



Impact of the parasitic helminth *Schistosoma mansoni* on host anti-viral vaccine responses: proof of concept from the anti-polio vaccine

by

Fungai Musaigwa

[BSc Hons (Microbiology and Biochemistry), MPath (Haematological pathology)]

Thesis Presented for the Degree of DOCTOR OF PHILOSOPHY in the Department of Pathology, Faculty of Health Science, University of Cape Town

Supervisor: Dr Justin Komguez Nono
Co-supervisor: Prof Frank Brombacher

**Department of Pathology
Division of Immunology (Cytokine and Disease Group)
International Centre for Genetic Engineering and Biotechnology (ICGEB)
Institute of Infectious Disease and Molecular Medicine Faculty of Health Science (IDM)
University of Cape Town
February 2022**

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I, Fungai Musaigwa, hereby declare that the work on which this thesis is based, is my original work and that neither the whole work nor any part thereof is being, has been, or is to be submitted for another degree in this or any other University.

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Signature:

**This thesis is dedicated to my mother, father, younger brother and older brother
Liober Musaigwa (née Mazai), Motsi Bula Musaigwa, Tawanda Musaigwa and
Simbarashe Musaigwa**

Acknowledgements

This work is made possible by the immeasurable support from my supervisors, **Dr Justin Komguez Nono** and **Prof Frank Brombacher**. I am immensely grateful for your dedication to my growth, your teaching patience, your encouragement when I faltered, and for providing funding, laboratory necessities and critical thinking guidance for the work that has culminated into this thesis. I am honoured and thankful to you for choosing me to be your mentee and student. Thank you for believing in me!

I am grateful to my research teammates who happily took me under their wing in several aspects of my research work. Special thanks to Dr Thabo Mpotje for getting me up to speed on the animal work and laboratory aspects that helped to generate data for this thesis. Your humbleness, kindness, and patience in your teachings, even through your own demanding research work, did not go unnoticed. I am grateful and humbled. Dr Severin Donald Kamdem, the human arm of my work could not have been possible without your hard work under field conditions that demanded resilience, perseverance, and passion to advance science and improve the health of the African child. Your help inside and outside the laboratory is deeply appreciated. Your happiness and dances made us all chuckle and brightened even the long nights spent on the laboratory bench. Dr Nada Abdel Aziz, regularly you challenged my thought process and helped me to master the finer details of several laboratory techniques. Thank you. Dr Paballo Mosala, your strength, resilience and determination in your research work had me believing that I too could it. Always kind and never too busy to assist in several of my laboratory assays. Dr Raphael Aruleba, chief, the Table Mountain hiking adventures were full of insightful discussions and were reaffirming of our science endeavours and visions. Thank you.

Thank you to supportive friends and colleagues of the Brombacher group (Cytokines and Disease group) for all the mind-chiselling discussions, your friendship, and timely assistance in navigating the laboratory; Dr Mumin Ozturk, Dr Jermaine Khumalo, Dr Sabelo Hadebe, Ms Sibongiseni Poswayo, Ms Nontobeko Mthembu, Dr Inssaf Berkiks, Ms Tshepiso Mbangiwa, Ms Solima Sabeel, Dr Bernard Osero, Dr Shandré Pillay, Ms Lwanda Ndwandwe, Dr Lorna Gcanga, Mr Robert Rousseau, Mr Rudranil

Hazra, Dr Sara Shelby Jones, Dr Martyna Scibiorek, Mr Paul Ikwegbue, Ms Zama Cele, Ms Priscilla Opare, Mr Bongani Motaung, Dr Emily Tangie, Dr Khanyisile Kgoadi, Dr Matthew Darby and Mr Timothy Reid (chief). I am also thankful to Ms Wendy Green, Ms Zarinah Sondag-Jattiem, Ms. Lizette Fick and Ms Munadia Ansarie for all the administrative and kind technical support. Thank you to Mr Rodney Lucas, Dr John Chipangura and all the staff from the Research Animal facility (RAF) for kind assistance with animal experiments and welfare.

I am honoured and thankful for the financial assistance I received towards this research from the National Research Foundation (NRF), University of Cape Town (UCT) International Student Scholarship award and the Polio Research Foundation (PRF). Opinions expressed and conclusions arrived at, are those of the author and are not necessarily to be attributed to the funders.

Lastly, I wish to extend my sincere gratitude to my family; mom, dad and my brothers, my sister Ms Patience Ndlovu (née Kamanga), my brother Mr Tendai Mpaso, my uncle and aunt Dr Tafadzwa Chakare and Ms Rejoice Chakare, my friends Mr Raymond Rukoro, Mr Blessing Mapfumo, Ms Constance Chihururu, and Mr Tafadzwa Mugadza. My pillars! We are here today because you went out of your way to support me!

To God be the glory!

Publications contributed

Published and accepted manuscripts, with notable contributions

Authors: Fungai Musaigwa, Severin Donald Kamdem, Thabo Mpotje, Paballo Mosala[#], Nada Abdel Aziz[#], De'Broski R. Herbert, Frank Brombacher and Justin Komguez Nono

Title of Contribution: *Schistosoma mansoni* induces plasmablast and plasma cell death in the bone marrow and accelerates the decline of host vaccine responses

Journal, Date : PLOS Pathogens, 2022

Publisher : PLOS

Format: Original research

DOI: 10.1371/journal.ppat.1010327

[#] Equal contributions

Authors: Justin Komguez Nono[#], Severin Donald Kamdem[#], Fungai Musaigwa, Chukwudi A Nnaji, Frank Brombacher

Title of Contribution: Influence of schistosomiasis on host vaccine responses

Journal, Date: Trends in Parasitology, 2022

Publisher: Elsevier Current Trends

Format: Review

DOI: 10.1016/j.pt.2021.07.009

[#] Equal contributions

Authors: Justin Komguez Nono, Thabo Mpotje, Paballo Mosala, Nada Abdel Aziz, Fungai Musaigwa, Lerato Hlaka, Thomas Spangenberg, Frank Brombacher

Title of Contribution: Praziquantel treatment of *Schistosoma mansoni* infected mice renders them less susceptible to reinfection

Journal, Date: Frontiers in Immunology, 2021

Publisher: Frontiers

Format: Original research

DOI: 10.3389/fimmu.2021.748387

Manuscripts under preparation, with notable contributions

Authors: Thabo Mpotje,[#] Justin Komguez Nono,^{*,#} Paballo Mosala, Nada Abdel Aziz, Donald Nyangahu, **Fungai Musaigwa**, Severin Donald Kamdem, Frank Brombacher,^{*,#}

Title of Contribution: The host microbiome trumps genetics in conferring protection against chronic schistosomiasis.

Format: Original research

[#] Equal contributions

^{*} Co-correspondence

Authors: Mireille Kameni, **Fungai Musaigwa**, Poppy Lamberton, Thomas Spangenberg, Justin Komguez Nono

Title of Contribution: Harnessing Schistosoma-associated Metabolites Changes in the Human Host to Assist Diagnostic and Morbidity Control

Format: Review

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List of Abbreviations

APCs	Antigen-presenting cells
CD	Cluster of differentiation
DAMPs	Damage-associated molecular patterns
DTPa-hepB- IPV-Hib	Diphtheria, Tetanus, Pertussis (acellular, component), hepatitis B, Inactivated Polio Vaccine, Haemophilus influenzae b
ELISA	Enzyme-linked immunosorbent assay
EPI	Extended program on immunization
Foxp3	Foxhead box P3
GC	Germinal center
hepB	Hepatitis B
Ig	Immunoglobulin
IL	interleukin
IFN- γ	Interferon gamma
IPV	Inactivated polio vaccine
MDA	Mass drug administration
MHC	Major histocompatibility class
NLRP3	NLR family pyrin domain containing 3
NLRs	NOD-like receptors
NK	Natural Killer
NTD	Neglected tropical disease
PAMPs	Pathogen-associated molecular patterns
PRR	Pattern recognition receptors
PZQ	Praziquantel
<i>S. guineensis</i>	<i>Schistosoma guineensis</i>
<i>S. haematobium</i>	<i>Schistosoma haematobium</i>
<i>S. intercalatum</i>	<i>Schistosoma intercalatum</i>
<i>S. japonicum</i>	<i>Schistosoma japonicum</i>
<i>S. mansoni</i>	<i>Schistosoma mansoni</i>
<i>S. mekongi</i>	<i>Schistosoma mekongi</i>
SEA	<i>Schistosoma mansoni</i> Soluble Egg Antigen
Sm	<i>Schistosoma mansoni</i>

spp.	Species
T _{CM}	Central memory T
T _{EM}	Effector memory T
T _{fh}	Follicular helper T
T _h	Helper T
TNF- α	Tumour necrosis factor alpha
Treg	Regulatory T
Vac	DTPa-hepB-IPV-Hib vaccinated
VPDs	Vaccine preventable diseases
WHO	World Health Organization

Abstract

Schistosomiasis is a devastating and neglected tropical disease caused by *Schistosoma* spp. parasites. The disease remains greatly neglected by global health control programmes thus classified as a top neglected tropical disease of humankind. One of the most common species of these parasites, *Schistosoma mansoni*, causes hepatosplenic schistosomiasis and distributes widely in sub-Saharan Africa. *Schistosoma mansoni* is physiologically debilitating and potentially deadly with a known potential as a master regulator of the host immune responses. However, the mechanistic bases and the scope of this immunoregulatory potential of *S. mansoni* still remain poorly understood. This study explored the influence of *S. mansoni*-driven schistosomiasis on vaccine-induced memory immunity in schoolchildren from a rural endemic area of Cameroon and in laboratory mice. As a proof of concept, a well-established anti-viral vaccination programme with wide global coverage, i.e., anti-polio vaccination, was evaluated for its sustainability in the face of schistosomiasis in human and mouse hosts. Our findings using the poliovirus specific enzyme-linked immunosorbent assay revealed a schistosomiasis-associated impairment of polio-specific serologic antibody memory responses in previously vaccinated schoolchildren and mice, as judged by significantly reduced serum anti-polio IgG antibody levels. To explore our findings further, cellular evaluations were conducted using flow cytometry in mouse models of vaccination and schistosomiasis. The reduction of anti-polio elicited antibody responses was paralleled by the general depletion, through reduced survival and increased cell death, of bone marrow plasma cells and plasmablasts in schistosomiasis-diseased mice. Notably, schistosomiasis reduced memory T cell responses as well in vaccinated hosts and considerably

impaired the expression of survival markers on antibody-producing long term plasma cells in the bone marrow. When attempting to control the disease by administration of the sole commercially available drug for schistosomiasis control, praziquantel, the parasite-driven depletion of the plasma cell and memory T cell compartment was restored followed by a partial recovery of antibody-producing abilities in vaccinated hosts. Taken together, our findings unprecedentedly demonstrate how schistosomiasis might negatively influence the efficacy and potentially the effectiveness of anti-viral vaccine memory immunity. Additionally, we present findings on how strategic therapeutic interventions against schistosomiasis might positively influence vaccine-elicited anti-viral immunity in schistosomiasis-diseased hosts. Our results have robust implications for future more informed deployment of effective vaccination campaigns, beyond the sole consideration of coverage and encourage frequent mass drug administration of praziquantel in schistosomiasis-endemic areas to ensure the sustainability of vaccine-elicited responses thus protection of the hosts against vaccine preventable diseases.

Chapter 1: Literature review and Introduction

1.1. The immune system – An overview

1.1.1. Innate immunity

Mammals are protected against a wide range of disease-causing pathogens through the activity of the immune system. The non-specific first line of host defence comprises of anatomical and physiological barriers such as the intact skin, low pH in the stomach, and lysozymes in saliva and tears (Turvey and Broide, 2010). Beyond these are two major arms of immunity, the non-specific innate immunity and the antigen-specific adaptive immunity, which are involved in complex interactions involving both forms of immunity to protect their host against invading pathogens (Parkin and Cohen, 2001, Murphy *et al.*, 2008).

Reinforcing the protection conferred by anatomic and physiological barriers, the innate immunity involves a rapidly acting array of immune components and cells such as macrophages, granulocytes, basophils, eosinophils, neutrophils, mast cells, natural killer (NK) cells, along with immunity influencing proteins that act as signals for example cytokines (Parkin and Cohen, 2001, Murphy *et al.*, 2008, Turvey and Broide, 2010) (Figure 1.1). Together, these cells act within minutes to hours when foreign antigen survives or invades through the anatomical and physiological barriers (Parkin and Cohen, 2001, Murphy *et al.*, 2008).

Innate immunity relies on limited genetically predetermined germline-encoded receptors that can identify conserved regions of invading microbes (Turvey and Broide, 2010). The innate receptors, the pattern recognition receptors (PRR), can detect molecules that are commonly found in pathogens, the pathogen associated molecular patterns (PAMPs), through intracellular NOD-like receptors (NLRs) like NOD2 and IPAF, or Toll-like receptors (TLRs) expressed by cells such as macrophages and dendritic cells (Murphy *et al.*, 2008, Jimenez-Dalmaroni *et al.*, 2016, Roh and Sohn, 2018). Differently, the PRRs can detect damage-associated molecular patterns (DAMPs), for example, NLR family pyrin domain containing 3 (NLRP3) and RAGE family receptors (Roh and Sohn, 2018). The DAMPs are identified in products of tissue damage and cell lysis, for example, uric acid

originating from pathogenic action or inflammation (Turvey and Broide, 2010, Roh and Sohn, 2018). Additionally, dendritic cells can also be activated by cytokines produced by inflammatory responses before (Murphy *et al.*, 2008).

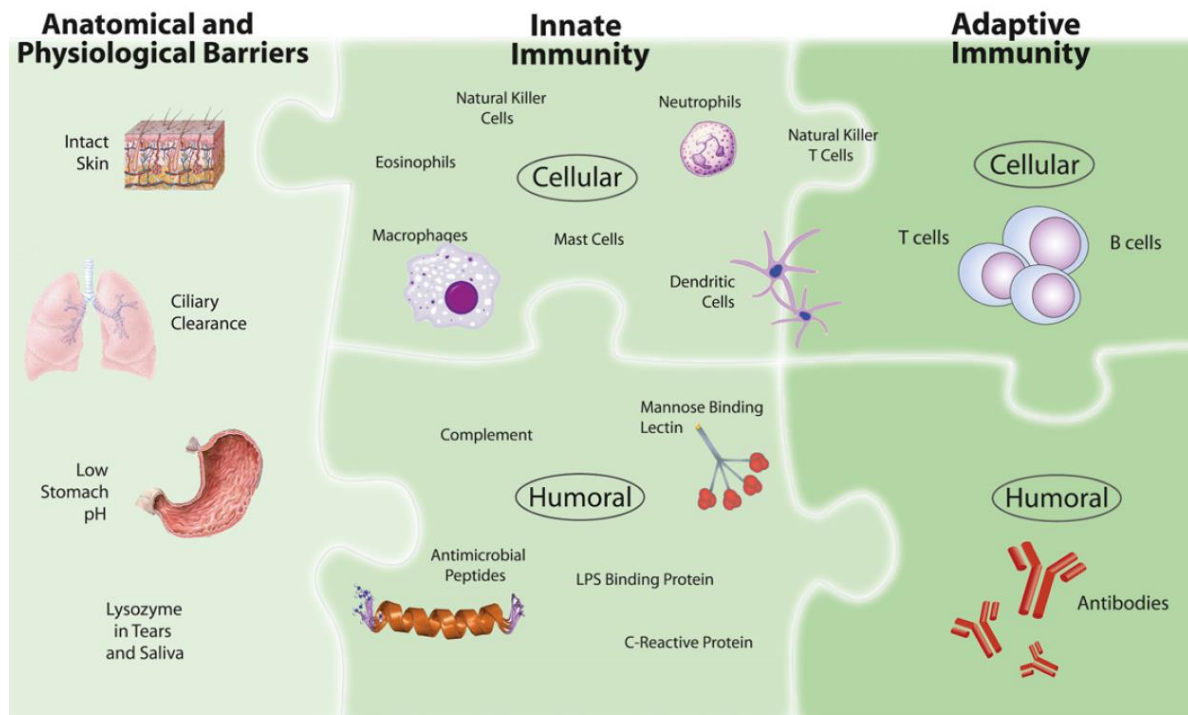


Figure 1. 1: The human immune system.
Figure extracted from (Turvey and Broide, 2010).

Innate immunity can also identify pathogens when its cellular system fails to recognise on their surface, molecules that are normally expressed on the host's healthy cells. The identification of 'self' molecules on the host's cells produces inhibition signals that limit the action of NK cells through major histocompatibility class (MHC) I molecules. Lack of these 'self' signals results in NK cell driven attacks on foreign objects through the MHC I proteins (Joncker and Raulet, 2008).

Complementing cellular responses, innate immunity also involves a humoral arm through several antimicrobial peptides such as defensins, complement proteins, C-reactive proteins, and LPS-binding proteins (Turvey and Broide, 2010) (Figure 1.1). These circulating complement proteins can sense microbes and induce effector mechanisms that enhance pathogen clearance, for example, through phagocytosis. This complement system mobilizes the action of antigen-presenting cells (APCs)

such as macrophages and dendritic cells (Murphy *et al.*, 2008, Turvey and Broide, 2010, Marshall *et al.*, 2018) (Figure 1.1).

Phagocytic monocytes circulate in the peripheral blood, and they become macrophages upon migration into tissues (Margolick *et al.*, 2014). Also migrating from the peripheral blood into the tissue are immature dendritic cells. Both dendritic cells and macrophages capture tissue antigens, process them and migrate to the lymphoid organs where they present antigen peptides through MHC I or MHC II molecules to naïve T cells (Murphy *et al.*, 2008, Margolick *et al.*, 2014). This marks the beginning of the adaptive arm of immunity.

1.1.2. Adaptive immunity – The development of long- term memory immunity

Augmenting the innate immunity, the adaptive immunity conducts its functions through antigen specific B and T cells (Parkin and Cohen, 2001, Murphy *et al.*, 2008) (Figure 1.1). While the fast-acting innate immunity is critical to the rapid sensing and destruction of pathogens in a non-specific manner, the adaptive immunity generally takes more time to generate a more refined immunity against different antigens from pathogens (Parkin and Cohen, 2001, Murphy *et al.*, 2008). The evolutionary advantage of the adaptive immunity is its ability to mount long lived T and B cell memory immunity which can elicit faster and more robust antigen specific responses upon reintroduction of the antigen or pathogen (Delves and Roitt, 2000, Parkin and Cohen, 2001, Murphy *et al.*, 2008).

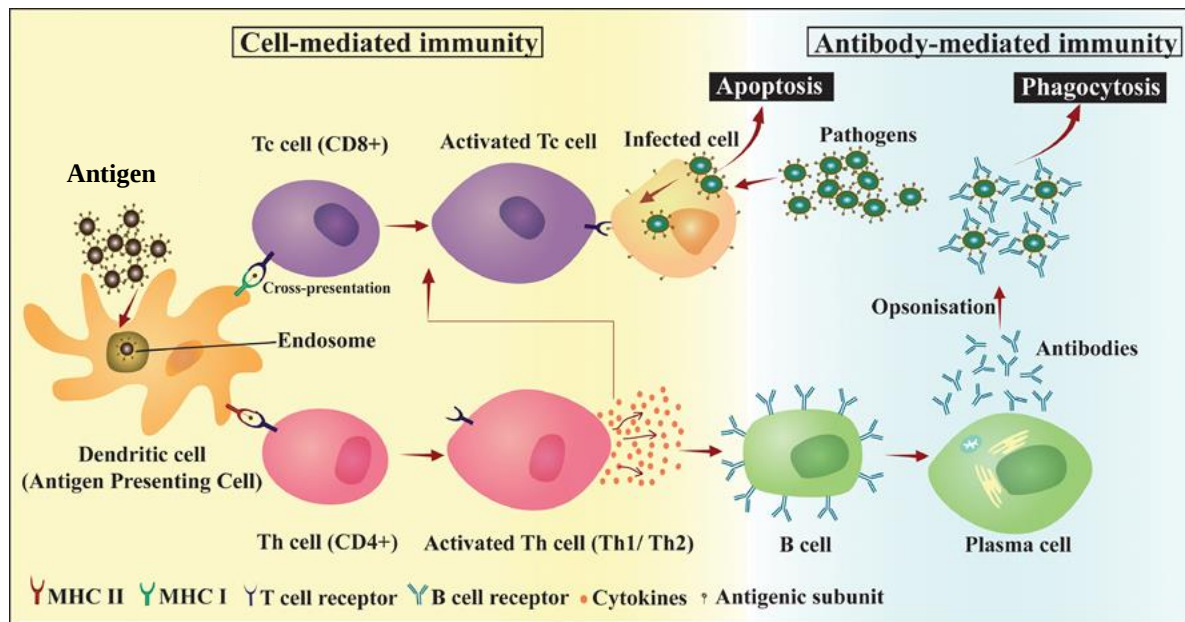


Figure 1. 2: Activation of adaptive immunity.

Figure adapted from (Pippa *et al.*, 2021)

The development of T cells in the thymus produces antigen naïve T cells which enter the circulation towards lymphoid organs for example lymph nodes and the spleen. Naïve T cell migration into lymphoid organs is mediated by chemokine attraction and cell-adhesion molecules such as integrins and selectins, for example, L-selectin (CD62L) (Murphy *et al.*, 2008). Selectins are responsible for ensuring naïve T cell homing in the lymphoid organs. In the lymphoid organs, the T-cell antigen receptors of naïve T cells are exposed to antigens attached on the surfaces of MHC molecules of APCs such as dendritic cells from the innate immunity (Murphy *et al.*, 2008, Margolick *et al.*, 2014). This activates the naïve T cell to proliferate (clonal expansion) and differentiate into several types of antigen specific effector and memory T cells. These cells can identify antigen peptides through MHC molecules on APCs and are specialised to induce specific immunological interactions to mount robust immunity against the antigen (Parkin and Cohen, 2001, Murphy *et al.*, 2008, Farber *et al.*, 2016, Pradeu and Du Pasquier, 2018).

Naïve CD8 T cells recognize antigens on MHC I molecules after which they differentiate into cytotoxic T cells (Figure 1.2). Upon activation of CD8 T cells, they differentiate into CD8 cytotoxic T cells in addition to memory CD8 T cells. Cytotoxic CD8 T cells migrate to the sites of infection where they perform effector functions

such as the killing of infected cells. The memory CD8 T cells, now primed for specific antigen recognition, can be found in lymphoid tissue. On the other hand, naïve CD4 T cells act through MHC II molecule mediated antigen presentation. Upon activation, CD4 T cells can differentiate into several types of helper T (Th) cells. Of importance in this thesis are Th1, Th2 cells, follicular helper T cells and Treg cells (Murphy *et al.*, 2008, Margolick *et al.*, 2014). Helper T cells can activate specific cell types, for example, B cells, directly or indirectly via cytokine signals to launch distinct antigen specific immunity whereas the Treg cells can control and limit Th effector function (Margolick *et al.*, 2014). CD4 Th 1 cells act mainly through inflammatory and cell mediated immunity whereas CD4 Th2 are critical in mediating B cell antigen specific immunity which, with the help of follicular helper T cells, eventually proliferate and differentiate into long lived memory B cells and antibody producing plasma cells (Murphy *et al.*, 2008, Clem, 2011). Additionally, the antigen activation of CD4 T cells produces memory T cells that act faster on secondary encounter with a similar primary antigen. Treg cells mediate control mechanisms that limit the extent of Th responses which inadvertently affects a range of immunological profiles such as cytokine production, B cell activation and maintenance, and inflammatory responses depending on Th type.

Activation of pre- follicular helper T cells localised in the lymphoid organs increases follicular helper T cells expression of the BCL-6 gene in mice and TGF- β , IL-12, IL-23 in humans (Ueno *et al.*, 2015, Qin *et al.*, 2018). Follicular helper T cells, via CD40L and IL-21 activation, support B cell progression through the germinal centre (GC) reaction (Qin *et al.*, 2018). Follicular B cells in secondary lymphoid organs such as the spleen are important in supporting T cell dependent reactions and GC reactions which give rise to class-switched memory B cells, for example, mature IgG1 memory B cells. Another group of cells in the spleen, the marginal zone (MZ) B cells, are the major producers of IgM in humans and have a lower activation affinity than follicular B cells. These cells commonly participate in T cell independent reactions to produce immunoglobulin (Ig) M antibodies with low antigen affinity and broad target specification (Weller *et al.*, 2004, Appelgren *et al.*, 2018, Bugya *et al.*, 2021). Interestingly, these cells can also be instrumental in T cell dependent reactions to produce high affinity and highly antigen specific antibodies through follicular pathways supported by follicular helper T cells from the GC (Cerutti *et al.*, 2013).

Within the GC reaction, antigen specific CD4 T cell activated B cells proliferate and undergo somatic hypermutation and class switch recombination from the IgM antibody isotype to high affinity and highly antigen specific antibodies, the IgG, IgA and IgE isotypes (Gatto and Brink, 2010). These processes produce B cells with increased antigen binding affinity, in addition to memory B cells, antibody secreting immature plasmablasts and antibody secreting long lived mature plasma cells (Gatto and Brink, 2010, Stebegg *et al.*, 2018).

High affinity memory B cells and mature plasma cells are critical in maintaining long term memory immunity which is faster and more robust than the initial adaptive responses on the second encounter with antigen. Memory B and T cells reside mostly in lymphoid organs along with some plasma cells. Plasmablasts are the proliferating-capable precursors of terminally differentiated plasma cells (Tarlinton *et al.*, 2008, Gatto and Brink, 2010). The transition of plasmablasts into mature non-proliferating plasma cells requires homing within the bone marrow (Nutt *et al.*, 2015). Hence after the GC reaction, plasmablasts migrate to the bone marrow where they differentiate into long-lived antibody secreting plasma cells (Nutt *et al.*, 2015, Pak *et al.*, 2018). Antibody secreting plasma cells are essential to human health as they provide antibody mediated immunity against pathogens on the first pathogen encounter, followed by a more robust and faster immunity on second antigen exposure to prevent or minimise the impact of disease on the host. For these reasons, memory B and T cells immunity along with antibody secreting plasma cells are critical to the success of many vaccines in preventing disease over prolonged periods (Nutt *et al.*, 2015).

1.2. Vaccines

1.2.1. Vaccination – An overview

A vaccine is a biological preparation that confers active acquired immunity to a specific pathogen without causing disease (Vetter *et al.*, 2018, Jiskoot *et al.*, 2019). Prophylactic vaccines represent one of the greatest public health triumphs as evidenced by their effectiveness in the prevention of highly fatal infections for over 2 centuries (Guimaraes *et al.*, 2015). With an estimated 2-3 million lives saved each year, vaccination is one of the most cost-effective life-saving medical intervention (Delany *et al.*, 2014).

The introduction of large-scale routine vaccination in the 20th century has controlled major infectious diseases such as smallpox, poliomyelitis, diphtheria, tetanus, pertussis, measles, *haemophilus influenza b*, rubella, typhoid, rabies, yellow fever and mumps (Plotkin and Plotkin, 2004, Tognotti, 2010, Delany *et al.*, 2014). Nowadays, over 30 different infectious diseases can be prevented by licensed vaccines with several of them having been combined into single vaccines or administered at a single vaccination visit (Delany *et al.*, 2014). These have reduced the associated mortality by 97–99% (Rappuoli *et al.*, 2002).

1.2.2. Vaccine immunobiology – Long term memory immunity induction and maintenance

Vaccination-mediated protection from infectious diseases occurs through complex interactions between the innate, lymphocyte-mediated and humoral Immunity (Pulendran and Ahmed, 2011). Cellular and humoral memory immunity induced in the adaptive phase is the stronghold of successful vaccination (Plotkin, 2010, Siegrist, 2013). Upon vaccination, ideally a robust and long-lasting protective immune response to the targeted pathogen is elicited with minimal risk of acquiring the disease and its potential complications (Gershwin, 2018, Vetter *et al.*, 2018) (Figure 1.3). Similar to natural infection, vaccines act by first activating the non-specific innate immune response, which in turn activates the vaccine antigen-specific adaptive immune response (Vetter *et al.*, 2018, Jiskoot *et al.*, 2019) (Figure 1.4).

