Ashland, OR). First, the lymphocyte population was gated on the basis of forward scatter and side scatter, and analyzed for CD4 and FOXP3. For the activation and proliferation markers HLA-DR and CD71, CD3⁺ cells were selected out of the lymphocyte population, followed by CD4⁺/CD71⁺ or CD4⁺/HLA-DR⁺ cells. Serum C-reactive protein was determined by analysis with a SYNCHRON CX system (Beckman Coulter, Fullerton, CA).

Statistical Analysis

The normality of data was assessed by the D'Agostino and Pearson omnibus normality test. Medians are given with the interquartile range (IQR) and means are presented with the standard deviation (SD). Paired parametric data were analyzed by Student paired *t* test or repeated-measures analysis of variance, and nonparametric paired data were analyzed by Wilcoxon matched-pairs test or by the Friedman test. Unpaired parametric variables were assessed by Student unpaired *t* test and nonparametric variables were assessed by Mann-Whitney U test.

RESULTS

We first compared the frequency of *M. tuberculosis* antigen-specific IFN- γ spot-forming cells (SFCs) cross-sectionally in TB–IRIS at presentation, non-IRIS after 2 weeks of cART, and untreated HIV+TB groups (Table 1). The TB–IRIS and non-IRIS groups were similar in age, tuberculosis disease form, duration of cART at sampling, baseline CD4⁺ cell count, and sex (see Table 1). They did, however, differ in the median interval between starting TB treatment and cART, which was shorter in the TB–IRIS group (55 d, IQR 30–82 vs. 80 d, IQR 44–141; *P* = 0.011). In the untreated HIV+TB group more TB diagnoses were microbiologically confirmed and the baseline CD4⁺ cell count was higher than for the both TB–IRIS and non-IRIS groups (see Table 1). In addition the median age of the untreated HIV+TB group was lower than that of the non-IRIS group (28 yr, IQR 25–36 vs. 35.6 yr, IQR 30–41; *P* = 0.018). In 25 of 30 (83%) patients with TB–IRIS for whom a result was available the convalescent (i.e., post-IRIS episode) viral load was below the detection limit. In the patients with a detectable load, no value exceeded 1,000 copies/ml. In addition, an increase in CD4⁺ cell count occurred in 31 of 33 (94%) patients, with a median increase of 130 cells/ μ l (IQR 63–209).

The antigens assayed included the secreted and species-specific ESAT-6 molecule (26, 27); a 38-kD cell wall antigen (28, 29); two small cytoplasmic heat shock proteins, Acr1 and Acr2 (25, 30); and PPD. The response to the 38-kD antigen was not assayed in the untreated HIV+TB group. Overall responses were highly heterogeneous with strong positive responses to some antigens in some non-IRIS patients and a lack of response to some antigens in some patients with TB–IRIS (Figure 1). However, the median frequency of IFN- γ SFCs was higher in the TB–IRIS group than in the non-IRIS group, with the

TABLE 1. BASELINE CHARACTERISTICS OF PATIENTS WITH TUBERCULOSIS-ASSOCIATED IMMUNE RECONSTITUTION INFLAMMATORY SYNDROME AND COMPARATOR NON-IRIS AND UNTREATED HIV+TB GROUPS

	TB-IRIS	Non-IRIS	Untreated HIV+TB
N	35	29	31
Median age, yr (IQR)	31.4 (25.8–36.2)	35.6 (30.3–41.0)	28 (25–36)*
Baseline CD4 ⁺ cell count, cells/ μ l (IQR)	51 (29–106)	45 (23–122)	195 (111–331) [†]
TB treatment before cART, d	55 (30–82)	78 (38–132) [‡]	N/A
Days of cART to IRIS or to sample	14 (8–21)	14 (14, 15)	N/A
Females, n (%)	23 (66)	19 (66)	18 (58)
Previous TB, n (%)	12 (34)	4 (14)	7 (23)
TB disease form, n (%)			
Pulmonary or pleural	21 (60)	22 (76)	31 (100)
Disseminated	11 (31)	4 (14)	
Lymphadenopathic	3 (9)	3 (10)	
Smear or culture confirmed?	19 (54)	16 (55)	30 (97) [§]

Definition of abbreviations: cART = combination antiretroviral therapy; N/A = not applicable; TB = tuberculosis; TB-IRIS = tuberculosis-associated immune reconstitution inflammatory syndrome.

* Significantly lower than non-IRIS: $P = 0.018$.

[†] Significantly higher than TB-IRIS and non-IRIS: $P < 0.0001$.

[‡] Significantly more than TB-IRIS: $P = 0.011$.

[§] Significantly more than TB-IRIS and non-IRIS: $P < 0.0001$.

exception of the response to the 38-kD antigen (ESAT-6: 1,312, IQR 322–1,908 vs. 624, IQR 0–1,481 [$P = 0.024$]; Acr1: 1,080, IQR 432–1,363 vs. 228, IQR 7–1,037 [$P = 0.019$]; Acr2: 1,313, IQR 196–1,785 vs. 281, IQR 0–1,032 [$P = 0.027$]; 38-kD: 1,026, IQR 222–1,761 vs. 710, IQR 0–1,340 [$P = 0.25$]; PPD: 1,047, IQR 347–1,620 vs. 168, IQR 34–1,333 [$P = 0.047$]). When the TB-IRIS group was compared with the untreated HIV+TB group (median ESAT-6: 353, IQR 99–977 [$P = 0.004$]; Acr1: 57, IQR 0–139 [$P = 0.0001$]; Acr2: 58, IQR 22–189 [$P = 0.0002$]; PPD: 279, IQR 130–693 [$P = 0.001$]) the higher response of patients with TB-IRIS was even more pronounced. By contrast, there were no significant differences between the non-IRIS and untreated HIV+TB groups in response to any antigen. An alternative to effector T cells as a marker of immune activation is the serum C-reactive protein (CRP). We therefore determined CRP concentrations in the TB-IRIS and non-IRIS groups. The median CRP concentration was three times higher in the TB-IRIS group than in the non-IRIS group (99 mg/L, IQR 80–169 vs.

32 mg/L, IQR 9–83; $P < 0.0001$). Elevation of CRP above the reference laboratory range (10 mg/L) was invariable in the patients with TB-IRIS, but it was also elevated in 20 of 29 (69%) of the non-IRIS patients. Although this difference in proportions is significant ($P = 0.0004$) these data again reflect considerable immune activation in the non-IRIS group in the absence of reported symptoms or signs of TB-IRIS.

The high response to several classes of *M. tuberculosis* antigens in the TB-IRIS group implies polyclonal, rather than oligoclonal, T-cell expansion. To further test this hypothesis we peptide-mapped responses to the two heat shock proteins Acr1 and Acr2, antigens that showed the greatest fold difference in recognition between the TB-IRIS and non-IRIS groups (see Figure 1). PBMCs from 13 patients with TB-IRIS known to respond to either Acr1, Acr2, or both were selected for this purpose. The median number of Acr1 peptides recognized per donor was 10 (IQR 3–14) and 6 (IQR 3–10) for Acr2. Immunodominant determinants (defined by a response exceed-

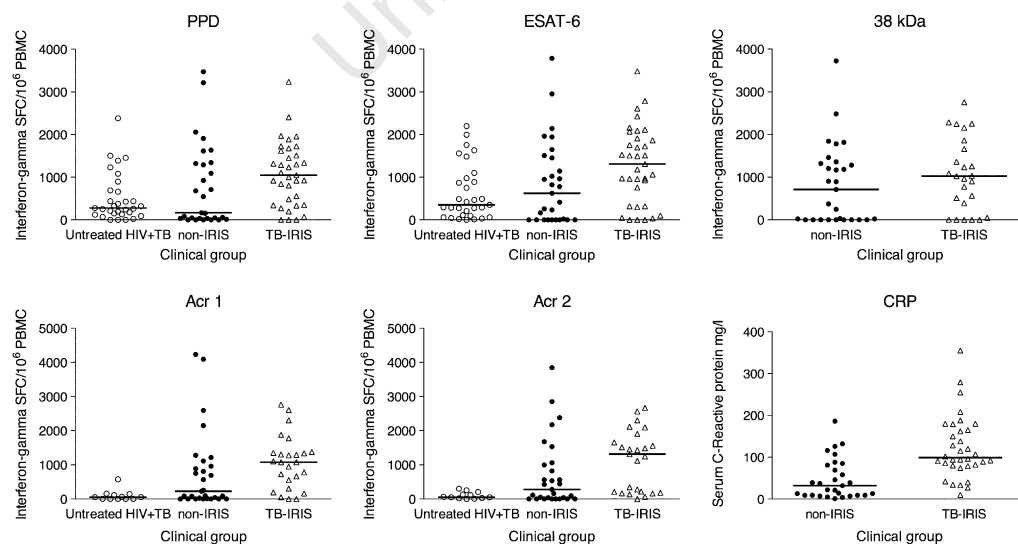


Figure 1. Frequency of *Mycobacterium tuberculosis* antigen-specific IFN- γ spot-forming cells (SFCs) in HIV-infected patients with tuberculosis-associated immune reconstitution inflammatory syndrome (TB-IRIS). Two comparator groups were patients with HIV-associated tuberculosis before treatment and not receiving combination antiretroviral therapy (cART) (untreated HIV+TB), and patients with treated HIV-associated TB also receiving cART for a similar duration to the patients with TB-IRIS (non-IRIS). The frequency of IFN- γ SFCs was higher in patients with TB-IRIS than in non-IRIS patients ($P \leq 0.03$), with the exception of the response to 38-kD cell wall antigen. The frequency of IFN- γ SFCs

was also higher in patients with TB-IRIS than in patients with untreated HIV+TB in every instance ($P \leq 0.004$). By contrast, the frequency of IFN- γ SFCs did not differ between the non-IRIS and untreated HIV+TB groups in response to any antigen.

ing 30 IFN- γ SFCs per 10^6 PBMCs) were distributed widely across both molecules (see Figure E1 in the online supplement). Taken together, these findings strongly suggest a polyclonal response.

It has been suggested that defective restoration of regulatory T-cell function may contribute to the pathogenesis of TB-IRIS (10, 18). This was examined by flow cytometric analysis of PBMCs from a subset of TB-IRIS ($n = 11$) and non-IRIS ($n = 8$) patients for whom sufficient cells were available. The percentage of unstimulated CD4⁺ cells positive for the transcription factor FoxP3, although higher in the TB-IRIS group, did not significantly differ between the groups (TB-IRIS: mean, 5.3 ± 4.5 ; vs. non-IRIS: mean, 2.46 ± 2.46 [$P = 0.13$]; Figure 2). hkh37Rv restimulation of PBMCs for 24 hours did not appreciably change the detection frequency in the TB-IRIS group or make the difference between groups significant (TB-IRIS: mean, 5.6 ± 4.6 ; vs. non-IRIS: mean, 2.9 ± 2.3 [$P = 0.15$]; see Figure 2). In these subjects we also evaluated lymphocyte activation as determined by CD71 (transferrin receptor) and HLA-DR expression (see Figure 2). The percentage of activated CD4⁺ cells did not differ between groups (TB-IRIS: mean CD4⁺CD71⁺, 26.6 ± 17.2 ; vs. non-IRIS: mean CD4⁺CD71⁺, 29.9 ± 11.2 [$P = 0.64$]; TB-IRIS: mean CD4⁺ DR⁺, 45.2 ± 22.3 vs. non-IRIS: mean CD4⁺ DR⁺, 38.0 ± 15.0 [$P = 0.44$]). There was also evidence of a similar degree of CD8⁺ cell activation (TB-IRIS: mean CD4⁺ DR⁺, 28.8 ± 18.9 vs. non-IRIS: mean CD4⁺ DR⁺, 22.4 ± 9.6 [$P = 0.40$]; TB-IRIS: mean CD8⁺ DR⁺, 32.4 ± 8.6 vs. non-IRIS: mean CD8⁺ DR⁺, 37.0 ± 12.1 [$P = 0.35$]), but again this did not differentiate the TB-IRIS group from the non-IRIS group.

Cross-sectional analyses only provide partial insight and we were therefore interested to determine whether our findings could be repeated in a prospective longitudinal analysis of patients with TB commencing cART. In the prospective analysis 8 of 62 participants had insufficient clinical follow-up to exclude IRIS, 1 was withdrawn because of drug-induced hepatitis, 1 declined further study involvement after Week 1, and 1 died of *Escherichia coli* septicemia 12 days after starting cART. Ten of 51 (19.6%) of those with sufficient follow-up developed TB-IRIS according to our case definitions (see Table E1 and Table 2). ELISpot data gathered at three or more time points was available for 39 participants, all patients with IRIS, and 29 of 41 non-IRIS patients. Responses showed marked dynamic changes within both groups (Figure 3). Within-category (non-IRIS and TB-IRIS) data in the longitudinal study were first assessed by repeated measures analysis (Friedman). The only significant trend was the increase in response to PPD in the TB-IRIS group over 8 weeks ($P = 0.041$). Within-category analysis by less stringent two-time point paired testing confirmed the increase in median PPD response in the TB-IRIS group, from 495 SFCs per 10^6 PBMCs (IQR 169–630) to 1,549 SFCs per 10^6 PBMCs (IQR 725–2432) between Weeks 0 and 8, to be significant ($P = 0.008$). In addition, the increase in PPD response in the non-IRIS group between Week 0 (66 SFCs per 10^6 PBMCs [IQR 10–358]) and both Week 2 (161 SFCs per 10^6 PBMCs [IQR 5–1414]; $P = 0.04$) and Week 4 (376 SFCs per 10^6 PBMCs [IQR 37–1140]; $P = 0.01$) was significant. Expansion of PPD-specific SFCs to more than twice baseline at any time was observed in 8 of 10 (80%) patients with TB-IRIS and in 18 of 29 (62%) non-IRIS patients ($P = 0.45$). No other significant intragroup changes occurred, despite the highly dynamic nature of these responses. When comparing IRIS and non-IRIS groups the only significant difference was between the 8-week response to PPD in the non-IRIS group (174 SFCs per 10^6 PBMCs; IQR 87–556) and the group that developed TB-IRIS (1,541 SFCs per 10^6 PBMCs; IQR 725–2432) ($P = 0.001$). Two or more CRP results were also available from all 10 TB-IRIS cases and 29 of

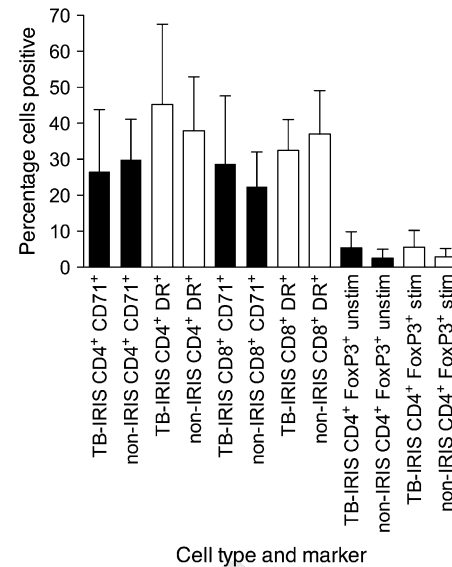


Figure 2. FoxP3 and T-cell activation markers in peripheral blood mononuclear cells (PBMCs) of patients with tuberculosis-associated immune reconstitution inflammatory syndrome (TB-IRIS). PBMCs from 11 TB-IRIS and 8 non-IRIS patients were analyzed. Although the proportion of activated T cells (both CD4⁺ and CD8⁺) was high in both groups, there were no significant differences between clinical groups. For FoxP3 analysis cells were either unstimulated (unstim) or restimulated (stim) with PPD for 24 hours. Again, there were no significant differences between TB-IRIS and non-IRIS groups. Error bars show the SD.

41 non-IRIS cases (see Figure 3). Repeated-measures tests did not confirm overall trends, partially because of data gaps at some time points that reduced the overall power of the analysis. Within-category analysis by two-time point paired testing showed the increase in median CRP response in the TB-IRIS group, from 10 mg/L (IQR 9–25) to 104 mg/L (IQR 56–175) between Weeks 0 and 2, to be significant ($P = 0.031$). In addition, the increase in CRP in the non-IRIS group between Week 0 (15 mg/L, IQR 7–20) and both Week 2 (39 mg/L, IQR 9–87; $P = 0.004$) and Week 4 (39 mg/L, IQR 12–78; $P = 0.006$) was significant. When comparing IRIS and non-IRIS groups significant differences were found between the 2-week response in the non-IRIS group (39 mg/L, IQR 9–87) and the group that developed TB-IRIS (104 mg/L, IQR 56–175) ($P = 0.027$). Of the 10 TB-IRIS patients shown in Figure 3, 4 were prescribed corticosteroid therapy and 2 were randomized to a double-blind trial of prednisone versus placebo for mild to moderate TB-IRIS. No obvious trend with respect to the effect of corticosteroid therapy on the Th1 expansions was observed.

