Lymphocyte-specific reconstitution of IL-4Rα-deficient mice: characterization and infectious disease studies.

Elmarie Myburgh

Thesis submitted to the University of Cape Town in fulfillment of the degree

Doctor of Philosophy

Department of Immunology

Faculty of Health Sciences

University of Cape Town

August 2006

Declaration

I, Elmarie Myburgh, hereby declare that the work on which this thesis is based, is my original work (except where acknowledgements indicate otherwise) and that neither the whole work or any part thereof has been, is being or is to be submitted for another degree in this or any other University.

I empower the University of Cape Town to reproduce for the purpose of research either the whole or any portion of the contents in any manner whatsoever.

Signed by candidate

Elmarie Myburgh

August 2006

This thesis is dedicated to the loving memory of my mother

Linette Myburgh

Acknowledgements

I would like to express my sincere gratitude to the many people who have supported me over the years and contributed to the completion of this thesis. I especially want to thank:

Prof. Frank Brombacher, my supervisor, who has encouraged and guided me throughout my research. His enthusiasm and knowledge of the field has been an inspiration.

To my colleagues and friends at the department of Immunology for continuous motivation, advise and friendly faces when I needed it most. To Berenice for all her patience and support while teaching me so many of the techniques I know today. To De'broski, Bill and Tony for their scientific input and willingness to help, especially with the critical review of this thesis. To Mark Simpson for his motivation and interesting discussions throughout the years. To all the technical and administrative staff at the department of Immunology, none of this would have been possible without them.

To the staff of the UCT animal facility for the friendly and helpful way they assisted me through the last few years and for providing an excellent service.

I am grateful to the Medical Research Council, National Research Foundation and German Academic Exchange Service (DAAD-Deutscher Akademischer Austauschdienst) for funding me throughout these studies.

Lastly, to my family and friends. Thank you for your continuous support, love and patience. With all of you in my life, nothing seems impossible.

Contents

Abbreviations

Ag	Antigen
APC	Antigen presenting cell
BSA	Bovine serum albumin
BSF	B cell stimulatory factor
CD	Cluster of differentiation
CTLA-4	Cytotoxic T lymphocyte associated antigen 4
°C	Degree Celsius
DC	Dendritic cell
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
FACS	Fluorescent activated cell sorting
FCS	Foetal calf serum
Fig	Figure
FITC	Flourescein isothiocyanate
G-CSF	Granulocyte-colony stimulating factor
GM-CSF	Granulocyte-macrophage colony stimulating factor
hIL-4	Human Interleukin-4
hrs	Hours
IFN	Interferon
Ig	Immunoglobulin
IL-	Interleukin
IL-4R	Interleukin-4 receptor
IMDM	Iscoves Modified Dulbeccos medium
IRS	Insulin receptor substrate
ISPF	α-isonitrosopropiophenone
JAK	Janus tyrosine kinase
kb	Kilobases
kD	Dissociation constant
kDA	kiloDalton

L. amazonensis	*Leishmania amazonensis*
L. brasiliensis	*Leishmania brasiliensis*
L. donovani	*Leishmania donovani*
L. major	*Leishmania major*
L. mexicana	*Leishmania mexicana*
L. tropica	*Leishmania tropica*
LACK	*Leishmania* receptor for activated C kinase
LPS	Lipopolysaccharide
MCP	Macrophage chemoattractant protein
M-CSF	Macrophage-colony stimulating factor
MHC	Major histocompatibility complex
mIL-4	Murine Interleukin-4
MIP	Macrophage inflammatory protein
n	Sample number
N. brasiliensis	*Nippostrongylus brasiliensis*
ND	Not detectable
NK	Natural killer
NO	Nitric oxide
NOS2	Type 2 nitric oxide synthase
OVA	Ovalbumin
PAR	Protease activated receptor
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PGE2	Prostaglandin E2
p.i.	Post infection
PI3K	Phosphatidylinositol 3-kinase
PNPP	p-nitrophenyl phosphate
RAG2	Recombination-activating gene 2
RANTES	Regulated on Activation, Normal T-cell Expressed and Secreted
RNA	Ribonucleic acid
rpm	Revolutions per minute

s.c.	subcutaneous
SCID	Severe combined immunodeficiency
SD	Standard deviation
SEM	Standard error of the mean
STAT	Signal transducer and activator of transcription
T-bet	T-box expressed in T cells
TCR	T cell receptor
Tg	Transgene
TGF	Transforming growth factor
Th	T helper
TMB	3, 3', 5, 5' -tetramethylbenzidine
TNF	Tumor necrosis factor
TRAIL	Tumor necrosis factor-related apoptosis-inducing ligand
Tregs	Regulatory T cells
VCAM	Vascular cell adhesion molecule
WHO	World Health Organization
WT	Wild-type
γc	Common gamma chain

List of figures and tables

Literature Review

Materials and Methods

Results

Appendix A

Abstract

Lymphocyte-specific reconstitution of IL-4Rα was recently established by intercrossing lymphocyte-specific human IL-4Rα transgenic mice with mIL-4Rα-deficient mice. Human IL-4Rα may bind to mouse γc resulting in a chimeric receptor specific for human IL-4 but not mouse IL-4. This provides us with an inducible IL-4 system. The aim of this study was to investigate the *in vitro* and *in vivo* characteristics of our novel hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mouse model. The integrity of the lymphocyte-specific hIL-4Rα expression in hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice was demonstrated by FACS analysis. Lymphocytes responded to hIL-4 but not mIL-4 or mIL-13 in proliferation and T helper differentiation assays, demonstrating the species-specificity and inducibility of the chimeric receptor *in vitro*. Non-lymphocytes like macrophages were unresponsive to mIL-4 and mIL-13 as assayed by IFN-γ/LPS-induced NO production and arginase activity. The *in vivo* characteristics of hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice were studied using 2 murine disease models: 1) the *L. major* model and 2) the *N. brasiliensis* model. In contrast to mIL-4Rα$^{-/-}$ mice, hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice developed Th2 cytokine and type 2 antibody responses during either *L. major* or *N. brasiliensis* infections, in the absence of hIL-4. Transgenic mice were unable to expel *N. brasiliensis* worms confirming unresponsiveness in non-lymphocytes. These results suggested that endogenous mIL-4 signaled through the chimeric receptor in hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice *in vivo*. Neutralization of endogenous mIL-4 resulted in an inhibition of *N. brasiliensis*-induced Th2 cytokine and total IgE production in transgenic mice. In a genetic approach, we depleted endogenous mIL-4 and mIL-13 by intercrossing hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice with mIL-4$^{-/-}$/ mIL-13$^{-/-}$ mice. The absence of mIL-4 and mIL-13 completely abrogated type 2 responses in both *N. brasiliensis* and *L. major* infections. These data confirmed that the chimeric receptor responded to mIL-4 *in vivo*. However, hIL-4Rα Tg/ mIL-4Rα$^{-/-}$ /mIL-4$^{-/-}$ /mIL-13$^{-/-}$ mice were susceptible to *L. major* infection, indicating that this mouse model could not be used in *L. major* infection studies.

Together, these data demonstrate that despite the unresponsiveness of the hIL-4Rα/ mγc chimeric receptor to mIL-4 and mIL-13 *in vitro*, the chimeric receptor responded to endogenous mIL-4 in 2 different *in vivo* disease models.

Literature Review

Literature Review

LR. 1 Introduction to immunity

Immunity (L. *immun*, safe, free of burden) is defined as the ability of an organism to protect itself against disease. This includes the identification and destruction of foreign substances or organisms but also of abnormal cells, such as cancer cells. The mammalian immune system consists of the innate immunity and the acquired or adaptive immunity. The innate immune response is the body's first line of defense. This type of immunity is present before exposure to pathogens and is especially important early in infections. Physical and chemical barriers such as the skin, acidic conditions in the gut and mucus and cilia in the airways are all components of the innate immunity. These exterior defenses prevent many infectious agents from entering the body. However, when entry is gained, cellular components of the innate immunity respond immediately to eliminate the invading organism. Cells of the innate immunity include granulocytes (eosinophils, basophils and neutrophils), monocytes/macrophages, dendritic cells (DCs) and natural killer (NK) cells (Beutler, 2004). Pathogens are recognized by common structures, for example bacteria contain "pathogen-associated molecular patterns" (PAMPs) such as lipopolysaccharide (LPS) and mannan. Innate cells can kill pathogens directly by the release of cytotoxic substances, or phagocytose organisms for intracellular killing mostly via free radicals and other toxic derivatives (Janeway and Medzhitov, 2002).

The adaptive immune response is a highly specific response that recognizes particular peptide components (antigens) of pathogens or other cells. This involves antibody producing B lymphocytes, constituting the humoral immunity, and T lymphocytes which form part of the cell-mediated immunity. B lymphocytes express specific antigen receptors on their surface, also known as immunoglobulins (Ig) (Raghaven and Bjorkman, 1996). When an antigen binds to its Ig receptor, B lymphocytes develop into plasma cells, which secrete large amounts of the receptor in a soluble form (Calame, 2001; Dal Porto et al., 2004). These receptor molecules are known as antibodies and circulate the blood and tissue fluids to specifically bind their antigens. Another part of the

antibody interacts with other factors of the immune system, thereby allowing these elements to recognize and destroy antibody-coated pathogens (Gessner et al., 1998; Monteiro and Van De Winkel, 2003; Hofer et al., 2006).

T lymphocytes recognize antigens by a specific receptor termed the T cell antigen receptor (TCR) (Rudolph et al., 2006). The TCR is structurally related to the surface receptors on B lymphocytes but does not bind free antigen as antibodies do. Antigens are only recognized when presented by host cells in combination with major histocompatibility complex (MHC) or CD1 molecules. MHC molecules are polymorphic cell-surface molecules that bind antigens which have been degraded inside the cell. They are divided into MHC class I and class II molecules depending on the type and source of the antigen presented (Watts, 1997; Cresswell et al., 2005; Stern et al., 2006). CD1 molecules are structurally related to MHC molecules but are not polymorphic and present lipid and glycolipid antigens (Brigl and Brenner, 2004). Recognition of antigens by T lymphocytes causes the release of soluble proteins, called cytokines, or the killing of cells bearing the antigen. T lymphocytes and their functions are discussed in more detail later.

There is a constant interaction, coordination and communication between the various parts of the immune system and most immune responses to invading organisms combine the components from both the innate and adaptive immunity.

LR. 2 Cytokines

Communication between the components of the immune system is facilitated by the release of chemical messengers such as cytokines. Cytokines are low molecular weight proteins that control, coordinate, and regulate various immune or inflammatory responses. They are important for inflammation, proliferation, differentiation of B and T lymphocytes and regulation of macrophage and lymphocyte functions (O'Shea et al., 2002). There are many cytokines and most of them have a multitude of functions. They include the chemokines, interleukins, interferons, growth factors, colony-stimulating factors and tumour necrosis factors. Chemokines are mainly involved in directing the migration of cells around the body and activating specific cell types. Examples of these proteins are MCP-1, RANTES MIP-1α and MIP-1β. Interleukins (IL-1 to IL-33) are

produced primarily by T cells and play a major role in the proliferation and differentiation of cells. Interferons, such as IFN-α and IFN-β are secreted by virally infected cells to induce resistance to viral infection in uninfected cells. IFN-γ is produced by T cells and has a whole range of functions.

Growth factors such as transforming growth factor (TGF) promote growth of fibroblasts and other cell types. Colony-stimulating factors mediate the proliferation and differentiation of mainly bone-marrow stem cells and leucocyte precursors but also macrophages and monocytes. Tumour necrosis factors like TNF-α and TNF-β have a major role in cytotoxicity and inflammation. This nomenclature is based mainly on the first function described for each cytokine but most of them have a multitude of functions and may belong to more than one of these groups (Borish and Steinke, 2003; Steinke and Borish, 2006). Cytokines exert their effects by binding to specific receptors and activating intracellular signaling pathways that will induce or inhibit transcription of cytokine regulated genes (Miyajima et al., 1992; Ihle et al., 1995). There are four major signaling pathways. The Ras/mitogen-activated protein kinase (Ras/MAPK) and phosphoinositide-3-kinase (PI-3-kinase) pathways are both important for cell proliferation and prevention of apoptosis (Leonard and O'Shea, 1998; Dong et al., 2002; Deane and Fruman, 2004). Cytokines like IL-2, IL-3, IL-4, IL-5 and GM-CSF activate these pathways. Signal transducer and activator of transcription (STAT) pathways stimulate gene transcription by activating cytoplasmic transcription factors. Receptor associated kinases such as Janus kinases (JAKs), phosphorylate different transcription factors (STATs 1-6, NF-IL6, NFκβ) resulting in their translocation to the nucleus and activation of genes (Hebenstreit et al., 2005). Another major group of pathways are those that lead to cell death. Members of the TNF family like TNF-α, TNF-β, Fas ligand, CD40 Ligand and TRAIL are especially important in the activation of these pathways (Aggarwal, 2003).

Some cytokines share receptor subunits and this partly explains their functional redundancy. Understanding the roles of individual cytokines and the importance of cytokine expression profiles for the outcome of disease has been a major undertaking in the scientific world. The use of gene-deficient (knockout) and transgenic mice has contributed significantly to our understanding of cytokine functions (Durum and Muegge,

1998). We now make use of mice that are deficient for cytokines or their receptor subunits, mice that are double, triple or even quadruple gene-deficient and more recently mice with cell-specific deletions or transgenes.

LR. 3 T lymphocytes

All T lymphocytes express CD3 surface marker and are divided into two major subpopulations: cytotoxic T (Tc) cells and T helper (Th) cells (Woodland and Dutton, 2003). Tc cells, which are identified by their expression of CD8 ($CD8^+$), specifically recognize antigenic peptides in combination with MHC class I molecules. The recognition of foreign peptides such as virus particles presented by MHC class I molecules will stimulate Tc cells to destroy infected host cells. Killing is mainly mediated by the release of preformed cytotoxic proteins such as perforin and granzyme, or the induction of apoptosis due to binding of Tc cell Fas ligand to Fas on host cells (Wong and Pamer, 2003; Russel and Ley, 2002).

On the other hand, Th cells, which express CD4, recognize antigens associated with MHC class II molecules. These molecules are largely present on immune cells such as B cells, dendritic cells and macrophages and present peptides which have been internalized and degraded by the cell. Upon stimulation the T helper cells produce cytokines that inhibit or activate responses in immune effector cells. $CD4^+$ T helper cells can be mainly subdivided into T helper 1 (Th1) and T helper 2 (Th2) cells (Mosmann et al., 1986b; Romagnani, 1996b). Both Th1 and Th2 cells originate from the same naïve IL-2 producing $CD4^+$ T cell precursors and differentiation of TCR stimulated $CD4^+$ T cells is mainly controlled by the cytokine environment (Fernandez-Botran et al., 1988; Sad and Mosmann, 1994; Swain et al., 1988). Exposure to IL-12 and IFN-γ will lead to Th1 differentiation while IL-4 induces Th2 differentiation (Hsieh et al., 1993; Swain et al., 1990) (Figure A). Other factors that influence T cell differentiation include the amount and type of antigen (Holland et al., 2000), the antigen presenting cell present (Ron and Sprent, 1987) and co-stimulatory molecules (Dubey et al., 1995).

Th1 cells secrete interleukin-2 (IL-2), lymphotoxin and interferon-γ (IFN-γ) which are associated with cytotoxicity and inflammatory responses to intracellular pathogens such

as *Leishmania major* and *Mycobacterium tuberculosis* (Cherwinski et al., 1987). Some of the effects mediated by Th1 cells are delayed type hypersensitivity (Cher and Mosmann, 1987), activation of macrophages, Tc and NK cells, and production of IgG2 antibodies (Stevens et al., 1988). Th2 cells produce IL-4, IL-5, IL-9 and IL-13 which are important for protection against extracellular parasites such as *Nippostrongylus brasiliensis* and *Trichinella spiralis*. The cytokines produced by Th2 cells mediate type 2 immunity that is characterized by enhanced IgG1 and IgE antibodies, infiltration of mast cells and basophils and eosinophilia (Katona et al., 1988; Romagnani, 1996a). The roles of IL-4, IL-13 and their receptors in type 2 immunity forms a central part of the studies presented here and is therefore discussed further in the following section.

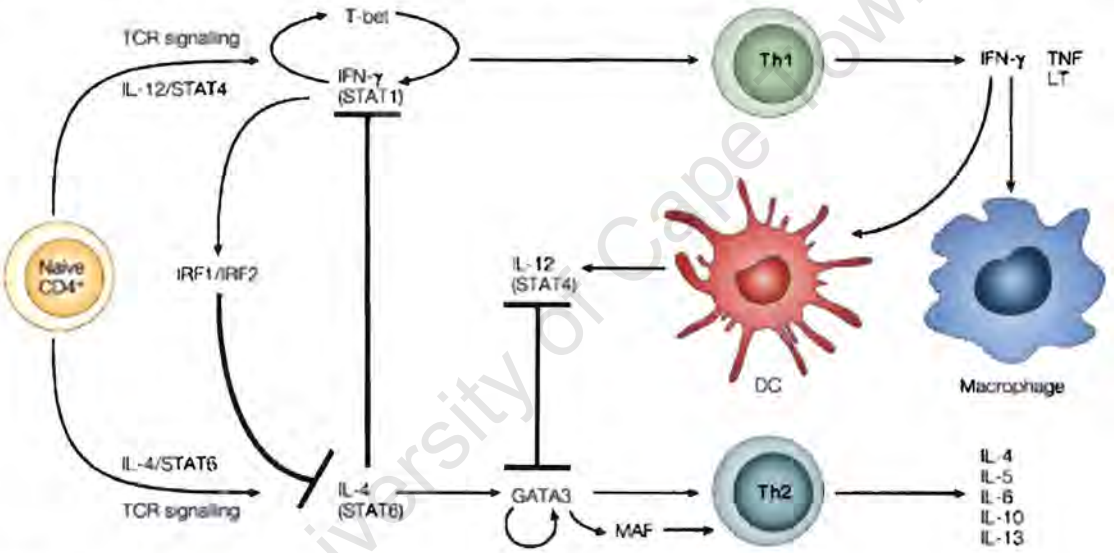

Figure A. Regulation of Th cell development.

Naive $CD4^+$ T helper (Th) cells can differentiate along the Th1 or the Th2 pathway. Interleukin-12 (IL-12) and interferon-γ (IFN-γ) are important for Th1 cell differentiation, while IL-4 is required for Th2 cell differentiation. IL-12 acting via the signal transducer and activator of transcription 4 (STAT4) induces IFN-γ production. IFN-γ promotes expression of IL-12 and the IL-12 receptor (IL-12R), and also activates Th1 cell-specific transcription factor T-bet, which, in turn, upregulates expression of IFN-γ. On the other hand, IFN-γ inhibits the Th2 pathway by suppressing *IL4* gene expression through the interferon regulatory factors IRF1 and IRF2. IL-4 signaling through STAT6 induces the Th2 cell-specific transcription factor GATA3 (GATA-binding protein 3). In turn, GATA3 regulates the expression of a range of Th2 cytokines but suppresses Th1 cytokine expression through repression of IL-12 signaling. The IL-4R–STAT6 and the IL-12R–STAT4 signaling pathways can downregulate expression of IFN-γ and GATA3, respectively, thereby promoting one response by inhibiting the other response. DC, dendritic cell; LT, lymphotoxin; TCR, T-cell receptor; TNF, tumour-necrosis factor (Li-Weber and Krammer, 2003).

Besides Th1 and Th2 cells, there are a few other subsets of T lymphocytes. Tr1, Th3 and natural $CD4^+CD25^+$ regulatory T cells (Tregs) regulate immune responses and play a crucial role in preventing autoimmunity and excessive effector responses to pathogens. While Tr1 and Th3 cells secrete immunosuppressive cytokines like IL-10 and TGF-β, Tregs exert their actions mainly through ligation of the T cell receptor and cell-cell contact (Maloy and Powrie, 2001; McGuirk and Mills, 2002; O'Garra et al., 2004; Sakaguchi et al., 1995; Shevach, 2002).

Th17 cells are IL-17 producing effector $CD4^+$ T cells that require TGF-β for development This newly described Th17 lineage has a role in autoimmunity and is probably involved in the clearance of pathogens that are not targeted by Th1 or Th2 cells (Bettelli et al., 2006; Bettelli and Kuchroo, 2005).

LR. 4 Interleukin-4 and Interleukin-13

LR. 4.1 Interleukin-4

IL-4 was originally identified in 1982 as a T cell-derived factor that stimulates proliferation of murine B cells exposed to anti-IgM antibodies (Howard et al., 1982). Actions on B cells lead to the name B cell stimulatory growth factor 1 (BSF-1) but when it became evident that this 15-19 kDa protein formed part of a greater family of cytokines, and had effects on other cells such as T and mast cells the name IL-4 was proposed (Lee et al., 1986; Mosmann et al., 1986a; Noma et al., 1986). IL-4 is also secreted by several other cell types including activated T cells, NKT cells, mast cells and basophils and it exerts a broad spectrum of effects on B cells, T cells, macrophages, NK cells, mast cells, fibroblasts, granulocytes and endothelial cells (Delespesse et al., 1989; Monroe et al., 1988; Sad and Mosmann, 1995; Spits et al., 1987; Thornhill and Haskard, 1990; Yoshimoto and Paul, 1994). IL-4 increases the expression of genes such as the low affinity IgE receptor (CD23) (Defrance et al., 1987), IL-4R (Ohara and Paul, 1988), MHC class II (Noelle et al., 1984), CD80 and CD86 (Stack et al., 1994) but down-regulates the expression of the IgG receptors, CD64, CD32 and CD16 (te Velde et al., 1990). In addition, IL-4 induces isotype switching to IgE and IgG1 in activated B cells

while suppressing the production of IgM, IgG2a, IgG2b and IgG3 (Coffman et al., 1986; Snapper et al., 1988; Vitetta et al., 1985). It enhances the generation of basophils, mast cells and eosinophils from progenitor cells (Favre et al., 1990; Rennick et al., 1987) and activates neutrophils for the killing and phagocytosis of opsonized cells (Boey et al., 1989). IL-4 inhibits the production of IL-1, IL-6, IL-8 and TNF by monocytes while stimulating the production of G-CSF and M-CSF (Essner et al., 1989; Miossec et al., 1992; Standiford et al., 1990; Wieser et al., 1989). On T cells, it plays an important role in the development of antigen-specific cytotoxic cells (Horohov et al., 1988) and, importantly, promotes the differentiation of naïve $CD4^+$ T cells into the Th2 phenotype (Le Gros et al., 1990; Swain et al., 1990).

LR. 4.2 Interleukin-13

IL-13 is a 10-14 kDa pleiotropic Th2 type cytokine belonging to the same α helix superfamily as IL-4 (Brown et al., 1989; McKenzie et al., 1993a; Minty et al., 1993). The human IL-13 gene is located on chromosome 5q31 only 12 kb distal from the IL-4 gene. The mouse IL-13 gene is found on chromosome 11 together with the genes for IL-3, IL-4, IL-5 and GM-CSF (Boulay and Paul, 1992; McKenzie et al., 1993b; Smirnov et al., 1995). IL-13 is produced by T cells, NK cells, mast cells, basophils, eosinophils and dendritic cells (Hoshino et al., 1999; McKenzie et al., 1993a). IL-4 and IL-13 have only about 25% homology but share many functional properties. IL-13 up-regulates MHC class II, CD23 and CD80/86 on monocytes and macrophages and inhibits monocytes production of proinflammatory cytokines and chemokines (de Waal Malefyt et al., 1993; McKenzie et al., 1993a; Punnonen et al., 1993). In addition, it induces vascular cell adhesion molecule (VCAM)-1 on vascular epithelium to direct migration of lymphocytes, monocytes, basophils and eosinophils to sites of inflammation (Bochner et al., 1995; Ying et al., 1997). IL-13 also has distinct functions. Unlike IL-4, it does not influence differentiation or STAT6 activation in T cells (de Waal Malefyt et al., 1995; Minty et al., 1997; Zurawski et al., 1993). Whereas IL-4 behaves similarly in human and mouse B cells, IL-13 acts differently in these species. In human B cells, IL-13 induces class switching to IgG4 and IgE as well as modulating expression of CD23 and MHC class II

(Punnonen et al., 1993). In contrast, mouse IL-13 does not exert such actions on B cells in the murine system (Welham et al., 1995; Zurawski and de Vries, 1994). There have been a few studies supporting a role for IL-13 on mouse B cells. IL-13-deficient mice had decreased IgE production while mice overexpressing IL-13 had elevated IgE levels (Emson et al., 1998; McKenzie et al., 1998b) Another study showed that treatment of mice with rIL-13 caused enhanced antibody production but no elevated IgE (Lai and Mosmann, 1999). The *in vivo* effects observed for IL-13 on murine IgE is probably indirect.

IL-13 plays an essential role in protective immunity against some parasite infections and a detrimental role in others. It is critical for resistance to gastrointestinal nematodes like *N. brasiliensis* and *T. muris* (Bancroft et al., 1998; Urban et al., 1998) while being the key mediator of hepatic fibrosis in schistosomiasis (Fallon et al., 2000). It was also found to be involved in tumor immunosurveillance (Terabe et al., 2000) and has a pivotal role in allergic asthma (Wills-Karp et al., 1998). Administration of IL-13 to mice and the overexpression of IL-13 in the lungs of transgenic mice induce many features of the allergic phenotype including airway inflammation, mucus cell hyperplasia, eosinophilic infiltration, nonspecific airway hyperresponsiveness and airway remodeling. On the other hand, blockade of IL-13 in allergen challenged mice prevented airway inflammation and mucus production (Grunig et al., 1998; Wills-Karp et al., 1998). With the availability of more gene-deficient mice additional roles for IL-13 are being discovered.

LR. 4.3 IL-4/IL-13 receptors

The overlapping biological functions of IL-4 and IL-13 can be explained by their shared receptor components. For signaling, both cytokines require IL-4Rα as part of their heterodimeric receptors. Treatment of mice with anti-IL-4Rα antibodies or IL-4 antagonists that specifically bind IL-4Rα, blocked responses of both IL-4 and IL-13 (Hilton et al., 1996; Zurawski et al., 1995; Zurawski et al., 1993). It is suggested that at least three receptor combinations exist for the IL-4 and IL-13 receptor complexes although some of the subunits may bind IL-4 or IL-13 as non-signaling monomers (Figure B).

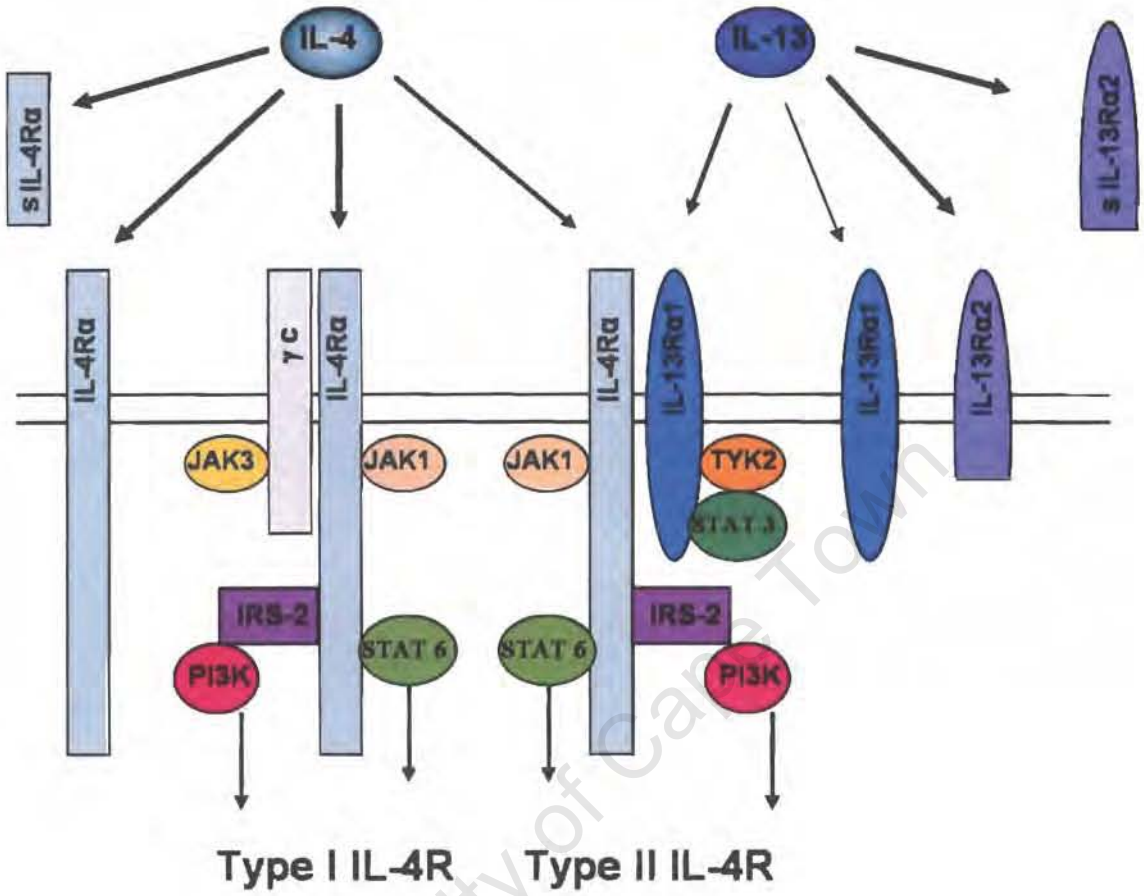

Figure B. IL-4 and IL-13 receptor complexes

IL-4 interacts with the IL-4Rα subunit in combination with either γc (Type I IL-4R) or IL-13Rα1 (Type II IL-4R). IL-13 interacts with IL-13Rα1 in combination with IL-4Rα or with IL-13Rα2. Increasing thickness of the arrows indicate increasing binding affinities (K_d) (Adapted from Brombacher, 2000)

The type I receptor consists of the common IL-2Rγ chain, γc and the 140 kDa IL-4Rα subunit (Mosley et al., 1989; Russell et al., 1993). The γc is also shared by the receptors for IL-2, IL-7, IL-9 and IL-15 (Giri et al., 1994; Noguchi et al., 1993; Russell et al., 1994; Takeshita et al., 1992). Neither the IL-4Rα nor the γc, separately or together binds IL-13 (Vita et al., 1995). The type I receptor is expressed predominantly on hematopoietic cells and binds IL-4 with high affinity (Kd ≈ 50-100pM) leading to the heterodimerization of

the two receptor subunits. IL-4Rα is expressed at low levels in most cell types examined but its expression is up-regulated with IL-4 or mitogenic stimulation (Lowenthal et al., 1988; Ohara and Paul, 1987; Ohara and Paul, 1988; Park et al., 1987a).

The type II receptor is composed of the IL-4Rα chain and the 70 kDa IL-13Rα1 chain. This receptor is found on both hematopoietic and nonhematopoietic cells and interacts with IL-4 and IL-13 with high affinity (Hilton et al., 1996; Miloux et al., 1997). The IL-4Rα chain binds only IL-4 whereas the IL-13Rα1 chain binds only IL-13 (Zurawski et al., 1993). The IL-13Rα1 chain by itself binds IL-13 with low affinity (Kd ≈ 10nM) and is unable to transduce a signal, but when combined with IL-4Rα it forms a high affinity IL-13 binding complex capable of signaling (Aman et al., 1996; Miloux et al., 1997). IL-13Rα1 expression was demonstrated on the majority of cell types examined except for human T cells and mouse T cells and B cells, explaining the lack of IL-13 responsiveness in these cell types (Akaiwa et al., 2001; Gauchat et al., 1997; Graber et al., 1998; Murata et al., 1998; Vita et al., 1995).

The third receptor found in both mice and humans is a 55-60 kDa protein termed IL-13Rα2, which specifically binds IL-13 with high affinity (kD ≈ 0.25nM) (Caput et al., 1996; Donaldson et al., 1998). The extracellular region of IL-13Rα2 is closely related to IL-13Rα1 but the cytoplasmic domain differs in that it does not posses signaling motifs or binding sequences for signaling molecules. The absence of signaling combined with high affinity ligand binding suggests that IL-13Rα2 may act as a decoy receptor similar to the IL-1 type II receptor (Colotta et al., 1994). Evidence for this was provided by studies using IL-13Rα2-deficient mice. It was shown that IL-13Rα2 expression is regulated by IL-13 and the absence of this receptor increased IL-13-dependent liver fibrosis during a *Schistosoma mansoni* infection (Chiaramonte et al., 2003). The majority of lymphoid populations examined thus far do not express IL-13Rα2 except for a subpopulation of germinal center cells and a plasmacytoid cell line that contain small amounts of mRNA for this protein (Guo et al., 1997).

In addition to the cell surface receptors, secreted forms of the IL-4 and IL-13 receptor subunits have been described. The soluble IL-4R (sIL-4R) is a 30-40 kDa truncated form of the membrane receptor that still binds IL-4 with the same affinity as the membrane IL-4Rα (Fanslow et al., 1990; Fernandez-Botran and Vitetta, 1990; Keegan et al., 1991; Mosley et al., 1989). The two forms of the receptor are encoded by different messages produced by alternative RNA splicing of transcripts from the same gene (Wrighton et al., 1992). The soluble IL-4R can act as a competitive inhibitor of IL-4 *in vitro* and *in vivo* indicating a role in regulation of IL-4 activity (Fanslow et al., 1991; Gessner et al., 1994; Mosley et al., 1989). It was furthermore suggested that sIL-4R may act as an agonistic carrier protein (Fernandez-Botran and Vitetta, 1991; Sato et al., 1993). IL-13Rα2 can also be secreted from the cell surface as a soluble form of the receptor (Zhang et al., 1997).

LR. 4.4 Mechanisms of IL-4 and IL-13 signaling

The components of the IL-4-IL-13 receptor complexes are constitutively associated with Janus tyrosine kinases (JAKs): IL-4Rα associates with JAK1 (Yin et al., 1994), γc with JAK3 (Russell et al., 1994) and IL-13Rα1 with JAK2 or TYK2 (Murata et al., 1996; Umeshita-Suyama et al., 2000). Upon ligand binding the receptor subunits dimerize leading to the activation of JAKs. Activated JAKs phosphorylate tyrosine residues in the cytoplasmic domain of the IL-4Rα, which can in their phosphorylated state act as docking sites for signaling molecules. The IL-4Rα dedicated signal transducer and activator of transcription 6 (STAT6) is recruited to the phosphorylated IL-4Rα where it also becomes phosphorylated by JAKs (Hou et al., 1994; Ryan et al., 1998). Phosphorylated STAT6 monomers dimerize and translocate to the nucleus to bind STAT6 binding elements in promoters of IL-4 and IL-13 responsive genes (Izuhara et al., 1999; Johnston et al., 1994; Miyazaki et al., 1994; Witthuhn et al., 1994). The cytoplasmic domain of IL-13Rα1 also binds to STAT3 and IL-4 or IL-13 binding leads to the phosphorylation of this transcription factor (Orchansky et al., 1999; Umeshita-Suyama et al., 2000).

Other groups of signaling molecule recruited to the phosphorylated IL-4Rα are regulatory phosphatases, which are involved in the negative regulation of signaling (Hanson et al.,

2003) and the insulin receptor substrate (IRS) family (Jiang et al., 2000). IRS proteins become phosphorylated and can associate with cytoplasmic signaling molecules such as phosphatidylinositol 3-kinase (PI3K) which is important for growth and regulatory signals through the IL-4Rα (Izuhara and Harada, 1993; 1996).

LR. 4.5 Species-specificity

Murine and human IL-4 display about 60% amino acid homology and mediate similar effects on T cells in their respective species (Lee et al., 1986; Yokota et al., 1986). Furthermore, the receptors for these two proteins share about 53% homology (Idzerda et al., 1990). Despite this homology the binding of murine and human IL-4 to their respective receptors is species-specific (Lowenthal et al., 1988; Nakajima et al., 1987; Ohara and Paul, 1987; Park et al., 1987a; 1987b). Human cell lines are able to respond to human IL-4 but not mIL-4, while mouse cell lines do not bind hIL-4 but respond to mIL-4 (Mosmann et al., 1987). However, hIL-4 is able to bind to receptors on monkey cell lines (Park et al., 1987b). The expression of hIL-4Rα in a mouse cell line confers responsiveness to hIL-4 indicating that hIL-4Rα is able to form a functional receptor with mouse signaling components (Idzerda et al., 1990). Later it was demonstrated that murine γc can complex with either mIL-4Rα or hIL-4Rα to form a functional type I receptor (Andrews et al., 2001). The same does not hold true for the type II receptor: the interaction between IL-4Rα and IL-13Rα1 is species-specific. In murine B cells, transfected hIL-4Rα was not able to associate with mIL-13Rα1 to form a functional IL-13R, whereas transfection of both hIL-4Rα and hIL-13Rα1 rendered cells responsive to IL-13. Interestingly, IL-13 itself is not species-specific and both human and murine IL-13 can signal through receptors of the other species (McKenzie et al., 1993a). However, IL-13 is species selective because although mouse IL-13 will act equally on mouse and human cells, human IL-13 has a greater effect on human cells than on mouse cells (de Vries, 1996; Minty et al., 1993).

LR. 5 *Leishmania major*

LR. 5.1 Brief history of *Leishmania*

Leishmania is the causative agent of the disease leishmaniasis. It was first discovered in 1903 in the spleens of patients with malaria-like symptoms and named as *Leishmania donovani* after its separate co-discoverers William Leishman and Charles Donovan. The protozoan parasites are transmitted by the sandfly vectors Phlebotomus (old world) or Lutzumyia (new world) which become infected with *Leishmania* when feeding on the blood of infected individuals or animals (Figure C). The *Leishmania* parasites live as amastigotes in macrophages but transform into motile flagellate promastigotes when released in the stomach of the insect (Killick-Kendrick, 1990). When the sandfly takes another blood meal, the metacyclic promastigotes are transferred into the skin of the host. The parasite promastigotes bind to host macrophages via surface molecules such as the complement receptor 1 and 3, mannose fucose receptor, the Fc receptor and the fibronectin receptor (Channon et al., 1984; Da Silva et al., 1989; Guy and Belosevic, 1993; Locksley et al., 1988; Ouaissi, 1988; Wilson and Pearson, 1986). The organisms are internalized into phagosomes which fuse with lysosomes to form the macrophage parasitophorous vacuole. Here, the parasites transform into replicating intracellular amastigotes and continue to spread to uninfected cells (Alexander and Russell, 1992; Antoine et al., 1998; Desjardins and Descoteaux, 1998).

The different species of the genus *Leishmania* produce a wide range of diseases differing in severity (Herwaldt, 1999; Pearson et al., 1983). In visceral leishmaniasis, caused by *L. donovani*, the parasite disseminates to spleen, liver and bone marrow. Cutaneous leishmaniasis is characterized by localized lesions with the parasites remaining in the cutaneous lesions and draining lymph nodes. This form of leishmaniasis is caused by *L. tropica* and *L. major* in the old world while *L. mexicana*, *L. amazonensis* and *L. brasiliensis* is responsible for cutaneous leishmaniasis in the new world.

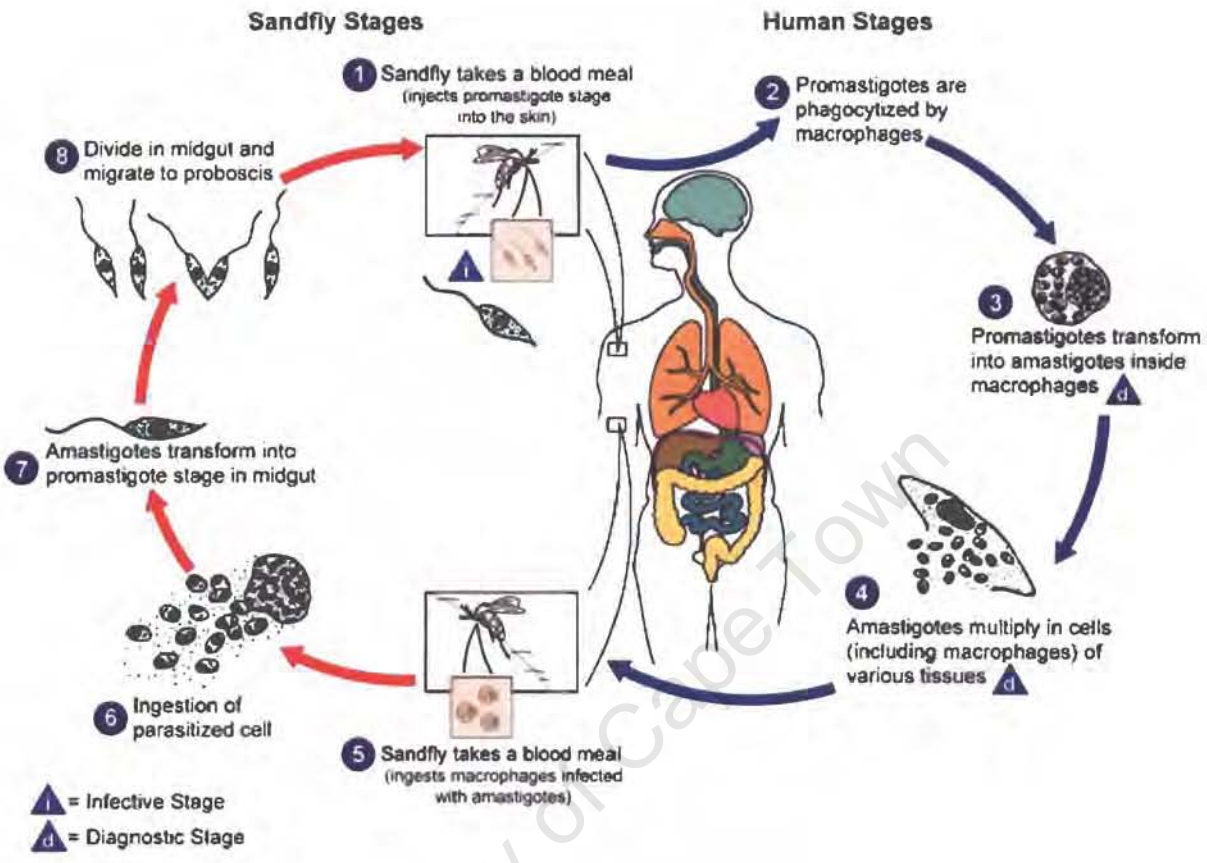

Figure C. Life cycle of the *Leishmania* species

Leishmania parasites are transmitted by female phlebotomine sandflies. The sandflies inject the infective stage, promastigotes, into the skin of the host during blood meals ❶ Promastigotes inside the skin are phagocytized by macrophages ❷and transform into amastigotes ❸. Amastigotes multiply in infected cells of various tissues depending on the *Leishmania* species ❹. Sandflies become infected when they ingest amastigote-infected macrophages during blood meals on an infected host (❺, ❻). Amastigote parasites differentiate into promastigotes in the midgut of the sandfly ❼, where they multiply and migrate to the proboscis ❽ (CDC).

LR. 5.2 The murine *L. major* model

The use of animal models is essential for the characterization of disease and its effects on the host. It is important to choose an animal model that matches the disease response in humans but is also easily available and maintained. The different *Leishmania* species cause clinically distinct symptoms in mice making them useful to study the spectrum of leishmaniasis observed in humans. The murine *Leishmania major* model is a well established model for cutaneous leishmaniasis and the disease observed in mice closely resembles that in humans, including varying susceptibility depending on the strain of inbred mouse used. Genetically susceptible BALB/c mice make a non-protective Th2 response while resistant C57BL/6 mice mount an early Th1 response that results in healing (Heinzel et al., 1991; 1989; Locksley et al., 1987). This has made the murine *L. major* model useful for studying the *in vivo* factors involved in T helper differentiation of $CD4^+$ T cells as well as the role of cytokines in the outcome of disease.

LR. 5.2.1 Influence of parasite strain on disease outcome

Many discrepancies in the results between research groups are due to the use of different *L. major* strains (Table A). IL-4-deficient BALB/c mice maintain a non-healing phenotype when infected with *L. major* Seidman while being able to control infection by other *L. major* strains like FEBNI, Friedlin and IR173. The reasons for these differences are not always clear but it seems that strains might be predisposed to induce a particular type of immune response. For example, *L. major* LV39 infection results in a non-healing phenotype when mice are treated with anti-IFN-γ or anti-IL-12 while lesions induced by *L. major* Friedlin are controlled under these conditions. It is possible that these two strains also express different levels of the LACK (for Leishmania homologue of the receptor for activated C kinase) antigen because proliferation of T cell clones to the LV39 strain is more than double that observed for *L. major* Friedlin (Hondowicz and Scott, 1999). Different results with IL-4-deficient mice using *L. major* LV39 may be due to strain differences between laboratories.

Table A. BALB/c susceptibility to different *Leishmania major* substrains

L. major substrains are indicated as follows: Seidman (WHOM/SN/74), FEBNI (MOHM/IL/81/FEBNI), Friedlin (MOHM/IL/80/Friedlin), IR 173 (WHOM/IR/-/173) and LV39 (MRHO/Sv/59/P-strain).

L.major strain	Anti-IL-4	IL-4$^{-/-}$	IL-13$^{-/-}$	IL-4/IL-13 $^{-/-}$ IL-4Rα $^{-/-}$ STAT6 $^{-/-}$	Note	Lab	Reference
Seidman		yes				Muller	Kropf et al., 1997, 1999, 2003
FEBNI	no					Gessner	Debus et al., 2003
		no					Kopf et al., 1996
		no				Muller	Kropf et al., 2003
Friedlin		*reduced*				Sacks	Belkaid et al., 1998
				reduced		Sacks	Noben-Trauth et al., 2003
		no		no		Staudt	Dent et al., 1999
IR 173	*reduced*	*reduced*		no	↑ parasite load	Sacks	Noben-Trauth et al., 1999, 2003
	reduced					Locksley	Heinzel et al., 1989
	no					Locksley	Sadick et al., 1990, 1991
	no					Locksley	Wakil et al., 1996
	no					Locksley	Stetson et al., 2002
	no					Coffman	Chatelain et al., 1992, 1999
	no					Coffman	von der Weid et al., 1996
	no	no				Maier	Heinzel & Maier 1999
	no					Reiner	Brown et al., 1996
LV39		no	no	no		McKenzie	Matthews et al., 2000
		reduced		*reduced*	↑ parasite load	Brombacher	Mohrs et al., 1999
	reduced	yes		yes		Sacks	Noben-Trauth et al., 1999, 2003
	reduced	yes				Muller	Noben-Trauth et al., 1996
		yes				Muller	Kropf et al., 2003

LR. 5.2.2 Other factors that influence susceptibility

It has become clear that susceptibility is controlled by more than one critical factor and that cytokine profile of the host alone can not predict disease outcome. Host strain differences play a major role in the outcome of *L. major* infection and resistant or susceptible strains have a bias towards a certain response due to intrinsic differences in factors that may influence differentiation or effector pathways. For example, BALB/c mice may have a bias towards a Th2 response due to inherent defects in Th1 differentiation. DC's from BALB/c mice produce low levels of IL-1 compared to resistant strains, which could result in insufficient T cell priming and defective Th1 differentiation (Filippi et al., 2003; von Stebut et al., 2003). A few studies have investigated genetic differences between resistant and susceptible strains and identified multiple genetic loci which by themselves have no effect but contribute to resistance to *L. major* infection (Beebe et al., 1997; Demant et al., 1996; Lipoldova et al., 2000; Vladimirov et al., 2003). Together with host strain differences, the parasite strain used (as discussed earlier), the site of infection and dose of the parasite also influence disease outcome. Most experimental infection models involve subcutaneous infection in the hind footpad or at the base of the tail. Although susceptibility in BALB/c mice appears to be unaffected by the inoculation site, SWR mice develop large lesions when infected at the base of the tail while resolving footpad lesions (Nabors and Farrell, 1994). Another example is the differences observed between intradermal infections in the ear and at the base of the tail. DBA/2 mice are susceptible when infected in the tail while they are able to cure infections in the ear. Furthermore, there is a lack of correlation between disease severity and cytokine profiles in CBA/H, C3H/HeN, C57BL/6J and DBA/2 mice (Baldwin et al., 2003). The dose of the parasite used for infection is particularly important in BALB/c mice. High doses favor a Th2 response and progressive disease while a low dose promotes Th1 development and healing. As expected, a high dose also renders normally resistant mice susceptible to infection (Bretscher, 1992; Menon and Bretscher, 1998). The influence of these factors on immune response and disease outcome emphasizes the importance of choosing a suitable infection model and taking these factors into account when interpreting results.

LR. 5.3 The immune response to *L. major*

Taking into account that various factors play a role in resistance and susceptibility to *L. major* infection, the study of this model has still provided crucial information about $CD4^+$ T cell development and the regulation of the Th1/Th2 balance.