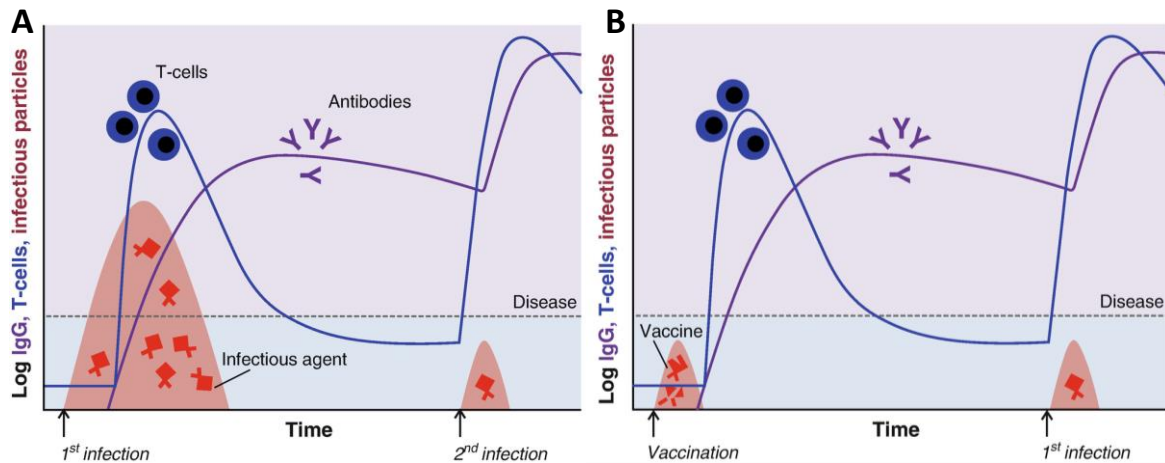


Figure 1. 3: Vaccine mediated elicitation of protection against disease.

A. First exposure to infectious agent in the absence of vaccine primed immunity results in the manifestation of disease. On secondary exposure to the infectious agent, memory immunity produced during the first infection responds robustly to prevent or significantly limit disease manifestation. **B.** Vaccine preparations mimic the infectious agent to induce memory immunity similar to the model shown in A but without causing disease. Subsequent exposure to infectious agent in vaccinated host results in robust immune responses that prevent or significantly limit the extent of disease. Image adapted from (Jiskoot *et al.*, 2019).

All vaccines have an antigen-adjuvant system which elicits an inflammatory immune response in a manner similar to PAMPs (Guimaraes *et al.*, 2015) which after recognition by TLR mainly on dendritic cells, triggers the adaptive immune response after antigen processing and presentation (Beverley, 2002, Bhurani *et al.*, 2018) (Figure 1.4). The type of stimuli on the TLRs determine DC cytokine production and hence the induction of either Th1 mediated immunity and/or Th2 mediated immunity (Kapsenberg, 2003). Vaccines may therefore induce immunity either via a type 1 immune response or via a type 2 immune response depending on vaccine type (Rosenthal and Zimmerman, 2006) (Figure 1.4).

Adjuvants are key initiators of immunity upon vaccine administration. As such, adjuvants ideally enhance immunogen uptake and thus immunity induction (Brewer, 2006, Rosenthal and Zimmerman, 2006). These adjuvants are also critical in the type of immunity produced by vaccines. The induction of Th2-type responses is usually facilitated by weakly DC activating adjuvants, for example the classical aluminium adjuvants, that are designed to improve immunogen uptake without robustly activating DCs to minimize the production of Th1 driving IL-12 (Rosenthal and

Zimmerman, 2006). Vaccines that induce Th1-type responses, for example the bacillus Calmette-Guérin (BCG), usually have powerful DC activating adjuvants that can be mixed with TLR ligands in mineral oil to promote phagocytosis and type 1 immunity (Rosenthal and Zimmerman, 2006). Thus, vaccine memory induction immunity can be mainly Th1 and Th2 type depending on vaccine components (Figure 1.4).

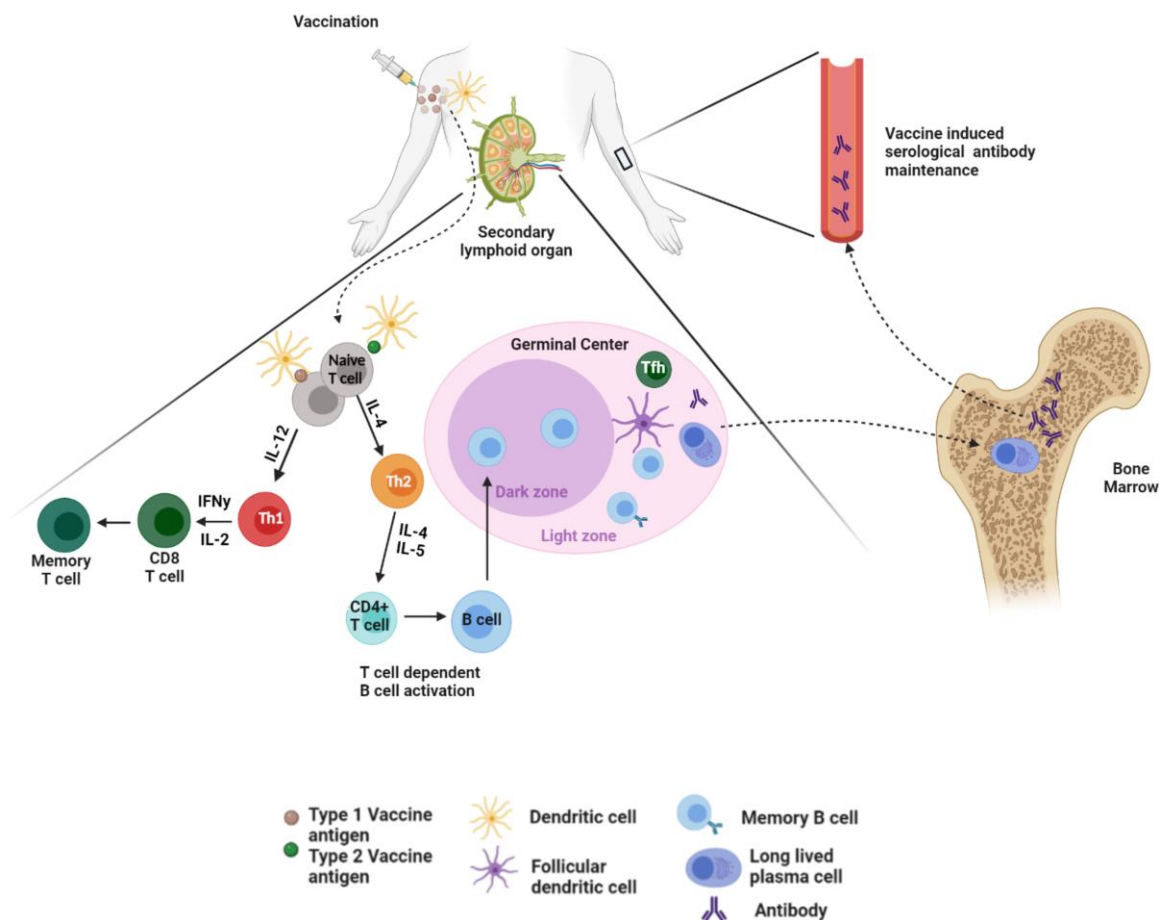


Figure 1. 4: The host immune response to vaccines.

A. Type 2 vaccine immune response: Type 2 antigen is recognised by DCs from the vaccine inoculation site. The activated DCs then migrate to the secondary lymphoid organs. Here, they activate naïve CD4+ T cells with help of IL-4, resulting in a Th2 profile. Further presence of Th2 produced IL-4 and IL-5 together with Th2 cells activate naïve B cells. Activated then enter the GC reaction where proliferate in the dark zone. In the GC compartment, somatic mutation and isotype switching of the activated antigen specific B cells also takes place. From here, antigen specific B cells enter the light zone of the GC, where FDC and IL-21 supported positive B cell selection takes place. These events lead to the production of high affinity antigen specific memory B cells and antibody producing plasma cells, antibodies. Long-lived plasma cells then migrate to their survival niches, the bone marrow. Here,

continuously produce antigen (vaccine) specific antibodies, and wait for antigen activated recall responses that trigger a rapid production of antibodies. **B. Type 1 vaccine immune response:** DCs are activated by Type 1 antigen from vaccine at site of inoculation. The activated DCs in turn activates naive CD4+ T cells towards Th1 profile through DC produced IL-12. Th1 cells produce IFN- γ and IL-2, cytokines which activate CD8+ T cells resulting in the production of vaccine specific memory CD8+ T cells. DC: Dendritic Cells; Th2: Type 2 Immune response; Th1: Type 1 immune response; IL: Interleukin; GC: Germinal centre; FDC: Follicular Dendritic Cells; Tfh: T follicular helper cells; IFN- γ : Interferon gamma. Image extracted from (Nono *et al.*, 2022).

Basically, each vaccine presents antigens that mimic the course of an infection by inducing a lymphocyte-mediated immunity through the production of highly specific subsets of T lymphocytes, and humoral immunity by stimulating B lymphocytes to produce antigen specific antibodies (Beverley, 2002, Siegrist, 2013) (Figure 1.4). Each phase of immunity, including both B and T cells, sensitizes the host immune system towards the antigen. As a result, the adaptive immune system normally establishes a pattern of immunological memory, the basis of long-term protection which is the desired objective of vaccinations. For most vaccines, long term protection is conferred through the production of antibodies and the generation of memory lymphocytes that can quickly and robustly reactivate following a subsequent exposure to the same pathogen (Plotkin, 2010, Siegrist, 2013, Bhurani *et al.*, 2018, Vetter *et al.*, 2018).

In the case of polio vaccination, both T cell and B cell compartments are mobilised to form long term memory immunity against the poliovirus (Wahid *et al.*, 2005). Both types of polio vaccination, the oral polio vaccine (OPV) and the inactivated polio vaccine (IPV) have been shown to induce long term memory CD4+ T cells, CD8+ T cells, memory B cells and antibody producing plasma B cells (Wahid *et al.*, 2005, Dey *et al.*, 2016, Bhaumik *et al.*, 2018). Likewise, both T and B cell immunity (including antibodies) have been demonstrated in individuals vaccinated for hepatitis B (Leroux-Roels *et al.*, 1994, Litjens *et al.*, 2008, Buchmann *et al.*, 2013) and measles (Ovsyannikova *et al.*, 2003, Kakoulidou *et al.*, 2013, Nono *et al.*, 2018). Hence, the induction of both cellular and humoral immunity involving vaccine antigen specific T and B cells as well as antibody- production is critical to the success of vaccination efforts to control different infections.

1.3. Schistosomiasis

1.3.1. Overview – *Schistosoma* spp. infections

Schistosomiasis (commonly known as bilharzia) is a debilitating and potentially fatal vector borne parasitic disease that has been classified by the World Health Organisation (WHO) as a Neglected Tropical Disease (NTD) (Gryseels *et al.*, 2006, WHO, 2021). The causative parasites for the disease belong to the genus *Schistosoma* (Gryseels *et al.*, 2006, WHO, 2021), and people usually get infected through percutaneous penetration on contact with *Schistosoma* spp. cercariae in contaminated freshwater sources (Weerakoon *et al.*, 2015). The disease is generally associated with poverty, poor sanitation and unsafe drinking water, in addition to insufficient open water and parasite control measures (Gryseels *et al.*, 2006, Colley *et al.*, 2014, McManus *et al.*, 2018, Aruleba *et al.*, 2019, WHO, 2021).

Table 1. 1: *Schistosoma* trematode worm species and their geographical distribution. Table adapted from (WHO, 2021).

Clinical Presentation	Species	Geographical distribution
Intestinal	<i>S. mansoni</i>	Africa, the Middle East, the Caribbean, Brazil, Venezuela and Suriname
	<i>S. japonicum</i>	China, Indonesia, the Philippines
	<i>S. mekongi</i>	Several districts of Cambodia and the Lao People's Democratic Republic
	<i>S. guineensis</i> and related <i>S. intercalatum</i>	Rain forest areas of central Africa
Urogenital	<i>S. haematobium</i>	Africa, the Middle East, Corsica (France)

Schistosomiasis is commonly classified as either an intestinal or a urogenital disease depending on clinical presentation (Table 1.1). These two major forms of the disease are often presentations that are dependent on different *Schistosoma* spp. invasions. A total of five human infecting *Schistosoma* spp. are currently identified (WHO, 2021) (Table 1.1). Of these, *S. mansoni*, *S. haematobium* and *S. Japonicum* are the three most common species reported, and their encounter are mainly dependent on geographic locations (Ross *et al.*, 2001, Gryseels *et al.*, 2006, Colley *et al.*, 2014) (Table 1.1). *Schistosoma mansoni* is primarily encountered in Africa while *S. haematobium* can be found in Africa and the Middle East. *Schistosoma japonicum* is

commonly found in Asia whereas the remaining species, *S. mekongi* and *S. intercalatum*, have been reported in Africa and South-East Asia, respectively (Ross *et al.*, 2001, WHO, 2021). In Cameroon, *S. mansoni* and *S. haematobium* are the most commonly reported *Schistosoma* spp. (Anchang-Kimbi *et al.*, 2017, Nono *et al.*, 2018, Kamdem *et al.*, 2019, Kamdem *et al.*, 2020).

1.3.2. The life cycle of schistosomiasis

The life cycle of schistosomiasis starts in freshwater bodies that become contaminated by schistosome eggs via urinal or faecal deposition from infected hosts. This situation is driven mostly by poor sanitary practices. At the forefront of poor sanitary behaviour is the general lack of standard sanitation facilities in often resource constrained settings.

In the urine or faeces of infected persons are commonly *Schistosoma* spp. eggs depending on stage of infective life cycle (Figure 1.5). Once deposited in the freshwater sources, *Schistosoma* spp. eggs hatch and release free-swimming miracidia. For the contamination to be continual, there is a need for an intermediate host in the contaminated water sources. These intermediate hosts, the water snails, are penetrated by the free-swimming miracidia.

Upon penetrating the snails, miracidia lose its cilia before developing further into sporocysts. The resulting sporocysts are capable of asexual reproduction which produces *Schistosoma* spp. larvae that are commonly referred to as cercariae (Figure 1.5). These cercariae remain resident in the snail until sunlight exposure and increases in environmental temperature. From here, the cercariae swim into the water where they freely swim until contact with the next host, the human or an animal, whom they penetrate through the skin (Figure 1.5).

Distinct species of snails act as intermediate hosts for different *Schistosoma* spp. cercaria. The *Biomphalaria* spp. snails are commonly known hosts for *S. mansoni* whereas, the *Bullinus* spp. snails propagate *S. haematobium*. *S. japonicum* cercariae are propagated in the *Oncomelania* spp. snails (McManus *et al.*, 2018).

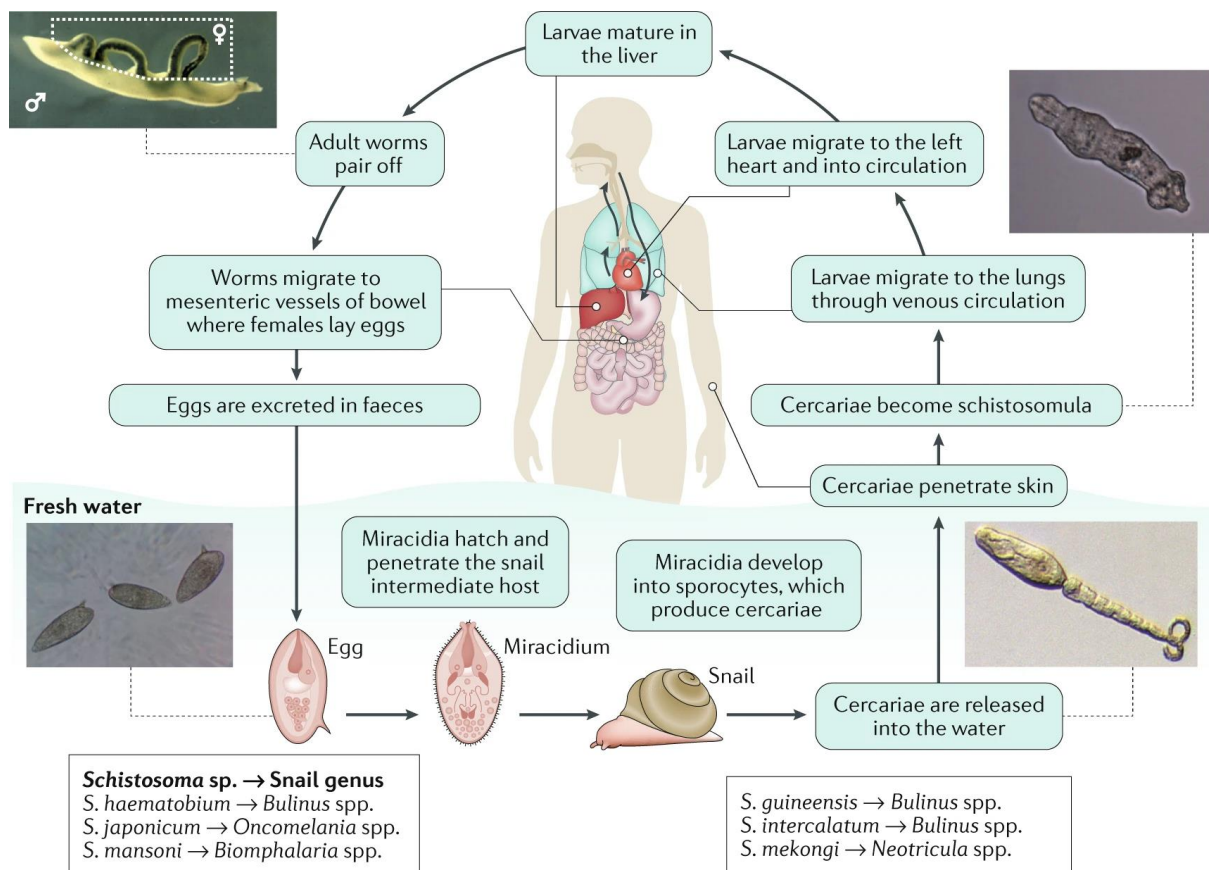


Figure 1. 5: The life cycle of schistosomiasis.

Infected persons excrete *Schistosoma* spp. eggs into open freshwater sources. The eggs hatch to produce miracidia which develops into cercariae using intermediary host freshwater snails. Under the right environmental conditions, the cercariae are released into the water where they can penetrate humans through the skin. From here, cercariae develops into schistosomula and migrate to the lungs, the heart and the liver through circulatory pipelines. In the liver, schistosomula matures into adult worms. Male and female worms pair off and migrate to the mesenteric vessels where they actively lay eggs that are eventually excreted into the environment by infected host through defecation. Worm lodging around the bladder results in egg excretion through urination. Figure extracted from (McManus *et al.*, 2018).

On contact with the human or animal skin, cercariae penetrates the skin through mechanisms involving proteolytic activity and peptidase secretions (Hansell *et al.*, 2008, Ingram *et al.*, 2011). Once in the host, cercariae develop further into schistosomula (Walker, 2011, McManus *et al.*, 2018). These schistosomula migrate to the host dermis and blood vessel walls to enter the capillary circulation. From here, schistosomula migrate into the hepatic portal vessels by passing through lung vessels (Walker, 2011). Once in the liver, schistosomula feeds on blood as they continue to develop into mature worms (Figure 1.5).

Intestinal disease causing schistosomula, for example *S. mansoni*, migrate further into mesenteric veins while urogenital disease causing schistosomula such as *S. haematobium* migrate towards the vesical vessels around the bladder (Walker, 2011). In these tissues, schistosomula grow into mature worms with developed teguments. In their mature forms, opposite sexes of schistosomula mate to produce *Schistosoma* spp. eggs (Walker, 2011, Gryseels, 2012). Adult worms of *S. mansoni* produce hundreds of eggs while those of *S. haematobium* produce thousands continuously. These eggs are excreted through the faeces or urine from *S. mansoni* or *S. haematobium* worms, respectively, to contaminate the environment (McManus *et al.*, 2018) (Figure 1.5).

The remaining eggs enter the blood circulation where their transport leads to lodging within the liver and sometimes the spleen (Gryseels, 2012). Here, eggs are responsible for an array of immunological responses mounted by the host, resulting in the formation of granulomas around the trapped eggs (McManus *et al.*, 2018). Granulomas are products of inflammatory cell collections such as macrophages, lymphocytes, eosinophils and neutrophils that are attracted to the site of the tissue trapped eggs (McManus *et al.*, 2018). It is generally accepted that these eggs are the major cause of disease, and their deposition hallmarks the chronic stage of schistosomiasis (Wilson *et al.*, 2007, Gryseels, 2012).

1.3.3. Epidemiology of schistosomiasis

Schistosomiasis is most common in Africa where more than 90% of people requiring treatment for the infection reside (Figure 1.6). The transmission of schistosomiasis has been reported for over 70 developing countries, mainly in sub-Saharan Africa (Figure 1.5). The disease is not common in developed countries. An estimated 236 million are affected globally (WHO, 2021). It is estimated that a range of 24 000 to 200 000 annual deaths are linked to schistosomiasis globally, and over 10 million disability adjusted life years (DALYs) are lost due to the disease (van der Werf *et al.*, 2003, WHO, 2021). Evidence from a systematic analysis showed that an estimated 700 million people remain at risk of developing schistosomiasis worldwide (Steinmann *et al.*, 2006).

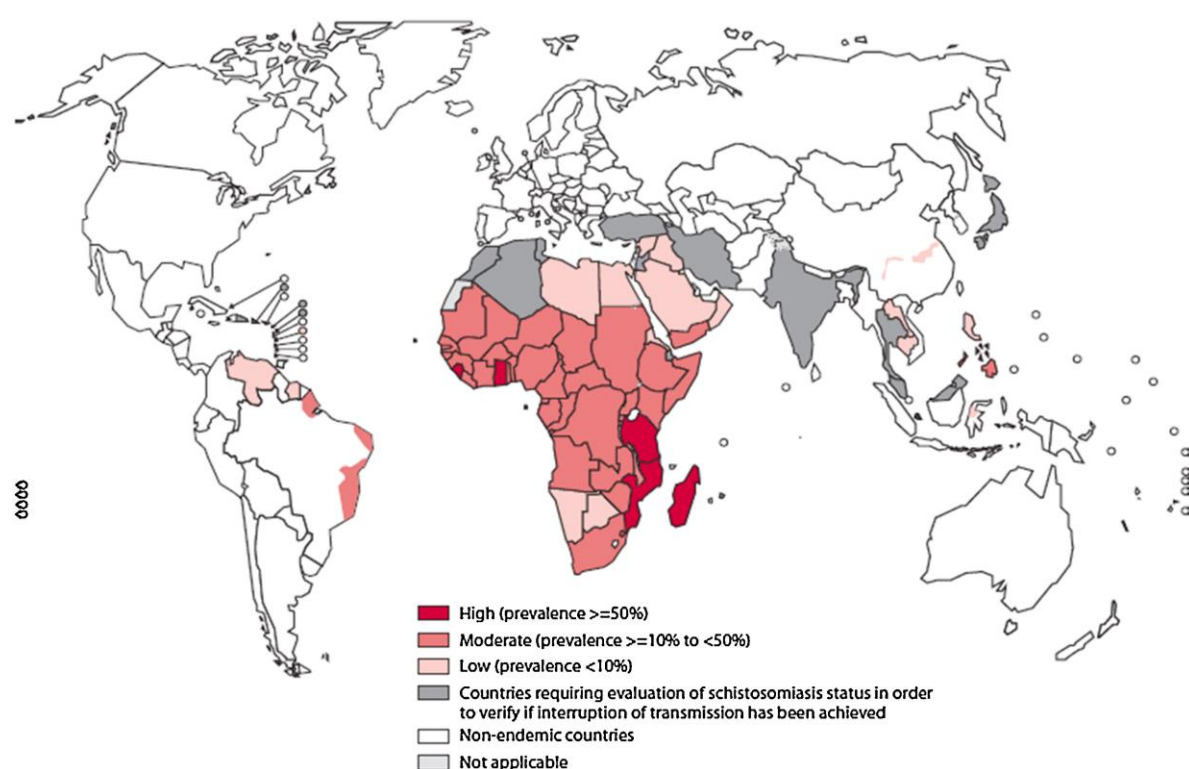


Figure 1. 6: Distribution of schistosomiasis in the world. Schistosomiasis is most common in sub-Saharan Africa.
Image extracted from (Ross *et al.*, 2017).

Mostly at risk of schistosomiasis are poor agricultural and fishing communities. Women are often infected during daily chores that require contact with open

contaminated water sources, for example, washing clothes, and both men and women risk getting infected by bathing in these waters. It has been reported that school-age children from endemic areas are highly vulnerable to schistosomiasis due to poor hygiene and activities such as swimming and fishing in *Schistosoma* spp. infested water bodies (WHO, 2021). In developing countries, mass population movement and migration to urban areas is instrumental in spreading the disease to new areas. Residents of developed countries are usually infected with schistosomiasis during their tourism visits to high-risk areas (WHO, 2021). In Cameroon, *S. mansoni*, with a prevalence ranging between 5 - >34%, is the most prevalent schistosome species followed by *S. haematobium* with a prevalence ranging from 1.7 - >25% (Sama *et al.*, 2007, Tchuem Tchuente *et al.*, 2012, Nono *et al.*, 2018, Kamdem *et al.*, 2019).

1.3.4. Clinical manifestation

Within a week after percutaneous cercarial invasion, maculopapular rash may manifest (Ross *et al.*, 2007, McManus *et al.*, 2020). Thereafter, schistosomiasis occurs mainly as two major phases, the acute phase and the chronic phase. Acute infections are commonly seen in tourists or travellers to endemic areas. Chronic infections are mainly reported in people who reside in poorly resourced endemic areas (Ross *et al.*, 2002, McManus *et al.*, 2020).

1.3.5. Acute schistosomiasis

Also known as Katayama fever, the acute phase normally occurs early after infection, commonly starting within 6 weeks after cercarial penetration (Ross *et al.*, 2007, McManus *et al.*, 2020). In some instances, particularly in people without prior exposure to the parasite, this phase can be delayed by several weeks to months (McManus *et al.*, 2020). Symptoms may be non-specific, for example, fever, urticaria and eosinophilia (Ross *et al.*, 2007, Coron *et al.*, 2016) in addition to malaise, fatigue, abdominal and muscle pain and haematuria depending on *Schistosoma* spp. infection (Ross *et al.*, 2007, McManus *et al.*, 2020).

1.3.6. Chronic schistosomiasis

Establishment of active infection is characterised by mature adult worms and egg production and excretion in human waste (McManus *et al.*, 2018). This stage of the disease is commonly seen in individuals from schistosomiasis endemic areas, starting from around week 5 onwards, and sets the stage for further development into the chronic phase of the disease. Symptoms in the established and chronic phase typically range from inflammatory reactions to the advanced hepatosplenomegaly and lesions that affect the bladder and the reproductive system (Colley *et al.*, 2014, McManus *et al.*, 2020).

1.3.7. Immunopathology of schistosomiasis and host control mechanisms

Evidence suggests that host immune responses occur within 2 weeks of *Schistosoma* spp. cercarial invasion (Figure 1.7). A type 1 immune response in this initial stage induces inflammatory cytokines IFN- γ and IL-12 and can last well into the mature worm development stage (McSorley and Maizels, 2012, LoVerde, 2019). However, these responses are usually not associated with significant immunopathology and are therefore commonly subclinical (McSorley and Maizels, 2012, Hams *et al.*, 2013, LoVerde, 2019). Detecting the infection at this stage is not common due to asymptomatic and unsuspecting hosts.