DISCUSSION

Cape Town has a high tuberculosis case notification rate (>1,000/100,000 per annum in the area in which this study was conducted), rising adult HIV prevalence (antenatal seroprevalence exceeds 30% in some areas where the study was conducted), and rapidly increasing access to cART with roll-out to more than 20,000 people in the city so far. This triple coincidence has led to a substantial increase in cases of TB-IRIS presenting to our service: we have been referred nearly 400 cases in the last 3 years. This situation is mirrored in other large urban areas of sub-Saharan Africa. TB-IRIS is an opportunity to better understand aberrant immunopathological immunity in tuberculosis

TABLE 2. CLINICAL CHARACTERISTICS OF TB-IRIS THAT AROSE DURING LONGITUDINAL FOLLOW-UP OF HIV-INFECTED PATIENTS WITH TUBERCULOSIS WHO RECEIVED COMBINATION ANTIRETROVIRAL THERAPY

Patient no.	Sex	Age (yr)	CD4 ⁺ Cell Count Nadir (cells/ μ l)	Presenting Form of TB	Days of cART to TB-IRIS	Presentation of IRIS
B13	F	38	92	Culture-positive PTB	13	Developed new right cervical lymphadenopathy
B20	M	36	92	Clinical PTB	28	Transient worsening of pulmonary symptoms
B28	M	46	30	Disseminated	7	Worsening pulmonary infiltrate, hepatomegaly, and tachycardia.
B31	F	37	71	Culture-positive disseminated	13	Hepatic enlargement and cholestatic LFT derangement: liver biopsy showed necrotizing granulomas
B30	F	42	42	Clinical PTB	14	Anemia and new cervical lymphadenopathy
B33	M	28	5	Culture-positive pleural	14	Worsening pulmonary infiltrate
B34	M	30	13	Culture-positive PTB	15	Pleurisy, cough, and high fever.
B38	M	37	10	Disseminated, smear positive	7	Fever, confusion, and lymphocytic meningitis
B49	F	36	113	Disseminated, smear positive	12	New headache and fever, found to have meningitis and a tuberculoma
B52	F	20	48	Disseminated	28	New bilateral cervical lymphadenopathy

Definition of abbreviations: cART = combination antiretroviral therapy; F = female; LFT = liver function test; M = male; PTB = pulmonary tuberculosis; TB-IRIS = tuberculosis-associated immune reconstitution inflammatory syndrome.

and thereby not only to treat TB-IRIS more effectively but also to reduce the tissue damage that is characteristic of tuberculosis in both HIV-infected and uninfected persons.

Cross-sectional analysis clearly confirmed that TB-IRIS is associated with high frequencies of *M. tuberculosis* antigen-specific IFN- γ -secreting T cells, as previously reported (16). These cells seem likely to contribute to the inflammatory reactions observed clinically. The biggest difference in recognition between TB-IRIS and non-IRIS was in the recognition of the small cytoplasmic heat shock proteins Acr1 and Acr2 (see Figure 1). Both are upregulated by *M. tuberculosis* during

conditions of stress (31, 32), and it is possible that the combined stresses of antituberculosis chemotherapy and an improving immune response lead to such upregulation, thereby rendering these molecules prominent antigenic targets. In this respect the general principle of antigenic specificity implied by the poor recognition of ESAT-6 in the patients with TB-IRIS studied by Bourgarit and coworkers is borne out (16). However, and contrary to those findings, we observed ESAT-6 to be a significant antigenic target in patients with TB-IRIS. This difference may relate to ethnicity, disease extent, or length of antituberculosis therapy, as the response to ESAT-6 tends to decline

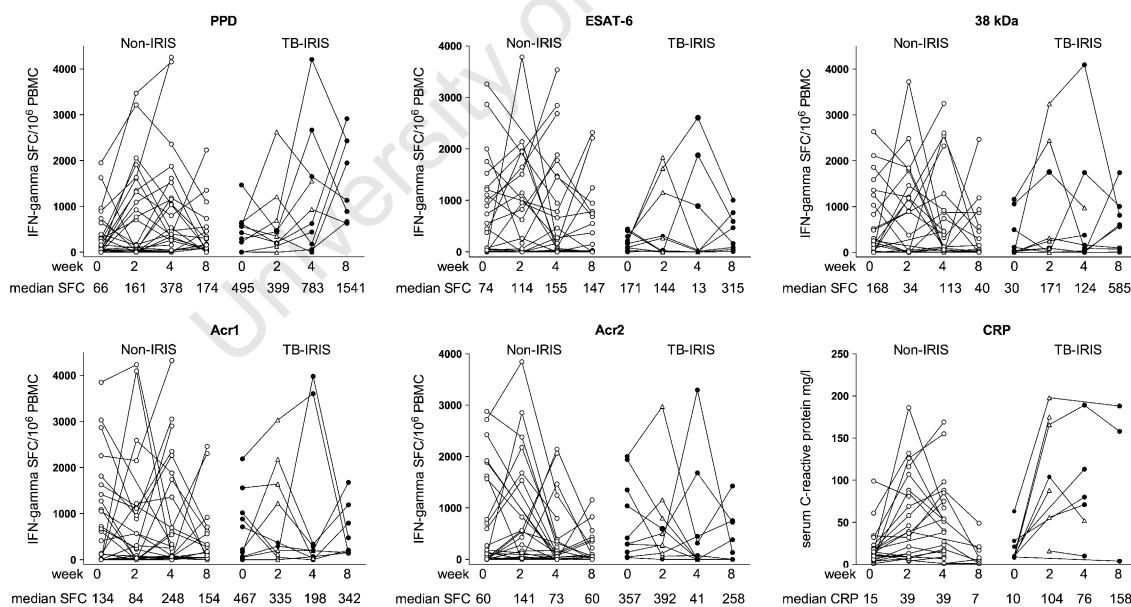


Figure 3. Longitudinal analysis of the frequency of *M. tuberculosis* antigen-specific IFN- γ spot-forming cells in response to five antigens and C-reactive protein (CRP) in HIV-TB patients receiving combination antiretroviral therapy (cART). An open triangle in the TB-IRIS group indicates the point at which tuberculosis-associated immune reconstitution inflammatory syndrome (TB-IRIS) was diagnosed. Repeated measures analysis demonstrated a significant increase in PPD response only in the TB-IRIS group ($P = 0.041$). Within-category analysis confirmed the increase in median PPD response in the TB-IRIS group between Weeks 0 and 8 to be significant ($P = 0.008$). In addition, the increase in PPD response in the non-IRIS group between Week 0 and both Weeks 2 and 4 was significant ($P \leq 0.04$). When comparing IRIS and non-IRIS groups the only significant difference was between the 8-week response to PPD ($P = 0.001$). The increase in median CRP response in the TB-IRIS group from between Weeks 0 and 2 was significant ($P = 0.031$). In addition, the increase in CRP in the non-IRIS group between Week 0 and both Weeks 2 and 4 ($P \leq 0.006$) was significant. When comparing IRIS and non-IRIS groups significant differences in CRP were found between the 2-week response in the non-IRIS group when compared with those who developed TB-IRIS ($P = 0.027$).

during successful treatment (33). We have also previously documented a high frequency of recognition of ESAT-6 (60–90%) in HIV-infected persons with both active and latent tuberculosis in this population (24, 34, 35).

Our flow cytometric analysis (see Figure 2) indicated that both CD4⁺ and CD8⁺ cells from patients with TB-IRIS (and non-IRIS patients) are activated, with a high percentage of cells expressing HLA-DR or CD71. We attribute the CD8⁺ cell activation to the restoration of effector responses to HIV-1 itself. Expression of the transcription factor FoxP3 has been associated with depression of *M. tuberculosis* antigen-specific effector T-cell responses (36–38), leading to the hypothesis that delayed cART-mediated restoration of such presumed regulatory T-cell responses underlies TB-IRIS (18). We found only low levels of FoxP3-expressing CD4⁺ T cells, perhaps in keeping with this idea but, if anything, their level was actually higher in patients with TB-IRIS than in non-IRIS patients. Flow cytometry was performed on a relatively small subset of 11 patients with TB-IRIS and 8 non-IRIS subjects; this, given the heterogeneous responses, limits power. However, we cannot readily ascribe the difference between clinical groups to CD4⁺ T cells that express FoxP3.

In the longitudinal analysis 10 patients developed TB-IRIS (see Figure 3). These data, however, illustrate an important point that was partially apparent from the cross-sectional analysis (see Figure 1). Dynamic expansion (and reductions) of *M. tuberculosis* antigen-specific T cells also occurred in the majority of non-IRIS patients, as did elevation of CRP. This had the consequence that stringent tests for trend (with the exception of the response to PPD in the TB-IRIS group) did not yield significant results. Overall, the highly variable patterns of fluctuation observed did not differ between persons who did and did not develop TB-IRIS. Although an association between dynamic T-cell expansions and TB-IRIS therefore exists, these data bring into question whether an explosion of tuberculin-specific Th1 responses is the cause of this syndrome (16). Our data favor the interpretation that during early cART in HIV-1-infected patients with tuberculosis, effector responses are highly dynamic, most likely reflecting a complex mixture of recirculation, immune restoration, and a variably falling mycobacterial antigen and HIV-1 viral load. The clinical heterogeneity of the syndrome (see Table 2) also cautions against drawing conclusions from limited numbers of patients.

In addition to clinical heterogeneity, a limitation of all studies of TB-IRIS is the selection of control subjects. We chose to study similar HIV-1-infected patients with tuberculosis sampled after a similar duration of cART (14 d, the characteristic time to onset of TB-IRIS) (see Table 1), but who did not develop the condition during 2 months of follow-up. However, the median duration of antituberculosis treatment was longer in the non-IRIS group (see Table 1). It is recognized that a risk factor for TB-IRIS is a short interval between the initiation of antituberculosis therapy and cART. As prescribing practice trends toward the earlier initiation of cART in patients with tuberculosis because of likely mortality benefits (7), we can expect the incidence and thus clinical importance of TB-IRIS to increase. Another limitation is that the study was conducted under program conditions and thus World Health Organization clinical case definitions of HIV-1-associated tuberculosis (23) and a clinical case definition of TB-IRIS (see Table E1) that excluded documentation of increased CD4⁺ cell count and a fall in HIV-1 viral load were in use. However, in patients with TB-IRIS for whom results were available 83% had complete HIV suppression in samples taken after the IRIS episode and 94% showed an increase in CD4⁺ cell count: highly consistent with general program conditions in which the HIV-1 viral load was

undetectable in 88.1% persons at 3 months and the median increase in CD4⁺ cell count was 134 cells/ μ l at 6 months (39). A final limitation is that, in keeping with many studies of the immunology of tuberculosis, we studied blood cells as a readily accessible compartment. It would be of interest to conduct similar analyses on cells from disease sites.

TB-IRIS is associated with high frequencies of *M. tuberculosis* antigen-specific IFN- γ -secreting T cells and invariably with CRP elevation, both indicative of vigorous cART-mediated immune restoration. However, dynamic expansion of *M. tuberculosis* antigen-specific T cells also occurred in the majority of non-IRIS patients, as did CRP elevation. The frequency of CD4⁺ cells expressing FoxP3 did not differ between persons who developed TB-IRIS and those who did not. Therefore the differences in immune regulation that mark orderly or dysregulated cART-mediated immune restoration in the context of antituberculosis treatment in HIV-infected persons remain to be fully elucidated.

Conflict of Interest Statement: None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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CHAPTER 6

Randomized placebo-controlled trial of prednisone for paradoxical tuberculosis-associated immune reconstitution inflammatory syndrome

Randomized placebo-controlled trial of prednisone for paradoxical tuberculosis-associated immune reconstitution inflammatory syndrome

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Objective: Paradoxical tuberculosis-associated immune reconstitution inflammatory syndrome (TB-IRIS) is a frequent complication of antiretroviral therapy in resource-limited countries. We aimed to assess whether a 4-week course of prednisone would reduce morbidity in patients with paradoxical TB-IRIS without excess adverse events.

Design: A randomized, double-blind, placebo-controlled trial of prednisone (1.5 mg/kg per day for 2 weeks then 0.75 mg/kg per day for 2 weeks). Patients with immediately life-threatening TB-IRIS manifestations were excluded.

Methods: The primary combined endpoint was days of hospitalization and outpatient therapeutic procedures, which were counted as one hospital day.

Results: One hundred and ten participants were enrolled (55 to each arm). The primary combined endpoint was more frequent in the placebo than the prednisone arm {median hospital days 3 [interquartile range (IQR) 0–9] and 0 (IQR 0–3), respectively; $P=0.04$ }. There were significantly greater improvements in symptoms, Karnofsky score, and quality of life (MOS-HIV) in the prednisone vs. the placebo arm at 2 and 4 weeks, but not at later time points. Chest radiographs improved significantly more in the prednisone arm at weeks 2 ($P=0.002$) and 4 ($P=0.02$). Infections on study medication occurred in more participants in prednisone than in placebo arm (27 vs. 17, respectively; $P=0.05$), but there was no difference in severe infections (2 vs. 4, respectively; $P=0.40$). Isolates from 10 participants were found to be resistant to rifampicin after enrolment.

Conclusion: Prednisone reduced the need for hospitalization and therapeutic procedures and hastened improvements in symptoms, performance, and quality of life. It is important to investigate for drug-resistant tuberculosis and other causes for deterioration before administering glucocorticoids.

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Introduction

The roll out of antiretroviral therapy (ART) in resource-limited countries has been associated with dramatic improvements in survival and quality of life. In these settings, a high proportion of patients commence ART while on treatment for active tuberculosis, resulting in a range of management challenges [1]. Paradoxical tuberculosis-associated immune reconstitution inflammatory syndrome (TB-IRIS) is increasingly recognized as an early complication of ART [2,3]. Paradoxical TB-IRIS is thought to result from restoration of tuberculosis-specific immune responses resulting in inflammation at disease sites of tuberculosis wherein antigen persists despite antitubercular treatment [4,5]. Typically, there is initial improvement on antitubercular therapy, then, after commencing ART, new, recurrent, or worsening tuberculosis symptoms, signs, or radiographic manifestations occur. Paradoxical TB-IRIS occurs in 8–43% patients starting ART while on antitubercular therapy [3,6–13]. Paradoxical TB-IRIS causes substantial morbidity, often resulting in hospitalization and/or the need for diagnostic and therapeutic procedures [13].

Glucocorticoids have been recommended for the treatment of paradoxical TB-IRIS [14,15], but there is little evidence for this recommendation, the largest study being a retrospective report of nine patients [7]. In tuberculous meningitis, glucocorticoids reduce mortality [16], in tuberculous pericarditis, reduce mortality and the need for repeat aspiration [17,18], and in pulmonary tuberculosis, cause modest clinical and radiographic improvement [19]. However, there is potential for harm when prescribing glucocorticoids in HIV-infected patients. Increased risk or progression of herpes zoster and Kaposi's sarcoma [20–22] have been reported. We have recently reported that a substantial proportion of patients presenting with suspected paradoxical TB-IRIS have undiagnosed drug-resistant *Mycobacterium tuberculosis* [23], another situation in which glucocorticoids may potentially cause harm.

We, therefore, conducted a randomized controlled trial of glucocorticoid therapy for patients with paradoxical TB-IRIS with the hypothesis that a 4-week course of prednisone would reduce morbidity without an excess of adverse events.

Methods

Study participants

Patients were recruited at GF Jooste Hospital, a secondary-level university-affiliated hospital in the Western Cape Province of South Africa serving communities with an antenatal HIV seroprevalence of up to 33% [24]. In 2006, the annual tuberculosis case

notification rate in the province was 1031/100 000 [25]. Most patients initiate antitubercular treatment and ART in primary care clinics. We have previously described the ART and antitubercular therapy regimens used in these clinics [23]. In accordance with national guidelines, antitubercular drug susceptibility testing (DST) is not routinely performed for new tuberculosis cases, but in patients receiving re-treatment or not responding to antitubercular treatment. Clinicians at the primary care ART clinics were informed of the study and encouraged to refer all patients with suspected paradoxical TB-IRIS for assessment.

Consecutive patients were screened using standardized case definitions for paradoxical TB-IRIS [23]. We limited enrolment to four TB-IRIS manifestations to reduce clinical heterogeneity and allow longitudinal radiographic comparison. Only patients with new or recurrent tuberculosis symptoms and at least one of the following TB-IRIS manifestations were enrolled: infiltrate on chest radiograph, enlarging lymph node(s), serous effusion, or cold abscess. Each participant underwent full clinical evaluation and chest radiography. Further investigations were conducted to exclude alternative reasons for clinical deterioration, according to presentation.

Exclusion criteria were age less than 18 years, known rifampicin-resistant tuberculosis, previous glucocorticoid therapy during this tuberculosis episode, prior ART exposure, pregnancy, uncontrolled diabetes mellitus, Kaposi's sarcoma, and immediately life-threatening TB-IRIS [defined as respiratory failure with arterial $pO_2 < 8$ kPa, altered level of consciousness, new focal neurological sign(s), or compression of a vital structure].

The study was approved by the University of Cape Town Research Ethics Committee (337/2004). Written informed consent was provided by all participants. The trial was registered on 17 August 2005 with the International Standard Randomised Controlled Trial Number Register (ISRCTN 21322548). The trial was conducted in accordance with the Helsinki Declaration.

Laboratory investigations

One or more clinical specimens (e.g., sputum, lymph node aspirate) were sent for tuberculosis microscopy, culture, and drug susceptibility testing. Specimens were also sent for rapid rifampicin resistance determination using a mycobacteriophage reporter system (FASTPlaque) [26]. Repeat samples were sent if deterioration occurred during follow-up.

Baseline investigations included electrolytes, urea and creatinine, random glucose, full blood count, liver function tests, calcium and albumin, random cortisol, C-reactive protein (CRP), CD4⁺ lymphocyte count (CD4 cell count), and hepatitis B surface antigen. Arterial blood gas determination was performed in patients with

respiratory distress. Routine follow-up investigations included CRP and glucose at each visit. CD4 cell count was repeated at week 4.

Treatment

Study medication consisted of prednisone tablets (5 mg) or matching placebo. Prior to the study, a randomization sequence assigning participants in a 1:1 ratio was generated using Excel by the study statistician and given to an independent pharmacist. Study medication was packaged according to sequence by the independent pharmacist off-site. The study medication was then transferred to the GF Jooste Hospital pharmacy. The hospital pharmacists, study clinicians, and participants remained blind to sequence and randomization throughout the trial. Participants were enrolled by the study clinicians and consecutive participants received the next study medication container from number 1 to 110. Participants received study medication 1.5 mg/kg per day for 2 weeks followed by 0.75 mg/kg per day for 2 weeks. The initial high dose of prednisone (1.5 mg/kg per day) was chosen because rifampicin induces prednisone metabolism [27]. Follow-up was at weeks 1, 2, 4, 8, and 12 with a full clinical assessment at each visit.

If significant clinical deterioration occurred after 2 weeks of follow-up, the study protocol allowed participants to be switched to open-label prednisone. If life-threatening deterioration occurred before 2 weeks, participants could be switched earlier. Unblinding, after switch to open-label prednisone, was considered only if this information influenced clinical management. Participants with significant relapse of TB-IRIS symptoms after completing 4 weeks of study medication could also receive open-label prednisone. Initiation of open-label prednisone required agreement between at least two senior clinical investigators. Participants were re-investigated at deterioration for alternative diagnoses.