LR. 5.3.1 The role of IL-12 for Th1 differentiation

The outcome of disease in *L. major* infections is determined by the balance of Th1 and Th2 responses. Resistance in C57BL/6 mice is associated with a shift towards the expansion of $CD4^+$ T cells that produce IFN-γ while non-healer BALB/c mice exhibit more $CD4^+$ T cells that produce IL-4 and IL-10 during infection (Heinzel et al., 1991; Heinzel et al., 1989; Sadick et al., 1986). The opposing roles of these distinct $CD4^+$ subsets in disease outcome was demonstrated with the transfer of *L. major* specific Th1 and Th2 cells in severe combined immunodeficiency (SCID) mice, resulting in healing or non-healing lesions, respectively (Holaday et al., 1991; Varkila et al., 1993). Although IFN-γ has a crucial role in the elimination of *L. major* in infected hosts it is not necessary for the development of a Th1 response (Swihart et al., 1995). Several studies identified IL-12 as a critical regulator for $CD4^+$ T cell differentiation. Administration of rIL-12 at the time of infection cures normally susceptible BALB/c mice (Heinzel et al., 1993b) while anti-IL-12 treatment exacerbates disease in C57BL/6 mice (Sypek et al., 1993). Direct evidence for the role of IL-12 in Th1 induction was provided with genetically resistant IL-12-deficient mice being susceptible to *L. major* and mounting a polarized Th2 response (Mattner et al., 1996).

IL-12 is primarily produced by dendritic cells, polymorphonuclear cells, macrophages and B cells, and stimulates the production of IFN-γ from T and NK cells (Gately et al., 1998; Trinchieri, 1998). Although various intracellular pathogens strongly induce macrophages to produce IL-12, *L. major* does not elicit this response (Cooper et al., 1995; Hsieh et al., 1993; Reiner et al., 1994; Tripp et al., 1993). *L. major* parasites are not only poor inducers of IL-12 in infected macrophages but selectively impair IL-12 production by these cells (Belkaid et al., 1998; Carrera et al., 1996).

Dendritic cells were shown to be the major source of IL-12 and play a pivotal role in the development of *Leishmania*-specific T cell responses (Sousa et al., 1997; Will et al., 1992). Resident macrophages phagocytose promastigote parasites at sites of infection resulting in the recruitment of various inflammatory cells including dendritic cells (DCs) (Belkaid et al., 1998; von Stebut et al., 1998). The DCs take up amastigotes, become activated to produce IL-12 and migrate to lymph nodes where they present *Leishmania* antigen to naïve T cells for the induction of Th1 differentiation (Moll et al., 1995; Moll et al., 1993; von Stebut et al., 1998). IL-12 also induces the production of innate IFN-γ by NK cells which promotes further IL-12 secretion by DC's and Th1 differentiation (Borg et al., 2004).

Many studies suggested that the difference between resistant and susceptible strains lies not in the levels of IL-12 produced but in IL-12 responsiveness due to the expression of its receptor subunits (Guler et al., 1996; Szabo et al., 1995). The IL-12 receptor (IL-12R) is composed of at least two subunits, IL-12Rβ1 and IL-12Rβ2 (Chua et al., 1995; Presky et al., 1996) and the inability of Th2 cells to respond to IL-12 is due to a lack of IL-12Rβ2 expression (Szabo et al., 1995). In susceptible BALB/c mice the early IL-4 production inhibits IL-12Rβ2 expression thereby promoting Th2 cell development whereas in C57BL/6 mice IL-12 receptor expression is maintained allowing the induction of Th1 differentiation (Himmelrich et al., 1998; Jones et al., 1998; Launois et al., 1997b; Szabo et al., 1997). However, a later study showed that transgenic BALB/c mice constitutively expressing IL-12Rβ2 still exhibit a non-healing phenotype to *L. major* infection indicating that IL-12 signaling alone can not explain differences in susceptibility (Nishikomori et al., 2001).

LR. 5.3.2 The role of other cytokines for Th1 differentiation

IL-18, IL-23 and IL-27 have also been identified as having roles in Th1 differentiation and the production of IFN-γ during *L. major* infection. IL-18 is produced by activated macrophages and has been shown to be critical for immunity against intracellular pathogens such as *Mycobacterium tuberculosis* and *Cryptococus neoformans* (Kawakami et al., 1997; Okamura et al., 1995; Sugawara et al., 1999). During *L. major* infection, IL-

18 may play a role early in infection but it is not essential for Th1 development and protection against the parasite. IL-18$^{-/-}$ C57BL/6 mice develop a Th1 response and are able to resolve *L. major* lesions as effectively as the wild-type mice (Monteforte et al., 2000). However, IL-18 may act synergistically with IL-12 to produce IFN-γ and induce protection against *L. major* infection in BALB/c mice (Li et al., 2004; Ohkusu et al., 2000). IL-23 is an IL-12-related cytokine consisting of a p19 protein and the p40 subunit of IL-12. This cytokine is mainly produced by activated dendritic cells and can induce proliferation and IFN-γ production from memory T cells (Oppmann et al., 2000). IL-23 may be important for the maintenance of IFN-γ production but can not by itself induce protection to *L. major* infection, as was demonstrated by IL-12p35$^{-/-}$ mice (Mattner et al., 1996). IL-27, a heterodimer consisting of an IL-12p40 related protein and p28, an IL-12p35 related protein, can synergize with IL-12 to induce IFN-γ production from naïve CD4^{+} T cells (Pflanz et al., 2002). C57BL/6 mice deficient in the IL-27 receptor, WSX-1, are more susceptible to *L. major* infection and have impaired IFN-γ production early in infection (Yoshida et al., 2001). More recently it was shown that the requirement for IL-27 in Th1 differentiation and protection to *L. major* infection is restricted to an environment in which IL-4 is produced (Artis et al., 2004).

LR. 5.3.3 The role of IL-4 in Th2 differentiation and susceptibility

The early IL-4 associated with the Th2 response in susceptible BALB/c mice is produced by MHC class II restricted Vβ4Vα8 CD4^{+} T cells that recognize the LACK antigen (Julia et al., 1996; Launois et al., 1997a; Mougneau et al., 1995). Vβ4-deficient BALB/c mice do not generate the early IL-4 burst and are resistant to *L. major* infection while rIL-4 treatment restores the Th2 development and susceptibility in these mice (Himmelrich et al., 2000). Neutralization of IL-4 in *L. major* infected BALB/c mice demonstrated that the presence of IL-4 within the first 48 hours of infection is crucial for susceptibility and the irreversible commitment of naïve T cells to the Th2 pathway (Sadick et al., 1991). These findings suggested that early IL-4 might be the controlling factor for a Th2 response and susceptibility. Contrary to this, it was found that resistant strains like B10.D2 and C57BL/6 also produce a burst of IL-4 in response to LACK antigen

implying that early IL-4 production can not predict disease outcome in all resistant and susceptible strains (Julia and Glaichenhaus, 1999; Reiner et al., 1994; Scott et al., 1996). The regulatory role of IL-4 in *L. major* infection was further questioned with the finding that IL-4-deficient BALB/c mice, although being more resistant, do not eliminate the parasite as efficiently as a resistant strain (Kopf et al., 1996b). Another study showed that IL-4$^{-/-}$ mice were susceptible to disease and proposed that other factors may contribute to susceptibility (Noben-Trauth et al., 1996). IL-4Rα$^{-/-}$ and STAT6$^{-/-}$ mice are more resistant than IL-4$^{-/-}$ mice suggesting that IL-13 also plays a role in susceptibility. Surprisingly, these mice develop an unimpaired Th2 phenotype, providing evidence that IL-4 and IL-13 are not required for Th2 differentiation in *L. major* infection. The original simplistic view of IL-4 as the sole regulator of Th2 differentiation has now given way to a more complex model where IL-4 and IL-13, as well other factors influence disease outcome. This model is further complicated by findings showing that the effect of IL-4 on disease outcome is determined not by its accumulation but by the timing of its presence during an infection. When present only during the initial priming of dendritic cells by *L. major*, IL-4 induces DC's to produce IL-12 leading to Th1 differentiation and resistance to disease. In contrast, the additional presence of IL-4 during T cell priming decreases the expression of IL-12Rβ2 resulting in Th2 differentiation and susceptibility (Biedermann et al., 2001).

However, IL-4Rα$^{-/-}$ and STAT6$^{-/-}$ mice are still more resistant to infection than BALB/c mice clearly indicating the important role of IL-4Rα signaling for control of *L. major* infection. Various cell-specific IL-4Rα$^{-/-}$ strains are currently under investigation to unravel the roles of IL-4Rα signaling in different cells of the immune system and the effects this may have on susceptibility to *L. major* infection.

LR. 5.3.4 The role of other Th2 cytokines in *L. major* infection

A role for IL-13 in disease outcome to *L. major* infection has been shown with studies using mice deficient for IL-4Rα, STAT6, IL-13 or both IL-4 and IL-13. IL-4Rα$^{-/-}$ mice and IL-4/IL-13 double-deficient mice are more resistant to *L. major* infection than mice deficient for only one of these cytokines (Matthews et al., 2000; Noben-Trauth et al.,

1999). This finding together with the increased susceptibility observed in C57BL/6 mice overexpressing IL-13, indicates that IL-13 can cooperate with IL-4 to induce Th2 differentiation and susceptibility to *L. major*. On the other hand, *L. major* infected IL-4Rα$^{-/-}$ mice develop progressive disease during the late (chronic) phase of infection while IL-4$^{-/-}$ mice remain resistant suggesting a protective role for IL-13 in chronic disease (Mohrs et al., 1999).

IL-9 is another Th2 cytokine produced in BALB/c mice during an infection with *L. major* (Gessner et al., 1993). Treatment of genetically susceptible mice with long lasting anti-IL-9 antibodies results in increased resistance to *L. major* and impaired Th2/type2 responses. These detrimental effects are dependent on IL-4Rα expression suggesting that IL-9 acts downstream from IL-4-mediated Th2 differentiation and may play a role in the maintenance of a Th2 response. The increased resistance observed for IL-4, IL-5, IL-9 and IL-13 quadruple-deficient mice compared to IL-4$^{-/-}$ or IL-4/IL-13 double-deficient mice further supports the notion that the Th2 cytokines are functionally redundant and play a combined role in susceptibility to *L. major* (Roberts et al., 2005).

LR. 5.3.5 The role of IL-10 in parasite survival

The strong correlation between IL-10 production and disease progression in humans with leishmaniasis points to an important role for this cytokine (Ghalib et al., 1993; Louzir et al., 1998). IL-10 transgenic C57BL/6 mice are unable to control *L. major* infection despite the development of a Th1 response. In this study it was suggested that IL-10 inhibits effector functions because susceptibility in transgenic mice is reversed by anti-IL-10 treatment (Belkaid et al., 2001; Groux et al., 1999). Conversely, BALB/c mice deficient in IL-10 or treated with anti-IL-10R antibody are more resistant to *L. major* infection (Kane and Mosser, 2001). Mice deficient for both IL-10 and IL-4Rα were strikingly more resistant than the single-deficient mice indicating that IL-4, IL-13 and IL-10 have a cumulative effect for susceptibility (Noben-Trauth et al., 2003).

Leishmania amastigotes induce the production of IL-10 by macrophages via host IgG and Fcγ receptors. IgG binds to the surface of amastigotes resulting in the ligation of macrophage FcγR and subsequent IL-10 production (Guy and Belosevic, 1993; Peters et

al., 1995; Sutterwala et al., 1998). In the absence of IgG, amastigotes lose their ability to induce IL-10. The important role of FcγR ligation in this induction was demonstrated with the reduced IL-10 production in γ-deficient mice (Kane and Mosser, 2001).

LR. 5.3.6 The role of $CD4^+CD25^+$ T cells

$CD4^+CD25^+$ regulatory T cells are a major source of IL-10 and play an important role in the regulation of immune responses. Tregs are essential for the suppression of detrimental pathogenic responses especially to self antigens but may also lead to the suppression of beneficial responses against infectious agents. In *L. major* infected C57BL/6 mice, $CD4^+CD25^+$ T cells are recruited to the site of infection where they suppress the ability of effector cells to eliminate parasites (Belkaid et al., 2002). The depletion of $CD25^+$ cells in this strain enhances IFN-γ production by $CD4^+$ T cells in lesions, resulting in more effective parasite clearance. Similarly, in BALB/c mice, treatment with anti-CD25 increases resistance to *L. major* infection (Heinzel et al., 1993a). However, other studies demonstrated that the transfer of naïve $CD4^+CD25^+$ T cells in SCID mice reconstituted with $CD4^+CD25^-$ T cells, suppressed *L. major* disease development. This suggests that $CD4^+CD25^+$ T cells also inhibit Th2 responses and susceptibility to *L. major* infection (Xu et al., 2003). Although more studies are needed to unravel the role of Tregs in *Leishmania* infection the current evidence suggests that the balance between Tregs and effector cells is critical for immunity to *Leishmania*.

LR. 5.3.7 Killing of *L. major* by macrophages

Macrophages are the main host cells for *L. major* parasites and the outcome of disease is thus dependent on the activation of macrophage effector mechanisms that kill the parasite. Killing involves the induction of type 2 nitric oxide synthase (NOS2) in classically activated macrophages which catalyzes the production of nitric oxide (NO) from the amino acid L-arginine. Inhibition of this process by NOS2 inhibitors abrogates the ability of resistant mice to control infection (Evans et al., 1993; Liew et al., 1990). In

addition, genetically resistant mice lacking NOS2 are susceptible to *L. major* infection (Wei et al., 1995).

The activation state of macrophages and its ability to produce NO is dependent on a variety of factors including the cytokine environment (Figure D). IFN-γ is critical for the induction of classically activated macrophages which produce NO and pro-inflammatory cytokines such as IL-1, IL-6 and TNF (Jorens et al., 1995; MacMicking et al., 1997). Treatment of macrophages with recombinant IFN-γ can enhance NOS2 expression and NO production resulting in the clearance of *L. major* (Ding et al., 1988; Green et al., 1990a; Green et al., 1990b). Tumor necrosis factor (TNF) is able to synergize with IFN-γ to enhance macrophage mediated microbicidal activity but can not activate macrophages by itself (Bogdan et al., 1990). Administration of TNF during infection results in increased parasite killing (Titus et al., 1989) but studies using $TNF^{-/-}$ and $TNFR^{-/-}$ mice showed that TNF is not required for NO production and clearance of *L. major* parasites (Nashleanas et al., 1998; Vieira et al., 1996; Wilhelm et al., 2001).

IL-4, IL-10, IL-13 and TGF-β have all been shown to inhibit classical macrophage activation and killing of *L. major* parasites. While IL-4 can synergize with IFN-γ to activate macrophages under certain conditions, it inhibits macrophage-mediated killing of *L. major* when present before IFN-γ activation (Bogdan et al., 1991; Bogdan et al., 1994; Liew et al., 1989). The inhibition of NO production by IL-4 and IL-13 is due to the induction of alternatively activated macrophages which have increased arginase I activity and express higher levels of the mannose receptor (Doyle et al., 1994; Modolell et al., 1995; Munder et al., 1999; Stein et al., 1992). Arginase I and NOS2 share L-arginine as a substrate and the increased activity of one enzyme depletes the available substrate for the other (Munder et al., 1998; Rutschman et al., 2001). Macrophage/neutrophil-specific IL-$4R\alpha^{-/-}$ mice show significantly delayed disease progression during *L. major* infection indicating that IL-4 and/or IL-13 effects on macrophages are important for early disease progression. In these mice macrophages are unable to respond to IL-4 and IL-13 resulting in increased NO production and parasite killing. The macrophage/neutrophil-specific IL-$4R\alpha^{-/-}$ mice became susceptible eventually while IL-$4R\alpha^{-/-}$ mice remained resistant

indicating that IL-4Rα signaling in other cells plays a role in later susceptibility (Hölscher et al., 2006).

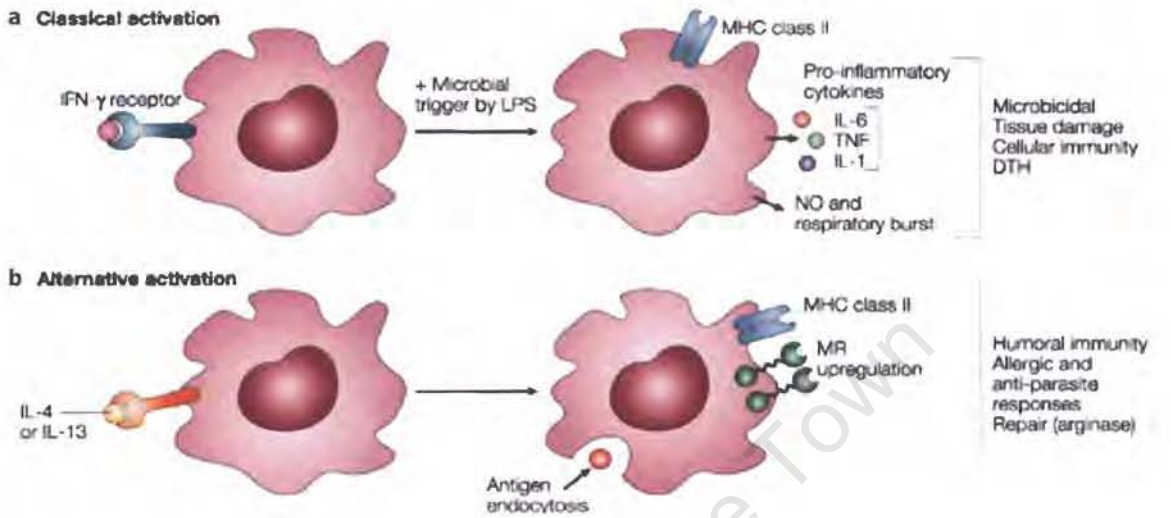

Figure D. Classical and alternative activation of macrophages

a. Priming by IFN-γ followed by a microbial trigger (lipopolysaccharide, LPS) results in classical activation of macrophages. **b**. Alternative activation is mediated by interleukin-4 (IL-4) and IL-13, signaling through the common IL-4Rα. DTH, delayed-type hypersensitivity; MR, mannose receptor; NO, nitric oxide; TNF, tumour-necrosis factor (Gordon, 2003).

IL-10 acts differently on macrophages to IL-4 and IL-13. It downregulates MHC class II expression, NOS2 activity and the release of pro-inflammatory cytokines by classically activated macrophages (Donnelly et al., 1999; Montaner et al., 1999). Treatment of *L. major* infected macrophages with rIL-10 results in the suppression of IL-12 and TNF production as well as failure to control parasite growth (Kane and Mosser, 2001; Vieth et al., 1994). In this regard the IL-10 induction by amastigotes (as discussed earlier) may be a way for the parasite to modify the host immune response and thereby enhance parasite survival.

TGF-β regulates macrophage function *in vitro* by inhibiting NOS2 protein expression and NO production. *In vivo* evidence for the suppressive effect of TGF-β was shown with the inverse correlation of TGF-β and NOS2 levels in lesions of *L. major* infected BALB/c and C57BL/6 mice (Stenger et al., 1994). Neutralization of TGF-β during *L. major* infection results in enhanced NO production in lesions and a decrease in parasite numbers leading to rapid healing (Li et al., 1999).

Besides having an important role in killing NOS2 also acts as a mediator of immunopathology. NO produced by macrophages inhibits the transcription of IL-12p40 and NOS2-deficient mice produce significantly higher levels of IL-12, resulting in the development of enhanced Th1 cell responses (Huang et al., 1998; Wei et al., 1995). A study by Diefenbach et al (1999) demonstrated that IL-12p40 and IFN-γ mRNA was significantly lower in NOS2-deficient mice up to 5 days after *L. major* infection.

Induction of arginase I favors the growth of *Leishmania* in macrophages not only due to the inhibition of NO production but also the increase in polyamine synthesis (Iniesta et al., 2001; Iniesta et al., 2002). The lethality observed in arginase-deficient *Leishmania* parasites demonstrated the essential role of arginase for parasite survival (Roberts et al., 2004). The addition of L-ornithine and putrescine to infected macrophages promotes the growth of *Leishmania in vitro* indicating that parasites can scavenge polyamines from their environment, and that increases in the host polyamine synthesis would be advantageous for the parasite. Thus, the development of a Th2 response, via its induction of alternatively activated macrophages and arginase I, would support the growth of *L. major*. IL-10 and TGF-β which are induced during *L. major* infection, have both been shown to enhance arginase activity (Chatelain et al., 1999a; Gantt et al., 2003). This may in part explain their important roles in parasite survival.

LR. 6 Parasitic nematodes

Parasitic helminth infections are among the most common infections of humans worldwide, with over two billion people chronically infected with intestinal parasites of whom 300 million suffer severe morbidity (Savioli et al., 2002). Helminths include trematodes, such as schistosomes, cestodes which include tapeworms, and nematodes (Table B). Medically important nematodes include *Ascaris lumbricoides*, the most common human parasite in the world, whipworm *Trichuris trichura* and the hookworms *Necator americanus* and *Ancylostoma duodenale*.

Gastrointestinal nematodes are unsegmented, spindle-shaped roundworms that have multistage life cycles. The life cycles of the various species differ but most of them undergo a series of moults through larval stages. The adult nematodes sexually reproduce in their definitive hosts leading to the release of eggs through urine or feces to the environment. In some cases like *Trichinella spiralis*, the eggs do not leave the host but develop to the next stage inside the body. Eggs hatch to release L1 larvae which develop through more larval phases (L2-L5). In most cases the L3 larvae is the form that infects the host by oral ingestion or skin penetration and migrates within the body as it matures into the adult parasite. Many nematodes persist within the host for a number of years due to their ability to reinfect the host and evade the host defenses (Bethony et al., 2006; Finkelman et al., 1997).

Table B. Parasitic helminth infections in humans

(Adapted from Bethony et al., 2006; Roitt et al., 2002)

Helminths	Disease	Estimated population infected (millions)
Trematodes		
Schistosoma mansoni *Schistosoma haematobium* *Schistosoma japonicum*	Schistosomiasis	200
Cestodes (tapeworms)	Tapeworm	10-30
Nematodes (roundworms)		
Trichuris trichura (whipworm)	Trichuriasis	604-795
Ascaris lumbricoides	Ascariasis	807-1221
Trichinella spiralis	Trichinosis	70-90
Ancylestoma duodenale Necator americanus	Hookworm	576-740
Wuchereria bancrofti Brugia malayi Dipetalonema perstans	Lymphatic filariasis	70-90
Onchocerca volvulus	River blindness	20-30

LR. 6.1 *Nippostrongylus brasiliensis* model

Gastrointestinal nematodes induce a strong type 2 immune response associated with Th2 cytokines like IL-4, IL-5, IL-9 and IL-13 and increased serum IgE antibody levels (Ogilvie and Jones, 1969; Urban et al., 1992). To investigate the mechanisms underlying this response, the rat nematode *Nippostrongylus brasiliensis* was experimentally adapted to the mouse and is now a well established model for studying Th2 responses (Jacobson and Reed, 1974). In this murine model third-stage larvae (L3) are injected subcutaneously into the skin of the mouse. The route of infection and subsequent migration of the worms mimics the natural infection of many intestinal roundworms and hookworms (Anderson, 2000). The parasites trigger a strong Th2 response resulting in the expulsion of worms by day 10 post infection. This has made *N. brasiliensis* a convenient and powerful model for studying the Th2 responses.

LR. 6.2 *N. brasiliensis* life cycle

N. brasiliensis eggs hatch in the feces of hosts to release L1 larvae which moult and increase in size to develop into the infective stage larvae (L3). The L3 larvae naturally enter the host by penetrating the skin or in the experimental model are injected subcutaneously into the skin. From here they migrate to the lungs and mature into L4 larvae. Studies showed that larvae migrate from the skin within 4 hours of subcutaneous inoculation and enter the lungs after 16-48 hrs (Kassai, 1982). L4 larvae migrate to the trachea and esophagus or are coughed up due to a strong eosinophilic response in the lungs. They are then swallowed by the host to reach the small intestine between 48-72 hrs after infection (Rosbottom et al., 2002). Here larvae attach to the gut wall of the jejunum and mature into egg-producing adults. Eggs are released into the feces for further development outside the host (Figure E).

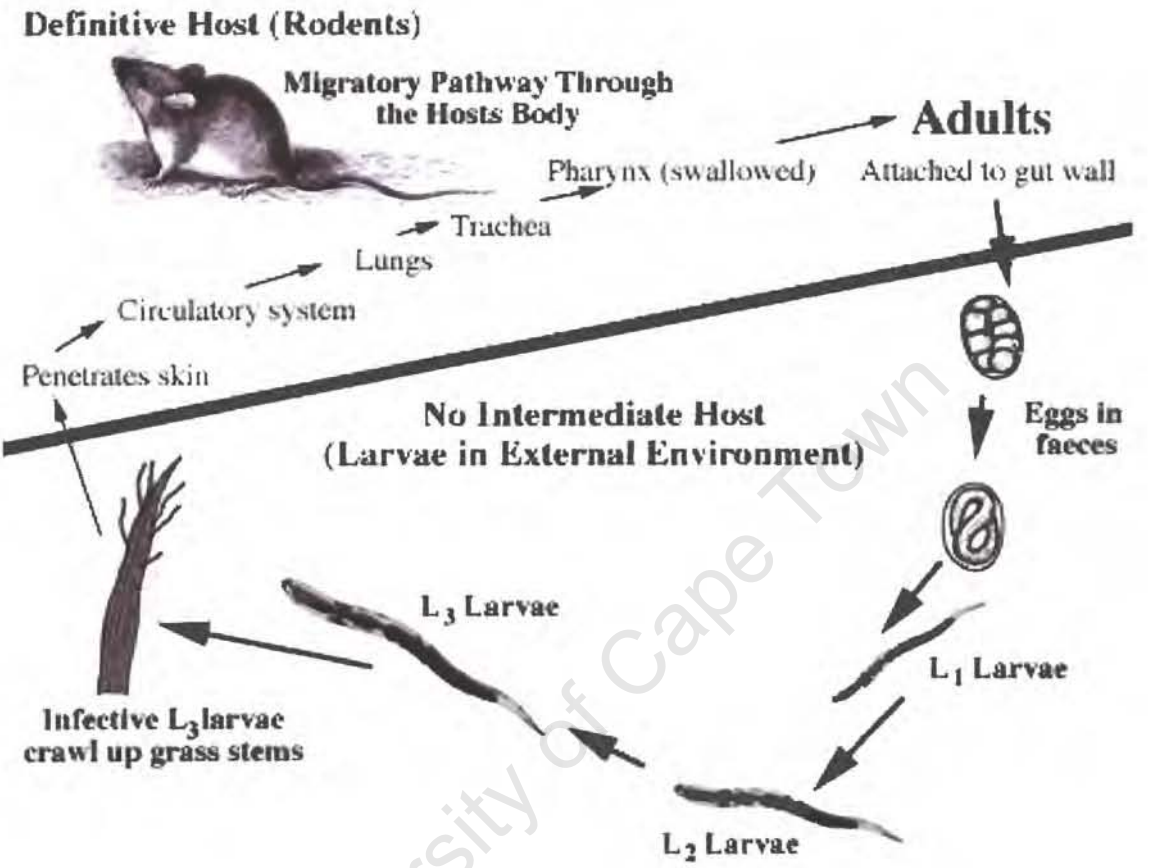

Figure E. Life cycle of *N. brasiliensis* (University of Cambridge, 2006)

LR. 6.3 Immune response to *N. brasiliensis*

Infection with *N. brasiliensis* induces a type 2 immune response that is associated with the production of the Th2 cytokines IL-4, IL-5, IL-9 and IL-13. The response results in elevated serum IgE, eosinophilia, intestinal mast cell hyperplasia and goblet cell proliferation (Else and Finkelman, 1998; Finkelman et al., 1997; Madden et al., 1991; Miller and Nawa, 1979). Clearance of the parasite is facilitated by a response termed "weep and sweep", referring to the increased intraluminal fluid due to mucus secretion, and hypercontractility of smooth muscle (Madden et al., 2002; Shea-Donohue and Urban, 2004; Zhao et al., 2003). Mast cells release soluble mediators such as PGE_2, histamine and leukotriene D which increase epithelial cell secretion and enhance smooth muscle contractility (Castro, 1985; Goldhill et al., 1997; Schwartz and Austen, 1984). The hypercontractility effects are mediated by alterations in the sensitivity of nerves in the gut wall and changes in cells that coordinate smooth muscle contraction (Faussone-Pellegrini et al., 2002; Khan et al., 2003). The responses to *N. brasiliensis* are made to infections with most other intestinal nematodes but different subsets of these responses are required for protection against a specific parasite. Various neutralizing antibodies and gene-deficient mice have been employed to determine the factors involved in worm expulsion and Th2 development for each of these parasites.

LR. 6.3.1 Requirements for worm expulsion

Immunocompetent BALB/c mice develop a strong Th2 response after infection with *N. brasiliensis* and expel worms 10 days after inoculation (Jacobson and Reed, 1974). Initial studies showed that *N. brasiliensis* infection induces the production of IgE antibodies but it was demonstrated that antibodies are not required for expulsion of the adult worms (Jacobson et al., 1977). Depletion of $CD4^+$ but not $CD8^+$ T cells with neutralizing antibodies completely blocked worm expulsion (Katona et al., 1988). It was suggested that this requirement for $CD4^+$ T cells is limited to the production of IL-4 because treatment of SCID and anti-CD4 treated mice with IL-4 resulted in expulsion of worms (Urban et al., 1995). However, parasites are expelled normally by IL-$4^{-/-}$ mice (Lawrence

et al., 1996). On the other hand, IL-4Rα$^{-/-}$ or STAT6$^{-/-}$ mice fail to expel the worms indicating a key role for IL-13 in *N. brasiliensis* expulsion (Barner et al., 1998; Urban et al., 1998). Furthermore, otherwise incompetent RAG2$^{-/-}$ mice expel the parasites after treatment with recombinant IL-13 and worm expulsion is suppressed in IL-13$^{-/-}$ mice despite a robust Th2 cytokine response (McKenzie et al., 1998a). IL-4 and IL-13 have similar effects due to their shared receptor components (Zurawski et al., 1993). Both, acting via STAT6, induce changes in intestinal epithelial cell function, smooth muscle hypercontractility and mucus secretion (Akiho et al., 2002; Madden et al., 2002; Zhao et al., 2003). The fact that either IL-4 or IL-13 mediates worm expulsion in anti-CD4 treated mice indicates that both these Th2 cytokines contribute to protection. Further evidence for this is that IL-4 treatment promotes worm expulsion in IL-13$^{-/-}$ mice (McKenzie et al., 1999). The greater role for IL-13 may be due to stronger activation of STAT6 by IL-13 or its greater production than IL-4 in the intestines of *N. brasiliensis* infected mice (Finkelman et al., 1999).

A recent study demonstrated that RAG$^{-/-}$ mice that normally fail to expel worms eliminated *N. brasiliensis* parasites when reconstituted with STAT6 or IL-4/IL-13-deficient CD4^{+} T cells. They concluded that worm expulsion is dependent on T cells and IL-4/IL-13 from non-T cells (Voehringer et al., 2006). Basophils and eosinophils are the major non-T cell IL-4 producers that are recruited to affected tissues during *N. brasiliensis* infection (Shinkai et al., 2002; Voehringer et al., 2004). Normal worm expulsion by eosinophil-deficient mice indicates that eosinophils are not the innate cells required for protective immunity (Yu et al., 2002). Basophils are a good candidate for the non-T cell source of IL-4/IL-13 needed for worm expulsion: they constitutively express IL-13 and IL-4 (Gessner et al., 2005), can generate more IL-4 than Th2 cells (Devouassoux et al., 1999; Mitre et al., 2004) and are recruited to tissues independent of IL-4/IL-13 signals but requiring other signals from CD4^{+} T cells (Min et al., 2004; Voehringer et al., 2004). More studies on basophils are required to assess this role for them as the IL-4/IL-13 producers required for *N. brasiliensis* protection.

To identify the IL-4/IL-13 responsive cells required for worm expulsion, mice expressing IL-4Rα on combinations of bone marrow and non-bone marrow-derived cells were examined. These studies revealed that *N. brasiliensis* expulsion requires IL-4Rα signaling on non-bone marrow-derived cells (Urban et al., 2001). It's been suggested that goblet cells are the major effector cells required for elimination of the worms. Studies in rats provided evidence for a chronological association between goblet cell hyperplasia and worm expulsion (Nawa et al., 1994). The host's immune system regulates alterations in sugar residues of goblet cell mucins which are important for the expulsion of worms (Ishikawa et al., 1993; 1994). Various studies have investigated the effects that IL-4 and IL-13 have on non-bone marrow derived cells. IL-4 and IL-13 promote smooth muscle cell contractility and induce STAT6 dependent changes in epithelial cell responses (Zhao et al., 2003). These epithelial changes all contribute to worm expulsion and include increases in responsiveness to prostaglandin E2 and intestinal permeability, as well as decreases in glucose absorption (Madden et al., 2004). A more recent study evaluated the roles of IL-4 and IL-13 on protease activated receptor 1 (PAR-1). This receptor is expressed on intestinal smooth muscle, epithelial, nerve and lamina propria cells and promotes smooth muscle contractility and gastrointestinal transit (Buresi et al., 2002; 2001; Kawabata et al., 2001). *N. brasiliensis* infection resulted in the IL-13 and STAT6 dependent up-regulation of PAR-1 and increased hypercontractility (Zhao et al., 2005). It is still not clear whether one of these cell types is more critical for worm expulsion or whether multiple cell types coordinate via IL-4 and IL-13 to expel the worms. Currently, studies are in progress using mice either expressing or deficient in IL-4Rα on one or multiple defined cell types. The observations made from these mice should shed more light on the cells involved in host protection. Preliminary studies with smooth muscle cell-specific IL-4Rα$^{-/-}$ mice indicates that IL-4/IL-13 signaling in these cells play a role in protection but are not critical. These mice were still able to expel *N. brasiliensis* worms but goblet cell hyperplasia and worm expulsion was delayed compared to wild-type mice (Horsnell et al., not published).

L.R. 6.3.2 The role of other cytokines and co-stimulatory molecules in worm expulsion

IL-5 and IL-9 are also produced by Th2 cells in response to *N. brasiliensis* infection suggesting that they may play important roles in host protection (Kopf et al., 1993; Urban et al., 1992). Although IL-5 is not essential for worm expulsion (Kopf et al., 1996a), IL-5 transgenic mice showed enhanced resistance to *N. brasiliensis* infection (Dent et al., 1999b). The overexpression of IL-5 did not influence the expulsion of worms but resulted in increased eosinophilia and a decrease in worm migration to the intestine. The additive role of this cytokine in protection to *N. brasiliensis* was shown by the use of multiple gene-deficient mice. Animals lacking IL-4, IL-13 and IL-5 showed a delay in worm expulsion compared to IL-4/IL-13$^{-/-}$ mice. A similar additive effect was observed for IL-9 with IL-4/IL-5/IL-9/IL-13-deficient mice harboring worms in intestines even after 60 days of infection. As observed for IL-5, a lack of IL-9 alone does not impair worm expulsion, but resulted in fewer goblet cells in the intestines of infected mice (Townsend et al., 2000).

The Th2 response to helminth infections, such as *Heligmosomoides polygyrus* and *Trichuris muris* infection, is dependent on B7-1/B7-2 co-stimulatory signals (Greenwald et al., 1997; Jankovic et al., 2001; Urban et al., 2000). However, mice deficient in these molecules were protected against *N. brasiliensis* infection (Liu et al., 2002). Furthermore, blockade of B7 co-stimulation with CTLA4-Ig treatment resulted in reduced IL-4 and IL-5 production but mice retained their ability to expelled *N. brasiliensis* worms (Harris et al., 1999).

Taking into account the role of Th2 cytokines in protection to *N. brasiliensis* infection, it is not surprising that Th1 cytokines exacerbate disease. Treatment of BALB/c mice with recombinant IL-12 suppresses the host protection against *N. brasiliensis* (Finkelman et al., 1994). This effect is mainly due to the induction of IFN-γ by IL-12 as the suppression is mostly but not completely reversed by anti-IFN-γ treatment.

LR. 6.3.3 Requirements for Th2 development during *N. brasiliensis* infection

A requirement for IL-4 in Th2 development was demonstrated by studies using IL-4$^{-/-}$ or STAT6$^{-/-}$ mice (Kopf et al., 1993; Takeda et al., 1996). In these gene-deficient strains the production of Th2 cytokines by CD4^{+} T cells from *N. brasiliensis* infected mice were significantly reduced but not completely absent. The IL-4$^{-/-}$ mice also showed reduced helminth induced eosinophilia which could be attributed to the reduction in IL-5 levels. Studies with IL-4Rα$^{-/-}$ mice confirmed these findings but revealed the presence of cells with IL-4 producing capacity independent of IL-4 signaling (Noben-Trauth et al., 1997). Th2 cytokine production is more severely impaired in IL-4Rα$^{-/-}$ mice than IL-4$^{-/-}$ mice suggesting that IL-13 also has a role in Th2 development possibly by down-regulating IL-12 (Barner et al., 1998). However, IL-13$^{-/-}$ mice make a strong Th2 response demonstrating that IL-13 is not required for Th2 development (McKenzie et al., 1998a). Contrary to the reduced eosinophilia in IL-4$^{-/-}$ mice, *N. brasiliensis* infected STAT6$^{-/-}$ mice develop eosinophilia comparable to the wild-type mice and a stronger mucosal mast cell response (Urban et al., 1998). IL-4/IL-13$^{-/-}$ mice also develop delayed elevated IL-5 and eosinophilia after *N. brasiliensis* infection (McKenzie et al., 1999). These data demonstrate that some effects associated with the Th2 response may still be induced by nematodes even in the absence of IL-4/IL-13 signaling.

Eosinophils and basophils infiltrate the *N. brasiliensis* infected lung independent of IL-4/IL-13 or STAT6 expression by CD4^{+} T cells (Voehringer et al., 2004). This suggests that recruitment does not require Th2 cells and that basophils or eosinophils may provide the initial IL-4 signal for the induction of Th2 differentiation. IL-4 production by basophils reaches a maximum after 10 days of infection implying that they probably do not provide the early IL-4 for Th2 induction but may prime newly recruited CD4^{+} T cells (Min et al., 2004). Eosinophils are also not essential for Th2 development because IL-5$^{-/-}$ mice still develop a Th2 response after injection of soluble nippostrongylus antigen. In the same study it was shown that Th2 induction is independent of B cells or CD8^{+} and NKT cells restricted to Class I molecules (Holland et al., 2005). A recent study demonstrated that Th2 differentiation occurs independently of IL-4/IL-13 from non-T

cells but that these cytokines were required for recruitment of Th2 cells to affected tissues (Voehringer et al., 2006).

LR. 6.3.4 Antibody production in response to *N. brasiliensis* infection

Infection with *N. brasiliensis* induces a striking increase in the production of IgG1 and IgE antibodies to both parasite and non-parasite antigens (Urban, 1982). High affinity IgE receptors are found on mast cells and basophils, and surface bound IgE antibodies can be cross-linked by antigen resulting in degranulation (Conrad et al., 1975; Ishizaka and Ishizaka, 1978; 1984). The mast cells release mediators that induce eosinophilia and intestinal hypermotility that contribute to damage and expulsion of worms (Askenase et al., 1980; Kojima et al., 1985a; 1985b). Eosinophils also express receptors for IgE and IgG and their binding to worms coated with antibodies increases the release of their contents onto the surface of the worms (Kojima et al., 1985a; McLaren et al., 1977). Macrophages express receptors for IgG antibodies and can be triggered by the binding and crosslinking of these antibodies resulting in cytotoxicity and the formation of multinucleate giant cells in the lungs of infected mice (Egwang et al., 1985; Gauldie et al., 1983). Early $CD4^+$ T cell depletion studies during *N. brasiliensis* infection demonstrated the requirement of these T helper cells for IgE production (Katona et al., 1988). Treatment of infected mice with anti-IL-4 antibodies inhibits IgE production by 99% (Finkelman et al., 1988). It was shown that IL-4 is required to generate and sustain established IgE responses during *N. brasiliensis* infection. In support of this mice lacking IL-4, IL-4 and IL-13, STAT6 or IL-4Rα make no detectable levels of IgE (Kuhn et al., 1991; McKenzie et al., 1999; Urban et al., 1998). More specifically, IgE production is dependent on IL-4Rα on bone-marrow derived cells and the production of IL-4/IL-13 by $CD4^+$ T cells (Urban et al., 2001; Voehringer et al., 2006). It was recently suggested that IL-13 induces IgE production in *N. brasiliensis* infected mice. Elevated IgE levels were observed in GATA3-Tg mice concurrent with elevated IL-13 but no increased IL-4 (Ozawa et al., 2005). However, no direct evidence has been provided for this role of IL-13 in IgE production.

The production of IgG1 and IgG2 occurs by a different mechanism. IL-$4^{-/-}$ and IL-4/IL-$13^{-/-}$ *N. brasiliensis* infected mice produce lower levels of IgG1 but higher IgG2 compared to wild type mice. On the other hand $STAT6^{-/-}$ mice make substantial IgG1 and IgG2 responses while IL-$4R\alpha^{-/-}$ mice make significant IgG2 but no detectable IgG1 antibodies. This indicates that IL-4-induced IgG1 production is dependent on IL-4Rα but through a STAT6 independent pathway. Furthermore, STAT6 signaling inhibits IgG2 production (Urban et al., 1998).

Aims of the project

- To investigate the lymphocyte-specific expression of hIL-4Rα in hIL-4Rα Tg/ mIL-4Rα$^{-/-}$ mice and test the species-specificity and inducibility of the chimeric receptor *in vitro*
- To study the *in vivo* characteristics of the hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mouse model during infection with *L. major*
- To study the *in vivo* characteristics of the hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mouse model during infection with *N. brasiliensis*
- To study the effect of anti-mIL-4 treatment in hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice during infection with *N. brasiliensis*
- To study the effect of IL-4 and IL-13 deletion in the hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mouse model during infection with *L. major* and *N. brasiliensis*

Materials and Methods

Materials and Methods

MM. 1 Mice

All experimental animals were maintained under specific pathogen-free (SPF) conditions in the animal facility of the University of Cape Town, South Africa. Mice were housed in filter top cages under light and temperature controlled conditions and provided with water and food *ad libitum*. Male and female mice between the ages of 8-12 weeks were used for all experiments. Genetically modified mice were generated on a pure BALB/c background and backcrossed for more than 10 generations. Lymphocyte-specific hIL-4RαTg/mIL-4Rα$^{-/-}$ mice were obtained from Dr. Masato Kubo of the Research Institute for Biological Sciences, Japan (Seki et al., 2004). BALB/c, C57BL/6, mIL-4Rα$^{-/-}$ and hIL-4RαTg/mIL-4Rα$^{-/-}$/ mIL-4$^{-/-}$/ mIL-13$^{-/-}$ mice were obtained from Prof Frank Brombacher, Institute of Infectious Disease and Molecular Medicine, University of Cape Town (Matthews et al., 2000; Mohrs et al., 1999). All experiments performed were in accordance with the guidelines of the Animal Research Ethics Board of the University of Cape Town, South Africa.

MM. 2 Genotyping of genetically modified mice

The genotypes of all genetically modified mice were confirmed by the polymerase chain reaction (PCR) of DNA obtained from tail biopsies. PCR reactions were performed under the following conditions: 94°C/1 minute; 40 cycles of 94°C/30 seconds, 57°C/30 seconds and 72°C/1 minute; 72°C/5 minutes and samples held at 4°C on an MJ thermocycler (Biozym, Hessissch Olderdorf, Germany). Primers were designed by Anita Schwegmann and PCR analysis performed by Erica Smit and Wendy Dryding, Institute of Infectious Disease and Molecular Medicine, University of Cape Town. The primers used for reactions are outlined in Table 1.

Table 1. PCR primer sequences for genotyping of mice

Primers for PCR screening of IL-4Rα deficient mice	**Expected product size**
Wild-type allele Exon 7 forward: 5' GTACAGCGCACATTGTTTTT 3' Exon 8 reverse: 5' CTGGGCGCACTGACCCATCT 3'	600 bp
Knockout allele IL-4Rα KO P1: 5' GGCTGCTGACCTGGAATAACC 3' IL-4Rα KO P2: 5' CCTTTGAGAACTGCGGGCT 3'	471 bp
Primers for PCR screening of hIL-4Rα transgene	
P1: 5' GCCGCCATGGGGTGGCTTTGCTCT 3' P2: 5' ATCTGCCGGGTCGTTTTCACTCCAA 3'	522 bp
Primers for PCR screening of IL-4/IL-13 deficient mice	
P5: 5' CCTGGATTCCCTGACCAACATC 3' P6: 5' GGCCTTGCGGTTACAGAGGCC 3' P7: 5' ACCACACTGCTCGACATTGGGTG 3'	80 bp (wild-type) 100 bp (IL-4/IL-13$^{-/-}$)

MM. 3 Flow cytometry

Naïve mice (3 mice per group) were sacrificed by a high anaesthetic dose of CO_2 and the skins removed carefully to expose the peritoneum. A 25G needle (B.Braun, Melsungen, Germany) was used to inject 8 ml of PBS supplemented with 10% Foetal Calf Serum (FCS) and the peritoneum was massaged. The peritoneal lavage fluid was harvested using a syringe and an 18G needle (B.Braun, Melsungen, Germany) and kept on ice. Lymph nodes and spleens were removed aseptically and placed in ice cold FACS buffer. Single cell suspensions of the lymph node cells and splenocytes were prepared and filtered through 70 µm nylon cell strainers (Becton-Dickinson, New Jersey). The peritoneal lavage fluid and single cell suspensions were centrifuged at 1200rpm at 4°C for 10 minutes and resuspended in 2ml FACS buffer. Aliquots of cells (10µl) were diluted in Trypan Blue and counted on a Neubauer haemocytometer to determine the cell concentrations of viable cells. For each staining $1x10^5$ cells were blocked for 15 minutes

in 100ul of FACS buffer containing 1% heat inactivated rat serum and 4ug/ml 2.4G2 (anti-FcγRII and -III). The cells were washed in 200ul FACS buffer and resuspended in 100ul FACS buffer containing one of the following antibodies: rat anti-mouse IL-4Rα mAb (M-1, Amgen Inc. Thousand Oaks, CA; gift from F. Finkelman, Cincinatti, OH), biotinylated mouse anti-human IL-4Rα mAb (hIL-4R-M57, BD Pharmingen, San Diego, CA) or biotinylated mouse IgG1 isotype control (MOPC-21, BD Pharmingen, San Diego, CA). After staining the cells for 30 minutes in the dark at 4°C, cells were washed and resuspended in 100µl FACS buffer containing the following detecting antibodies: FITC labeled goat anti-rat F(ab')2 antibody fragments (Southern Biotechnology, Birmingham, AL) to detect anti-mouse IL-4Rα mAb and streptavidin-APC (BD Pharmingen, San Diego, CA) to detect biotinylated anti-human IL-4Rα mAb and mouse IgG1 isotype control. Cells were stained for 30 minutes in the dark at 4°C, washed and stained with antibodies to cell surface markers. Anti-mouse CD3-FITC, CD4-PE, CD8-PE, B220-PE, CD19-PE, pan NK-PE (DX5), MHC class II-FITC, GR-1-FITC, CD11c-PE, CD11b-PE (BD Pharmingen, San Diego, CA) and F4/80-PE (Serotec, Oxford, UK) mAbs were used to identify different cell subsets. Cells were incubated for 30 minutes in the dark at 4°C and washed. Non-viable cells were stained by 7-AAD or propidium iodide (Sigma, St. Louis, MO) in 500µl FACS buffer and excluded from analysis.

For FACS analysis of purified $CD4^+$ T cells, cells were blocked as described above and stained with CD4-FITC, CD8-PE or CD19-PE. Cells were washed and resuspended in 500µl FACS buffer.

Acquisition was performed using FACSCaliber and cells were analyzed using Cellquest (Becton-Dickinson, Ferndale, South Africa). 100 000 cells were acquired for analysis of lymphocyte populations while 200 000 cells were acquired for analysis of non-lymphocyte populations.

MM. 4 Cell proliferation assay

Naïve mice were sacrificed by cervical dislocation. Lymph nodes were removed aseptically and placed in ice cold Iscoves Modified Dulbeccos Medium (IMDM) supplemented with 10% FCS (Life Technologies, Gaithersburg, MI). Single cell

suspensions were prepared and filtered through 70 µm nylon cell strainers (Becton-Dickinson, New Jersey). Cells contaminated with red blood cells were resuspended in 5ml Red cell lysis buffer and underlayed carefully with 2ml heat inactivated FCS. The cells were centrifuged at 1200rpm at 4°C for 10 minutes and resuspended in 2ml IMDM supplemented with 10% FCS (IMDM/10% FCS). Aliquots of cells (10µl) were diluted in Trypan Blue and counted on a Neubauer haemocytometer. The cell concentrations of non-stained viable cells were determined as Trypan Blue stains dead cells. Cell volumes were adjusted to attain cell concentrations of $5x10^5$ cells /ml. PMA (Sigma) was added to cells at 10ng/ml for a final concentration of 5ng/ml. Triplicates of 100µl from each group of cells were plated out in round bottom 96 well tissue culture plates (Nalge Nunc International, Naperville, IL, USA). Working dilutions of stimulants were prepared as follows in IMDM/10%FCS: 2000U/ml rIL-2 (BD Pharmingen), 2000U/ml rIL-4 (BD Pharmingen), 2000ng/ml and 200ng/ml rhIL-4 (gift from Dr. Masato Kubo), 400ng/ml rIL-13 (R&D Systems, Minneapolis, MN). A series of eleven 2-fold dilutions were made for each stimulant in IMDM/10%FCS. Each dilution or medium alone was added to a row of cells at 100ul per well and the cells were cultured for 30 hours at 37°C in a humidified 5% CO_2 incubator. [^{3}H]-thymidine (AEC Amersham, Uppsala, Sweden) was added in 20µl at 1µCi per well and cultured for another 18 hours. The cells were harvested onto filter paper using a semiautomated sample harvester. The paper was dried, placed in scintillation fluid and samples counted in a β liquid scintillation counter to determine the amount of thymidine incorporated. Results were referred to as counts per minute (cpm) for each stimulant dilution.