Schistosome worms induce type 1 immunity that increases Th1 cell production about 5 weeks into the infection (Llanwarne and Helmby, 2021) (Figure 1.7). This potentially fatal increase in inflammatory Th1 cells initiates the development of granulomas (Hoffmann *et al.*, 2000, Pearce and MacDonald, 2002, Llanwarne and Helmby, 2021). In addition, studies in mice have shown that granuloma formation can also be mediated by tumour necrosis factor alpha (TNF- α), a type 1 inflammatory cytokine (Brunet *et al.*, 1998, Maizels *et al.*, 1999). As the worms mature and lay eggs, there is a shift from worm induced type 1 immunity towards egg induced type 2 biased immune responses (Schwartz and Fallon, 2018) (Figure 1.7).

Human and animal studies have demonstrated that schistosomiasis disease heightened burden is a result of the impact of eggs and not necessarily worms (Fallon, 2000, Dessein *et al.*, 2004, Abath *et al.*, 2006, Burke *et al.*, 2009, Kamdem *et al.*, 2018, McManus *et al.*, 2018, Llanwarne and Helmby, 2021). Infection with *S. mansoni* and *S. japonicum* results in egg excretion into the environment through the bowel faecal route, while in *S. haematobium* infection, eggs are lodged in the bladder and urogenital system. Not all eggs are excreted, some remain lodged in the intestines, liver, bladder and/or urogenital system of hosts depending on parasite species (McManus *et al.*, 2020).

Trapped in the organs, the eggs secrete type 2 immunity inducing factors such as proteolytic enzymes. These factors elicit eosinophilic inflammatory responses which cause cell damage and induce potent immune responses that lead to the development of granulomas and fibrosis (Kamdem *et al.*, 2018, McManus *et al.*, 2020).

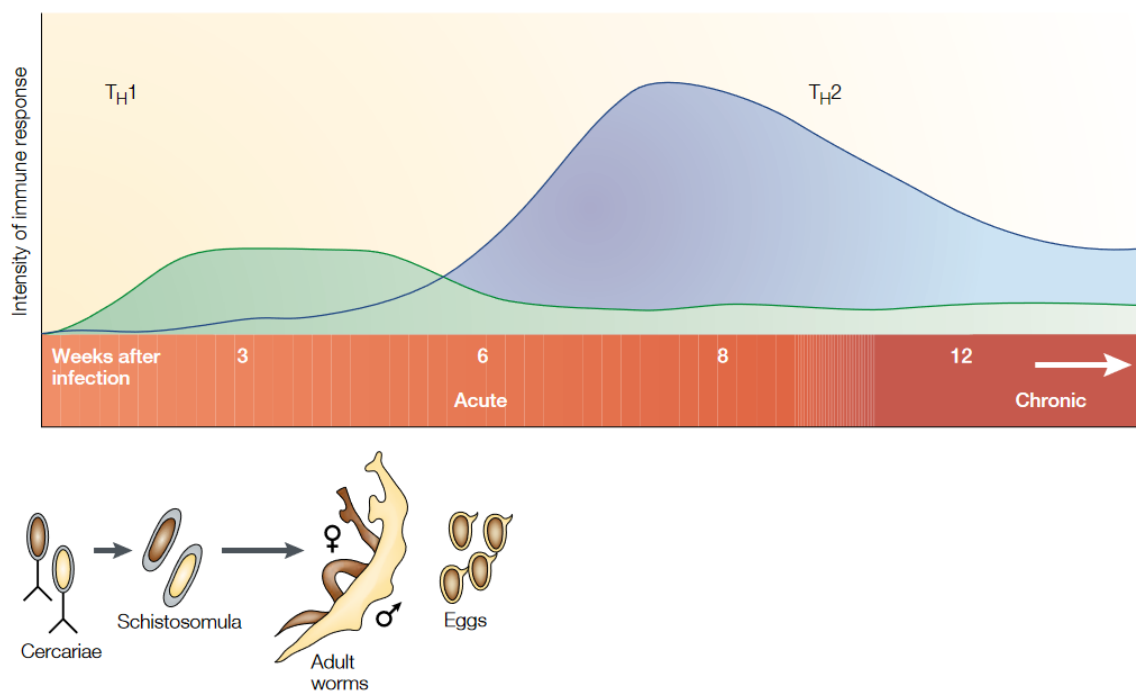


Figure 1. 7: Immunity induction against schistosomiasis.

Type 1 immunity hallmarked by Th1 cells is mounted in the first 3 to 5 weeks of *Schistosoma* spp. infection. This stage is inflammatory owing to the Th1 immune profile in host. Worm maturation leads to mating and production of eggs. This stage commences around weeks 5 and 6 post infection and results in the reduction of type 1 immunity. In favour of an intense type 2 immunity in response to egg products. By this time granulomas are developed. As the infection progresses, the intense Th2

immunity is regulated by regulatory mechanisms and granuloma formation slows down. At this stage, a balance between type 1/type 2 immunity is reached and granulomas are maintained in the chronic phase of the disease. The continued persistence of granulomas results in prolonged deposition of collagen and the development of fibrosis and portal hypertension. Image extracted from (Pearce and MacDonald, 2002).

The induction of a strong type 2 immunity upregulates the expansion of Th2 cells e.g., CD4⁺ T cells, myeloid cells e.g., basophils and eosinophils, cytokines IL-5, IL-13 and IL-4, and antibodies such as IgG and IgE, some of which may be associated worsening pathology without treatment or with improving pathology post treatment (McSorley and Maizels, 2012, Colley and Secor, 2014, McManus *et al.*, 2020). The host's immunity becomes polarized to produce alternatively activated macrophages and a cascade of events lead to the surrounding of schistosome eggs by a wall of immune cells to block their toxic secretions and destroy them (Pearce and MacDonald, 2002, Kamdem *et al.*, 2018, Schwartz and Fallon, 2018). These walls of cells become granulomas and eventually fibrosis (Kamdem *et al.*, 2018, Llanwarne and Helmby, 2021).

Granulomas and fibrosis are the major contributors of pathology in schistosomiasis (Caldas *et al.*, 2008, Colley *et al.*, 2014, McManus *et al.*, 2020). In the absence of the Th1 restricting strong type 2 immunity induction, animal studies have shown that type 1 immunity associated pro-inflammatory responses drive hepatotoxicity, endotoxemia and wasting, ultimately leading to death (Fallon *et al.*, 2000, Zheng *et al.*, 2020). On the other hand, Th2 immunity, particularly IL-13 cytokine production, can also facilitate fibrotic activity and increased pathology in the spleen and liver (Magalhaes *et al.*, 2004, Wynn and Barron, 2010, LoVerde, 2019). Activation of IL-13 from CD4⁺ T cells induces the expression of arginase which induces the production of L-ornithine from L-arginine to eventually induce proline. Proline facilitates the production of collagen and aids the development of fibrosis and pathology (Hesse *et al.*, 2001, Angeles *et al.*, 2020, Masamba and Kappo, 2021). Additionally, IL-13 initiates the differentiation of hepatic stellate cells (HSCs), a major source of source hepatic collagen and hence a major contributor to fibrosis (Anthony *et al.*, 2012, Angeles *et al.*, 2020).

Further, severe disease is indicated by elevated levels of Th2 derived IgG antibodies usually in chronic disease (McSorley and Maizels, 2012). In contrast, resistance or reduced pathology to schistosomiasis is also associated with Th2 derived antibodies, IgE, thus reflecting the complexity of factors driving established disease course regardless of *Schistosoma* spp. (Hagan *et al.*, 1991, Rihet *et al.*, 1991, Zhang *et al.*, 1997, McSorley and Maizels, 2012). Other fibrosis inducing key players, Th17 cells and Kupfer cells produce pro-inflammatory IL-17 which is instrumental in both inflammatory mediated parasite expulsion and pathology (McSorley and Maizels, 2012, Mpotje, 2021, Santana *et al.*, 2021). However, IL-17's role in schistosomiasis is still poorly understood (Santana *et al.*, 2021).

As the infection progresses, the strong type 2 phenotype is downmodulated to establish a balance between Th1 and Th2 responses. It has been shown that regulatory T cells produce regulatory cytokines such as IL-10 and TGF β which limit Th2 responses and restrict the extent of damage from egg deposition (Schwartz and Fallon, 2018). Interestingly, IL-10 driven modulation of type 1 immunity has also been shown to orchestrate the transition from the acute phase to the chronic phase of schistosomiasis (Sadler *et al.*, 2003). Additionally, although disputed, suggestions have been made that in addition to type 2 regulatory T cells, type 1 CD8⁺ T cells may also limit the extent of granuloma pathology through interferon gamma (IFN- γ) production, failure of which may result in death (Pedras-Vasconcelos and Pearce, 1996, Yap *et al.*, 1997, Zheng *et al.*, 2020).

The regulatory stage of schistosomiasis results in smaller granulomas that can persist throughout the chronic phase. In their persistence, Th2 driven granulomas are associated with the continual deposition of collagen, a condition which result in tissue repair but also fibrosis. Continued fibroblast recruitment and hence, fibrosis, results in excessive scar tissue formation and eventually severely limits liver function and adds to significant pathology (Sandler *et al.*, 2003, Kamdem *et al.*, 2018, Schwartz and Fallon, 2018, Mpotje, 2021). Interestingly, proinflammatory cytokine IL-17 has also been implicated in granuloma formation and fibrosis in mice and humans (Zhang *et al.*, 2012, Mbow *et al.*, 2013, Zhang *et al.*, 2015)

As the disease progresses, old granulomas resolve as new ones develop facilitated by continuous egg deposition. As old granulomas resolve, fibrotic plaque may remain

as the *Schistosoma* spp. eggs die. These changes cause a reduction in the portal effusion capacity and increases in portal blood pressure due to elevated liver fibrosis and congestion. The portal vein becomes harder to perfuse, thus causing portal hypertension (Pearce and MacDonald, 2002). As a result, the host may experience an accumulation of fluid in the peritoneal cavity (ascites development), and portal systemic venous shunts may develop. Exacerbated by heavy schistosome worm and egg burden, these events can lead to vein rupture and internal bleeding, both of which can be fatal without appropriate medical attention (Pearce and MacDonald, 2002, Andrade, 2009).

1.3.8. Schistosome driven immunomodulation

Failure to modulate granuloma formation leaves the host vulnerable to severe pathology, for example, hepatosplenic disease, intense fibrosis and lung trapped eggs (Colley and Secor, 2014). Schistosomiasis driven immune regulatory mechanisms are therefore essential in improving host survival by dampening heightened immunopathology. However, the dampening of immunity through regulatory mechanisms also facilitates the survival of the parasite in addition to attenuated immune responses to unrelated antigens (Figures 1.7 and 1.8). In this manner, the disease is maintained for prolonged periods with a critical balance between host survival and parasite life cycle maintenance through egg production and excretion into the environment. This parasite orchestrated effective manipulation of the host immune system occurs through an array of evolutionary immunomodulatory factors (Maizels *et al.*, 2018).

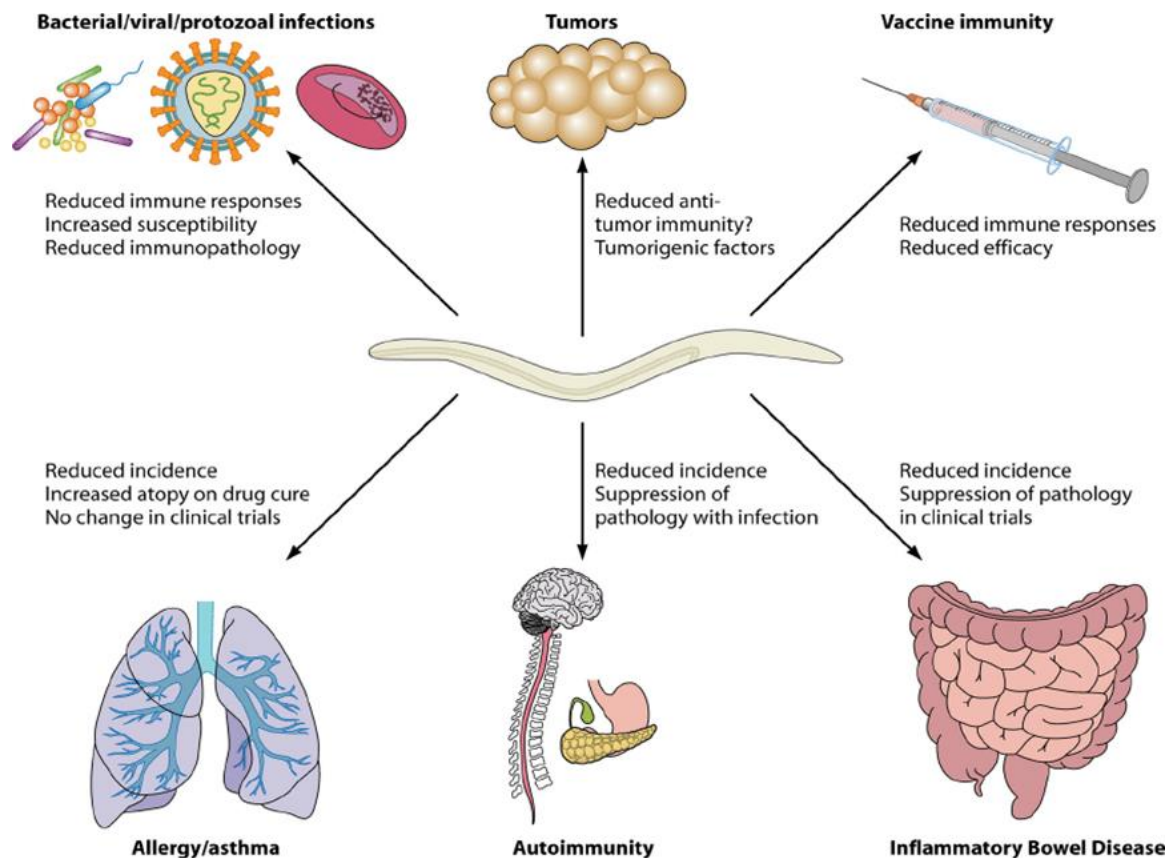


Figure 1. 8: Some parasitic helminths, such as schistosomes, modulate host immune responses for several vaccines, pathogens and allergies.
Figure extracted from (McSorley and Maizels, 2012).

Despite their host directed survival advantages, regulatory mechanisms can also suppress host immune responses for unrelated pathogens and antigens (McSorley and Maizels, 2012). Mature *S. mansoni* harbour a TLR2 ligand, phospholipid lysophosphatidylserine (LysoPS), in their teguments (Retra *et al.*, 2015, Maizels *et al.*, 2018) which can stimulate TLRs on dendritic cells to induce T cell proliferation suppressive IL-10 producing T cells (van der Kleij *et al.*, 2002, Maizels *et al.*, 2018). Induction of regulatory cytokine IL-10 restricts type 1 inflammatory immune responses (McSorley and Maizels, 2012). Cysteine proteases such as *S. mansoni* Cathepsin B (SmCB1) (Soloviova *et al.*, 2019) have been shown to suppress MyD88-independent TRIF (TIR-domain-containing adapter-inducing interferon- β)-dependent signalling on type 1 TLR4 and TLR3 of myeloid cells (Donnelly *et al.*, 2010). In addition, the schistosome glutathione-S-transferase protein (P28GST) upregulates Th2 responses which downregulate Th1 and Th17 inflammatory responses in mice (Driss *et al.*, 2016, Maizels *et al.*, 2018). Another protein, *S. mansoni* chemokine

binding protein (SmCKBP) can inhibit the action of chemokines CX3CL1, CCL2, CCL3, CCL5 and IL-8. Blockage of these chemokines inhibits neutrophil migration to sites of inflammation (Maizels *et al.*, 2018).

Schistosomiasis also drives immunomodulatory responses through egg glycoprotein dependent reactions (Meevissen *et al.*, 2012, Maizels *et al.*, 2018). In addition to SEA driven Th2 polarization through DCs (Okano *et al.*, 1999, Kaisar *et al.*, 2018), lactose-N-fucopentose III (LNFPIII) from *S. mansoni* alternatively activate APCs to induce anti-inflammatory responses *in vivo* (Srivastava *et al.*, 2014). Schistosome SEA can modulate macrophages to release IL-10 and TGF- β , cytokines involved in the suppression of proliferation of naïve CD4⁺ T cells (Terrazas *et al.*, 2001, Maizels *et al.*, 2018). It has also been shown that LNFPIII can modulate activation of DCs in a manner similar to SEA (Thomas *et al.*, 2005) along with *in vivo* B cell induction to upregulate production of IL-10, a potent immunomodulator (Velupillai and Harn, 1994, Maizels *et al.*, 2018).

Omega-1 (ω -1), a glycoprotein from *S. mansoni* eggs, is recognised by DCs via mannose receptors which bind to its tetrasaccharide carbohydrate component, Lewis-X. Upon binding with DCs, ω -1 induces degradation of ribosomal RNA and messenger RNA to interfere protein syntheses and drive Th2 polarization (Everts *et al.*, 2012, Maizels *et al.*, 2018). Additionally, *S. mansoni* and *S. japonicum* ω -1 DC binding also induces the production of regulatory Foxp3⁺ T cells (Tregs) (Zaccone *et al.*, 2011, Ke *et al.*, 2017). Studies have shown that schistosomiasis induced Foxp3⁺ T cells can suppress host immune responses to non-related antigens, for example, in allergic responses and asthma (Pacifico *et al.*, 2009, Layland *et al.*, 2013) (Figure 1.8).

In addition to Tregs, *S. mansoni* eggs also induce the production IL-10 producing Regulatory B cells (Bregs) (Haeberlein *et al.*, 2017, Maizels *et al.*, 2018). The induction of Bregs in schistosomiasis is still poorly understood, however, it has been demonstrated that a glycoprotein from SEA, Interleukin-4-inducing principle of *S. mansoni* eggs (IPSE), can induce IL-10 producing B cells that can stimulate Treg production *in vitro* (Haeberlein *et al.*, 2017). Interestingly ω -1 did not induce Bregs *in vitro* (Haeberlein *et al.*, 2017), showing the differential specificity of SEA glycoproteins in inducing regulatory cells, and SEA's ability to induce different types

of regulatory B and T cells. Schistosome soluble egg antigen also stimulates a Th2 immunity through the IgE dependent but antigen independent stimulation of basophils to produce IL-4 without uptake of IPSE (Schramm *et al.*, 2007, Maizels *et al.*, 2018).

Blocking inflammatory and Th1 immune responses leave the host susceptible to intracellular pathogens such as HIV, *Bordetella pertussis* and mycobacteria (Elias *et al.*, 2007, Donnelly *et al.*, 2010). Interestingly, cysteine proteases have been demonstrated to limit Th1 immunity induction by *B. pertussis* vaccine administration in mice (Donnelly *et al.*, 2010). Further, studies have shown that schistosomiasis infected people are more vulnerable to viruses such as HIV (Kjetland *et al.*, 2006). The parasitic infection has also been linked with immunological failure and lower levels of CD4+ T cell recovery upon ART (anti-retroviral treatment) initiation in comparison to non-schistosome-infected counterparts (Efraim *et al.*, 2013). Schistosomiasis induced Th1 immunity downregulation in HIV and *mycobacterium tuberculosis* infections is a major public health concern because these infections are also prevalent in already disadvantaged and poorly resourced areas where schistosomiasis may be endemic (Kjetland *et al.*, 2006, Walson and John-Stewart, 2008, Hewitson *et al.*, 2009, Babu and Nutman, 2016, Filbey *et al.*, 2019) (Figure 1.8).

Schistosomiasis driven immunomodulation can also limit immune responses against viral vaccination (Figure 1.8). Vaccination against measles has been reported to be impaired in association with schistosomiasis (Nono *et al.*, 2018, Tweyongyere *et al.*, 2019, Nono *et al.*, 2022). Similar findings for other vaccines were reported in other regions of Africa where schistosomiasis is endemic, although disputing observations were also noted (Nono *et al.*, 2022). The influence of the parasite on several vaccines for example polio vaccines, remain unexplored. Additionally, for those vaccines where schistosomiasis has been shown to downmodulate host responses to unrelated antigen immunity, mechanisms behind these findings are largely unexplored, particularly the parasite's impact on vaccine induced long lived antibody producing plasma cell maintenance in their long term survival niches, for example, the bone marrow (Nono *et al.*, 2022).

This evidence highlights the critical need for investigating unrelated infections and vaccinations in light of schistosomiasis co-infection, especially in regions where the parasites are endemic, 90% of which are sub-Saharan regions. Noteworthy, poorly resourced countries significantly rely on vaccinations to control a range of serious but vaccine preventable infections which if uncontrolled could cause significant morbidity and can be fatal, in addition to substantial demands on resources which are poorly accessible or unavailable for poor communities which are mostly at risk of infections. As such, the immunoregulatory impact of schistosomiasis on vaccine immunology is a poorly understood but critical puzzle that remains to be solved. This critical area of vaccinology requires prompt evaluation for the informed and strategic implementation of vaccination policies in schistosomiasis vulnerable populations.

1.3.9. Control measures against schistosomiasis

Schistosomiasis is controlled mainly through the use of one drug, praziquantel (PZQ), a highly effective treatment against mature *Schistosoma* spp. worms (Ross *et al.*, 2001, Mduluzza and Mutapi, 2017, Schneeberger *et al.*, 2018, Fukushige *et al.*, 2021). Praziquantel (2-cyclohexylcarbonyl-1,2,3,6,7,11b-hexa-hydro-4H-pyrazino-(2,1- α)), commercially known as Biltricide, is a stable and safe chemotherapeutic drug that has been used to treat schistosomiasis for decades (Aruleba *et al.*, 2019). For forty years, PZQ has been the sole commercially available therapeutic drug against all forms of schistosomiasis (Aruleba *et al.*, 2019, Fukushige *et al.*, 2021, Spangenberg, 2021). The drug remains the stronghold treatment against *Schistosoma* spp. owing to its ease of accessibility, high efficacy rates, remarkable safety profile and low cost of acquisition (Fenwick *et al.*, 2003, Hagan *et al.*, 2004, Vale *et al.*, 2017, Aruleba *et al.*, 2019).

The WHO recommended dosage for the drug is pegged at 40mg/kg, with cure rates ranging between 73% and 95% achieved for different *Schistosoma* spp. (Zwang and Olliaro, 2017, Aruleba *et al.*, 2019, Spangenberg, 2021). In schistosomiasis endemic areas, PZQ mass drug administration (MDA) is a common and recommended public health intervention to control the parasitic disease, especially targeting schoolchildren (Spangenberg, 2021). The use of the drug is also recommended in

pre-school aged children in whom its safety and efficacy were strongly demonstrated (Mutapi *et al.*, 2011, Mduluzza and Mutapi, 2017). Demonstrating its importance beyond simple parasite induced morbidity control, praziquantel use is also recommended as a potent control measure for use in remote areas where schistosomiasis is closely linked with co-infections such as HIV, as recommended for rural Zimbabwe (Kjetland *et al.*, 2006).

Mass treatment campaigns have a coverage rate just above the 60% mark (Spangenberg, 2021). This incomplete drug coverage coupled with reinfections and the inability of PZQ to kill juvenile *Schistosoma* spp. worms render MDA campaigns insufficient as sole control methods to effectively control schistosomiasis in endemic areas (Nono *et al.*, 2018, Sircar *et al.*, 2018). In their study, Nono *et al.* advocated for reinforcing schistosomiasis control strategies after observing notable prevalences of the parasitic infections in an endemic region of Cameroon despite dedicated annual PZQ MDA campaigns (Nono *et al.*, 2018). Additionally, although disputed (Fukushige *et al.*, 2021), increasing *Schistosoma* spp. resistance against PZQ highlights the critical need for alternative treatment and control strategies against the parasites (Wang *et al.*, 2012).

Hence, additional control measures are important to curb schistosomiasis endemicity. Water, sanitation and hygiene (WASH), along with information, education and communication (IEC) are recommended useful tools in efforts aimed at schistosomiasis control and elimination (Monnier *et al.*, 2020). In addition, the control of schistosomiasis requires strong surveillance and information systems including key ground personnel who can identify human, animal and environmental sources of *Schistosoma* spp. to aid targeted intervention planning and vector control. Vector control has been demonstrated an effective tool in the control and elimination of schistosomiasis, as shown in Japan, People's Republic of China, and Puerto Rico where snail control yielded successful control of the parasites (Monnier *et al.*, 2020). Another way of limiting schistosomiasis involves *Schistosoma* spp. host snail predators such as fish, crustacean species and insects, and biological methods such introduction of nematode *Angiostrongylus cantonensis*, trematode of the genus *Echinostoma* or *Plagiorchis* , or bacterial pathogens such as species of *Paenibacillus*, which can limit *Schistosoma* spp. snail growth and fertility. However, biological control methods could be detrimental to human and animal health (Duval

et al., 2015). Alternative control measures such as direct host snail control programs may demand expensive resources and specialised human resources in addition to specialised infrastructure to identify and diagnose infected snails (Leonardo *et al.*, 2020, Kamel *et al.*, 2021) and to sustainably implement control strategies in endemic remote areas (Tenorio and Molina, 2021). Additionally, the use of snail killing chemicals, for example the WHO recommended niclosamide (Dai *et al.*, 2014), may be damaging to the environment (Leonardo *et al.*, 2020, Esquilla *et al.*, 2021, Tenorio and Molina, 2021).

Together, despite the PZQ associated shortcomings, the importance of the drug in controlling schistosomiasis remains the stronghold in a setup where limited alternative strategies are barely at reach for many resource-constrained developing countries.

1.3.10. Chronic schistosomiasis influences host immune responses to unrelated antigens

Despite effective vaccination programs which have resulted in a dramatic decrease in mortality from infectious diseases (Aristegui *et al.*, 2003), some vaccine-preventable diseases (VPDs) remain a serious concern in developing countries (Levine, 2003) particularly in children (WHO, 2014). It is estimated that 1-2 million children continue to die each year from VPDs, which makes up nearly 17% of the global mortality of children below the age of 5 years (Saitoh and Aizawa, 2016, Nanni *et al.*, 2017).

Hence, to overcome this issue, the WHO launched the Expanded Programme on Immunization (EPI) in May 1974. The EPI was created with the aim to expand large scale delivery of vaccines against VPDs (Cherian and Mantel, 2020) especially in new-borns and children (Saso and Kampmann, 2017, Cherian and Mantel, 2020). Additionally, the widespread use of a vaccine indirectly protects other unvaccinated children and adults within a community through “herd immunity” (Anderson *et al.*, 2018, Haire *et al.*, 2018).

Extensive literature reviewing by our research group revealed a lack of consensus on the impact of schistosomiasis on vaccine immunology, particularly its impact on memory immunity maintenance (Nono *et al.*, 2022). This was rather unsurprising, owing to the discrepancies of reports on several vaccines with differing results even for similar vaccines from different areas and within different studies (Nono *et al.*, 2022). For those vaccines that were shown to be negatively influenced by schistosomiasis, *S. mansoni* appeared more likely to impair vaccine responses in multiple studies, although contrasting evidence was presented in others (Nono *et al.*, 2022). It is therefore critical to continue investigations that improve our understanding of this potentially catastrophic phenomenon as far as vaccination in schistosomiasis vulnerable populations is concerned. However, it is acknowledged that mechanistic investigations in humans may be hindered partly due to ethical considerations and partly due to drastically limited control of potentially confounding factors such as intrinsic hereditary factors. Where human studies are not readily available or severely limited, mechanistic evaluations are recommended in animal studies, for example in mice models of schistosomiasis which are well established to mimic the natural

course of the disease in humans (Abdul-Ghani and Hassan, 2010, Nono *et al.*, 2017, Abdel Aziz *et al.*, 2018, Nono *et al.*, 2020, Nono *et al.*, 2021).

In addition to earlier described schistosomiasis driven immunoregulatory mechanisms, the need for co-infection and/or unrelated vaccine targeted research in schistosomiasis endemic areas is further strengthened by recent evidence that demonstrated that schistosomiasis promotes bone loss in mice (Li *et al.*, 2021). Li and colleagues showed that schistosome infection induces CD4⁺ T and B cells to produce high levels of bone loss associated receptor activator of nuclear factor- κ B (NF- κ B) Ligand (RANKL) (Li *et al.*, 2021). This increased production of RANKL during schistosomiasis could be driven by the parasite's immunomodulatory mechanics (Li *et al.*, 2021), however, evidence is currently limited. The impact of schistosomiasis induced bone tissue loss raises the question of whether bone marrow mediated vaccine memory immunity maintenance is affected by schistosomiasis, particularly in the context of antibody producing plasma B cells which are known to reside on the bone marrow compartment for extended periods. This poorly researched immunological dilemma requires mechanistic investigative efforts.