Most patients with respiratory presentations were prescribed broad-spectrum antibiotics prior to enrolment. If such patients experienced symptom resolution on antibiotics, the diagnosis of TB-IRIS was reconsidered and the patient was not enrolled. Nonsteroidal anti-inflammatory drugs were not prescribed.

Assessment of outcome

The primary endpoint was cumulative days of hospital admission during the 12-week study period, combined with outpatient therapeutic procedures (including aspiration of lymph nodes, cold abscesses, and serous effusions) that were assigned a value of one hospital day. Procedures performed prior to or at enrolment were not included.

There were several secondary outcome measures. At each study visit, participants were asked about the TB-IRIS symptoms they had presented with and the study clinician enquired about any new TB-IRIS symptoms. The study

clinician, blinded to treatment allocation, graded TB-IRIS symptom response at week 2 and 4 visits in relation to the symptoms described at study entry. Symptom response was graded in one of three categories: deteriorated, no change, or improved/resolved. All patients who developed new TB-IRIS symptoms were graded as 'deteriorated'. Participants who switched to open-label prednisone within 2 weeks or between 2 and 4 weeks had their symptoms scored at the time of switching for their 2-week and 4-week scores, respectively. Symptoms of participants who switched to open-label prednisone at or before week 2 were not scored at week 4. The Medical Outcomes Study-HIV (MOS-HIV) Health Survey [28] and Karnofsky performance score were performed at each visit. Participants were assessed for glucocorticoid adverse drug reactions and new infections.

Two radiologists, blinded to study allocation, compared chest radiographs at weeks 2 and 4 with baseline (week 0). They utilized a three-point scale (deteriorated, no change, or improved/resolved). If there was a disagreement, they met to agree on a final consensus score. Ultrasound scans (measuring lymph node diameter or pericardial effusion width) were also scored at the same time points using a three-point scale: more than 25% increase, less than 25% increase or decrease, and more than 25% decrease in size.

Statistical analysis

Defervescence in paradoxical TB-IRIS is reported to occur in 50% by 2 weeks [6]. We based our sample size calculation on the assumption that spontaneous resolution of paradoxical TB-IRIS at 2 weeks would occur in 50% of the participants who received placebo. We estimated resolution in 80% of the participants on prednisone by 2 weeks. A sample size of 90 would be required to detect these rates of resolution for an α of 0.05 and β of 0.2. Therefore, we planned recruitment of 100 patients, assuming a 10% drop-out rate. Sample size was subsequently increased to 110, as we found that approximately 10% of our participants had unsuspected rifampicin-resistant tuberculosis [23].

A data and safety monitoring board (DSMB) of three clinical researchers and an independent statistician reviewed the study results after 50 participants had completed the study follow-up. They advised continuing based on predetermined stopping rules.

The analysis of the primary endpoint included all participants, according to the intention-to-treat principle. Analysis of the primary combined endpoint was performed using the Wilcoxon rank-sum test. Other comparisons between the two groups were made using Wilcoxon rank-sum, chi-squared, and Fisher's exact tests, as appropriate. Quantile regression was performed to adjust the primary endpoint for baseline differences between the two groups in duration from start of

antitubercular therapy to initiation of ART and random cortisol level. Kaplan–Meier methods were used to construct time-to-event curves for the two groups and the Gehan–Breslow–Wilcoxon test was used for comparison. Reported *P* values are two-sided.

Results

Two hundred and eighty-seven patients were screened and 110 were enrolled (55 to prednisone, 55 to placebo). Progress of participants through the trial is shown in Fig. 1. There were six protocol deviations (Supplementary Table 4, <http://links.lww.com/QAD/A85>).

Seventy (64%) were women and the median age was 31.6 years (range 19–56). Median CD4 cell count prior to ART was 53 cells/ μ l and at enrolment was 116 cells/ μ l. Table 1 shows baseline characteristics comparing the two arms. Median duration from antitubercular therapy to ART initiation was significantly longer in the prednisone

arm. Random cortisol was significantly lower in the prednisone arm, but no participant had a value below reference range. Otherwise the arms were evenly matched. Forty-four participants received antibiotics prior to enrolment.

Initial tuberculosis diagnosis was made by culture of *M. tuberculosis* in 46 (42%), a positive smear for acid-fast bacilli in 26 (24%), and was empiric based on clinical and radiographic findings in 38 (35%). Fourteen of the 38 participants with an initial empiric tuberculosis diagnosis had microbiologic confirmation at some stage during the study (seven culture positive and seven smear positive).

Outcomes are shown in Table 2 and Fig. 2. The median cumulative number of hospital days (with outpatient therapeutic procedures counted as one additional day) was 0 [interquartile range (IQR) 0–3] in the prednisone arm and 3 (IQR 0–9) in the placebo arm ($P=0.04$). In a multivariate regression model controlling for baseline differences between the two arms, this difference remained significant ($P=0.009$).

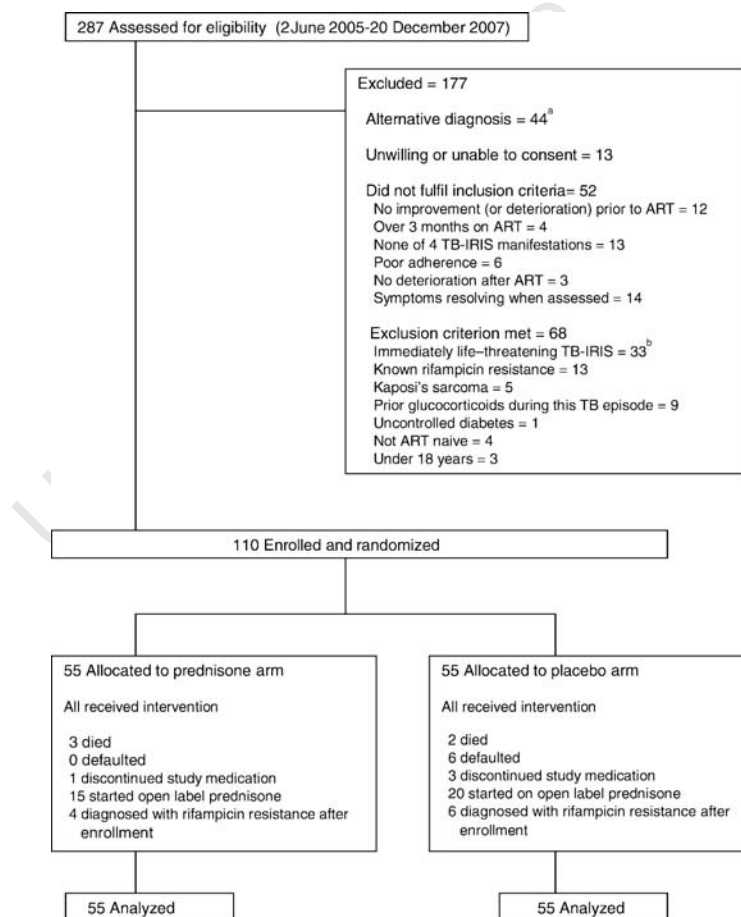


Fig. 1. Progress of participants through the trial. ^aCommon alternative diagnoses were drug reaction (nine), bacterial infection (eight), cryptococcosis (four), diarrhoeal illness (four), and heart failure (four). ^bAmong these 33 patients, the most frequent immediately life-threatening manifestation was neurological tuberculosis-associated immune reconstitution inflammatory syndrome (TB-IRIS) ($n=25$).

Table 1. Participant characteristics at enrolment.

	Placebo arm (N=55)	Prednisone arm (N=55)	P value for comparison
Age	31.6 (19–56.9)	31.5 (19.1–46)	0.82
Female sex	32 (58%)	38 (69%)	0.23
Previous tuberculosis	10 (18%)	15 (27%)	0.26
CD4 cell count prior to ART (cells/ μ l)	48 (20–92)	56 (30–103)	0.15
WHO stage 4 at ART initiation	33 (60%)	29 (53%)	0.44
Duration antitubercular therapy to ART in days	43.5 (23.8–76)	66 (35–84)	0.02
Duration ART to TB-IRIS in days	10 (7–19)	14 (7–21)	0.21
Duration TB-IRIS to enrolment in days	14 (8–23.5)	12.5 (7–21)	0.24
TB-IRIS manifestations			
New/recurrent lymphadenopathy	28 (51%)	19 (35%)	0.10
New/recurrent cold abscess	1 (2%)	1 (2%)	1.0
New/worsening pulmonary infiltrate	16 (29%)	19 (35%)	0.54
New/worsening serous effusion	9 (16%)	9 (16%)	1.0
Recurrent symptoms and consistent radiography, but without baseline radiography available for comparison	14 (25%)	15 (27%)	0.83
CD4 cell count at enrolment (cells/ μ l) (n=97)	109 (55–190)	138 (78–243)	0.07
Random glucose (mmol/l, 4.1–11.1) (n=108)	5.3 (4.8–5.7)	5.1 (4.8–6)	0.79
Haemoglobin (g/dl, male 13–17, female: 12–15) (n=107)	9.2 (7.8–10.1)	9.1 (8.1–10.3)	0.79
Albumin (g/l, 35–52) (n=108)	23 (19.5–26.5)	23 (20–26)	0.62
C-reactive protein (mg/l, 0–10) (n=108)	106 (79–172)	104 (50–150)	0.18
Random cortisol (nmol/l, 138–690) (n=97)	559.5 (405.8–774)	471 (350–614)	0.03
Hepatitis B surface antigen positive (n=94)	3/42 (7%)	3/52 (6%)	0.79
Weight (kg)	52.2 (46.6–58.8)	51.6 (48.1–56.5)	0.69
Hospitalized at enrolment	19 (35%)	14 (25%)	0.30
Antibiotics prior to enrolment	19 (35%)	25 (45%)	0.24
Karnofsky performance score (n=107)	70 (30–80)	70 (30–80)	0.96
MOS-HIV Health Survey (n=106)			
Physical health summary score	37.9 (32.8–44.9)	36.3 (33.4–43.1)	0.97
Mental health summary score	49.8 (39.1–56.9)	49.7 (44.5–56)	0.75

Values shown are medians (interquartile range) or numbers (%). Reference ranges for laboratory tests are shown in brackets. ART, antiretroviral therapy; MOS, Medical Outcomes Study; TB-IRIS, tuberculosis-associated immune reconstitution inflammatory syndrome.

The symptom score showed more rapid improvement in the prednisone arm at 2 weeks ($P=0.001$) and 4 weeks ($P=0.03$) (Fig. 2a). The chest radiograph score demonstrated greater improvement in the prednisone arm at 2 and 4 weeks (Fig. 2b). The ultrasound score ($n=29$) demonstrated no significant difference at either time point (data not shown). There were significantly greater improvements in MOS-HIV physical and mental health summary scores, Karnofsky performance score, and CRP at weeks 2 and 4 in the prednisone arm, but not at later time points.

Five participants switched to open-label prednisone during the period of study medication (first 4 weeks) in the prednisone arm and 18 in the placebo arm ($P=0.002$) (Fig. 3). Three such participants had study allocation unblinded. There was concern of hepatitis B flare in one, oesophagitis due to herpes virus (not confirmed) in another, and pancreatitis in a third. All three had been allocated placebo. Ten participants in the prednisone arm and two in the placebo arm were started on open-label prednisone after completing the 4 weeks of study medication ($P=0.01$) due to ongoing deterioration or, more frequently, relapse after having improved on study medication. Participants who initiated open-label prednisone were weaned according to response. Median duration of open-label prednisone was 84 days (IQR 60–126).

Eight participants in the prednisone arm and three in the placebo arm had events that could potentially be attributed to a glucocorticoid adverse drug reaction while on study medication ($P=0.11$). Infections while on study medication occurred in 27 participants in the prednisone arm and 17 in the placebo arm ($P=0.05$). The majority of these infections were mild, mainly oral and vaginal candidiasis, and uncomplicated herpes simplex (Supplementary Table 1, <http://links.lww.com/QAD/A82>). Severe infections, defined as invasive bacterial infections or new World Health Organisation stage 4 conditions, occurred in two participants in the prednisone arm and four in the placebo arm during the 12-week study period ($P=0.40$). These severe infections were a *Klebsiella* wound infection complicated by fatal sepsis syndrome, oesophageal candidiasis, pneumocystis pneumonia, and cryptococcal meningitis in the placebo arm. The participant who developed oesophageal candidiasis was on open-label prednisone when this occurred. In the prednisone arm, the severe infections were fatal pneumonia and cytomegalovirus retinitis.

There were three deaths in the prednisone arm and two in the placebo arm ($P=0.65$). Causes of death are shown in Supplementary Table 2 (<http://links.lww.com/QAD/A83>). Six participants defaulted follow-up for more than 7 days (all in the placebo arm; $P=0.01$). Five subsequently returned to care.

Table 2. Primary and secondary outcomes^a.

	Placebo arm (N = 55)	Prednisone arm (N = 55)	P value for comparison
Cumulative days hospitalized and outpatient therapeutic procedures	3 (0–9)	0 (0–3)	0.04
Number of participants hospitalized	25 (45%)	17 (31%)	0.12
Cumulative number of days hospitalized	463	282	–
Median number of days hospitalized (all participants)	0 (0–8)	0 (0–3)	0.07
Median number of days hospitalized (among 42 participants who were hospitalized)	12 (5–30)	4 (3–29)	0.26
Number of participants who had outpatient therapeutic procedure performed	12 (22%)	12 (22%)	1.0
Cumulative number of outpatient therapeutic procedures ^b	28	24	–
Karnofsky performance score			
Week 2 (n = 92)	70 (50–90)	90 (80–90)	<0.001
Week 4 (n = 86)	80 (60–90)	90 (80–100)	<0.001
Week 8 (n = 91)	90 (75–100)	90 (90–100)	0.33
Week 12 (n = 89)	90 (90–100)	100 (90–100)	0.16
MOS-HIV Health Survey			
Physical health summary score			
Week 2 (n = 98)	44.5 (36.7–52.4)	51.5 (44.5–54.5)	0.01
Week 4 (n = 94)	48.3 (39.7–55)	51.2 (46.6–56.8)	0.04
Week 8 (n = 96)	52.9 (44.2–55.5)	53.3 (42.9–56)	0.97
Week 12 (n = 88)	52.4 (48.1–56.1)	52.4 (48.9–55.5)	0.93
Mental health summary score			
Week 2 (n = 98)	57.4 (48.8–60)	59.9 (54.2–62.1)	0.02
Week 4 (n = 94)	55.5 (50.3–60)	58.5 (55.6–62.2)	0.01
Week 8 (n = 96)	60.6 (55.8–62.6)	60.6 (54.4–62.7)	0.79
Week 12 (n = 88)	61.7 (58.6–63)	60.7 (58.6–63.7)	0.68
CRP (mg/l)			
Week 2 (n = 101)	96.5 (53.8–122.5)	35 (13.8–60.3)	<0.001
Week 4 (n = 94)	63 (40.5–117.5)	34 (16.3–57)	0.001
Week 8 (n = 95)	42 (20–83.5)	39 (13–84.3)	0.49
Week 12 (n = 97)	36 (17–80)	25 (9–60.5)	0.12
Week 4 CD4 cell count (cells/ μ l) (n = 75)	145 (61–224)	154 (78–248)	0.51
Glucocorticoid adverse drug reaction ^c while on study medication	3 (5%)	8 (15%)	0.11
Infections while on study medication	17 (31%)	27 (49%)	0.05
Death	2 (4%)	3 (5%)	0.65

Values shown are medians (IQR) or numbers (%). ART, antiretroviral therapy; CRP, C-reactive protein; IQR, interquartile range; MOS, Medical Outcomes Study.

^aSymptom and radiographic scores are not shown here, but are shown in Fig. 2.

^bOutpatient therapeutic procedures performed during the 12 week study period are shown in Supplementary Table 3, <http://links.lww.com/QAD/A84>.

^cDefined as clinical events that could potentially be attributed to glucocorticoid adverse drug reaction: blood pressure higher than 140/90 mmHg, glucose higher than 11.1 mmol/l, oedema, hypomania, Cushingoid features, acne or gastritis symptoms.

Drug resistance

Ten cases of rifampicin-resistant tuberculosis were diagnosed after study enrolment. In eight, it was diagnosed after completion of study medication. Three received open-label prednisone. In the placebo arm, there were six cases [five multidrug resistant (MDR) and one rifampicin mono-resistant] and in the prednisone arm four (two MDR, one rifampicin mono-resistant, and one rifampicin resistant on FASTPlaque assay, but other drug susceptibility testing could not be done due to contamination) ($P = 0.50$). INH-mono-resistant tuberculosis was present in one participant in the placebo arm (diagnosed at tuberculosis diagnosis) and one in the prednisone arm (diagnosed at TB-IRIS presentation).

Discussion

We found that a 4-week course of prednisone reduced the primary combined endpoint of days hospitalized and

outpatient therapeutic procedures in patients presenting with paradoxical TB-IRIS. Mortality was not chosen as a primary outcome, as death due to paradoxical TB-IRIS is infrequent in reported series [3,12,13,29]. Furthermore, exclusion of patients with immediately life-threatening manifestations reduced the likelihood that we would demonstrate a significant difference. Additional benefits of prednisone were seen across a range of secondary outcome measures including symptom and Karnofsky performance scores, quality-of-life assessments, radiographic response, and reduction in CRP. The greatest effects were seen at the 2-week visit. Thereafter, the effect size and significance diminished, likely due to the combined effect of cross-overs from placebo to open-label prednisone for symptom deterioration and the self-limiting nature of most cases of paradoxical TB-IRIS in placebo group.