MM. 5 T cell differentiation assay

Naïve mice were sacrificed by cervical dislocation. Lymph nodes were removed aseptically and placed in ice cold RPMI 1640 supplemented with 10% FCS (Gibco Life Technologies, Paisley, UK). Single cell suspensions were prepared and filtered through 70 µm nylon cell strainers (Becton-Dickinson, New Jersey). Cells contaminated with red blood cells were resuspended in 5ml Red cell lysis buffer and underlayed carefully with 2ml heat inactivated FCS. The cells were centrifuged at 1200rpm at 4°C for 10 minutes

and resuspended in 2ml RPMI 1640. Aliquots of cells (10µl) were diluted in Trypan Blue and counted on a Neubauer haemocytometer to determine the cell concentration of viable cells. $CD4^+$ T cells were purified by magnetic CD4 microbeads using the MiniMACS system as instructed by the manufacturer (Miltenyi Biotec, Auburn, CA). The purity of the cells was ~97% as determined by FACScan analysis. The $CD4^+$ T cells were plated at $2x10^5$ cells per well in complete RPMI 1640 medium supplemented with 10% FCS (Gibco Life Technologies, Paisley, UK), 50µM B2-mercaptoethanol (Sigma, St. Louis, MO), 1mM sodium pyruvate, non-essential amino acids (Gibco Life Technologies, Paisley, UK) in flat bottom 96 well plates (Nalge Nunc International, Naperville, IL, USA). The wells were previously coated overnight at 4°C with 10µg/ml anti-CD3 (BD Pharmingen) and 5µg/ml anti-CD28 (BD Pharmingen) in sterile filtered PBS. For Th1 polarization cells were incubated with 5ng/ml IL-12 (BD Pharmingen) and 50µg/ml anti-IL-4 (11B11). Th2 polarization was induced by 50µg/ml anti-IFN-γ (R4-6A2, BD Pharmingen) and either 50ng/ml mouse IL-4 (BD Pharmingen), 100ng/ml mouse IL-13 (R&D Systems, Minneapolis, MN) or 100ng/ml human IL-4 (gift from Dr. Masato Kubo). Medium alone was added for the neutral condition. The cells were cultured in a final volume of 200µl in a 37°C, 5% CO2 incubator for 72 hours. After culturing, cells were transferred to a round bottom 96 well plate (Nalge Nunc International, Naperville, IL, USA) and washed three times in RPMI 1640 supplemented with 10% FCS. Cells were resuspended in fresh complete medium containing appropriate cytokines and antibodies as used before, supplemented with IL-2 (20U/ml, BD Pharmingen) and cultured for another 48 hours. The cells were washed with fresh medium and replated in triplicate at $2x10^5$ cells per well in a flat bottom 96 well plate precoated overnight with 20µg/ml anti-CD3. Supernatants were harvested after 48 hours of stimulation and frozen at -70°C until cytokine analysis using ELISA.

MM. 6.1 Peritoneal macrophages

Naïve mice were injected intraperitoneal with 500µl 3% (w/v) Brewer thioglycollate (Difco Laboratories, USA). After 5 days mice were sacrificed and the skins removed carefully to expose the peritoneum. A 25G needle (B.Braun, Melsungen, Germany) was

used to inject 8 ml of IMDM supplemented with 10% FCS and the peritoneum was massaged. The peritoneal lavage fluid was harvested using a syringe and an 18G needle (B.Braun, Melsungen, Germany). The samples were kept on ice throughout and centrifuged at 1200rpm at 4°C for 8 minutes. The exudate cells were resuspended in 5ml IMDM/10% FCS and counted using a Neubauer haemocytometer. Cells were plated in triplicate at $4x10^6$ cells per ml in a volume of 100µl in flat bottom 96 well plates (Nalge Nunc International, Naperville, IL, USA) and incubated for 4 hrs at 37°C in a 5% CO_2 incubator. The non-adherent cells were removed and the wells washed 2 times with 200µl IMDM/10% FCS. The adherent macrophages were incubated for 16 hrs with either 100µl of medium, 100µl of rIL-4 (1000U/ml, BD Pharmingen) or 100µl of rIL-13 (200ng/ml, R&D Systems). The cells were subsequently stimulated with 100µl of medium or 100µl of IFN-γ (200U/ml, BD Pharmingen) and LPS (20ng/ml, Sigma). After culturing cells for 48 hrs at 37°C in a 5% CO2 incubator, the plates were centrifuged at 1200rpm for 10 minutes. Supernatant were removed and frozen at -80°C for NO determination. The remaining cells were washed with PBS and resuspended in 50µl of 0.1% Triton X-100. Plates were stirred on a shaker for 30 minutes at room temperature, mixed well and stored at -80°C for measurement of arginase activity.

MM. 6.2 Nitric oxide determination

The levels of nitric oxide (NO) in supernatants from peritoneal macrophages were measured by the Griess reaction (Oswald et al., 1992). Briefly, for a standard curve a series of six 3-fold dilutions were made from a 2mM NO_2 solution in IMDM and 50µl of each dilution added to a microtiter 96 well plate (Nalge Nunc International, Naperville, IL, USA). The samples were diluted 3-fold in IMDM and 50µl added in triplicate to the plate. To each well, 25µl of Solution 1 (1% sulfanilamide in 2.5% phosphoric acid) was added causing the IMDM to turn yellow. An equal volume (25µl) of Solution 2 (0.1%napthyl-ethylene-diamene in 2% phosphoric acid) was subsequently added to the wells and the presence of NO indicated by a purple-pink colour. The absorbances of samples were measured at 540nm and a reference at 690nm using a Versamax spectrophotometer (Molecular Devices, Sunnyvale, CA, USA).

MM. 6.3 Measurement of arginase activity

Arginase I activity in macrophages was measured as previously described (Corraliza et al., 1994). After lysis of peritoneal macrophages, 50µl of 10mM $MnCl_2$, 50mM Tris-HCl, pH 7.5 were added and the enzyme was activated for 10 minutes at 55°C. Arginine hydrolysis was carried out in Eppendorf tubes by the addition of 25µl of 0.5M arginine, pH 9.7 to 25µl of the activated cell lysates. The samples were incubated at 37°C for 60 minutes and the reaction stopped by the addition of 400µl of an acid mixture consisting of H_2SO_4, H_3PO_4 and H_2O (1:3:7). Aliquots of 25µl of freshly made 9% ISPF dissolved in 100% EtOH was added to each sample and heated at 100°C for 45 minutes. After leaving samples for 10 minutes in the dark, 200µl of each was transferred to a 96 well microtiter plate (Nalge Nunc International, Naperville, IL, USA). A standard curve was prepared by making 3-fold dilutions of 1mg/ml of urea in H_2O. The standards were treated as the samples from the addition of the acid mixture. The absorbances of samples and standards were measured at 540nm in the microtiter plate using a Versamax spectrophotometer (Molecular Devices, Sunnyvale, CA, USA). The urea concentration measured for each sample was used as a measure of arginase activity.

MM. 7 Cytokine ELISA

ELISA antibodies and recombinant proteins were obtained from BD Pharmingen or R&D Systems (ELISA reagents, Table 1). 96 well Maxisorp microtiter plates (Nalge Nunc International, Naperville, IL, USA) were coated overnight at 4°C with 50µl capturing antibody diluted in PBS. The coated plates were washed once and incubated overnight at 4°C with 200µl Blocking buffer. Three-fold dilutions of the samples were prepared in dilution buffer ranging from an undiluted sample to a 1/9 dilution while recombinant standards were diluted 3-fold from either 100ng/ml or 250ng/ml. After blocking, the plates were washed 4 times and 50µl of the diluted standards and samples added. The plates were incubated overnight at 4°C and washed 4 times. For each cytokine, 50µl of the corresponding biotinylated anti-mouse detecting antibody was added and the plates incubated at 37°C for 3 hours. The plates were washed 4 times and 50µl of streptavidin-

alkaline phosphatase (1/1000 dilution) or streptavidin horse radish peroxidase (1/5000 dilution, for IL-13) added to the wells. After incubating at 37°C for 1 hour, the plates were washed 4 times and 50µl of substrate was added. TMB was added to detect IL-13 whereas p-nitrophenyl phosphate (1mg/ml) was used for the other cytokines. The absorbances were read between 405nm and 495nm on a Versamax microplate spectrophotometer (Molecular Devices, Sunnydale, CA, USA).

MM. 8 *Leishmania major* methods

MM. 8.1 Culturing of *L.major*

L. major LV39 (MRHO/Sv/59/P-strain) and (MHOM/IL/81/FEBNI) (Mattner et al., 1996) strains were maintained by continuous passage in BALB/c mice. Anesthetized mice were infected subcutaneously into the left hind footpad with $2x10^6$ stationary phase metacyclic *L. major* promastigotes in a volume of 50 µl of Hank's Balanced Salt Solution (HBSS) (Gibco, Life Technologies). Parasites were isolated from skin lesions of infected mice by incubating the infected footpads and draining popliteal lymph nodes in Schneider's insect medium (Sigma) supplemented with 20% FCS for 1 week at room temperature. After 3-4 days motile parasites became visible and were counted daily to assess their growth curve. Stationary phase parasites were used for infection of experimental mice.

MM. 8.2 Preparation of *L. major*

The culture medium containing stationary phase *L. major* promastigotes was centrifuged at 2500 rpm for 10 minutes to pellet the parasites. The parasite pellets were washed twice in sterile HBSS (Gibco, Life Technologies), counted and finally resuspended in HBSS at $4x10^7$ parasites per ml.

MM. 8.3 Infection of mice

The infection of mice was carried out in an Animal Biosafety Level 2 Laboratory. Mice were anaesthetized by intraperitoneal injection of 200µl anaesthetic consisting of 100mg/ml Anaket-V (Centaur Labs, RSA) and 2% Rompun (Bayer, Germany) in PBS. The mice were injected subcutaneously into the left hind footpad with $2x10^6$ stationary phase metacyclic *L. major* parasites in a volume of 50µl.

MM. 8.4 Footpad measurements

Disease progression was monitored by measuring the thickness of both infected and uninfected footpads weekly using a Mitutoyo micrometer caliper (Japan). The footpad swelling (mm) was determined by subtracting the footpad size (mm) of the uninfected right footpad from the infected left footpad. These measurements were carried out from week 1 post infection until the mice were sacrificed.

MM. 8.5 Quantification of parasite burden

The parasite limiting dilution assay was used to determine the number of viable *L. major* parasites in infected organs. The experimental mice were sacrificed at the desired time points and the infected draining popliteal lymph nodes (pLN) and footpads harvested from individual mice. Single cell suspensions were prepared from the pLN and the cells were resuspended in 6.4 ml of Schneider's insect medium (Sigma) supplemented with 20% FCS. The individual footpads were weighed and homogenized in 6.4 ml Schneider's insect medium using an X620 CAT homogenizer (Staufen, Germany). An aliquot of 200µl of the cell suspensions from each organ was transferred to flat bottom 96 well plates and 24, 2-fold serial dilutions made in Schneider's insect medium supplemented with 20% FCS. The plates were incubated in a humidified chamber at 26 – 28°C for ~7 days after which each well was microscopically examined for parasite growth. Minimal estimates of the number of viable *L. major* parasites per lymph node or per gram of

footpad were calculated from the single hit Poisson model equation using the χ^2 minimization method as described previously (Kropf et al., 2002).

MM. 8.6 Preparation of *L. major* soluble antigen

Stationary phase *L. major* parasites were prepared as described in MM.7.1 Culturing of *L. major*. The culture medium was washed three times by centrifuging at 2500 rpm for 10 minutes and resuspending the pellet in 10ml PBS. After the last wash, the *L. major* pellet was resuspended in 2ml PBS and counted to determine the parasite concentration. The suspension was rapidly frozen in liquid nitrogen and thawed at 37°C ten times. After ensuring that the suspension contained no motile parasites, the freeze-thawed (F/T) antigen preparation was frozen at -80°C until required.

MM. 8.7 Restimulation of lymph node cells

Mice were sacrificed at the desired time points by cervical dislocation and the draining popliteal lymph nodes collected in ice cold IMDM supplemented with 10% FCS (IMDM/10%FCS). Single cell suspensions were prepared by straining the cells through metal sieves. Contaminating red blood cells were removed by resuspending cells in 5 ml Red cell lysis buffer and underlaying carefully with 2ml heat-inactivated FCS. The cells were centrifuged at 1200rpm at 4°C for 10 minutes and resuspended in 2ml IMDM/10%FCS. Aliquots of cells (10µl) were diluted in Trypan Blue and counted on a Neubauer haemocytometer to determine the cell concentration of non-stained viable cells. The cell volumes were adjusted to cell concentrations of $1x10^6$ cells/ml in IMDM/10%FCS and triplicates of 500µl of cells were plated in flat bottom 48 well plates (Costar). For anti-CD3 stimulation, wells were precoated overnight at 4°C with 20µg/ml anti-CD3 (clone 145-2C11) in PBS and the antibodies removed before adding cells. For a control PBS was used to coat wells. The plates were incubated at 37°C for 72 hours in a 5% CO_2 incubator. Supernatant were removed and aliquots of 100µl frozen at -80°C for cytokine analysis using ELISA.

MM. 8.8 *Leishmania*-specific antibody ELISA

The tails of experimental mice were heated under an infrared light and a small incision was made across the lateral tail vein. Blood was collected in microtainer serum separator tubes and kept on ice. The samples were centrifuged at 4500rpm for 15 minutes and stored at -20°C until antibody analysis using ELISA. Blood samples were collected at indicated times and the antibodies of all time points measured simultaneously.

Leishmania-specific IgG1, IgG2a and IgG2b and total IgE was measured to determine the antibody responses of infected mice. For the IgG antibodies, 96 well Maxisorp microtiter plates (Nalge Nunc International, Naperville, IL, USA) were coated overnight at 4°C with 10ug/ml of soluble *Leishmania* antigen in PBS. For total IgE determination the plates were coated with 10ug/ml coating IgE antibody (clone 84.1C) in PBS. The coated plates were washed 3 times with Washing buffer and incubated overnight at 4°C with 200µl Blocking buffer. After blocking, the plates were washed 4 times and 50µl of the samples were added in 5-fold dilutions in Dilution buffer ranging from 1/10 to $1/10^7$. For the IgE plates the sample dilutions were diluted 3-fold starting at 1/10 and IgE standards (BD Pharmingen) were added in 3-fold dilutions ranging from 1µg/ml. The plates were incubated overnight at 4°C and washed 4 times. For each isotype a 1:1000 dilution of anti-mouse isotype-specific antibody labeled with polyclonal alkaline phosphatase was prepared in Dilution buffer and 50µl added to the plates. After incubating at 37°C for 3 hours the plates were washed 4 times and 50µl of p-nitrophenyl phosphate (1mg/ml) (Boehringer Manheim, Germany) added. The absorbances of samples were measured between 405nm and 494nm using a Versamax microplate spectrophotometer (Molecular Devices, Sunnydale, CA, USA) and plotted as OD against dilution.

MM. 9 *Nippostrongylus brasiliensis* methods

MM. 9.1 Culturing of *N. brasiliensis*

N. brasiliensis worms were passaged routinely in Witstar rats. Live L3 larvae were prepared in 0.9% NaCl at 10 000 worms per ml. Six to eight rats were injected subcutaneously in the flank of the leg with 500µl of the worm solution using 20G needles (Braun, Melsungen, Germany). After 5 days of infection, rats were transferred to gridded cages for fecal collection and the bottom of the cages lined with damp paper to keep fecal pellets moist. Fecal pellets from infected rats were collected daily from day 6 to day 12 post infection and soaked each day in MilliQ water containing Fungizone (Gibco Life Technologies). Pellets were mixed with an equal volume of granular charcoal (Sigma) to form a paste-like consistency and spread onto prepared culture dishes. A culture dish consisted of a large petri dish containing a moist filter paper and a wet piece of square gauze underneath it to act as reservoir. The dishes were incubated in humidified containers at 25°C for at least 5 days to allow ex-sheathed L3 larvae to migrate to the edge of the moist filter paper. The outer layers of filter paper containing the worms were removed and transferred to fresh culture dishes for further incubation until worms were used for infection.

MM. 9.2 Preparation of worms and infection

Pieces of filter paper containing fresh ex-sheathed L3 larvae were placed in water for 10 minutes to dislodge the worms. The worms were counted in a gridded petri dish and a solution of 3750 worms per ml prepared in 0.9% NaCl. The solution was continuously mixed to prevent worms from settling at the bottom. The infection of mice was carried out in an Animal Biosafety Level 2 Laboratory. Mice were injected subcutaneously with 750 worms in 200µl NaCl using a 20G needle (B.Braun, Melsungen, Germany).

MM. 9.3 Fecal collection and egg counts

Infected mice were separated into individual cages containing no bedding on day 5 post infection. From day 6 to day 15 about 20 fecal pellets were collected from each mouse and weighed. Cages were cleaned every day for the collection of fresh feces on the following day. The counting of eggs was carried out using a McMaster counting chamber (Advanced Equine Product, USA). The pellets were soaked in 5ml MilliQ water overnight to emulsify the feces and 10ml of saturated NaCl added. The emulsion was mixed and centrifuged at 1300rpm for 5 minutes. An aliquot of 2ml was taken from the top of each sample and transferred to clean tubes to ensure that all eggs were included. The aliquots were mixed thoroughly and a sample from each mixture added to both chambers of the McMaster slide. After waiting 30 seconds the eggs were counted under both of the etched areas on the slide. The volume under the etched area of each chamber was 0.15ml, thus a total volume of 0.3ml was examined per sample. The total number of eggs in the two chambers was divided by 0.3, multiplied by 2, and finally divided by the weight of each sample to obtain the eggs per gram feces.

MM. 9.4 Intestinal worm counts

Infected mice were sacrificed on day 7 or day 14 post infection using cervical dislocation. The intestines from individual mice were removed aseptically and placed in 50ml of 0.65% NaCl. The intestines were split longitudinally, placed on gauze and the gauze bags submerged in the same 0.65% NaCl. The tubes were incubated at 37°C for 4 hours to allow worms to migrate out. The fluid was transferred to gridded petri dishes and the worms counted.

MM. 9.5 Restimulation of mesenteric lymph node cells

After sacrificing mice the mesenteric lymph nodes were removed under sterile conditions and pooled for each group in IMDM supplemented with 10% FCS (IMDM/10%FCS). Single cell suspensions were prepared and filtered through 70µm nylon cell strainers. The

cells were centrifuged at 1200rpm for 10 minutes and resuspended in 2ml IMDM/10%FCS. Aliquots of cells (10µl) were diluted in Trypan Blue and viable cells counted on a Neubauer haemocytometer. $CD4^+$ T cells were purified by magnetic CD4 microbeads using the MiniMACS system as instructed by the manufacturer (Miltenyi Biotec, Auburn, CA). FACScan analysis was used to confirm that the purity of the cells was above 95%. Purified $CD4^+$ T cells were resuspended in IMDM/10% FCS at a concentration of $1x10^6$ cells/ml and triplicates of 500µl of cells were plated in flat bottom 48 well plates (Costar). The plates were previously coated overnight at 4°C with 200µl of 20µg/ml anti-CD3 (clone 145-2C11) in PBS or PBS alone as control. The cells were cultured at 37°C for 72 hours in a 5% CO_2 incubator. Supernatant were removed and stored at -80°C for cytokine analysis using ELISA.

MM. 9.6 Anti-mIL-4 treatment

Anti-mouse IL-4 antibody (11B11, rat IgG1) was diluted in PBS to 1mg/ml. Designated mice were injected intraperitoneally with 1mg of anti-mIL-4 at 1 day before infection and 7 days post infection.

MM. 9.7 Analysis of antibody responses

The tails of experimental mice were heated under an infrared light and a small incision was made across the lateral tail vein. Blood was collected in microtainer serum separator tubes and kept on ice. The samples were centrifuged at 4500rpm for 15 minutes and stored at -20°C until antibody analysis using ELISA. Blood samples were collected from naïve mice and at day 7 and day 14 p.i. from infected mice. The antibodies of all time points were measured simultaneously.

Total IgG1, IgG2a, IgG2b and IgE were measured to determine the antibody responses in *N. brasiliensis*-infected mice. The goat anti-mouse antibodies (Southern Biotechnology Associates, Inc, Brimingham, AL, USA) for each isotype were diluted 1:1000 in PBS and added at 50µl per well to 96 well Maxisorp plates (Nalge Nunc International, Naperville,

IL, USA). The plates were incubated overnight at 4°C, washed once and 200µl Blocking buffer added. After blocking overnight at 4°C, the plates were washed 4 times and 50ul of diluted samples and standards added. Samples were diluted 3-fold in Dilution buffer ranging from 1/100 for IgG isotypes (Southern Biotechnology Associates, Inc, Brimingham, AL, USA) and diluted 10-fold from 1/10 for IgE (BD Paharmingen). The standards were diluted 3-fold from 10ug/ml for the IgG isotypes and 1ug/ml for IgE. The plates were incubated overnight at 4°C and washed 4 times. A 1:1000 dilution of goat anti-mouse antibody labeled with alkaline phosphatase was prepared for each isotype (Biotechnology Associates, Inc, Brimingham, AL, USA) and 50µl added to the wells. The plates were incubated at 37°C for 3 hours, washed 4 times and 50µl of p-nitrophenyl phosphate (1mg/ml) diluted in Substrate buffer added. The absorbances of samples were measured between 405nm and 495nm on a Versamax microplate reader (Molecular Devices, Sunnydale, CA, USA).

MM. 10 Statistical analyses

Values are given as mean ± SEM and significant differences were determined using the unpaired two-tailed Student's t-test (Prism software). A ρ value of < 0.05 was considered significant.

Results

Results

1.1 Generation of lymphocyte-specific hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice

IL-4 plays a key role in the differentiation of naïve CD4^{+} T cells into Th2 cells *in vitro* (Swain et al., 1990; Murphy and Reiner, 2002). Upon activation of the TCR, IL-4 acts via the STAT6 signaling pathway to up-regulate the transcription factor GATA3, which in turn is essential for the maintenance of the Th2 phenotype (Zheng and Flavell 1997; Zhang et al., 1997). To investigate the individual roles of IL-4 signaling and TCR activation during Th2 lineage commitment, transgenic mice expressing hIL-4Rα under the control of a lymphocyte-specific intronic enhancer of the IgH chain (Eμ) locus promoter were recently established (Seki et al., 2004). The hIL-4Rα subunit may interact with mouse γc to form a functional chimeric type I IL-4R that is responsive to hIL-4 (Idzerda et al., 1990; Andrews et al., 2001). On the other hand, mIL-4 is unable to signal through the chimeric receptor because IL-4 binds to its receptor in a species-specific manner (Mosmann et al., 1987). No chimeric type II IL-4R is formed due to both the lack of IL-13Rα1 in mouse lymphocytes, and the inability of hIL-4Rα to associate with mIL-13Rα1 (Zurawski and de Vries, 1994; Gauchat et al., 1997; Andrews et al., 2001). The hIL-4Rα Tg mice, backcrossed to a BALB/c background for more than 10 generation, were intercrossed with mIL-4Rα$^{-/-}$ mice (Mohrs et al., 1999) to generate lymphocyte specific hIL-4Rα Tg/mIL-4Rα$^{-/-}$ (transgenic) mice (Fig. 1). The novel mice thus express the chimeric IL-4R in lymphocytes while being completely deficient for mIL-4R, allowing for the control of IL-4 signaling in lymphocytes independent of endogenous mIL-4 signaling. This novel mouse model provides us with an inducible IL-4 system to study the time dependent role of lymphocyte-specific IL-4 signaling in Th2 development and disease outcome.

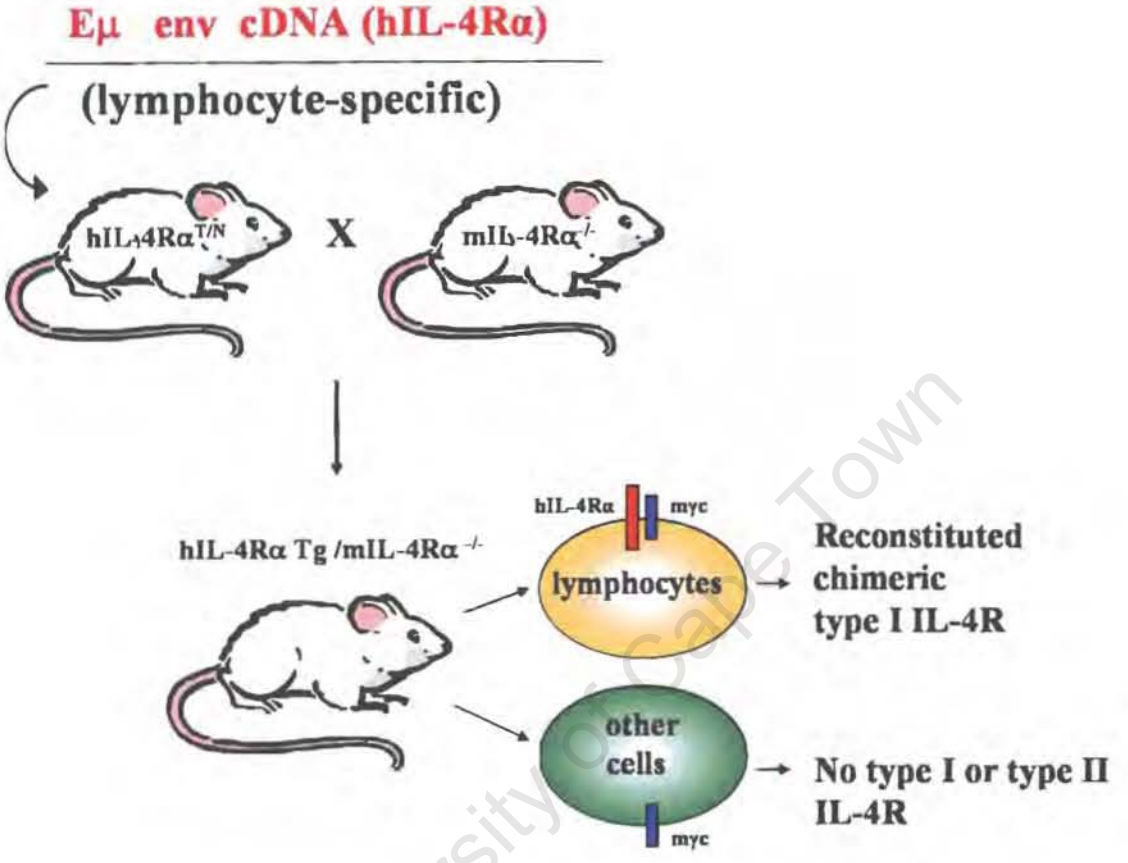

Figure 1. Generation of lymphocyte-specific hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice

Transgenic BALB/c mice expressing lymphocyte-specific hIL-4Rα were intercrossed with mIL-4Rα$^{-/-}$ mice to generate lymphocyte-specific hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice. Lymphocytes from transgenic mice express chimeric type I IL-4R while other cells lack type I and type II IL-4R.

1.2 Genotyping

To discern between hIL-4Rα Tg/mIL-4Rα$^{-/-}$ and the littermate control mIL-4Rα$^{-/-}$, all mice from these strains were genotyped using PCR analysis (Fig. 2). The presence of the mIL-4Rα gene was determined by using two sets of primers, one that amplifies the wild-type allele and another that amplifies the "knockout allele". In a mIL-4Rα$^{-/-}$ mouse exons 7, 8 and 9 of the mIL-4Rα gene are deleted. The forward primer to the wild-type allele binds in exon 7 and the reverse primer in exon 8 resulting in the amplification of a 600bp band from DNA of a wild-type mouse containing these exons. On the other hand, the forward primer of the knockout allele binds in exon 6 and the reverse primer in exon 10. In a PCR reaction using DNA from mIL-4Rα$^{-/-}$ mice, these primers would amplify a 471bp band, but the 3000bp section between exon 6 and 10 in a wild-type mouse is too big to amplify. Therefore, whereas the PCR products from BALB/c mice contain the 600bp wild-type band, PCR products from hIL-4Rα Tg/ mIL-4Rα$^{-/-}$ and mIL-4Rα$^{-/-}$ mice do not contain this band. On the other hand, the 471bp knockout allele was amplified from DNA of hIL-4Rα Tg/mIL-4Rα$^{-/-}$ and mIL-4Rα$^{-/-}$ mice but not from BALB/c mice (Fig. 2A). The presence of the hIL-4Rα transgene was assessed by amplifying a 522bp segment from the transgene (Fig. 2B). The PCR products from hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice were positive for this 522bp band while this segment was not amplified in DNA from BALB/c and mIL-4Rα$^{-/-}$ mice. The negative control in both reactions had no amplification products.

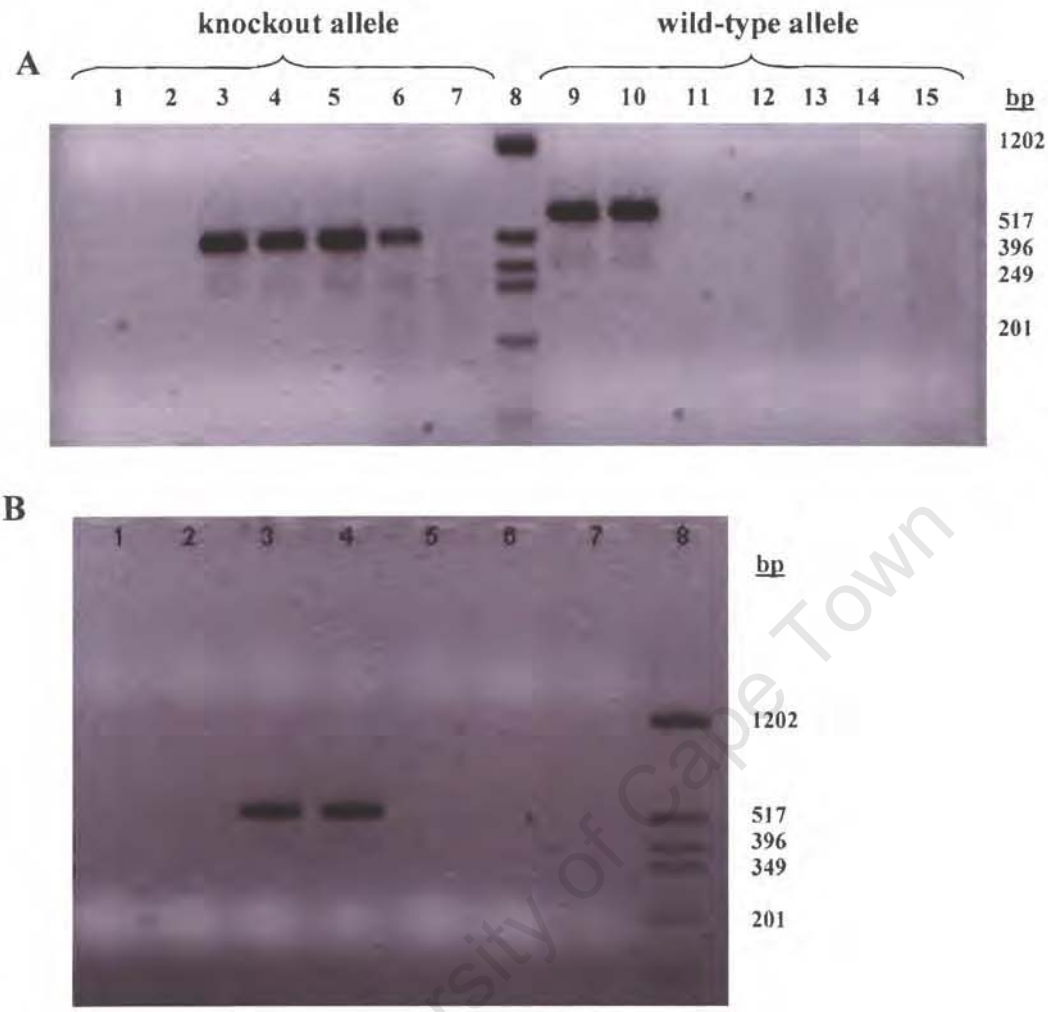

Figure 2. Genotyping of gene-deficient and transgenic mice

Three PCR reactions were used to identify hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice. **A.** PCR reactions using primers to the 600bp wild-type allele and primers to the 471bp knockout allele. Lanes 1, 2, 9 and 10 contain PCR products from WT BALB/c mice. Lanes 3, 4, 11 and 12 contain PCR products from hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice while lanes 5, 6, 13 and 14 contain PCR products from mIL-4Rα$^{-/-}$ mice. A DNA marker was loaded in lane 8 and the negative controls for each reaction in lanes 7 and 15. **B.** PCR reactions using primers to the hIL-4Rα transgene. Lanes 1 and 2 contain the PCR products from WT BALB/c mice, lanes 3 and 4 contain PCR products from hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice and lanes 5 and 6 contain PCR products from mIL-4Rα$^{-/-}$ mice. A DNA marker was loaded in lane 8 and a negative control in lane 7. The sizes (in bp) of the DNA marker are indicated on the right of each gel.

1.3 Analysis of cell surface expression of mIL-4Rα and hIL-4Rα

The cell surface expression of mIL-4Rα and hIL-4Rα was analyzed using four colour flow cytometry to verify that transgenic mice express lymphocyte-specific hIL-4Rα but no mIL-4Rα. Different cell populations from naïve lymph node, peritoneal exudate cells and splenocytes were identified by staining with fluorescence labeled antibodies to various cell surface molecules. The correct isotype controls for these antibodies were used to ensure their specificity. Cells were further incubated with 7-AAD or propidium iodide to ensure the analysis of viable cells. mIL-4Rα surface expression was absent on $CD3^+$, $CD4^+$ or $B220^+$ lymph node cells from hIL-4Rα Tg/mIL-4Rα$^{-/-}$ similar as mIL-4Rα$^{-/-}$ strain compared to BALB/c positive control (Fig. 3).

To confirm that transgenic mice express lymphocyte-specific hIL-4Rα, lymphocyte and non-lymphocyte subpopulations were analyzed for expression of this receptor subunit. An isotype control antibody was used to verify the specificity of the anti-hIL-4Rα antibody and function as negative control. BALB/c and mIL-4Rα$^{-/-}$ strains were included as additional negative controls. $CD3^+CD4^+$ (Fig. 4A) and $CD3^+CD8^+$ (Fig. 4B) T cell subsets expressed hIL-4Rα in the hIL-4Rα Tg/mIL-4Rα$^{-/-}$ strain. Of the $CD3^+CD4^+$ cells, 62% were positive for hIL-4Rα expression while 69% of $CD3^+CD8^+$ T cells expressed the subunit. A smaller NK-T cell subset (data not shown) with high CD3 and CD49b (DX5) expression showed hIL-4Rα expression as did 37% of $CD19^+$ B cells (Fig. 4C).

For surface expression in non-lymphocytes, macrophages, NK cells, dendritic cells (DC) and granulocytes were analyzed. MHC II^- $F4/80^+$ (Fig. 5A) and MHC II^+ $F4/80^+$ (Fig. 5B) macrophages showed no expression of hIL-4Rα. $CD3^-$ $DX5^+$ NK cells (Fig. 5C) and MHC II^+CD11c^+ DC's (Fig. 5D) were mostly negative for hIL-4Rα expression but 9% and 8% of each respective gated population expressed the receptor subunit. GR-1^+ $CD11b^+$ granulocytes (Fig. 5E) showed no expression of hIL-4Rα. Cells from BALB/c and mIL-4Rα$^{-/-}$ strains had no hIL-4Rα expression in any of the lymphocyte or non-lymphocyte subpopulations studied as compared to the isotype control.

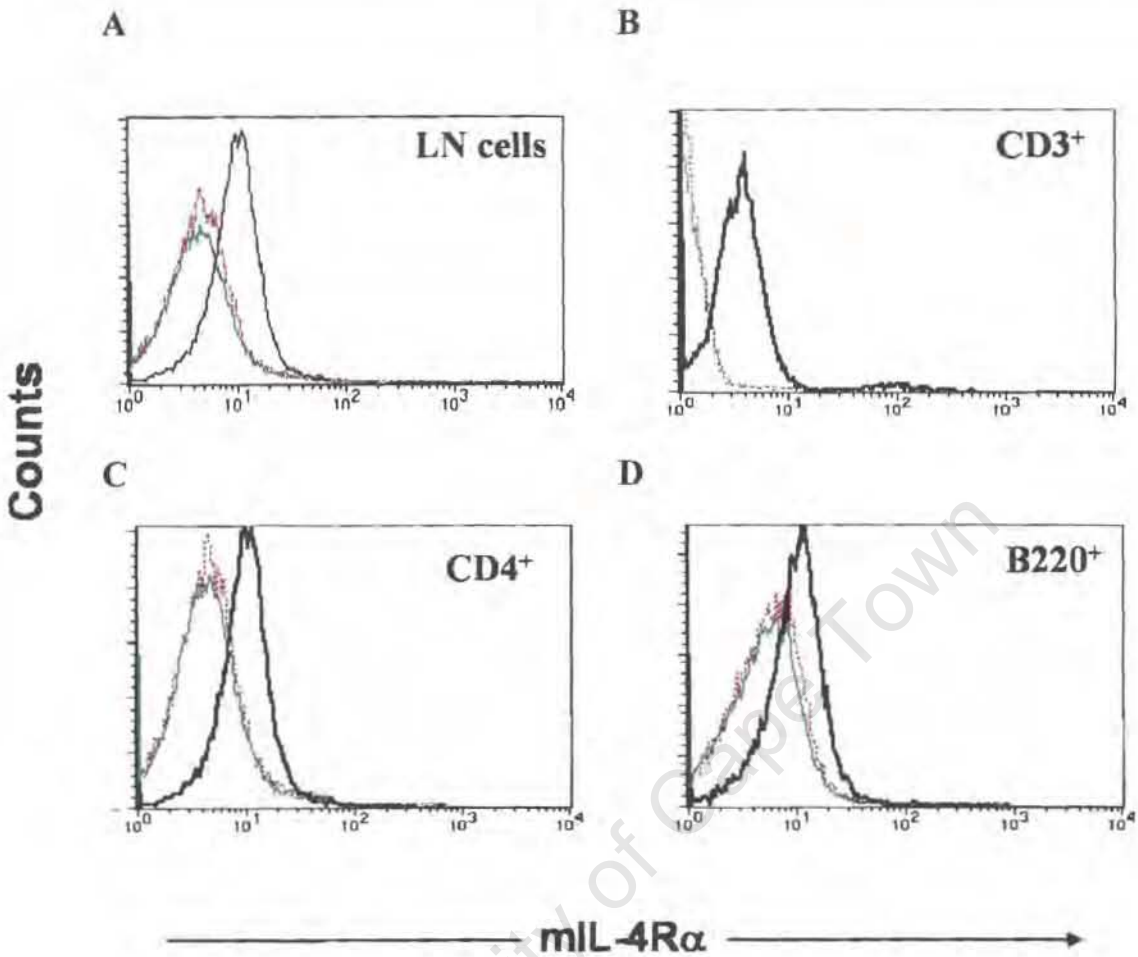

Figure 3. Surface expression of mIL-4Rα on lymphocytes.

Lymph node cells were isolated from naïve BALB/c (black line), hIL-4Rα Tg/mIL-4Rα$^{-/-}$ (green line) and mIL-4Rα$^{-/-}$ (red line) mice and analyzed by FACS using various cell surface molecules. Whole lymph node (LN) cells, CD3$^+$ T cells, CD4$^+$ T cells and B220$^+$ lymphocytes were examined for the expression of mIL-4Rα. Cells were pooled from 3 mice per group with results representing 1 of 2 independent experiments.

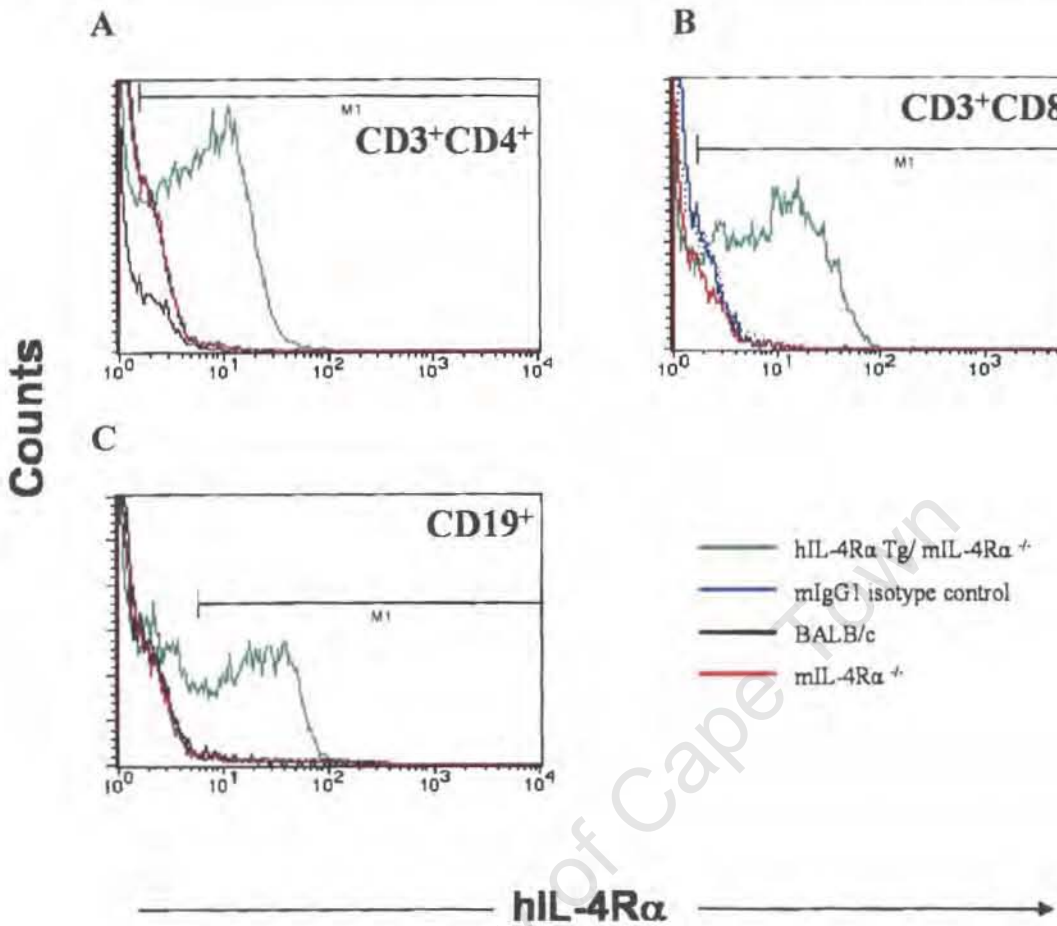

Figure 4. Surface expression of hIL-4Rα on lymphocytes.

Lymph node cells were isolated from naïve BALB/c (black line), hIL-4Rα Tg/mIL-4Rα$^{-/-}$ (green line) and mIL-4Rα$^{-/-}$ (red line) mice and analyzed by FACS for the expression of hIL-4Rα. Cells from hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice were stained with the appropriate isotype control antibody (blue line). CD3$^+$CD4$^+$ and CD3$^+$CD8$^+$ T cell subsets, as well as CD19$^+$ B cells were examined for hIL-4Rα expression. M1 indicates surface expression of hIL-4Rα above that detected for the isotype and negative controls. Cells were pooled from 3 mice per group with results representing 1 of 3 independent experiments.

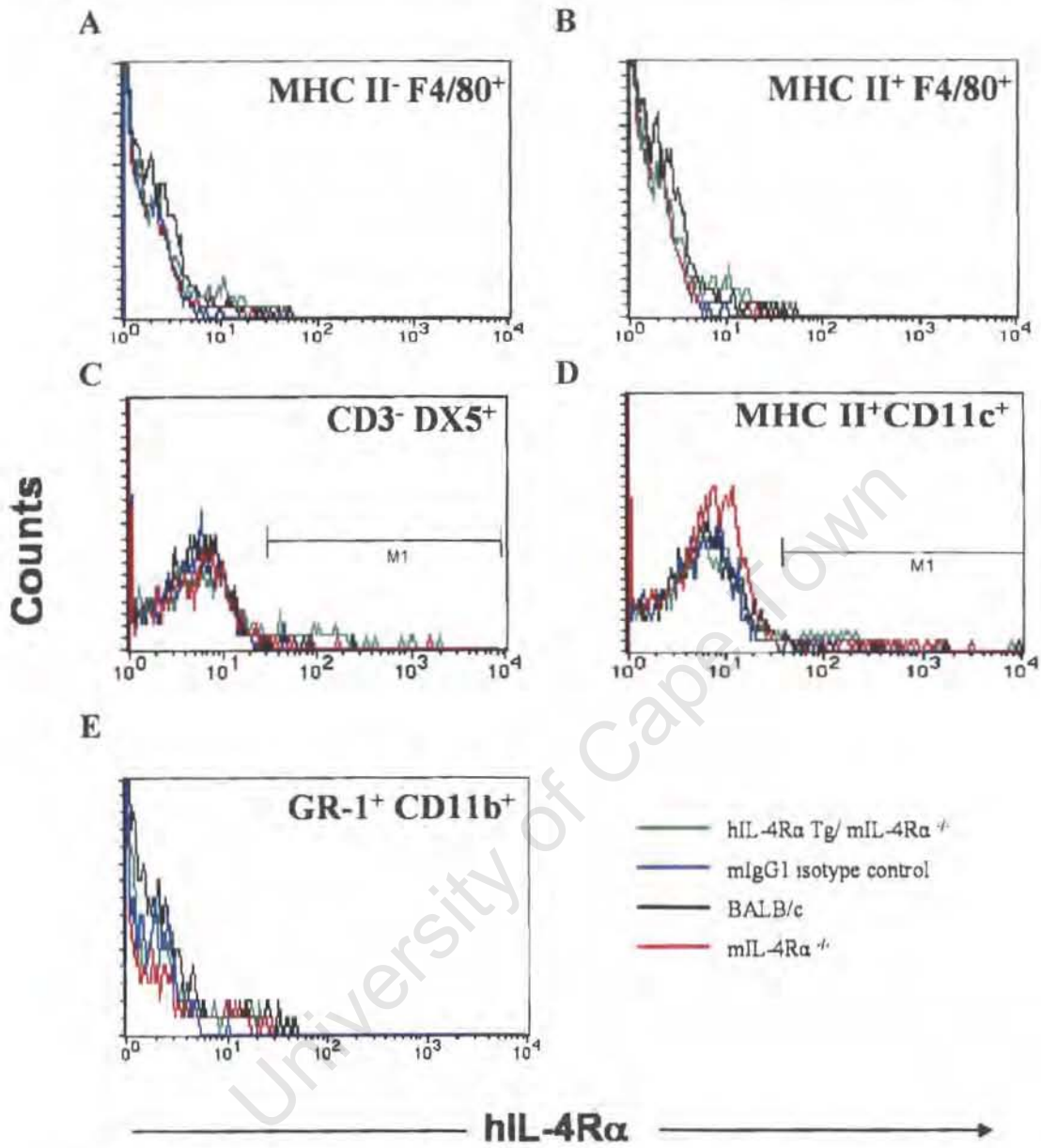

Figure 5. Surface expression of hIL-4Rα on non-lymphocytes.

Splenocytes and peritoneal exudate cells were isolated from naïve BALB/c (black line), hIL-4Rα Tg/mIL-4Rα$^{-/-}$ (green line) and mIL-4Rα$^{-/-}$ (red line) mice and assessed for surface expression of hIL-4Rα. Cells from hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice were stained with the appropriate isotype control antibody (blue line). Non-lymphocyte populations were identified as MHC II$^-$ F4/80$^+$ and MHC II$^+$ F4/80$^+$ for macrophages, CD3$^-$ DX5$^+$ for NK cells, MHC II$^+$CD11c$^+$ for dendritic cells and GR-1$^+$CD11b$^+$ for granulocytes. M1 indicates surface expression of hIL-4Rα above that detected for the isotype and negative controls. Cells were pooled from 3 mice per group with results representing 1 of 3 independent experiments.