1.4. Study rationale – Investigating the influence of schistosomiasis on poliovirus vaccination

Although on the verge of global eradication, poliomyelitis is a serious, WHO notifiable and highly contagious disease of international significance. The causative virus, poliovirus, is a human enterovirus with an RNA genome (7.5 kbp) and a capsid protein (Grassly, 2013, Cochi *et al.*, 2014, Minor, 2016). The three types of polioviruses, type 1, type 2 and type 3 are transmitted through the faecal-oral route and the virus is shed into the environment via faecal excretion by infected individuals (Grassly, 2013, Cochi *et al.*, 2014, Minor, 2016). Infection with polioviruses can affect nerves leading to partial or full paralysis and a significantly reduced quality of host's life, mostly in children.

There is no specific treatment nor cure for poliomyelitis. Currently, polio vaccines are the primary preventive measures employed to control and eradicate the disease (Grassly, 2013, Cochi *et al.*, 2014, Minor, 2016). Two different polio vaccines have been in use for over six decades. The first of the vaccines, the OPV, contains a mixture of three different and attenuated polioviruses with mutations to decrease their virulence while the second vaccine is a formalin killed IPV (Grassly, 2013, Cochi *et al.*, 2014, Minor, 2016).

Despite the availability of these effective polio vaccines since the 1950s, over 350,000 children in 125 countries were at risk of being paralyzed by polio every year by the 1980s (Grassly, 2013, Cochi *et al.*, 2014, Minor, 2016). To address this debilitating polio morbidity burden on a global scale, the Global Polio Eradication Initiative (GPEI) was launched in 1988. The overall goal of GPEI is to completely eradicate and contain all wildtype, vaccine-related and Sabin polio viruses worldwide (Cochi *et al.*, 2014). By 1999, the GPEI had been instrumental in the reduction of annual polio cases by 99% and the successful eradication of the type 2 poliovirus. In addition, the eradication of all polioviruses from 3 of the 6 WHO polio high risk regions was achieved by 1999 (Minor, 2016).

Despite this notable success, the era of the oral polio vaccines did not come without limitations. In the past decade, the suspension of the oral polio vaccines in countries at high risk of the disease led to new outbreaks of polio in 20 countries that had been

previously certified free of polio (Cooper *et al.*, 2022). Furthermore, failure to maintain or reach complete polio vaccination coverage in these high-risk countries' populations exacerbated the polio outbreak with thousands of children becoming paralyzed due to the disease (Grassly, 2013, Cochi *et al.*, 2014, Mangal *et al.*, 2014, Akil and Ahmad, 2016, Minor, 2016). The remaining virulent poliovirus strains were last reported in Nigeria, Afghanistan and Pakistan (Minor, 2016).

In addition to problems in policy management and vaccination coverage, attenuated vaccine strains (OPV) posed a further challenge as the weakened live virus can escape the acid and enzymes in the human gut to replicate efficiently. This can result in vaccine-associated paralytic poliomyelitis and vaccine-derived polioviruses (VDPVs), a major public health concern especially in poorly resourced regions of the world (Mangal *et al.*, 2014, Minor, 2016). Because of the reverse virulence associated with the use of OPV, it was decided to progressively reduce OPV vaccines in favour of the killed IPV which excludes the possibility of vaccine-associated paralytic poliomyelitis and vaccine-derived polioviruses in the absence of wild poliovirus (Mangal *et al.*, 2014, Minor, 2016).

The current global polio eradication strategy emphasizes the essentiality of switching from OPV to IPV use in areas where wild viruses' transmission has been halted (Grassly, 2013, Minor, 2016). The potential of the IPV-based vaccine for eradication is supported by its strong ability to induct the host immune memory responses against poliovirus-associated morbidities while providing herd protection to residents of sufficiently vaccinated communities (Mangal *et al.*, 2014, Minor, 2016). However, the continuance of OPV administration together with IPV has been suggested as a potentially effective strategy to eradicate wild-type poliovirus in countries where the virus remains a considerable risk (Grassly, 2013).

The 65th Assembly of the World Health Organization (WHO) declared that the eradication of poliomyelitis is a global public health emergency that can be completed by careful strategic vaccination planning (Mangal *et al.*, 2014, Minor, 2016). The WHO and GPEI Polio Endgame strategic plan insists on the need to detect and stop all wild-type and live vaccine related poliovirus transmissions. A core manifest of this strategy is the planned progressive withdrawal of the OPV, the source of vaccine-derived viruses, and the worldwide inclusion of the IPV into immunization routine

programs (Grassly, 2013, Mangal *et al.*, 2014, Minor, 2016). As does the OPV, the IPV induces a protective antibody response that protects the host from poliomyelitis manifestation

Host protection against poliomyelitis from IPV-based vaccination is generally illustrative of vaccine-mediated anti-viral immunization. For several vaccines, including both types of anti-polio vaccines, successful immunization is largely IgG antibody-mediated (Grassly, 2013, Mangal *et al.*, 2014, Akil and Ahmad, 2016, Minor, 2016, Xiao and Daniell, 2017) in addition to cellular immunity (Wahid *et al.*, 2005, Van Damme *et al.*, 2019, Pollard and Bijker, 2021). Notably, however, antibody and cellular immune responses mounted by vaccination against polio, and generally for other diseases, can be influenced by a variety of endogenous factors such as genetics and age, and exogenous factors, for example, infectious pathogens such as unrelated viruses and parasites (Triki *et al.*, 1997, Van Loveren *et al.*, 2001, Nono *et al.*, 2022). Effective vaccination programs require the strategic consideration of these potentially confounding factors for successful vaccination program implementation.

The transmission of *Schistosoma* spp. parasites and polioviruses are both associated with poor sanitary conditions. Indeed, the parasites may be endemic in poliovirus infection high risk countries, for example, in specific regions in Nigeria, Afghanistan and Pakistan (Grassly, 2013, Mangal *et al.*, 2014, Akil and Ahmad, 2016, Minor, 2016, Xiao and Daniell, 2017). In some of these countries, particularly where the wildtype poliovirus is still circulating, any risk of impaired polio vaccination efficiency could ignite a polio public health concern that could potentially become global. However, despite increasing evidence on the diminishing immunoregulatory capacity of schistosomiasis on unrelated antigens including some vaccines, the influence of *Schistosoma* spp. on the efficiency and sustainability of anti-polio vaccination has not been addressed. Hence in our current research, we sought to address whether *S. mansoni* infection, and its control using PZQ treatment, could influence the long term IPV-induced protective antibodies in schoolchildren and mice, in addition to *in vivo* cellular responses in mice. Here, polio vaccination represented a proxy for general vaccination where similar immunity induction criteria are expected.

1.5. Hypothesis and Objectives

The overall aim of this study was to assess the influence of *S. mansoni* infection on anti-viral vaccination using anti-polio vaccination in children as a proxy and to investigate further this influence at a mechanistic level by employing laboratory mouse models. Our goal is to integrate our findings into the current anti-viral vaccination strategy globally where suitable and particularly in schistosomiasis-diseased areas.

Specifically, our study sought:

1. To investigate the influence of schistosomiasis on serological antibody memory from polio vaccination in schoolchildren from a schistosomiasis endemic area of Cameroon
2. To assess the impact of treating schistosomiasis by using PZQ, the sole commercially available drug against the disease, on serological antibody memory from polio vaccination in schoolchildren from a schistosomiasis endemic area of Cameroon
3. To establish an IPV vaccinated mouse model, confirm anti-polio IgG specific antibodies maintenance following the subcutaneous injection of laboratory mice in our setting, and investigate the influence of *S. mansoni* infection on the serum titres of IPV-elicited polio-specific IgGs in the established IPV vaccinated laboratory mice
4. To investigate if PZQ treatment against *S. mansoni* influences the IPV vaccination responses in *S. mansoni* infected laboratory mice
5. To assess the influence of schistosomiasis and PZQ treatment on spleen and bone marrow B and T cells with particular interest on antibody producing plasma B cells.

Chapter 2: Materials and Methods

2.1 Human study

2.1.1. Ethics statement

Ethical approval to conduct this study using schoolchildren as participants was granted by the Cameroon National Ethics committee for Human Health Research (Ethics Approval no. 2018/02/976/CE/CNERSH/SP). Additional authorization to conduct the study at school premises was provided by the Ministries of Basic Education (Ethics Approval no. 3/105/L/MINEDUB/DREB-C/SDAG/IMS) and Ministry of Public Health of Cameroon (Ethics Approval no. 030-571/L/MINSANTE/SG/DROS/CRC/NPN/TMC/ 631-12.18). This study was authorised by local, regional, and school authorities. Schoolchildren and parents' or legal guardians were thoroughly informed of this study's objectives and the methodology through a process facilitated with assistance from schoolteachers. Each child's participation was informed and voluntary, and all schoolchildren and parents/legal guardians gave informed written consent before the study was conducted. To protect the privacy and ensure confidentiality of participants, collected data were deidentified before any downstream analyses were conducted. As recommended by the Cameroon National program for the control of Schistosomiasis and Soil transmitted Helminthiasis, all schoolchildren, whether participating in the study or not, were treated with praziquantel regardless of their parasitological status.

2.1.2. Study areas and populations

We conducted a cross-sectional study with participants recruited from a total of five public schools in the *S. mansoni* endemic region of Bokito, a rural region located approximately 100km from Yaoundé, the capital city of Cameroon. Participants were recruited from schools from 5 different villages within the Bokito region namely, Yoro 1, Yoro 2, Bongando, Ediolomo and Kedia, respectively. This study area has been previously described (Nono *et al.*, 2018, Kamdem *et al.*, 2020).

2.1.3. Pre-screening using interview questionnaire

Each child was interviewed by a member of the research staff assisted by their class teacher and parents/legal guardians after informed consent was attained. The interviews were conducted and recorded on questionnaires (Appendix 1) designed to collect demographic information such as age and gender as well as self-reported and/or parent/legal guardian confirmed general health and vaccination status. In Cameroon, children are vaccinated with the polio vaccines; OPV at birth, 1 month, 2 months and OPV/IPV 3 months (Appendix 2). These deidentified data are available upon request.

2.1.4. Recruitment of participants: Inclusion and exclusion criteria

The study was conducted in two sub-phases. In the first sub-phase, a total of 64 participating schoolchildren under the age of 18 years were voluntarily and randomly recruited from Yoro 1 in the year 2016. In the second sub-phase, a follow up of the study was conducted and a larger sample size of 189 children from five different public schools were voluntarily and randomly recruited in the year 2018. In the year 2018, schoolchildren within the initial age ranges as in 2016 were recruited from Yoro 1, Yoro 2, Bongando, Ediolomo and Kedia. As an inclusion criterion, all participating schoolchildren were confirmed to have resided within the endemic area for a minimum of 6 months. Gender was not used as determinant for participant selection. However, the gender of each child was recorded. Informed voluntary written consent of all recruits was obtained.

Both the 2016 and the 2018 studies were conducted in a period falling at least 5 months post praziquantel treatment of all children within the Bokito region under the MDA program of Cameroon. This MDA program is aimed at controlling schistosomiasis in endemic regions of Cameroon using PZQ treatment. Dosage of PZQ in MDA campaigns is based on the WHO recommended tablet dose pole strategy. Each child's dose was calculated based on their body height, the dose-pole method, to achieve an optimal dosage of 40 mg/kg (Montresor *et al.*, 2005, Baan *et*

al., 2016). Additionally, all children contacted during our study efforts were treated with a single dosage of 40 mg/kg praziquantel regardless of their schistosomiasis status.

2.1.5. Blood sample collection and screening for Malaria and Hepatitis infections

Between 2.5ml to 5ml of whole blood was aseptically collected from each child (depending on age) by venepuncture into Heparin coated tubes by experienced and authorised phlebotomists. All children were provided with standard post-puncture care to minimise risk of infection. Malaria infection status was assessed by thick smears as previously described (Ohrt *et al.*, 2002). Briefly, a spot of well mixed blood was placed on a clean slide. Using the edge of another clean slide, red blood cells were carefully lysed into a thick smear to release the *Plasmodium* spp. parasite. The smear was air-dried followed by Giemsa staining. Analysis was conducted using an optical microscope. Hepatitis B and hepatitis C viral infection statuses were evaluated using the DiaSpot HBsAg and DiaSpot HCV-Ab test kits (DiaSpot, Jakarta, Indonesia), respectively, according to their manufacturer's instructions.

2.1.6. Parasitological survey including *S. mansoni* screening

All participating children were given 2 prelabelled 50ml screw-cap tube which they used to collection fresh morning stool samples on 2 separate days. The two collections were separated by a 5-day gap. On each day of collection, the stool samples were processed in the field and evaluated for parasitological analyses using the Kato-Katz technique to identify and quantify eggs from *Schistosoma* spp. and other geohelminths as previously shown (Katz *et al.*, 1972, Moyou-Somo *et al.*, 2003). Briefly, 41.7mg of stool was prepared for each sample, 2 stools per child, and assessed by microscopy by 2 independent laboratory technologists for the detection and quantification of *S. mansoni* eggs. Urine samples (10ml) were collected from each child and assessed by filtration method to identify *S. haematobium* eggs as previously described.

2.1.7. Anti-polio specific IgG antibody ELISA of selected participants

Collected blood (2.5 – 5ml) was centrifuged to isolate plasma. Aliquots of isolated plasma were stored at -80°C until use. The quantification of total anti-poliovirus IgGs in plasma samples was carried out by sandwich ELISA using a commercial kit (Human Anti-Poliomyelitis Virus 1-3 IgG ELISA Kit, Alpha Diagnostic International, Tx, USA) following the manufacturer's instructions.

2.2 Animal study

2.2.1 Ethics statement

Authorization to conduct experiments on animals was approved by the Animal Research Ethics committee of the Faculty of Health Science of the University of Cape Town (Protocol Approval no. 018/029). All researchers and animal handlers were authorised to handle animals by the Animal Research Ethics committee of the Faculty of Health Science of the University of Cape Town and the South African Veterinary Council. All animal experiments were performed ensuring the minimisation animal suffering in accordance with the guidelines of the Animal Research Ethics committee of the Faculty of Health Science of the University of Cape Town, the South African Veterinary Council and the South African National Standard (SANS 10386:2008).

2.2.2 Generation of BALB/c Mice

Mice on a BALB/c background were used. The mice were maintained under specific pathogen-free animal conditions at the University of Cape Town, in accordance with the guidelines established by the Animal Research Ethics committee of the Faculty of Health Science of the University of Cape Town and the South African Veterinary Council.

2.2.3 Life cycle maintenance for *S. mansoni*

Live cercariae were generated from *Biomphalaria glabrata* snails (a gift from Adrian Mountford, York, UK) that are maintained in our laboratory. Naval Medical Research Institute (NMRI) female mice were used to maintain and propagate the *S. mansoni* parasite larvae.

2.2.4 Induction of anti-poliovirus immunity in mice

Age and gender matched mice aged 6-8 weeks were injected subcutaneously with a commercial hexavalent vaccine against infectious diseases diphtheria, tetanus, pertussis (acellular, component), hepatitis b, inactivated polio vaccine, haemophilus influenzae b (DTPa-hepB-IPV-Hib) (Sanofi Pasteur, Lyon, France) at 2 sites i.e., 150ul on the neck and 100ul on the abdomen, at day 0 (primary vaccination) and at day 30 (secondary vaccination / booster).

2.2.5 Infection of DTPa-hepB-IPV-Hib vaccinated mice with *S. mansoni*

DTPa-hepB-IPV-Hib vaccinated mice were anesthetized and infected with a low dose of 35 live *S. mansoni* cercariae for 30 minutes by percutaneous abdomen exposure as previously described (Abdel Aziz *et al.*, 2018).

2.2.6 Preparation of praziquantel solution and administration in the treatment of *S. mansoni* infected mice

Praziquantel (Merck KGaA, Darmstadt, Germany) solution was prepared as previously described (Nono *et al.*, 2020, Nono *et al.*, 2021). Briefly, PZQ was weighted at quantities sufficient for 400 mg/kg of animal body weight and mixed with 10 parts 70% Tween + 30% Ethanol using a magnetic stirrer. Next, 90 parts of distilled sterile water were slowly added and stirring to obtain a homogeneous suspension. Administration of 200ul of the homogeneous suspensions were administered within 3 h after preparation. Between week 9 and week 10 post *S. mansoni* exposure, infected mice were treated with a single daily oral dose of 400mg/kg of PZQ solution or mock treated for 7 days.

2.2.7 Scheduled mouse blood collection and serum collection

From week 4 post *S. mansoni* infection, 50ul tail vein blood was collected from all mice at week 4, 8, and finally cardiac blood at week 18, the experimental endpoint when mice were euthanized. Blood was centrifuged in serum separator tubes (BD Bioscience, San Diego, CA) at $8\,000 \times g$ for 10 min at 4°C to retrieve the serum. The upper aqueous serum phase was aliquoted into tubes and stored at -80°C until further use.

2.2.8 Anti-poliovirus specific IgG ELISA (enzyme-linked immunosorbent assay) for anti-polio IgG titre kinetics

Anti-poliovirus specific total IgG titres were quantified by sandwich ELISA using a commercial kit (Mouse Anti-Poliovirus Virus 1-3 IgG ELISA Kit, Alpha Diagnostic International, Tx, USA) according to the manufacturer's instructions.

2.2.9 Anti- SEA specific IgG ELISA for anti-SEA IgG titre kinetics

Ninety-six well plates (Nalge Nunc International, USA) were coated with 20ug/mL of SEA (Theodor Bilharz Research Institute, Giza, Egypt) diluted in 1X PBS buffer. The plates were incubated overnight at 4°C. The following day, the plates were washed with wash buffer (20g KCl, 800g NaCl, 20g KH₂HPO₄.2H₂O, 50ml Tween-20, dissolved in 5 litres, then diluted 1:20 in ddH₂O) 4 times. Two hundred microliters of blocking buffer with 2% of milk powder was added followed by a 2-hour incubation at 37°C. Thereafter, the plates were washed with wash buffer. Serum samples were added, 50ul per well, in 10-fold dilutions in 1% BSA diluent. The plates were incubated overnight at 4°C. The plates were washed the following day and Secondary biotinylated IgG antibodies were added to the plates followed by a 2–3-hour incubation at 37°C. Thereafter, the plates were washed in wash buffer followed by the addition of streptavidin substrate conjugated with alkaline phosphatase was added at 1/1000 dilution. The plates were washed in wash buffer followed by the

addition of 1mg/ml of 4 Nitro-phenyl phosphate disodium salt hexahydrate (PNP) (Merck, Germany) diluted in AP substrate buffer to each well. The plates were then incubated at 37°C for 10 minutes in the dark to develop the plates. Optical density was measured on the VERSAmax microplate reader (Molecular devices, USA) at absorbance 405nm over 490nm.

2.2.10 Serum cytokine measurement by ELISA

Ninety-six well plates (Nalge Nunc International, USA) were coated with cytokine antibody of interest diluted in 1X PBS buffer. The plates were incubated overnight at 4°C. The following day, the plates were washed with wash buffer (20g KCl, 800g NaCl, 20g KH₂HPO₄.2H₂O, 50ml Tween-20, dissolved in 5 litres, then diluted 1:20 in ddH₂O) 4 times. Two hundred microliters of blocking buffer with 2% of milk powder was added followed by a 2-hour incubation at 37°C. Thereafter, the plates were washed with wash buffer. Samples were added at 50ul per well. The plates were incubated overnight at 4°C. The plates were washed the following day and Secondary biotinylated antibodies of interest were added to the plates followed by a 2–3-hour incubation at 37°C. Thereafter, the plates were washed in wash buffer followed by the addition of streptavidin substrate conjugated with alkaline phosphatase was added at 1/1000 dilution. The plates were washed in wash buffer followed by the addition of 1mg/ml of 4 Nitro-phenyl phosphate disodium salt hexahydrate (PNP) (Merck, Germany) diluted in AP substrate buffer to each well. The plates were then incubated at 37°C for 10 minutes in the dark to develop the plates. Optical density was measured on the VERSAmax microplate reader (Molecular devices, USA) at absorbance 405nm over 490nm.

2.2.11 Bone marrow single cell isolation

Following euthanasia at week 18 post infection, the rear left femur bone was collected for each mouse. The collected bones were placed in 15ml tube with cold 5mL Iscove's Modified Dulbecco's Medium (IMDM) (Gibco, USA) supplemented with 10% Fetal Bovine Serum (Roche Diagnostics, Germany) and 0.5% Penicillin-Streptomycin

(50units/mL penicillin and 50ug/mL streptomycin diluted from an undiluted antibiotic solution containing 10,000 units/mL of penicillin/ 10,000 µg/mL of Streptomycin in a 10 mM citrate buffer) (Gibco, USA). Uncompromised bones were cleaned in cold ethanol followed by cold PBS and again in supplemented IMDM. The bones were cut swiftly on each end with a sharp scissors. Bone marrow was washed out of the bone into a 70µm sieve (Thermo Scientific, USA) using a 10ml syringe filled with supplemented IMDM. The bone marrow / IMDM solution was sieved into a sterile petri dish followed by a second sieving through a 70µm thresh into a newly labelled 50mL tube. The samples were centrifuged for 10 minutes at 1200rpm at 4°C. The supernatant was discarded, and the cells were resuspended in 5mL red blood cell (RBC) lysis buffer and incubated for 10 minutes at room temperature. Five millilitres of IMDM media supplemented with 10% Fetal Bovine Serum and 0.5% Penicillin-Streptomycin was then added to the solution followed by centrifugation for 10 minutes at 1200rpm at 4°C. The supernatant was discarded, and the samples were resuspended 3mL of supplanted IMDM. The cells were the counted under a microscope using tryptophan blue at 1:10 dilution. The prepared single cell suspensions of bone marrow were immediately used for downstream flow cytometry analyses.

2.2.12 Spleen single cell isolation

Spleens were collected in 15ml tube with cold 5mL IMDM media supplemented with 10% Fetal Bovine Serum and 0.5% Penicillin-Streptomycin. The samples were sieved through a 70µm sieve into a sterile petri dish followed by a second sieving through a 70µm thresh into a newly labelled 50mL tube. The samples were centrifuged for 10 minutes at 1200rpm at 4°C. The supernatant was discarded, and the cells were resuspended in 5mL red blood cell (RBC) lysis buffer and incubated for 10 minutes at room temperature. Five millilitres of IMDM media supplemented with 10% Fetal Bovine Serum and 0.5% Penicillin-Streptomycin was then added to the solution followed by centrifugation for 10 minutes at 1200rpm at 4°C. The supernatant was discarded, and the samples were resuspended 3mL of IMDM media

supplemented with 10% Fetal Bovine Serum and 0.5% Penicillin-Streptomycin. The cells were counted under a microscope using tryptophan blue at 1:10 dilution.

2.2.13 Flow cytometry

Two hundred microliter of IMDM media supplemented with 10% Fetal Bovine Serum and 0.5% Penicillin-Streptomycin containing 2 million isolated single cells (bone marrow or spleen) were pipetted into a V bottom 96 well plate (Nalge Nunc International, USA) and centrifuged at 1500 rpm for 5 minutes at 4°C. The supernatant was discarded to remain with a cell pellet, which was subsequently resuspended in an amine based live/dead marker diluted in 1x cold PBS. Cells were incubated at room temperature for 15 minutes. The cells were then washed with 1x PBS and centrifuged at 1500 rpm for 5 minutes at 4°C, after which the supernatant was discarded. The cell pellet was resuspended in surface antibody staining cocktail of interest (Appendix 2) mixed in FACS buffer (0.1% BSA in 1x PBS buffer), 2% heat inactivated rat serum (iRS) and 1% FcyRIII. The cells were incubated in the fridge at 4°C for 30mins on ice. Stained cells were then washed with FACS buffer before resuspension in 1x Annexin V binding buffer, centrifuged at 1500 rpm for 5 minutes at 4°C, and resuspended in Annexin V antibody mix in Annexin V binding buffer before incubation for 15 minutes at room temperature in the dark right. The cells were acquired on the BD LSR Fortessa (BD Immunocytometry system, San Jose, CA, USA), and data analysis was performed using FlowJo software (Treestar, Ashland, OR, US). For intracellular or intranuclear staining on specific antibody panels, the cell pellet was washed with 200ul fixation-permeabilization wash buffer and centrifuged at 1500 rpm for 5 minutes at 4°C. The supernatant was discarded and 100ul of fixation-permeabilization solution added to the cell pellet followed by incubation for 1 hour at 4°C. Thereafter, cells were washed in 100ul of fixation-permeabilization wash buffer followed by centrifugation 1500 rpm for 5 minutes at 4°C. The cell pellet was resuspended in antibody cocktail of interest mixed in fixation-permeabilization wash buffer and incubated for 45 minutes in the fridge at 4°C. The cells were then washed with 200ul of fixation-permeabilization wash buffer followed by centrifugation 1500 rpm for 5 minutes at 4°C. The cells were acquired on

the BD LSR Fortessa (BD Immunocytometry system, San Jose, CA, USA), and data analysis was performed using FlowJo software (Treestar, Ashland, OR, US).

2.2.14 Histology

At experimental endpoint, collected liver and small intestinal samples were immediately placed in formalin solution, neutral buffered. During sample processing, approximately 5µm sample sections were collected and stained with the haematoxylin and eosin (H&E) stain on glass microscopy slides. Using specialised microscopy, granuloma size was quantified as product of granuloma circumference minus single egg circumference using a computerized morphometry analysis program. A minimum of 80 granulomas were quantified for each mouse treatment group.

2.2.15 *Schistosoma mansoni* egg counts

Liver and gut samples were excised and collected from each mouse at experimental endpoint were weighed digested in 4% aqueous potassium hydroxide (KOH) for 18 h, as previously described (Nono et al., 2017). Schistosome eggs were counted using an inverted microscope.

2.3 Statistical analyses

Data was analysed using GraphPad Prism 8.0 software (<http://www.prism-software.com>). Data was summarised using simple descriptive tools (means $\pm$ S.D or means $\pm$ S.E.M, frequencies (%) and counts). Data normality was assessed using Shapiro-Wilk test. All other statistical evaluations were performed using one of the following tests depending on data distribution: unpaired Student's t-test for equal or unequal variances or Mann-Whitney test, and Kruskal-Wallis test followed by the Dunn's multiple comparisons, ANOVA followed by Bonferroni's multiple comparisons or FDR for multiple comparisons (McDonald, 2009). A p-value threshold of less than

0.05 was considered as statistically significant. Statistical significance was shown as *($p < 0.05$), **($p < 0.01$) and ***($p < 0.001$).

Chapter 3: Influence of *S. mansoni* and its treatment with praziquantel, on poliovirus specific serological memory in anti-poliovirus vaccinated schoolchildren from a schistosomiasis endemic area.

3.1 Results

3.1.1 Impact of *S. mansoni* infection status on anti-polio IgG serological memory in schoolchildren

In the year 2016, schoolchildren were randomly recruited to assess the impact of schistosomiasis on vaccine-specific anti-polio immunity. With informed consent assisted by parents or legal guardians, children were voluntarily interviewed for background data collections. Each child's polio vaccination status was confirmed by consultation of public health program distributed vaccination cards and parents' or legal guardians' recall. Of the vaccinated children, 2 stool samples were collected on 2 separate days. On each day, each stool sample was split into 2 and processed by 2 qualified health laboratory technologists to identify *S. mansoni* eggs by the Kato Katz technique. All children provided between 2.5ml and 5ml blood samples depending on their age. Children were excluded from the study if they had missing questionnaire data, refused consent for the study or if found to be infected with Hepatitis B or C, Malaria and other geohelminths aside from *S. mansoni*. A total of 64 children (age range: 9.2 – 12.7 years old) were recruited.