Switching to open-label prednisone while on study medication occurred significantly more frequently in the

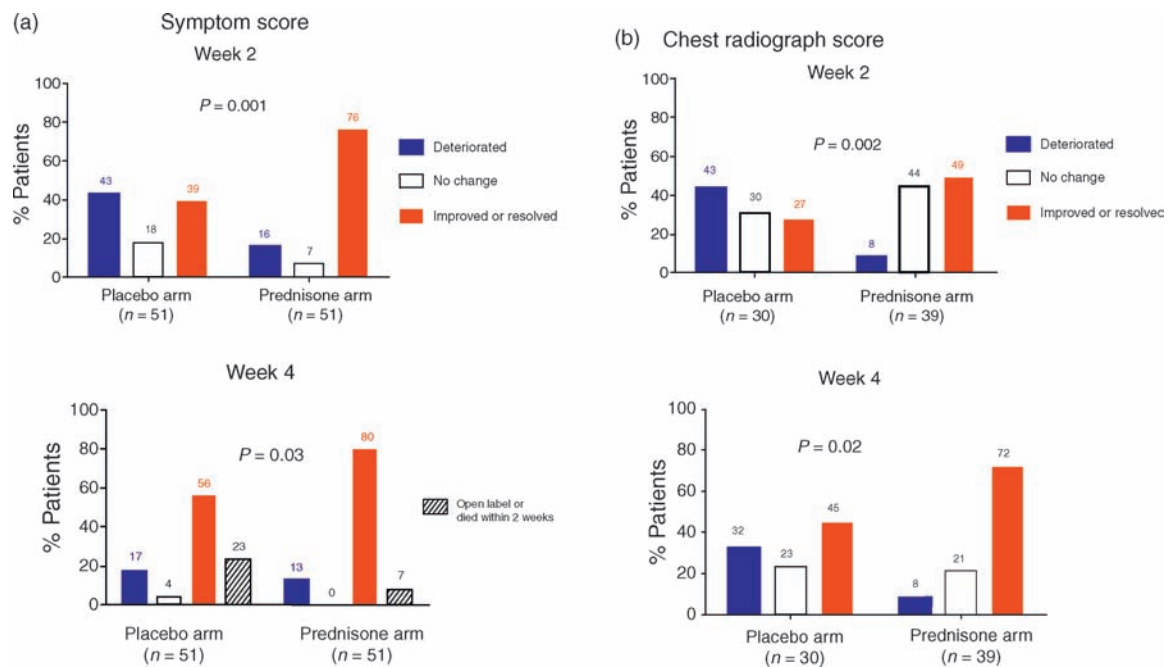


Fig. 2. Symptom and chest radiograph scores. (a) Symptom score at weeks 2 and 4. The distribution of symptom scores in percentage at weeks 2 and 4 in three categories (deteriorated, no change, improved/resolved) is shown. Participants who switched to open-label prednisone at or before week 2 were not scored at week 4 (they are shown in a separate category together with those who died within 2 weeks on the week 4 graph). There were significant differences between the two arms at week 2 ($P = 0.001$) and week 4 ($P = 0.03$). (b) Chest radiograph scores at weeks 2 and 4. Participants who had a pulmonary infiltrate on chest radiograph at baseline (week 0) had a chest radiograph score assigned at weeks 2 and 4 by two radiologists. Scores were allocated in three categories (deteriorated, no change, improved/resolved) in comparison with the week 0 chest radiograph. The distribution of scores in percentage is shown. There were significant differences between the two arms at week 2 ($P = 0.002$) and week 4 ($P = 0.02$).

placebo arm. This is further evidence of the benefit of prednisone. The fact that 10 participants in the prednisone arm needed to restart prednisone after 4 weeks suggests that this course was too short for a subset. Some participants were treated with open-label prednisone for several months. Paradoxical TB-IRIS is a heterogeneous condition with variable natural history and the glucocorticoid regime should in clinical practice be tailored to severity and response.

Prednisone was well tolerated. There was no excess of glucocorticoid adverse drug reactions while on study medication in the prednisone arm. More infections occurred in the prednisone arm while on study drug. The majority of these were mild infections and there was no difference in the incidence of severe infections by study arm.

When considering glucocorticoid therapy for paradoxical TB-IRIS, it is crucial to exclude alternative diagnoses, especially new infections or drug-resistant tuberculosis [23], because glucocorticoids may cause harm if the diagnosis of paradoxical TB-IRIS is incorrect. Most patients who had a respiratory presentation were treated with a broad-spectrum antibiotic prior to enrolment, as a bacterial chest infection is an important differential

diagnosis in this context. An alternative diagnosis was made in 44 of the paradoxical TB-IRIS suspects screened, and rifampicin-resistant tuberculosis was diagnosed in a further 10 participants after enrolment, even though we excluded patients with known rifampicin-resistant tuberculosis and patients who had not symptomatically improved prior to ART. There is currently no diagnostic test for paradoxical TB-IRIS. In resource-limited settings, where most cases of paradoxical TB-IRIS occur, it is difficult to exclude alternative diagnoses and drug-resistant tuberculosis. Some caution, therefore, has to be exercised when prescribing glucocorticoids in resource-limited settings. In any setting, it is prudent to avoid or defer glucocorticoids until the diagnosis of paradoxical TB-IRIS is firmly established and reassess the diagnosis of paradoxical TB-IRIS should a patient further deteriorate while being treated with glucocorticoids.

A major challenge in management and research of paradoxical TB-IRIS is that there is no confirmatory diagnostic test. We [23] have previously reported that CRP is almost universally elevated in paradoxical TB-IRIS and that its levels are higher in paradoxical TB-IRIS suspects who are subsequently diagnosed with rifampicin-resistant TB. However, CRP is unlikely to have diagnostic utility, as most of the differential diagnoses for

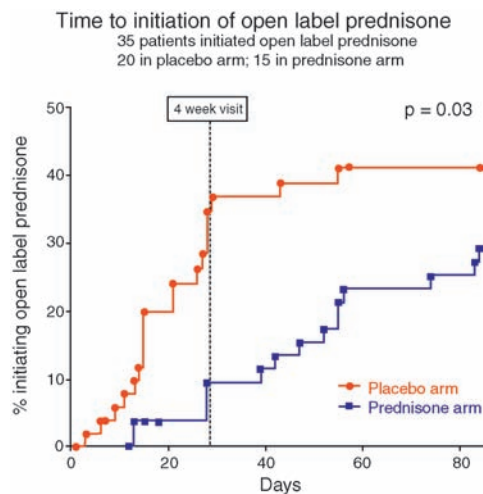


Fig. 3. Thirty-five participants were started on open-label prednisone (20 in the placebo arm and 15 in the prednisone arm). This Kaplan–Meier graph demonstrates the differences in the time that open-label prednisone was started between the two arms. In the first four weeks, while participants were receiving study medication, 18 in the placebo arm (33%) compared with five in the prednisone arm (9%) were switched to open-label prednisone ($P=0.002$) because of significant symptom deterioration. After 4 weeks, two in the placebo arm (4%) compared with 10 in the prednisone arm (18%) were started on open-label prednisone ($P=0.01$) mainly because of relapse of tuberculosis-associated immune reconstitution inflammatory syndrome (TB-IRIS) symptoms.

paradoxical TB-IRIS also cause elevations of CRP. Interferon-gamma release assays (IGRAs) have been proposed as possible diagnostic tools. Certain studies [4,30] have demonstrated that IGRAs, with purified protein derivative as the antigen stimulus, differentiate paradoxical TB-IRIS cases from controls. Our own study [5] suggested that IGRAs do not sufficiently differentiate cases from controls to be considered as a diagnostic test. Other approaches being explored are the identification of a characteristic cytokine profile or gene expression signature for paradoxical TB-IRIS. In the interim, diagnosis relies upon the use of clinical case definitions [2].

The development of Kaposi's sarcoma in HIV-infected patients treated with glucocorticoids has been reported [20,21]. Kaposi's sarcoma was an exclusion criterion in our study and no cases occurred in our study, possibly due to the protective effect of ART. We recommend avoidance of glucocorticoids in patients with Kaposi's sarcoma, as life-threatening exacerbation may occur [22].

Our study has several limitations. It was conducted at a single site with a relatively small sample size that did not permit subgroup analyses. Radiography from the time of initial tuberculosis diagnosis and ART initiation was unavailable in some participants. In these participants, the

diagnosis of paradoxical TB-IRIS was made on the basis of recurrent tuberculosis symptoms and the presence of compatible radiographic tuberculosis manifestations (pulmonary infiltrates, visceral lymphadenopathy, or serous effusions), but we did not know for certain whether the radiographic manifestations were worsening. Furthermore, it is possible that certain of the subjective measures of improvement (symptom score, quality-of-life assessment, and Karnofsky performance score) may have been influenced by the euphoric effect associated with high-dose glucocorticoids. Although the treatment allocation was randomized, two characteristics were found not to be evenly matched between the two arms (random cortisol and duration of antitubercular therapy to ART). These variables could thus potentially have confounded study findings, but no such effect could be found on the primary endpoint when these two variables were included in a multivariate regression model. An additional limitation was that the tuberculosis diagnosis was confirmed by culture in only 48% of participants. This reflects the practice in a programmatic setting in South Africa where culture is limited and not routinely performed in new tuberculosis cases.

In conclusion, a 4-week course of prednisone reduced days hospitalized and outpatient therapeutic procedures and resulted in more rapid improvements in symptoms, radiography, markers of inflammation, performance, and quality of life. An important caveat is that clinicians should be certain of the diagnosis of paradoxical TB-IRIS and investigate for antitubercular drug resistance when considering glucocorticoid therapy. Knowing that there is effective symptomatic therapy for paradoxical TB-IRIS may make clinicians less reluctant to start ART early in patients with tuberculosis and advanced immunosuppression [31].

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Authors' contributions: G.M., G.M., and R.J.W. designed and co-ordinated the study. G.M., D.J.P., K.R., M.X.R., and T.O. were involved in patient recruitment and follow-up and collected clinical outcomes data. Data management and analysis were performed by C.M. and G.M. G.M. wrote the manuscript, which was reviewed by all authors critically.

There are no conflicts of interest.

The study was presented previously at 16th Conference on Retroviruses and Opportunistic Infections, Montreal, Canada, 8–11 February 2009 (abstract 34) and published in CROI 2009 Program & Abstracts, CROI, 2009.

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CHAPTER 6 SUPPLEMENTARY TABLES

Supplementary Table 1: New or recurrent infections while on study medication

	Placebo arm	Prednisone arm
Oral or vaginal candidiasis	8	21
Tinea	4	4
Molluscum contagiosum	0	2
Herpes simplex	3	6
Warts	0	3
Pneumonia	0	1
Cystitis	1	0
Otitis media	1	0
Cryptococcal meningitis	1	0
TOTAL	18	37

While on study medication, in the placebo arm 18 infections occurred in 17 participants and in the prednisone arm there were 37 infections in 27 participants.

Supplementary Table 2: Causes of death (n=5)

Cause of death	Study arm	Study week
Community-acquired pneumonia	Prednisone	3
Wasting syndrome and TB-IRIS	Prednisone	2
Wasting syndrome and abdominal TB-IRIS	Prednisone	6
Multidrug-resistant tuberculous meningitis	Placebo	1
Warfarin skin necrosis, klebsiella wound infection complicated by systemic sepsis	Placebo	7

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Supplementary Table 3: Outpatient therapeutic procedures performed after enrollment and during the 12 week study period

	Placebo arm	Prednisone arm
Aspiration of lymph node or cold abscess	23	22
Aspiration of serous effusion	5	1
Intercostal drain insertion	0	1
TOTAL	28	24

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Supplementary Table 4: Six protocol deviations

Protocol deviation	Study arm
Participant found to have been ART non-naïve after study	Prednisone
After enrollment found that participant had previous adjunctive prednisone exposure, prior to ART, for tuberculous meningitis	Prednisone
Participant under-dosed in the first week due to misunderstanding	Prednisone
Participant had started ART 2 weeks before tuberculosis diagnosis then developed paradoxical reaction after starting antitubercular therapy. This sequence of events only became known after enrollment as the referral letter had stated ART was started after tuberculosis diagnosis.	Placebo
Participant received the study medication at the higher dose (1.5mg/kg) for week 3	Placebo
Participant took an overdose of study medication on day 1 due to a misunderstanding	Placebo

CHAPTER 7

Corticosteroid modulated immune activation in HIV / tuberculosis-associated immune reconstitution inflammatory syndrome

Corticosteroid modulated immune activation in HIV / tuberculosis-associated immune reconstitution inflammatory syndrome

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Key words: HIV, tuberculosis, immune reconstitution inflammatory syndrome, glucocorticoids

The authors have no conflicts of interest to declare

Abstract

Rationale

HIV-tuberculosis-associated immune reconstitution inflammatory syndrome (TB-IRIS) is an immunopathological reaction to mycobacterial antigens induced by antiretroviral therapy (ART). Prednisone reduces morbidity in TB-IRIS, but the mechanisms are unclear.

Objectives

To determine the effect of prednisone on the inflammatory response in TB-IRIS (antigen-specific effector T cells, cytokines and chemokines). Samples from participants in a randomized placebo-controlled trial of prednisone for TB-IRIS, taken at 0, 2 and 4 weeks, were analysed using ELISpot, RT-PCR and Luminex multiplex cytokine analysis.

Findings

58 participants with TB-IRIS (31 prednisone, 27 placebo) were included. No differences in ELISpot responses comparing prednisone and placebo groups were shown in response to ESAT-6, Acr1, Acr2, 38kDa or heat killed H37Rv *M. tuberculosis*. PPD ELISpot responses increased over 4 weeks in prednisone group and decreased in placebo group ($p=0.007$). In serum, significant decreases in IL-6, IL-10, IL-12p40, TNF α , IFN γ and IP-10 concentrations during prednisone, but not placebo, treatment were observed.

Conclusions

The beneficial effects of prednisone in TB-IRIS appear mediated via suppression of predominantly innate pro-inflammatory cytokine responses, not via a reduction of the numbers of antigen-specific T cells.

Introduction

Inflammatory pathology driven by the immune response to mycobacteria is a characteristic feature of treatment in tuberculosis (TB), leprosy and other non-tuberculous mycobacterial infections. Paradoxical reactions after commencement of TB treatment (1, 2), immune reconstitution inflammatory syndrome (IRIS) after initiation of antiretroviral therapy (ART) (3-5), and type 1 and 2 reactions in patients being treated for leprosy (6, 7) are all examples of treatment-induced immunopathology. Corticosteroids are used to modulate these immunopathological reactions and in certain forms of TB, such as meningitis and pericarditis, adjunctive treatment with corticosteroids has been shown to improve clinical outcomes (8-10). The benefit of corticosteroids is likely related to reducing pathological inflammation caused by the immune response, but mechanisms are not well characterized.

Paradoxical TB-IRIS occurs in 8-43% of HIV-infected patients who start ART while on treatment for TB (3). Within a few weeks of starting ART patients develop recurrent symptoms of TB and worsening of clinical and radiographic features of TB, such as enlargement of TB lymphadenitis (3, 4). We have recently reported a randomised placebo-controlled clinical trial of prednisone for the treatment of paradoxical TB-IRIS (11). A 4-week course of prednisone reduced the duration of hospitalization and the number of outpatient therapeutic procedures. In addition, participants on prednisone experienced significant improvement in TB-IRIS symptoms, chest radiographs and more rapid reduction in C-reactive protein (CRP). There was no mortality difference and no excess of severe infections in the prednisone-treated participants in this trial.

The immunological mechanisms underlying paradoxical TB-IRIS are partially elucidated. Expansion of mycobacterial-specific effector T cells that produce interferon-gamma (IFN γ) after starting ART have been associated with TB-IRIS in several studies (12-14). However, we have questioned the causal role of such expansions because we observed similar expansions in control TB patients starting ART who did not develop TB-IRIS and some TB-IRIS patients did not demonstrate expansions at the time of IRIS onset (14). Pro-inflammatory cytokines, in particular TNF α , IFN γ and IL-6, likely play an important role in pathogenesis as their concentrations are significantly raised in TB-IRIS patients (15). Defective T regulatory cell function is also speculated to play a role (16, 17).

In this study we analysed samples collected during the randomised controlled trial of prednisone for paradoxical TB-IRIS (11) to assess the effect of prednisone on T cell expansions, cytokine and chemokine gene expression and protein concentrations after *in vitro* stimulation and cytokine/chemokine concentrations *in vivo*. The aim was to determine the effect of corticosteroids on the aberrant immune response in TB-IRIS.

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Methods

Setting. The study was conducted at GF Jooste Hospital, a community-based referral hospital, in Cape Town, South Africa, that serves communities with a high prevalence of HIV infection and TB. Most patients are commenced on TB treatment and ART in community primary care clinics, but are referred to GF Jooste Hospital when complications occur for investigations and/or admission. Patients with a first episode of TB are treated with rifampicin, isoniazid, ethambutol and pyrazinamide for 2 months followed by rifampicin and isoniazid for 4 months. For subsequent episodes the treatment duration is 8 months and includes streptomycin for the first 2 months. At the time of the study the preferred ART regimen for patients on TB treatment was stavudine, lamivudine and efavirenz.

Clinical trial. Between 2 June 2005 and 20 December 2007 we enrolled participants into a randomized placebo-controlled clinical trial of prednisone for the treatment of paradoxical TB-IRIS. The methods have been described in detail (11), but are summarized here. Consecutive patients with suspected paradoxical TB-IRIS referred to the hospital were screened using standardized case definitions for paradoxical TB-IRIS (3). We limited enrollment to four TB-IRIS manifestations to reduce heterogeneity and allow longitudinal radiographic comparison. Only patients with new or recurrent tuberculosis symptoms and ≥ 1 of the following manifestations were enrolled: (1) infiltrate on chest radiograph, (2) enlarging lymph node/s, (3) serous effusion/s or (4) cold abscess/es. Patients with immediately life threatening TB-IRIS were excluded. The TB diagnosis was made on the basis of culture, smear microscopy or clinico-radiological diagnosis (18, 19).

Participants were randomized to receive study medication, either prednisone at 1.5mg/kg/day for 2 weeks followed by 0.75mg/kg/day for 2 weeks or identical placebo. If significant clinical deterioration occurred after 2 weeks of follow up, the study protocol allowed participants to be switched to open label prednisone or earlier if life-threatening deterioration occurred. Follow-up was for 12 weeks. The primary endpoint of the study was cumulative number of days hospitalized and number of outpatient therapeutic procedures performed counted as one additional hospital day. Secondary endpoints of efficacy included symptom score, quality of life score, Karnofsky performance score, chest radiography and C-reactive

protein. Mortality, metabolic side effects of prednisone and new infections were other secondary endpoints (11).