1.4 *In vitro* functional analysis of chimeric IL-4R responsiveness

1.4.1 Cell proliferation

Proliferation of murine B and T lymphocytes *in vitro* can be induced by IL-4 via the type I IL-4R (Zurawski et al., 1995; Kondo et al., 1993). Cell proliferation assays on lymph node cells were performed to determine whether hIL-4Rα expressed in lymphocytes forms a functional chimeric receptor with mouse γc. Radiolabeled (^{3}H) thymidine incorporation during DNA synthesis was used as a measure of cell proliferation. Cells were stimulated with mIL-2 as positive control and medium alone as negative control. Recombinant hIL-4, mIL-4 and mIL-13 were used to confirm that hIL-4Rα functions in a species-specific manner. Cells from BALB/c and mIL-4Rα$^{-/-}$ strains were used as additional positive and negative controls. When stimulated with mIL-2 (Fig. 6A), cells from all three strains proliferated in a dose dependent manner. Stimulation with mIL-4 (Fig. 6B) induced proliferation in BALB/c cells but not in cells from hIL-4Rα Tg/mIL-4Rα$^{-/-}$ and mIL-4Rα$^{-/-}$ strains. On the other hand, hIL-4 stimulation (Fig. 6C) resulted in proliferation of lymph node cells from hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice but not in cells from BALB/c and mIL-4Rα$^{-/-}$ mice. The addition of mIL-13 (Fig. 6D) did not induce proliferation of lymph node cells from any of the strains. Assays done without PMA showed the same results but with lower thymidine incorporation (data not shown). These data confirm the species-specificity and function of the chimeric IL-4R and the deletion of mIL-4R *in vitro*.

1.4.2 T cell differentiation

IL-4 induces naïve T helper (Th) cells to differentiate into Th2 cells *in vitro* (Le Gros et al., 1990) while IL-12 drives Th1 differentiation (Hsieh et al., 1993; Manetti et al., 1993; Seder et al., 1993). In order to ascertain whether hIL-4 stimulates the differentiation of CD4$^+$ T cells from hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice into Th2 cells, Th1/Th2 differentiation was analyzed *in vitro*.

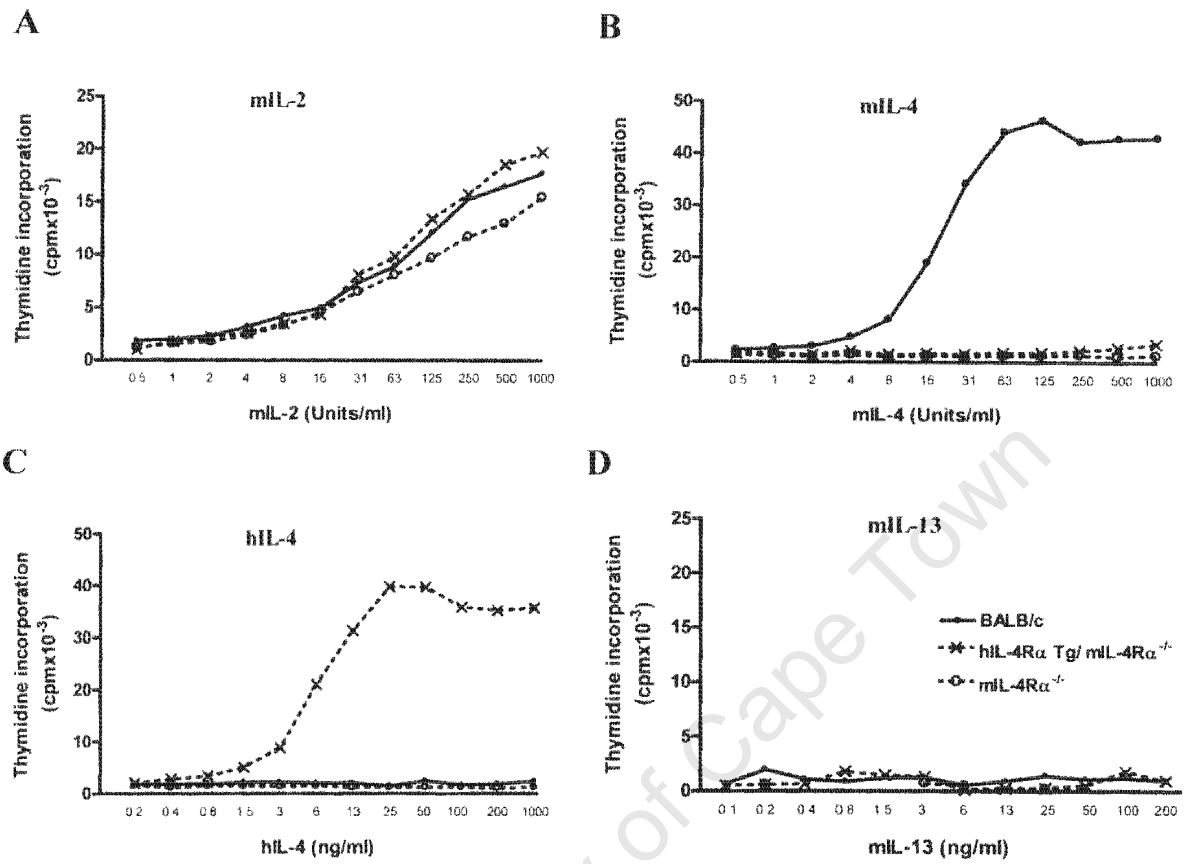

Figure 6. Cell proliferation of lymph node cells stimulated with mIL-2, mIL-4, hIL-4 or mIL-13.

Lymph node cells were isolated from naïve BALB/c (—●—), hIL-4Rα Tg/mIL-4Rα$^{-/-}$ (--✕--) and mIL-4Rα$^{-/-}$ (---○---) mice and stimulated with mIL-2 (panel A), mIL-4 (panel B), hIL-4 (panel C) or mIL-13 (panel D) in the presence of PMA (5ng/ml) for 48 hrs. The incorporation of [^{3}H] thymidine during the last 18 hrs of culture was used as a measure of proliferation. The results are representative of 4 independent experiments. (n = 4 mice per strain)

Cells from BALB/c and mIL-4Rα$^{-/-}$ mice were used as controls. Purified CD4$^+$ T cells isolated from naïve lymph node cells were activated with plate bound anti-CD3/anti-CD28. Cells were cultured for 72 hours in the presence of IL-2 (20 U/ml) with mIL-4, hIL-4 or mIL-13 and anti-IFN-γ for Th2 differentiation, or with IL-12 and anti-IL-4 for Th1 differentiation. After restimulation of cells for an additional 48 hours with anti-CD3, Th1 and Th2 cell differentiation were assayed by measuring the IFN-γ and IL-4 secretion, respectively. The results presented in Fig. 7 (left panel) illustrate that T cells from all

three mouse strains showed equivalent ability to differentiate in Th1 cells after stimulation with IL-12 and anti-IL-4. IL-4 production in the right panel (Fig. 7) indicates that $CD4^+$ T cells from hIL-4Rα Tg/mIL-4Rα$^{-/-}$ had impaired Th2 differentiation after culturing with mIL-4 as did mIL-4Rα$^{-/-}$ cells. In contrast, hIL-4 induced Th2 differentiation in hIL-4Rα Tg/mIL-4Rα$^{-/-}$ but not in BALB/c and mIL-4Rα$^{-/-}$ cells. IL-13 had no visible effect on either IFN-γ or IL-4 production in $CD4^+$T cells from the three strains confirming previous reports that IL-13 does not influence T cell differentiation *in vitro* (Zurawski et al., 1993; de Waal Malefyt et al., 1995; Sornasse et al., 1996). In conclusion, hIL-4 but not mIL-4 induces Th2 differentiation of naive $CD4^+$ T cells from hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice *in vitro*.

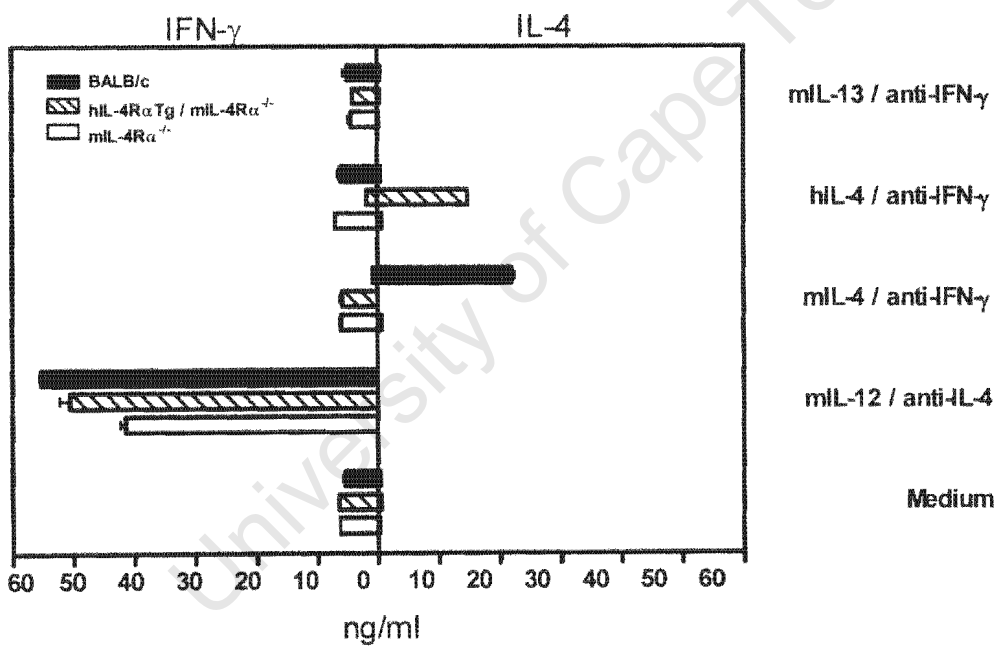

Figure 7. *In vitro* Th1/Th2 differentiation of $CD4^+$ T cells from BALB/c, hIL-4Rα Tg/mIL-4Rα$^{-/-}$ and mIL-4Rα$^{-/-}$ mice.

$CD4^+$ T cells were purified (>95% purity) from pooled lymph node cells of naïve BALB/c, hIL-4Rα Tg/mIL-4Rα$^{-/-}$ and mIL-4Rα$^{-/-}$ mice (n = 3 mice per group). Cell were activated by plate-bound anti-CD3/anti-CD28 in the presence of IL-2 and cultured with mIL-12 and anti-mIL-4 for Th1 differentiation or, for Th2 differentiation, with anti-IFN-γ and mIL-4, hIL-4 or mIL-13. After 72 hrs of culture, cells were restimulated with anti-CD3 for 48 hrs and the levels of IFN-γ (left panel) and IL-4 (right panel) in the supernatant measured by ELISA. The results represent 1 of 3 independent experiments and show the average ± SEM of triplicate values.

1.4.3 Macrophage responsiveness to mIL-4 and mIL-13

IFN-γ/LPS induce the production of NO in classically activated macrophages (Ding et al 1988; Green et al., 1990a). This induction is counterregulated by IL-4Rα-dependent mechanisms leading to alternative activated macrophages that have decreased NO production and increased arginase I activity (Doyle et al., 1994; Corraliza et al., 1995; Modolell et al., 1995; Takeda et al., 1996; Hölscher et al., 2006). Results from the genotyping and FACS analysis showed that hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice were deficient in mIL-4Rα. To confirm that macrophages from hIL-4Rα transgenic mice are unresponsive to mIL-4 and mIL-13, IFN-γ/LPS induced NO production and arginase I activity were analyzed in macrophage cultures. Thioglycollate-elicited peritoneal macrophages from BALB/c, hIL-4Rα Tg/mIL-4Rα$^{-/-}$ and mIL-4Rα$^{-/-}$ mice were preincubated with medium, mIL-4 or mIL-13 and subsequently stimulated with IFN-γ/LPS. Macrophages were assayed for nitric oxide (NO) and for urea production as a measure of arginase I activity. IFN-γ/LPS induced NO production by BALB/c macrophages (Fig. 8A) was significantly suppressed by preincubation with mIL-4 or mIL-13 while arginase I (Fig. 8B) and thus urea production were induced. mIL-4 and mIL-13 were unable to stimulate urea production and suppress NO production induced by IFN-γ/LPS in hIL-4Rα Tg/mIL-4Rα$^{-/-}$ and mIL-4Rα$^{-/-}$ macrophages. These data confirm that macrophages from transgenic mice lack functional IL-4/IL-13 receptors.

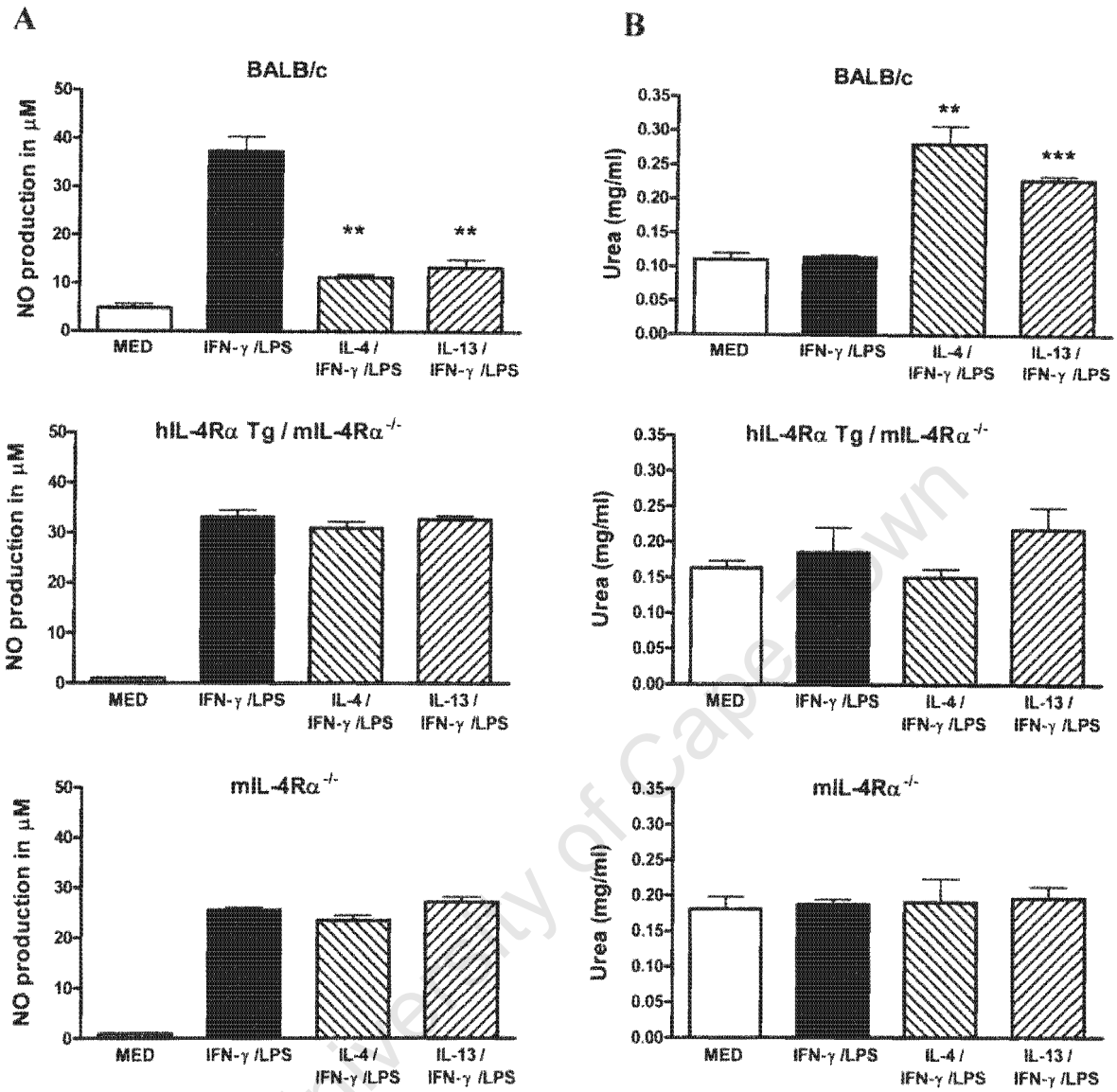

Figure 8. Macrophage responsiveness to mIL-4 and mIL-13.

Pooled peritoneal exudate cells from naïve BALB/c, hIL-4Rα Tg/mIL-4Rα$^{-/-}$ and mIL-4Rα$^{-/-}$ mice (n = 5 mice per group) were stimulated with medium alone or preincubated for 16 hrs with medium, mIL-4 or mIL-13 and subsequently stimulated with IFN-γ/LPS. After 48 hrs the NO production (panel A) was determined by the Griess reaction. Urea production (panel B) after the addition of arginine was used as a measure of arginase activity. One experiment representative of 2 is shown with data representing mean ± SEM of triplicate cultures. Statistical differences compared to the IFN-γ/LPS-stimulated cultures are indicated as ** $p< 0.01$ and *** $p< 0.001$.

2. *In vivo* disease studies

2.1. *Leishmania major* infection

2.1.1 *L. major* disease progression

Susceptibility of BALB/c mice to *L. major* infection is associated with a Th2 response and the production of type 2 antibodies (Heinzel et al., 1989). In the absence of IL-4Rα, BALB/c mice show increased resistance to *L. major* infection and a shift towards the production of type 1 antibodies (Noben-Trauth et al., 1999; Mohrs et al., 1999). The *L. major* infection model is thus well suited to test the *in vivo* specificity of the chimeric receptor in hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice. Establishing this *in vivo* model would allow the investigation of the IL-4-dependent type 2 responses during *L. major* infection. Furthermore, the inducibility of the system may shed light on how the timing of IL-4 signaling influences Th2 or type 2 responses.

BALB/c, C57BL/6, hIL-4Rα Tg/mIL-4Rα$^{-/-}$ and the littermate control mIL-4Rα$^{-/-}$ mice were infected subcutaneously (s.c.) with $2x10^6$ *L. major* LV39 promastigotes in the hind footpads and lesion development monitored by measuring footpad swelling. BALB/c mice developed progressive non-healing lesions resulting in ulceration while C57BL/6 mice were able to control lesion development with no ulceration (Fig. 9). The mIL-4Rα$^{-/-}$ animals showed increased resistance compared with BALB/c with a moderate footpad swelling. The hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice developed disease progression with increasing footpad swelling and ulceration similar to that in BALB/c mice.

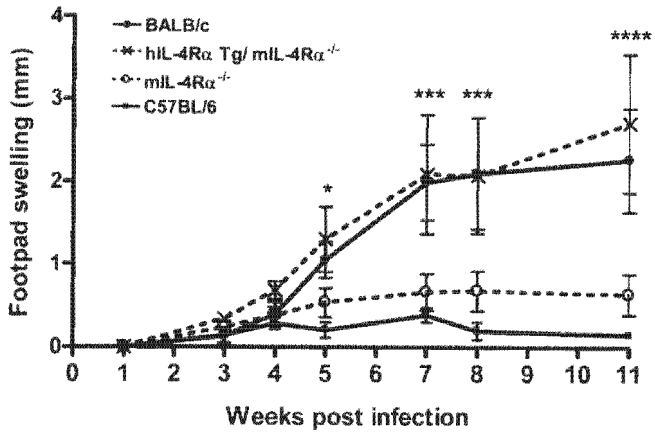

Figure 9. Footpad lesion development during *L. major* infection.

BALB/c, C57BL/6, hIL-4Rα Tg/mIL-4Rα$^{-/-}$ and mIL-4Rα$^{-/-}$ mice (n = 4 mice per strain) were infected with 2x10^6 *L. major* LV39 promastigotes in the hind footpads. Lesion development was monitored weekly and footpad swelling calculated as the difference between the infected and uninfected footpads. One of 4 representative experiments is shown with data representing mean footpad swelling ± SEM. The occurrence of ulceration and necrosis from individual mice is indicated with an asterisk (*).

2.1.2 Increased parasites burden in hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice

To evaluate the ability of *L. major*-infected mice in controlling parasite growth, parasite burdens were determined in infected footpads and lymph nodes at week 4 and week 12 p.i. Whole footpad homogenates and lymph node cell suspensions from single mice were serially diluted, incubated for 10 days and examined for viable parasites. After 4 weeks (Fig. 10) of infection parasite burdens in BALB/c and hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice were similarly enhanced in footpads as well as in draining lymph nodes. This was consistent with the comparable lesion development observed for these two strains throughout the infection. The parasite burdens in footpads from C57BL/6 and mIL-4Rα$^{-/-}$ mice were significantly reduced compared with BALB/c mice. In the draining lymph nodes, C57BL/6 mice had reduced parasites while the mIL-4Rα$^{-/-}$ mice showed a high parasite burden comparable to the levels in BALB/c and hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice. At 12 weeks p.i. the parasite burdens were in accordance with the lesion development observed in the different strains. The susceptible BALB/c and hIL-4Rα Tg/mIL-4Rα$^{-/-}$ strains

showed equally enhanced parasite loads in both footpads and draining lymph nodes while resistant C57BL/6 mice had markedly reduced parasite loads. The mIL-4Rα$^{-/-}$ mice also showed reduced parasite loads in both footpads and draining lymph nodes compared with BALB/c and hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice. As was observed with the moderate lesion development, mIL-4Rα$^{-/-}$ mice had higher parasite burdens compared to C57BL/6 mice. These results demonstrate that hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice, in the absence of hIL-4, maintain a susceptible phenotype to *L. major*, comparable to that of BALB/c mice. Furthermore, hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice are significantly more susceptible than the littermate control mIL-4Rα$^{-/-}$ mice.

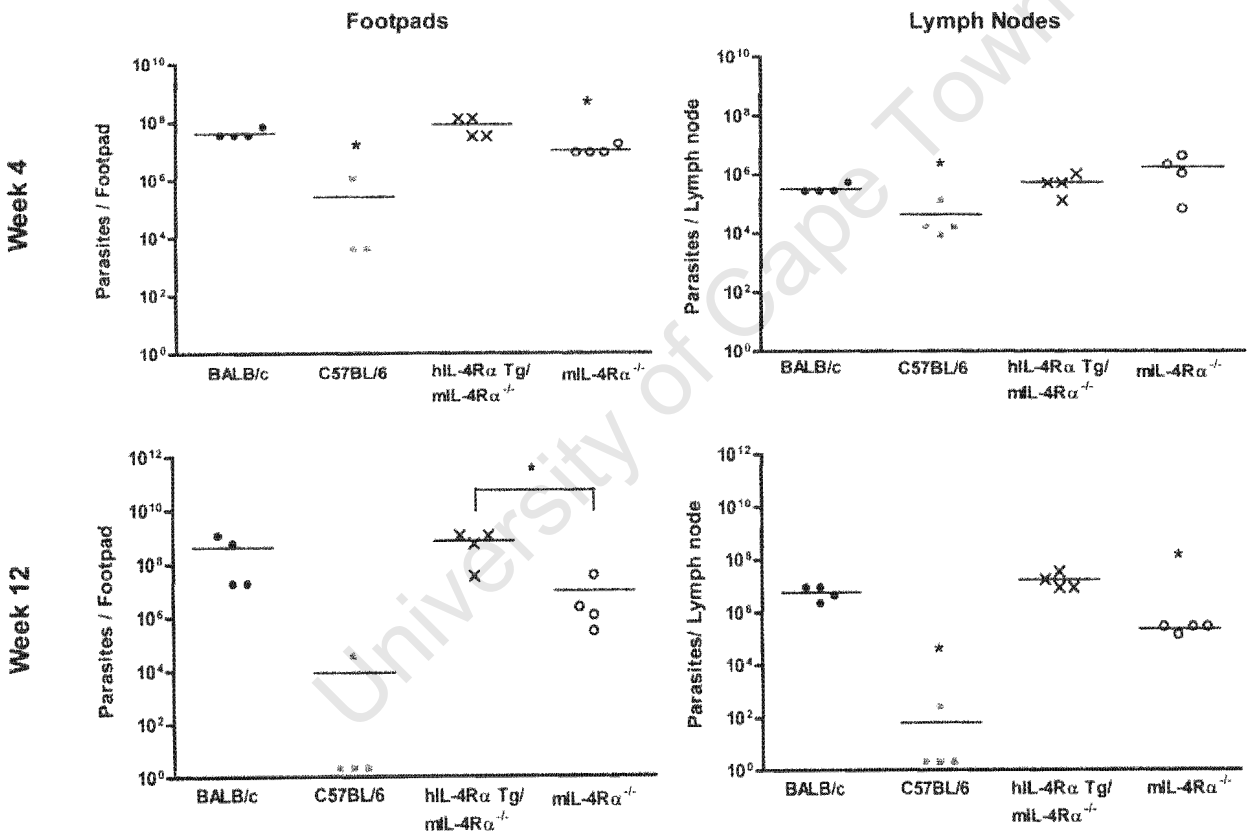

Figure 10. Parasite loads in footpads and lymph nodes of *L. major*-infected mice.

Footpads homogenates and lymph node single cell suspensions were prepared from single *L. major*-infected mice (n = 4 mice per strain) at week 4 and 12 p.i. Limiting dilution assays were performed and the dilutions were inspected for viable parasites after 10 days of culture. Each data point represents the parasite burden in the organ of a single mouse with the bars indicating arithmetic means. One representative of 2 experiments is shown. Statistical differences compared to the BALB/c mice are indicated as * $p < 0.05$. For the parasite burden in footpads at week 12 statistical difference between hIL-4Rα Tg/mIL-4Rα$^{-/-}$ and mIL-4Rα$^{-/-}$ mice are shown.

2.1.3 Type 2 antibody responses to *L. major* infection

IL-4 induces the production of type 2 antibodies IgG1 and IgE in mouse B cells (Coffman et al., 1986; Snapper and Paul, 1987). During *L. major* infection BALB/c mice produce type 2 antibodies which are significantly reduced in mice that lack IL-4Rα (Mohrs et al., 1999, 2000). Furthermore, a deficiency in IL-4Rα results in a shift towards the production of type 1 antibodies IgG2a and IgG2b. It was expected that the lack of mIL-4Rα in transgenic mice would result in increased type 1 and reduced type 2 antibody responses. Sera samples from *L. major*-infected mice were collected at day 0, week 4 and week 10 post infection and the *L. major*-specific IgG1, IgG2a and IgG2b, as well as total IgE antibodies were measured by ELISA. Antibody levels of antigen-specific IgG1 (Fig. 11A), IgG2a (Fig. 11B) and IgG2b (Fig. 11C) were low and similar in all groups at week 4 p.i.

After 10 weeks of infection, *L. major*-specific IgG1 production was increased in BALB/c and hIL-4Rα Tg/mIL-4Rα$^{-/-}$ strains but impaired in C57BL/6 and mIL-4Rα$^{-/-}$ mice (Fig. 11A). BALB/c mice also produced high IgG2a and IgG2b while C57BL/6 mice made low amounts of both these two antibody isotypes (Fig. 11B & Fig. 11C). Type1 IgG2a and IgG2b antibody production was equally elevated in hIL-4Rα Tg/mIL-4Rα$^{-/-}$ and mIL-4Rα$^{-/-}$ mice. Total IgE (Fig. 12) was increased in BALB/c and hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice before infection with *L. major*. The levels of this type 2 antibody increased with time in BALB/c mice while remaining constant in hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice. On the other hand, IgE antibody production was low throughout the infection in C57BL/6 and completely abrogated in mIL-4Rα$^{-/-}$ mice.

These data demonstrate that transgenic mice make both type 1 and type 2 antibodies in response to *L. major* infection. IgE production is dependent on IL-4 signaling (Shimoda et al., 1996) indicating that B lymphocytes in transgenic mice respond to mIL-4 *in vivo* via the chimeric hIL-4Rα/mγc receptor.

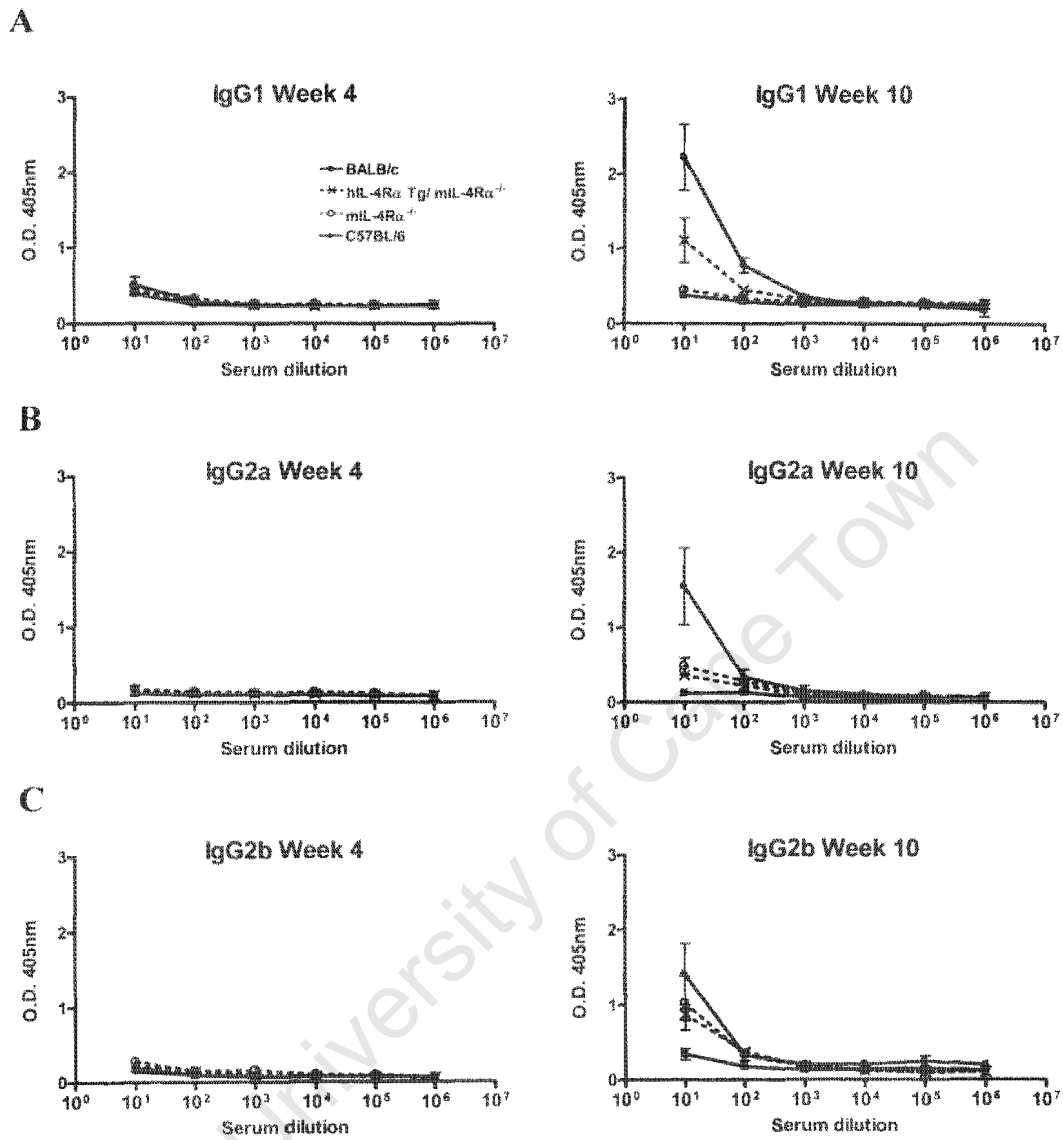

Figure 11. Antigen-specific antibody responses in *L. major*-infected mice.

BALB/c, C57BL/6, hIL-4Rα Tg/mIL-4Rα$^{-/-}$ and mIL-4Rα$^{-/-}$ mice were infected with 2x10^6 *L. major* promastigotes in the hind footpads and sera collected at day 0, week 4 and week 10 post infection. *L. major*-specific IgG1, IgG2a and IgG2b titers were determined by ELISA. One representative of 3 experiments is shown with values representing mean ± SEM of 4 mice per group.

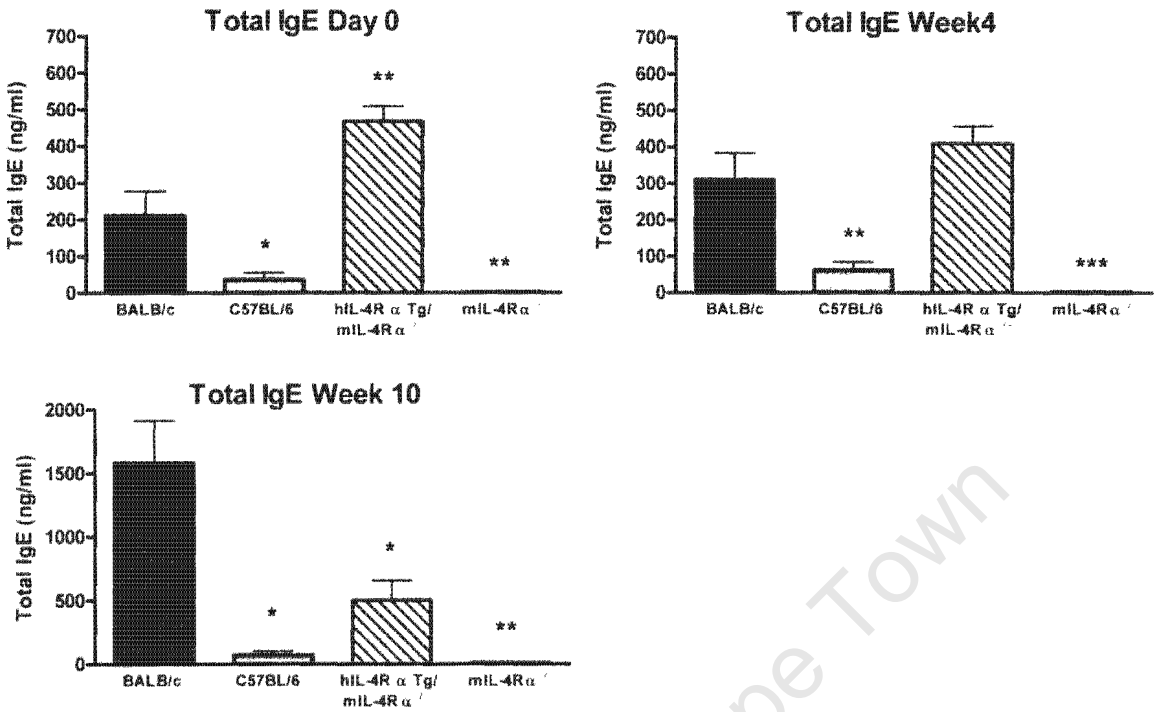

Figure 12. Total IgE production in response to *L. major* infection.

Sera from *L. major*-infected mice were collected at day 0, week 4 and week 10 post infection and the total IgE antibody levels determined by ELISA. One representative of 3 experiments is shown. The data is expressed as mean ± SEM of 4 mice per group. Statistical differences compared to BALB/c values are indicated as * $p < 0.05$ and ** $p < 0.01$.

2.1.4 Cytokine response to *L. major* infection

Susceptibility of BALB/c mice to *L. major* is associated with the production of Th2 cytokines such as IL-4, IL-13 and IL-10 (Heinzel et al., 1989, 1991; Reiner et al., 1994). To test whether the susceptibility observed in hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice is accompanied with a dominant Th2 response, the cytokine production of lymphocytes were analyzed after 4 weeks of infection with *L. major*. Single cell suspensions were made from pooled draining lymph nodes of infected mice and restimulated with anti-CD3 for 72 hours. The cell supernatants were analyzed by ELISA for IL-4, IL-5, IL-13 and IFN-γ. Cells from BALB/c mice produced increased amounts of IL-4 compared to C57BL/6 and mIL-4Rα$^{-/-}$ mice while hIL-4Rα Tg/mIL-4Rα$^{-/-}$ cells expressed strikingly

higher amounts of this cytokine (Fig. 13A). The hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice also expressed increased levels of IL-5 (Fig. 13B), similar to BALB/c mice, but significantly higher concentrations of IL-13 (Fig. 13C). In contrast, C57BL/6 and mIL-4Rα$^{-/-}$ mice made lower amounts of both IL-5 and IL-13. The IFN-γ production was elevated in C57BL/6 mice while being lower in BALB/c and hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice and even more reduced in mIL-4Rα$^{-/-}$ mice (Fig. 13D).

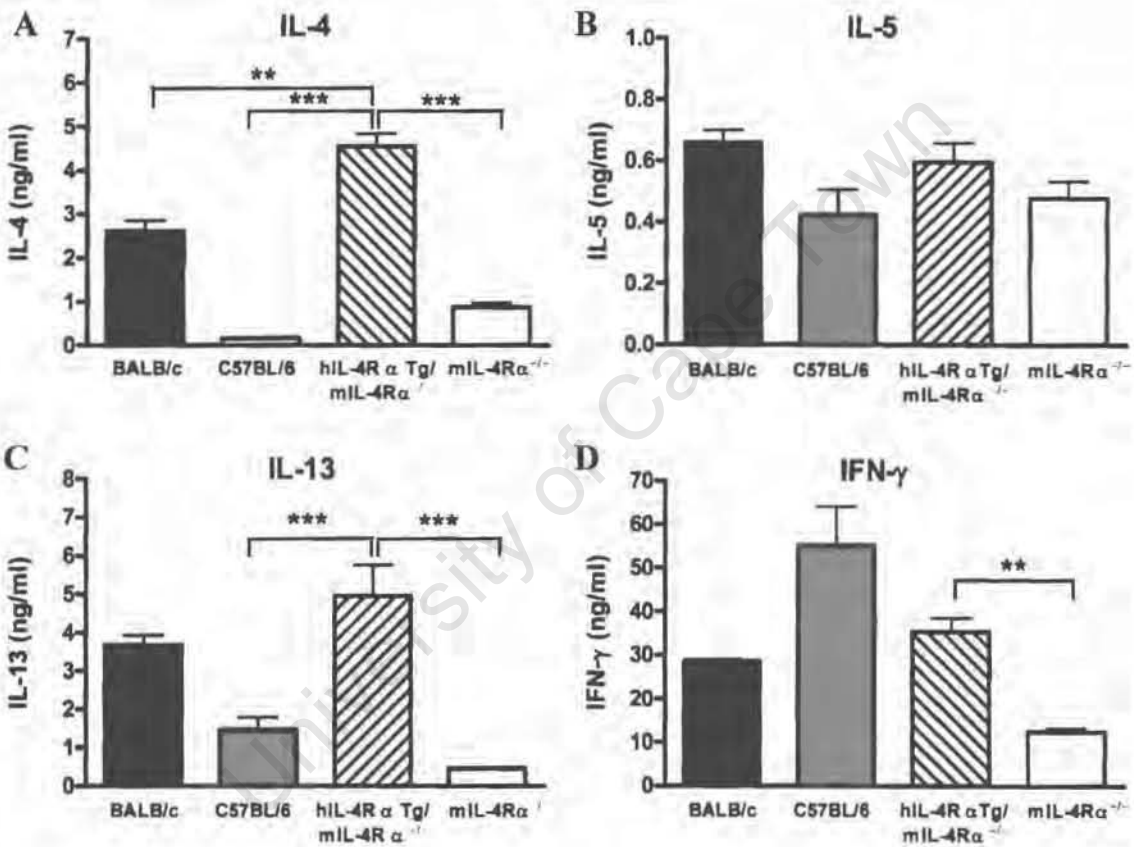

Figure 13. Cytokine production in lymph node cells from *L. major*-infected mice.

Single cell suspensions were prepared from pooled lymph nodes of week 4 *L. major*-infected mice (n = 4 mice per group) and restimulated with anti-CD3 for 72 hrs. The levels of IL-4, IL-5, IL-13 and IFN-γ in cell supernatants were measured by ELISA. One representative of 3 experiments is shown with the values representing mean ± SEM of triplicate cultures. Statistical differences between groups are indicated as ** $p < 0.01$ and *** $p < 0.001$.

2.1.5 Effect of hIL-4 treatment on susceptibility to *L. major* infection

To determine whether the hIL-4R/myc chimeric receptor in hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice functions as an inducible system, the response of hIL-4-treated transgenic mice to *L. major* infection was investigated. For *in vivo* treatment of mice, IL-4 at concentrations of 1-10μg has been used previously with increased IgE responses observed with 10μg of IL-4. Treatment of mice with IL-4 during the first 64 hrs of *L. major* infection is sufficient to induce Th2 development and susceptibility (Biederman et al., 2001; Sato et al., 1993; Himmelrich et al., 2000). Therefore, transgenic mice were injected with 10 μg of hIL-4 in PBS intraperitoneal one day before infection, at the time of infection and day 2 post infection. The treated mice as well as untreated transgenic and mIL-4Rα$^{-/-}$ mice were infected with 2x10^6 *L. major* LV39 promastigotes in the left hind footpads and lesion development monitored by measuring footpad swelling. As previously observed (Mohrs et al., 1999; Hölscher et al., 2006), mIL-4Rα$^{-/-}$ mice developed slight footpad swelling but were capable of controlled disease till week 12 of the *L. major* infection (Fig. 14). Both hIL-4Rα Tg/mIL-4Rα$^{-/-}$ groups developed progressive footpad swelling. Treatment with hIL-4 had no significant effect on the susceptibility of transgenic mice to *L. major*.

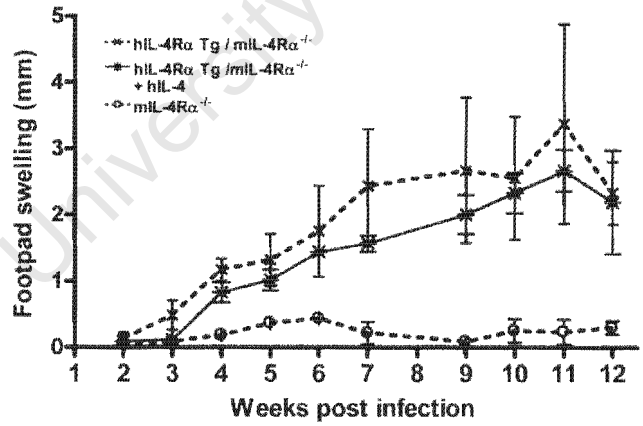

Figure 14. Effect of hIL-4 treatment on lesion development during *L. major* infection.

Mice from hIL-4Rα Tg/mIL-4Rα$^{-/-}$ and mIL-4Rα$^{-/-}$ strains were infected with 2x10^6 *L. major* in the hind footpads. A group of hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice was treated with hIL-4 on day -1, day 0 and day 2 post infection. The infection was monitored weekly by measuring footpads and footpad swelling calculated as the difference between the infected and uninfected footpad. Data represents mean ± SEM of 3 mice per group.

2.1.6 Type 2 antibody responses in *L. major*-infected mice

To analyze the effect of hIL-4 treatment on antibody responses in *L. major*-infected mice, serum was collected one day before infection (day -1) and at week 7 and week 12 post infection. *L. major*-specific IgG1, IgG2a and IgG2b titers as well as total IgE were measured by ELISA. Whereas there was no increase in IgG1 levels (Fig. 15A) in mIL-4Rα$^{-/-}$ mice at both week 7 and week 12 p.i., hIL-4-treated and untreated hIL-4Rα Tg/mIL-4Rα$^{-/-}$ groups produced higher amounts of IgG1 at week 7 which was further increased at week 12 after infection. The mIL-4Rα$^{-/-}$ and transgenic mice had increased levels of *L. major*-specific IgG2a (Fig. 15B) and IgG2b (Fig. 15C) after infection. As expected no *L. major*-specific antibodies were detected in sera of mice from day -1 (data not shown). Consistent with the previous antibody data naïve untreated hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice produced total IgE (Fig. 15D). Both hIL-4-treated and untreated groups showed similar levels of IgE at week 7 and week 12 p.i., but these antibodies were not increased compared to naïve IgE levels. On the other hand, IgE production was completely abrogated in mIL-4Rα$^{-/-}$ mice during all time points examined.

These data clearly show that hIL-4 treatment of transgenic mice does not affect type 2 responses observed during *L. major* infection. The presence of the hIL-4Rα transgene, even in the absence of exogenous hIL-4, results in susceptibility and type 2 antibody responses. Treatment of transgenic mice with hIL-4 was discontinued to further investigate why the chimeric receptor signals *in vivo* in the absence of hIL-4.

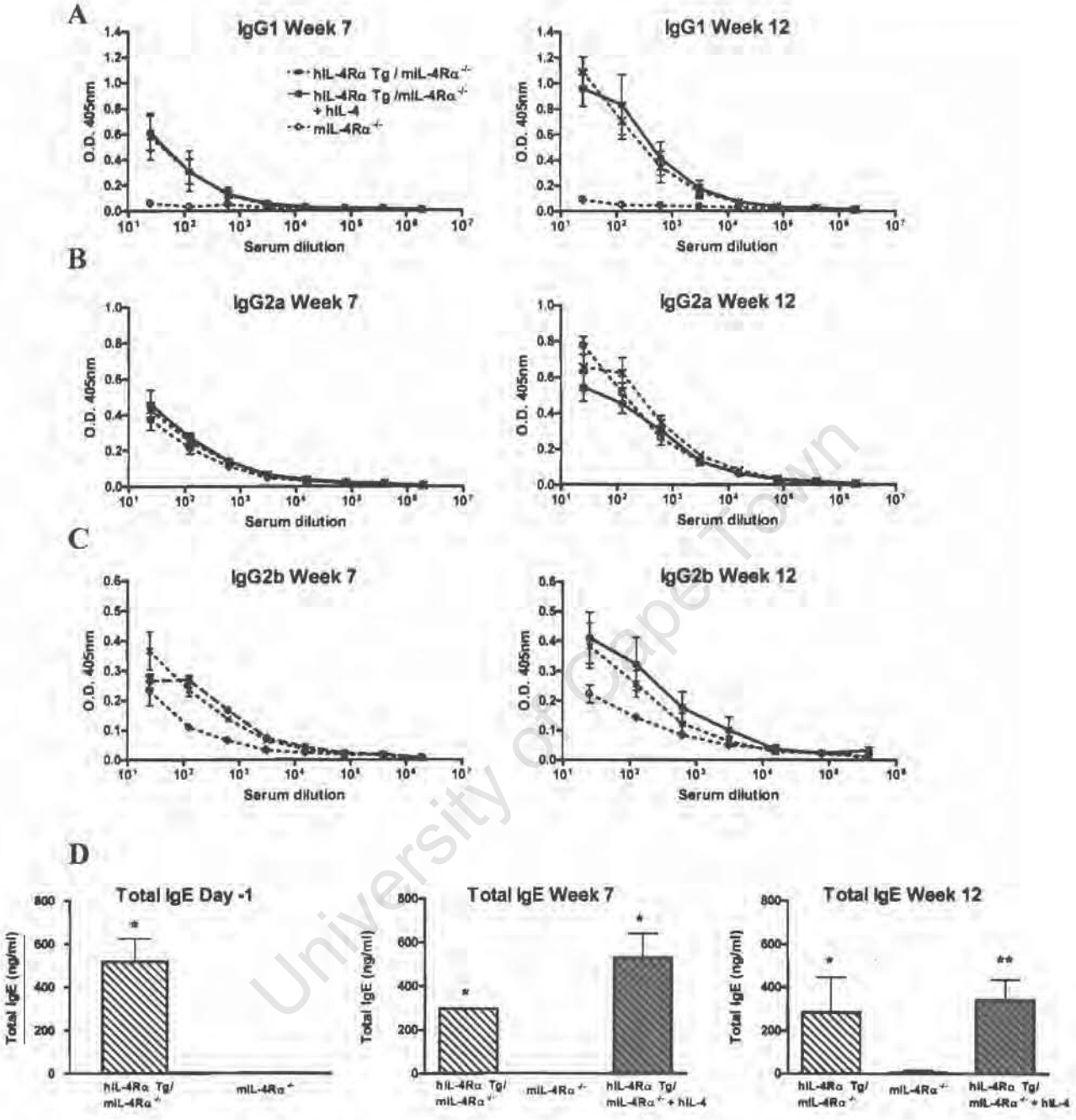

Figure 15. Effect of hIL-4 treatment on antibody responses in mice infected with *L. major*.

Experimental mice were infected s.c. with $2x10^6$ *L. major* and sera collected at day -1, week 7 and week 12 post infection. Antigen-specific IgG1, IgG2a and IgG2b as well as total IgE titers were determined by ELISA. Data points represent mean ± SEM of 3 mice per group. For total IgE values, statistical analysis was performed using unpaired Student *t* test defining differences to untreated mIL-4Rα$^{-/-}$ mice as significant (* $p< 0.05$, ** $p< 0.01$).

2.2 *Nippostrongylus brasiliensis* infection

2.2.1 Worm expulsion

Expulsion of *N. brasiliensis* is IL-13 dependent and requires IL-4Rα expression in non-bone marrow derived cells (Urban et al., 1998, 2001; McKenzie et al., 1998b). The hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice were shown to be deficient in mIL-4Rα and it was thus expected that transgenic mice would not expel *N. brasiliensis* worms. BALB/c, hIL-4Rα Tg/mIL-4Rα$^{-/-}$ and mIL-4Rα$^{-/-}$ mice were infected with 750 L3 stage *N. brasiliensis* larvae and compared for their worm expulsion and fecundity. As expected, wild-type BALB/c mice completely expelled their worms by day 14 p.i. whereas the hIL-4Rα Tg/mIL-4Rα$^{-/-}$ and mIL-4Rα$^{-/-}$ animals had intestinal worms present at this time point (Fig. 16A). No worm eggs could be detected in feces of wild-type mice after day 10 p.i. while the presence of eggs in hIL-4Rα Tg/mIL-4Rα$^{-/-}$ and mIL-4Rα$^{-/-}$ strains indicates to an inability of these mice to expel egg-producing *N. brasiliensis* adult worms (Fig. 16B). These data confirm that transgenic mice do not express IL-4Rα on non-bone marrow derived cells.