3.1.2 *Schistosoma mansoni* infection suppresses anti-polio IgG serological memory of vaccinated schoolchildren

Data assessments showed an 82.8% (53/64) prevalence of *S. mansoni* infection among the randomly selected participants. Results from anti-polio IgG specific ELISA showed significantly reduced vaccine-induced anti-polio IgG titres in children with *S. mansoni* infection in comparison to the non-infected control group ($p < 0.01$) (Figure 3.1).

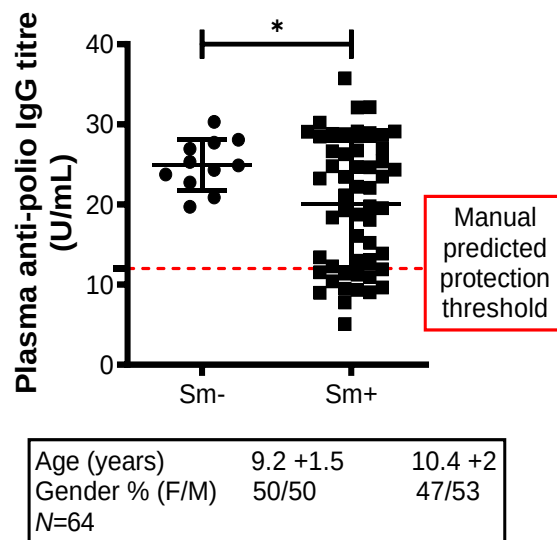


Figure 3. 1: Impact of schistosomiasis on anti-poliovirus IgG levels in schoolchildren in the village of Yoro, Bokito in Cameroon in the year 2016. Data are expressed as mean ± S.D; Data was analysed by Shapiro-Wilk test followed by the two-tailed unpaired Student t test for unequal variances; * $p < 0.05$, ; Sm, *S. mansoni*.

3.1.3 *Schistosoma mansoni* infection selectively suppresses anti-polio IgG serological memory of vaccinated schoolchildren from different schistosomiasis endemic areas within the same region

Following up on our initial findings that schistosomiasis is associated with reduced anti-polio IgG titres in schoolchildren from Yoro in the 2016 cohort (Figure 3.1), we sought to explore further these findings in a larger cohort of children. Using the same participant selection criteria described above (section 3.1.1), a follow-up study was conducted in the year 2018 with a larger study number of participants. A total of 189

schoolchildren were recruited from 5 different schools within the schistosomiasis endemic Bokito region of rural Cameroon (Figure 3.2 and Table 3.1).

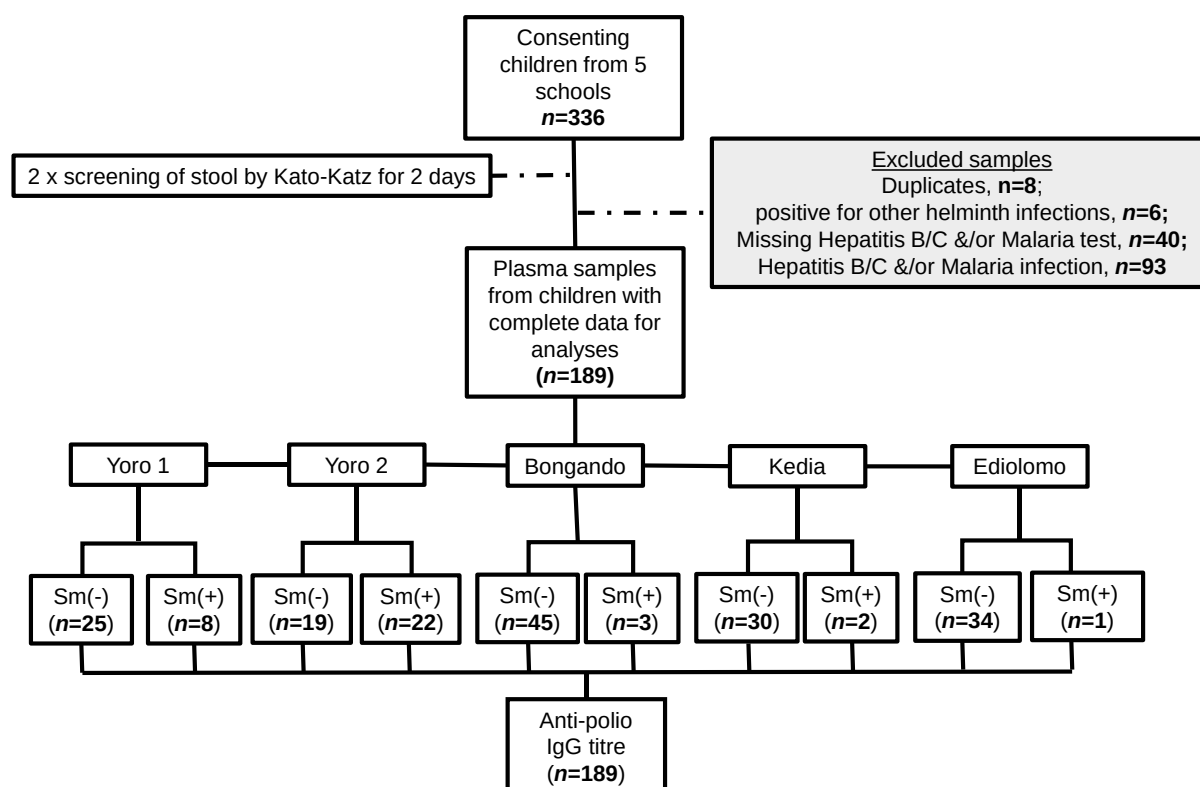


Figure 3. 2: Flowchart showing the recruitment and assessment of children from 5 public schools in Bokito, a schistosomiasis endemic rural region of Cameroon.

Table 3. 1. Demographics and baseline characteristics of selected participants

School	Yoro 1		Yoro 2		Bongando		Kedia		Ediolomo	
Total cases	N = 33		N = 41		N = 48		N = 32		N = 35	
Infection status	Sm(-)	Sm(+)	Sm(-)	Sm(+)	Sm(-)	Sm(+)	Sm(-)	Sm(+)	Sm(-)	Sm(+)
All cases, N (%)	25 (75.8)	8 (24.2)	19 (46.3)	22 (53.7)	45 (93.75)	3 (6.25)	30 (93.75)	2 (6.25)	34 (97.1)	1 (2.9)
Sex: M/F	14/11	7/1	9/10	11/11	17/28	0/3	15/15	0/2	17/17	1/0
Age groups, N (%)										
<10 years	1 (100)	0 (0)	5 (35.7)	9 (64.3)	10 (100)	0 (0)	7 (87.5)	1 (12.5)	8 (100)	0 (0)
(10 -14) years	23 (82.1)	5 (17.9)	14 (56)	11 (44)	34 (91.9)	3 (8.1)	22 (95.7)	1 (4.3)	23 (95.8)	1 (4.2)
>14 years	1 (25)	3 (75)	0 (0)	2 (100)	1 (100)	0 (0)	1 (100)	0 (0)	3 (100)	0 (0)

The children recruited in the 2018 study were between the ages of 7 and 16 years. The study participants' modal age range for all schools was fell between 10 and 14 years old (Table 3.1). A total of thirty-three children were recruited from the original 2016 study site, Yoro village school 1 (Y1). Forty-one children were recruited from a different school within the Yoro village school 2, Yoro 2 (Y2), 48 children were recruited from Bongando village school (BG), 32 children joined the study from Kedia village school (KD), and the remaining 35 children were recruited from Ediolomo village school (ED). The prevalence of *S. mansoni* infection was 24.2% (8/33) at Y1, 53.7% (22/41) at Y2, 6.25% (3/48) at BG, 6.25% (2/32) at KD and 2.86% (1/35) at ED (Table 3.1).

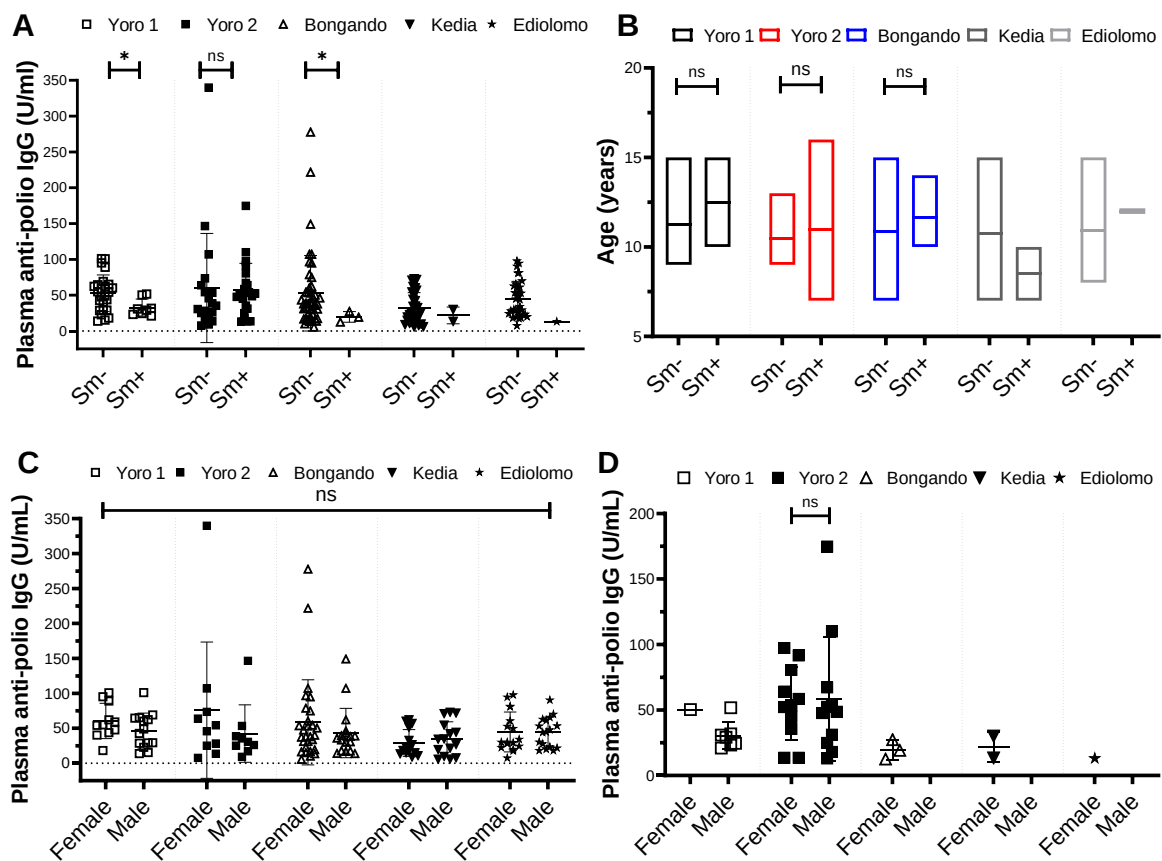


Figure 3. 3: Influence of *S. mansoni* infection and age on vaccine-induced anti-poliovirus responses in schoolchildren from five schools in a rural region of Cameroon.

A. Anti-poliovirus IgG titres of schoolchildren presented for each school surveyed in the larger study conducted in different villages in the Bokito region in Cameroon in the year 2018. **B.** Age distribution by *S. mansoni* prevalence in children from different schools. **C.** Anti-poliovirus IgG titre distribution by gender in non-infected children from different schools. **D.** Anti-poliovirus IgG titre distribution by gender in *S. mansoni* infected children from different schools. Data are expressed as mean $\pm$ S.D; Data was analysed by Shapiro-Wilk test followed by two-tailed unpaired Student t test for

equal or unequal variances or Mann-Whitney test; ns, $p > 0.05$; * $p < 0.05$; Sm, *S. mansoni*; PZQ, praziquantel.

Quantitative evaluation of vaccine-induced blood plasma anti-poliovirus specific IgG revealed significantly reduced anti-poliovirus IgG antibody titres in children infected with *S. mansoni* infected children at Y1 ($p < 0.01$) and BG ($p < 0.01$) (Figure 3.3A) when compared to non-infected control children for each site. Additionally, a reduction in anti-polio IgG titres was observed in children from KD and ED, however, sample size limitations could not allow for robust statistical evaluations (Figure 3.3A). With contrasting indications, *S. mansoni* appeared to have no impact on anti-polio IgG titres in children from Y2 ($p > 0.05$). Further analysis for each site showed no influence of neither gender nor age on anti-polio IgG titres (Figure 3.3B-C). As such, we concluded that schistosomiasis was associated with the reduction of vaccine-induced anti-polio IgG titres in children from Y1 and BG but not in children from Y2.

3.1.4 *Schistosoma mansoni* infection suppresses anti-polio IgG serological memory of vaccinated schoolchildren over the age of 14 years for all sites combined

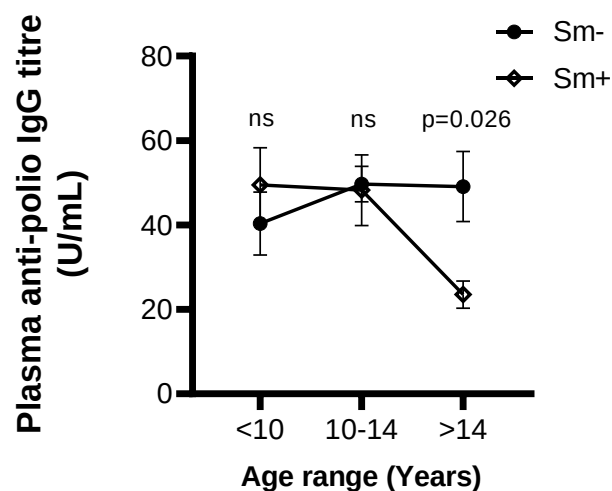


Figure 3. 4: Distribution of anti-poliovirus IgG titres stratified by *S. mansoni* infection status and age groups in all children from the five surveyed schools. Data expressed as mean $\pm$ S.D; Data was analysed by Shapiro-Wilk test followed by the two-tailed unpaired Student t test for unequal variances; ns, $p > 0.05$; Sm, *S. mansoni*.

Further analyses showed that the presence of *S. mansoni* infection in older children over the age of 14 years was associated with reduced blood plasma anti-polio IgG titres for all the 5 schools combined ($p < 0.05$) (Figure 3.4). Analyses of participants' gender and body mass index (BMI) ($\text{weight [kg]} \div \text{height}^2 [\text{meters}]$) as a measure of general wellbeing indicated no influence of this parameter on observations made between *S. mansoni* infected children and non-infected children from each of the 5 schools and all the 5 schools combined ($p > 0.05$) (Figure 3.5A-F).

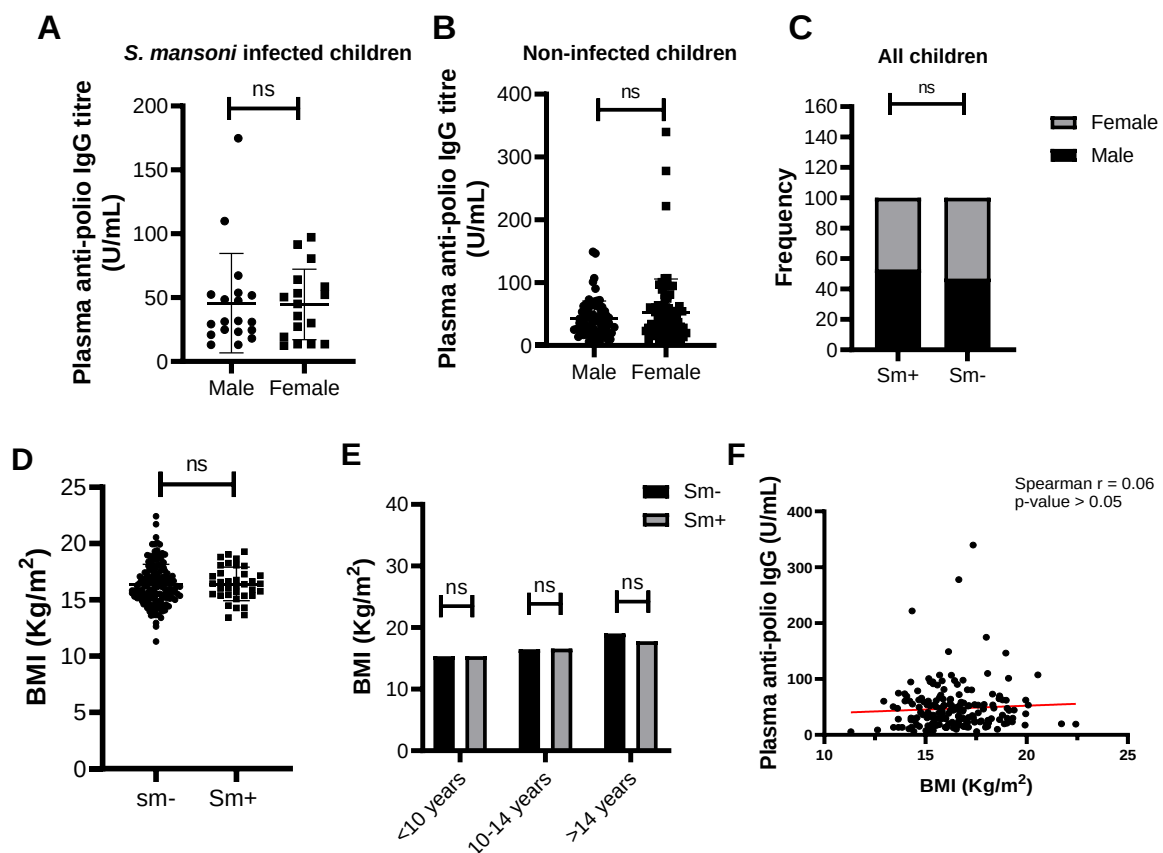


Figure 3. 5: Distribution of antipolio IgG titres by gender and BMI in all children from the five surveyed schools.

A. Anti-poliovirus IgG titre distribution by gender in *S. mansoni* infected children **B.** Anti-poliovirus IgG titre distribution by gender in non-infected children. **C.** Anti-poliovirus IgG titre distribution by gender in infected and non-infected children combined. **D.** BMI distribution in all infected and non-infected children. **E.** BMI distribution in infected and non-infected children stratified by age ranges. **F.** Correlation analysis between BMI and anti-poliovirus IgG titres in all participating school children. Data are expressed as mean $\pm$ S.D; Data was analysed by Shapiro-Wilk test followed by two-tailed unpaired Student t test for equal or unequal variances or Mann-Whitney test (**A** and **B**); Chi-square test (**C**); Spearman's correlation (**F**); ns, $p > 0.05$; Sm, *S. mansoni*.

Taken together, our observations emanating from two separate field surveys indicate that *S. mansoni* infection may be associated with reductions in vaccine-elicited anti-poliovirus serological memory immunity in schoolchildren residing in *S. mansoni* endemic regions. However, due to the limitations and conflections of the available data among different schools and other vaccines as shown in our recent literature (Nono *et al.*, 2022), our present findings in their current state are considered suggestive and not conclusive. As such, they warrant further in-field prospective investigations in other studies with long-term continuous data collection in schistosomiasis endemic areas.

3.1.5 Impact of anthelmintic chemotherapy, praziquantel, treatment on serological memory of anti-poliovirus vaccinated schoolchildren infected with *S. mansoni* infections

Currently, the only WHO-recommended treatment for schistosomiasis is a curative drug, PZQ. As described earlier, in schistosomiasis endemic areas, PZQ mass treatment is the leading control measure against schistosomiasis (Wang *et al.*, 2012). We, therefore, sought to assess the impact of PZQ treatment on schistosomiasis's reductive influence on vaccine-specific anti-polio IgG titres in children from Y1. We conducted a follow-up investigation of children from Y1 who took part in both the 2016 and 2018 arms of our study. Eight children were identified with all required information in both the years 2016 and 2018 from the pool of participants (Figure 3.6). *Schistosoma mansoni* infection was identified in 6/8 children in 2016. In 2018, this group of children had been cured of schistosomiasis. The remaining 2/8 children were found without *S. mansoni* infection in 2016 but at follow up in 2018, they were found to be infected with the parasitic disease.

3.1.6 Anthelmintic chemotherapy, praziquantel, restores anti-polio IgG titres suppressed in association with *S. mansoni* infection in schoolchildren from schistosomiasis endemic areas

As part of efforts to control and eliminate schistosomiasis in endemic regions, the government of Cameroon mass-administers single doses of the PZQ (40mg/kg) drug to all schoolchildren in *S. mansoni* endemic regions, such as Bokito, once annually. Between the years 2016 and 2018, children recruited for the 2018 follow-up study received a total of 3 single PZQ doses under this mass drug administration (MDA) program. Further, each child enrolled in our study was given a single dose of 40mg/kg PZQ after sample collection. Thus, the follow-up study participants received a total of 4x PZQ treatment cycles between 2016 and 2018 (Figure 3.6). Stored plasma samples were interrogated by ELISA to quantify their anti-polio specific IgG antibody titres.

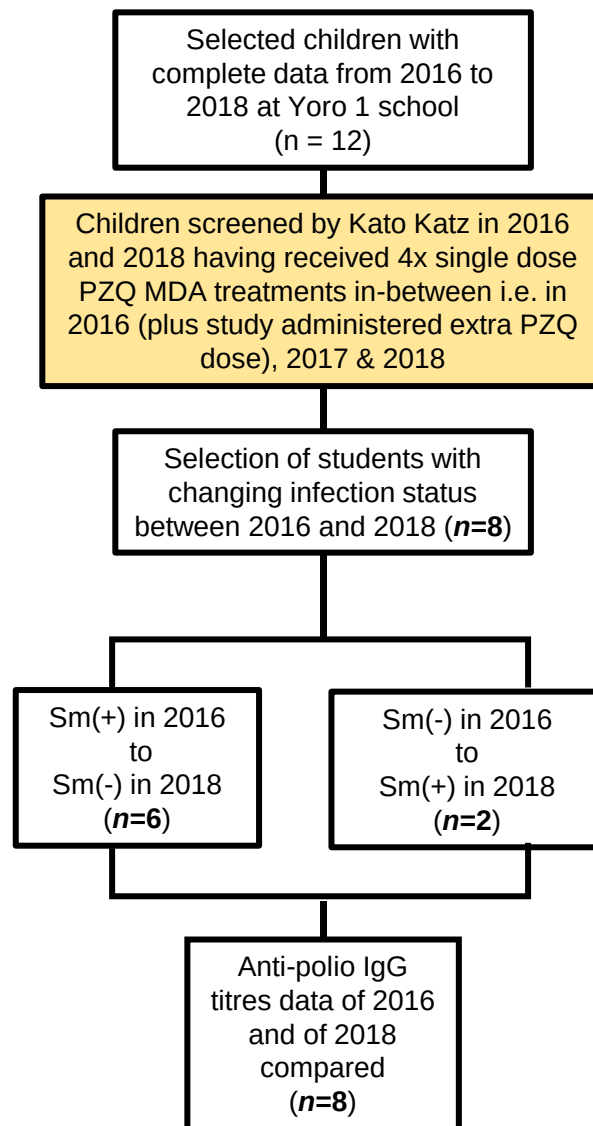


Figure 3. 6: The follow-up recruitment of children between the years 2016 and 2018 to evaluate the impact of schistosomiasis and treatment using PZQ on anti-poliovirus IgG plasma titres of vaccinated children.

Our analyses directed at periods before and after PZQ treatment in relation to the presence of *S. mansoni* infection or not indicated that children who were previously infected with *S. mansoni* in the year 2016 had their anti-poliovirus specific IgG antibody titres significantly raised in the year 2018 post curative treatment i.e., clearance of the *S. mansoni* parasite ($p < 0.05$) (Figure 3.7A). On the other hand, no significant differences were observed for children who started without *S. mansoni* free in 2016 and were found infected in 2018. However, there was a clear trend in the reduction of anti-polio specific IgG antibodies in this second group of children in their diseased state in comparison to their non-schistosomiasis state (Figure 3.7B).

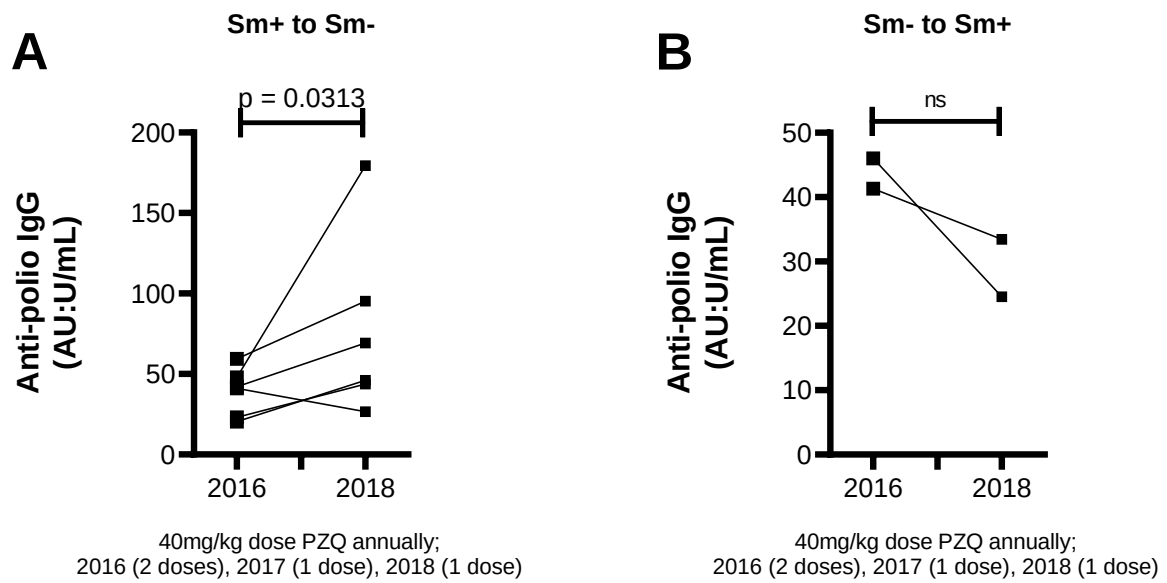


Figure 3. 7. Influence of *S. mansoni* infection and its treatment with PZQ on vaccine mounted anti-poliovirus responses in schoolchildren from five schools in the schistosomiasis endemic Bokito region of Cameroon:

A; B. Effect of Schistosomiasis and its treatment with praziquantel, on vaccine-induced plasma anti-poliovirus IgG titres. Data are expressed as mean $\pm$ S.D; Data was analysed by Shapiro-Wilk test followed by the two-tailed Wilcoxon signed-rank test or paired Student's t test (D-E); ns, $p > 0.05$; Sm, *S. mansoni*; PZQ, praziquantel.

Overall, we concluded that the clearance of *S. mansoni* infection with PZQ treatment could restore poliovirus vaccine-induced serological memory in previously schistosomiasis infected children.

3.2 Discussion: Influence of schistosomiasis and its treatment with PZQ on polio vaccination in schoolchildren from schistosomiasis endemic rural region of Cameroon

Anti-poliovirus vaccines confer long term memory immunity, demonstrable at as long as 18 years post inoculation, in vaccinees (Bottiger, 1990, Mulders *et al.*, 1999, Wahid *et al.*, 2005, Dey *et al.*, 2016, Bhaumik *et al.*, 2018). As such, these vaccines are administered early in childhood in several countries globally. In Cameroon and several other African countries, poliovirus vaccines are administered as recommended under the WHO and local health service-driven extended program on immunization (EPI) program (Keja *et al.*, 1988, Andre *et al.*, 2014, Ewang *et al.*,

2020). Reduced levels of polio memory immunity increase the likelihood of reduced protection against polio and consequently increases the risk of poliovirus infections in specific areas (Andre *et al.*, 2014, Patel *et al.*, 2019, GPEI, 2021, Cooper *et al.*, 2022). Hence, effective control of the poliovirus and the maintenance of successes against the debilitating poliomyelitis disease requires robust surveillance and effective preventive measures such as vaccination, the leading cost-effective disease prevention tool globally (Andre *et al.*, 2014, Cochi *et al.*, 2014, Zaidi *et al.*, 2016, Patel *et al.*, 2019).