Timing of blood samples. Blood samples were taken from participants enrolled in the clinical trial prior to starting study medication (week 0) and then at week 2 and week 4 on the trial. Placebo-treated participants who were switched to open-label prednisone were not excluded from this immunology study, but any sample taken after switch to prednisone was excluded. For all experiments, laboratory workers were blinded to the treatment allocation of participants.

Participants. 110 patients were enrolled in the clinical trial (55 received placebo and 55 prednisone). Blood samples were taken from the first 73 participants in the clinical trial for this immunology study provided that laboratory staff were available to process specimens during working hours. If at least one repeat specimen (at 2 or 4 weeks) was available then the participant was included in these analyses. The inclusion and exclusion of participants was not subject to systematic bias because the treatment allocation was randomized and unknown to clinical or laboratory staff at the time of specimen processing. To analyze ELISpot results by treatment response participants were classified as improvers (symptoms improving at week 2 and no new symptoms) or non-improvers (symptoms unchanged or worsened at week 2 in comparison with week 0 symptoms) irrespective of treatment allocation.

Ethical permission and trial registration. The study was approved by the Research Ethics Committee of the Faculty of Health Sciences of the University of Cape Town (337/2004 and 049/2009). Written informed consent was obtained from all participants. The trial was registered with the International Standard Randomised Controlled Trial Number register (ISRCTN 21322548).

PBMC isolation. PBMC were isolated from 30ml of blood collected in Na-heparin BD vacutainers using the Ficoll separation method on day of collection.

ELISPOT assay. ELISpot analysis was performed as previously described (14, 20-22) on the day the sample was taken. Antigenic stimuli were endotoxin free and were assayed in duplicate wells. The concentrations used were 5 µg/mL of early secreted antigen target-6

(ESAT-6), 10 µg/mL of α-crystallin 1, 20 µg/mL of α-crystallin 2 and 2.5 µg/mL of 38kDa antigen. PPD was used at 200 IU/mL (4.6 µg/mL) and heat-killed (hk) H37Rv *Mycobacterium tuberculosis* at a multiplicity of infection (MOI) of 1:1 (200 000 H37Rv: 200 000 PBMC). Control wells included phytohaemagglutinin (5 µg/mL) and no antigenic stimulus. The number of IFN γ spot forming cells/10⁶ PBMC on ELISPOT plates were counted on an Immunospot Series 3B Analyzer (Cellular Technology) and plates were retained for visual inspection and confirmation in case of anomaly. Forty-one participants were included in the ELISPOT analysis (16 placebo-treated and 25 prednisone-treated).

RNA isolation and quantitative RT-PCR following *Mycobacterium tuberculosis*

stimulation of PBMC. After PBMC isolation, cells at 2.5 x 10⁶ /ml in RPMI/10% FCS were rested overnight in an incubator at 37°C in 5% CO₂. Thereafter the PBMC were restimulated with hkH37Rv *Mycobacterium tuberculosis* for 24 hrs by infecting PBMC at an MOI 1:1 and unstimulated cultures were also incubated. After restimulation, PBMC were harvested and lysed in 350 µl of RLT-lysing buffer (Qiagen, Valencia, CA). Lysates were collected for RNA analysis and tissue culture supernatants were preserved at -80°C until used for the Luminex multiplex experiments (described below).

RNA was extracted from PBMC lysates using the RNeasy Mini Kit Spin Protocol for isolation of total RNA from Animal Cells (Qiagen) as per manufacturer's instructions and stored at -80°C until further use. Primers and probes for RT-PCR were purchased from Applied Biosystems as predesigned inventoried assay reagents. We used the following TaqMan® Gene Expression Assays: IL-1 β Catalogue Number, Hs00174097_m1; IL-2, Hs00174114_m1; IL-4, Hs00174122_m1; IL-6, Hs00985639_m1; IL-8, Hs01038788_m1; IL-10, Hs00174086_m1; IL-12p40, Hs01011518_m1; IL-13, Hs00174379_m1; IL-15, Hs00542562_m1; IL-17A, Hs00174383_m1; IL-22, Hs00220924_m1; IL-27, Hs00377399_m1; TNF α , Hs00174128_m1; IFN γ , Hs00174143_m1; GM-CSF, Hs00171266_m1; CCL3, Hs00234142_m1; CCL4, Hs99999148_m1; CCL5, Hs00174575_m1. RNA concentration was determined by Nanodrop and samples diluted to give a RNA working solution concentration of approximately 10ng/µl. RT-PCR was performed using the TaqMan® RNA-Ct 1 Step kit protocol (Applied Biosystems, Foster City, CA). The reaction mixture was prepared using the following outlined procedure: 1µl of Taqman Gene Expression assay, 10µl of 2X buffer, 0.5µl of RT-enzyme and 8.5µl of diluted

mRNA for each reaction. Beta-actin was used as an endogenous control throughout. RT-PCR was performed on an AB Prism 7000 platform under the following universal thermal cycling conditions: reverse transcription at 48°C for 15 minutes, enzyme activation at 95°C for 15 seconds (40 cycles), annealing/primer extension at 60°C for 1 minute (40 cycles). Transcript abundance was calculated by subtracting the cycle threshold (Ct) of β -actin from the Ct of the gene of interest to derive a Δ Ct value. Fold induction of genes in response to HkH37Rv stimulation was calculated using the $\Delta\Delta$ method: the Δ Ct of the unstimulated sample was subtracted from the Δ Ct of the stimulated sample and 2 was then raised to the power of $-\Delta\Delta$ Ct. Values obtained were normalized by \log_{10} transformation and these values are reported. RT-PCR was performed in 25 participants (9 placebo-treated and 16 prednisone-treated), who had cells available for RNA extraction at week 0 and at least one additional timepoint.

Luminex multiplex assay for cytokine/chemokine concentrations in supernatants and serum. Supernatants from stimulated and unstimulated 24 hr cultures (see above) were later assayed for the following cytokines and chemokines: IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p40, IL-13, IL-15, TNF- α , IFN- γ , IP-10, GM-CSF, CCL3, CCL4 and CCL5. These assays were performed in 96-well filter plates on the Bio-Plex platform (Bio-Rad Laboratories, Hercules, CA, USA) using customized Milliplex™ kits (Millipore, St Charles, Missouri, USA) according to manufacturer's instructions. Assays were performed in 29 participants (12 placebo-treated and 17 prednisone-treated). The change in concentrations subtracting background from stimulated was used for comparisons.

Serum was stored at -80°C and using the same protocols the following cytokines/chemokines were assayed in serum samples at a later time point: IL-1 β , IL-2, IL-6, IL-8, IL-10, IL-12p40, TNF- α , IFN- γ , IP-10, GM-CSF, CCL3 and CCL4. Assays were performed in 58 participants (27 placebo-treated and 31 prednisone-treated). In all Luminex multiplex experiments samples from the same patient were included on the same 96-well plate.

IL-8 sandwich ELISA. Human IL-8 ELISA Core Kit (Koma Biotechnology, Seoul, Korea) was used and the assay performed according to manufacturer's instructions.

Statistical analysis. Medians are presented with interquartile range (IQR) and means \pm standard deviation (SD). For clinical data, categorical variables were compared using Chi-square or Fisher's exact test and continuous variables with the Wilcoxon signed-rank test. Unpaired and paired normally distributed variables were compared using Student's unpaired and paired t-tests as appropriate. Paired non-parametric variables were analysed by paired Wilcoxon signed-rank test. To factor multiple comparisons p values were multiplied by n-1 (Bonferroni correction) where n = number of comparisons. STATA 10.1 (Stata Corporation, USA) and GraphPad Prism® Version 5.0a Software (USA) were used for statistical tests.

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Results

Serial samples were available from 58 participants (27 placebo-treated and 31 prednisone-treated). Among these 58 participants, 33 were female (57%) and the median age was 31 years (IQR 26 - 35). The median CD4+ lymphocyte count prior to ART was 56 cells/ μ l (IQR 29 - 94) and at study enrollment median CD4 count was 116 cells/ μ l (IQR 60 - 234). There were no significant differences between the placebo- and prednisone-treated participants in terms of demographics, TB and HIV disease characteristics (Table 1). ELISpot was performed in 41 participants, RT-PCR in 25 participants, Luminex multiplex on supernatants of cultures stimulated with hkH37Rv MTB of 29 participants and Luminex multiplex on serum of 58 participants.

ELISpot

There were no significant differences in median number of spot forming cells comparing placebo- and prednisone-treated participants at each of the three time points (week 0, 2 and 4 weeks) for any of the 6 antigenic stimuli (Figure 1). Longitudinal within group analysis using paired Wilcoxon signed-rank test to compare week 0 with 2, week 0 with 4 and week 2 with week 4 revealed a difference only in the PPD stimulated wells: there was an increase in the median number of spot forming cells (SFC) from 449 SFC/ 10^6 PBMC (IQR 197 – 1368) at week 2 to 800 (IQR 188 – 2078) at week 4 ($p = 0.03$) in prednisone-treated participants.

Next, delta values ($\Delta = \text{week 4 ELISpot value} - \text{week 0 ELISpot value}$) were calculated and delta values for each antigenic stimulus were compared for placebo and prednisone-treated participants. The only significant difference was for the PPD-stimulated samples: between week 0 and 4 in placebo-treated participants median ELISpot value decreased by 496 SFC/ 10^6 PBMC (IQR – 1623 to +217) and in prednisone-treated participants the median ELISpot value increased by 455 SFC/ 10^6 PBMC (IQR -140 to +807) ($p = 0.007$) (Figure 2).

To assess the relationship between ELISpot responses and change in symptoms over time, the median ELISpot value at week 0 and at week 2 were compared for those who did, or did not, experience improvement by week 2 irrespective of whether they were randomized to prednisone or placebo. Thereafter, within group analyses comparing 0 and 2 week values were performed. No significant differences were shown apart from borderline significant

difference for 38 kDa comparing the values of those who improved to those who did not improve at week 2. In improvers the median was 580 SFC/10⁶ PBMC (IQR 30 – 1435) and in non-improvers 10 SFC/10⁶ PBMC (IQR 0 - 678) (p = 0.049) (Figure 3).

RT-PCR for cytokine/chemokine gene upregulation in response to MTB stimulation

There were significant reductions in mean log fold-induction in placebo-treated participants at week 4 (compared with week 0) for IL-1 β , IL-6, IL-13, GM-CSF and CCL3 (Table 2). There was a significant increase in mean log fold-induction for IL-22 at week 4 in placebo-treated participants and decrease in prednisone-treated participants at week 4 for IL-4 and IL-10 genes (all compared with week 0). After Bonferroni correction the only change that remained significant was the decrease in mean log fold induction from 0.32 (\pm 0.42) at week 0 to -0.26 (\pm 0.29) for IL-4 at week 4 in prednisone-treated participants (p corr = 0.03).

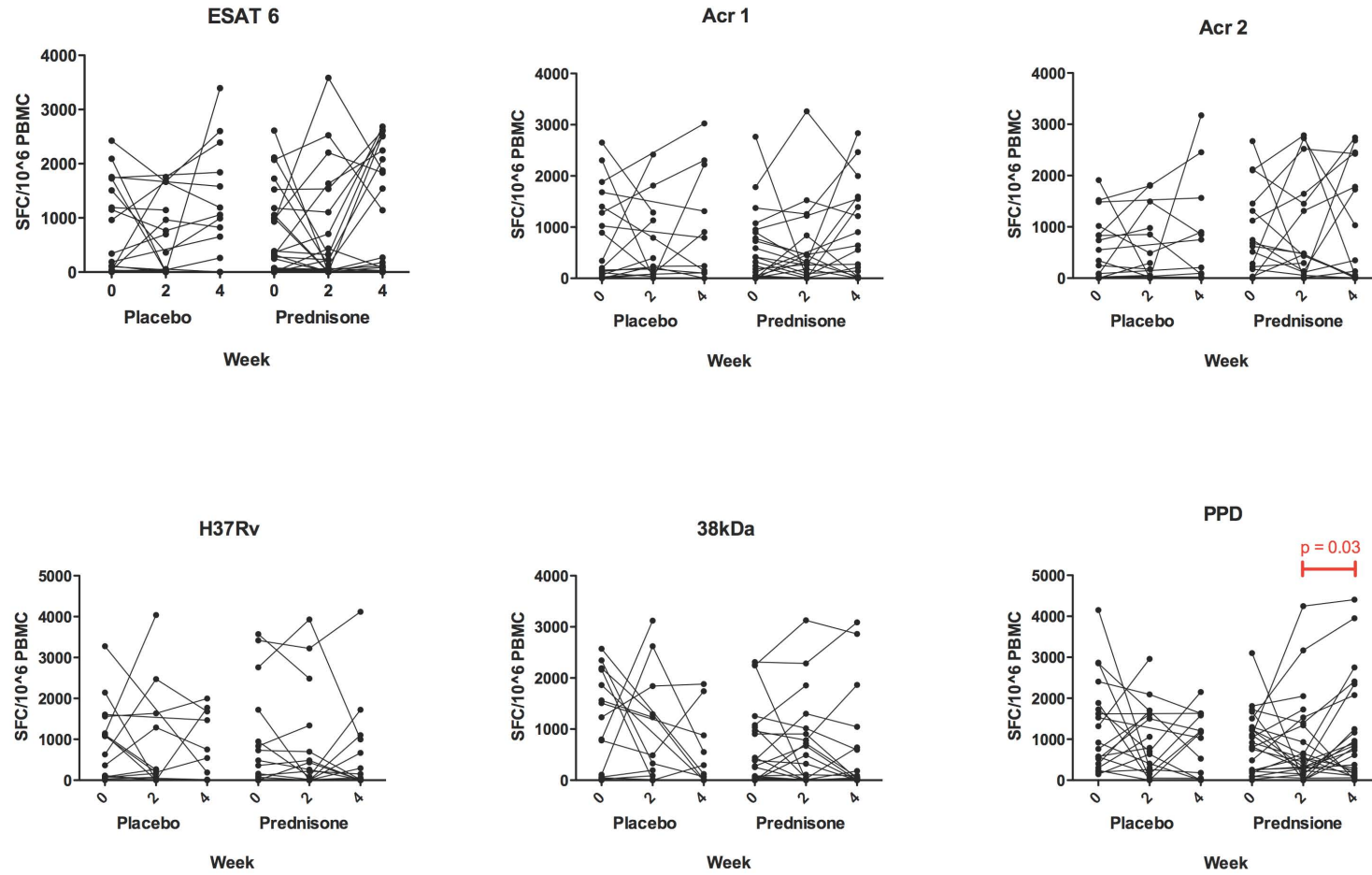
Luminex multiplex analysis of tissue culture supernatants

For placebo-treated participants in relation to week 0 there were increases in concentrations of IFN γ at week 2, decreased IL-13 at week 2, and decreased IL-1 β , IL-6, IL-10, TNF α , GM-CSF, CCL3 and CCL4 at week 4 (Table 3). In prednisone-treated participants GM-CSF and CCL3 decreased at week 2 and IL-1 β and IL-6 decreased at both week 2 and week 4 in relation to week 0 value. After Bonferroni correction, none of these differences remained significant.

Luminex multiplex analysis of serum

Luminex multiplex analysis of serum from prednisone-treated participants showed significant declines from week 0 to week 2 and week 0 to week 4, after correcting for multiple comparisons, for IL-6, IL-10, IL-12p40, IP-10 and TNF α concentrations (Table 4, Figure 4). For IFN γ there was a significant decrease from week 0 to week 4, but not at week 2. There was no significant decrease in IL-8, CCL3 or CCL4 in prednisone-treated participants after correction for multiple comparisons. In placebo-treated participants none of these cytokines or chemokines declined significantly at the week 2 nor at week 4 timepoints compared with week 0 after correction for multiple comparisons. Concentrations of IL-1 β , IL-2 and GM-CSF were undetectable in most samples and there was no significant change in values over 4 weeks in either treatment group.

Figure 1



Legend for Figure 1: ELISpot results in response to overnight restimulation of PBMC with six different antigen stimuli at 0, 2 and 4 weeks comparing placebo and prednisone-treated participants.