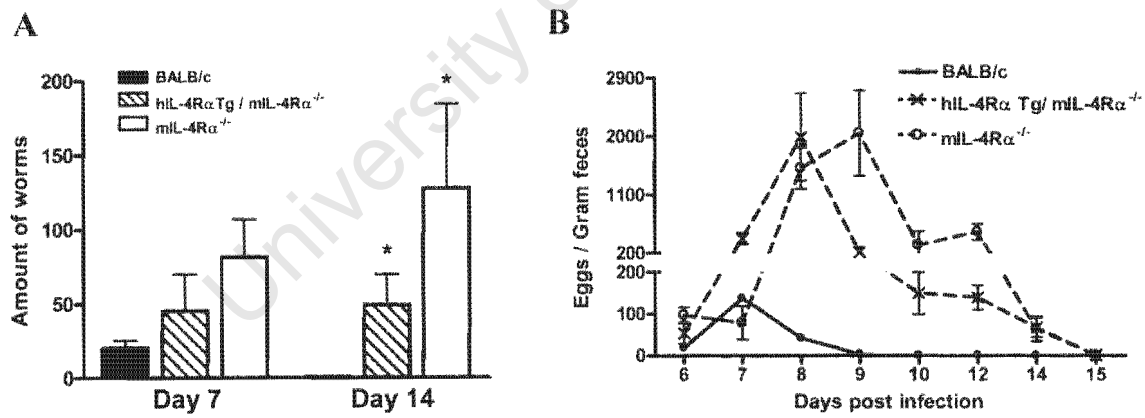

Figure 16. Expulsion of *N. brasiliensis* worms.

BALB/c, hIL-4Rα Tg/mIL-4Rα$^{-/-}$ and mIL-4Rα$^{-/-}$ mice were infected s.c. with 750 L3 stage *N. brasiliensis* larvae. **A.** At day 7 and day 14 after infection the number of worms in intestines of individual mice were determined. **B.** Worm egg production was determined daily from individual mice and expressed as eggs per gram feces. One representative of 3 experiments is shown and data represents mean ± SEM of 4 mice per group. Statistical differences compared to BALB/c values are indicated as * $p < 0.05$.

2.2.2 Antibody responses to *N. brasiliensis* infection

Infection with *N. brasiliensis* provokes an IL-4 and STAT6 dependent polyclonal IgE response in BALB/c mice (Finkelman et al., 1988; Urban et al., 1998). Although antibody production is not required for worm expulsion (Jacobson et al., 1977), the absence of IL-4, IL-13, IL-4Rα or STAT6 alters the antibody profile induced by *N. brasiliensis*. Both IL-4$^{-/-}$/IL-13$^{-/-}$ and IL-4Rα$^{-/-}$ strains make undetectable IgE and increased IgG2 levels after *N. brasiliensis* infection (McKenzie et al., 1999; Urban et al., 1998). To ascertain whether the antibody responses in hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice were similar to those reported for mIL-4Rα$^{-/-}$ animals, total serum IgG1, IgG2b and IgE antibodies were measured at day 14 after infecting with *N. brasiliensis*. BALB/c and mIL-4Rα$^{-/-}$ mice had a similar increase in total IgG1 antibodies after infection (Fig. 17A). Transgenic mice responded with significantly higher levels of IgG1. The mIL-4Rα$^{-/-}$ and hIL-4Rα Tg/mIL-4Rα$^{-/-}$ strains both produced increased IgG2b (Fig. 17B). Elevated IgE was observed in BALB/c mice as well as in hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice while mIL-4Rα$^{-/-}$ animals made no detectable levels of this antibody (Fig. 17C). Thus, although BALB/c and mIL-4Rα$^{-/-}$ animals mounted the expected antibody responses, transgenic mice made high IgE and IgG1 antibodies indicating signaling through the chimeric receptor in B lymphocytes *in vivo*.

2.2.3 Cytokine response to *N. brasiliensis* infection

In BALB/c mice the Th2 response induced by *N. brasiliensis* is characterized by CD4$^+$ T cells that produce high levels of IL-4, IL-5, IL-10 and IL-13 but low levels of IFN-γ. On the other hand, IL-4Rα$^{-/-}$ mice have a markedly reduced production of Th2 cytokines (Kopf et al., 1993, Barner et al., 1998). To determine whether hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice have impaired Th2 cytokine responses, the cytokine production after 7 days of a *N. brasiliensis* infection was analyzed. CD4$^+$ T cells were purified from pooled mesenteric lymph nodes of infected mice and stimulated with medium or anti-CD3. The cytokine levels in cell supernatants were measured by ELISA. As expected, cells from BALB/c mice produced high IL-4 with lower IFN-γ while CD4$^+$ T cells from infected mIL-4Rα$^{-/-}$

mice had reduced IL-4 but higher IFN-γ levels (Fig. 18). However, the IL-5 and IL-13 levels were similar in these two strains. In contrast, the hIL-4Rα Tg/mIL-4Rα$^{-/-}$ cells did not respond like mIL-4Rα$^{-/-}$ but produced significantly higher IL-4, IL-5 and lower IFN-γ than the littermate controls. Although IL-13 levels were higher in hIL-4Rα Tg/mIL-4Rα$^{-/-}$ compared to mIL-4Rα$^{-/-}$ cells, the differences were not significant. The data suggests that transgenic mice develop Th2 cytokine responses *in vivo* due to signaling through the chimeric receptor.

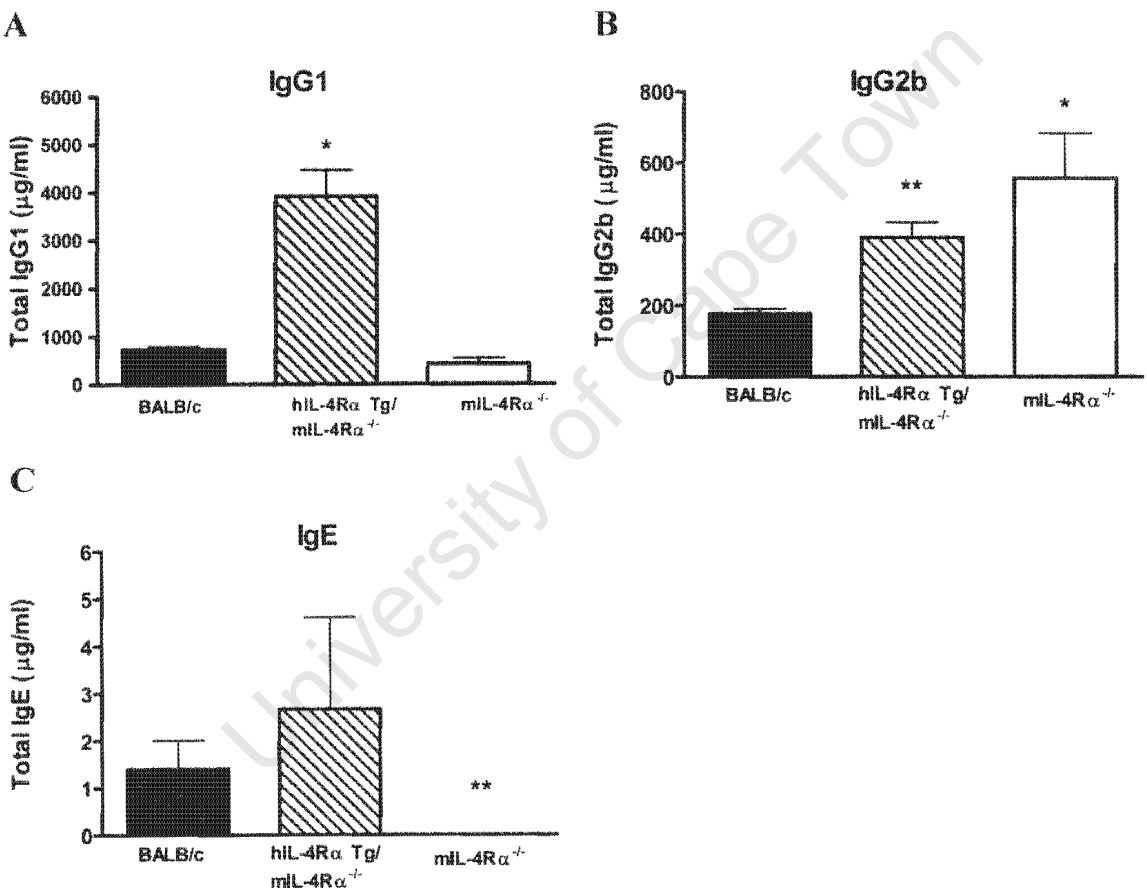

Figure 17. Antibody responses in *N. brasiliensis*-infected mice.

Sera samples were collected from *N. brasiliensis*-infected mice at day 14 post infection and the total IgG1, IgG2b and IgE antibodies measured by ELISA. One representative of 3 experiments is shown and data is expressed as mean ± SEM of 4 mice per group. Statistical differences compared to BALB/c values are indicated as * $p < 0.05$ and ** $p < 0.01$.

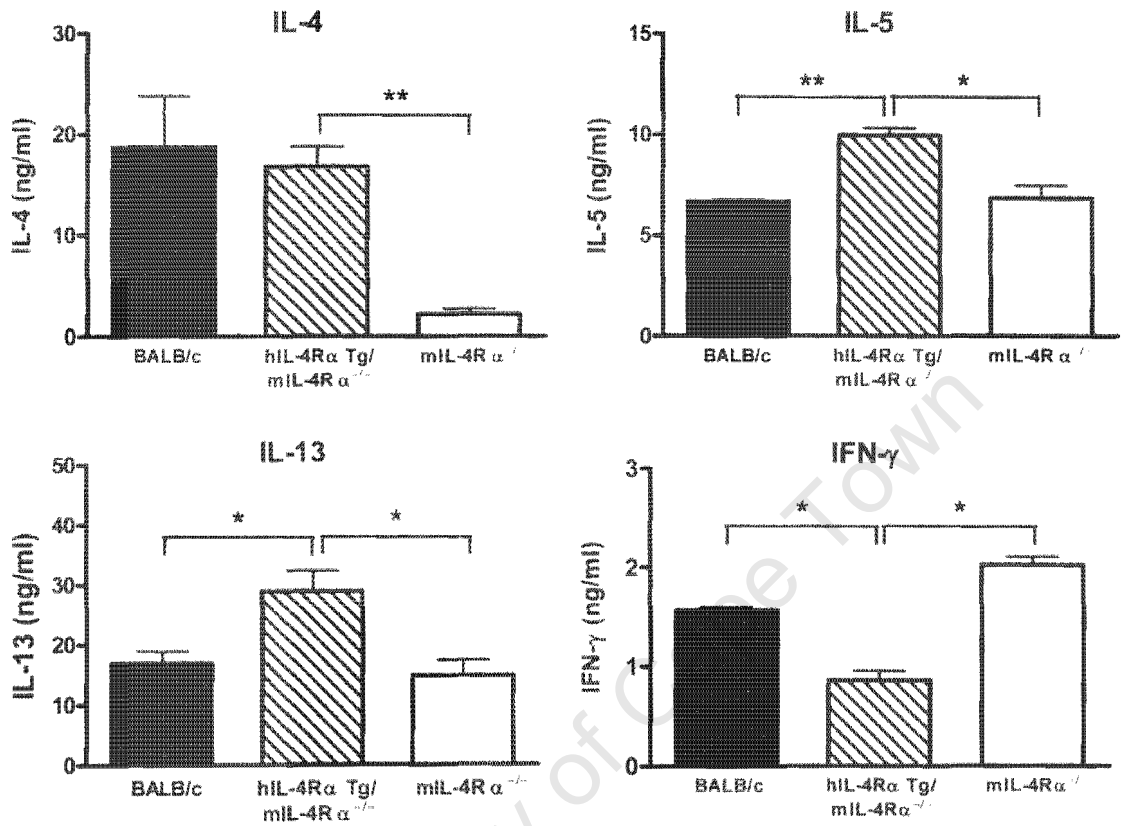

Figure 18. Cytokine production of CD4$^+$ T cells from *N. brasiliensis*-infected mice.

CD4$^+$ T cells were purified from pooled mesenteric lymph nodes of day 7 *N. brasiliensis*-infected mice (n = 4 mice per group). The cells were restimulated with anti-CD3 for 72 hrs and the levels of IL-4, IL-5, IL-13 and IFN-γ measured in supernatants by ELISA. Data is expressed as mean ± SEM of triplicate cultures with results representing 3 independent experiments. Statistical differences between groups are indicated as * $p< 0.05$ and ** $p< 0.01$.

2.3 Neutralization of mIL-4

2.3.1 Worm expulsion of *N. brasiliensis*

The IL-4 signaling by $CD4^+$ T cells and B lymphocytes in transgenic mice suggest that endogenous mIL-4 signals through the chimeric receptor *in vivo*. To address this hypothesis, mIL-4 was neutralized using anti-mIL-4 antibodies during *N. brasiliensis* infection.

Treatment of mice with 0.5 – 1mg of anti-mIL-4 at the time of infection and at weekly intervals thereafter is sufficient to suppress IgE levels in mice infected with *N. brasiliensis* (Finkelman et al., 1986) and *L. major*, respectively (Heinzel et al., 1989; Debus et al., 2003). Thus, BALB/c and hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice were injected intraperitoneally with 1mg of anti-mIL-4 one day prior to infection with 750 L3 stage *N. brasiliensis* and again 7 days later. An untreated group of mIL-4Rα$^{-/-}$ mice was included as control. As expected, the treated and untreated hIL-4Rα Tg/mIL-4Rα$^{-/-}$ and mIL-4Rα$^{-/-}$ mice were unable to expel worms while both BALB/c groups expelled the parasites by day 14 after infection (Fig. 19A). A similar result was seen in the egg counts (Fig. 19B). Whereas no more eggs could be detected in the feces of BALB/c mice by day 14 post infection, fecal eggs were present in hIL-4Rα Tg/mIL-4Rα$^{-/-}$ and mIL-4Rα$^{-/-}$ strains at this time point. Consistent with published data about IL-4-deficient mice, the anti-mIL-4 treatment was unable to suppress egg production and worm expulsion in BALB/c mice (Lawrence et al., 1996, Urban et al., 1998).

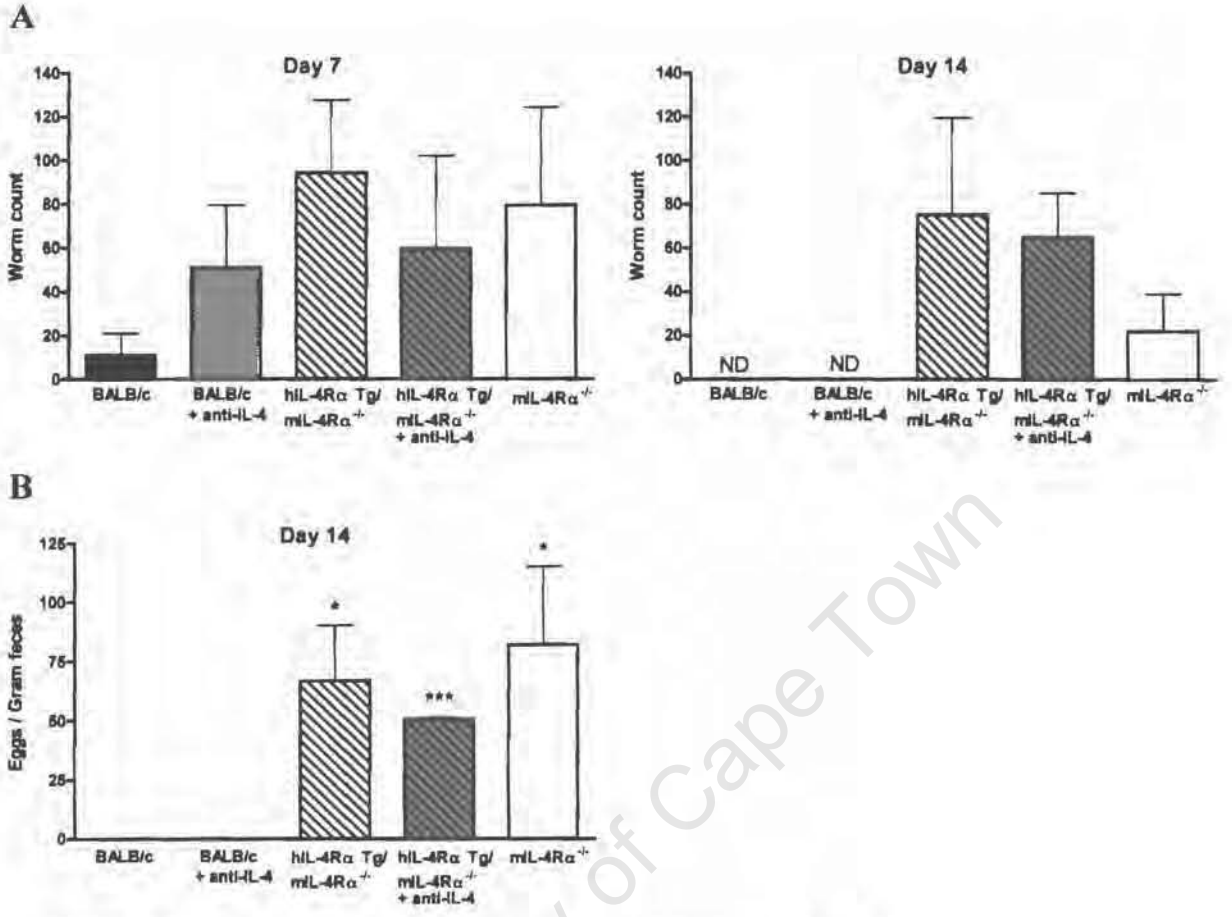

Figure 19. Expulsion of *N. brasiliensis* after neutralization of mIL-4.

BALB/c and hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice were injected intraperitoneal with anti-mIL-4 (+ anti-IL-4) and infected subcutaneously with *N. brasiliensis* one day later. Treated mice received a second dose of anti-mIL-4 at day 7 after infection. As control an untreated group of mIL-4Rα$^{-/-}$ was infected with *N. brasiliensis*. **A.** The number of worms in intestines of individual mice was counted on day 7 and day 14 post infection. **B.** The fecal eggs were counted daily in samples from single mice with the values for day 14 shown. The results are representative of 2 independent experiments with values representing mean ± SEM of 4 mice per group. Statistical differences compared to BALB/c values are indicated as * $p< 0.05$ and *** $p< 0.001$.

2.3.2 Antibody responses to *N. brasiliensis* after neutralization of mIL-4

To determine the effects of IL-4 neutralization on antibody responses in hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice, total IgG1, IgG2a, IgG2b and IgE production were analyzed. *N. brasiliensis*-infected mice were bled on day 14 post infection and their total serum antibodies measured by ELISA. There were no significant differences in the levels of

IgG1 between strains and anti-mIL-4 treatment had no observable effect on the production of this antibody (Fig. 20A). As previously demonstrated, total IgG2a was significantly higher in mIL-4Rα$^{-/-}$ mice compared to BALB/c mice (Fig. 20B). Anti-mIL-4 treatment had no effect on serum levels of IgG2a in BALB/c mice but increased levels in hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice so that it was significantly higher than in BALB/c. The levels of IgG2b were higher in hIL-4Rα Tg/mIL-4Rα$^{-/-}$ and mIL-4Rα$^{-/-}$ mice than in BALB/c animals but these differences did not reach statistical significance (Fig. 20C). The production of this antibody isotype was not affected by anti-mIL-4 treatment. Neutralization of mIL-4 had a more pronounced effect on the total IgE levels measured. The higher levels of IgE in both BALB/c and hIL-4Rα Tg/mIL-4Rα$^{-/-}$ strains were significantly decreased in animals treated with anti-mIL-4. As shown before no IgE was detected in the mIL-4Rα$^{-/-}$ mice (Fig. 20D). These results were consistent in two experiments and clearly indicate that IgE production observed in hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice is IL-4 dependent.

2.3.3 Effect of anti-mIL-4 treatment on cytokine production

The effect of mIL-4 neutralization on cytokine production was analyzed, to determine whether endogenous mIL-4 drives the Th2 responses observed in *N. brasiliensis*-infected transgenic mice. Purified CD4$^+$ T cells from pooled mesenteric lymph nodes of day 7 infected mice were restimulated with anti-CD3 for 72 hrs. The levels of IL-4, IL-5, IL-13 and IFN-γ were measured in cell supernatants by ELISA. Untreated BALB/c and hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice produced more IL-4, IL-5 and IL-13 than mIL-4Rα$^{-/-}$ mice while IFN-γ production was increased in both mIL-4Rα$^{-/-}$ and hIL-4Rα Tg/mIL-4Rα$^{-/-}$ strains (Fig. 21). The increased Th2 cytokine production in hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice corresponded to the previous findings. Anti-mIL-4 treatment reduced the levels of IL-4, IL-5 and IL-13 in BALB/c and hIL-4Rα Tg/mIL-4Rα$^{-/-}$ strains. IFN-γ production was slightly increased in BALB/c animals treated with anti-mIL-4 while being decreased in the hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice. These data show that anti-mIL-4 treatment reduces Th2 cytokine responses in transgenic mice confirming that endogenous mIL-4 signals through the chimeric receptor.

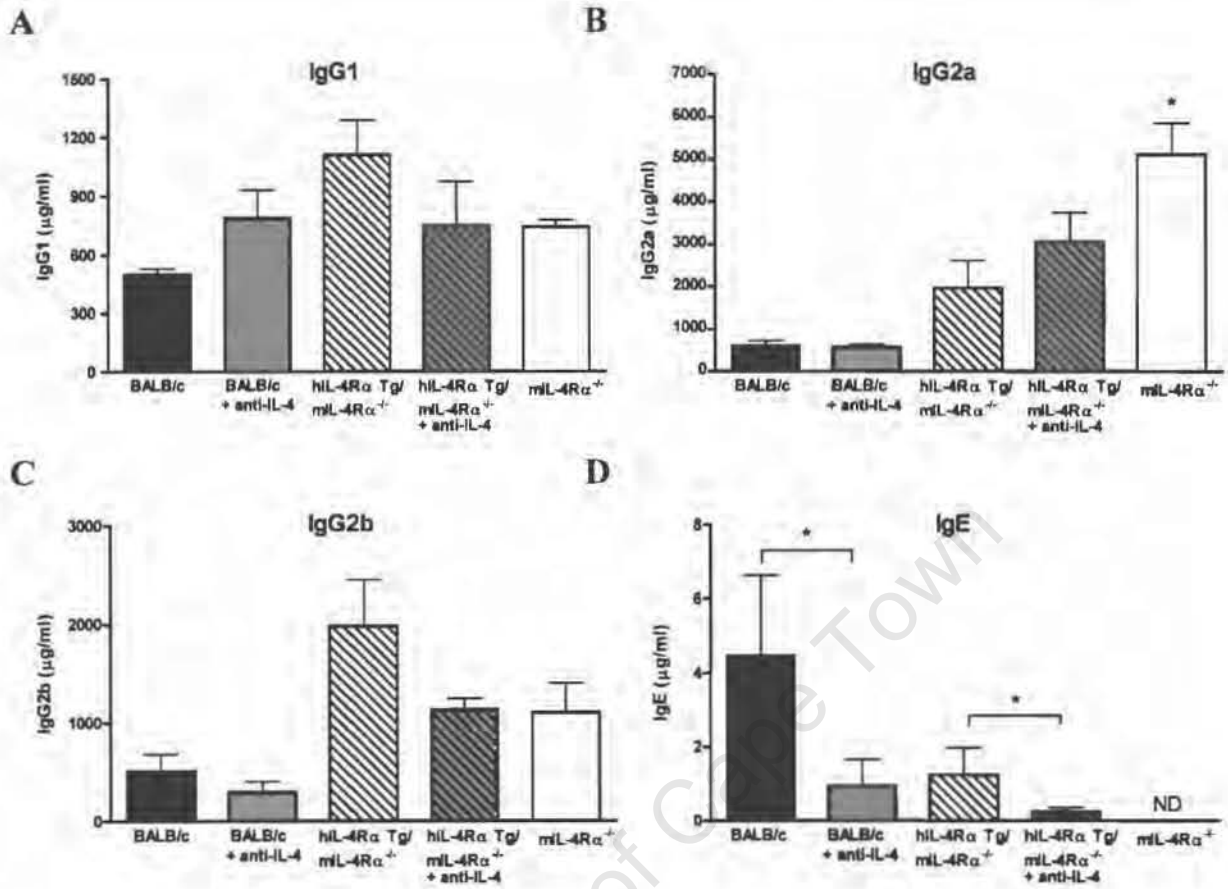

Figure 20. Antibody production in *N. brasiliensis*-infected mice treated with anti-mIL-4.

BALB/c, hIL-4Rα Tg/mIL-4Rα$^{-/-}$ and mIL-4Rα$^{-/-}$ mice were infected subcutaneously with 750 L3 stage *N. brasiliensis* larvae. Groups of BALB/c and hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice were treated with anti-mIL-4 one day prior to infection and at day 14 after infection (+ anti-IL-4). Sera samples were collected at day 14 post infection and the total IgG1, IgG2a, IgG2b and IgE antibodies measured by ELISA. The results are representative of 2 independent experiments with values representing the mean ± SEM of 4 mice per group. Unpaired Student *t* test was used to determine statistical differences compared to BALB/c mice or to untreated groups where indicated (* $p < 0.05$).

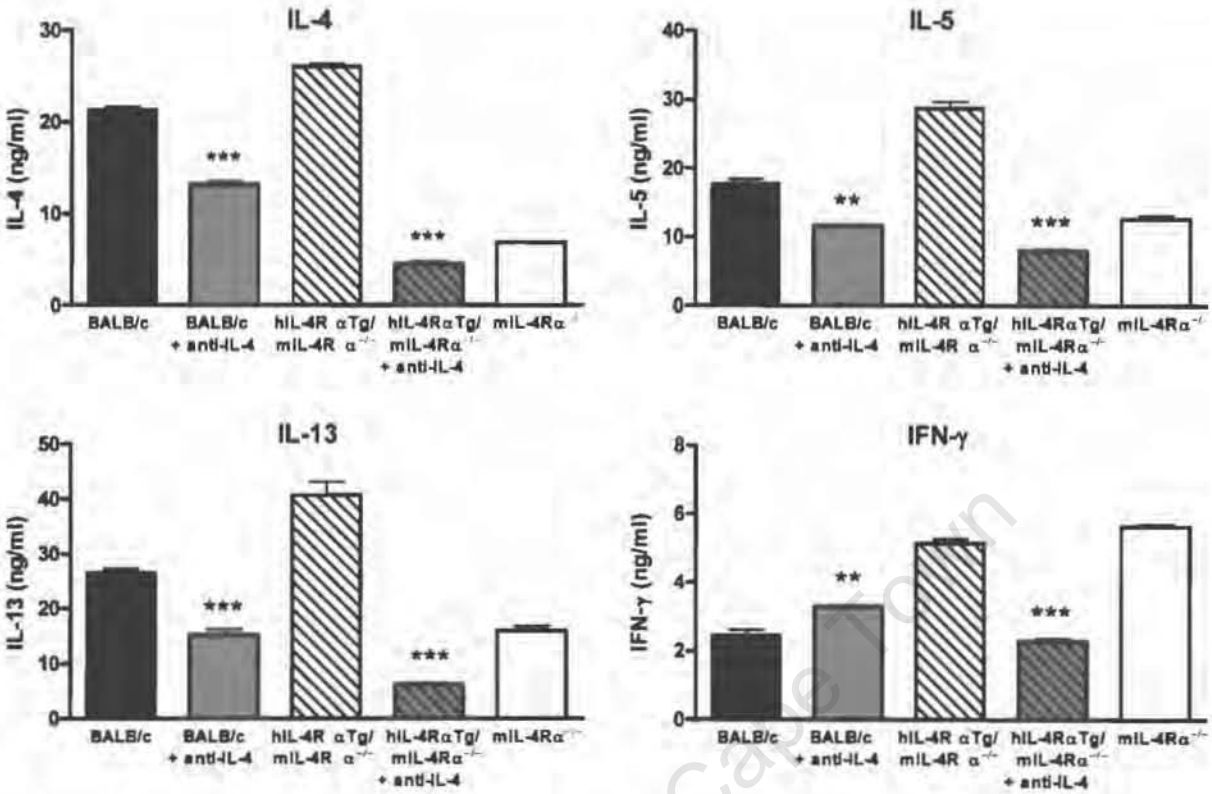

Figure 21. Cytokine production in anti-mIL-4 treated mice infected with *N. brasiliensis*

BALB/c, hIL-4Rα Tg/mIL-4Rα$^{-/-}$ and mIL-4Rα$^{-/-}$ mice were infected subcutaneously with 750 L3 stage *N. brasiliensis* larvae. Groups of BALB/c and hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice were treated with anti-mIL-4 one day prior to infection and at day 14 after infection (+ anti-IL-4). CD4^{+} T cells were purified from pooled mesenteric lymph nodes of day 7 infected mice (n = 4 mice per group) and restimulated with anti-CD3 for 72 hrs. The levels of IL-4, IL-5, IL-13 and IFN-γ in cell supernatants were measured by ELISA. The results are representative of 2 independent experiments with data representing mean ± SEM of triplicate cultures. Unpaired Student *t* test was used to determine statistical differences compared to untreated groups where indicated (** $p < 0.01$, *** $p < 0.001$).

3 *In vivo* analysis of hIL-4Rα Tg/ mIL-4Rα$^{-/-}$ /mIL-4$^{-/-}$ /mIL-13$^{-/-}$ mice

3.1 Generation and genotypic analysis of hIL-4Rα Tg/mIL-4Rα$^{-/-}$/ mIL-4$^{-/-}$ /mIL-13$^{-/-}$ mice

The mIL-4 neutralization studies indicated that mIL-4 is responsible for the increased IgE production and Th2 cytokine profile observed in *N. brasiliensis*-infected hIL-4Rα Tg/ mIL-4Rα$^{-/-}$ mice. In a genetic approach, to confirm that IL-4 signals through the chimeric receptor, hIL-4Rα Tg/ mIL-4Rα$^{-/-}$ mice were intercrossed with mIL-4/mIL-13 double-deficient mice. The use of mice that are also deficient in IL-13 further eliminates possible IL-13 signaling through the chimeric receptor. Both strains had previously been backcrossed onto the BALB/c background for more than 10 generations. The hIL-4Rα Tg/ mIL-4Rα$^{-/-}$ /mIL-4$^{-/-}$ /mIL-13$^{-/-}$ mice were identified by PCR analysis of DNA from tail biopsies (Fig. 22). The presence of a 522bp PCR product using hIL-4Rα primers and a 471bp product using mIL-4Rα knockout allele primers, as described before for the hIL-4Rα Tg/mIL-4Rα mice, was used as positive identification. To verify the deletion of IL-4 and IL-13 three primers were used. The amplification of an 80bp product indicated a wild-type mouse while the amplification of 100bp product identified mIL-4/mIL-13$^{-/-}$ mice. The amplification of both products are observed in DNA from mIL-4/mIL-13$^{-/+}$ mice. PCR products from hIL-4Rα Tg/ mIL-4Rα$^{-/-}$ /mIL-4$^{-/-}$ /mIL-13$^{-/-}$ mice contained the 100bp PCR product while PCR product from BALB/c mice contained the 80bp product.

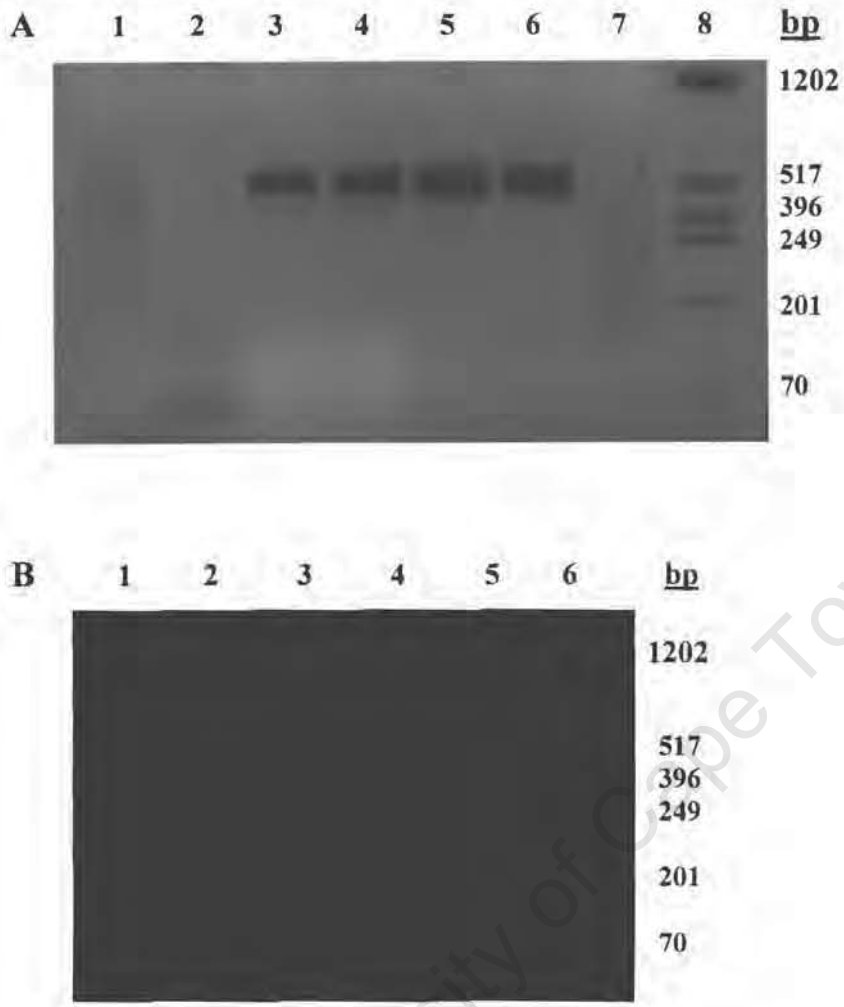

Figure 22. Genotyping of hIL-4RαTg/mIL-4Rα$^{-/-}$/mIL-4$^{-/-}$/mIL-13$^{-/-}$ mice

Four PCR reactions were used to identify hIL-4Rα Tg/mIL-4Rα$^{-/-}$/mIL-4$^{-/-}$/mIL-13$^{-/-}$ mice.
A. PCR reactions using primers to the 600bp wild-type allele, the 471bp knockout allele and the hIL-4Rα transgene. Lanes 1 and 2 contain PCR products from transgenic mice for wild-type allele; lanes 3 and 4 contain PCR products for knockout allele and lanes 5 and 6 contain PCR products for hIL-4Rα transgene. A DNA marker was loaded in lane 8 and a negative control in lanes 7. **B.** PCR reactions using primers to the mIL-4/IL-13 allele. Lanes 1 and 2 contain the PCR products from IL-4/IL-13$^{-/-}$ mice, lane 3 contains DNA from a hIL-4RαTg/mIL-4Rα$^{-/-}$/mIL-4$^{-/-}$/mIL-13$^{-/-}$ mouse and lane 4 contains PCR products from WT BALB/c mice. A DNA marker was loaded in lane 6 and a negative control in lane 5. The sizes (in bp) of the DNA marker are indicated on the right of each gel.

3.2 Immune response to *N. brasiliensis* infection

3.2.1 Worm expulsion

To determine whether IL-4 and IL-13-deficiency had an effect on the immune response elicited in transgenic mice, BALB/c, hIL-4Rα Tg/mIL-4Rα$^{-/-}$, mIL-4Rα$^{-/-}$ and hIL-4Rα Tg/ mIL-4Rα$^{-/-}$ /mIL-4$^{-/-}$ /mIL-13$^{-/-}$ mice were infected with 750 L3 stage *N. brasiliensis* larvae. BALB/c mice had intestinal worms present at day 7 p.i. but had expelled all worms by day 14 (Fig. 23). In contrast, hIL-4Rα Tg/mIL-4Rα$^{-/-}$, mIL-4Rα$^{-/-}$ and hIL-4Rα Tg/mIL-4Rα$^{-/-}$ /mIL-4$^{-/-}$ /mIL-13$^{-/-}$ mice were unable to expel the parasites by this time point. As observed with the mIL-4 neutralization, the absence of mIL-4 and mIL-13 had no effect on worm expulsion in mice containing the hIL-4Rα transgene.

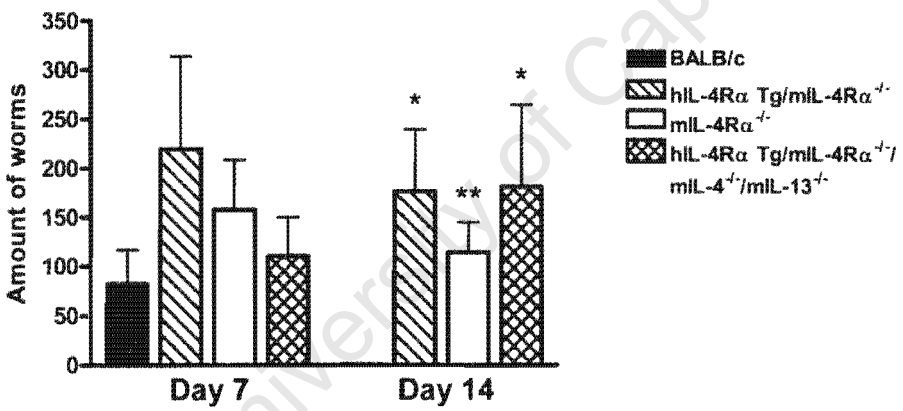

Figure 23. Worm expulsion of *N. brasiliensis* in hIL-4Rα Tg/mIL-4Rα$^{-/-}$ /mIL-4$^{-/-}$ /mIL-13$^{-/-}$ mice.

BALB/c, hIL-4Rα Tg/mIL-4Rα$^{-/-}$, mIL-4Rα$^{-/-}$ and hIL-4Rα Tg/mIL-4Rα$^{-/-}$ /mIL-4$^{-/-}$ /mIL-13$^{-/-}$ mice were infected with 750 L3 stage *N. brasiliensis* larvae. The numbers of worms in intestines of individual mice were counted on day 7 and day 14 post infection. Values represent mean ± SEM of 4 mice per group. Statistical differences compared to BALB/c values are indicated as * $p< 0.05$ and ** $p< 0.01$.

3.2.2 Antibody response to *N. brasiliensis* infection

Total IgG1, IgG2b and IgE levels were measured in sera of *N. brasiliensis*-infected animals to determine the effect of endogenous mIL-4 or IL-13 on antibody production in hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice. Importantly, hIL-4Rα Tg/mIL-4Rα$^{-/-}$ /mIL-4$^{-/-}$ /mIL-13$^{-/-}$ mice had no detectable levels of IgE (Fig. 24). The absence of mIL-4 and mIL-13 in these mice resulted in a deficiency to generate IgE as was observed for mIL-4Rα$^{-/-}$ mice. Both BALB/c and hIL-4Rα Tg/mIL-4Rα$^{-/-}$ strains showed an increase in IgE and IgG1 at day 14 p.i. whereas these antibodies were either low or undetectable in mIL-4Rα$^{-/-}$ and hIL-4Rα Tg/mIL-4Rα$^{-/-}$ /mIL-4$^{-/-}$ /mIL-13$^{-/-}$ mice. On the other hand, hIL-4Rα Tg/mIL-4Rα$^{-/-}$, mIL-4Rα$^{-/-}$ and hIL-4Rα Tg/mIL-4Rα$^{-/-}$ /mIL-4$^{-/-}$ /mIL-13$^{-/-}$ mice responded with significantly higher type 1 IgG2b antibodies. These data confirm that mIL-4 or mIL-13 signals through the chimeric receptor in B lymphocytes of transgenic mice.

3.2.3 Cytokine production

To confirm that hIL-4Rα Tg/mIL-4Rα$^{-/-}$ /mIL-4$^{-/-}$ /mIL-13$^{-/-}$ mice made no detectable levels of IL-4 or IL-13 during a *N. brasiliensis* infection, cytokine production was measured at day 7 p.i. CD4^{+} T cells were purified from pooled mesenteric lymph nodes of infected mice and restimulated with anti-CD3 for 72hrs. The levels of IL-4 and IL-13 in cell supernatants were determined by ELISA. As observed previously, BALB/c and hIL-4Rα Tg/ mIL-4Rα$^{-/-}$ mice made higher amounts of IL-4 compared to mIL-4Rα$^{-/-}$ mice (Fig. 25). IL-13 levels were higher in BALB/c mice while being similarly reduced in hIL-4Rα Tg/ mIL-4Rα$^{-/-}$ and mIL-4Rα$^{-/-}$ mice. As expected, hIL-4Rα Tg/mIL-4Rα$^{-/-}$ /mIL-4$^{-/-}$ /mIL-13$^{-/-}$ mice showed undetectable levels of both IL-4 and IL-13 confirming a deficiency in the expression of these cytokines.

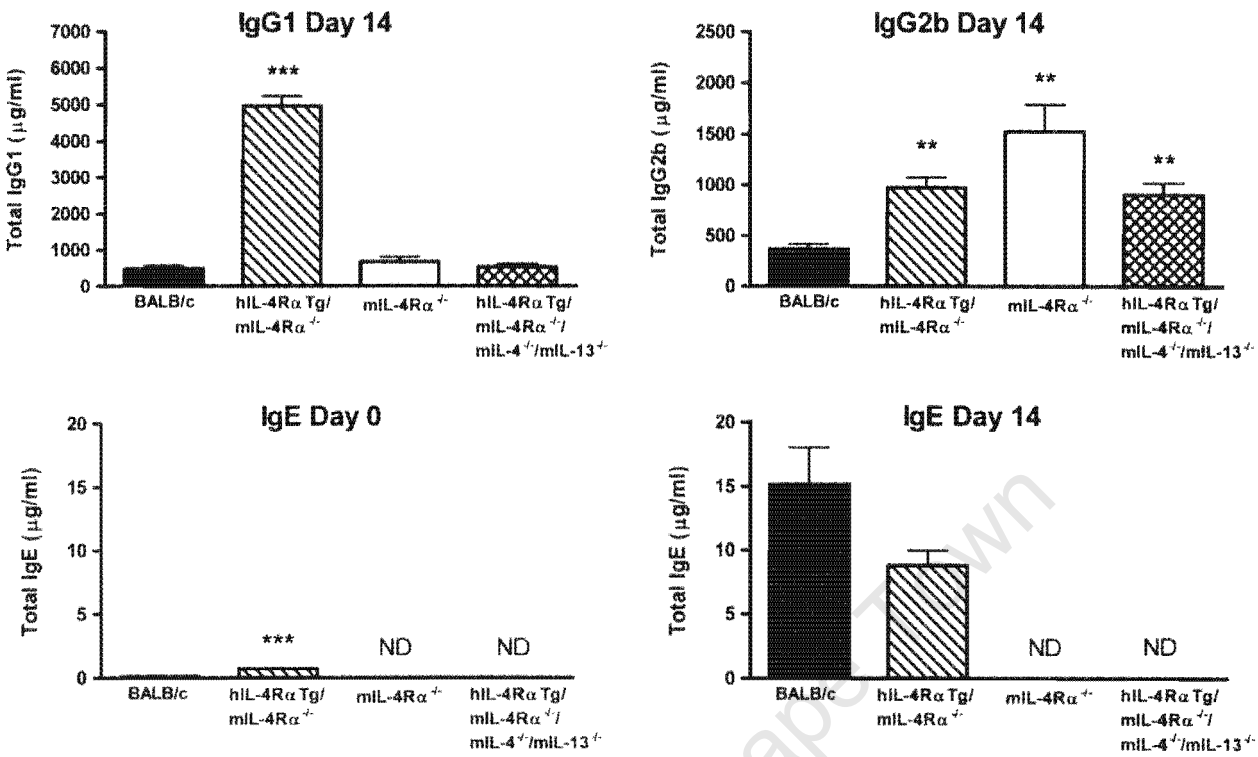

Figure 24. Antibody responses in hIL-4Rα Tg/mIL-4Rα$^{-/-}$ /mIL-4$^{-/-}$ /mIL-13$^{-/-}$ mice infected with *N. brasiliensis*

BALB/c, hIL-4Rα Tg/mIL-4Rα$^{-/-}$, mIL-4Rα$^{-/-}$ and hIL-4Rα Tg/ mIL-4Rα$^{-/-}$ /mIL-4$^{-/-}$ /mIL-13$^{-/-}$ mice were infected with 750 L3 stage *N. brasiliensis* larvae. Sera samples were collected at day 0 and day 14 post infection and the total IgG1, IgG2b and IgE antibodies measured by ELISA. Values represent mean ± SEM of 4 mice per group. Statistical differences compared to BALB/c values are indicated as ** $p < 0.01$ and *** $p < 0.001$.

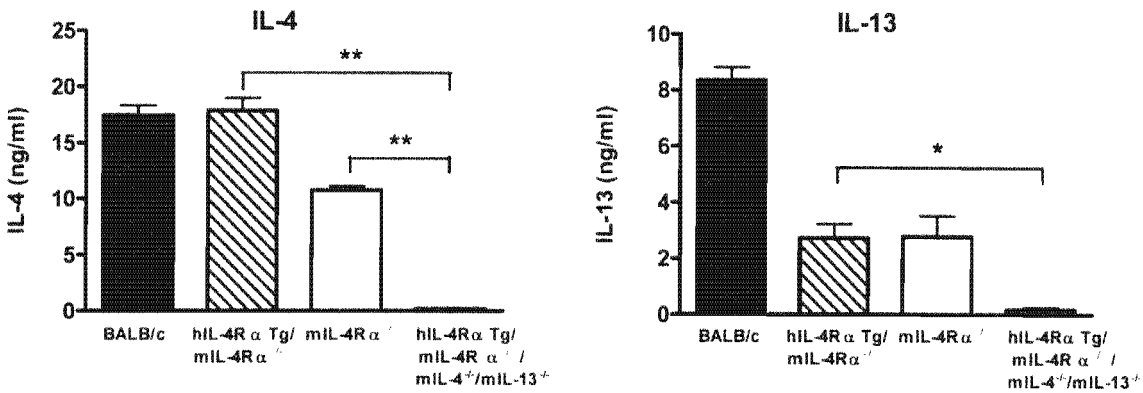

Figure 25. Cytokine production of CD4$^+$ T cells from hIL-4Rα Tg/mIL-4Rα$^{-/-}$ /mIL-4$^{-/-}$ /mIL-13$^{-/-}$ mice infected with *N. brasiliensis*

CD4$^+$ T cells were purified from pooled mesenteric lymph nodes of day 7 *N. brasiliensis*-infected mice (n = 4 mice per group). Cells were restimulated with anti-CD3 for 72 hrs and the levels of IL-4 and IL-13 in cell supernatants measured by ELISA. Data represent mean ± SEM of triplicate cultures. Statistical differences between groups are indicated as * $p< 0.05$ and ** $p< 0.01$.

3.3 Immune response to *L. major* infection

3.3.1 Footpad lesion development after infection with *L. major*

To determine whether the absence of mIL-4 and mIL-13 rendered hIL-4Rα Tg/ mIL-4Rα$^{-/-}$ mice resistant to *L. major* infection, BALB/c, C57BL/6, hIL-4Rα Tg/mIL-4Rα$^{-/-}$, mIL-4Rα$^{-/-}$ and hIL-4Rα Tg/mIL-4Rα$^{-/-}$ /mIL-4$^{-/-}$ /mIL-13$^{-/-}$ mice were infected with $2x10^6$ *L. major* promastigotes in the hind footpads. Due to time constraints mice were infected with the more virulent *L. major* substrain MOHM/IL/81/FEBNI. BALB/c mice infected with this strain develop more rapid footpad swelling compared to mice infected with *L. major* LV 39, MRHO/Sv/59/P (Biederman et al., 2001). Disease progression was followed by weekly footpad measurements. Surprisingly, hIL-4Rα Tg/ mIL-4Rα$^{-/-}$ /mIL-4$^{-/-}$ /mIL-13$^{-/-}$ mice were as susceptible to *L. major* infection as hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice indicating that the presence of the hIL-4Rα transgene is sufficient to induce susceptibility in these mice (Fig. 26).

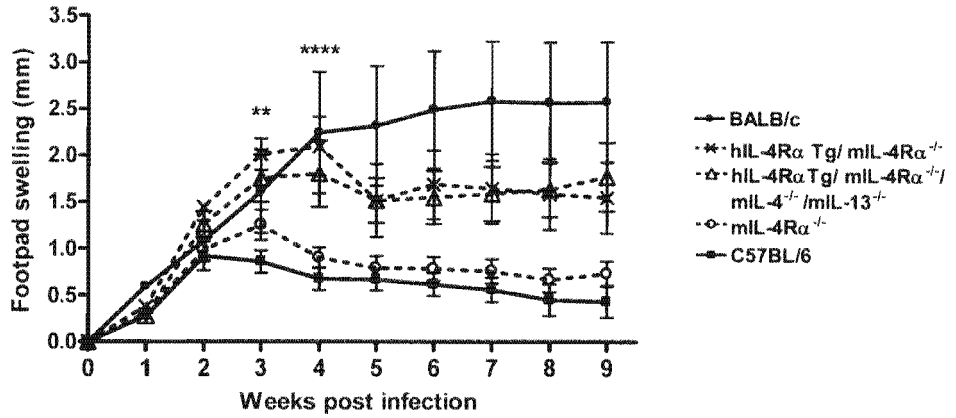

Figure 26. Footpad lesion development in the absence of IL-4 and IL-13 during *L. major* infection.