Previous studies have demonstrated that commonly used and licensed vaccines are standardised by their ability to induce protective blood antibodies that can kill bacteria, inactivate viruses and neutralise toxins (Robbins *et al.*, 1995, Clem, 2011, Hawken and Troy, 2012). Although several types of antibodies can be elicited due to vaccination, it has been indicated that one isotype, IgG, is common and effective for several vaccines and as such is one of the most important antibodies in vaccinology (Robbins *et al.*, 1995, Clem, 2011, Hawken and Troy, 2012). Indeed, for polio, both OPV and IPV induce potent IgG antibodies whereas the OPV alone induces IgA antibodies, a sharp contrast to IPV which does not primarily induce IgA antibodies (Xiao and Daniell, 2017, Zhao *et al.*, 2020). Immunoglobulin A antibodies are mainly notable on mucosal surfaces in their dimeric form within secretions, hence offering mainly localised protection for specific vaccines (Clem, 2011), although monomeric forms of IgA can be detected in the plasma. Mucosal IgA may not necessarily translate to systemic immunological protection, whereas the induction of monomeric IgA is limited in comparison to the quantity and systemic activity of IgG antibodies (Macpherson *et al.*, 2008, Clem, 2011), especially when considering different types of vaccines, many of which induce robust IgG antibodies. As such, since our study was set as a proof-of-concept evaluation aimed at informing the impact of schistosomiasis on general vaccination and to widen the potential scope of our findings relative to the expected immunological similarities among different vaccines, we assessed the impact of schistosomiasis on polio vaccine induced IgG antibodies. Additionally, the GPEI recommends the future use of an IPV mediated strategy for poliomyelitis control globally. Since IPV primarily elicit IgG antibodies for protection, we sought to evaluate IgG based systemic protection against polioviruses as a

feasible measure of this recommended intervention for under resourced areas where schistosomiasis is endemic.

Having ruled out the potentially confounding effects of geohelminths, malaria and viral co-infections by exclusion (Figure 3.2), our observations showed a significant negative association between *S. mansoni* infection and the parasite host's ability to maintain vaccine-induced serological IgG antibody memory immunity against the poliovirus at Y1 and BG. At Y1 it is notable that polio vaccine specific IgG titres were reduced at two different timepoints that were spaced 3 years apart in randomly selected children. These results strongly suggest a negative impact of the parasite on polio vaccine immunity. At one school, however, Y2, schistosomiasis had no influence on vaccine immunity.

Our findings highlight the complexity of factors associated with the influence of schistosome parasites on unrelated immunity. At this stage, the factors influencing contrasting findings at Y1 and BG versus Y2 are unclear. However, we postulate that host specific morbidity associated intrinsic factors such as genetic predispositions could be at play (Abel *et al.*, 1991, Mohamed-Ali *et al.*, 1999, Mawa *et al.*, 2021). These require further detailed evaluations on ideal platforms to unravel this complexity, for example, metagenomics and microbiome analyses. Another potential contributor to the discrepancy could be the influence of reinfection cycles which have been shown to increase protective and pathology limiting IgE levels (Hagan *et al.*, 1991, Rihet *et al.*, 1991, Zhang *et al.*, 1997, Karanja *et al.*, 2002). This factor could potentially reduce the extent of *S. mansoni* on the serological vaccine immunity in Y2. In our study however, we could not investigate the influence of IgE due to sample limitations.

Our findings indicating the depletory impact of schistosomiasis on vaccine induced humoral immunity are similar to our previous findings in measles, where *S. mansoni* negatively impacted measles virus vaccine induced IgG antibody responses in schoolchildren from the schistosomiasis endemic Bokito region of Cameroon (Nono *et al.*, 2018, Nono *et al.*, 2022). Concurring with our findings in both this study and our measles study, *S. mansoni* infected children were found with weakened memory immunity responses to the measles virus vaccine in Uganda (Tweyongyere *et al.*,

2019), and for vaccine immunity against the hepatitis B virus in Uganda (Riner *et al.*, 2016) and Egypt (Ghaffar *et al.*, 1990).

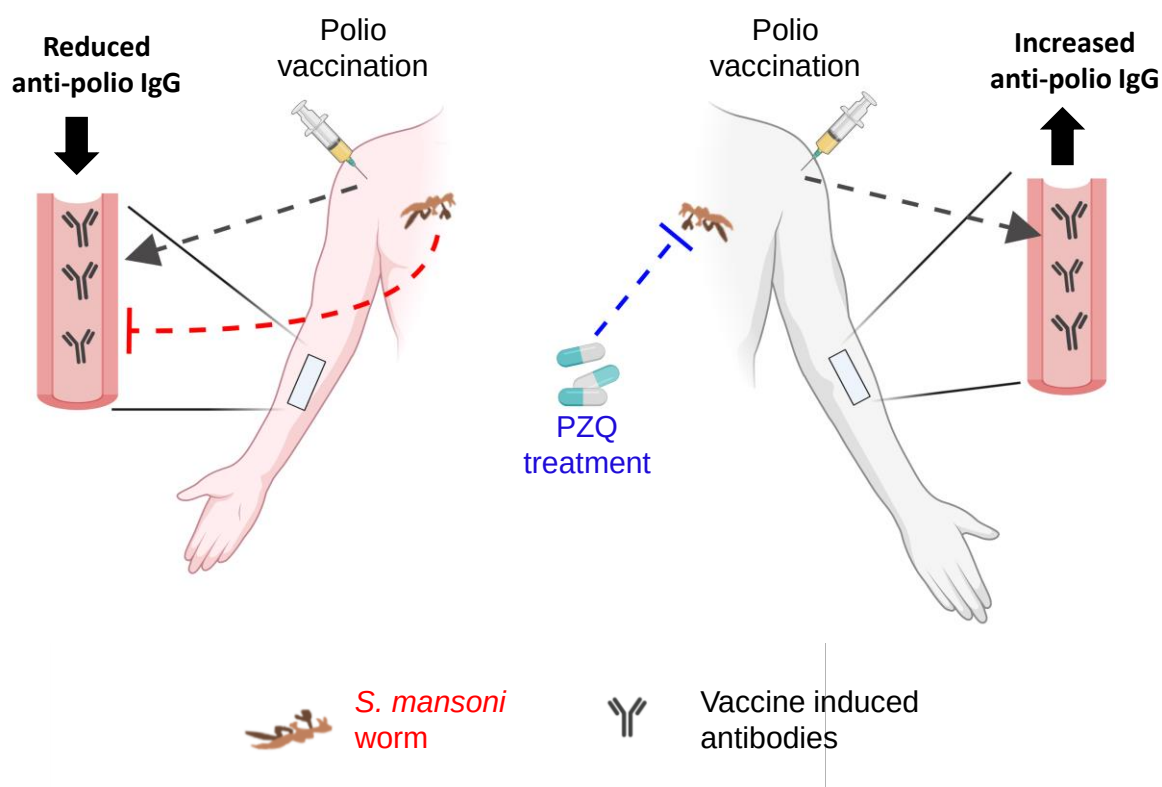


Figure 3. 8: Impact of schistosomiasis and the influence of PZQ treatment of schistosomiasis infected in children vaccinated against poliomyelitis.

Figure made using Biorender.com.

Together, the commonality of these observations on immunity for several and different viral vaccines suggests, logically, a generalised schistosomiasis driven weakening of serological immunity for viral vaccines in children from schistosomiasis endemic areas (Figure 3.8). Additionally, our observations that older children infected with schistosomiasis had significantly reduced anti-polio specific antibody titres suggest an increased likelihood of the negative consequence of *S. mansoni* infection with increasing age. As such, we recommend the strengthening of serologic polio immunity surveillance and the consideration of evidence-led polio catchup mass vaccinations for older children in areas where schistosomiasis is endemic. This strategy could strengthen polio immunity among vulnerable age groups in schistosomiasis endemic areas. Intriguingly, our study also shows the robustness of the current polio vaccination strategy in Cameroon as indicated by the identification

of sufficient polio IgG antibodies in children older than 14 years, a strong indication of the longevity of polio immunity in resource constrained settings.

We also noted, with interest, that *S. mansoni* infection prevalence was higher (>54%) in children from Y1 in the year 2016, while a considerably lower *S. mansoni* prevalence of less than 24.2% was observed for the same enrolment site in the year 2018. Considering that our findings show that 75% (6/8) of our study's follow-up participants were found to have cleared the infection within the same timeframe between the years 2016 to 2018, our findings suggest that the reduction in *S. mansoni* prevalence in Y1 could be attributed to the efficiency of the MDA programs in the area. However, there remains a permanent risk of reinfection with the *Schistosoma* parasite (Nono *et al.*, 2018) which needs consolidated efforts not only through treatment but community educative interactions involving schoolchildren.

Successfully treating schistosomiasis with PZQ, as indicated by lack of *S. mansoni* eggs in the stool at follow up of previously infected children post PZQ treatment, was associated with restored anti-viral vaccine-specific antibody serological memory. Similar findings have been reported in Uganda where PZQ treatment of schistosomiasis was associated with improved responses to the measles catch-up vaccination in vulnerable preschool children (Tweyongyere *et al.*, 2019). Together, these findings strongly point towards the beneficial impact of PZQ treatment where vaccine immunity maintenance is concerned in schistosomiasis endemic settings (Figure 3.8).

However, whether this impact of PZQ is intrinsically a PZQ driven mechanism or rather due to its anti-parasitic worm killing effect in addition to reduced host pathology remains to be determined. Such findings also seem logical owing to the previously demonstrated immunosuppressive nature of schistosomiasis (Duvaux-Miret *et al.*, 1992) whose effects could have been counteracted upon by the multi-round PZQ orchestrated parasite worm killing. At present, curative PZQ treatment's potential in restoring vaccine induced serologic immunity maintenance has been observed (Figure 3.8). However, these findings require further evaluations at a mechanistic level that may be too invasive for human studies. Hence, further investigations in our study employed the use of mouse models of polio vaccinated mice infected with schistosomiasis and treated with PZQ, to decisively assess the parasites' impact on

unrelated vaccine immunity as well as the drug's impact on parasite infected hosts' unrelated vaccine immunity.

Chapter 4: Schistosomiasis drives impairment of DTPa-hepB-IPV-Hib vaccine immune responses, and the influence of praziquantel treatment in mice.

4.1 Results

4.1.1 Establishing a hexavalent (DTPa-hepB-IPV-Hib) vaccination mouse model: DTPa-hepB-IPV-Hib vaccine induces elevated levels of long-lasting anti-polio IgG titres in BALB/c mice

To address the causality in the above-observed and suggested negative effect of schistosomiasis on anti-polio vaccine responses in children, laboratory mice were experimentally used for advanced mechanistic studies. We assessed the impact of schistosomiasis on IPV induced anti-polio serological immunity in mice. This vaccine had been shown to induce serological anti-poliovirus IgG antibodies that were maintained at elevated levels for up to 400 days in mice (Xiao and Daniell, 2017). We, therefore, sought to establish an IPV mouse vaccination model in our setting by vaccinating mice with an IPV containing DTPa-hepB-IPV-Hib vaccine.

Age (6-8 weeks) and gender matched BALB/c mice were injected twice, a prime dose and a booster dose, with a commercial DTPa-hepB-IPV-Hib vaccine (Hexaxim, Sanofi Pasteur, Lyon, France) or placebo vaccinated (naïve group) with phosphate buffered saline at day 0 and day 30 (Figure 4.1A). Starting at 2 weeks post booster dose administration, tail vein blood was collected once every 2 weeks for 16 weeks with subsequent immediate isolation of serum. The serum was evaluated for anti-poliovirus specific IgG antibodies using ELISA.

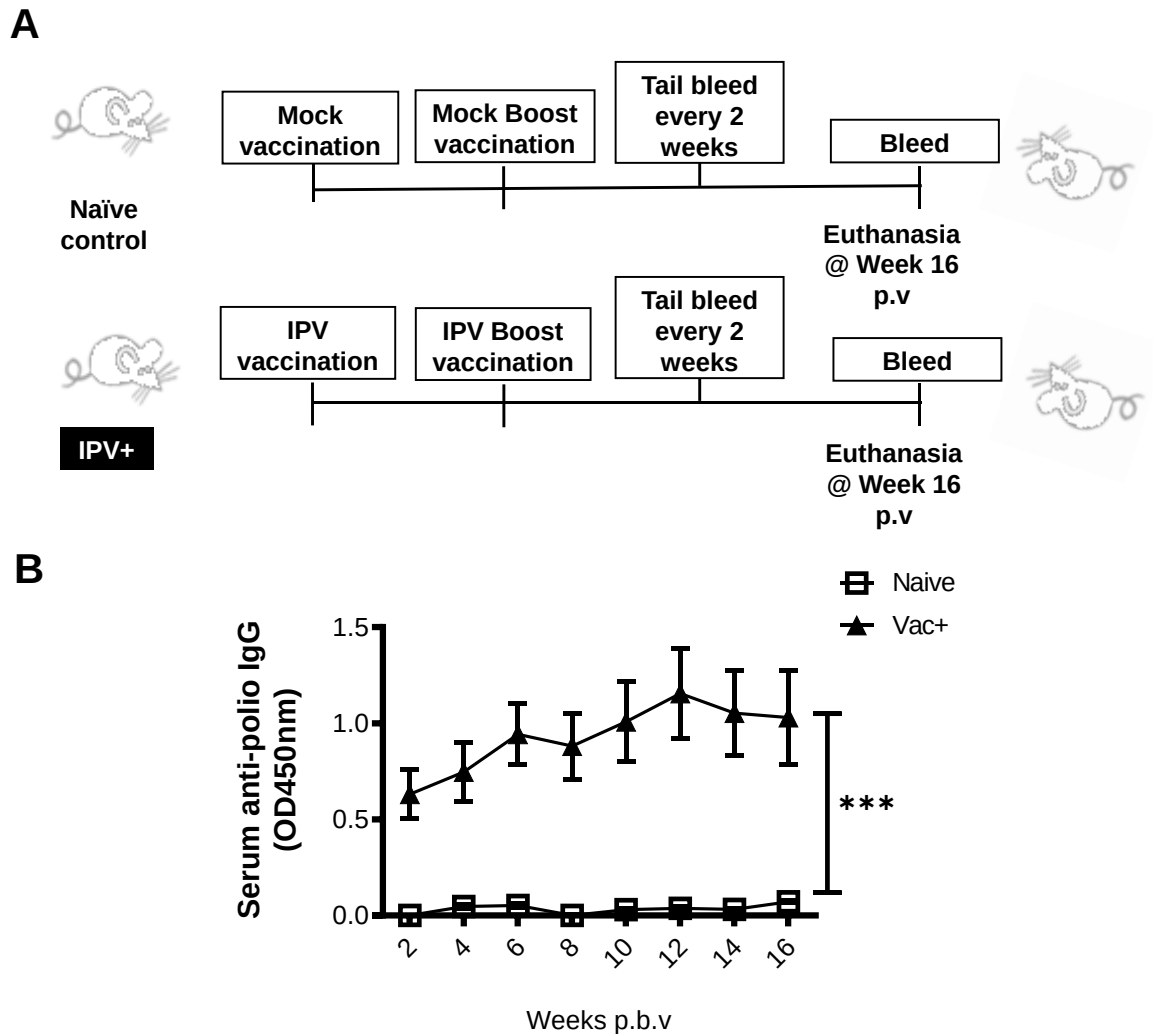


Figure 4. 1: Experimental design and serum anti-polio serological immunity evaluation to establish a mouse model of anti-poliovirus vaccination in our setting.

A. Experimental design: BALB/c mice (6 to 8 weeks old and acclimatized for 1 week before procedure) were mock vaccinated (naïve) or injected subcutaneously with the DTPa-hepB-IPV-Hib vaccine as at 2 sites i.e., 150ul on the neck and 150ul on the abdomen at day 0 and day 30. Blood was collected from tail vein (approximately 50ul) every two weeks until day 142 (week 16 after second vaccine dose). The animals were sacrificed at week 16, terminally bled and their spleens were collected.

B. Serum was evaluated by ELISA for anti-polio virus IgG titre kinetics. Data are expressed as means $\pm$ S.E.M and representative of 2 independent experiments; n = 6 -10 mice per group; Data was analysed by two-way ANOVA followed by FDR corrected multiple comparisons; *** p< 0.0001; Vac, DTPa-hepB-IPV-Hib; p.b.v, post-booster-vaccination.

Our findings revealed significantly higher anti-poliovirus IgG antibody titres in vaccinated mice when compared to naïve mice throughout the 16-weeks post booster vaccine dose ($P < 0.0001$) (Figure 4.1B). A conclusion was therefore reached

that vaccination of BALB/c mice with the IPV containing DTPa-hepB-IPV-Hib vaccine induces long-term poliovirus specific serological memory immunity for mice in our study setting.

4.1.2 DTPa-hepB-IPV-Hib vaccine induces the expansion of memory B cell in BALB/c mice

The same mice groups described above (Figure 4.1A) were also harnessed to establish further the impact of vaccination on mice systemic immunity. At 16 weeks post booster dose administration, the mice were euthanised and their spleens were excised. Splenocytes were isolated and splenic memory B cell responses were measured by flow cytometry (Figure 4.2A) to evaluate if vaccination with the DTPa-hepB-IPV-Hib vaccine could induce B cell-specific systemic responses.

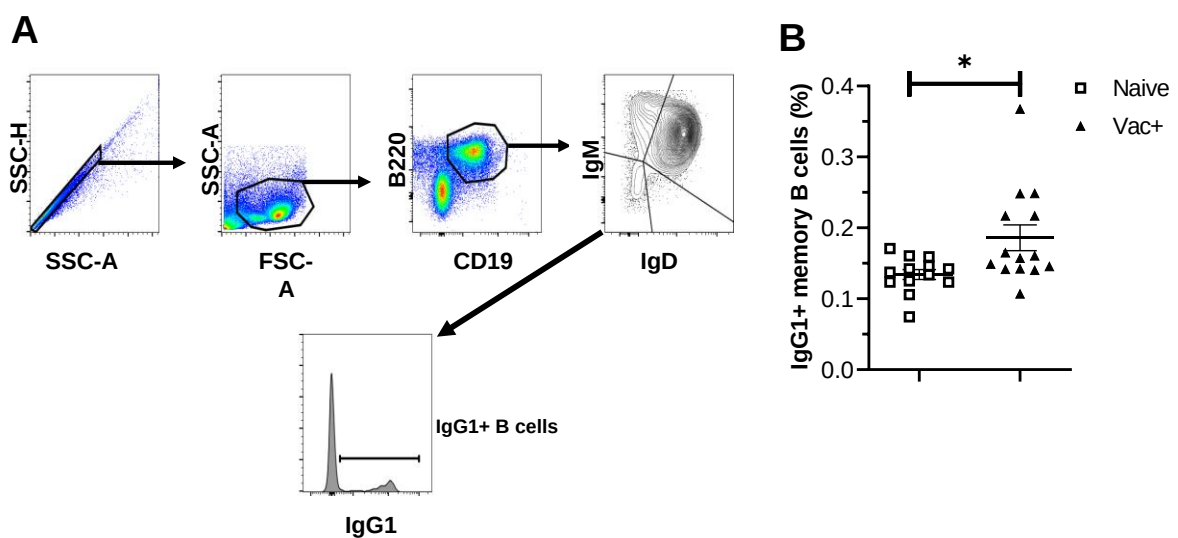


Figure 4. 2: Assessment of IgG1 memory B cells in DTPa-hepB-IPV-Hib vaccinated mice.

A. Gating strategy for class switched IgG1 memory B cell gating. **B.** IgG1 memory B cells were significantly elevated in vaccinated mice in comparison to naïve mice. Data are expressed as mean $\pm$ S.E.M and representative of 2 independent experiments; $n = 15 - 20$ mice per group; Data analysed by Mann-Whitney test or Two-way ANOVA followed by FDR corrected multiple comparisons; * $p < 0.05$; Vac, DTPa-hepB-IPV-Hib; p.v, post-vaccination.

Immunoglobulin G and its sub-isotype IgG1 represent the most common antibody isotype in blood and its presence generally correlates with long term immunity

stemming from germinal centre reactions, as observed in vaccine induced immunity (Thomson, 2016, Jacofsky *et al.*, 2020). To measure long-term memory B cell immunity induction by the vaccine in our mouse model, we measured splenic IgG1 memory B cells of vaccinated versus unvaccinated mice. We found significantly increased levels of IgG1+ memory B cells in vaccinated mice in comparison to non-vaccinated naïve mice ($P < 0.05$) (Figure 4.2B). Based on our findings, we concluded that vaccination with the DTPa-hepB-IPV-Hib vaccine induces long term systemic IgG1 memory B cells in the spleens of BALB/c mice in our study setting.

4.1.3 Impact of chronic *S. mansoni*, and its subsequent treatment with anthelmintic chemotherapy, praziquantel, on IPV induced anti-polio IgG in the serum of BALB/c mice

Upon the successful establishment of the hexavalent (DTPa-hepB-IPV-Hib) mouse vaccination model in our study setting, we went on to investigate the impact of chronic *S. mansoni* infection, and the impact of treating the infection using PZQ, on polio specific serological memory immunity induced by the DTPa-hepB-IPV-Hib vaccine in BALB/c mice.

In the field, since children are often at increased risk of schistosomiasis during their school-going age, and the vaccine against the poliovirus is administered within one year from birth, our experimental strategy in mice was designed to assess how schistosomiasis infection post-polio vaccine administration would influence the sustenance of vaccine responses. In our study design, we created four groups of age and gender matched BALB/c mice (Figure 4.3). The first group (Figure 4.3A) represented the placebo vaccinated naïve mice which were handled similarly to vaccinated mice. The remaining three groups of mice were all vaccinated (priming dose and booster dose 30 days apart) subcutaneously with the hexavalent vaccine (DTPa-hepB-IPV-Hib) per our established mouse vaccination model (Figure 4.2A, Figure 4.3B-C). The first of the remaining three vaccinated mice groups was designated the vaccinated only control (Vac+) (Figure 4.3B). At 5 days post booster vaccine administration, chronic schistosomiasis was established in the remaining two groups of DTPa-hepB-IPV-Hib vaccinated mice via percutaneous exposure to a low dose of *S. mansoni* cercariae (35 cercariae) (Nono *et al.*, 2020). One of these two

groups was designated the Vac+Sm+ group (Figure 4.3C). The remaining vaccinated and *S. mansoni* infected group was then treated with PZQ at week 10 post *S. mansoni* infection (Vac+Sm+PZQ+) (Figure 4.3D).

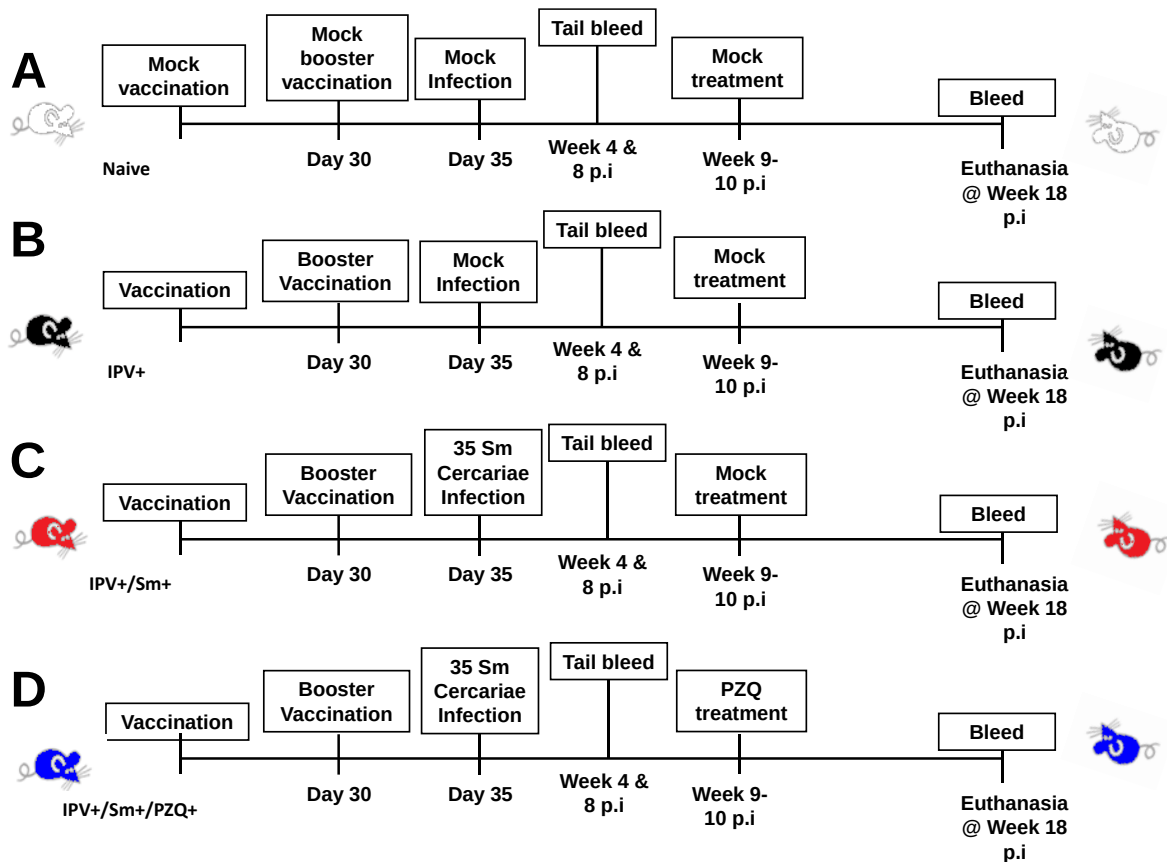


Figure 4. 3: Experimental design to evaluate the impact of schistosomiasis on anti-poliovirus vaccination in mice.

Age and gender matched BALB/c mice (6-8 weeks old) were placed into 4 experimental groups. **A.** was injected with phosphate buffered saline (PBS). **B., C.,** and **D.** were injected subcutaneously with a commercialized hexavalent vaccine, DTPa-hepB-IPV-Hib, at 2 sites i.e., 150ul on the neck and 100ul on the abdomen, at day 0 and day 30. Five days after the second vaccine dose (day 35), Naïve (A) and IPV-Vaccinated control mice (B) were mock infected with PBS while groups (c) (vaccinated then Sm infected i.e., Vac+Sm+) and (D) (Vaccinated then Sm infected then PZQ treated i.e., Vac+Sm+PZQ+) were percutaneously infected with a low dose of *S. mansoni* (35 cercariae) to establish a chronic disease course. At the beginning of week 10 after infection (end of week 9 p.i), (**A., B.** and **C.**) were mock treated with PBS while d. was treated with PZQ once daily for a week at 400mg/kg. Mice were bled at week 4, 8 and 18 post *S. mansoni* infection to obtain serum. Animals were monitored for schistosomiasis disease progression (daily weight over time) from day zero of infection. At week 18 post infection, all mice were euthanised and cardiac blood and organs (spleen, bone marrow, liver, and gut) were collected for downstream analyses. Vac, DTPa-hepB-IPV-Hib; Sm, *S. mansoni*; PZQ, praziquantel; p.i, post infection.

4.1.4 Analysis of weight changes and spleen and liver pathology in vaccinated mice, schistosomiasis infected and PZQ treated mice

We evaluated mouse weight changes over time as an indicator of their general wellbeing to assess the impact of different treatments depending on designated groups as described earlier (Figure 4.4A-D).

Our assessments revealed that while naïve mice and Vac+ mice continuously gained and maintained weight over time, mice infected with schistosomiasis (Vac+Sm+ and Vac+Sm+PZQ+) (Figure 4.3C-D) started losing weight starting at end of week 8 into week 9 post infection (Figure 4.4A). However, when one of the schistosomiasis infected mice groups was treated with 400mg/kg of PZQ for 7x days starting between week 9 and 10 post infection (Sm+Vac+PZQ+), the mice quickly recovered from weight loss and were able to maintain this recovered weight until the week 18 post infection (Figure 4.1A). On the other hand, non-treated Vac+Sm+ mice continued to lose their weight steadily until the experimental endpoint at week 18 post *S. mansoni* infection (Figure 4.1A).