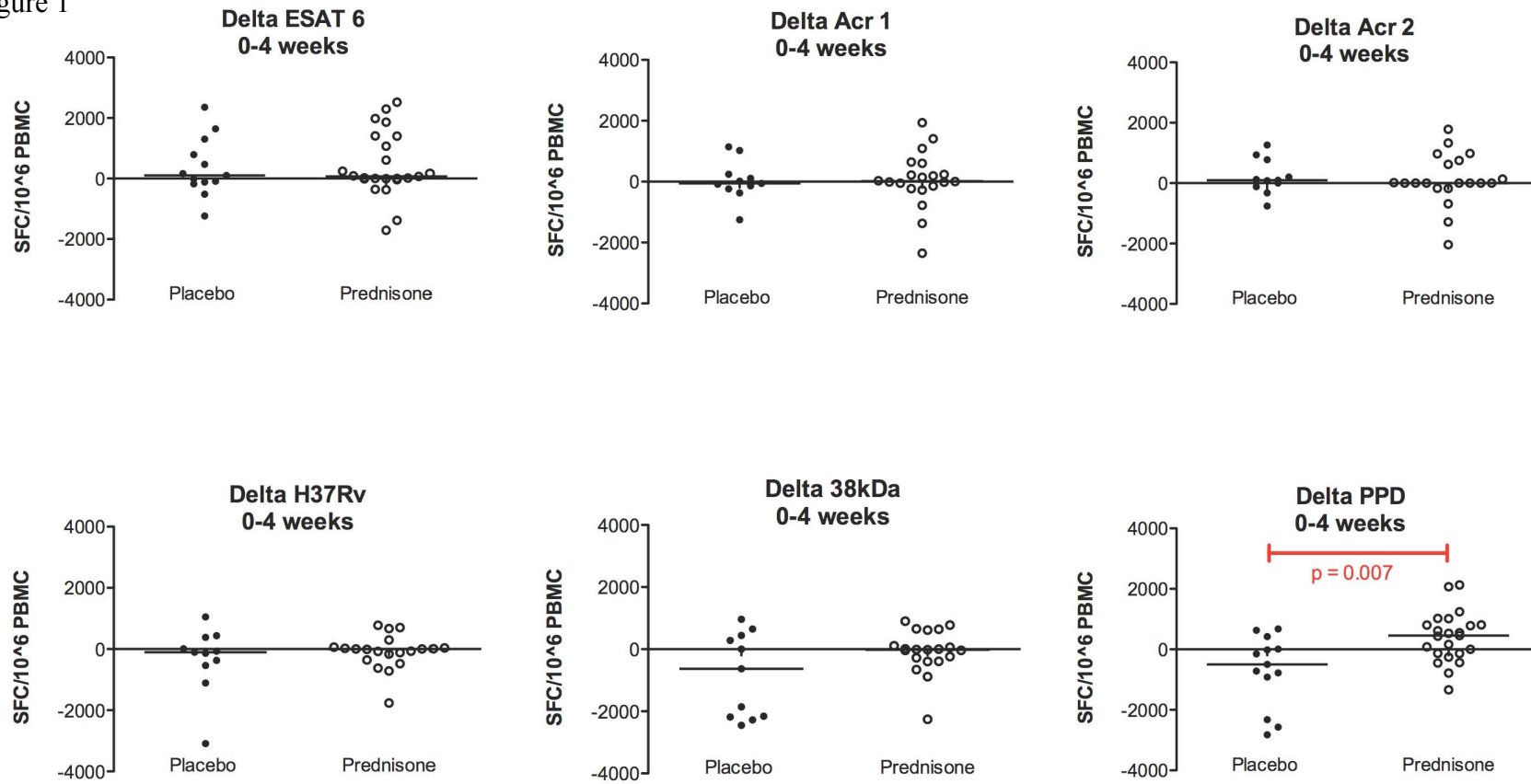
Antigen stimuli used were: 5 µg/mL of early secreted antigen target-6 (ESAT-6), 10 µg/mL of α-crystallin 1, 20 µg/mL of α-crystallin 2 and 2.5 µg/mL of 38kDa antigen. PPD was used at 200 IU/mL (4.6 µg/mL) and heat-killed H37Rv *Mycobacterium tuberculosis* at a multiplicity of infection (MOI) of 1:1 (200 000 H37Rv: 200 000 PBMC).

Statistical comparisons of median values for each time point comparing placebo and prednisone-treated participants showed no significant differences. Within group paired comparisons between time points (0 vs 2 weeks, 0 vs 4 weeks and 2 vs 4 weeks) revealed only one significant difference ($p < 0.05$): a rise in the number SFC to PPD in the prednisone-treated participants from 2 to 4 weeks (from 449 SFC/ 10^6 PBMC (IQR 197 – 1368) at week 2 to 800 (IQR 188 – 2078) at week 4 ($p = 0.03$)).

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Figure 2

Figure 1

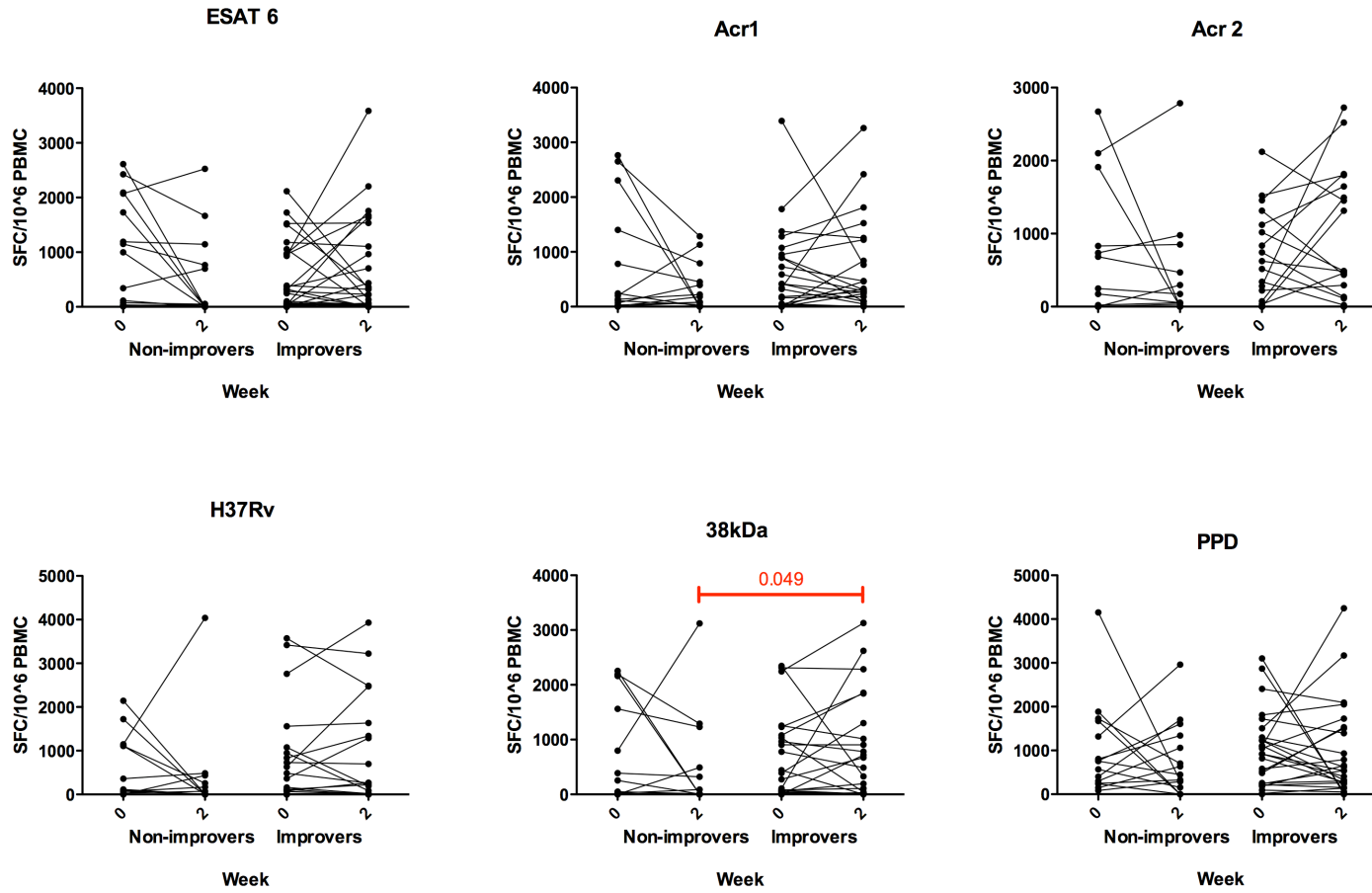


Legend for Figure 2: Changes in ELISpot responses between week 0 and week 4 comparing placebo and prednisone-treated patients.

These results relate to the same experiments as Figure 1, but show the change (delta) in responses over four weeks in placebo and prednisone-treated participants. The only significant difference ($p < 0.05$) was PPD: between week 0 and 4 in placebo-treated participants median ELISpot value decreased by 496 SFC/ 10^6 PBMC (IQR – 1623 to +217) and in prednisone-treated participants the median ELISpot value increased by 455 SFC/ 10^6 PBMC (IQR -140 to +807) ($p=0.007$).

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Figure 3

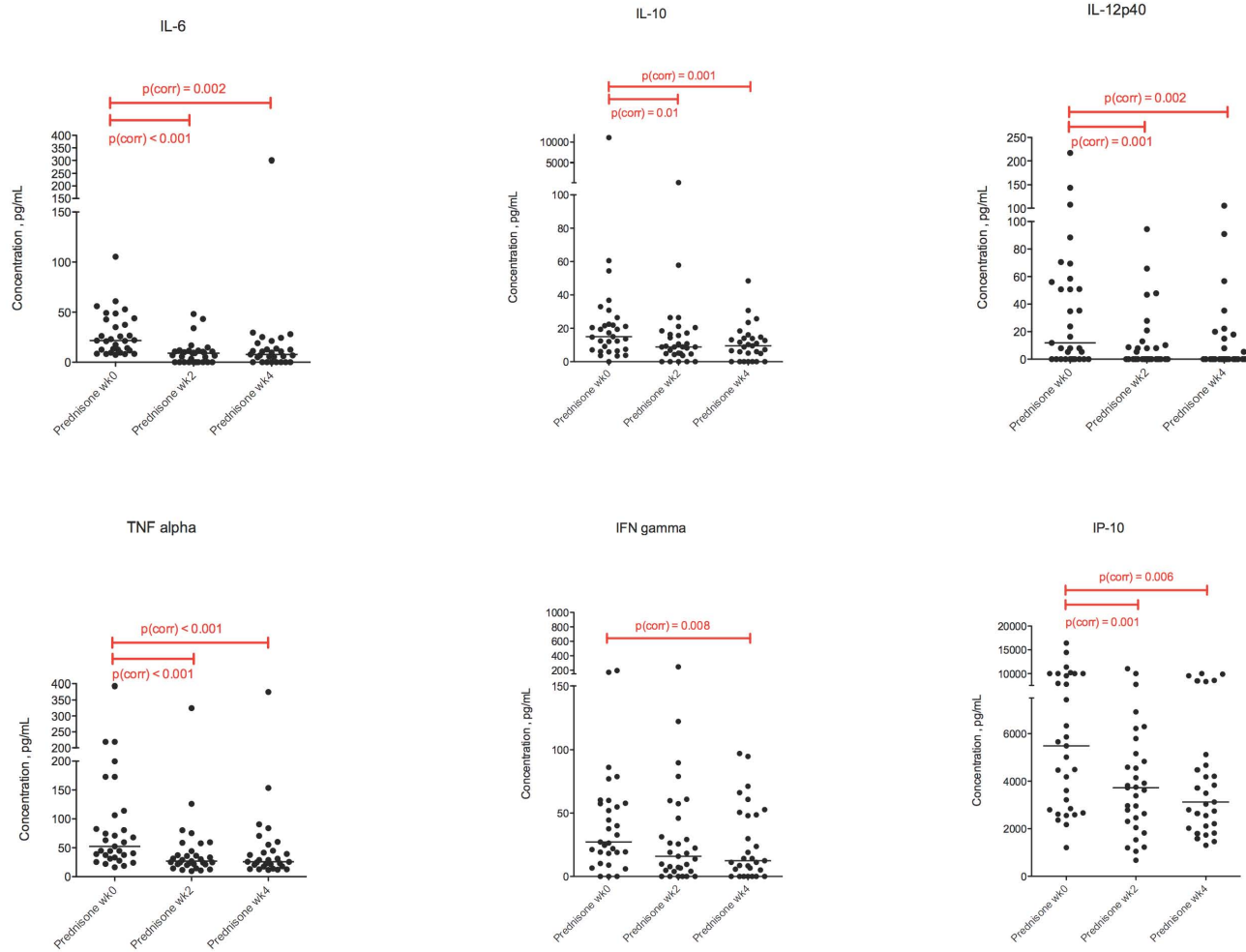


Legend for Figure 3: ELISpot responses at week 0 and week 2 comparing participants who had no improvement in symptoms during this time with patients who reported symptom improvement, regardless of treatment allocation.

Statistical comparisons were made between non-improvers and improvers at each time point and within group paired analyses comparing 0 and 2 week time points. There was a significant difference ($p < 0.05$) for 38 kDa comparing values of responders to non-responders at week 2. In improvers the median was 580 SFC/ 10^6 PBMC (IQR 30 – 1435) and in non-improvers 10 SFC/ 10^6 PBMC (IQR 0 - 678) ($p = 0.049$).

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Figure 4



Legend for Figure 4: Serum Luminex multiplex assay showing results for IL-6, IL-10, IL-12p40, TNF- α , IFN- γ and IP-10 concentrations in prednisone-treated participants at week 0, 2 and 4.

Statistical comparisons were performed using the matched pairs Wilcoxon test and corrected for multiple comparisons with the Bonferroni correction. There were significant reduction (pcorr < 0.05) between week 0 and 2 for all these cytokines/chemokine apart from IFN- γ . There were significant reductions for all between week 0 and 4. Full results of these experiments are shown in Table 4.

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Table 1: Clinical and demographic characteristics of participants included in immunological analyses

Variable	Placebo-treated N=27	Prednisone-treated N=31	p-value
Gender			
Male	13 (48%)	12 (39%)	0.8
Female	14 (52%)	19 (61%)	
Age	31 (27 - 36)	31 (26 - 35)	0.6
Previous TB	6 (22%)	10 (32%)	0.6
Basis of TB diagnosis			
Cultured <i>Mycobacterium tuberculosis</i>	20 (74%)	17 (55%)	0.07
Acid-fast bacilli smear positive	5 (19%)	4 (13%)	
Clinico-radiological diagnosis	2 (7%)	10 (32%)	
Extrapulmonary TB at TB diagnosis	15 (56%)	16 (52%)	0.8
CD4 count prior to ART (cells/ μ l)	58 (20 – 95)	55 (31 – 94)	0.6
CD4 count at enrollment (cells/ μ l) ^A	107 (49 – 202)	117 (73 - 278)	0.2
C-reactive protein at enrollment (mg/l)	100 (80 -143)	94 (42 -150)	0.6
Duration TB treatment to ART (days)	43 (26 - 79)	69 (35 - 96)	0.09
Duration ART to TB-IRIS symptom onset (days)	10 (7 - 17)	14 (7 - 22)	0.1
TB-IRIS clinical manifestations ^B			
New or enlarging lymph node(s)	11 (41%)	9 (29%)	0.4
New or expanding pulmonary infiltrate(s)	6 (22%)	9 (29%)	0.8
New enlarging serous effusion(s)	3 (11%)	2 (6%)	0.7
Recurrent symptoms and infiltrate(s) on CXR but without baseline CXR available for comparison	10 (37%)	14 (45%)	0.6

Legend for Table 1

Values shown are numbers (percentage) or median (interquartile range). P-values compare placebo and prednisone-treated participants.

CXR = Chest radiograph

^A CD4 count at enrollment available for 24 placebo-treated and 27 prednisone-treated participants

^B Participants may have had more than 1 TB-IRIS manifestation, thus manifestations sum to more than 100%

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Table 2: Log fold gene induction after 24 hrs restimulation of PBMC with heat-killed H37Rv *Mycobacterium tuberculosis* at weeks 0, 2 and 4 on clinical trial for placebo-treated and prednisone-treated participants

PLACEBO-TREATED						PREDNISONE-TREATED				
Cytokine/ chemokine	Week 0 N = 9	Week 2 N = 8	Week 4 N = 6	p-value (0 vs 2)	p-value (0 vs 4)	Week 0 N = 16	Week 2 N = 14	Week 4 N = 15	p-value (0 vs 2)	p-value (0 vs 4)
IL-1β	2.25 (0.80)	2.35 (0.41)	1.95 (0.76)	0.46	0.03 *	2.29 (1.11)	2.28 (0.94)	2.14 (0.74)	0.89	0.34
IL-2	0.97 (0.68)	0.99 (0.43)	0.97 (0.81)	0.57	0.76	0.78 (0.74)	0.97 (0.55)	0.68 (0.67)	0.45	0.61
IL-4	-0.13 (0.49)	-0.09 (0.34)	0.04 (0.30)	0.75	0.79	0.32 (0.42)	0.12 (0.52)	-0.26 (0.29)	0.43	0.002 *
IL-6	2.63 (1.37)	2.48 (1.10)	2.46 (1.15)	0.94	0.01 *	2.75 (1.56)	2.69 (1.19)	2.26 (1.11)	0.68	0.18
IL-8	1.29 (0.54)	1.15 (0.45)	1.14 (0.52)	0.91	0.26	1.52 (0.83)	1.33 (0.62)	1.12 (0.66)	0.34	0.09
IL-10	0.24 (0.84)	-0.04 (0.82)	0.41 (1.05)	0.23	0.93	0.38 (0.80)	0.13 (0.69)	-0.72 (1.08)	0.46	0.006 *
IL-12p40	2.02 (0.80)	2.24 (0.62)	2.47 (0.44)	0.41	0.62	2.40 (0.82)	2.04 (1.11)	2.21 (1.23)	0.45	0.84
IL-13	2.24 (1.06)	2.17 (0.96)	1.94 (0.96)	0.98	0.004 *	2.29 (0.86)	1.89 (0.90)	1.97 (0.75)	0.31	0.22
IL-15	0.43 (0.36)	0.24 (0.54)	0.63 (0.44)	0.17	0.38	0.67 (0.53)	0.73 (0.40)	0.50 (0.49)	0.51	0.33
IL-17A	1.53 (0.64)	1.46 (0.75)	1.52 (0.83)	0.79	0.27	1.28 (1.07)	1.11 (0.65)	1.10 (0.93)	0.63	0.65
IL-22	1.98 (0.89)	1.98 (0.66)	2.27 (0.80)	0.68	0.02 *	1.90 (1.11)	1.76 (0.77)	1.83 (0.82)	0.67	0.80

IL-27	1.42 (0.62)	1.28 (0.49)	2.00 (0.73)	0.95	0.37	1.39 (0.79)	1.52 (0.66)	1.34 (1.21)	0.82	0.72
TNFα	1.60 (0.30)	1.27 (0.80)	1.47 (0.42)	0.33	0.26	1.45 (0.65)	1.45 (0.32)	1.31 (0.64)	0.74	0.42
IFNγ	1.50 (0.78)	1.82 (0.53)	1.78 (0.42)	0.09	0.35	1.45 (0.88)	1.43 (0.70)	1.25 (0.71)	0.73	0.44
GM-CSF	2.55 (1.43)	2.62 (1.09)	2.00 (1.10)	0.88	0.049 *	3.01 (1.45)	2.74 (1.19)	2.48 (0.91)	0.32	0.15
CCL3	1.46 (0.51)	1.41 (0.30)	1.09 (0.68)	0.74	0.04 *	1.50 (0.73)	1.40 (0.54)	1.33 (0.58)	0.48	0.29
CCL4	1.11 (0.32)	0.93 (0.22)	0.82 (0.35)	0.31	0.09	1.17 (0.59)	1.02 (0.45)	0.95 (0.54)	0.41	0.26
CCL5	-0.09 (0.16)	-0.21 (0.26)	-0.11 (0.15)	0.21	0.73	-0.02 (0.27)	0.00 (0.30)	-0.17 (0.39)	0.61	0.24

Legend for Table 2

Mean log fold-induction (+/- standard deviation) in gene expression of 18 cytokines and chemokines after 24 hrs restimulation of PBMC with heat-killed H37Rv *Mycobacterium tuberculosis* are shown for placebo and prednisone-treated patients at weeks 0, 2 and 4 on the clinical trial. P-values were calculated for comparisons between 0 and 2 weeks values and 0 and 4 week values for each treatment group separately using Student's paired t-tests. Two patients in the placebo-treated arm switched to open label prednisone at week 2 and their week 4 samples were thus excluded from analysis.