BALB/c, C57BL/6, hIL-4Rα Tg/mIL-4Rα$^{-/-}$, mIL-4Rα$^{-/-}$ and hIL-4Rα Tg/mIL-4Rα$^{-/-}$ /mIL-4$^{-/-}$ /mIL-13$^{-/-}$ mice were infected with 2x10^{6} *L. major* MOHM/IL/81/FEBNI promastigotes in the hind footpads. Lesion development was monitored weekly and footpad swelling calculated as the difference between infected and uninfected footpads. Data represents the mean footpad swelling ± SEM of 8 mice per group. For each group 4 mice were killed at week 4 p.i. and the rest at week 9 p.i. The occurrence of ulceration and necrosis from individual mice is indicated with an asterisk (*).

3.3.2 Parasite burdens of transgenic mice in the absence of IL-4 and IL-13

To determine whether the increased footpad swelling in IL-4/IL-13-deficient transgenic mice correlated with an inability to control parasite growth, parasite burdens were determined in infected draining popliteal lymph nodes at week 4 and week 9 p.i. As observed previously, the parasite burdens in lymph nodes after 4 weeks of infection were similar in BALB/c, mIL-4Rα and both hIL-4Rα transgenic strains (data not shown). Consistent with the lesion development, BALB/c lymph nodes contained high numbers of parasites at week 9 p.i., while C57BL/6 mice showed no parasites at this time point (Fig. 27). The parasite burdens in the two hIL-4Rα transgenic strains were equally enhanced and were significantly higher than that observed for mIL-4Rα$^{-/-}$ mice. As reported previously, mIL-4Rα$^{-/-}$ mice harbored more parasites in lymph nodes compared to C57BL/6 mice. These data confirm that transgenic mice, even in the absence of mIL-4 and mIL-13, are unable to control *L. major* infection.

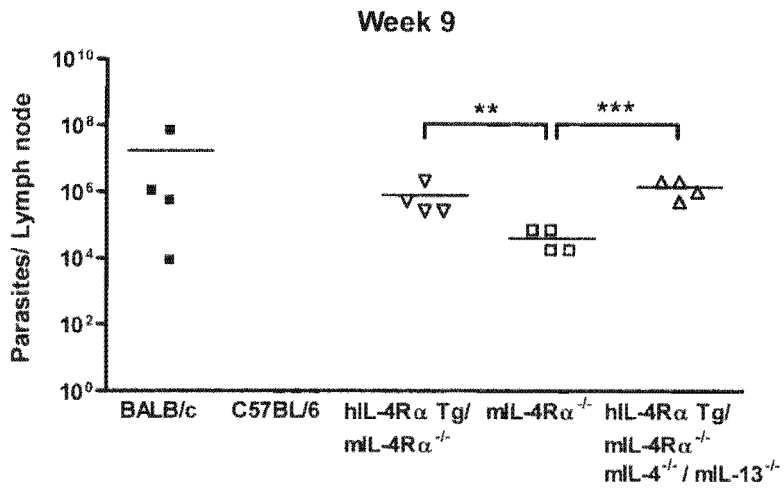

Figure 27. Parasite loads in lymph nodes of *L. major*-infected mice.

Single cell suspensions were prepared of lymph nodes from individual *L. major*-infected mice (n = 3-4 mice per strain) at week 9 post infection and limiting dilution assays performed. After 7 days of culture the dilutions were inspected for viable parasites. Each data point represents the parasite burden in the lymph node of a single mouse with the bars indicating arithmetic means. Statistical differences compared to mIL-4Rα$^{-/-}$ mice are indicated as ** $p < 0.01$ and *** $p < 0.001$.

3.3.3 Antibody responses in mice infected with *L. major*

The data above showed that the lack of mIL-4 and mIL-13 in transgenic mice did not affect susceptibility to *L. major* infection. To determine whether antibody responses were influenced by the mIL-4/mIL-13-deficiency, *L. major*-specific IgG1 and IgG2a, and total IgE antibodies were analyzed. The absence of endogenous mIL-4 and mIL-13 completely abrogated total IgE and *L. major*-specific IgG1 responses observed for hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice (Fig. 28). The increased IgG2b production remained unchanged by the deficiency for IL-4 and IL-13. These data confirm the findings from the *N. brasiliensis* infection, showing that endogenous mIL-4 or mIL-13 is responsible for the type 2 immune responses in transgenic mice. This unexpected *in vivo* responsiveness of the hIL-4Rα chimeric receptor to mIL-4 or mIL-13 does not explain the susceptibility of these mice to *L. major* infection.

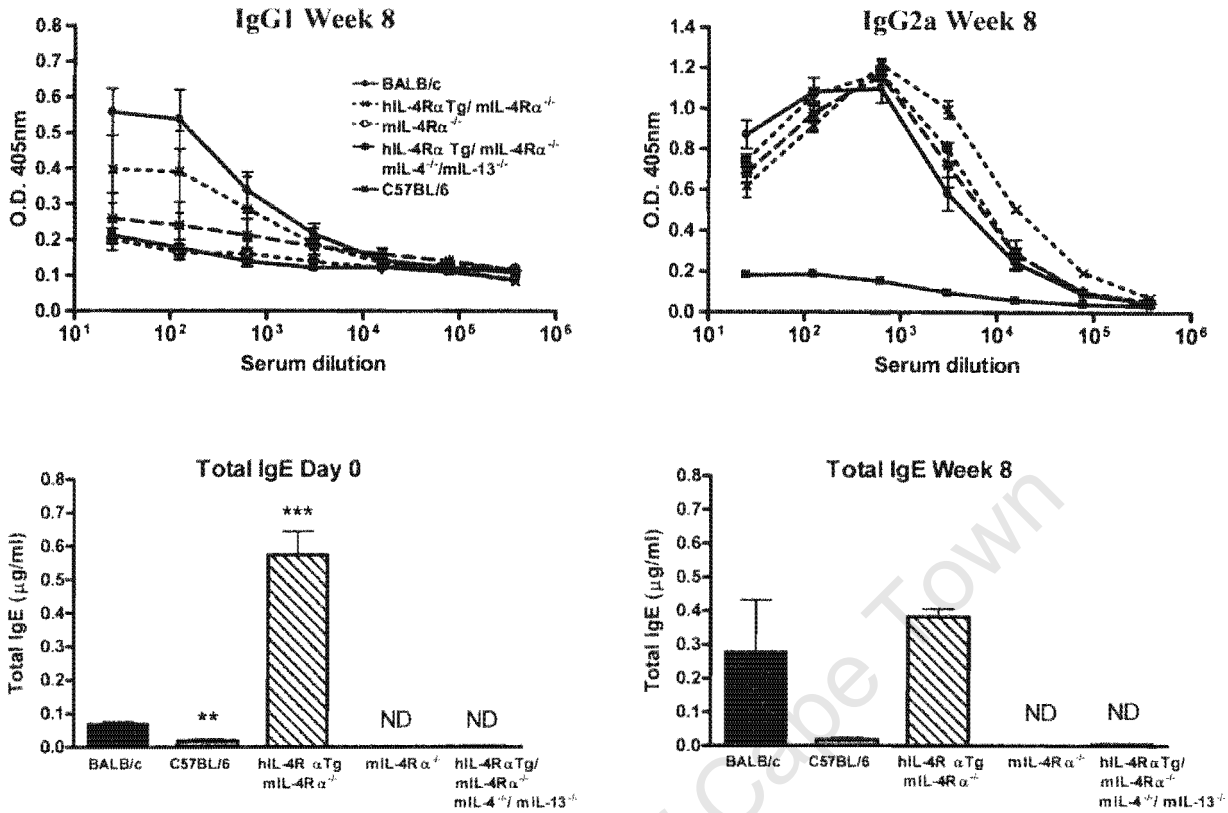

Figure 28. Antibody responses in hIL-4Rα Tg/mIL-4Rα$^{-/-}$ /mIL-4$^{-/-}$ /mIL-13$^{-/-}$ mice infected with *L. major*

BALB/c, C57BL/6, hIL-4Rα Tg/mIL-4Rα$^{-/-}$, mIL-4Rα$^{-/-}$ and hIL-4Rα Tg/mIL-4Rα$^{-/-}$ /mIL-4$^{-/-}$ / mIL-13$^{-/-}$ mice were infected with 2x10^6 *L. major* MOHM/IL/81/FEBNI promastigotes in the hind footpads. Sera samples were collected at day 0 and week 8 post infection and the levels of *L. major*-specific IgG1 and IgG2a antibodies as well as total IgE measured by ELISA. Data represents mean ± SEM of 4 mice per group. Statistical differences compared to BALB/c values are indicated as ** $p < 0.01$ and *** $p < 0.001$.

Discussion

Discussion

Transgenic BALB/c mice expressing human IL-4Rα under the control of an intronic enhancer element of the Ig H chain (Eμ) locus promoter, were recently generated to study the temporal role of IL-4 signaling in Th2 differentiation *in vitro* (Seki et al., 2004). The Eμ element functions in B and T cells (Grosschedl et al., 1984; Kemp et al., 1980), and in transgenic mice, linkage of Eμ to genes (such as chloramphenicol acetyl transferase or Thy-1) has been shown to provide B and T cell-specific expression (Chen et al., 1987; Reik et al., 1987). In the study by Seki et al (2004) the hIL-4Rα gene linked to Eμ was introduced into the germline of BDF1 mice and backcrossed with BALB/c mice, resulting in lymphocyte-specific expression of hIL-4Rα on a BALB/c background. In lymphocytes, hIL-4Rα may interact with mγc to form a functional chimeric type I IL-4R (Andrews et al., 2001). Furthermore, due to the species-specific binding of murine and human IL-4 to their respective receptors, the hIL-4Rα/mγc chimeric receptor may be responsive to hIL-4 but not mIL-4 (Lowenthal et al., 1988; Park et al., 1987a; 1987b). The species-specificity and function of the hIL-4Rα/mγc chimeric receptor in hIL-4Rα Tg mice were confirmed by the hIL-4-induced proliferation of B and T cells from transgenic mice. On the other hand, cells from BALB/c mice could not proliferate in response to hIL-4 (Seki et al., 2004). In addition, hIL-4 stimulated the Th2 differentiation of $CD4^+$ T from hIL-4Rα Tg mice similarly to mIL-4-induced Th2 differentiation of $CD4^+$ T cells from BALB/c mice. In order to eliminate signaling via mIL-4 in hIL-4Rα Tg mice and generate a fully hIL-4 inducible system, hIL-4Rα Tg mice were backcrossed to mIL-4Rα$^{-/-}$ BALB/c mice. The resulting hIL-4Rα Tg/ mIL-4Rα$^{-/-}$ mice allowed for the Th2 differentiation of TCR activated $CD4^+$ T cells only when stimulated with hIL-4. It was demonstrated that hIL-4 added to $CD4^+$ T cells in the initial 48 hrs after TCR activation resulted in Th2 lineage commitment. Delaying hIL-4 treatment to later time points resulted in impaired Th2 differentiation, clearly showing temporal restrictions on IL-4-induced GATA3 expression, and induction of Th2 differentiation *in vitro*. Comparable results were observed in cells from hIL-4Rα Tg/ mIL-4Rα$^{-/-}$ mice and anti-mIL-4 treated cells from hIL-4Rα Tg mice. In that study it was demonstrated that the

hIL-4Rα/mγc chimeric receptor in hIL-4Rα Tg/ mIL-4Rα$^{-/-}$ mice functions in an inducible and species-specific manner *in vitro*.

In the present study we characterized the hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mouse model as an applicable *in vivo* model for studying the temporal role of lymphocyte-specific IL-4 signaling in Th2 differentiation and disease outcome. Although IL-4 acting via STAT6 signaling is critical for Th2 differentiation *in vitro* (Le Gros et al., 1990; Swain et al., 1990), the role of IL-4 for Th2 development *in vivo* is more complicated and controversial. Early studies using anti-IL-4 antibodies suggested a requisite role for IL-4 in mediating Th2 differentiation (Saddick et al., 1990; Heinzel et al., 1991; Fernandez-Botran et al., 1986). However, studies with IL-4Rα$^{-/-}$ or STAT6$^{-/-}$ mice indicated that Th2 responses can be induced independently of IL-4 and IL-13 (Mohrs et al., 1999; Brewer et al., 1999; Urban et al., 1998).
This inducible *in vivo* hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mouse model may firstly, elucidate the role of lymphocyte-specific IL-4 signaling for Th2 differentiation at different time points after infection. Secondly, the requirement for lymphocyte-specific IL-4 signaling for disease progression, in the absence of IL-4Rα in non-lymphocytes could be investigated. Thirdly, the inducible reconstitution of IL-4R could indicate when IL-4 signaling in lymphocytes plays a role in disease outcome and development of type 2 responses.

Briefly, this study demonstrated that the lymphocyte-specific hIL-4Rα/mγc chimeric receptor functions as a hIL-4 inducible system *in vitro*, as described previously (Seki et al., 2004). However, *in vivo* studies using *L. major* and *N. brasiliensis* infection models demonstrated that endogenous mIL-4 could signal through the chimeric receptor in the presence or absence of hIL-4. Neutralization of mIL-4 or the genetic deletion of mIL-4 and mIL-13 abrogated the signaling of the chimeric receptor in transgenic mice.

Before establishing the *in vivo* characteristics of the hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mouse model, *in vitro* analysis was performed to confirm the lymphocyte-specific expression of hIL-4Rα and the induction of IL-4-mediated responses in lymphocytes by hIL-4. Genotypic analysis of hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice confirmed the disruption of mIL-4Rα

and the presence of the hIL-4Rα transgene. Phenotypic analysis showed that hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice did not express mouse IL-4Rα but did express hIL-4Rα in the lymphocyte populations, CD4$^+$, CD8$^+$ T cells, and CD19$^+$ B cells. However, hIL-4Rα surface expression was not observed in all lymphocytes. About 62% of CD3$^+$CD4$^+$ T cells, 69% of CD3$^+$CD8$^+$ T cells and 37% of CD19$^+$ B cells expressed hIL-4Rα. The non-lymphocyte populations NK cells, granulocytes and macrophages showed no surface expression of hIL-4Rα but some hIL-4Rα expression was detected in 8% of dendritic cells and 9% of NK cells. In the study by Seki et al (2004), the investigators stated that the hIL-4Rα transgenic line selected expressed hIL-4R similarly to mIL-4R in B and T cells. However, it was not specified how expression was quantified and it is possible that their expression studies were done under different conditions. Furthermore, the study was restricted to lymphocyte populations and no mention was made about hIL-4Rα expression in non-lymphocyte populations. It is not surprising that hIL-4Rα is expressed differently to mIL-4Rα as the hIL-4Rα transgene is under the control of Eμ and not the mIL-4Rα promoter. Most studies using Eμ-linked transgenes show no detectable levels of the transgenes in non-lymphocyte populations (Moroy et al., 1990). Some low levels of transgene expression in mice with an Eμ-controlled N-myc transgene were attributed to infiltrating lymphocytes in the organs studied (Dildrop et al., 1989). Another study showed that an Eμ-linked transplantation antigen gene (H-2Kb) was not only expressed in B and T cells but also in a subset of splenic epithelium (Allison et al., 1990).

Our functional analysis confirmed the lymphocyte-specific expression and function of hIL-4Rα, as described before (Seki et al., 2004) et al). Stimulation with hIL-4 resulted in proliferation of lymphocytes and Th2 differentiation of murine CD4$^+$ T cells while mouse IL-4 had no effect. The lack of IL-13 signaling confirmed previous findings that mouse T and B cells do not respond to IL-13 (Zurawski et al., 1993), and that hIL-4Rα can not form a functional type II IL-4R with mIL-13Rα1 (Andrews et al., 2001).

IL-4 and IL-13 are able to suppress IFNγ/LPS-induced NO production of BALB/c macrophages by an IL-4Rα-dependent mechanism (Doherty et al., 1993; Doyle et al., 1994; Modolell et al., 1995). Macrophages from IL-4Rα$^{-/-}$ mice show no suppression of NO production by IL-4 and IL-13. In our studies, the failure of IL-4 and IL-13 to

suppress NO production and induce arginase I activity in the non-lymphocyte cell type macrophages verified the functional deletion of mIL-4Rα. The inability of macrophages from transgenic mice to respond to mIL-4 and mIL-13 corresponded to the impairment of alternatively activated macrophages in macrophage/neutrophil-specific IL-4Rα$^{-/-}$ mice (Herbert et al., 2004). Those studies also demonstrated that the deficiency of IL-4Rα in macrophages resulted in impaired suppression of NO by IL-4 and IL-13.

For the *in vivo* studies two well established disease models were chosen: 1) the *Leishmania major* model and 2) the *Nippostrongylus brasiliensis* model. Infection of BALB/c mice with either of these pathogens induces a strong Th2/type 2 response and important roles for IL-4Rα in *L. major* and *N. brasiliensis* infections are well characterized (Mohrs et al., 1999; Barner et al., 1998; Urban et al., 1998). These models are ideal to test the functional species-specificity and inducibility of the chimeric IL-4 receptor, and investigate the importance of lymphocyte-specific IL-4 signaling during the course of infection.

The *in vitro* data clearly showed that hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice were deficient for mIL-4Rα and that recombinant mIL-4 alone did not stimulate lymphocytes from transgenic mice through the chimeric receptor. We therefore expected transgenic mice to behave as the littermate control mIL-4Rα$^{-/-}$ mice *in vivo* in the absence of hIL-4. However, in both *L. major* and *N. brasiliensis* infections hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice responded differently to infection compared to mIL-4Rα$^{-/-}$ mice, clearly demonstrating that the mouse model does not function *in vivo* as expected. The major differences observed between hIL-4Rα Tg/mIL-4Rα$^{-/-}$ and mIL-4Rα$^{-/-}$ mice, were enhanced Th2 cytokine and IgE production by *L. major* or *N. brasiliensis*-infected transgenic mice, compared to reduced Th2 cytokine levels and undetectable IgE in mIL-4Rα$^{-/-}$ mice. Furthermore, hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice were as susceptible to *L. major* infection as BALB/c mice while mIL-4Rα$^{-/-}$ mice showed increased resistance compared to BALB/c mice. Treatment of transgenic mice with hIL-4 at the doses described in this study had no effect on susceptibility or antibody responses to *L. major* infection.

Expulsion of *N. brasiliensis* by BALB/c mice is critically dependent on IL-4Rα expression on non-bone marrow derived cells (Urban et al., 2001). In our studies hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice were unable to expel *N. brasiliensis* worms as was observed for mIL-4Rα$^{-/-}$ mice. These data confirmed previous findings that mIL-4Rα$^{-/-}$ mice show impaired worm expulsion (Barner et al., 1998; Urban et al., 1998) and that IL-4Rα in non-lymphocytes is required for worm expulsion (Urban et al., 2001). This was also recently shown in T cell-specific IL-4Rα$^{-/-}$ mice where the lack of IL-4Rα in CD4^{+} T cells had no effect on *N. brasiliensis* expulsion (Hoving et al., unpublished data). The inability of hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice to expel worms in our studies furthermore indicated that the problem with our *in vivo* hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mouse model lies not in the mIL-4Rα-deficiency. Our studies further demonstrated that even in the presence of a strong Th2/type 2 response, *N. brasiliensis* worms could not be expelled normally in the absence of IL-4Rα in non-lymphocytes.

The strong Th2 response in *N. brasiliensis*-infected transgenic mice, in the absence of hIL-4, was unexpected. Previous studies with mIL-4Rα$^{-/-}$ and mIL-4/IL-13$^{-/-}$ mice clearly demonstrated IL-4/IL-13-dependent Th2 development during *N. brasiliensis* infection (Barner et al., 1998; McKenzie et al., 1999). In the absence of IL-4/IL-13 signaling the production of Th2 cytokines were reduced while IFN-γ was elevated. The reduced IL-4 and increased IFN-γ production by anti-CD3 stimulated CD4^{+} T cells from mIL-4Rα$^{-/-}$ mice in our studies confirmed the requirement of IL-4Rα for Th2 development. However, similar levels of IL-5 and IL-13 were observed for BALB/c and mIL-4Rα$^{-/-}$ mice. This difference between our data and a previous study (Barner et al., 1998) may be due to the longer 72 hrs anti-CD3 stimulation in our studies compared to 48 hrs in theirs. In contrast to mIL-4Rα$^{-/-}$ mice, CD4^{+} T cells from transgenic mice produced enhanced IL-4, IL-5 and IL-13 with lower IFN-γ. This suggested that the hIL-4Rα/mγc chimeric receptor in transgenic mice signaled *in vivo* in *N. brasiliensis* infection and that endogenous mIL-4 could be responsible for the IL-4R signaling.

In accordance with our observations in the *N. brasiliensis* model, transgenic mice produced elevated Th2 cytokines during *L. major* infection compared to BALB/c mice. Th2 development might be expected in transgenic mice in the *L. major* disease model as studies using mIL-4Rα$^{-/-}$ mice demonstrated that *L. major* infection can induce Th2

differentiation independent of IL-4 and IL-13 signaling (Noben-Trauth et al., 1999; Mohrs et al., 2000). Indeed, Th2 development in the absence of IL-4Rα in T cells was recently demonstrated using $CD4^+$ T cell-specific IL-4Rα$^{-/-}$ mice (Radwanska et al., unpublished data). However, IL-4 and IL-13-dependent mechanisms may also influence Th2 development and disease outcome. Here, we showed that anti-CD3 stimulated lymph node cells from mIL-4Rα$^{-/-}$ mice produced lower Th2 cytokines compared to BALB/c mice. A reduction in Th2 cytokines by anti-CD3 stimulated cells from mIL-4Rα$^{-/-}$ mice was also shown by others (Noben-Trauth et al., 1999; Mohrs et al., 2000). However, despite this impaired Th2 cytokine production by mIL-4Rα$^{-/-}$ mice, hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice produced elevated Th2 cytokines. This confirmed that the hIL-4Rα/myc chimeric receptor signals *in vivo* during *L. major* infection.

These *in vivo* data clearly showed that in both *L. major* and *N. brasiliensis* infection models, hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice produced Th2 responses that were not observed in mIL-4Rα$^{-/-}$ mice. We could conclude that the chimeric receptor in transgenic mice was able to signal *in vivo* in T lymphocytes.

The enhanced IgE and IgG1 production in *L. major* or *N. brasiliensis*-infected transgenic mice, in the absence of hIL-4, supported the notion that endogenous mIL-4 was responsible for the signaling of the chimeric receptor observed *in vivo*. *N. brasiliensis*-infected IL-4Rα$^{-/-}$ mice produced significantly lower amounts of IgG1 and undetectable IgE antibodies, as reported previously (Barner et al., 1998; Urban et al., 1998). IgE production absolutely requires IL-4 signaling and others have shown that in the absence of IL-4, STAT6 or IL-4Rα, *N. brasiliensis*-infected mice produced no detectable IgE antibodies (Kuhn et al., 1991; Urban 1998). The high levels of IgG1 and IgE production in hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice in contrast to the low IgG1 and undetectable IgE in mIL-4Rα$^{-/-}$ mice indicated that endogenous mIL-4 signaled through the chimeric IL-4R in B lymphocytes *in vivo*. Similarly, *L. major*-infected hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice produced high *L.major*-specific IgG1 and total IgE antibodies, in the presence or absence of hIL-4. In *L major* infection, type 2 IgG1 and IgE antibody responses strictly require IL-4 signaling and are abrogated in both IL-4$^{-/-}$ and IL-4Rα$^{-/-}$ mice (Mohrs et al., 1999; 2000). The impairment of IgG1 and IgE production in mIL-4Rα$^{-/-}$ mice in our *L. major* infection

studies confirmed the requirement for IL-4Rα for type 2 antibody responses. The high type 2 responses in *L. major*-infected transgenic mice clearly indicated that the chimeric receptor in B lymphocytes was responsive to mIL-4 *in vivo*.

The observed increased Th2/type 2 responses suggested that endogenous mIL-4 could bind to the hIL-4Rα/myc chimeric receptor *in vivo* resulting in signaling. Due to the short transit time of *N. brasiliensis* through the host and the rapid development of Th2 responses (Finkelman et al., 1997), this model was chosen to investigate whether neutralization of mIL-4 would abrogate Th2/type 2 responses in transgenic mice. In our study, treatment of hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice with anti-mIL-4 antibodies during *N. brasiliensis* infection resulted in decreased Th2 cytokine production and IgE antibodies. IgE production was not completely blocked but this could be attributed to insufficient mIL-4 neutralization resulting in low levels of mIL-4 still present after anti-mIL-4 treatment. These data clearly indicated that mIL-4 signaled through the chimeric receptor *in vivo* despite the species-specificity observed *in vitro*. Some studies have implicated a role for IL-13 in IgE production making it possible that mouse IL-13 may signal through the chimeric receptor (McKenzie et al., 1999; Emson et al., 1998). However, this is improbable as no mIL-13Rα1 expression has been shown in murine B cells and hIL-4Rα does not bind to mIL-13Rα1 *in vitro* (Andrews et al., 2001). Furthermore, the significant decrease in Th2 cytokine and IgE production after neutralization of mIL-4 in our studies confirmed that mIL-4 was responsible for the observed Th2/type2 responses in *N. brasiliensis*-infected transgenic mice.

To rule out a role for mIL-4 or mIL-13 in signaling via the chimeric receptor, we used a genetic approach. The hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice were intercrossed with mIL-4$^{-/-}$/mIL-13$^{-/-}$ animals (McKenzie et al., 1999) and investigated in the *L. major* and *N. brasiliensis* *in vivo* models. Genotypic analysis confirmed the disruption of mIL-4 and mIL-13 genes while cytokine responses during *N. brasiliensis* infection showed that IL-4 and IL-13 were not expressed in novel hIL-4Rα Tg/mIL-4Rα$^{-/-}$/mIL-4$^{-/-}$/mIL-13$^{-/-}$mice. In both *L. major* and *N. brasiliensis* infection models the absence of IL-4 and IL-13 resulted in abrogation of type 2 antibody responses. These data conclusively demonstrated that

endogenous mIL-4 signaled through the hIL-4Rα/mγc chimeric receptor *in vivo* leading to enhanced Th2/type 2 responses in transgenic mice.

In an attempt to explain the discrepancy between the *in vitro* and *in vivo* signaling of the chimeric receptor we revisited earlier studies. All reports demonstrating species-specificity of IL-4R signaling were performed *in vitro* and correspond to our *in vitro* data (Mosmann et al., 1987; Seki et al., 2004). However, there are no other reports showing species-specificity of IL-4R signaling *in vivo*. The differences between *in vitro* and *in vivo* signaling may be explained by the measurement of different responses. In this study, and as demonstrated previously (Seki et al., 2004), mIL-4 could not signal though the chimeric receptor in B lymphocytes as assessed by B cell proliferation. Earlier studies made use of human cell lines and radio-labeled IL-4 to show that mIL-4 does not bind hIL-4R (Nakajima et al., 1987; Park et al., 1987b). More recently, species-specific IL-4-induced signaling in B cells *in vitro* was demonstrated by induction of CD23 and class II MHC surface expression, as well as STAT6 phosphorylation (Andrews et al., 2001). However, in our *in vivo* studies, IL-4 signaling in B cells was assessed by B cell antibody responses. In T cells a similar approach was used to measure signaling of the chimeric receptor *in vitro* and *in vivo*. The inability of mIL-4 to signal via the chimeric receptor *in vitro* was shown by us and others in T cell proliferation and Th1/Th2 differentiation assays (Lowenthal et al., 1988; Seki et al., 2004). Whereas mIL-4 could not induce Th2 cytokine production *in vitro*, mIL-4 signaling *in vivo* resulted in T cells producing Th2 cytokines. Although some IL-4 producing T cells were also observed in mIL-4Rα$^{-/-}$ mice the Th2 cytokine production was significantly enhanced in hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice.

The possibility that another factor may signal through the chimeric receptor *in vivo* could be ruled out, because blockade of mIL-4 and deletion of mIL-4 and mIL-13 abrogated the type 2 responses observed in transgenic mice. A factor present only *in vivo* may contribute to the IL-4 signaling through the chimeric receptor. It is possible that a parasite-induced factor stimulates or enhances IL-4 signaling during *L. major* or *N. brasiliensis* infection. Several parasites including *Leishmania* and helminths release factors in the host which modulate the host immune responses to establish an infection

(Mottram et al., 1996; Alexander et al., 1998; Mangan et al., 2004). Cysteine proteases produced by *L. mexicana* specifically inhibit Th1 responses in C3H mice and induce Th2 responses in BALB/c mice (Buxbaum et al., 2003; Pollock et al., 2003). Soluble proteins produced by *L. major* were found to induce the activation of B cells and the production of antibodies while down-regulating Th1 cytokine production (Bohme et al., 1986; Cordeiro-Da Silva et al., 2001). Preliminary evidence from OVA/alum sensitization studies demonstrated that transgenic mice produced total IgE but no OVA-specific IgE in response to OVA immunization. This indicated that an inert antigen such as OVA did not result in increased antigen-specific responses while *L. major* and *N. brasiliensis* infections induced strong Th2 and both antigen-specific and non-specific type 2 responses.

Another possible explanation for differences observed *in vivo* and *in vitro* is that recombinant mIL-4 used *in vitro* is glycosylated differently to natural mIL-4. For example, mouse and human IL-4 are hyper-glycosylated by yeast hosts (Park et al., 1987b). Nevertheless, early studies showed that additional glycosylation does not alter the binding of either human or mouse IL-4 to their respective receptors (Park et al., 1987a; 1987b). Furthermore, in a comparative study using both natural and recombinant mIL-4, neither of these proteins had an effect on proliferation of human T cells (Mosmann et al., 1987). However, these comparative studies were performed *in vitro* and differences in glycosylation may only have an effect *in vivo*. The recombinant mIL-4 used in our studies was produced in baculovirus-transfected insect cells. It is well documented that insect cells have a different N-glycosylation pathway to eucaryotic cells, resulting in the production of recombinant proteins that lack some oligosaccharide side chains found in the mammalian-produced protein (Jarvis and Summers, 1992). These differences in the side chains may affect protein-protein interactions and more specifically in our case the binding of IL-4 to its receptor. Also, *in vivo*, other factors such as structural changes or myristylation of the chimeric receptor or mIL-4, and associated proteins may affect binding of mIL-4 to the chimeric receptor.

The Eμ element linked to the hIL-4Rα transgene may control transcription of the gene differently *in vitro* and *in vivo* resulting in differences in expression and function of the chimeric receptor. A study using an Eμ-controlled green fluorescent protein (GFP)

transgene investigated the expression of GFP by flow cytometry *in vitro*, using cell lines and *in vivo* using spleen and bone-marrow from transgenic mice (Guglielmi et al., 2003). Whereas GFP expression was constitutive *in vitro*, no GFP^+ cells or transcripts were observed *in vivo*, clearly showing differences between *in vitro* and *in vivo* transcriptional control by Eμ.

Besides the discrepancy between the *in vivo* and *in vitro* function of the chimeric receptor, an additional major problem with the *in vivo* hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mouse model was the observed susceptibility of these transgenic mice to *L. major* infection. We expected that in the absence of hIL-4, transgenic mice would behave as mIL-4Rα$^{-/-}$ mice *in vivo*, and control *L. major* infection in the acute phase. In some studies *L. major*-infected mIL-4Rα$^{-/-}$ showed increased resistance to acute *L. major* infection compared to BALB/c mice (Mohrs et al., 1999; Radwanska et al., unpublished data), while others showed either susceptibility or slight increased resistance (Noben-Trauth et al., 1999). These studies highlighted that IL-4/IL-13-dependent and IL-4/IL-13-independent mechanism may control disease outcome. Consistent with reports by our group (Hölscher et al., 2006; Mohrs et al., 1999; Radwanska et al., unpublished data), we demonstrated that mIL-4Rα$^{-/-}$ mice were more resistant to *L. major* infection compared to BALB/c mice. However, *L. major*-infected transgenic mice developed similar lesion progression and parasite burdens as observed for BALB/c mice, in the absence of hIL-4. The treatment of transgenic mice with hIL-4 had no influence on the susceptibility of these mice to *L. major* infection indicating that no hIL-4 induction of the chimeric receptor occurred *in vivo*. These data demonstrated that the hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mouse model did not function *in vivo* as expected.

Surprisingly, transgenic mice were highly susceptible to infection even though macrophages from these mice were unable to respond to IL-4 and IL-13. This suggests that other factors such as IL-10 or TGFβ may play a role in the observed susceptibility of transgenic mice. Both of these cytokines have been shown to inhibit classically activated macrophages and NO production during *L. major* infection (Kane and Mosser, 2001; Li et al., 1999). Our studies revealed no noticeable differences in the IL-10 production of anti-CD3 stimulated lymph node cells from the different groups (data not shown).

However, IL-10 levels at sites of infection were not investigated and may be more informative considering that *Leishmania* amastigotes directly induce IL-10 production by macrophages (Guy and Belosevic, 1993; Kane and Mosser, 2001). Preliminary data indicated that lymph node cells from *L. major*-infected transgenic mice produced more TGFβ than BALB/c and IL-4Rα$^{-/-}$ mice (data not shown). Neutralization of TGFβ during infection may shed more light on the role of this cytokine in susceptibility of hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice to *L. major*.

The finding that mIL-4 signaled through the chimeric receptor suggested that deletion of IL-4 and IL-13 in transgenic mice may result in increased resistance to *L. major* infection as observed for mIL-4Rα$^{-/-}$ and mIL-4/mIL-13$^{-/-}$ mice (Matthews et al., 2000; Mohrs et al., 1999). However, hIL-4Rα Tg/mIL-4Rα$^{-/-}$ /mIL-4$^{-/-}$/mIL-13$^{-/-}$mice were susceptible to *L. major* already during acute infection, which was in contrast to mIL-4Rα$^{-/-}$ mice. We concluded that the hIL-4Rα transgenic mouse model, even in the absence of mIL-4 and mIL-13 can not be used as an inducible system in *L. major* infection studies. The genetic background of transgenic mice may influence susceptibility to *L. major* infection. The original hIL-4Rα transgenic line was generated in a BDF1 strain which is the first progeny of a cross between C57BL/6 and DBA/2 mice (Manning and Thompson, 1976). C57BL/6 and DBA/2 mice are resistant to *L. major* infection (Saddick et al., 1986; Nashed et al., 2000; Onishi et al., 2004). This suggested two possible explanations for the observed susceptibility of transgenic mice to *L. major* infection in the absence of mIL-4 and mIL-13. A resistance gene may have been disrupted by the incorporation of the transgene into the germline of BDF1 mice. The other possibility is that the transgene may be flanked by susceptibility genes as the resistant strains DBA/2 and C57BL/6 may also have susceptibility genes in their genome. Although transgenic mice were backcrossed with BALB/c mice for more than 10 generations, the transgene is always selected for and any gene closely linked to it may be transferred to the next generation. The combination of the transgene-linked sequences together with the disruption of IL-4Rα may induce susceptibility to *L. major* infection or create an "on" switch for susceptibility genes.

In conclusion, *in vitro* we showed that hIL-4 but not mIL-4 or mIL-13 stimulated lymphocyte proliferation and Th2 differentiation of CD4$^+$ T cells from hIL-4Rα Tg/mIL-

$4R\alpha^{-/-}$ mice. However, in contrast to the species-specific signaling of the chimeric receptor *in vitro*, endogenous mIL-4 signaled through this receptor *in vivo*. In the absence of hIL-4, *N. brasiliensis* or *L. major* infection induced Th2/type 2 responses in transgenic mice. These responses were abrogated by the neutralization of mIL-4 or the genetic deletion of mIL-4 and mIL-13 in transgenic mice, indicating that endogenous mIL-4 signaled through the chimeric receptor *in vivo*. Our findings highlight the importance of extending *in vitro* studies to *in vivo* models and the additional complexity this may bring.

Future studies

Further investigation is required to explain how endogenous mIL-4 signals through the chimeric receptor. Some studies have implicated a role for IL-13 in IgE production *in vivo* (McKenzie et al., 1999; Emson et al., 1998). The abrogation of Th2/type 2 responses in transgenic mice treated with anti-mIL-4 antibodies demonstrated that mIL-4 was responsible for the signaling observed in hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice. The intercrossing of transgenic mice with animals deficient in either mIL-4 or mIL-13 could confirm that mIL-4 and not mIL-13 signaled through the chimeric receptor. In another approach, the reconstitution of mIL-4 in hIL-4Rα Tg/mIL-4Rα$^{-/-}$ /mIL-4$^{-/-}$/mIL-13$^{-/-}$ mice using recombinant protein will confirm the role of mIL-4 in transducing a signal through the chimeric receptor. The measurement of IgE responses after treatment of OVA/alum immunized mice with either recombinant mIL-4 or PBS could be used as an *in vivo* approach. Immuno-precipitation studies may provide evidence for the proteins that bind to the hIL-4Rα/mγc chimeric receptor and identify whether other factors are involved in the observed IL-4 signaling *in vivo*. Additional FACS analysis is required to explore the differences in hIL-4Rα and mIL-4Rα expression in lymphocyte populations and to further investigate hIL-4Rα surface expression in DC's.

The novel hIL-4Rα Tg/mIL-4Rα$^{-/-}$ /mIL-4$^{-/-}$/mIL-13$^{-/-}$ mouse model can be used as an inducible system to study the time-dependent role of IL-4 signaling in *N. brasiliensis* infection. Treatment of mice with hIL-4 at various time points could uncover when IL-4 influences Th2 differentiation in this *in vivo* model. The hIL-4 treatment of transgenic mice during *L. major* infection had no effect on susceptibility or type 2 antibody responses. It is possible that the doses of hIL-4 used were insufficient to induce signaling via the chimeric receptor. Further investigation is needed to find the optimal dose of hIL-4 for chimeric receptor signaling. The combination of hIL-4 and shIL-4R may provide stronger and longer lasting *in vivo* responses as has been previously shown for mIL-4 (Finkelman et al., 1993). Due to the lack of IL-4 and IL-13, other markers for Th2 responses would be examined. These include the transcription factors, GATA3 and T-bet,

other Th2 cytokines such IL-5 and IL-9, type 2 responses such as eosinophilia, and type 2 antibodies, IgG1 and IgE.

Genetic analysis of transgenic mice is required to identify where the transgene was incorporated into the mouse genome. The known sequences of the hIL-4Rα transgene could be used to design radio-labeled probes for Southern blot analysis. DNA from transgenic mice could be digested with restriction enzymes and bands containing the transgene identified in Southern blots. The cloning of identified products into vectors and subsequent sequencing would allow for comparative analysis with BALB/c sequences. These studies may show whether a disrupted or transferred gene explains the susceptibility of hIL-4Rα Tg/mIL-4Rα$^{-/-}$ mice to *L. major* infection. These data could also identify new resistance or susceptibility factors in *L. major* infection for future studies.

References

References

Abbas A. K., Murphy K. M. and Sher A. (1996) Functional diversity of helper T lymphocytes. *Nature* **383**, 787-93.

Aggarwal B. B. (2003) Signalling pathways of the TNF superfamily: a double-edged sword. *Nat Rev Immunol* **3**(9), 745-56.

Akaiwa M., Yu B., Umeshita-Suyama R., Terada N., Suto H., Koga T., Arima K., Matsushita S., Saito H., Ogawa H., Furue M., Hamasaki N., Ohshima K. and Izuhara K. (2001) Localization of human interleukin 13 receptor in non-haematopoietic cells. *Cytokine* **13**, 75-84.

Akiho H., Blennerhassett P., Deng Y. and Collins S. M. (2002) Role of IL-4, IL-13, and STAT6 in inflammation-induced hypercontractility of murine smooth muscle cells. *Am J Physiol Gastrointest Liver Physiol* **282**, G226-32.

Alexander J., Coombs G. H. and Mottram J. C. (1998) *Leishmania mexicana* cysteine proteinase-deficient mutants have attenuated virulence for mice and potentiate a Th1 response. *J Immunol* **161**, 6794-801.

Alexander J. and Russell D. G. (1992) The interaction of *Leishmania* species with macrophages. *Adv Parasitol* **31**, 175-254.

Allison J., Mullbacher A., Cox K., Morahan G., Boyd R., Scollay R., Blanden R. V. and Miller J. F. (1990) Selection of the T-cell repertoire in transgenic mice expressing a transplantation antigen in distinct thymus subsets. *Proc Biol Sci* **241**, 170-8.

Aman M. J., Tayebi N., Obiri N. I., Puri R. K., Modi W. S. and Leonard W. J. (1996) cDNA cloning and characterization of the human interleukin 13 receptor alpha chain. *J Biol Chem* **271**, 29265-70.

Anderson R. C. (2000) *Nematode Parasites of vertebrates: Their Development and Transmission*. CABI Publishing, CAB International, Wallingford Oxon, UK.

Andrews R. P., Rosa L. R., Daines M. O. and Khurana Hershey G. K. (2001) Reconstitution of a Functional Human Type II IL-4/IL-13 Receptor in Mouse B Cells: Demonstration of Species Specificity. *J Immunol* **166**, 1716-1722.

Antoine J. C., Prina E., Lang T. and Courret N. (1998) The biogenesis and properties of the parasitophorous vacuoles that harbour *Leishmania* in murine macrophages. *Trends Microbiol* **6**, 392-401.

Artis D., Johnson L. M., Joyce K., Saris C., Villarino A., Hunter C. A. and Scott P. (2004) Cutting Edge: Early IL-4 Production Governs the Requirement for IL-27-WSX-1 Signaling in the Development of Protective Th1 Cytokine Responses following *Leishmania major* Infection. *J Immunol* **172**, 4672-4675.

Askenase P. W., Bursztajn S., Gershon M. D. and Gershon R. K. (1980) T cell-dependent mast cell degranulation and release of serotonin in murine delayed-type hypersensitivity. *J Exp Med* **152**, 1358-74.

Baldwin T. M., Elso C., Curtis J., Buckingham L. and Handman E. (2003) The Site of *Leishmania major* Infection Determines Disease Severity and Immune Responses. *Infect. Immun.* **71**, 6830-6834.

Bancroft A. J., McKenzie A. N. J. and Grencis R. K. (1998) A Critical Role for IL-13 in Resistance to Intestinal Nematode Infection. *J Immunol* **160**, 3453-3461.

Barner M., Mohrs M., Brombacher F. and Kopf M. (1998) Differences between IL-4R alpha-deficient and IL-4-deficient mice reveal a role for IL-13 in the regulation of Th2 responses. *Curr Biol* **8**, 669-72.

Beebe A. M., Mauze S., Schork N. J. and Coffman R. L. (1997) Serial backcross mapping of multiple loci associated with resistance to *Leishmania major* in mice. *Immunity* **6**, 551-7.

Belkaid Y., Butcher B. and Sacks D. L. (1998) Analysis of cytokine production by inflammatory mouse macrophages at the single-cell level: selective impairment of IL-12 induction in *Leishmania*-infected cells. *Eur J Immunol* **28**, 1389-400.

Belkaid Y., Hoffmann K. F., Mendez S., Kamhawi S., Udey M. C., Wynn T. A. and Sacks D. L. (2001) The role of interleukin (IL)-10 in the persistence of *Leishmania major* in the skin after healing and the therapeutic potential of anti-IL-10 receptor antibody for sterile cure. *J Exp Med* **194**, 1497-506.

Belkaid Y., Piccirillo C. A., Mendez S., Shevach E. M. and Sacks D. L. (2002) CD4+CD25+ regulatory T cells control *Leishmania major* persistence and immunity. *Nature* **420**, 502-507.

Bethony J., Brooker S., Albonico M., Geiger S. M., Loukas A., Diemert D. and Hotez P. J. (2006) Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. *Lancet* **367**, 1521-32.

Bettelli E., Carrier Y., Gao W., Korn T., Strom T. B., Oukka M., Weiner H. L. and Kuchroo V. K. (2006) Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature* **441**, 235-8.

Bettelli E. and Kuchroo V. K. (2005) IL-12- and IL-23-induced T helper cell subsets: birds of the same feather flock together. *J Exp Med* **201**, 169-71.

Beutler B. (2004) Innate immunity: an overview. *Mol Immunol* **40**(12), 845-59.

Biedermann T., Zimmermann S., Himmelrich H., Gumy A., Egeter O., Sakrauski A. K., Seegmuller I., Voigt H., Launois P., Levine A. D., Wagner H., Heeg K., Louis J. A. and Rocken M. (2001) IL-4 instructs TH1 responses and resistance to *Leishmania major* in susceptible BALB/c mice. **2**, 1054-1060.

Bochner B., Klunk D., Sterbinsky S., Coffman R. and Schleimer R. (1995) IL-13 selectively induces vascular cell adhesion molecule-1 expression in human endothelial cells. *J Immunol* **154**, 799-803.

Boey H., Rosenbaum R., Castracane J. and Borish L. (1989) Interleukin-4 is a neutrophil activator. *J Allergy Clin Immunol* **83**, 978-84.

Bogdan C., Moll H., Solbach W. and Rollinghoff M. (1990) Tumor necrosis factor-alpha in combination with interferon-gamma, but not with interleukin 4 activates murine macrophages for elimination of *Leishmania major* amastigotes. *Eur J Immunol* **20**, 1131-5.

Bogdan C., Stenger S., Rollinghoff M. and Solbach W. (1991) Cytokine interactions in experimental cutaneous leishmaniasis. Interleukin 4 synergizes with interferon-gamma to activate murine macrophages for killing of *Leishmania major* amastigotes. *Eur J Immunol* **21**, 327-33.

Bogdan C., Vodovotz Y., Paik J., Xie Q. W. and Nathan C. (1994) Mechanism of suppression of nitric oxide synthase expression by interleukin-4 in primary mouse macrophages. *J Leukoc Biol* **55**, 227-33.

Bohme M. W., Evans D. A., Miles M. A. and Holborow E. J. (1986) Occurrence of autoantibodies to intermediate filament proteins in human visceral leishmaniasis and their induction by experimental polyclonal B-cell activation. *Immunology* **59**, 583-8.

Borg C., Jalil A., Laderach D., Maruyama K., Wakasugi H., Charrier S., Ryffel B., Cambi A., Figdor C., Vainchenker W., Galy A., Caignard A. and Zitvogel L. (2004) NK cell activation by dendritic cells (DCs) requires the formation of a synapse leading to IL-12 polarization in DCs. *Blood* **104**, 3267-3275.

Borish L. C. and Steinke J. W. (2003) 2. Cytokines and chemokines. *J Allergy Clin Immunol* **111**(2 Suppl), S460-75.

Boulay J.-L. and Paul W. E. (1992) The interleukin-4 family of lymphokines. *Current Opinion in Immunology* **4**, 294-298.

Bretscher P. A. (1992) An hypothesis to explain why cell-mediated immunity alone can contain infections by certain intracellular parasites and how immune class regulation of the response against such parasites can be subverted. *Immunol Cell Biol* **70 (Pt 5)**, 343-51.

Brewer J. M., Conacher M., Hunter C. A., Mohrs M., Brombacher F. and Alexander J. (1999) Aluminium hydroxide adjuvant initiates strong antigen-specific Th2 responses in the absence of IL-4- or IL-13-mediated signaling. *J Immunol* **163**, 6448-54.

Brigl M. and Brenner M. B. (2004) CD1: antigen presentation and T cell function. *Annu Rev Immunol* **22**, 817-90.

Brombacher F. (2000) The role of interleukin-13 in infectious diseases and allergy. *Bioessays* **22**, 646-56.

Brown D., Fowell D., Corry D., Wynn T., Moskowitz N., Cheever A., Locksley R. and Reiner S. (1996) Beta 2-microglobulin-dependent NK1.1+ T cells are not essential for T helper cell 2 immune responses. *J. Exp. Med.* **184**, 1295-1304.

Brown K. D., Zurawski S. M., Mosmann T. R. and Zurawski G. (1989) A family of small inducible proteins secreted by leukocytes are members of a new superfamily that includes leukocyte and fibroblast-derived inflammatory agents, growth factors, and indicators of various activation processes. *J Immunol* **142**, 679-87.

Buresi M. C., Buret A. G., Hollenberg M. D. and MacNaughton W. K. (2002) Activation of proteinase-activated receptor 1 stimulates epithelial chloride secretion through a unique MAP kinase- and cyclo-oxygenase-dependent pathway. *Faseb J* **16**, 1515-25.

Buresi M. C., Schleihauf E., Vergnolle N., Buret A., Wallace J. L., Hollenberg M. D. and MacNaughton W. K. (2001) Protease-activated receptor-1 stimulates Ca(2+)-dependent Cl(-) secretion in human intestinal epithelial cells. *Am J Physiol Gastrointest Liver Physiol* **281**, G323-32.

Buxbaum L. U., Denise H., Coombs G. H., Alexander J., Mottram J. C. and Scott P. (2003) Cysteine protease B of *Leishmania mexicana* inhibits host Th1 responses and protective immunity. *J Immunol* **171**, 3711-7.

Calame K. L. (2001) Plasma cells: finding new light at the end of B cell development. *Nat Immunol* **2**(12), 1103-8.