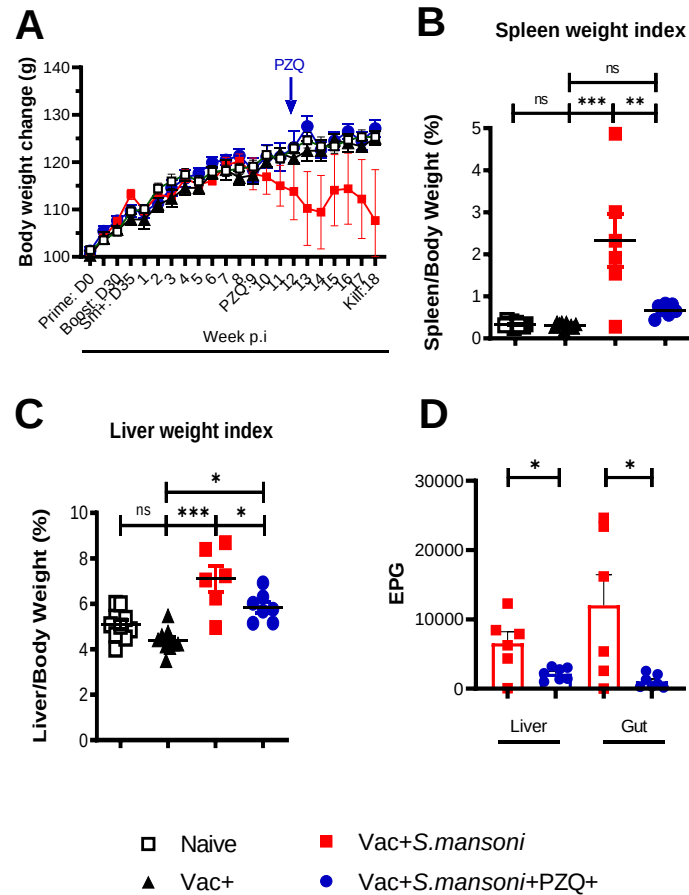


Figure 4. 4: Body weights, organ weights and histopathological profile of DTPa-hepB-IPV-Hib vaccinated mice with chronic *S. mansoni* infection and after treatment with praziquantel.

Age and gender matched BALB/c mice (6-8 weeks old) were placed into 4 experimental groups. The first group (Naïve) was injected with PBS. The remaining 3 groups were injected subcutaneously with a commercialized hexavalent vaccine, DTPa-hepB-IPV-Hib, at 2 sites i.e., 150ul on the neck and 100ul on the abdomen, at day 0 and day 30. Five days after the second vaccine dose (day 35), Naïve and IPV-vaccinated control mice (Vac+) were mock infected with PBS while the remaining 2 groups were percutaneously infected with 35 *S. mansoni* cercariae to establish a chronic disease course (Vac+Sm+) . One group from the two Vac+Sm+ groups was then treated with 400mg/kg PZQ once daily for one week at 400mg/kg (Vac+Sm+PZQ+) starting at the beginning of week 10 after infection (end of week 9 p.i). Naïve, Vac+ and Vac+Sm+ mice were mock treated with PBS in the same period. All mice were bled at week 4, 8 and 18 post *S. mansoni* infection to obtain serum. **A.** Body weight changes over time following infection with *S. mansoni* and treatment with PZQ. Note a drastic weight drop in the Vac+ Sm+ group that is rescued in the Vac+Sm+PZQ+ group. **B.** Spleen/body weight index. **C.** Liver/body weight index. **D.** Number of eggs per gram of liver or gut. Data are expressed as mean $\pm$ S.E.M and representative of 2 independent experiments; n = 6 - 10 mice per group; Data was analysed by one way ANOVA followed by the Bonferroni's multiple comparisons test (B-C) and two tailed Student's t test for unequal variances (D); ns, $p > 0.05$; * $p < 0.05$, ** $p < 0.001$, *** $p < 0.0001$; Vac, DTPa-hepB-IPV-Hib; Sm, *S. mansoni*; PZQ, praziquantel.

Concurrent measurements of liver and spleen weights relative to the mice body weights were conducted to further assess mouse wellbeing. Results showed similarities in the weights of these organs in naïve and Vac+ groups (Figure 4.4B-C). In contrast, these organs were found inflamed in *S. mansoni* infected Vac+Sm+ mice, as indicated by significantly increased spleen and liver weights in comparison to the non-infected Vac+ mice ($p < 0.0001$, respectively). Further assessments showed that PZQ treated mice, the Vac+Sm+PZQ+ group had reduced spleen and liver inflammation than Vac+Sm+ mice ($p < 0.001$ and $p < 0.05$, respectively; Figure 4.4B-C). Interestingly, there were no significant differences in the spleen weights of Vac+Sm+PZQ+ mice when compared to Vac+ mice ($p > 0.05$) (Figure 4.4B). However, the liver weights of Vac+Sm+PZQ+ mice remained significantly higher, although minimally, than that of Vac+ mice ($p < 0.05$) (Figure 4.4C).

We isolated and quantified *S. mansoni* eggs from the liver and gut of infected mice groups Vac+Sm+ and Vac+Sm+PZQ+ mice (Figure 4.4D). The successful isolation of parasite eggs in both mice groups confirmed the chronicity of schistosomiasis in both mice groups. In addition, our results showed that for both organs, Vac+Sm+ mice had significantly higher frequencies of *S. mansoni* eggs than their PZQ treated counterparts ($p < 0.05$, respectively) (Figure 4.4D). These findings revealed the efficiency of PZQ treatment in our study, and the significant impact PZQ had in reducing the disease burden associated with *S. mansoni* eggs in both the liver and the gut.

Together, our findings showed that vaccination did not significantly impact the general wellbeing of mice as shown by comparable body weight kinetics with naïve mice. Additionally, vaccination-induced no spleen or liver associated pathologies as indicated by similarities with naïve mice, representative of base level weights. In contrast, our results showed that while schistosomiasis resulted in deteriorating mouse general wellbeing along with increased liver and spleen pathology, treatment of schistosomiasis with PZQ was associated with a restoration of the mouse weight, hence indicating wellbeing restoration, by week 8 post PZQ administration. Furthermore, PZQ treatment significantly reduced the *S. mansoni* egg burden in both the liver and the gut, signifying the partial restorative impact of PZQ treatment in reducing the burden of schistosomiasis disease in mice.

4.1.5 *Schistosoma mansoni* infection in DTPa-hepB-IPV-Hib vaccinated mice induces liver granuloma formation whereas PZQ treatment reduces granuloma size

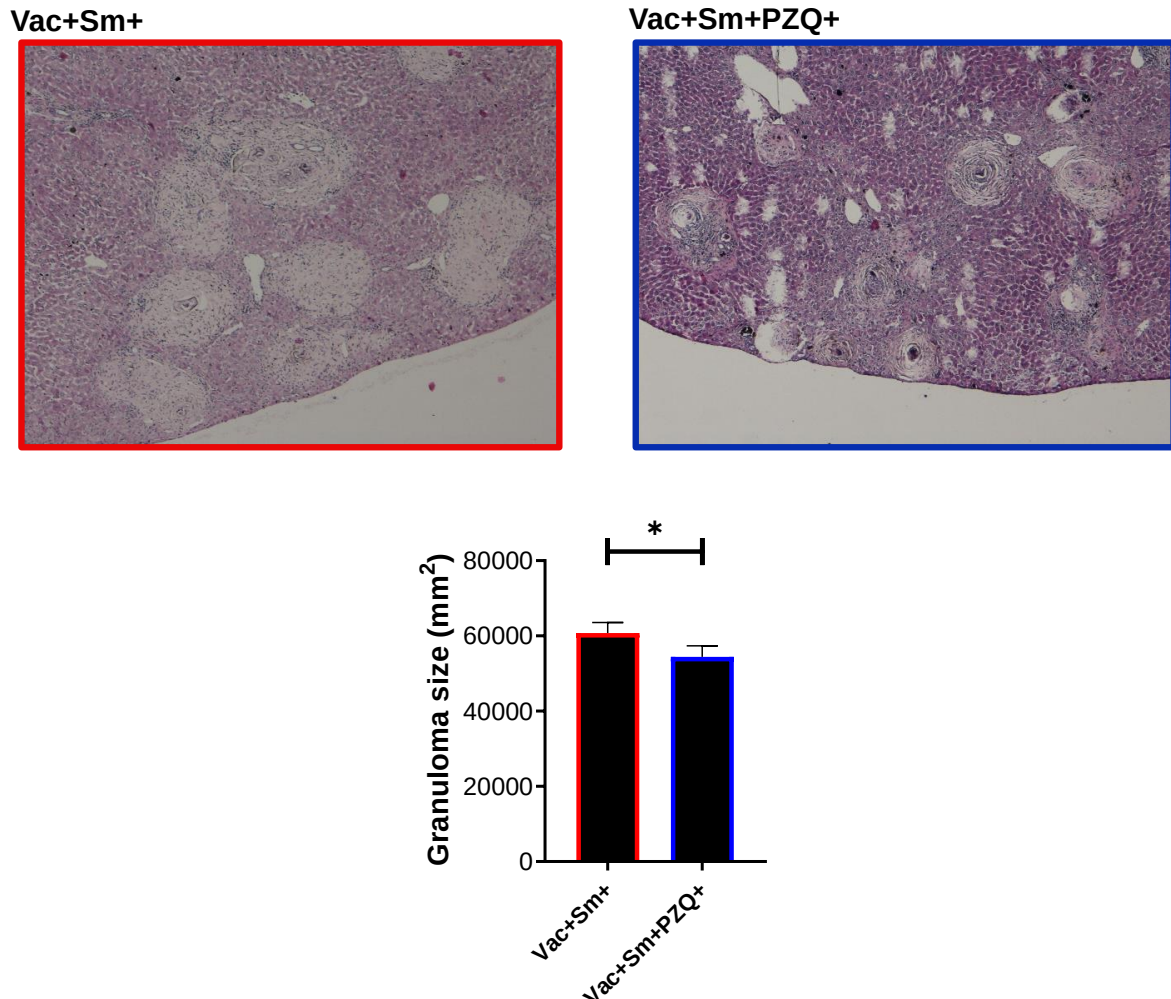


Figure 4. 5: Praziquantel treatment of DTPa-hepB-IPV-Hib vaccinated mice infected with schistosomiasis reduces liver granuloma size.

Data are expressed as mean $\pm$ S.E.M and representative of 2 independent experiments; n = 5 -10 mice per group; Data was analysed by Shapiro-Wilk test followed by one tailed Mann-Whitney test; * p< 0.05; Slides viewed at x100 magnification; Vac, DTPa-hepB-IPV-Hib; Sm, *S. mansoni*; PZQ, praziquantel.

We further evaluated the extent of schistosomiasis induced pathology by assessing granuloma formation in the livers of the two groups of schistosomiasis infected mice, Vac+Sm+ and Vac+Sm+PZQ+. Results showed that schistosomiasis caused liver granuloma formations in both group of mice. We also noted that PZQ treatment significantly diminished granuloma size in comparison to the non-treated group

($p < 0.05$; Figure 4.5). Together, these results indicated, further, the effectiveness of our PZQ treatment regimen in mice infected with schistosomiasis.

4.1.6 Schistosomiasis reduces serum anti-polio IgG titres in vaccinated mice, and treatment with PZQ partially restores vaccine-induced antibodies

Next, we investigated the influence of *S. mansoni* infection and its treatment with PZQ on serological memory induced by polio vaccination in mice. Tail vein blood was collected for serum isolation from all mice groups described above at weeks 4, 8 and 18 post *S. mansoni* infection (Figure 4.3). Using this serum, anti-polio specific antibody titres were quantified by ELISA to evaluate the impact of chronic *S. mansoni* infection on IPV induced anti-polio serological immunity over time in mice.

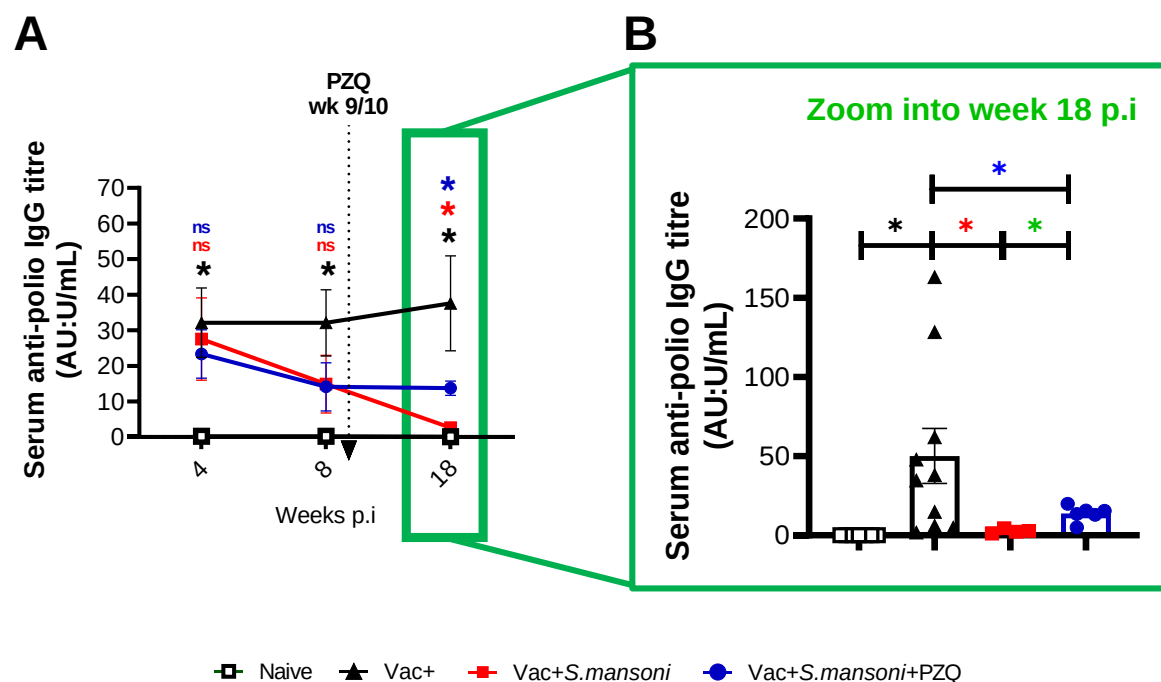


Figure 4. 6: Kinetics of humoral response to poliovirus vaccination in schistosomiasis-infected mice.

BALB/c mice (6-8 weeks old) were separated into 4 groups. The first group was mock vaccinated (naïve controls) while the other 3 groups were injected subcutaneously with 150ul and 100ul of DTPa-hepB-IPV-Hib at 2 sites, i.e., neck and abdomen respectively, at day 0 and day 30. One vaccinated group was designated as the vaccinated control group (Vac+) as the reference control group by excellence for

schistosomiasis impact on vaccine responses. The remaining 2 vaccinated groups (test groups) were infected with 35 *S. mansoni* cercariae (Vac+/S. *mansoni*+) at day 35. At week 9 post infection (p.i), one group of infected mice was treated with 400mg/kg of praziquantel once a day for 7 days (Vac+/S. *mansoni*+/PZQ+). From week 4 p.i., 50ul of blood was collected from the tail vein for serum isolation for all mice at week 4, 8, paused during treatment of one group and then continued at week 18 (experimental endpoint). Serum was probed by ELISA to establish a poliovirus vaccine-induced anti-poliovirus IgG titre kinetics for all mice groups **A.** Anti-poliovirus IgG titres over time **B.** Anti-poliovirus IgG titres at week 18 after infection i.e., the experimental endpoint. Data are expressed as mean $\pm$ S.E.M and representative of 2 independent experiments; n = 5-10 mice per group; For (**A**) the Vac+ group is used as reference for statistical comparison. Data was analysed by two-way ANOVA followed by FDR corrected multiple comparisons; ns, $p > 0.05$; * $p < 0.05$; PZQ, praziquantel.

Similar to our initial observations in which the mouse vaccination model was established for our setting (Figure 4.1B), we found that from week 4 to 18 post *S. mansoni* infection (corresponding to week 5 post booster vaccination), anti-polio IgG antibodies were significantly increased in non-infected Vac+ mice in comparison to Naïve mice ($p < 0.05$) (Figure 4.6A). Additionally, at week 4 and 8 post infection, anti-polio IgG titres of vaccinated / *S. mansoni* infected mice were significantly higher than naïve mice ($p < 0.05$) (Figure 4.6A). However, a notable reduction in anti-polio IgG titres was apparent in *S. mansoni* infected mice at week 8 post infection in comparison to Vac+ mice, although without statistical significance ($p > 0.05$) (Figure 4.6A).

At week 18 post *S. mansoni* infection, the anti-poliovirus specific IgG antibody titres in *S. mansoni* infected mice, Vac+Sm+ and Vac+Sm+PZQ+, were found significantly lower than their non-infected counterparts Vac+ ($p < 0.05$, respectively; Figure 4.6A-B). Intriguingly, we noted that at week 18, anti-polio IgG antibody titres of *S. mansoni* infected and subsequently PZQ treated mice, Vac+Sm+PZQ+, were substantially higher than that of their non-treated counterparts, Vac+Sm+, at week 18 post infection ($p < 0.01$; Figure 4.6A-B).

Altogether, these results indicated that chronic *S. mansoni* infection reduces IPV specific anti-polio IgG titres in BALB/c mice over time. Importantly, schistosomiasis treatment, PZQ, partially reduces the depth of this chronic *S. mansoni* driven reduction of anti-polio IgG titres 8 weeks post treatment administration.

4.1.7 Increasing serum SEA IgG and organ specific schistosome egg burden associate negatively with vaccine-specific anti-polio IgG antibodies in mice infected with chronic schistosomiasis.

In animal studies, the extent and burden of schistosomiasis disease can be measured in terms of *Schistosoma* spp SEA specific IgG levels in the blood and egg burden in the liver and gut respectively (Suzuki *et al.*, 2006). We wondered if the extent and intensity of the infection were associated with prior observed schistosomiasis associated reductions in IPV induced serum anti-polio IgG titres in mice. To investigate, the same mice groups employed throughout this study were assessed (Figure 4.3A). Serum SEA specific IgG was measured by ELISA and *S. mansoni* eggs were counted in the liver and gut of all Vac+Sm+ and Vac+Sm+PZQ+ mice by microscopy. The SEA specific IgG and *S. mansoni* egg burdens were then separately plotted against each corresponding mouse's serum anti-polio IgG to assess the relationship between infection extent or intensity and IPV induced anti-polio IgG titres.

Pearson's correlation showed that at weeks 4 and 8 post infection of vaccinated mice, there was no significant correlation between serum SEA specific IgG and anti-polio IgG titres ($r=0.17$ and $r=-0.08$, $p>0.05$, respectively; Figure 4.7A-B). However, at week 18 post infection, a significant negative correlation showing that increases in SEA specific IgG titres were associated with reduced anti-polio IgG titres was observed for all infected mice ($r=-0.82$, $p<0.05$; Figure 4.7C).

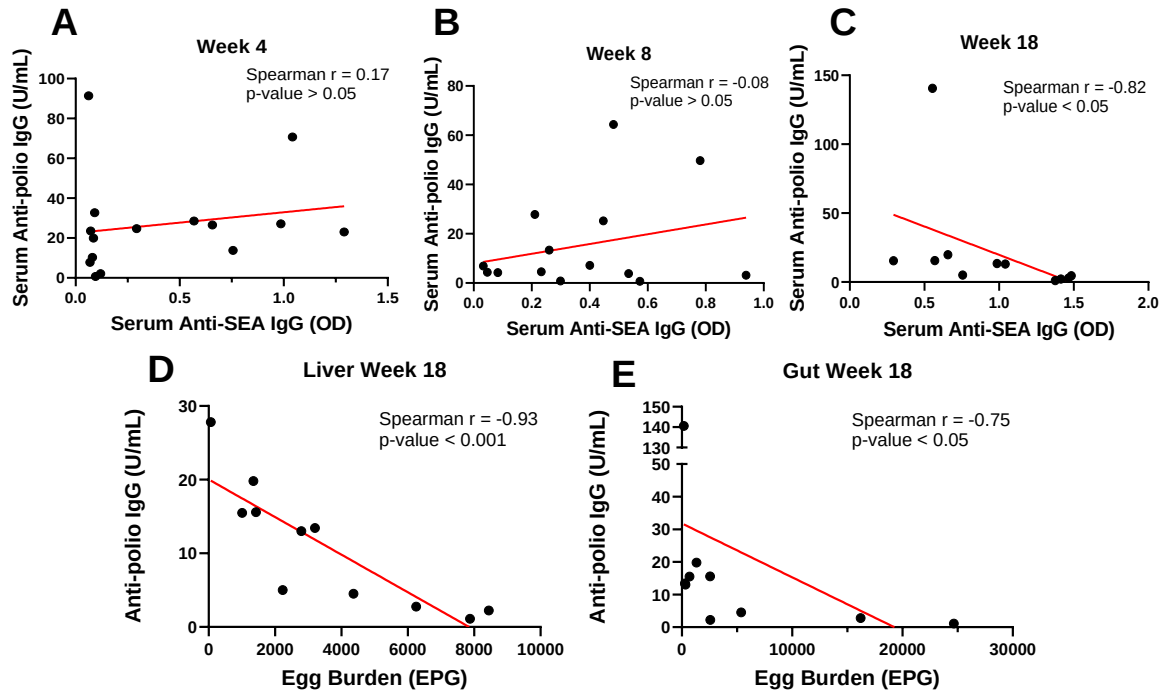


Figure 4. 7. Elevated serum SEA IgG titers and liver and gut *S. mansoni* egg burden are associated with reduced serum anti-polio vaccine-induced IgG titers in mice.

A. Anti-polio IgG titres vs anti-SEA IgG titres at week 4 p.i. **B.** Anti-polio IgG titres vs anti-SEA IgG titres at week 8 p.i. **C.** Anti-polio IgG titres vs anti-SEA IgG titres at week 18 p.i. **D.** Anti-polio IgG titres vs liver EPG at week 18 p.i. **E.** Anti-polio IgG titres vs gut EPG at week 18 p.i. Data are expressed as mean $\pm$ S.E.M and representative of 2 independent experiments with 6-10 mice; The Vac+ group is used throughout as reference for statistical comparison. Data was analysed by two-way ANOVA followed by FDR corrected multiple comparisons against the Vac+Sm+ group; PZQ, praziquantel; p.i, post *S. mansoni* infection.

Looking at the impact of egg burden as a measure of infection intensity, Pearson correlation measurements revealed a significant negative correlation between increased *S. mansoni* egg burden and anti-polio IgG titres. Increases in egg burden correlated with reduced serum anti-polio IgG titres for both the liver ($r=-0.93$, $p<0.0001$; Figure 4.7D) and gut ($r=-0.75$, $p<0.05$; Figure 4.7E) of schistosomiasis infected mice.

Together, these results suggest that increased schistosomiasis intensity is associated with reduced IPV induced anti-polio IgG titres in DTPa-hepB-IPV-Hib vaccinated mice.

4.1.8 Impact of chronic *S. mansoni*, and its subsequent treatment with anthelmintic chemotherapy, praziquantel, on bone marrow and spleen B cell populations: Schistosomiasis reduces plasma B cells in the bone marrow but not in the spleen of DTPa-hepB-IPV-Hib vaccine immunised mice

The broader scientific community agrees that vaccine-specific blood antibodies generally serve as strong – even though not exclusive – correlates of protection which may indicate the extent of protection against disease for several vaccines (Plotkin, 2010). Our findings indicating that chronic schistosomiasis drives reductions of anti-poliovirus vaccine-induced antibodies in mice, and the restorative influence of PZQ treatment in mice, prompted us to investigate further the mechanistic bases behind these observations. Hence, we investigated the impact of schistosomiasis, and its treatment with PZQ, on terminally differentiated antibody-producing plasma cell populations in the bone marrow and the spleen.

An experimental strategy similar to our earlier described experimental design was employed for mice groupings based on single or combination treatments including, naïve, vaccination, schistosomiasis and PZQ treatment (Figure 4.3). Briefly, four groups of mice were created, namely naïve, Vac+, Vac+Sm+ and Vac+Sm+PZQ+. All mice were euthanised at week 18 post *S. mansoni* infection and the left femur bone marrow and spleens were collected for single cell isolation and flow cytometric staining for CD138+ plasma B cells and analysis (Figure 4.8A).

Results showed that CD138+ plasma B cell populations (Figure 4.8A) were significantly increased in the bone marrow of Vac+ mice in comparison to naïve mice ($p < 0.01$; Figure 4.8B). Although these results were not necessarily vaccine antigen-specific, they nonetheless confirmed that DTPa-hepB-IPV-Hib vaccination induced a general increase in plasma cell populations in the bone marrow of mice. Assessments on the impact of chronic schistosomiasis on bone marrow plasma cells revealed that the parasite was associated with reduced plasma cell levels in Vac+Sm+ mice when compared to Vac+ mice ($p < 0.05$; Figure 4.8B). In schistosomiasis infected mice that were treated with PZQ (Vac+Sm+PZQ+) at week 9/10 post infection, we found significantly increased bone marrow plasma cells in comparison to their non-treated counterparts (Vac+Sm+) ($p < 0.05$; Figure 4.8B).

Further analyses revealed that there were no significant differences between plasma cell populations in the bone marrow of Vac+ mice and Vac+Sm+PZQ+ mice ($p>0.05$).

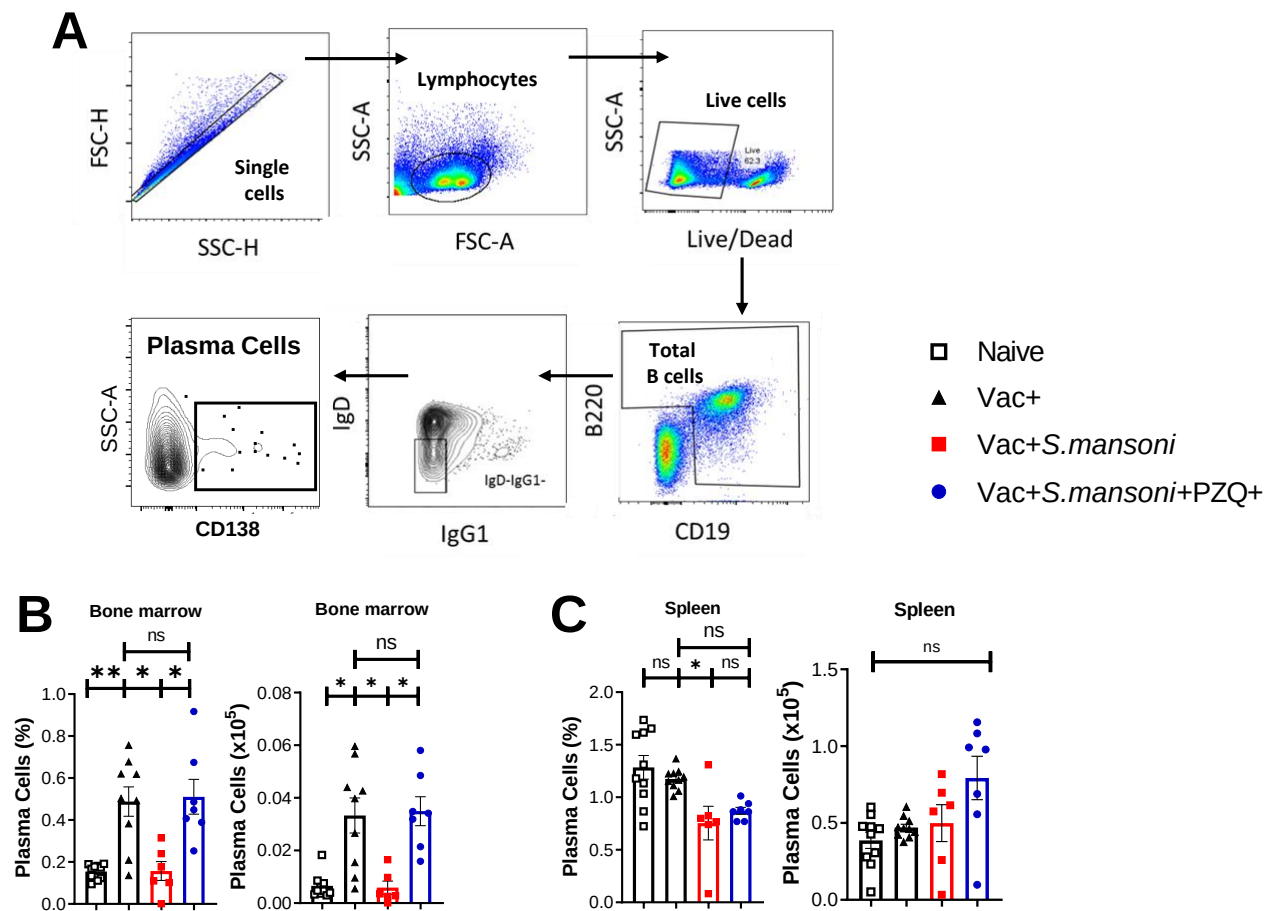


Figure 4. 8: Lymphocyte and plasma B cell frequencies and counts at 18 weeks post schistosomiasis in the bone marrows and spleens of anti-Poliovirus vaccinated mice.