Significant differences are indicated with *. After Bonferroni correction for multiple comparisons ($p_{corr} = p \times 18 - 1 = p \times 17$) only the change in IL-4 in prednisone-treated patients between week 0 and 4 remained significant ($p_{corr} = 0.03$).

Table 3: Changes in concentrations of cytokines and chemokines in supernatants of cell cultures after restimulation of PBMC with heat-killed H37Rv *Mycobacterium tuberculosis* for 24 hrs assessed by Luminex multiplex assay

PLACEBO-TREATED						PREDNISONE-TREATED				
Cytokine/ chemokine	Week 0 N = 12	Week 2 N = 11	Week 4 N = 9	p-value (0 vs 2)	p-value (0 vs 4)	Week 0 N = 17	Week 2 N = 15	Week 4 N = 15	p-value (0 vs 2)	p-value (0 vs 4)
IL-1β	1556 298.1 - 5182	495 226.4 - 2915	184.6 65 - 1788	0.70	0.01 *	2375 112.2 - 3623	112.4 0 - 699.4	235.8 98 - 456.8	0.01 *	0.03 *
IL-2	94.6 9.1 - 491.3	101.8 51.7 - 295.5	215.9 69.8 - 529.8	0.28	0.82	55.1 9.9 - 291	64.8 13.7 - 206.3	65.2 5.1 - 126	0.45	0.71
IL-4	0 0 - 0	0 0 - 0	0 0 - 0	0.75	0.50	0 0 - 1.6	0 0 - 0	0 0 - 0	0.63	0.13
IL-5	0 0 - 0	0 0 - 0	0 0 - 0	0.50	1.0	0 0 - 0	0 0 - 0	0 0 - 0	1.0	1.0
IL-6	17957 5088 - 29776	13026 2954 - 24499	6884 1119 - 15462	0.64	0.01 *	17084 3805 - 33389	3213 868.2 - 16139	6496 3411 - 12239	0.02 *	0.04 *
IL-10	249.1 139.3 - 2082	465.9 130.5 - 1297	190.4 77.1 - 1617	1.0	0.02 *	751.1 160.9 - 1591	124.3 55.4 - 963.7	311.5 187 - 638.8	0.14	0.06
IL-12p40	41.3 27.2 - 207.4	54.2 1.0 - 103.3	29.0 4.3 - 59.4	0.83	0.73	7.4 0 - 327.7	7.1 0 - 76.4	3.2 0 - 85.1	0.08	0.91
IL-13	10.1 0.2 - 62	9.4 2.8 - 67.9	3.5 -5.1 - 13.8	0.03 *	0.20	3.8 2 - 14.3	5.4 2.1 - 7.1	5.3 0.6 - 14	0.49	0.98
IL-15	0 0 - 2.6	0 0 - 3.8	0 0 - 0.3	1.0	0.81	0 0 - 0.4	0 0 - 0	0 0 - 0	0.20	0.84
TNFα	3799 1010 - 16035	4749 947 - 13480	1372 875.6 - 11891	0.90	0.01 *	6375 1017 - 11679	2727 598.3 - 3712	2627 1448 - 5406	0.06	0.07
GM-CSF	214.7 76.5 - 2584	314.8 175 - 492.2	116.8 48.9 - 2747	1.0	0.02 *	486.9 152.5 - 1516	147.2 42 - 427.2	245.8 175 - 595.5	0.03 *	0.06

IFNγ	257.0	835.8	467.3	0.02 *	0.65	233.4	133.9	416.4	0.06	0.39
	37.6 - 5157	266.9 - 5830	219.6 - 983.8			28.5 - 2524	49.1 - 983	29 - 807.8		
IP-10	1430	11596	1201	0.17	0.43	1282	20133	9222	0.23	0.76
	157.4 - 36114	398.1 - 18355	315.6 - 25330			-30.8 - 17084	4289 - 36155	3964 - 18476		
CCL3	29972	29608	13635	0.52	0.03 *	27820	9218	20029	0.03 *	0.08
	8888 - 95230	12920 - 65686	3968 - 24054			5995 - 49179	4119 - 33611	10720 - 26486		
CCL4	14046	8279	5293	0.64	0.01 *	8037	6860	6993	0.45	0.25
	3428 - 20732	4556 - 11353	2165 - 8015			2947 - 16094	3128 - 11886	4756 - 11283		
CCL5	2179	3210	2580	0.28	0.20	4122	2288	4443	0.76	0.42
	486.2 - 7919	1719 - 7681	1486 - 3616			871.8 - 7449	636.5 - 14529	1981 - 16442		
IL-8 (Elisa) ng/ml	54	54.5	47	0.77	0.69	57	52	50	0.43	0.55
	13 - 63	0 - 64.3	0 - 59.8			39 - 65.5	34 - 62	39 - 62		

Legend for Table 3

Results shown are cytokine/chemokine concentrations assayed with Luminex multiplex subtracting concentration in unstimulated PBMC cultures from concentration in cultures of PBMC stimulated with heat-killed H37Rv for 24 hrs. Median and interquartile range values are presented and units are pg/ml. The exception was IL-8 which was assayed using ELISA and units are in ng/ml. P-values were calculated for comparisons between 0 and 2 weeks values and 0 and 4 week values for each treatment group separately using Wilcoxon matched pairs test. Two patients in the placebo-treated arm switched to open label prednisone at week 2 and thus their week 4 sample was excluded from analysis.

Significant differences are indicated with *. After Bonferroni correction for multiple comparisons ($p_{corr} = p \times 17 - 1 = p \times 16$) none of the differences remained significant.

Table 4: Luminex multiplex assay of cytokine and chemokine concentrations in serum comparing participants treated with placebo vs patients treated with prednisone at weeks 0, 2 and 4 on the trial

	PLACEBO-TREATED							PREDNISONE-TREATED						
	Wk 0 N=27	Wk 2 N=24	Wk 4 N=18	P 0-2	Pcorr 0-2	P 0-4	Pcorr 0-4	Wk 0 N=31	Wk 2 N=31	Wk 4 N=29	P 0-2	Pcorr 0-2	P 0-4	Pcorr 0-4
IL-1β	0 0-0	0 0-0	0 0-0	1.0		1.0		0 0-0	0 0-0	0 0-0	1.0		0.5	
IL-2	0 0-0	0 0-0	0 0-0	1.0		1.0		0 0-0	0 0-0	0 0-0	0.55		0.53	
IL-6	35.5 20.4- 57.1	17.2 6.8- 30.2	17.2 5.0- 37.0	0.005 *	0.05	0.03 *	0.36	21.7 11.8- 42.7	9.0 0- 11.5	7.7 0- 16.3	<0.0001 *	<0.001 *	0.0002 *	0.002 *
IL-8	70.6 40.7- 146.0	72.7 31.0- 132.9	66.9 26.2- 150.9	0.25		0.26		50.6 35.2- 117.1	49.9 20.7- 72.5	50.8 26.6- 85.6	0.008 *	0.09	0.12	
IL-10	8.8 4.6- 25.7	10.1 4.1- 22.4	8.5 3.6- 17.9	0.16		0.56		15.0 7.0- 22.5	8.8 4.6- 17.2	9.5 2.5- 14.4	0.001 *	0.01 *	0.0001 *	0.001 *
IL-12p40	25.0 11.9- 55.8	13.0 0- 32.8	25.5 4.4- 219.9	0.13		0.94		11.9 0- 56.1	0 0- 10.2	0 0- 16.5	0.0001 *	0.001 *	0.0002 *	0.002 *
TNFα	51.1 34.4- 105.1	47.3 22.0- 95.6	41.3 24.4- 102.9	0.08		0.24		52.4 33.1- 106.2	26.9 21.1- 44.3	25.9 17.7- 50.2	<0.0001 *	<0.001 *	<0.0001 *	<0.001 *
IFNγ	27.4 12.8- 68.9	24.6 8.2- 71.9	29.9 15.7- 52.9	0.10		0.54		27.2 18.2- 57.8	15.9 4.1- 45.9	12.4 2.5- 49.6	0.10		0.0007 *	0.008 *

IP-10	3801 2299-9840	3330 1972-4229	2736 1943-3648	0.07		0.008 *	0.09	5487 2798-9998	3728 2309-5162	3126 2068-4896	0.0001 *	0.001 *	0.0005 *	0.006 *
GMCSF	0 0-0	0 0-0	0 0-0	0.50		1.0		0 0-0	0 0-0	0 0-0	0.94		0.38	
CCL3	29.8 0-78.6	23.3 0-66.8	35.2 3.3-169.6	0.79		0.04 *	0.45	26.6 0-50.4	15.5 0-47.2	24.3 0-85.1	0.66		0.63	
CCL4	71.6 40.8-100.4	53.0 37.4-78.8	69.1 56.1-132.1	0.03 *	0.37	0.19		78.3 55.0-110.7	81.9 44.6-100.1	81.8 53.4-115.5	0.02 *	0.26	0.73	

Legend for Table 4

Results shown are cytokine/chemokine concentrations assayed with Luminex multiplex in serum. Median and interquartile range values are presented and units are pg/ml. P-values were calculated for comparisons between 0 and 2 weeks values and 0 and 4 week values for each treatment group separately using Wilcoxon matched pairs test. Four patients in the placebo-treated arm switched to open label prednisone at week 2 and thus their week 4 sample was excluded from analysis.

Significant differences are indicated with *. Uncorrected p-values are shown and where the uncorrected p-value was < 0.05 a corrected p-value is also shown. The Bonferroni correction for multiple comparisons ($p_{\text{corr}} = p \times 12 - 1 = p \times 11$) was used.

Discussion

We have previously demonstrated in a randomized clinical trial that corticosteroid therapy results in clinical benefit in patients with paradoxical TB-IRIS (11). We have also previously demonstrated that TB-IRIS is associated with hypercytokinaemia (15) with the most consistently elevated cytokines being IL-6, TNF α and IFN γ . In the current study we sought to identify the immune mechanism of action of prednisone in TB-IRIS and thus potentially gain insight into the pathogenesis of TB-IRIS.

Corticosteroids are known to exert anti-inflammatory effects on immune cells through direct effects on transcription of inflammatory mediators via the Glucocorticoid Responsive Element, indirect genomic effects via interference with other transcriptional factors such as NF- κ B and AP-1, and non-genomic effects on anti-inflammatory proteins (23). These effects result in increased transcription of a number of anti-inflammatory mediators and decreased transcription of pro-inflammatory cytokines, chemokines, enzymes, receptors and adhesion molecules. In addition corticosteroids have been shown to reduce T cell survival by enhancing apoptosis (24).

Our most important finding was that serum concentrations of IL-6, IL-10, IL-12p40, IP-10 and TNF α were reduced in prednisone-treated participants at 2 and 4 weeks on the trial compared with baseline after correcting for multiple comparisons. The decrease in IFN γ occurred later at the week 4 time point. Similar decreases were not seen in placebo-treated participants. Many of these cytokines are of myeloid origin (IL-10, IL-12p40, IP-10) or of combined myeloid and lymphoid origin (TNF α and IL-6). These findings concur with reports that suggest myeloid cells may play an important role in TB-IRIS pathogenesis (25, 26).

We have previously reported that among patients treated with prednisone in this trial the CRP fell from a median 104 mg/l at week 0 to 35 mg/l at week 2. In placebo-treated patients the decrease was more gradual (11). This corresponds with our finding here that there is a significant fall in IL-6 in prednisone, but not placebo treated patients, as IL-6 is the major stimulus of CRP production (27). The reduction in the regulatory cytokine IL-10 may have been a direct corticosteroid effect or as a consequence of decreased inflammatory activity.

We have previously reported highly dynamic and heterogenous expansions and contractions of tuberculosis-specific IFN γ secreting T cells in both TB –IRIS cases and controls who do not develop IRIS (14). Similar ELISpot patterns were noted during the 4 weeks course of treatment among patients on this trial and these were largely unaffected by prednisone therapy. The only significant finding was the difference in PPD-specific cells with an increase in prednisone-treated participants and a decrease in placebo-treated participants from week 0 to 4. A plausible explanation for this was that the anti-inflammatory effect of prednisone at tissue sites of IRIS resulted in recirculation of these effector cells into peripheral blood and reduced recruitment. We thus found no evidence that the effect of prednisone in TB-IRIS was mediated via a reduction in numbers of antigen-specific effector T cells in peripheral blood, although it did reduce IFN γ production at week 4. It is possible that this effect was mediated at a transcriptional level although we did not demonstrate this in *ex vivo* restimulation assays.

The findings of the 24 hr stimulation experiments, which assessed changes in cytokine/chemokine gene expression by RT-PCR and protein concentration by Luminex multiplex in response to MTB stimulation, showed few differences and only a reduction in gene expression of IL-4 at week 4 in prednisone-treated participants remained significant after correcting for multiple comparisons. We interpret this to indicate that prednisone administered to patients does not have a significant effect that persists in tissue culture experiments during 24 hr *ex vivo* restimulation.

A clinical trial conducted by Thwaites and colleagues in Vietnam demonstrated that adjunctive dexamethasone for TB meningitis reduced mortality (10). In their trial, as was the case in our study, there was no significant effect of corticosteroid therapy on ELISpot responses to ESAT-6 (and in their study no effect on PPD responses either) and there were no effects of corticosteroid therapy on monocyte-derived cytokine concentrations after *ex vivo* restimulation of whole blood with mycobacterial antigens (28). In contrast to our findings, dexamethasone did not significantly alter concentrations of a number of cytokines and chemokines assayed. However, in their study concentrations were assayed in cerebrospinal fluid (CSF) rather than serum. Similarly, in children with TBM, prednisone did not

significantly alter TNF α , IL-1 β and IFN γ concentrations in CSF (29). The difference in our findings could be that we analysed blood. TB-IRIS frequently causes a systemic inflammatory syndrome characterized by fever, tachycardia and weight loss and this is reflected in high pro-inflammatory cytokine concentrations in blood (15) which are beneficially modulated by corticosteroids. A previous study of high dose prednisolone in patients with HIV-associated smear positive pulmonary TB and CD4 counts \geq 200 cells/ μ l demonstrated a reduction in serum TNF α in patients taking prednisolone (30).

Strengths of our study were that treatment allocation was randomized reducing potential biases, there were no significant differences in demographic and disease characteristics between treatment groups and the patients were extensively investigated to exclude alternative causes for deterioration and confirm the diagnosis of paradoxical TB-IRIS. An important limitation was the small sample size in the 24 hr stimulation assays because of insufficient serial samples being available. This may have limited power to detect significant differences.

Our findings suggest that the mechanism of corticosteroid action in TB-IRIS is at least partially mediated by a reduction in concentrations of pro-inflammatory cytokines. It raises the question as to whether more targeted anti-inflammatory therapy with TNF α blockers (etanercept, infliximab, adalumimab, certolizumab or golimumab) or an IL-6 blocker (tocilizumab) (31) may be effective in TB-IRIS for severe cases such as enlarging intracerebral tuberculomas that are potentially life threatening and may respond poorly to corticosteroids (32). In addition, our findings may shed light on the mechanisms of action of corticosteroids in reducing pathological inflammation in mycobacterial infections in general.

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CHAPTER 8

Conclusions

Paradoxical TB-IRIS has rapidly emerged in the last decade in South Africa, and many other sub-Saharan African countries, as a common medical condition as a result of the convergence of the epidemics of HIV and TB together with the massive scale up of ART for HIV-infected people. In South Africa, a major contributing factor is that many patients (up to 42% in some clinics (1)) start ART while on treatment for active TB. The clinical services in Cape Town, given the historically high rates of TB in the city together with the rapid and successful scale-up of the public sector ART programme in the Western Cape, provided an ideal setting for the conduct of these studies that investigated paradoxical TB-IRIS.

This thesis reports studies that investigated distinct clinical and immunological aspects of paradoxical TB-IRIS. In Chapter 3, the consensus case definitions for TB-IRIS developed at a meeting of the International Network for the Study of HIV-associated IRIS (INSHI) are presented. Chapter 4 reports a study on the clinical diagnosis and features of TB-IRIS, Chapter 5 reports on two studies which aimed to define the role of Th1 and regulatory T cells in pathogenesis, Chapter 6 reports the randomized clinical trial that assessed a 4-week course of prednisone for the treatment of TB-IRIS, and Chapter 7 examines the effect that prednisone has on mediators of the immune response in paradoxical TB-IRIS.