Caput D., Laurent P., Kaghad M., Lelias J. M., Lefort S., Vita N. and Ferrara P. (1996) Cloning and characterization of a specific interleukin (IL)-13 binding protein structurally related to the IL-5 receptor alpha chain. *J Biol Chem* **271**, 16921-6.

Carrera L., Gazzinelli R. T., Badolato R., Hieny S., Muller W., Kuhn R. and Sacks D. L. (1996) *Leishmania* promastigotes selectively inhibit interleukin 12 induction in bone marrow-derived macrophages from susceptible and resistant mice. *J Exp Med* **183**, 515-26.

Carter K. C., Gallagher G., Baillie A. J. and Alexander J. (1989) The induction of protective immunity to *Leishmania major* in the BALB/c mouse by interleukin 4 treatment. *Eur J Immunol* **19**, 779-82.

Castro G. A. (1985) Myeloperoxidase and intestinal inflammation. *Gastroenterology* **88**, 1294-5.

CDC. *Leishmania* life cycle. Centers of Disease Control. http://www.dpd.cdc.gov/dpdx/HTML/Leishmaniasis.htm

Channon J. Y., Roberts M. B. and Blackwell J. M. (1984) A study of the differential respiratory burst activity elicited by promastigotes and amastigotes of *Leishmania donovani* in murine resident peritoneal macrophages. *Immunology* **53**, 345-55.

Chatelain R., Mauze S. and Coffman R. L. (1999a) Experimental *Leishmania major* infection in mice: role of IL-10. *Parasite Immunol* **21**, 211-8.

Chatelain R., Mauze S., Varkila K. and Coffman R. L. (1999b) *Leishmania major* infection in interleukin-4 and interferon-gamma depleted mice. *Parasite Immunol* **21**, 423-31.

Chatelain R., Varkila K. and Coffman R. L. (1992) IL-4 induces a Th2 response in *Leishmania major*-infected mice. *J Immunol* **148**, 1182-7.

Chen S., Botteri F., van der Putten H., Landel C. P. and Evans G. A. (1987) A lymphoproliferative abnormality associated with inappropriate expression of the Thy-1 antigen in transgenic mice. *Cell* **51**, 7-19.

Cher D. J. and Mosmann T. R. (1987) Two types of murine helper T cell clone. II. Delayed-type hypersensitivity is mediated by TH1 clones. *J Immunol* **138**, 3688-94.

Cherwinski H. M., Schumacher J. H., Brown K. D. and Mosmann T. R. (1987) Two types of mouse helper T cell clone. III. Further differences in lymphokine synthesis between Th1 and Th2 clones revealed by RNA hybridization, functionally monospecific bioassays, and monoclonal antibodies. *J Exp Med* **166**, 1229-44.

Chiaramonte M. G., Mentink-Kane M., Jacobson B. A., Cheever A. W., Whitters M. J., Goad M. E., Wong A., Collins M., Donaldson D. D., Grusby M. J. and Wynn T. A. (2003) Regulation and function of the interleukin 13 receptor alpha 2 during a T helper cell type 2-dominant immune response. *J Exp Med* **197**, 687-701.

Chua A. O., Wilkinson V. L., Presky D. H. and Gubler U. (1995) Cloning and characterization of a mouse IL-12 receptor-beta component. *J Immunol* **155**, 4286-94.

Coffman R. L., Ohara J., Bond M. W., Carty J., Zlotnik A. and Paul W. E. (1986) B cell stimulatory factor-1 enhances the IgE response of lipopolysaccharide-activated B cells. *J Immunol* **136**, 4538-41.

Colotta F., Re F., Muzio M., Polentarutti N., Minty A., Caput D., Ferrara P. and Mantovani A. (1994) Interleukin-13 induces expression and release of interleukin-1 decoy receptor in human polymorphonuclear cells. *J. Biol. Chem.* **269**, 12403-12406.

Conrad D. H., Bazin H., Sehon A. H. and Froese A. (1975) Binding parameters of the interaction between rat IgE and rat mast cell receptors. *J Immunol* **114**, 1688-91.

Cooper A. M., Roberts A. D., Rhoades E. R., Callahan J. E., Getzy D. M. and Orme I. M. (1995) The role of interleukin-12 in acquired immunity to *Mycobacterium tuberculosis* infection. *Immunology* **84**, 423-32.

Cordeiro-Da-Silva A., Borges M. C., Guilvard E. and Ouaissi A. (2001) Dual role of the *Leishmania major* ribosomal protein S3a homologue in regulation of T- and B-cell activation. *Infect Immun* **69**, 6588-96.

Corraliza I. M., Campo M. L., Soler G. and Modolell M. (1994) Determination of arginase activity in macrophages: a micromethod. *J Immunol Methods* **174**, 231-5.

Corraliza I. M., Soler G., Eichmann K. and Modolell M. (1995) Arginase induction by suppressors of nitric oxide synthesis (IL-4, IL-10 and PGE2) in murine bone-marrow-derived macrophages. *Biochem Biophys Res Commun* **206**, 667-73.

Cresswell P. (2005) Antigen processing and presentation. *Immunol Rev* **207**, 5-7.

Dal Porto J. M., Gauld S. B., Merrell K. T., Mills D., Pugh-Bernard A. E., and Cambier J. (2004) B cell antigen receptor signaling 101. *Mol Immunol* **41**(6-7), 599-613.

Da Silva R. P., Hall B. F., Joiner K. A. and Sacks D. L. (1989) CR1, the C3b receptor, mediates binding of infective *Leishmania major* metacyclic promastigotes to human macrophages. *J Immunol* **143**, 617-22.

Deane J. A. and Fruman D. A. (2004) Phosphoinositide 3-kinase: diverse roles in immune cell activation. *Annu Rev Immunol* **22**, 563-98.

de Vries J. E. (1996) Molecular and biological characteristics of interleukin-13. *Chem Immunol* **63**, 204-18.

de Waal Malefyt R., Abrams J. S., Zurawski S. M., Lecron J. C., Mohan-Peterson S., Sanjanwala B., Bennett B., Silver J., de Vries J. E. and Yssel H. (1995) Differential regulation of IL-13 and IL-4 production by human CD8+ and CD4+ Th0, Th1 and Th2 T cell clones and EBV-transformed B cells. *Int Immunol* **7**, 1405-16.

de Waal Malefyt R., Figdor C. G., Huijbens R., Mohan-Peterson S., Bennett B., Culpepper J., Dang W., Zurawski G. and de Vries J. E. (1993) Effects of IL-13 on phenotype, cytokine production, and cytotoxic function of human monocytes. Comparison with IL-4 and modulation by IFN-gamma or IL-10. *J Immunol* **151**, 6370-81.

Debus A., Glasner J., Rollinghoff M. and Gessner A. (2003) High levels of susceptibility and T helper 2 response in MyD88-deficient mice infected with *Leishmania major* are interleukin-4 dependent. *Infect Immun* **71**, 7215-8.

Defrance T., Aubry J. P., Rousset F., Vanbervliet B., Bonnefoy J. Y., Arai N., Takebe Y., Yokota T., Lee F., Arai K. and et al. (1987) Human recombinant interleukin 4 induces Fc epsilon receptors (CD23) on normal human B lymphocytes. *J Exp Med* **165**, 1459-67.

Delespesse G., Sarfati M. and Peleman R. (1989) Influence of recombinant IL-4, IFN-alpha, and IFN-gamma on the production of human IgE-binding factor (soluble CD23). *J Immunol* **142**, 134-8.

Demant P., Lipoldova M. and Svobodova M. (1996) Resistance to *Leishmania major* in mice. *Science* **274**, 1392-3.

Dent A. L., Doherty T. M., Paul W. E., Sher A. and Staudt L. M. (1999a) BCL-6-deficient mice reveal an IL-4-independent, STAT6-dependent pathway that controls susceptibility to infection by *Leishmania major*. *J Immunol* **163**, 2098-103.

Dent L. A., Daly C. M., Mayrhofer G., Zimmerman T., Hallett A., Bignold L. P., Creaney J. and Parsons J. C. (1999b) Interleukin-5 transgenic mice show enhanced resistance to primary infections with *Nippostrongylus brasiliensis* but not primary infections with Toxocara canis. *Infect Immun* **67**, 989-93.

Desjardins M. and Descoteaux A. (1998) Survival strategies of *Leishmania donovani* in mammalian host macrophages. *Res Immunol* **149**, 689-92.

Devouassoux G., Foster B., Scott L. M., Metcalfe D. D. and Prussin C. (1999) Frequency and characterization of antigen-specific IL-4- and IL-13- producing basophils and T cells in peripheral blood of healthy and asthmatic subjects. *J Allergy Clin Immunol* **104**, 811-9.

Diefenbach A., Schindler H., Röllinghoff M., Yokoyama W. M. and Bogdan C. (1999) Requirement for Type 2 NO Synthase for IL-12 Signaling in Innate Immunity. *Science* **284**, 951-955.

Dildrop R., Ma A., Zimmerman K., Hsu E., Tesfaye A., DePinho R. and Alt F. W. (1989) IgH enhancer-mediated deregulation of N-myc gene expression in transgenic mice: generation of lymphoid neoplasias that lack c-myc expression. *Embo J* **8**, 1121-8.

Ding A. H., Nathan C. F. and Stuehr D. J. (1988) Release of reactive nitrogen intermediates and reactive oxygen intermediates from mouse peritoneal macrophages. Comparison of activating cytokines and evidence for independent production. *J Immunol* **141**, 2407-12.

Doherty T. M., Kastelein R., Menon S., Andrade S. and Coffman R. L. (1993) Modulation of murine macrophage function by IL-13. *J Immunol* **151**, 7151-60.

Donaldson D. D., Whitters M. J., Fitz L. J., Neben T. Y., Finnerty H., Henderson S. L., O'Hara R. M., Jr., Beier D. R., Turner K. J., Wood C. R. and Collins M. (1998) The murine IL-13 receptor alpha 2: molecular cloning, characterization, and comparison with murine IL-13 receptor alpha 1. *J Immunol* **161**, 2317-24.

Dong C., Davis R. J., and Flavell R. A. (2002) MAP kinases in the immune response. *Annu Rev Immunol* **20**, 55-72.

Donnelly R. P., Dickensheets H. and Finbloom D. S. (1999) The interleukin-10 signal transduction pathway and regulation of gene expression in mononuclear phagocytes. *J Interferon Cytokine Res* **19**, 563-73.

Doyle A. G., Herbein G., Montaner L. J., Minty A. J., Caput D., Ferrara P. and Gordon S. (1994) Interleukin-13 alters the activation state of murine macrophages *in vitro*: comparison with interleukin-4 and interferon-gamma. *Eur J Immunol* **24**, 1441-5.

Dubey C., Croft M. and Swain S. L. (1995) Costimulatory requirements of naive CD4+ T cells. ICAM-1 or B7-1 can costimulate naive CD4 T cell activation but both are required for optimum response. *J Immunol* **155**, 45-57.

Durum S. K. and Muegge K. (1998) *Cytokine Knockouts*. Humana Press, New Jersey.

Egwang T. G., Richards C. D., Stadnyk A. W., Gauldie J. and Befus A. D. (1985) Multinucleate giant cells in murine and rat lungs during *Nippostrongylus brasiliensis* infections. A study of the kinetics of the response *in vivo*, cytochemistry, IgG- and C3-mediated functions. *Parasite Immunol* **7**, 11-8.

Else K. J. and Finkelman F. D. (1998) Intestinal nematode parasites, cytokines and effector mechanisms. *Int J Parasitol* **28**, 1145-58.

Elser B., Lohoff M., Kock S., Giaisi M., Kirchhoff S., Krammer P. H. and Li-Weber M. (2002) IFN-gamma represses IL-4 expression via IRF-1 and IRF-2. *Immunity* **17**, 703-12.

Emson C. L., Bell S. E., Jones A., Wisden W. and McKenzie A. N. (1998) Interleukin (IL)-4-independent induction of immunoglobulin (Ig)E, and perturbation of T cell development in transgenic mice expressing IL-13. *J Exp Med* **188**, 399-404.

Essner R., Rhoades K., McBride W. H., Morton D. L. and Economou J. S. (1989) IL-4 down-regulates IL-1 and TNF gene expression in human monocytes. *J Immunol* **142**, 3857-61.

Evans T., Thai L., Granger D. and Hibbs J., Jr. (1993) Effect of *in vivo* inhibition of nitric oxide production in murine leishmaniasis. *J Immunol* **151**, 907-915.

Fallon P. G., Richardson E. J., McKenzie G. J. and McKenzie A. N. (2000) Schistosome infection of transgenic mice defines distinct and contrasting pathogenic roles for IL-4 and IL-13: IL-13 is a profibrotic agent. *J Immunol* **164**, 2585-91.

Fanslow W. C., Clifford K., VandenBos T., Teel A., Armitage R. J. and Beckmann M. P. (1990) A soluble form of the interleukin 4 receptor in biological fluids. *Cytokine* **2**, 398-401.

Fanslow W. C., Clifford K. N., Park L. S., Rubin A. S., Voice R. F., Beckmann M. P. and Widmer M. B. (1991) Regulation of alloreactivity *in vivo* by IL-4 and the soluble IL-4 receptor. *J Immunol* **147**, 535-40.

Faussone-Pellegrini M. S., Gay J., Vannucchi M. G., Corsani L. and Fioramonti J. (2002) Alterations of neurokinin receptors and interstitial cells of Cajal during and after jejunal inflammation induced by *Nippostrongylus brasiliensis* in the rat. *Neurogastroenterol Motil* **14**, 83-95.

Favre C., Saeland S., Caux C., Duvert V. and De Vries J. E. (1990) Interleukin-4 has basophilic and eosinophilic cell growth-promoting activity on cord blood cells. *Blood* **75**, 67-73.

Fernandez-Botran R., Krammer P. H., Diamantstein T., Uhr J. W. and Vitetta E. S. (1986) B cell-stimulatory factor 1 (BSF-1) promotes growth of helper T cell lines. *J Exp Med* **164**, 580-93.

Fernandez-Botran R., Sanders V. M., Mosmann T. R. and Vitetta E. S. (1988) Lymphokine-mediated regulation of the proliferative response of clones of T helper 1 and T helper 2 cells. *J Exp Med* **168**, 543-58.

Fernandez-Botran R. and Vitetta E. (1990) A Soluble, High-Affinity, Interleukin-4-Binding Protein is Present in the Biological Fluids of Mice. *PNAS* **87**, 4202-4206.

Fernandez-Botran R. and Vitetta E. S. (1991) Evidence that natural murine soluble interleukin 4 receptors may act as transport proteins. *J Exp Med* **174**, 673-81.

Filippi C., Hugues S., Cazareth J., Julia V., Glaichenhaus N. and Ugolini S. (2003) CD4+ T cell polarization in mice is modulated by strain-specific major histocompatibility complex-independent differences within dendritic cells. *J Exp Med* **198**, 201-9.

Finkelman F. D., Katona I. M., Urban J. F., Jr., Holmes J., Ohara J., Tung A. S., Sample J. V. and Paul W. E. (1988) IL-4 is required to generate and sustain *in vivo* IgE responses. *J Immunol* **141**, 2335-41.

Finkelman F. D., Katona I. M., Urban J. F., Jr., Snapper C. M., Ohara J. and Paul W. E. (1986) Suppression of *in vivo* polyclonal IgE responses by monoclonal antibody to the lymphokine B-cell stimulatory factor 1. *Proc Natl Acad Sci U S A* **83**, 9675-8.

Finkelman F. D., Madden K. B., Cheever A. W., Katona I. M., Morris S. C., Gately M. K., Hubbard B. R., Gause W. C. and Urban J. F., Jr. (1994) Effects of interleukin 12 on immune responses and host protection in mice infected with intestinal nematode parasites. *J Exp Med* **179**, 1563-72.

Finkelman F. D., Madden K. B., Morris S. C., Holmes J. M., Boiani N., Katona I. M. and Maliszewski C. R. (1993) Anti-cytokine antibodies as carrier proteins. Prolongation of *in vivo* effects of exogenous cytokines by injection of cytokine-anti-cytokine antibody complexes. *J Immunol* **151**, 1235-44.

Finkelman F. D., Shea-Donohue T., Goldhill J., Sullivan C. A., Morris S. C., Madden K. B., Gause W. C. and Urban J. F., Jr. (1997) Cytokine regulation of host defense against parasitic gastrointestinal nematodes: lessons from studies with rodent models. *Annu Rev Immunol* **15**, 505-33.

Finkelman F. D., Shea-Donohue T., Morris S. C., Gildea L., Strait R., Madden K. B., Schopf L. and Urban J. F., Jr. (2004) Interleukin-4- and interleukin-13-mediated host protection against intestinal nematode parasites. *Immunol Rev* **201**, 139-55.

Finkelman F. D., Wynn T. A., Donaldson D. D. and Urban J. F. (1999) The role of IL-13 in helminth-induced inflammation and protective immunity against nematode infections. *Curr Opin Immunol* **11**, 420-6.

Gantt K. R., Schultz-Cherry S., Rodriguez N., Jeronimo S. M., Nascimento E. T., Goldman T. L., Recker T. J., Miller M. A. and Wilson M. E. (2003) Activation of TGF-beta by *Leishmania chagasi*: importance for parasite survival in macrophages. *J Immunol* **170**, 2613-20.

Gately M. K., Renzetti L. M., Magram J., Stern A. S., Adorini L., Gubler U. and Presky D. H. (1998) The interleukin-12/interleukin-12-receptor system: role in normal and pathologic immune responses. *Annu Rev Immunol* **16**, 495-521.

Gauchat J. F., Schlagenhauf E., Feng N. P., Moser R., Yamage M., Jeannin P., Alouani S., Elson G., Notarangelo L. D., Wells T., Eugster H. P. and Bonnefoy J. Y. (1997) A novel 4-kb interleukin-13 receptor alpha mRNA expressed in human B, T, and endothelial cells encoding an alternate type-II interleukin-4/interleukin-13 receptor. *Eur J Immunol* **27**, 971-8.

Gauldie J., Richards C. and Lamontagne L. (1983) Fc receptors for IgA and other immunoglobulins on resident and activated alveolar macrophages. *Mol Immunol* **20**, 1029-37.

Gessner A., Blum H. and Rollinghoff M. (1993) Differential regulation of IL-9-expression after infection with *Leishmania major* in susceptible and resistant mice. *Immunobiology* **189**, 419-35.

Gessner J. E., Heiken H., Tamm A., and Schmidt R. E. (1998) The IgG Fc receptor family. *Ann Hematol* **76**(6), 231-48.

Gessner A., Mohrs K. and Mohrs M. (2005) Mast cells, basophils, and eosinophils acquire constitutive IL-4 and IL-13 transcripts during lineage differentiation that are sufficient for rapid cytokine production. *J Immunol* **174**, 1063-72.

Gessner A., Schroppel K., Will A., Enssle K. H., Lauffer L. and Rollinghoff M. (1994) Recombinant soluble interleukin-4 (IL-4) receptor acts as an antagonist of IL-4 in murine cutaneous Leishmaniasis. *Infect Immun* **62**, 4112-7.

Ghalib H. W., Piuvezam M. R., Skeiky Y. A., Siddig M., Hashim F. A., el-Hassan A. M., Russo D. M. and Reed S. G. (1993) Interleukin 10 production correlates with pathology in human *Leishmania donovani* infections. *J Clin Invest* **92**, 324-9.

Giri J. G., Ahdieh M., Eisenman J., Shanebeck K., Grabstein K., Kumaki S., Namen A., Park L. S., Cosman D. and Anderson D. (1994) Utilization of the beta and gamma chains of the IL-2 receptor by the novel cytokine IL-15. *Embo J* **13**, 2822-30.

Goldhill J., Morris S. C., Maliszewski C., Urban J. F., Jr., Funk C. D., Finkelman F. D. and Shea-Donohue T. (1997) Interleukin-4 modulates cholinergic neural control of mouse small intestinal longitudinal muscle. *Am J Physiol* **272**, G1135-40.

Gordon S. (2003) Alternative activation of macrophages. *Nat Rev Immunol* **3**, 23-35.

Graber P., Gretener D., Herren S., Aubry J. P., Elson G., Poudrier J., Lecoanet-Henchoz S., Alouani S., Losberger C., Bonnefoy J. Y., Kosco-Vilbois M. H. and Gauchat J. F. (1998) The distribution of IL-13 receptor alpha1 expression on B cells, T cells and monocytes and its regulation by IL-13 and IL-4. *Eur J Immunol* **28**, 4286-98.

Green S. J., Crawford R. M., Hockmeyer J. T., Meltzer M. S. and Nacy C. A. (1990a) *Leishmania major* amastigotes initiate the L-arginine-dependent killing mechanism in IFN-gamma-stimulated macrophages by induction of tumor necrosis factor-alpha. *J Immunol* **145**, 4290-7.

Green S. J., Meltzer M. S., Hibbs J. B., Jr. and Nacy C. A. (1990b) Activated macrophages destroy intracellular *Leishmania major* amastigotes by an L-arginine-dependent killing mechanism. *J Immunol* **144**, 278-83.

Greenwald R. J., Lu P., Halvorson M. J., Zhou X., Chen S., Madden K. B., Perrin P. J., Morris S. C., Finkelman F. D., Peach R., Linsley P. S., Urban J. F., Jr. and Gause W. C. (1997) Effects of blocking B7-1 and B7-2 interactions during a type 2 *in vivo* immune response. *J Immunol* **158**, 4088-96.

Grosschedl R., Weaver D., Baltimore D. and Costantini F. (1984) Introduction of a mu immunoglobulin gene into the mouse germ line: specific expression in lymphoid cells and synthesis of functional antibody. *Cell* **38**, 647-58.

Groux H., Cottrez F., Rouleau M., Mauze S., Antonenko S., Hurst S., McNeil T., Bigler M., Roncarolo M.-G. and Coffman R. L. (1999) A Transgenic Model to Analyze the Immunoregulatory Role of IL-10 Secreted by Antigen-Presenting Cells. *J Immunol* **162**, 1723-1729.

Grunig G., Warnock M., Wakil A. E., Venkayya R., Brombacher F., Rennick D. M., Sheppard D., Mohrs M., Donaldson D. D., Locksley R. M. and Corry D. B. (1998) Requirement for IL-13 independently of IL-4 in experimental asthma. *Science* **282**, 2261-3.

Guglielmi L., Le Bert M., Cogne M. and Denizot Y. (2003) Effect of the Emu IgH enhancer on expression of a GFP reporter gene in transfected B cells and transgenic mice. *Immunol Lett* **86**, 77-83.

Guler M. L., Gorham J. D., Hsieh C. S., Mackey A. J., Steen R. G., Dietrich W. F. and Murphy K. M. (1996) Genetic susceptibility to *Leishmania*: IL-12 responsiveness in TH1 cell development. *Science* **271**, 984-7.

Guo J., Apiou F., Mellerin M. P., Lebeau B., Jacques Y. and Minvielle S. (1997) Chromosome mapping and expression of the human interleukin-13 receptor. *Genomics* **42**, 141-5.

Guy R. A. and Belosevic M. (1993) Comparison of receptors required for entry of *Leishmania major* amastigotes into macrophages. *Infect Immun* **61**, 1553-8.

Hanson E. M., Dickensheets H., Qu C. K., Donnelly R. P. and Keegan A. D. (2003) Regulation of the dephosphorylation of Stat6. Participation of Tyr-713 in the interleukin-4 receptor alpha, the tyrosine phosphatase SHP-1, and the proteasome. *J Biol Chem* **278**, 3903-11.

Harris N. L., Peach R. J. and Ronchese F. (1999) CTLA4-Ig inhibits optimal T helper 2 cell development but not protective immunity or memory response to *Nippostrongylus brasiliensis*. *Eur J Immunol* **29**, 311-6.

Heinzel F., Sadick M., Mutha S. and Locksley R. (1991) Production of Interferon γ, Interleukin 2, Interleukin 4, and Interleukin 10 by CD4+ lymphocytes *in vivo* during healing and progressive murine leishmaniasis. *PNAS* **88**, 7011-7015.

Hebenstreit D., Horejs-Hoeck J., and Duschl A. (2005) JAK/STAT-dependent gene regulation by cytokines. *Drug News Perspect* **18**(4), 243-9.

Heinzel F. P. and Maier R. A., Jr. (1999) Interleukin-4-independent acceleration of cutaneous leishmaniasis in susceptible BALB/c mice following treatment with anti-CTLA4 antibody. *Infect Immun* **67**, 6454-60.

Heinzel F. P., Rerko R. M., Hatam F. and Locksley R. M. (1993a) IL-2 is necessary for the progression of leishmaniasis in susceptible murine hosts. *J Immunol* **150**, 3924-31.

Heinzel F. P., Sadick M. D., Holaday B. J., Coffman R. L. and Locksley R. M. (1989) Reciprocal expression of interferon gamma or interleukin 4 during the resolution or progression of murine leishmaniasis. Evidence for expansion of distinct helper T cell subsets. *J Exp Med* **169**, 59-72.

Heinzel F. P., Schoenhaut D. S., Rerko R. M., Rosser L. E. and Gately M. K. (1993b) Recombinant interleukin 12 cures mice infected with *Leishmania major*. *J Exp Med* **177**, 1505-9.

Herbert J. M., Savi P., Laplace M. C., Lale A., Dol F., Dumas A., Labit C. and Minty A. (1993) IL-4 and IL-13 exhibit comparable abilities to reduce pyrogen-induced expression of procoagulant activity in endothelial cells and monocytes. *FEBS Lett* **328**, 268-70.

Herwaldt B. L. (1999) Leishmaniasis. *Lancet* **354**, 1191-9.

Hilton D. J., Zhang J. G., Metcalf D., Alexander W. S., Nicola N. A. and Willson T. A. (1996) Cloning and characterization of a binding subunit of the interleukin 13 receptor that is also a component of the interleukin 4 receptor. *Proc Natl Acad Sci U S A* **93**, 497-501.

Himmelrich H., Launois P., Maillard I., Biedermann T., Tacchini-Cottier F., Locksley R. M., Rocken M. and Louis J. A. (2000) In BALB/c mice, IL-4 production during the initial phase of infection with *Leishmania major* is necessary and sufficient to instruct Th2 cell development resulting in progressive disease. *J Immunol* **164**, 4819-25.

Himmelrich H., Parra-Lopez C., Tacchini-Cottier F., Louis J. A. and Launois P. (1998) The IL-4 rapidly produced in BALB/c mice after infection with *Leishmania major* down-regulates IL-12 receptor {beta}2-chain expression on CD4+ T cells resulting in a state of unresponsiveness to IL-12. *J Immunol* **161**, 6156-6163.

Hofer T., Muehlinghaus G., Moser K., Yoshida T., H E. M., Hebel K., Hauser A., Hoyer B., E O. L., Dorner T., Manz R. A., Hiepe F., and Radbruch A. (2006) Adaptation of humoral memory. *Immunol Rev* **211**, 295-302.

Holaday B. J., Sadick M. D., Wang Z. E., Reiner S. L., Heinzel F. P., Parslow T. G. and Locksley R. M. (1991) Reconstitution of *Leishmania* immunity in severe combined immunodeficient mice using Th1- and Th2-like cell lines. *J Immunol* **147**, 1653-8.

Holland M. J., Harcus Y. M., Balic A. and Maizels R. M. (2005) Th2 induction by *Nippostrongylus* secreted antigens in mice deficient in B cells, eosinophils or MHC Class I-related receptors. *Immunol Lett* **96**, 93-101.

Holland M. J., Harcus Y. M., Riches P. L. and Maizels R. M. (2000) Proteins secreted by the parasitic nematode *Nippostrongylus brasiliensis* act as adjuvants for Th2 responses. *Eur J Immunol* **30**, 1977-87.

Hölscher C., Arendse B., Schwegmann A., Myburgh E. and Brombacher F. (2006) Impairment of Alternative Macrophage Activation Delays Cutaneous Leishmaniasis in Nonhealing BALB/c Mice. *J Immunol* **176**, 1115-1121.

Hondowicz B. and Scott P. (1999) Influence of host and parasite factors on the innate immune response and Th2 stability following infection with *Leishmania major*. *Microbes and Infection* **1**, 65-71.

Horohov D. W., Crim J. A., Smith P. L. and Siegel J. P. (1988) IL-4 (B cell-stimulatory factor 1) regulates multiple aspects of influenza virus-specific cell-mediated immunity. *J Immunol* **141**, 4217-23.

Hoshino T., Winkler-Pickett R. T., Mason A. T., Ortaldo J. R. and Young H. A. (1999) IL-13 production by NK cells: IL-13-producing NK and T cells are present *in vivo* in the absence of IFN-gamma. *J Immunol* **162**, 51-9.

Hou J., Schindler U., Henzel W. J., Ho T. C., Brasseur M. and McKnight S. L. (1994) An interleukin-4-induced transcription factor: IL-4 Stat. *Science* **265**, 1701-6.

Howard M., Farrar J., Hilfiker M., Johnson B., Takatsu K., Hamaoka T. and Paul W. E. (1982) Identification of a T cell-derived b cell growth factor distinct from interleukin 2. *J Exp Med* **155**, 914-23.

Hsieh C. S., Macatonia S. E., Tripp C. S., Wolf S. F., O'Garra A. and Murphy K. M. (1993) Development of TH1 CD4+ T cells through IL-12 produced by Listeria-induced macrophages. *Science* **260**, 547-9.

Huang F. P., Xu D., Esfandiari E. O., Sands W., Wei X. Q. and Liew F. Y. (1998) Mice defective in Fas are highly susceptible to *Leishmania major* infection despite elevated IL-12 synthesis, strong Th1 responses, and enhanced nitric oxide production. *J Immunol* **160**, 4143-7.

Idzerda R. L., March C. J., Mosley B., Lyman S. D., Vanden Bos T., Gimpel S. D., Din W. S., Grabstein K. H., Widmer M. B., Park L. S. and et al. (1990) Human interleukin 4 receptor confers biological responsiveness and defines a novel receptor superfamily. *J Exp Med* **171**, 861-73.

Ihle J. N., Witthuhn B. A., Quelle F. W., Yamamoto K., and Silvennoinen O. (1995) Signaling through the hematopoietic cytokine receptors. *Annu Rev Immunol* **13**, 369-98.

Iniesta V., Gomez-Nieto L. C. and Corraliza I. (2001) The Inhibition of Arginase by $N^{(\omega)}$ - Hydroxy-L-Arginine Controls the Growth of *Leishmania* Inside Macrophages. *J. Exp. Med.* **193**, 777-784.

Iniesta V., Gomez-Nieto L. C., Molano I., Mohedano A., Carcelen J., Miron C., Alonso C. and Corraliza I. (2002) Arginase I induction in macrophages, triggered by Th2-type cytokines, supports the growth of intracellular *Leishmania* parasites. *Parasite Immunol* **24**, 113-8.

Ishikawa N., Horii Y. and Nawa Y. (1993) Immune-mediated alteration of the terminal sugars of goblet cell mucins in the small intestine of *Nippostrongylus brasiliensis*-infected rats. *Immunology* **78**, 303-7.

Ishikawa N., Horii Y., Oinuma T., Suganuma T. and Nawa Y. (1994) Goblet cell mucins as the selective barrier for the intestinal helminths: T-cell-independent alteration of goblet cell mucins by immunologically 'damaged' *Nippostrongylus brasiliensis* worms and its significance on the challenge infection with homologous and heterologous parasites. *Immunology* **81**, 480-6.

Ishizaka T. and Ishizaka K. (1978) Triggering of histamine release from rat mast cells by divalent antibodies against IgE-receptors. *J Immunol* **120**, 800-5.

Ishizaka T. and Ishizaka K. (1984) Activation of mast cells for mediator release through IgE receptors. *Prog Allergy* **34**, 188-235.

Izuhara K., Arinobu Y., Sumimoto H., Nunoi H., Takeya R., Higuchi K., Takeshige K., Hamasaki N. and Harada N. (1999) Association of the interleukin-4 receptor alpha chain with p47phox, an activator of the phagocyte NADPH oxidase in B cells. *Mol Immunol* **36**, 45-52.

Izuhara K. and Harada N. (1993) Interleukin-4 (IL-4) induces protein tyrosine phosphorylation of the IL-4 receptor and association of phosphatidylinositol 3-kinase to the IL-4 receptor in a mouse T cell line, HT2. *J Biol Chem* **268**, 13097-102.

Izuhara K. and Harada N. (1996) Interleukin-4 activates two distinct pathways of phosphatidylinositol-3 kinase in the same cells. *Biochem Biophys Res Commun* **229**, 624-9.

Jacobson R. H. and Reed N. D. (1974) The immune response of congenitally athymic (nude) mice to the intestinal nematode *Nippostrongylus brasiliensis*. *Proc Soc Exp Biol Med* **147**, 667-70.

Jacobson R. H., Reed N. D. and Manning D. D. (1977) Expulsion of *Nippostrongylus brasiliensis* from mice lacking antibody production potential. *Immunology* **32**, 867-74.

Janeway C. A., Jr. and Medzhitov R. (2002) Innate immune recognition. *Annu Rev Immunol* **20**, 197-216.

Jankovic D., Sher A. and Yap G. (2001) Th1/Th2 effector choice in parasitic infection: decision making by committee. *Curr Opin Immunol* **13**, 403-9.

Jarvis D. L. and Summers M. D. (1992) Baculovirus expression vectors. In *Recombinant DNA vaccines: rationale and strategies* (Edited by Isaacson R. E.), p. 265-291. Marcel Dekker, Inc., New York.

Jiang H., Harris M. B. and Rothman P. (2000) IL-4/IL-13 signaling beyond JAK/STAT. *J Allergy Clin Immunol* **105**, 1063-70.

Johnston J. A., Kawamura M., Kirken R. A., Chen Y. Q., Blake T. B., Shibuya K., Ortaldo J. R., McVicar D. W. and O'Shea J. J. (1994) Phosphorylation and activation of the Jak-3 Janus kinase in response to interleukin-2. *Nature* **370**, 151-3.

Jones D., Elloso M. M., Showe L., Williams D., Trinchieri G. and Scott P. (1998) Differential Regulation of the Interleukin-12 Receptor during the Innate Immune Response to *Leishmania major*. *Infect. Immun.* **66**, 3818-3824.

Jorens P. G., Boelaert J. R., Halloy V., Zamora R., Schneider Y. J. and Herman A. G. (1995) Human and rat macrophages mediate fungistatic activity against Rhizopus species differently: *in vitro* and *ex vivo* studies. *Infect Immun* **63**, 4489-94.

Julia V. and Glaichenhaus N. (1999) CD4+ T Cells Which React to the *Leishmania major* LACK Antigen Rapidly Secrete Interleukin-4 and Are Detrimental to the Host in Resistant B10.D2 Mice. *Infect. Immun.* **67**, 3641-3644.

Julia V., Rassoulzadegan M. and Glaichenhaus N. (1996) Resistance to *Leishmania major* Induced by Tolerance to a Single Antigen. *Science* **274**, 421-423.

Kane M. M. and Mosser D. M. (2001) The Role of IL-10 in Promoting Disease Progression in Leishmaniasis. *J Immunol* **166**, 1141-1147.

Kaplan M. H., Sun Y. L., Hoey T. and Grusby M. J. (1996) Impaired IL-12 responses and enhanced development of Th2 cells in Stat4-deficient mice. *Nature* **382**, 174-7.

Kassai T. (1982) *Handbook of Nippostrongylus brasiliensis (nematode)*. Commonwealth Agricultural Bureau, Slough, United Kingdom.

Katona I. M., Urban J. F., Jr. and Finkelman F. D. (1988) The role of L3T4+ and Lyt-2+ T cells in the IgE response and immunity to *Nippostrongylus brasiliensis*. *J Immunol* **140**, 3206-11.

Kawabata A., Kawao N., Kuroda R., Tanaka A., Itoh H. and Nishikawa H. (2001) Peripheral PAR-2 triggers thermal hyperalgesia and nociceptive responses in rats. *Neuroreport* **12**, 715-9.

Kawakami K., Qureshi M. H., Zhang T., Okamura H., Kurimoto M. and Saito A. (1997) IL-18 protects mice against pulmonary and disseminated infection with *Cryptococcus neoformans* by inducing IFN-gamma production. *J Immunol* **159**, 5528-34.

Keegan A. D., Beckmann M. P., Park L. S. and Paul W. E. (1991) The IL-4 receptor: biochemical characterization of IL-4-binding molecules in a T cell line expressing large numbers of receptors. *J Immunol* **146**, 2272-9.

Kemp D. J., Wilson A., Harris A. W. and Shortman K. (1980) The immunoglobulin mu constant region gene is expressed in mouse thymocytes. *Nature* **286**, 168-70.

Khan W. I., Richard M., Akiho H., Blennerhasset P. A., Humphreys N. E., Grencis R. K., Van Snick J. and Collins S. M. (2003) Modulation of intestinal muscle contraction by interleukin-9 (IL-9) or IL-9 neutralization: correlation with worm expulsion in murine nematode infections. *Infect Immun* **71**, 2430-8.

Killick-Kendrick R. (1990) The life cycle of *Leishmania* in the sandfly with special reference to the form infective to the vertebrate host. *Ann Parasitol Hum Comp* **65 Suppl 1**, 37-42.

Kojima S., Yamamoto N., Kanazawa T. and Ovary Z. (1985a) Monoclonal IgE-dependent eosinophil cytotoxicity to haptenated schistosomula of *Schistosoma japonicum*: enhancement of the cytotoxicity and expression of Fc receptors for IgE by *Nippostrongylus brasiliensis* infection. *J Immunol* **134**, 2719-22.

Kojima S., Yamamoto N., Kanazawa T., Shigematsu H. and Ovary Z. (1985b) Enhancement of IgE-dependent eosinophil cytotoxicity to dinitrophenylated schistosomula by a nematode infection. *Int Arch Allergy Appl Immunol* **76**, 91-4.

Kondo M., Takeshita T., Ishii N., Nakamura M., Watanabe S., Arai K. and Sugamura K. (1993) Sharing of the interleukin-2 (IL-2) receptor gamma chain between receptors for IL-2 and IL-4. *Science* **262**, 1874-7.

Kopf M., Brombacher F., Hodgkin P. D., Ramsay A. J., Milbourne E. A., Dai W. J., Ovington K. S., Behm C. A., Kohler G., Young I. G. and Matthaei K. I. (1996a) IL-5-deficient mice have a developmental defect in CD5+ B-1 cells and lack eosinophilia but have normal antibody and cytotoxic T cell responses. *Immunity* **4**, 15-24.

Kopf M., Brombacher F., Kohler G., Kienzle G., Widmann K. H., Lefrang K., Humborg C., Ledermann B. and Solbach W. (1996b) IL-4-deficient Balb/c mice resist infection with *Leishmania major*. *J Exp Med* **184**, 1127-36.

Kopf M., Le Gros G., Bachmann M., Lamers M. C., Bluethmann H. and Kohler G. (1993) Disruption of the murine IL-4 gene blocks Th2 cytokine responses. *Nature* **362**, 245-8.

Kropf P., Etges R., Schopf L., Chung C., Sypek J. and Muller I. (1997) Characterization of T cell-mediated responses in nonhealing and healing *Leishmania major* infections in the absence of endogenous IL-4. *J Immunol* **159**, 3434-43.

Kropf P., Herath S., Weber V., Modolell M. and Muller I. (2003) Factors influencing *Leishmania major* infection in IL-4-deficient BALB/c mice. *Parasite Immunol* **25**, 439-47.

Kropf P., Schopf L. R., Chung C. L., Xu D., Liew F. Y., Sypek J. P. and Muller I. (1999) Expression of Th2 cytokines and the stable Th2 marker ST2L in the absence of IL-4 during *Leishmania major* infection. *Eur J Immunol* **29**, 3621-8.

Kuhn R., Rajewsky K. and Muller W. (1991) Generation and analysis of interleukin-4 deficient mice. *Science* **254**, 707-710.

Lai Y. H. and Mosmann T. R. (1999) Mouse IL-13 Enhances Antibody Production *In Vivo* and Acts Directly on B Cells *In Vitro* to Increase Survival and Hence Antibody Production. *J Immunol* **162**, 78-87.

Launois P., Maillard I., Pingel S., Swihart K. G., Xenarios I., Acha-Orbea H., Diggelmann H., Locksley R. M., MacDonald H. R. and Louis J. A. (1997a) IL-4 rapidly produced by V beta 4 V alpha 8 CD4+ T cells instructs Th2 development and susceptibility to *Leishmania major* in BALB/c mice. *Immunity* **6**, 541-9.

Launois P., Swihart K. G., Milon G. and Louis J. A. (1997b) Early production of IL-4 in susceptible mice infected with *Leishmania major* rapidly induces IL-12 unresponsiveness. *J Immunol* **158**, 3317-24.

Lawrence R. A., Gray C. A., Osborne J. and Maizels R. M. (1996) *Nippostrongylus brasiliensis*: cytokine responses and nematode expulsion in normal and IL-4-deficient mice. *Exp Parasitol* **84**, 65-73.

Le Gros G., Ben-Sasson S. Z., Seder R., Finkelman F. D. and Paul W. E. (1990) Generation of interleukin 4 (IL-4)-producing cells *in vivo* and *in vitro*: IL-2 and IL-4 are required for *in vitro* generation of IL-4-producing cells. *J Exp Med* **172**, 921-9.

Lee F., Yokota T., Otsuka T., Meyerson P., Villaret D., Coffman R., Mosmann T., Rennick D., Roehm N., Smith C., Zlotnik A. and Arai K.-I. (1986) Isolation and characterization of a mouse Interleukin cDNA clone that expresses B-cell stimulatory factor 1 activities and T-cell-and mast-cell-stimulating activities. *PNAS* **83**, 2061-2065.

Leonard W. J. and O'Shea J. J. (1998) Jaks and STATs: biological implications. *Annu Rev Immunol* **16**, 293-322.

Li J., Hunter C. A. and Farrell J. P. (1999) Anti-TGF-beta treatment promotes rapid healing of *Leishmania major* infection in mice by enhancing *in vivo* nitric oxide production. *J Immunol* **162**, 974-9.

Li Y., Ishii K., Hisaeda H., Hamano S., Zhang M., Nakanishi K., Yoshimoto T., Hemmi H., Takeda K., Akira S., Iwakura Y. and Himeno K. (2004) IL-18 gene therapy develops Th1-type immune responses in *Leishmania major*-infected BALB/c mice: is the effect mediated by the CpG signaling TLR9? *Gene Ther* **11**, 941-8.

Liew F., Millott S., Parkinson C., Palmer R. and Moncada S. (1990) Macrophage killing of *Leishmania* parasite *in vivo* is mediated by nitric oxide from L-arginine. *J Immunol* **144**, 4794-4797.

Liew F. Y., Millott S., Li Y., Lelchuk R., Chan W. L. and Ziltener H. (1989) Macrophage activation by interferon-gamma from host-protective T cells is inhibited by interleukin (IL)3 and IL4 produced by disease-promoting T cells in leishmaniasis. *Eur J Immunol* **19**, 1227-32.

Lipoldova M., Svobodova M., Krulova M., Havelkova H., Badalova J., Nohynkova E., Holan V., Hart A. A., Volf P. and Demant P. (2000) Susceptibility to *Leishmania major* infection in mice: multiple loci and heterogeneity of immunopathological phenotypes. *Genes Immun* **1**, 200-6.

Liu Z., Liu Q., Pesce J., Whitmire J., Ekkens M. J., Foster A., VanNoy J., Sharpe A. H., Urban J. F., Jr. and Gause W. C. (2002) *Nippostrongylus brasiliensis* can induce B7-independent antigen-specific development of IL-4-producing T cells from naive CD4 T cells *in vivo*. *J Immunol* **169**, 6959-68.

Li-Weber M. and Krammer P. H. (2003) Regulation of *IL4* gene expression by T cells and therapeutic perspectives. *Nature Reviews Immunology* **3**, 534-543.

Locksley R. M., Heinzel F. P., Fankhauser J. E., Nelson C. S. and Sadick M. D. (1988) Cutaneous host defense in leishmaniasis: interaction of isolated dermal macrophages and epidermal Langerhans cells with the insect-stage promastigote. *Infect Immun* **56**, 336-42.

Locksley R. M., Heinzel F. P., Sadick M. D., Holaday B. J. and Gardner K. D., Jr. (1987) Murine cutaneous leishmaniasis: susceptibility correlates with differential expansion of helper T-cell subsets. *Ann Inst Pasteur Immunol* **138**, 744-9.

Louzir H., Melby P. C., Ben Salah A., Marrakchi H., Aoun K., Ben Ismail R. and Dellagi K. (1998) Immunologic determinants of disease evolution in localized cutaneous leishmaniasis due to *Leishmania major*. *J Infect Dis* **177**, 1687-95.

Lowenthal J. W., Castle B. E., Christiansen J., Schreurs J., Rennick D., Arai N., Hoy P., Takebe Y. and Howard M. (1988) Expression of high affinity receptors for murine interleukin 4 (BSF-1) on hemopoietic and nonhemopoietic cells. *J Immunol* **140**, 456-64.

MacMicking J., Xie Q. W. and Nathan C. (1997) Nitric oxide and macrophage function. *Annu Rev Immunol* **15**, 323-50.

Madden K. B., Urban J. F., Jr., Ziltener H. J., Schrader J. W., Finkelman F. D. and Katona I. M. (1991) Antibodies to IL-3 and IL-4 suppress helminth-induced intestinal mastocytosis. *J Immunol* **147**, 1387-91.

Madden K. B., Whitman L., Sullivan C., Gause W. C., Urban J. F., Jr., Katona I. M., Finkelman F. D. and Shea-Donohue T. (2002) Role of STAT6 and mast cells in IL-4- and IL-13-induced alterations in murine intestinal epithelial cell function. *J Immunol* **169**, 4417-22.

Madden K. B., Yeung K. A., Zhao A., Gause W. C., Finkelman F. D., Katona I. M., Urban J. F., Jr. and Shea-Donohue T. (2004) Enteric nematodes induce stereotypic STAT6-dependent alterations in intestinal epithelial cell function. *J Immunol* **172**, 5616-21.

Maloy K. J. and Powrie F. (2001) Regulatory T cells in the control of immune pathology. *Nat Immunol* **2**, 816-22.

Manetti R., Parronchi P., Giudizi M. G., Piccinni M. P., Maggi E., Trinchieri G. and Romagnani S. (1993) Natural killer cell stimulatory factor (interleukin 12 [IL-12]) induces T helper type 1 (Th1)-specific immune responses and inhibits the development of IL-4-producing Th cells. *J Exp Med* **177**, 1199-204.

Mangan N. E., Fallon R. E., Smith P., van Rooijen N., McKenzie A. N. and Fallon P. G. (2004) Helminth infection protects mice from anaphylaxis via IL-10-producing B cells. *J Immunol* **173**, 6346-56.

Manning A. and Thompson M. L. (1976) Postcastration retention of sexual behaviour in the male BDF1 mouse: the role of experience. *Anim Behav* **24**, 523-33.

Matthews D. J., Emson C. L., McKenzie G. J., Jolin H. E., Blackwell J. M. and McKenzie A. N. (2000) IL-13 is a susceptibility factor for *Leishmania major* infection. *J Immunol* **164**, 1458-62.

Mattner F., Magram J., Ferrante J., Launois P., Di Padova K., Behin R., Gately M. K., Louis J. A. and Alber G. (1996) Genetically resistant mice lacking interleukin-12 are susceptible to infection with *Leishmania major* and mount a polarized Th2 cell response. *Eur J Immunol* **26**, 1553-9.

McGuirk P. and Mills K. H. (2002) Pathogen-specific regulatory T cells provoke a shift in the Th1/Th2 paradigm in immunity to infectious diseases. *Trends Immunol* **23**, 450-5.

McKenzie A. N., Culpepper J. A., de Waal Malefyt R., Briere F., Punnonen J., Aversa G., Sato A., Dang W., Cocks B. G., Menon S. and et al. (1993a) Interleukin 13, a T-cell-derived cytokine that regulates human monocyte and B-cell function. *Proc Natl Acad Sci U S A* **90**, 3735-9.

McKenzie A. N., Li X., Largaespada D. A., Sato A., Kaneda A., Zurawski S. M., Doyle E. L., Milatovich A., Francke U., Copeland N. G. and et al. (1993b) Structural comparison and chromosomal localization of the human and mouse IL-13 genes. *J Immunol* **150**, 5436-44.

McKenzie G. J., Bancroft A., Grencis R. K. and McKenzie A. N. (1998a) A distinct role for interleukin-13 in Th2-cell-mediated immune responses. *Curr Biol* **8**, 339-42.

McKenzie G. J., Emson C. L., Bell S. E., Anderson S., Fallon P., Zurawski G., Murray R., Grencis R. and McKenzie A. N. (1998b) Impaired development of Th2 cells in IL-13-deficient mice. *Immunity* **9**, 423-32.

McKenzie G. J., Fallon P. G., Emson C. L., Grencis R. K. and McKenzie A. N. (1999) Simultaneous disruption of interleukin (IL)-4 and IL-13 defines individual roles in T helper cell type 2-mediated responses. *J Exp Med* **189**, 1565-72.

McLaren D. J., Mackenzie C. D. and Ramalho-Pinto F. J. (1977) Ultrastructural observations on the *in vitro* interaction between rat eosinophils and some parasitic helminths (*Schistosoma mansoni*, *Trichinella spiralis* and *Nippostrongylus brasiliensis*). *Clin Exp Immunol* **30**, 105-18.