The study reported in **Chapter 4** involved screening consecutive patients referred or presenting to GF Jooste Hospital with suspected paradoxical TB-IRIS. These were patients who had recently started ART while on treatment for active TB and subsequently deteriorated with clinical features compatible with worsening TB. Standardised case definitions for paradoxical TB-IRIS were used when screening patients. The case definitions we developed formed the framework for the INSHI consensus case definitions presented in Chapter 3. During screening patients were investigated for alternative causes of clinical deterioration (these investigations were directed by the organ system affected) and TB culture and drug susceptibility testing was requested on clinical specimens (sputum and other body fluids). Of the 100 patients, 7 were found to have alternative opportunistic diseases, 4 were known to have rifampicin-resistant TB and a further 9 were found to have previously undiagnosed rifampicin resistance (7 of whom also had isoniazid resistance and thus had multi-drug resistant TB). Thus undiagnosed rifampicin-resistant TB was present in 10.1% of patients with suspected TB-IRIS once known rifampicin resistance and patients with alternative

diagnoses were excluded. Importantly there were no clinical or radiological features (apart from more frequent lymphadenopathy on chest radiograph) that differentiated these patients from those who presented with TB-IRIS without rifampicin-resistant TB. Based on these observations we proposed that paradoxical TB-IRIS and drug-resistant TB are not mutually exclusive diagnoses and that in patients with undiagnosed drug-resistant TB-IRIS may occur and accelerate clinical deterioration. These findings have important implications for clinical practice in our setting: in all patients presenting with suspected TB-IRIS drug susceptibility testing should be performed. This should preferably be done using a rapid rifampicin resistance assay. The FASTplaque assay we used in our studies performed poorly on fresh specimens, however newer PCR-based rapid tests such as the Genotype MTBDR*plus* and GeneXpert assays have shown more promising results (2, 3). In terms of clinical features, important findings of this study were that new or expanding lymph nodes were the most frequent TB-IRIS manifestation (present in 44%) and 56% of patients had hepatomegaly, many with cholestatic liver function derangement, suggesting that TB-IRIS frequently involves the liver.

The first substantial immunological study of paradoxical TB-IRIS was conducted by investigators in France, a longitudinal study of 19 patients with HIV-associated TB starting ART, 7 of whom developed TB-IRIS (4). They demonstrated expansions of PPD-specific IFN γ -producing T cells using ELISpot in TB-IRIS cases that were not seen in controls. Only low ESAT-6-specific expansions were seen in 3 TB-IRIS cases and no antigen 85B-specific expansions were observed. There were higher Th1 and non-specific inflammatory cytokine/chemokine concentrations in cases than controls after PPD stimulation. The authors concluded that Th1 T cell expansions were the cause of IRIS. However, association does not mean causation. Important limitations of their study were that comparisons between cases and controls were performed at 0, 3 and 6 months on ART whereas most IRIS cases occur within the first 4 weeks of ART, and the relatively small sample size. We thus set out to investigate antigen-specific T lymphocyte populations in a larger cohort that compared TB-IRIS cases with controls at earlier time points. We also wished to assess the role of regulatory T cells in pathogenesis, the hypothesis being that deficiencies in regulatory T cell numbers may contribute to the development of TB-IRIS. In **Chapter 5** two studies that investigated the immunopathogenesis of paradoxical TB-IRIS are described: one was a cross-sectional

study that included 35 TB-IRIS cases and two control groups and the other a longitudinal study over the first 8 weeks of ART in patients with HIV-associated TB. In the cross-sectional study patients with TB-IRIS sampled after 2 weeks on ART were found to have higher median ELISpot responses to a broad range of mycobacterial antigens including ESAT-6 (in contrast to Bourgarit's findings (4)) when compared to the two controls groups. However, the responses were heterogenous in both cases and controls: some cases showed no expansions and some controls (who did not develop TB-IRIS on ART) showed high levels of IFN γ -producing T cells in response to mycobacterial antigens. Thus, even though mycobacterial-specific T cells likely play a role in the development of TB-IRIS, we questioned whether they were truly causal as our data suggested they were neither a sufficient nor necessary factor in the development of the condition. This conclusion was reinforced by the findings of the longitudinal study in which dynamic expansions and contractions of mycobacterial-specific IFN γ -producing T cells were seen over the first 8 weeks of ART in both cases and controls with few significant differences between the groups. With respect to T regulatory cells, we found that TB-IRIS cases in fact had higher numbers than controls in flow cytometry experiments (although this difference was non-significant). Since the publication of these findings another study showed no association between T cell expansions and IRIS (5) and other studies have confirmed our finding that TB-IRIS is not associated with reduced numbers of T regulatory cells (6, 7).

In **Chapter 6** a randomized placebo-controlled trial of prednisone for the treatment of paradoxical TB-IRIS is reported. Prior to this clinical trial there were anecdotal reports in the literature of patients with paradoxical TB-IRIS experiencing symptomatic benefit from corticosteroid treatment (8). There was also an extensive body of literature on the use of corticosteroids as adjunctive treatment for TB as described in Chapter 2, but substantial clinical benefit has only been conclusively demonstrated for TB meningitis (9, 10). Studies had shown that adjunctive corticosteroids for TB in HIV-negative patients were generally safe (11), but there are concerns regarding the safety profile in HIV-infected patients (12-15). Thus there was equipoise to justify a clinical trial of corticosteroids in paradoxical TB-IRIS. However, the decision was taken during the trial design phase to exclude patients with immediately life-threatening TB-IRIS, mainly those with neurological manifestations, from the trial and these patients were treated within the clinical service with corticosteroids. We

reasoned that for patients with immediately life-threatening TB-IRIS there was sufficient indirect evidence (as described in Chapter 2) to support the potential benefit of corticosteroids, and that potential harms were likely to be outweighed by the benefits. The main hypothesis of the clinical trial was that prednisone would reduce morbidity related to paradoxical TB-IRIS without an excess of corticosteroid side effects or infections. Mortality was not chosen as the primary endpoint of the trial because mortality due to paradoxical TB-IRIS is infrequent (as discussed in Chapter 2) and patients with immediately life-threatening disease, including all those with neurological TB-IRIS, were excluded from the trial. The predetermined primary endpoint chosen for the clinical trial was a composite endpoint of total number of days hospitalized plus outpatient therapeutic procedures (for example therapeutic aspirations of suppurative lymph nodes), which were counted as one additional day. This primary endpoint was chosen because it allowed quantification of morbidity across a range of TB-IRIS presentations. Given the heterogeneity of TB-IRIS it was difficult to identify another endpoint that would adequately capture morbidity across the spectrum of potential manifestations.

110 participants were enrolled in the trial (55 in each arm). The primary endpoint was significantly more frequent in the placebo arm than the prednisone arm (median 3 hospital days (IQR = 0-9) versus 0 hospital days (IQR = 0-3) respectively, $p = 0.04$). Patients on prednisone experienced significantly more rapid improvement in symptoms, quality of life, performance status, and a chest radiology score. There was also rapid reduction in C-reactive protein concentration observed in those treated with prednisone: the median C-reactive protein concentration fell from 104 mg/l to 34 mg/l within one week in those on prednisone. It took 12 weeks for the C-reactive protein to decline to similar concentrations in those in the placebo arm. There was no significant excess of steroid metabolic side effects or severe infections (defined as invasive bacterial infections and new World Health Organisation stage 4 conditions) in the prednisone arm. There were more mild infections in those who received prednisone, such as oral or vaginal candidiasis and uncomplicated herpes simplex. A subgroup of participants required a prolonged course of open-label prednisone to control symptoms for a median of 84 days (IQR = 60-126) (the study design allowed for crossover to open label prednisone in patients deteriorating despite study drug and those who relapsed after completing the 4-week course).

Corticosteroids thus provide clinical benefit in paradoxical TB-IRIS reducing morbidity and improving symptoms. An understanding of their mechanism of action could therefore contribute to our understanding of the pathogenesis of paradoxical TB-IRIS. This question was addressed in the study reported in **Chapter 7**. Prior to this study our group had demonstrated that TB-IRIS is associated with higher gene expression levels and serum concentrations of a broad range of cytokines and chemokines, but most consistently IL-6, TNF α and IFN γ (16). Many of the cytokines we observed to be elevated in TB-IRIS are predominantly of myeloid origin. In the study described in Chapter 7, we analysed samples taken from 31 prednisone-treated and 27 placebo-treated participants at weeks 0, 2 and 4 on the clinical trial. The experiments performed were: ELISpot using a range of mycobacterial antigens, RT-PCR for gene expression levels following stimulation with heat-killed *Mycobacterium tuberculosis* and multiplex for cytokine/chemokine concentrations in the corresponding tissue culture supernatants and in unstimulated serum. The most important finding was that prednisone, but not placebo, suppressed concentrations of IL-6, IL-10, IL12p40, TNF α , IFN γ and IP-10 in unstimulated serum. Prednisone did not significantly decrease numbers of IFN γ -producing T cells in ELISpot experiments, suggesting that the mechanism of action of prednisone in TB-IRIS is mediated through suppression of the pro-inflammatory cytokine responses that we had shown characterized TB-IRIS in the earlier study (16).

Impact of the studies on the field

Until 2004, the literature on TB-IRIS comprised of case reports and case series as well as cohort studies which reported the incidence and risk factors for the condition. There were anecdotal reports of treatments used and response. Standard case definitions were not used across studies making comparison difficult.

Our clinical trial reported in Chapter 6 was the first clinical trial to assess a treatment strategy in any form of IRIS and showed that there was clinical benefit from a 4-week course of corticosteroids without an excess risk of severe infections or metabolic side effects. These findings have influenced clinical guidelines and practice. An important caveat based on

findings presented in Chapter 4 is that patients with undiagnosed rifampicin-resistant TB may present with paradoxical TB-IRIS indistinguishable from patients without rifampicin-resistant TB. If corticosteroids are prescribed to patients with undiagnosed rifampicin-resistant TB, who are not on optimal TB treatment, they may potentially do harm. Hence our recommendation is that drug susceptibility testing be performed on paradoxical TB-IRIS suspects wherever feasible.

Our group made a major contribution to the development of consensus case definitions for TB-IRIS under the auspices of INSHI. I (Graeme Meintjes) was the head of the writing committee and first author on the publication of these case definitions presented in Chapter 3. The main rationale for these case definitions was to promote standardization and comparability of studies investigating TB-IRIS. Since publication, they have been widely implemented in clinical and immunological studies of TB-IRIS. Four publications have specifically validated the performance of these case definitions in different settings using either expert opinion or other case definitions as the comparator (17-20).

Over the last 5 years several groups across the world have investigated the immunopathogenesis of TB-IRIS (reviewed in Chapter 2). Our immunology studies presented in Chapter 4 raised questions regarding the causal role of mycobacterial-specific IFN γ -producing T cells in the pathogenesis of TB-IRIS and others have cited this study as one of the reasons for exploring the role of the innate immune system in pathogenesis (21). Our findings, together with those of others (6, 7), have shown that there is no deficiency of regulatory T cell numbers in TB-IRIS suggesting that if defects in regulatory components of the immune system do play a role it is rather functional. The study presented in Chapter 7 is yet to be published.

Research priorities in TB-IRIS

Ongoing research in the field of paradoxical TB-IRIS includes studies of immunopathogenesis, predictive markers, diagnosis, prevention and treatment.

Much research is being undertaken to define biomarkers for TB-IRIS that could be used in prediction and diagnosis and also contribute to understanding pathogenesis (22). Candidate biomarkers include serum cytokine and chemokine concentrations and combinations or ratios of these, ELISpot assays, concentrations of other inflammatory proteins such as C-reactive protein and gene expression profiles using microarrays. Although these biomarkers may help in understanding the immunopathogenesis of TB-IRIS and may help in predicting those at risk, they are likely to perform poorly as a diagnostic test because the specificity in differentiating paradoxical TB-IRIS from the important clinical differential diagnoses (most importantly bacterial infections, opportunistic diseases and drug resistant TB) is likely to be low. The reason for this is that most TB-IRIS clinical mimics are also characterized by inflammatory responses. In addition, even if diagnostic biomarkers were discovered they may be difficult to implement in resource limited settings for cost and feasibility reasons, but could potentially be used in research.

It is thus likely that paradoxical TB-IRIS will remain a clinical diagnosis of exclusion and research regarding the most clinically and cost-effective diagnostic work-up algorithms of patients with suspected TB-IRIS is a priority. Such algorithms would vary according to the organ system involved. For example, the work-up of a patient with a pulmonary presentation will be different to that of a patient with meningitis. Clinical prediction rules for the diagnosis of paradoxical TB-IRIS taking into account baseline data prior to ART, presenting features and diagnostic tests done at the time of presentation with suspected paradoxical TB-IRIS would assist clinicians in busy operational settings provided they are kept simple and usable. Given that drug-resistant TB is the most important diagnosis to exclude in patients presenting with suspected paradoxical TB-IRIS in our setting (as described in Chapter 4), rapid diagnostic tests to ascertain drug susceptibility results would assist in appropriate management of these patients. The novel GeneXpert test has shown promising results in facilitating rapid initial diagnosis of rifampicin-resistant TB in fresh clinical specimens and the assay takes 2 hours to perform (3). Studies to assess its performance in patients presenting with suspected TB-IRIS in a range of clinical specimens, including sputum and pus from tuberculous abscesses, are required.

While clinical trials have demonstrated that delaying ART to prevent paradoxical TB-IRIS in patients with CD4 counts < 50 cells/mm³ is not appropriate because this results in a higher incidence of AIDS progression and mortality (23-25), delaying ART to two months may be appropriate in patients with CD4 counts > 50 cells/mm³. Other preventive strategies that need to be studied include the role of immunomodulatory agents. It has been hypothesized that vitamin D deficiency may predispose to paradoxical TB-IRIS and that Vitamin D supplementation in deficient patients may thereby prevent TB-IRIS (26). Further studies to confirm this underlying hypothesis are required before taking investigations of vitamin D supplementation further. Maraviroc, doxycycline and statins have also been shown to have immunomodulatory properties and may be candidates for study (27-30). Our clinical trial demonstrated that corticosteroids provide symptomatic benefit and reduce morbidity in the treatment of paradoxical TB-IRIS, and it may be that they could have a role in the prevention of TB-IRIS among high-risk patients. We have, however, documented cases of TB-IRIS occurring in patients with TB meningitis despite patients being on high dose corticosteroids when ART is started (31). But even if corticosteroids do not prevent TB-IRIS they may decrease its severity or delay its onset (32). The role of corticosteroids in the prevention of TB-IRIS needs to be tested in a clinical trial. The risk-benefit equation for their role in prevention may be different to that of treatment as this would involve treating far more patients with corticosteroids, many of whom would not develop TB-IRIS.

While we showed that prednisone provides clinical benefit in TB-IRIS, research into more targeted approaches to treatment is needed. In Chapter 7 we showed that the effect of corticosteroids was mediated at least in part through reduction in the concentrations of pro-inflammatory cytokines including TNF α and IL-6. We suggested that there may be a role for specific blockers of these cytokines (for example the TNF α blocker infliximab or the IL-6 blocker tocilizumab) in the treatment for TB-IRIS. Given the cost of these agents and the potential for TNF α blockers to precipitate fungal, bacterial, non-tuberculous mycobacterial and viral infections (33-35) it is likely that their use would only be warranted in life threatening cases, such as severe neurological TB-IRIS. The prevention and treatment of neurological TB-IRIS is the major clinical research priority given that it is relatively common and is the most life threatening form of TB-IRIS (36, 37) as discussed in Chapter 2.

A central issue in the treatment of TB-IRIS is that most of treatments that have been used (including corticosteroids) or suggested (such as TNF α blockers) are immunosuppressive with the risk of causing other infections and Kaposi's sarcoma (13-15). The exception is non-steroidal anti-inflammatory drugs that, however, have their own potential toxicities. The discovery of immunomodulatory agents that are not immunosuppressive that could be used to prevent and treat TB-IRIS would overcome this problem.

In certain patients paradoxical TB-IRIS has a prolonged course of several months and long duration of corticosteroids are required to control symptoms, as described in the clinical trial (Chapter 6). Predicting which patients are likely to have prolonged TB-IRIS and the optimal treatment for such patients, including duration of corticosteroids and the role of other treatment modalities, requires research. Prediction of prolonged TB-IRIS may be possible based on baseline markers such as C-reactive protein, the extent of TB-IRIS in terms of number of organs involved or organ sites of TB-IRIS.

In terms of the immunopathogenesis of TB-IRIS, the role of cells of the innate immune system has been relatively underexplored in research to date. While preliminary studies have suggested a role for monocytes and macrophages and cytokines produced by these cells (outlined in Chapter 2) these need to be confirmed and further defined in larger prospective studies. Our group's findings that high neutrophil counts in cerebrospinal fluid both predict and characterize TB meningitis IRIS (31), warrant further study to explore the role of neutrophils in pathogenesis particularly at sites of disease using tissue and body fluid specimens.

Looking ahead

Recent clinical trial data presented in Chapter 2 supports starting ART within 2 weeks of TB treatment in patients with HIV-associated TB and a CD4 count < 50 cells/mm³. This will impact on guidelines and clinician practice and will result in patients starting ART sooner after TB diagnosis where operationally feasible. In such patients with low CD4 counts starting ART 2 weeks after TB treatment the risk of TB-IRIS was increased 2-3 fold in these clinical trials (23-25). For this reason paradoxical TB-IRIS is likely to become more common

in the immediate future. However, if broader public health policies are successful in achieving wider uptake of HIV testing and thereby earlier diagnosis of HIV and earlier initiation of ART prior to advanced immunosuppression and prevention of TB disease through scale-up of isoniazid preventive therapy (IPT), improved infection control practices, intensified case finding and ART, it could be anticipated that in the longer term the incidence of paradoxical TB-IRIS may decline. Improved diagnostics for TB in HIV-infected people (3) that allow TB diagnosis prior to dissemination of TB may reduce the incidence of TB-IRIS, as disseminated TB and extrapulmonary TB have shown to be risk factor for TB-IRIS presumably related to antigen burden (38-41).

However, there are over 20 million people living with HIV globally who have not yet accessed ART. There is a global effort to make ART accessible to these people when it is required. Many of these people live in regions of the world where TB is both prevalent and the most common cause of morbidity and mortality among HIV-infected people. Despite public health efforts to promote earlier HIV diagnosis and prevention of TB many people in these regions will continue to access health care late in the course of their HIV disease when they have active TB, partly due to health system access issues. They will thus need treatment of TB followed by ART while on TB treatment putting them at risk of paradoxical TB-IRIS. It is therefore predicted that paradoxical TB-IRIS will remain an important cause of morbidity in ART treatment programmes for decades to come particularly in resource limited settings. While important in itself, the study of paradoxical TB-IRIS also provides an important window into the immune pathways and effector mechanisms involved in immunopathological responses to TB and mycobacterial diseases in general.

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