Menon J. N. and Bretscher P. A. (1998) Parasite dose determines the Th1/Th2 nature of the response to *Leishmania major* independently of infection route and strain of host or parasite. *European Journal of Immunology* **28**, 4020-4028.

Miller H. R. and Nawa Y. (1979) Immune regulation of intestinal goblet cell differentiation. Specific induction of nonspecific protection against helminths? *Nouv Rev Fr Hematol* **21**, 31-45.

Miloux B., Laurent P., Bonnin O., Lupker J., Caput D., Vita N. and Ferrara P. (1997) Cloning of the human IL-13R alpha1 chain and reconstitution with the IL4R alpha of a functional IL-4/IL-13 receptor complex. *FEBS Lett* **401**, 163-6.

Min B., Prout M., Hu-Li J., Zhu J., Jankovic D., Morgan E. S., Urban J. F., Jr., Dvorak A. M., Finkelman F. D., LeGros G. and Paul W. E. (2004) Basophils produce IL-4 and accumulate in tissues after infection with a Th2-inducing parasite. *J Exp Med* **200**, 507-17.

Minty A., Asselin S., Bensussan A., Shire D., Vita N., Vyakarnam A., Wijdenes J., Ferrara P. and Caput D. (1997) The related cytokines interleukin-13 and interleukin-4 are distinguished by differential production and differential effects on T lymphocytes. *Eur Cytokine Netw* **8**, 203-13.

Minty A., Chalon P., Derocq J. M., Dumont X., Guillemot J. C., Kaghad M., Labit C., Leplatois P., Liauzun P., Miloux B. and et al. (1993) Interleukin-13 is a new human lymphokine regulating inflammatory and immune responses. *Nature* **362**, 248-50.

Miossec P., Briolay J., Dechanet J., Wijdenes J., Martinez-Valdez H. and Banchereau J. (1992) Inhibition of the production of proinflammatory cytokines and immunoglobulins by interleukin-4 in an ex vivo model of rheumatoid synovitis. *Arthritis Rheum* **35**, 874-83.

Mitre E., Taylor R. T., Kubofcik J. and Nutman T. B. (2004) Parasite antigen-driven basophils are a major source of IL-4 in human filarial infections. *J Immunol* **172**, 2439-45.

Miyajima A., Kitamura T., Harada N., Yokota T., and Arai K. (1992) Cytokine receptors and signal transduction. *Annu Rev Immunol* **10**, 295-331.

Miyazaki T., Kawahara A., Fujii H., Nakagawa Y., Minami Y., Liu Z. J., Oishi I., Silvennoinen O., Witthuhn B. A., Ihle J. N. and et al. (1994) Functional activation of Jak1 and Jak3 by selective association with IL-2 receptor subunits. *Science* **266**, 1045-7.

Modolell M., Corraliza I. M., Link F., Soler G. and Eichmann K. (1995) Reciprocal regulation of the nitric oxide synthase/arginase balance in mouse bone marrow-derived macrophages by TH1 and TH2 cytokines. *Eur J Immunol* **25**, 1101-4.

Mohrs M., Ledermann B., Kohler G., Dorfmuller A., Gessner A. and Brombacher F. (1999) Differences between IL-4- and IL-4 receptor alpha-deficient mice in chronic leishmaniasis reveal a protective role for IL-13 receptor signaling. *J Immunol* **162**, 7302-8.

Moll H., Flohe S. and Rollinghoff M. (1995) Dendritic cells in *Leishmania major*-immune mice harbor persistent parasites and mediate an antigen-specific T cell immune response. *Eur J Immunol* **25**, 693-9.

Moll H., Fuchs H., Blank C. and Rollinghoff M. (1993) Langerhans cells transport *Leishmania major* from the infected skin to the draining lymph node for presentation to antigen-specific T cells. *Eur J Immunol* **23**, 1595-601.

Monroe J. G., Haldar S., Prystowsky M. B. and Lammie P. (1988) Lymphokine regulation of inflammatory processes: interleukin-4 stimulates fibroblast proliferation. *Clin Immunol Immunopathol* **49**, 292-8.

Montaner L. J., da Silva R. P., Sun J., Sutterwala S., Hollinshead M., Vaux D. and Gordon S. (1999) Type 1 and Type 2 cytokine regulation of macrophage endocytosis: differential activation by IL-4/IL-13 as opposed to IFN-{gamma} or IL-10. *J Immunol* **162**, 4606-4613.

Monteforte G. M., Takeda K., Rodriguez-Sosa M., Akira S., David J. R. and Satoskar A. R. (2000) Genetically resistant mice lacking IL-18 gene develop Th1 response and control cutaneous *Leishmania major* infection. *J Immunol* **164**, 5890-3.

Monteiro R. C. and Van De Winkel J. G. (2003) IgA Fc receptors. *Annu Rev Immunol* **21**, 177-204.

Moroy T., Fisher P., Guidos C., Ma A., Zimmerman K., Tesfaye A., DePinho R., Weissman I. and Alt F. W. (1990) IgH enhancer deregulated expression of L-myc: abnormal T lymphocyte development and T cell lymphomagenesis. *Embo J* **9**, 3659-66.

Mosley B., Beckmann M. P., March C. J., Idzerda R. L., Gimpel S. D., VandenBos T., Friend D., Alpert A., Anderson D., Jackson J. and et al. (1989) The murine interleukin-4 receptor: molecular cloning and characterization of secreted and membrane bound forms. *Cell* **59**, 335-48.

Mosmann T. R., Bond M. W., Coffman R. L., Ohara J. and Paul W. E. (1986a) T-cell and mast cell lines respond to B-cell stimulatory factor 1. *Proc Natl Acad Sci U S A* **83**, 5654-8.

Mosmann T. R., Cherwinski H., Bond M. W., Giedlin M. A. and Coffman R. L. (1986b) Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J Immunol* **136**, 2348-57.

Mosmann T. R. and Coffman R. L. (1989) TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annu Rev Immunol* **7**, 145-73.

Mosmann T. R., Yokota T., Kastelein R., Zurawski S. M., Arai N. and Takebe Y. (1987) Species-specificity of T cell stimulating activities of IL 2 and BSF-1 (IL 4): comparison of normal and recombinant, mouse and human IL 2 and BSF-1 (IL 4). *J Immunol* **138**, 1813-6.

Mottram J. C., Souza A. E., Hutchison J. E., Carter R., Frame M. J. and Coombs G. H. (1996) Evidence from disruption of the lmcpb gene array of *Leishmania mexicana* that cysteine proteinases are virulence factors. *Proc Natl Acad Sci U S A* **93**, 6008-13.

Mougneau E., Altare F., Wakil A. E., Zheng S., Coppola T., Wang Z. E., Waldmann R., Locksley R. M. and Glaichenhaus N. (1995) Expression cloning of a protective *Leishmania* antigen. *Science* **268**, 563-6.

Munder M., Eichmann K. and Modolell M. (1998) Alternative metabolic states in murine macrophages reflected by the nitric oxide synthase/arginase balance: competitive regulation by CD4+ T cells correlates with Th1/Th2 phenotype. *J Immunol* **160**, 5347-5354.

Munder M., Eichmann K., Moran J. M., Centeno F., Soler G. and Modolell M. (1999) Th1/Th2-regulated expression of arginase isoforms in murine macrophages and dendritic cells. *J Immunol* **163**, 3771-3777.

Murata T., Husain S. R., Mohri H. and Puri R. K. (1998) Two different IL-13 receptor chains are expressed in normal human skin fibroblasts, and IL-4 and IL-13 mediate signal transduction through a common pathway. *Int Immunol* **10**, 1103-10.

Murata T., Noguchi P. D. and Puri R. K. (1996) IL-13 induces phosphorylation and activation of JAK2 Janus kinase in human colon carcinoma cell lines: similarities between IL-4 and IL-13 signaling. *J Immunol* **156**, 2972-8.

Murphy K. M. and Reiner S. L. (2002) The lineage decisions of helper T cells. *Nat Rev Immunol* **2**, 933-44.

Nabors G. S. and Farrell J. P. (1994) Site-specific immunity to *Leishmania major* in SWR mice: the site of infection influences susceptibility and expression of the antileishmanial immune response. *Infect Immun* **62**, 3655-62.

Nakajima K., Hirano T., Koyama K. and Kishimoto T. (1987) Detection of receptors for murine B cell stimulatory factor 1 (BSF1): presence of functional receptors on CBA/N splenic B cells. *J Immunol* **139**, 774-779.

Nashed B. F., Maekawa Y., Takashima M., Zhang T., Ishii K., Dainichi T., Ishikawa H., Sakai T., Hisaeda H. and Himeno K. (2000) Different cytokines are required for induction and maintenance of the Th2 type response in DBA/2 mice resistant to infection with *Leishmania major*. *Microbes Infect* **2**, 1435-43.

Nashleanas M., Kanaly S. and Scott P. (1998) Control of *Leishmania major* Infection in Mice Lacking TNF Receptors. *J Immunol* **160**, 5506-5513.

Nawa Y., Ishikawa N., Tsuchiya K., Horii Y., Abe T., Khan A. I., Bing S., Itoh H., Ide H. and Uchiyama F. (1994) Selective effector mechanisms for the expulsion of intestinal helminths. *Parasite Immunol* **16**, 333-8.

Nishikomori R., Gurunathan S., Nishikomori K. and Strober W. (2001) BALB/c mice bearing a transgenic IL-12 receptor beta 2 gene exhibit a nonhealing phenotype to *Leishmania major* infection despite intact IL-12 signaling. *J Immunol* **166**, 6776-83.

Noben-Trauth N., Kropf P. and Muller I. (1996) Susceptibility to *Leishmania major* infection in interleukin-4-deficient mice. *Science* **271**, 987-90.

Noben-Trauth N., Lira R., Nagase H., Paul W. E. and Sacks D. L. (2003) The relative contribution of IL-4 receptor signaling and IL-10 to susceptibility to *Leishmania major*. *J Immunol* **170**, 5152-8.

Noben-Trauth N., Paul W. E. and Sacks D. L. (1999) IL-4- and IL-4 receptor-deficient BALB/c mice reveal differences in susceptibility to *Leishmania major* parasite substrains. *J Immunol* **162**, 6132-40.

Noben-Trauth N., Shultz L. D., Brombacher F., Urban J. F., Jr., Gu H. and Paul W. E. (1997) An interleukin 4 (IL-4)-independent pathway for CD4+ T cell IL-4 production is revealed in IL-4 receptor-deficient mice. *Proc Natl Acad Sci U S A* **94**, 10838-43.

Noelle R., Krammer P. H., Ohara J., Uhr J. W. and Vitetta E. S. (1984) Increased expression of Ia antigens on resting B cells: an additional role for B-cell growth factor. *Proc Natl Acad Sci U S A* **81**, 6149-53.

Noguchi M., Nakamura Y., Russell S. M., Ziegler S. F., Tsang M., Cao X. and Leonard W. J. (1993) Interleukin-2 receptor gamma chain: a functional component of the interleukin-7 receptor. *Science* **262**, 1877-80.

Noma Y., Sideras P., Naito T., Bergstedt-Lindquist S., Azuma C., Severinson E., Tanabe T., Kinashi T., Matsuda F., Yaoita Y. and et al. (1986) Cloning of cDNA encoding the murine IgG1 induction factor by a novel strategy using SP6 promoter. *Nature* **319**, 640-6.

O'Garra A., Vieira P. L., Vieira P. and Goldfeld A. E. (2004) IL-10-producing and naturally occurring CD4+ Tregs: limiting collateral damage. *J Clin Invest* **114**, 1372-8.

Ogilvie B. M. and Jones V. E. (1969) Reaginic antibodies and helminth infections. In *Cellular and Humoral Mechanisms in Anaphylaxis and Allergy* (Edited by Movat H. Z.), p. 13. Karger, Basel, New York.

Ohara J. and Paul W. E. (1987) Receptors for B-cell stimulatory factor-1 expressed on cells of haematopoietic lineage. *Nature* **325**, 537-40.

Ohara J. and Paul W. E. (1988) Up-Regulation of Interleukin 4/B-Cell Stimulatory Factor 1 Receptor Expression. *PNAS* **85**, 8221-8225.

Ohkusu K., Yoshimoto T., Takeda K., Ogura T., Kashiwamura S., Iwakura Y., Akira S., Okamura H. and Nakanishi K. (2000) Potentiality of interleukin-18 as a useful reagent for treatment and prevention of *Leishmania major* infection. *Infect Immun* **68**, 2449-56.

Okamura H., Tsutsi H., Komatsu T., Yutsudo M., Hakura A., Tanimoto T., Torigoe K., Okura T., Nukada Y., Hattori K. and et al. (1995) Cloning of a new cytokine that induces IFN-gamma production by T cells. *Nature* **378**, 88-91.

Onishi K., Li Y., Ishii K., Hisaeda H., Tang L., Duan X., Dainichi T., Maekawa Y., Katunuma N. and Himeno K. (2004) Cathepsin L is crucial for a Th1-type immune response during *Leishmania major* infection. *Microbes Infect* **6**, 468-74.

Oppmann B., Lesley R., Blom B., Timans J. C., Xu Y., Hunte B., Vega F., Yu N., Wang J., Singh K., Zonin F., Vaisberg E., Churakova T., Liu M., Gorman D., Wagner J., Zurawski S., Liu Y., Abrams J. S., Moore K. W., Rennick D., de Waal-Malefyt R., Hannum C., Bazan J. F. and Kastelein R. A. (2000) Novel p19 protein engages IL-12p40 to form a cytokine, IL-23, with biological activities similar as well as distinct from IL-12. *Immunity* **13**, 715-25.

Orchansky P. L., Kwan R., Lee F. and Schrader J. W. (1999) Characterization of the Cytoplasmic Domain of Interleukin-13 Receptor-alpha. *J. Biol. Chem.* **274**, 20818-20825.

O'Shea J. J., Ma A., and Lipsky P. (2002) Cytokines and autoimmunity. *Nat Rev Immunol* **2**(1), 37-45.

Ouaissi M. A. (1988) Role of the RGD sequence in parasite adhesion to host cells. *Parasitol Today* **4**, 169-73.

Ozawa H., Tamauchi H., Ito M., Terashima M., Inoue M., Hozumi K., Habu S. and Watanabe N. (2005) Immune responses to *Nippostrongylus brasiliensis* and tuberculin protein in GATA-3-transgenic mice. *Immunol Lett* **99**, 228-35.

Park L. S., Friend D., Grabstein K. and Urdal D. L. (1987a) Characterization of the high-affinity cell-surface receptor for murine B-cell-stimulating factor 1. *Proc Natl Acad Sci U S A* **84**, 1669-73.

Park L. S., Friend D., Sassenfeld H. M. and Urdal D. L. (1987b) Characterization of the human B cell stimulatory factor 1 receptor. *J Exp Med* **166**, 476-88.

Pearson R. D., Wheeler D. A., Harrison L. H. and Kay H. D. (1983) The immunobiology of leishmaniasis. *Rev Infect Dis* **5**, 907-927.

Peters C., Aebischer T., Stierhof Y. D., Fuchs M. and Overath P. (1995) The role of macrophage receptors in adhesion and uptake of *Leishmania mexicana* amastigotes. *J Cell Sci* **108 (Pt 12)**, 3715-24.

Pflanz S., Timans J. C., Cheung J., Rosales R., Kanzler H., Gilbert J., Hibbert L., Churakova T., Travis M., Vaisberg E., Blumenschein W. M., Mattson J. D., Wagner J. L., To W., Zurawski S., McClanahan T. K., Gorman D. M., Bazan J. F., de Waal Malefyt R., Rennick D. and Kastelein R. A. (2002) IL-27, a heterodimeric cytokine composed of EBI3 and p28 protein, induces proliferation of naive CD4(+) T cells. *Immunity* **16**, 779-90.

Pollock K. G., McNeil K. S., Mottram J. C., Lyons R. E., Brewer J. M., Scott P., Coombs G. H. and Alexander J. (2003) The *Leishmania mexicana* cysteine protease, CPB2.8, induces potent Th2 responses. *J Immunol* **170**, 1746-53.

Presky D. H., Minetti L. J., Gillessen S., Gubler U., Chizzonite R., Stern A. S. and Gately M. K. (1996) Evidence for multiple sites of interaction between IL-12 and its receptor. *Ann N Y Acad Sci* **795**, 390-3.

Punnonen J., Aversa G., Cocks B. G., McKenzie A. N., Menon S., Zurawski G., de Waal Malefyt R. and de Vries J. E. (1993) Interleukin 13 induces interleukin 4-independent IgG4 and IgE synthesis and CD23 expression by human B cells. *Proc Natl Acad Sci U S A* **90**, 3730-4.

Raghavan M. and Bjorkman P. J. (1996) Fc receptors and their interactions with immunoglobulins. *Annu Rev Cell Dev Biol* **12**, 181-220.

Reik W., Williams G., Barton S., Norris M., Neuberger M. and Surani M. A. (1987) Provision of the immunoglobulin heavy chain enhancer downstream of a test gene is sufficient to confer lymphoid-specific expression in transgenic mice. *Eur J Immunol* **17**, 465-9.

Reiner S., Zheng S., Wang Z., Stowring L. and Locksley R. (1994) *Leishmania* promastigotes evade interleukin 12 (IL-12) induction by macrophages and stimulate a broad range of cytokines from CD4+ T cells during initiation of infection. *J. Exp. Med.* **179**, 447-456.

Rennick D., Yang G., Muller-Sieburg C., Smith C., Arai N., Takabe Y. and Gemmell L. (1987) Interleukin 4 (B-cell stimulatory factor 1) can enhance or antagonize the factor-dependent growth of hemopoietic progenitor cells. *Proc Natl Acad Sci U S A* **84**, 6889-93.

Roberts M. T. M., Stober C. B., McKenzie A. N. and Blackwell J. M. (2005) Interleukin-4 (IL-4) and IL-10 Collude in Vaccine Failure for Novel Exacerbatory Antigens in Murine *Leishmania major* Infection. *Infect. Immun.* **73**, 7620-7628.

Roberts S. C., Tancer M. J., Polinsky M. R., Gibson K. M., Heby O. and Ullman B. (2004) Arginase plays a pivotal role in polyamine precursor metabolism in *Leishmania*: characterization of gene deletion mutants. *J. Biol. Chem.* **279**, 23668-23678.

Roitt I., Brostoff J. and Male D. (2002) Immunology. Mosby, London.

Romagnani S. (1996a) Th1 and Th2 in human diseases. *Clin Immunol Immunopathol* **80**, 225-35.

Romagnani S. (1996b) Understanding the role of Th1/Th2 cells in infection. *Trends Microbiol* **4**, 470-3.

Ron Y. and Sprent J. (1987) T cell priming *in vivo*: a major role for B cells in presenting antigen to T cells in lymph nodes. *J Immunol* **138**, 2848-56.

Rosbottom A., Knight P. A., McLachlan G., Thornton E. M., Wright S. W., Miller H. R. and Scudamore C. L. (2002) Chemokine and cytokine expression in murine intestinal epithelium following *Nippostrongylus brasiliensis* infection. *Parasite Immunol* **24**, 67-75.

Rudolph M. G., Stanfield R. L., and Wilson I. A. (2006) How TCRs bind MHCs, peptides, and coreceptors. *Annu Rev Immunol* **24**, 419-66.

Russell J. H. and Ley T. J. (2002) Lymphocyte-mediated cytotoxicity. *Annu Rev Immunol* **20**, 323-70.

Russell S., Keegan A., Harada N., Nakamura Y., Noguchi M., Leland P., Friedmann M., Miyajima A., Puri R., Paul W. and et a. (1993) Interleukin-2 receptor gamma chain: a functional component of the interleukin-4 receptor. *Science* **262**, 1880-1883.

Russell S. M., Johnston J. A., Noguchi M., Kawamura M., Bacon C. M., Friedmann M., Berg M., McVicar D. W., Witthuhn B. A., Silvennoinen O. and et al. (1994) Interaction of IL-2R beta and gamma c chains with Jak1 and Jak3: implications for XSCID and XCID. *Science* **266**, 1042-5.

Rutschman R., Lang R., Hesse M., Ihle J. N., Wynn T. A. and Murray P. J. (2001) Cutting edge: Stat6-dependent substrate depletion regulates nitric oxide production. *J Immunol* **166**, 2173-7.

Ryan J. J., McReynolds L. J., Huang H., Nelms K. and Paul W. E. (1998) Characterization of a mobile Stat6 activation motif in the human IL-4 receptor. *J Immunol* **161**, 1811-21.

Sad S. and Mosmann T. (1995) Interleukin (IL) 4, in the absence of antigen stimulation, induces an anergy-like state in differentiated CD8+ TC1 cells: loss of IL-2 synthesis and autonomous proliferation but retention of cytotoxicity and synthesis of other cytokines. *J. Exp. Med.* **182**, 1505-1515.

Sad S. and Mosmann T. R. (1994) Single IL-2-secreting precursor CD4 T cell can develop into either Th1 or Th2 cytokine secretion phenotype. *J Immunol* **153**, 3514-22.

Sadick M. D., Heinzel F. P., Holaday B. J., Pu R. T., Dawkins R. S. and Locksley R. M. (1990) Cure of murine leishmaniasis with anti-interleukin 4 monoclonal antibody. Evidence for a T cell-dependent, interferon gamma-independent mechanism. *J Exp Med* **171**, 115-27.

Sadick M. D., Locksley R. M., Tubbs C. and Raff H. V. (1986) Murine cutaneous leishmaniasis: resistance correlates with the capacity to generate interferon-gamma in response to *Leishmania* antigens *in vitro*. *J Immunol* **136**, 655-61.

Sadick M. D., Street N., Mosmann T. R. and Locksley R. M. (1991) Cytokine regulation of murine leishmaniasis: interleukin 4 is not sufficient to mediate progressive disease in resistant C57BL/6 mice. *Infect Immun* **59**, 4710-4.

Sakaguchi S., Sakaguchi N., Asano M., Itoh M. and Toda M. (1995) Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J Immunol* **155**, 1151-64.

Sato T. A., Widmer M. B., Finkelman F. D., Madani H., Jacobs C. A., Grabstein K. H. and Maliszewski C. R. (1993) Recombinant soluble murine IL-4 receptor can inhibit or enhance IgE responses *in vivo*. *J Immunol* **150**, 2717-23.

Savioli L., Stansfield S., Bundy D. A., Mitchell A., Bhatia R., Engels D., Montresor A., Neira M. and Shein A. M. (2002) Schistosomiasis and soil-transmitted helminth infections: forging control efforts. *Trans R Soc Trop Med Hyg* **96**, 577-9.

Schwartz L. B. and Austen K. F. (1984) Structure and function of the chemical mediators of mast cells. *Prog Allergy* **34**, 271-321.

Scott P., Eaton A., Gause W. C., di Zhou X. and Hondowicz B. (1996) Early IL-4 Production Does Not Predict Susceptibility to *Leishmania major*. *Experimental Parasitology* **84**, 178-187.

Seder R. A., Gazzinelli R., Sher A. and Paul W. E. (1993) Interleukin 12 acts directly on CD4+ T cells to enhance priming for interferon gamma production and diminishes interleukin 4 inhibition of such priming. *Proc Natl Acad Sci U S A* **90**, 10188-92.

Shea-Donohue T. and Urban J. F., Jr. (2004) Gastrointestinal parasite and host interactions. *Curr Opin Gastroenterol* **20**, 3-9.

Shevach E. M. (2002) CD4+ CD25+ suppressor T cells: more questions than answers. *Nat Rev Immunol* **2**, 389-400.

Shimoda K., van Deursen J., Sangster M. Y., Sarawar S. R., Carson R. T., Tripp R. A., Chu C., Quelle F. W., Nosaka T., Vignali D. A., Doherty P. C., Grosveld G., Paul W. E. and Ihle J. N. (1996) Lack of IL-4-induced Th2 response and IgE class switching in mice with disrupted Stat6 gene. *Nature* **380**, 630-3.

Shinkai K., Mohrs M. and Locksley R. M. (2002) Helper T cells regulate type-2 innate immunity *in vivo*. *Nature* **420**, 825-9.

Smirnov D. V., Smirnova M. G., Korobko V. G. and Frolova E. I. (1995) Tandem arrangement of human genes for interleukin-4 and interleukin-13: resemblance in their organization. *Gene* **155**, 277-81.

Snapper C. M., Finkelman F. D., Stefany D., Conrad D. H. and Paul W. E. (1988) IL-4 induces co-expression of intrinsic membrane IgG1 and IgE by murine B cells stimulated with lipopolysaccharide. *J Immunol* **141**, 489-98.

Snapper C. M. and Paul W. E. (1987) B cell stimulatory factor-1 (interleukin 4) prepares resting murine B cells to secrete IgG1 upon subsequent stimulation with bacterial lipopolysaccharide. *J Immunol* **139**, 10-7.

Sornasse T., Larenas P. V., Davis K. A., de Vries J. E. and Yssel H. (1996) Differentiation and stability of T helper 1 and 2 cells derived from naive human neonatal CD4+ T cells, analyzed at the single-cell level. *J Exp Med* **184**, 473-83.

Sousa C. R. e., Hieny S., Scharton-Kersten T., Jankovic D., Charest H., Germain R. N. and Sher A. (1997) *In Vivo* Microbial Stimulation Induces Rapid CD40 Ligand-independent Production of Interleukin 12 by Dendritic Cells and their Redistribution to T Cell Areas. *J. Exp. Med.* **186**, 1819-1829.

Spits H., Yssel H., Takebe Y., Arai N., Yokota T., Lee F., Arai K., Banchereau J. and de Vries J. E. (1987) Recombinant interleukin 4 promotes the growth of human T cells. *J Immunol* **139**, 1142-7.

Stack R. M., Lenschow D. J., Gray G. S., Bluestone J. A. and Fitch F. W. (1994) IL-4 treatment of small splenic B cells induces costimulatory molecules B7-1 and B7-2. *J Immunol* **152**, 5723-33.

Standiford T. J., Strieter R. M., Chensue S. W., Westwick J., Kasahara K. and Kunkel S. L. (1990) IL-4 inhibits the expression of IL-8 from stimulated human monocytes. *J Immunol* **145**, 1435-9.

Stein M., Keshav S., Harris N. and Gordon S. (1992) Interleukin 4 potently enhances murine macrophage mannose receptor activity: a marker of alternative immunologic macrophage activation. *J Exp Med* **176**, 287-92.

Steinke J. W. and Borish L. (2006) 3. Cytokines and chemokines. *J Allergy Clin Immunol* **117**(2 Suppl Mini-Primer), S441-5.

Stenger S., Thuring H., Rollinghoff M. and Bogdan C. (1994) Tissue expression of inducible nitric oxide synthase is closely associated with resistance to *Leishmania major*. *J Exp Med* **180**, 783-93.

Stern L. J., Potolicchio I., and Santambrogio L. (2006) MHC class II compartment subtypes: structure and function. *Curr Opin Immunol* **18**(1), 64-9.

Stetson D. B., Mohrs M., Mallet-Designe V., Teyton L. and Locksley R. M. (2002) Rapid expansion and IL-4 expression by *Leishmania*-specific naive helper T cells *in vivo*. *Immunity* **17**, 191-200.

Stevens T. L., Bossie A., Sanders V. M., Fernandez-Botran R., Coffman R. L., Mosmann T. R. and Vitetta E. S. (1988) Regulation of antibody isotype secretion by subsets of antigen-specific helper T cells. *Nature* **334**, 255-8.

Sugawara I., Yamada H., Kaneko H., Mizuno S., Takeda K. and Akira S. (1999) Role of interleukin-18 (IL-18) in mycobacterial infection in IL-18-gene-disrupted mice. *Infect Immun* **67**, 2585-9.

Sutterwala F. S., Noel G. J., Salgame P. and Mosser D. M. (1998) Reversal of proinflammatory responses by ligating the macrophage Fcgamma receptor type I. *J Exp Med* **188**, 217-22.

Swain S. L., McKenzie D. T., Weinberg A. D. and Hancock W. (1988) Characterization of T helper 1 and 2 cell subsets in normal mice. Helper T cells responsible for IL-4 and IL-5 production are present as precursors that require priming before they develop into lymphokine-secreting cells. *J Immunol* **141**, 3445-55.

Swain S. L., Weinberg A. D., English M. and Huston G. (1990) IL-4 directs the development of Th2-like helper effectors. *J Immunol* **145**, 3796-806.

Swihart K., Fruth U., Messmer N., Hug K., Behin R., Huang S., Del Giudice G., Aguet M. and Louis J. (1995) Mice from a genetically resistant background lacking the interferon gamma receptor are susceptible to infection with *Leishmania major* but mount a polarized T helper cell 1-type CD4+ T cell response. *J. Exp. Med.* **181**, 961-971.

Sypek J., Chung C., Mayor S., Subramanyam J., Goldman S., Sieburth D., Wolf S. and Schaub R. (1993) Resolution of cutaneous leishmaniasis: interleukin 12 initiates a protective T helper type 1 immune response. *J. Exp. Med.* **177**, 1797-1802.

Szabo S. J., Dighe A. S., Gubler U. and Murphy K. M. (1997) Regulation of the Interleukin (IL)-12R beta 2 Subunit Expression in Developing T Helper 1 (Th1) and Th2 Cells. *J. Exp. Med.* **185**, 817-824.

Szabo S. J., Jacobson N. G., Dighe A. S., Gubler U. and Murphy K. M. (1995) Developmental commitment to the Th2 lineage by extinction of IL-12 signaling. *Immunity* **2**, 665-675.

Szabo S. J., Kim S. T., Costa G. L., Zhang X., Fathman C. G. and Glimcher L. H. (2000) A novel transcription factor, T-bet, directs Th1 lineage commitment. *Cell* **100**, 655-69.

Takeda K., Tanaka T., Shi W., Matsumoto M., Minami M., Kashiwamura S., Nakanishi K., Yoshida N., Kishimoto T. and Akira S. (1996) Essential role of Stat6 in IL-4 signalling. *Nature* **380**, 627-30.

Takeshita T., Ohtani K., Asao H., Kumaki S., Nakamura M. and Sugamura K. (1992) An associated molecule, p64, with IL-2 receptor beta chain. Its possible involvement in the formation of the functional intermediate-affinity IL-2 receptor complex. *J Immunol* **148**, 2154-8.

Tanaka T., Hu-Li J., Seder R. A., Fazekas de St Groth B. and Paul W. E. (1993) Interleukin 4 suppresses interleukin 2 and interferon gamma production by naive T cells stimulated by accessory cell-dependent receptor engagement. *Proc Natl Acad Sci U S A* **90**, 5914-8.

te Velde A. A., Huijbens R. J., de Vries J. E. and Figdor C. G. (1990) IL-4 decreases Fc gamma R membrane expression and Fc gamma R-mediated cytotoxic activity of human monocytes. *J Immunol* **144**, 3046-51.

Terabe M., Matsui S., Noben-Trauth N., Chen H., Watson C., Donaldson D. D., Carbone D. P., Paul W. E. and Berzofsky J. A. (2000) NKT cell-mediated repression of tumor immunosurveillance by IL-13 and the IL-4R-STAT6 pathway. *Nat Immunol* **1**, 515-20.

Thornhill M. and Haskard D. (1990) IL-4 regulates endothelial cell activation by IL-1, tumor necrosis factor, or IFN-gamma. *J Immunol* **145**, 865-872.

Titus R., Sherry B. and Cerami A. (1989) Tumor necrosis factor plays a protective role in experimental murine cutaneous leishmaniasis. *J. Exp. Med.* **170**, 2097-2104.

Townsend J. M., Fallon G. P., Matthews J. D., Smith P., Jolin E. H. and McKenzie N. A. (2000) IL-9-deficient mice establish fundamental roles for IL-9 in pulmonary mastocytosis and goblet cell hyperplasia but not T cell development. *Immunity* **13**, 573-83.

Trinchieri G. (1998) Interleukin-12: a cytokine at the interface of inflammation and immunity. *Adv Immunol* **70**, 83-243.

Tripp C. S., Wolf S. F. and Unanue E. R. (1993) Interleukin 12 and tumor necrosis factor alpha are costimulators of interferon gamma production by natural killer cells in severe combined immunodeficiency mice with listeriosis, and interleukin 10 is a physiologic antagonist. *Proc Natl Acad Sci U S A* **90**, 3725-9.

Umeshita-Suyama R., Sugimoto R., Akaiwa M., Arima K., Yu B., Wada M., Kuwano M., Nakajima K., Hamasaki N. and Izuhara K. (2000) Characterization of IL-4 and IL-13 signals dependent on the human IL-13 receptor alpha chain 1: redundancy of requirement of tyrosine residue for STAT3 activation. *Int Immunol* **12**, 1499-509.

University of Cambridge. (2006) Department of Pathology: Nippostrongylus brasiliensis life cycle. http://www.path.cam.ac.uk/~schisto/Parasitology_Practicals/Practical_figures/Nippo_Life.gif

Urban J. F., Jr. (1982) Cellular basis of the non-specific potentiation of the immunoglobulin E response after helminth parasite infection. *Vet Parasitol* **10**, 131-40.

Urban J. F., Jr., Madden K. B., Svetic A., Cheever A., Trotta P. P., Gause W. C., Katona I. M. and Finkelman F. D. (1992) The importance of Th2 cytokines in protective immunity to nematodes. *Immunol Rev* **127**, 205-20.

Urban J. F., Jr., Maliszewski C. R., Madden K. B., Katona I. M. and Finkelman F. D. (1995) IL-4 treatment can cure established gastrointestinal nematode infections in immunocompetent and immunodeficient mice. *J Immunol* **154**, 4675-84.

Urban J. F., Jr., Noben-Trauth N., Donaldson D. D., Madden K. B., Morris S. C., Collins M. and Finkelman F. D. (1998) IL-13, IL-4Ralpha, and Stat6 are required for the expulsion of the gastrointestinal nematode parasite *Nippostrongylus brasiliensis*. *Immunity* **8**, 255-64.

Urban J. F., Jr., Noben-Trauth N., Schopf L., Madden K. B. and Finkelman F. D. (2001) Cutting edge: IL-4 receptor expression by non-bone marrow-derived cells is required to expel gastrointestinal nematode parasites. *J Immunol* **167**, 6078-81.

Urban J. F., Jr., Schopf L., Morris S. C., Orekhova T., Madden K. B., Betts C. J., Gamble H. R., Byrd C., Donaldson D., Else K. and Finkelman F. D. (2000) Stat6 signaling promotes protective immunity against *Trichinella spiralis* through a mast cell- and T cell-dependent mechanism. *J Immunol* **164**, 2046-52.

Varkila K., Chatelain R., Leal L. M. and Coffman R. L. (1993) Reconstitution of C.B-17 scid mice with BALB/c T cells initiates a T helper type-1 response and renders them capable of healing *Leishmania major* infection. *Eur J Immunol* **23**, 262-8.

Vieira L. Q., Goldschmidt M., Nashleanas M., Pfeffer K., Mak T. and Scott P. (1996) Mice lacking the TNF receptor p55 fail to resolve lesions caused by infection with *Leishmania major*, but control parasite replication. *J Immunol* **157**, 827-35.

Vieth M., Will A., Schroppel K., Rollinghoff M. and Gessner A. (1994) Interleukin-10 inhibits antimicrobial activity against *Leishmania major* in murine macrophages. *Scand J Immunol* **40**, 403-9.

Vita N., Lefort S., Laurent P., Caput D. and Ferrara P. (1995) Characterization and comparison of the interleukin 13 receptor with the interleukin 4 receptor on several cell types. *J Biol Chem* **270**, 3512-7.

Vitetta E. S., Ohara J., Myers C. D., Layton J. E., Krammer P. H. and Paul W. E. (1985) Serological, biochemical, and functional identity of B cell-stimulatory factor 1 and B cell differentiation factor for IgG1. *J Exp Med* **162**, 1726-31.

Vladimirov V., Badalova J., Svobodova M., Havelkova H., Hart A. A., Blazkova H., Demant P. and Lipoldova M. (2003) Different genetic control of cutaneous and visceral disease after *Leishmania major* infection in mice. *Infect Immun* **71**, 2041-6.

Voehringer D., Reese T. A., Huang X., Shinkai K. and Locksley R. M. (2006) Type 2 immunity is controlled by IL-4/IL-13 expression in hematopoietic non-eosinophil cells of the innate immune system. *J Exp Med* **203**, 1435-46.

Voehringer D., Shinkai K. and Locksley R. M. (2004) Type 2 immunity reflects orchestrated recruitment of cells committed to IL-4 production. *Immunity* **20**, 267-77.

von der Weid T., Beebe A. M., Roopenian D. C. and Coffman R. L. (1996) Early production of IL-4 and induction of Th2 responses in the lymph node originate from an MHC class I-independent CD4+NK1.1- T cell population. *J Immunol* **157**, 4421-7.

von Stebut E., Belkaid Y., Jakob T., Sacks D. L. and Udey M. C. (1998) Uptake of *Leishmania major* amastigotes results in activation and Interleukin 12 release from murine skin-derived dendritic cells: Implications for the initiation of anti-*Leishmania* immunity. *J. Exp. Med.* **188**, 1547-1552.

von Stebut E., Ehrchen J. M., Belkaid Y., Kostka S. L., Molle K., Knop J., Sunderkotter C. and Udey M. C. (2003) Interleukin 1α Promotes Th1 Differentiation and Inhibits Disease Progression in *Leishmania major*-susceptible BALB/c Mice. *J. Exp. Med.* **198**, 191-199.

Wakil A. E., Wang Z. E. and Locksley R. M. (1996) *Leishmania major*: targeting IL-4 in successful immunomodulation of murine infection. *Exp Parasitol* **84**, 214-22.

Watts C. (1997) Capture and processing of exogenous antigens for presentation on MHC molecules. *Annu Rev Immunol* **15**, 821-50.

Wei X.-q., Charles I. G., Smith A., Ure J., Feng G.-j., Huang F.-p., Xu D., MullersWerner, Moncada S. and Liew F. Y. (1995) Altered immune responses in mice lacking inducible nitric oxide synthase. **375**, 408-411.

Welham M. J., Learmonth L., Bone H. and Schrader J. W. (1995) Interleukin-13 signal transduction in lymphohemopoietic cells. Similarities and differences in signal transduction with interleukin-4 and insulin. *J Biol Chem* **270**, 12286-96.

Wieser M., Bonifer R., Oster W., Lindemann A., Mertelsmann R. and Herrmann F. (1989) Interleukin-4 induces secretion of CSF for granulocytes and CSF for macrophages by peripheral blood monocytes. *Blood* **73**, 1105-8.

Wilhelm P., Ritter U., Labbow S., Donhauser N., Rollinghoff M., Bogdan C. and Korner H. (2001) Rapidly fatal leishmaniasis in resistant C57BL/6 mice lacking TNF. *J Immunol* **166**, 4012-9.

Will A., Blank C., Rollinghoff M. and Moll H. (1992) Murine epidermal Langerhans cells are potent stimulators of an antigen-specific T cell response to *Leishmania major*, the cause of cutaneous leishmaniasis. *Eur J Immunol* **22**, 1341-7.

Wills-Karp M., Luyimbazi J., Xu X., Schofield B., Neben T. Y., Karp C. L. and Donaldson D. D. (1998) Interleukin-13: central mediator of allergic asthma. *Science* **282**, 2258-61.

Wilson M. and Pearson R. (1986) Evidence that *Leishmania donovani* utilizes a mannose receptor on human mononuclear phagocytes to establish intracellular parasitism. *J Immunol* **136**, 4681-4688.

Witthuhn B. A., Silvennoinen O., Miura O., Lai K. S., Cwik C., Liu E. T. and Ihle J. N. (1994) Involvement of the Jak-3 Janus kinase in signalling by interleukins 2 and 4 in lymphoid and myeloid cells. *Nature* **370**, 153-7.

Woodland D. L. and Dutton R. W. (2003) Heterogeneity of CD4(+) and CD8(+) T cells. *Curr Opin Immunol* **15**(3), 336-42.

Wong P. and Pamer E. G. (2003) CD8 T cell responses to infectious pathogens. *Annu Rev Immunol* **21**, 29-70.

Wrighton N., Campbell L. A., Harada N., Miyajima A. and Lee F. (1992) The murine interleukin-4 receptor gene: genomic structure, expression and potential for alternative splicing. *Growth Factors* **6**, 103-18.

Xu D., Liu H., Komai-Koma M., Campbell C., McSharry C., Alexander J. and Liew F. Y. (2003) CD4+CD25+ Regulatory T cells suppress differentiation and functions of Th1 and Th2 cells, *Leishmania major* infection, and colitis in mice. *J Immunol* **170**, 394-399.

Yin T., Tsang M. L. and Yang Y. C. (1994) JAK1 kinase forms complexes with interleukin-4 receptor and 4PS/insulin receptor substrate-1-like protein and is activated by interleukin-4 and interleukin-9 in T lymphocytes. *J Biol Chem* **269**, 26614-7.

Ying S., Meng Q., Barata L. T., Robinson D. S., Durham S. R. and Kay A. B. (1997) Associations between IL-13 and IL-4 (mRNA and protein), vascular cell adhesion molecule-1 expression, and the infiltration of eosinophils, macrophages, and T cells in allergen-induced late-phase cutaneous reactions in atopic subjects. *J Immunol* **158**, 5050-7.

Yokota T., Otsuka T., Mosmann T., Banchereau J., DeFrance T., Blanchard D., Vries J. E. D., Lee F. and Arai K.-I. (1986) Isolation and characterization of a human Interleukin cDNA clone, homologous to mouse B-cell stimulatory factor 1, that expresses B-cell- and T-cell-stimulating activities. *PNAS* **83**, 5894-5898.

Yoshida H., Hamano S., Senaldi G., Covey T., Faggioni R., Mu S., Xia M., Wakeham A. C., Nishina H., Potter J., Saris C. J. and Mak T. W. (2001) WSX-1 is required for the initiation of Th1 responses and resistance to *L. major* infection. *Immunity* **15**, 569-78.

Yoshimoto T. and Paul W. (1994) CD4pos, NK1.1pos T cells promptly produce interleukin 4 in response to *in vivo* challenge with anti-CD3. *J. Exp. Med.* **179**, 1285-1295.

Yu C., Cantor A. B., Yang H., Browne C., Wells R. A., Fujiwara Y. and Orkin S. H. (2002) Targeted deletion of a high-affinity GATA-binding site in the GATA-1 promoter leads to selective loss of the eosinophil lineage *in vivo*. *J Exp Med* **195**, 1387-95.

Zhang J. G., Hilton D. J., Willson T. A., McFarlane C., Roberts B. A., Moritz R. L., Simpson R. J., Alexander W. S., Metcalf D. and Nicola N. A. (1997) Identification, purification, and characterization of a soluble interleukin (IL)-13-binding protein. Evidence that it is distinct from the cloned Il-13 receptor and Il-4 receptor alpha-chains. *J Biol Chem* **272**, 9474-80.

Zhao A., McDermott J., Urban J. F., Jr., Gause W., Madden K. B., Yeung K. A., Morris S. C., Finkelman F. D. and Shea-Donohue T. (2003) Dependence of IL-4, IL-13, and nematode-induced alterations in murine small intestinal smooth muscle contractility on Stat6 and enteric nerves. *J Immunol* **171**, 948-54.

Zhao A., Morimoto M., Dawson H., Elfrey J. E., Madden K. B., Gause W. C., Min B., Finkelman F. D., Urban J. F., Jr. and Shea-Donohue T. (2005) Immune regulation of protease-activated receptor-1 expression in murine small intestine during *Nippostrongylus brasiliensis* infection. *J Immunol* **175**, 2563-9.

Zheng W. and Flavell R. A. (1997) The transcription factor GATA-3 is necessary and sufficient for Th2 cytokine gene expression in CD4 T cells. *Cell* **89**, 587-96.

Zurawski G. and de Vries J. E. (1994) Interleukin 13, an interleukin 4-like cytokine that acts on monocytes and B cells, but not on T cells. *Immunol Today* **15**, 19-26.

Zurawski S. M., Chomarat P., Djossou O., Bidaud C., McKenzie A. N., Miossec P., Banchereau J. and Zurawski G. (1995) The primary binding subunit of the human interleukin-4 receptor is also a component of the interleukin-13 receptor. *J Biol Chem* **270**, 13869-78.

Zurawski S. M., Vega F., Jr., Huyghe B. and Zurawski G. (1993) Receptors for interleukin-13 and interleukin-4 are complex and share a novel component that functions in signal transduction. *Embo J* **12**, 2663-70.

APPENDIX A

Reagents

All chemical reagents used were of analytical grade and were purchased from the following companies unless otherwise stated:

BDH Chemicals Ltd, Poole, England

Merck Laboratory Supplies, South Africa

Sigma-Aldrich, South Africa

The composition of the general reagents used is described below.

Phosphate Buffered Saline

- 8g NaCl
- 0.2g KCl
- 1.44g Na_2HPO_4
- 0.24g KH_2PO_4

Dissolve reagents in 900ml distilled H_2O. Adjust to pH 7.4 with HCl. Adjust the volume to 1000ml. Filter through a 0.22µm filter (Millipore Corporation, Bedford, USA).

ELISA reagents

Table 2. Antibodies used in cytokine ELISA

Cytokine	Capturing Antibody Clone number	Detecting Antibody Clone number (biotinylated)	Source
IL-4	BVD4 – 1D11	BVD6-24G2	BD Pharmingen
IL-5	TRFK5	TRFK4	BD Pharmingen
IL-13	38213	Matched to capturing antibody	R&D Systems
IFN-γ	R4-6A2	XMG1.2	BD Pharmingen

Blocking buffer

- 20g Bovine Serum Albumin (Boehringer Manheim, Germany)
- 0.2g NaN_3

Dissolve reagents in 800ml PBS (pH 7.4). Adjust the volume to 1000ml and store at 4°C.

Dilution buffer

- 10g Bovine Serum Albumin (Boehringer Manheim, Germany)
- 0.2g NaN_3

Dissolve reagents in 800ml PBS (pH 7.4). Adjust the volume to 1000ml. Filter through a 0.22µm filter (Millipore Corporation, Bedford, USA) and store at 4°C.

Substrate buffer

- 0.2g NaN_3
- 0.8g MgCl

Dissolve reagents in 700ml distilled H_2O. Add 97ml of liquefied diethanolamine. Adjust to pH 9.8 with concentrated HCl. Adjust the volume to 1000ml with distilled H_2O. Filter through a 0.22µm filter (Millipore Corporation, Bedford, USA) and store in dark at 4°C.

20x Washing buffer

- 20g KCl
- 20g KH_2PO_4
- 144g $Na_2HPO_4.2H_2O$
- 800g NaCl

Dissolve reagents in 4.5 liters distilled H_2O. Add 50ml Tween 20 and 100ml 10% NaN_3 solution. Adjust volume to 5 liters with distilled H_2O and store at room temperature.

FACS buffer

- 1g Bovine Serum Albumin (Boehringer Manheim, Germany)
- 0.05g NaN3

Dissolve reagents in 900ml PBS (pH 7.4). Adjust volume to 1000ml. Filter through a 0.22µm filter (Millipore Corporation, Bedford, USA) and store at 4°C.

Iscoves Modified Dulbeccos Medium

Dissolve 1 tube (for 1liter) of IMDM (Gibco/BRL #4302200) powder in 800ml distilled H_2O. Add 3.024g $NaHCO_3$ and 2ml of 500x penicillin/streptomycin. Using 1M NaOH, adjust to pH between 7.2 and 7.4. Adjust volume to 1000ml with distilled H_2O. Filter through a 0.22µm filter (Millipore Corporation, Bedford, USA) and store at 4°C.

500x Penicillin/Streptomycin

Dissolve 754.38mg Penicillin G and 0.5g Streptomycin in 50ml distilled water. Filter through a 0.22µm filter (Millipore Corporation, Bedford, USA) and store at -20°C.

Red cell lysis buffer

- 8.34g NH_4Cl
- 0.037g EDTA
- 1g $NaHCO_3$

Dissolve reagents in 1000ml distilled H_2O. Filter through a 0.22µm filter (Millipore Corporation, Bedford, USA) and store at 4°C.

RPMI 1640

Dissolve 1 tube (for 1liter) of RPMI 1640 (Gibco/ #31800-022) powder in 800ml distilled H_2O. Add 0.85g $NaHCO_3$ and 2ml of 500x penicillin/streptomycin. Using NaOH, adjust to pH 7.6 and adjust volume to 1000ml with distilled H_2O. Filter through a 0.22µm filter (Millipore Corporation, Bedford, USA) and store at 4°C.

Schneider's insect medum

While stirring gently, add 1 tube (for 1liter) of Schneider's insect medium (Sigma/S9895) powder to 800ml distilled H_2O until dispersed. Add 0.4g $NaHCO_3$ to medium and stir until dissolved. Using HCl, adjust the pH to 6.9 while stirring. Slowly add a calcium chloride solution (0.6g anhydrous calcium chloride dissolved in 50ml distilled H_2O) while stirring rapidly to prevent precipitation. While stirring, adjust to pH 7.2 and adjust volume to 1000ml with distilled H_2O. Filter through a 0.22µm filter (Millipore Corporation, Bedford, USA) and store at 4°C.