A. Representative gating strategy for plasma B cells. **B.** Bone marrow plasma B cells. **C.** Spleen plasma B cells. Data are expressed as mean $\pm$ S.E.M and representative of 2 independent experiments; $n=6-10$ mice per group; Data was analysed by the Shapiro-Wilk test followed by either one way ANOVA with Bonferroni's multiple comparisons test or by Kruskal Wallis followed by the Dunn's multiple comparisons test; ns, $p>0.05$; * $p<0.05$, ** $p<0.001$; Vac, DTPa-hepB-IPV-Hib; Sm, *S. mansoni*; PZQ, praziquantel.

In the spleen, there were no significant differences in the frequencies of plasma cells in vaccinated mice and naïve mice. However, we found significantly reduced plasma cell frequencies in the bone marrow of schistosomiasis infected mice (Vac+Sm+) in comparison to Vac+ mice ($p<0.05$; Figure 4.8B), although cell number plots showed

increased cell numbers in the spleens of infected mice with or without treatment. There were no significant differences in the frequencies of plasma cells of infected mice regardless of PZQ treatment although PZQ treated mice had higher cells numbers ($p>0.05$; Figure 4.8C).

Together, these findings suggest that chronic schistosomiasis is associated with reduced plasma cell populations in both the bone marrow and the spleen. However, the impact is more pronounced in the bone marrow, which incidentally is critical to the maintenance of long-term vaccine-specific serological antibody maintenance (Slifka *et al.*, 1995, Ellyard *et al.*, 2004, Chernova *et al.*, 2014, Lightman *et al.*, 2019). Although schistosomiasis reduces plasma cell frequencies in the spleen when compared to vaccinated only cells, this pattern is not seen in plasma cell numbers, a contrast to observations in the bone marrow. Further, our results demonstrate that PZQ treatment of schistosomiasis infected mice restores the plasma cell frequencies in the bone marrow whereas no difference was observed for splenic plasma cell frequencies.

4.1.9 Schistosomiasis reduces the bone marrow plasma B cell survival marker, CD93, in DTPa-hepB-IPV-Hib vaccinated mice

Next, having observed significant reductions of plasma cells in the bone marrow, we questioned if the survival of plasma cells in the bone marrow of vaccinated mice infected with chronic schistosomiasis was reduced. In our investigation, we measured the expression of a well characterised marker for plasma cell survival in the bone marrow, CD93 expression by bone marrow plasma cells (Chevrier *et al.*, 2009, Nutt *et al.*, 2015).

Our results showed that in Vac+Sm+ mice, schistosomiasis was associated with significantly reduced CD93 expression by plasma cells in comparison to the non-infected Vac+ mice ($p<0.05$; Figure 4.9). We also noted that there was a higher expression of CD93 in PZQ treated Vac+Sm+PZQ+ mice, in comparison to non-treated Vac+Sm+ mice, although no significant difference was observed statistically. In addition, there was no difference between CD93 expression in Vac+ mice and Vac+Sm+PZQ+ mice.

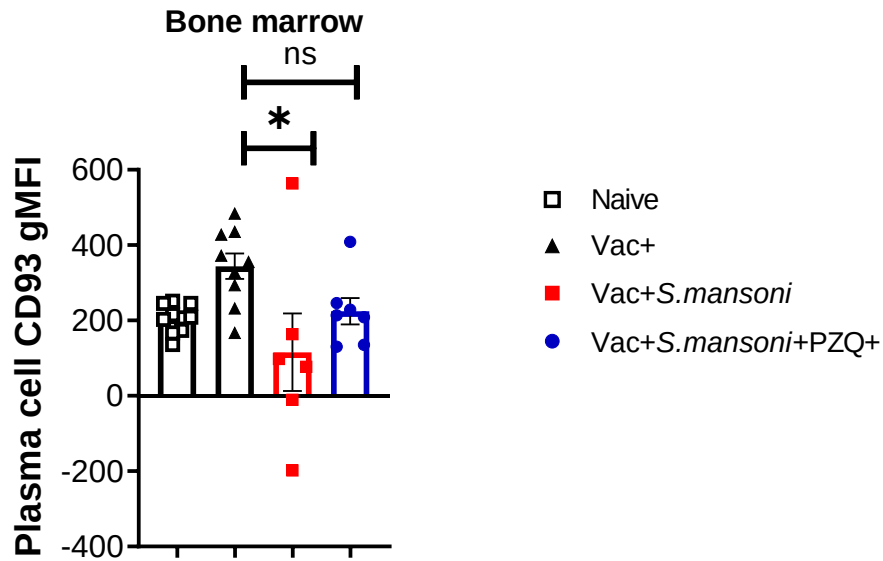


Figure 4. 9: Bone marrow plasma cell expression of survival marker, CD93, at week 18 post infection and subsequent PZQ treatment, of DTPa-hepB-IPV-Hib vaccinated mice.

Data are expressed as mean $\pm$ S.E.M and representative of 2 independent experiments; n=6-10 mice per group; Data was analysed by the Shapiro-Wilk test followed by either one-way ANOVA with Bonferroni's multiple comparisons test; ns, $p > 0.05$; * $p < 0.05$. Vac, DTPa-hepB-IPV-Hib; Sm, *S. mansoni*; PZQ, praziquantel; CD93, cluster of differentiation 93.

Taken together, our results suggest that chronic schistosomiasis is associated with reduced expression of CD93, a plasma cell survival marker, in bone marrow plasma cells of *S. mansoni* infected mice. These results support further our earlier reported findings where schistosomiasis is associated with the reduction of plasma cells in the bone marrow of infected mice.

4.1.10 Increasing serum anti-SEA IgG titres are associated with reduced bone marrow plasma cell frequencies while increasing anti-polio IgG titres are associated with increasing bone marrow and spleen plasma cells.

Because of our findings in plasma cell populations, we wondered if there was a relationship between plasma cells titres and IgG antibodies specific for SEA and poliovirus in our experimental model. To answer, we investigated correlation patterns between serum anti-SEA IgG titres and plasma cells in the bone marrow and spleens of the corresponding vaccinated mice at week 18 post *S. mansoni* infection despite PZQ treatment. In the bone marrow, we found a significantly negative correlation between serum anti-SEA IgG titres and plasma cells ($p < 0.05$) (Figure 4.10A). In the spleen, we found no significant correlation between the two parameters ($p > 0.05$; Figure 4.10B).

We also evaluated correlation patterns between serum anti-polio IgG titres and plasma cells in the bone marrow and spleen of the corresponding vaccinated mice at week 18 post *S. mansoni* infection. In both the bone marrow and spleen, we found significantly positive correlation between serum anti-polio IgG titres and plasma cells (bone marrow, $r = 0.53$, $p < 0.05$; spleen, $r = 0.66$, $p < 0.05$; Figure 4.10C-D).

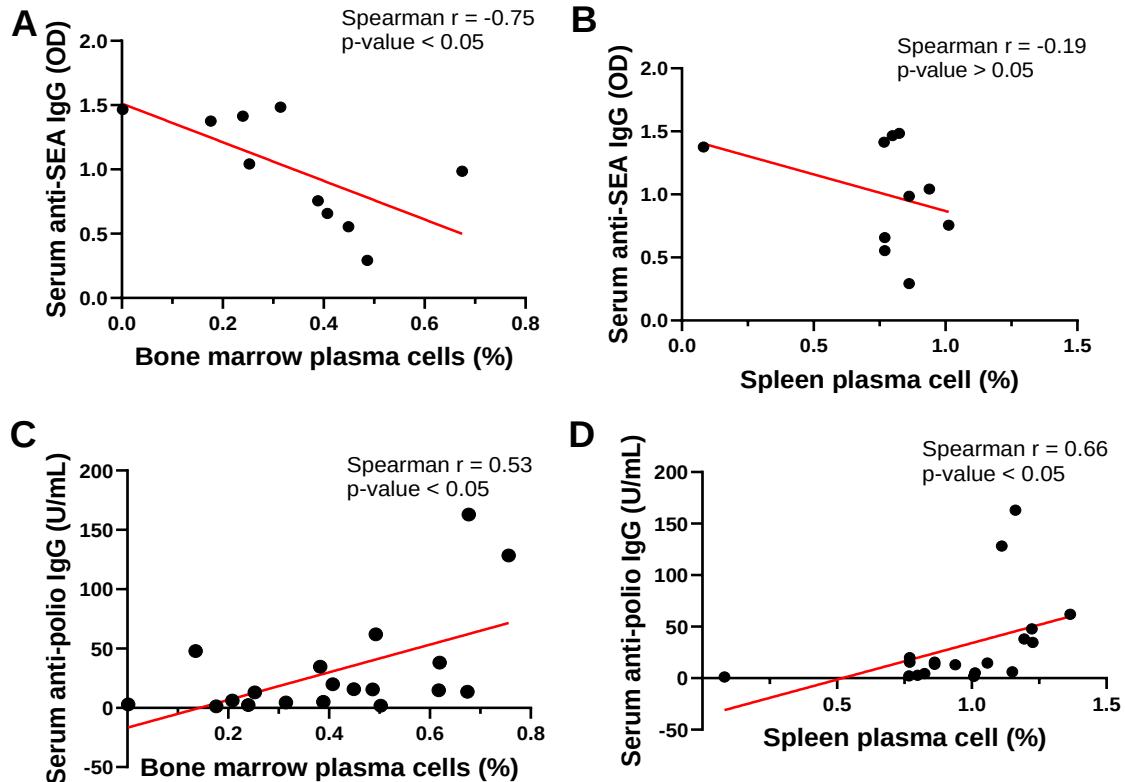


Figure 4. 10: Higher serum anti-SEA IgG titres are associated with lower bone marrow plasma cell frequencies and higher anti-polio IgG titres are associated with higher bone marrow plasma cells.

A. Anti-SEA IgG titres vs bone marrow plasma cell frequencies of schistosomiasis infected Vac+Sm+ and Vac+Sm+PZQ+ mice at week 18 p.i. **B.** Anti-SEA IgG titres vs spleen plasma cell frequencies of Vac+Sm+ and Vac+Sm+PZQ+ mice combined. **C.** Anti-polio IgG titres vs bone marrow plasma cell frequencies of schistosomiasis infected Vac+, Vac+Sm+ and Vac+Sm+PZQ+ mice at week 18 p.i. **D.** Anti-polio IgG titres vs spleen plasma cell frequencies of Vac+, Vac+Sm+, and Vac+Sm+PZQ+ mice combined. Data are representative of 2 independent experiments; $n=10\text{-}20$ mice; Data was analysed by Spearman's correlation. Vac, DTPa-hepB-IPV-Hib; Sm, *S. mansoni*; PZQ, praziquantel; p.i, post *S. mansoni* infection.

Together, these results show that increasing serum anti-SEA IgG titres, a proxy for the advancement of the infection burden, were significantly associated with reducing bone marrow plasma cells but not spleen plasma cells. In contrast, our results demonstrate that serum anti-polio IgG titres were significantly associated with increasing bone marrow and spleen plasma cells. This further confirmed a negative association of infection intensity, i.e., *S. mansoni* egg burden, with plasma cell levels, in contrast to a positive association between polio vaccine induced IgG titres and plasma cell levels.

4.1.11 Schistosomiasis reduces total B cells in the bone marrow and in the spleen of DTPa-hepB-IPV-Hib vaccinated mice

Based on our observations where plasma cell frequencies were reduced significantly in the bone marrow and spleens of mice infected with chronic schistosomiasis, we wondered if B cell populations were generally affected by *S. mansoni* infection in the bone marrow and the spleen.

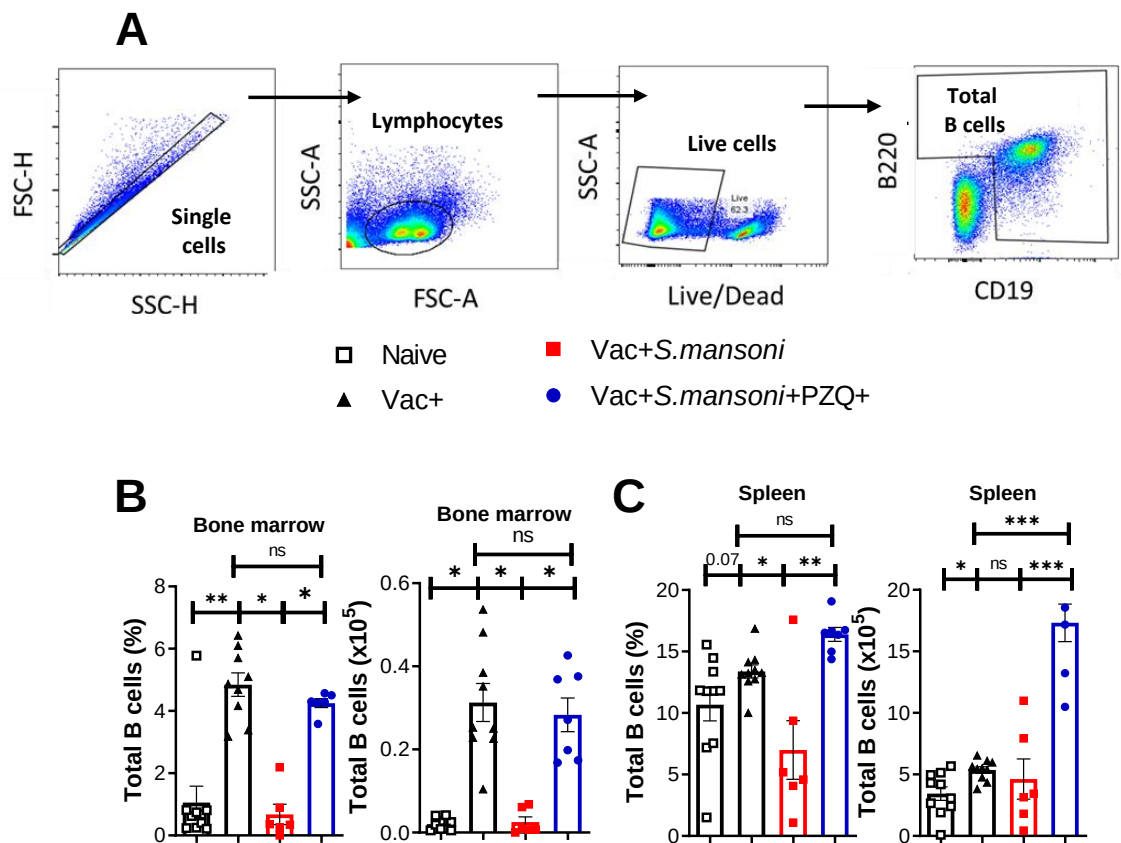


Figure 4. 11: Bone marrow and spleen total B cell frequencies and counts at 18 weeks post *S. mansoni* infection, and PZQ treatment in DTPa-hepB-IPV-Hib vaccinated mice.

A. Representative gating strategy for total B cells. **B.** Bone marrow total B cell frequencies and total numbers. **C.** Spleen B cell frequencies and total numbers. **C.** Representative gating strategy for plasma B cells. Data are expressed as mean $\pm$ S.E.M and representative of 2 independent experiments; n=6-10 mice per group; Data was analysed by the Shapiro-Wilk test followed by either one way ANOVA with Bonferroni's multiple comparisons test or by Kruskal Wallis followed by the Dunn's multiple comparisons test; ns, p> 0.05; * p< 0.05, ** p< 0.001, *** p< 0.0001; Vac, DTPa-hepB-IPV-Hib; Sm, *S. mansoni*; PZQ, praziquantel.

Flow cytometric measurements (Figure 4.11A) revealed that B220+CD19+ total B cells in both the bone marrow and spleens of schistosomiasis infected mice, Vac+Sm+, were significantly reduced in comparison to Vac+ mice ($p < 0.05$, respectively; Figure 4.11B-C). This negative impact of schistosomiasis on total B cell frequencies in Vac+Sm+ mice was significantly reversed in the bone marrow and spleens of PZQ treated mice, Vac+Sm+PZQ+, ($p < 0.05$; $p < 0.01$, respectively; Figure 4.11B-C). The total B cell frequencies in Vac+Sm+PZQ+ treated mice were comparable to Vac+ mice in both the bone marrow and spleens ($p > 0.05$, respectively). However, the absolute total B cell counts in the spleens showed no differences between Vac+ and Vac+Sm+ mice ($p > 0.05$) while Vac+Sm+PZQ+ had significantly higher cell populations than Vac+ mice ($p < 0.0001$; Figure 4.11C). Intriguingly, we observed significantly elevated total B cell counts in Vac+Sm+PZQ+ mice in comparison to Vac+ mice ($p < 0.0001$; Figure 4.11C).

Hence, we concluded that schistosomiasis reduces total B cell population frequencies in the bone marrow and spleens of DTPa-hepB-IPV-Hib vaccinated mice, and treatment with PZQ restores the total B cell populations to levels comparable with vaccinated and non-infected mice.

4.1.12 Influence of chronic schistosomiasis on follicular B cells, IgG1 memory B cells and marginal zone B cells in the spleens of DTPa-hepB-IPV-Hib vaccinated mice

Follicular B cells, memory B cells and MZ B cells are important B cells to consider in vaccine immunity, although currently, more attention is given to follicular B and IgG memory B cells. We analysed the frequencies of these B cell populations in the spleens of our mice groups (Figure 4.3) using flow cytometry (Figure 4.12A).

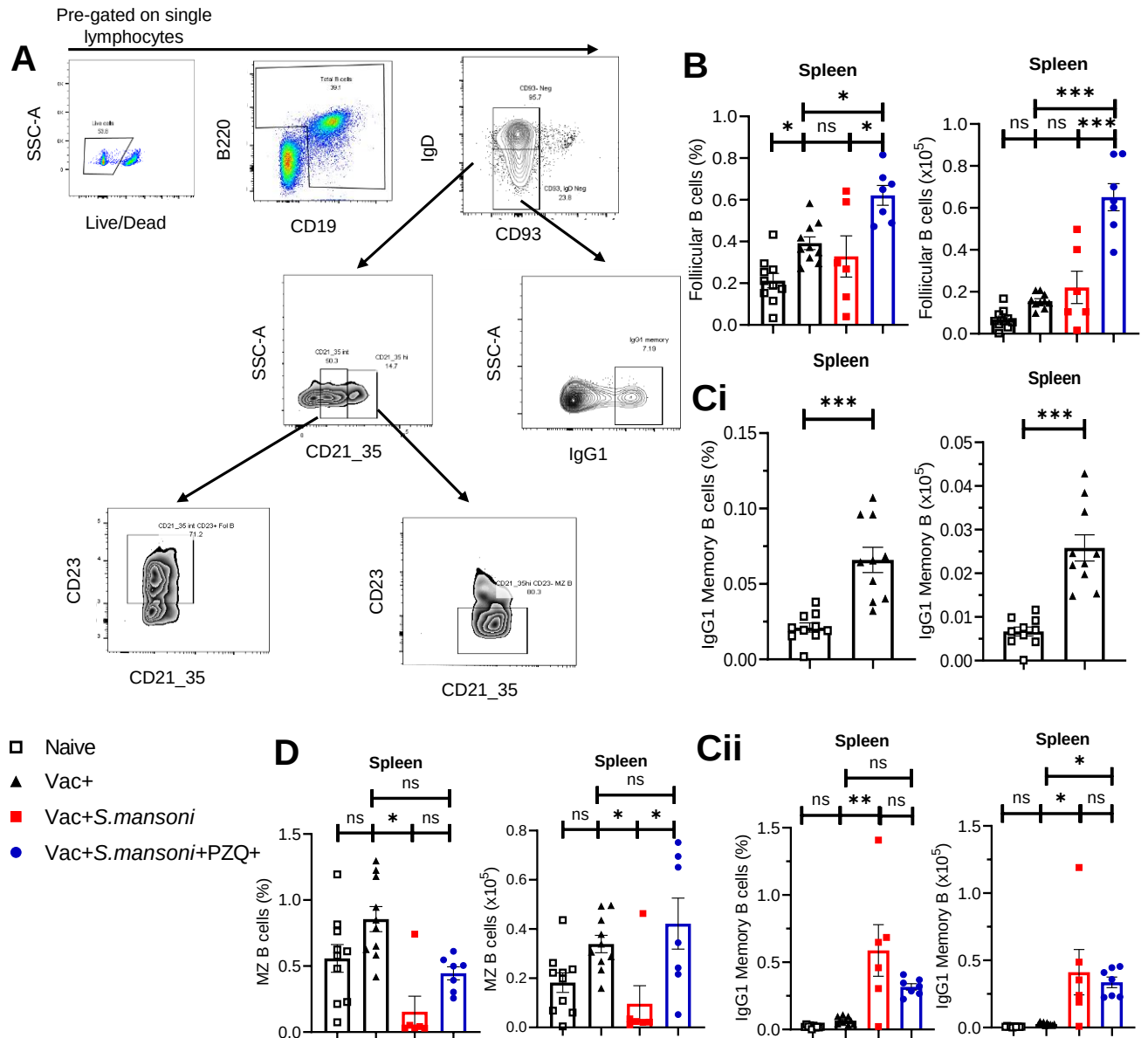


Figure 4.12: Frequencies and counts of Follicular B cells, IgG1 memory B cells and Marginal zone B cells.

A. Representative flow cytometry analysis of splenic follicular B cells, IgG1 memory B cells and marginal zone B cells, **B.** B200+CD19+CD93- CD21Int CD23+ Follicular B cells. **C & Cii.** CD19+B220+IgD- CD93- IgG1+ memory B cells. **D.** B220+CD19+CD93- CD21high CD23- Marginal zone B cells. Data are expressed as mean $\pm$ S.E.M and representative of 2 independent experiments; n=6-10 mice per group; Data was analysed by the Shapiro-Wilk test followed by either one way ANOVA with Bonferroni's multiple comparisons test or by Kruskal Wallis followed by the Dunn's multiple comparisons test; ns, $p > 0.05$; * $p < 0.05$, ** $p < 0.001$, *** $p < 0.0001$; Vac, DTPa-hepB-IPV-Hib; Sm, *S. mansoni*; PZQ, praziquantel; SSC, Side scatter; FSC, forward scatter; CD19, cluster of differentiation 19; IgG1, Immunoglobulin G1; IgD, Immunoglobulin D; B220, B cell isoform of 220 kDa; CD93, cluster of differentiation 93; CD21/35, cluster of differentiation 21 and 35; CD23, cluster of differentiation 23.

Results showed that although follicular B cell frequencies were significantly elevated in vaccinated (Vac+) mice in comparison to naïve mice ($p < 0.05$; Figure 4.12B), there were no significant differences between Vac+ mice and schistosomiasis infected Vac+Sm+ mice ($p > 0.05$). Also, no significant differences in follicular B cell counts were observed for these mice groups (Figure 4.12B). Interestingly, Vac+Sm+PZQ+ had significantly higher frequencies ($p < 0.05$) and absolute counts ($p < 0.0001$) of follicular B cells than both the Vac+ and Vac+Sm+ mice (Figure 4.12B).

Confirming the impact of vaccination on IgG1 memory B cells, a direct comparison of IgG1 memory B cell frequencies between naïve mice and Vac+ mice showed their significant increase in the spleens of Vac+ mice ($p < 0.0001$; Figure 4.12Ci). Further analyses showed that IgG1 memory B cell frequencies in schistosomiasis infected Vac+Sm+ mice were significantly elevated in comparison to Vac+ mice ($p < 0.001$; Figure 4.12Ci), while they were similar to their PZQ treated counterparts, Vac+Sm+PZQ+ ($p > 0.05$; Figure 4.12Cii). On the other hand, Vac+Sm+PZQ+ mice had IgG1 memory B cell frequencies that were visibly higher than Vac+ mice but lower than Vac+Sm+ mice, although without significance in both instances ($p > 0.05$, respectively; Figure 4.12Cii). Further, IgG1 memory B cell counts in Vac+Sm+PZQ+ mice were similar to Vac+Sm+ mice ($p > 0.05$) but significantly higher than that of Vac+ mice ($p < 0.05$; Figure 4.12Cii).

Marginal zone B cell frequencies and absolute counts were found significantly elevated in Vac+ mice than in naïve mice ($p < 0.05$, respectively; Figure 4.12D). Infection with schistosomiasis was associated with significantly reduced MZ B cell frequencies and counts in Vac+Sm+ mice when compared to Vac+ mice ($p < 0.05$, respectively; Figure 4.12D) while treatment of the infection with PZQ resulted in restored MZ B cell frequencies but not cell counts in Vac+Sm+PZQ+ versus Vac+Sm+ mice ($p < 0.05$, $p > 0.05$, respectively; Figure 4.12D). Furthermore, Vac+Sm+PZQ+ MZ B cell frequencies and counts were at similar levels to those of Vac+ mice ($p > 0.05$; Figure 4.12D).

Taken together, our findings suggest that while vaccination with DTPa-hepB-IPV-Hib increases follicular B cells and IgG1 memory B cells in mice, schistosomiasis did not negatively impact these populations, although our results are generic due to our study limitation of lack of B cell specificity to the vaccine. The results also

demonstrate that treatment of schistosomiasis with PZQ increases follicular B cells to levels higher than vaccinated only mice. These results suggest that PZQ may influence follicular B cell immunity independently. On the other hand, we concluded that schistosomiasis is associated with reduced MZ B cell populations in vaccinated mice while PZQ treatment is restorative. Interestingly and similar to our findings, MZ B cell induction has also been shown after vaccination with the pneumococcal polysaccharide vaccine (Tuomanen *et al.*, 2001, Weller *et al.*, 2004). These cells are generally associated with T cell independent immunity induction (Weller *et al.*, 2004, Bugya *et al.*, 2021). Proving their complexity, MZ B cells activity in T cell dependent reaction has also been reported (Cerutti *et al.*, 2013). Despite this intriguing puzzle, owing to the necessity for T cell dependency in mounting long term memory immunity in our vaccine model, the role of MZ B cell induction in our vaccination scheme remains unclear.

4.1.13 Chronic schistosomiasis increases expressions of the regulatory marker Foxp3 on CD4+ T cells and reduces follicular T cell populations in the spleens of DTPa-hepB-IPV-Hib vaccinated mice

Vaccine immunity is launched through adaptive immune responses that require the help of T cells to assist B cell reactions into and in GC processes which ultimately produce class-switched memory B cells and long-lived antibody producing plasma cells (Nutt *et al.*, 2015). As these reactions can occur in the spleen, we wondered if schistosomiasis also influenced Th cells and follicular helper T cells as had been observed for some B cell populations in this organ. To answer this question, we assessed, by flow cytometry, the influence of schistosomiasis, and its treatment with PZQ, on CD4+ Th cells and follicular helper T cells of vaccinated mice according to our experimental strategy (Figure 4.3 and Figure 4.13, respectively).

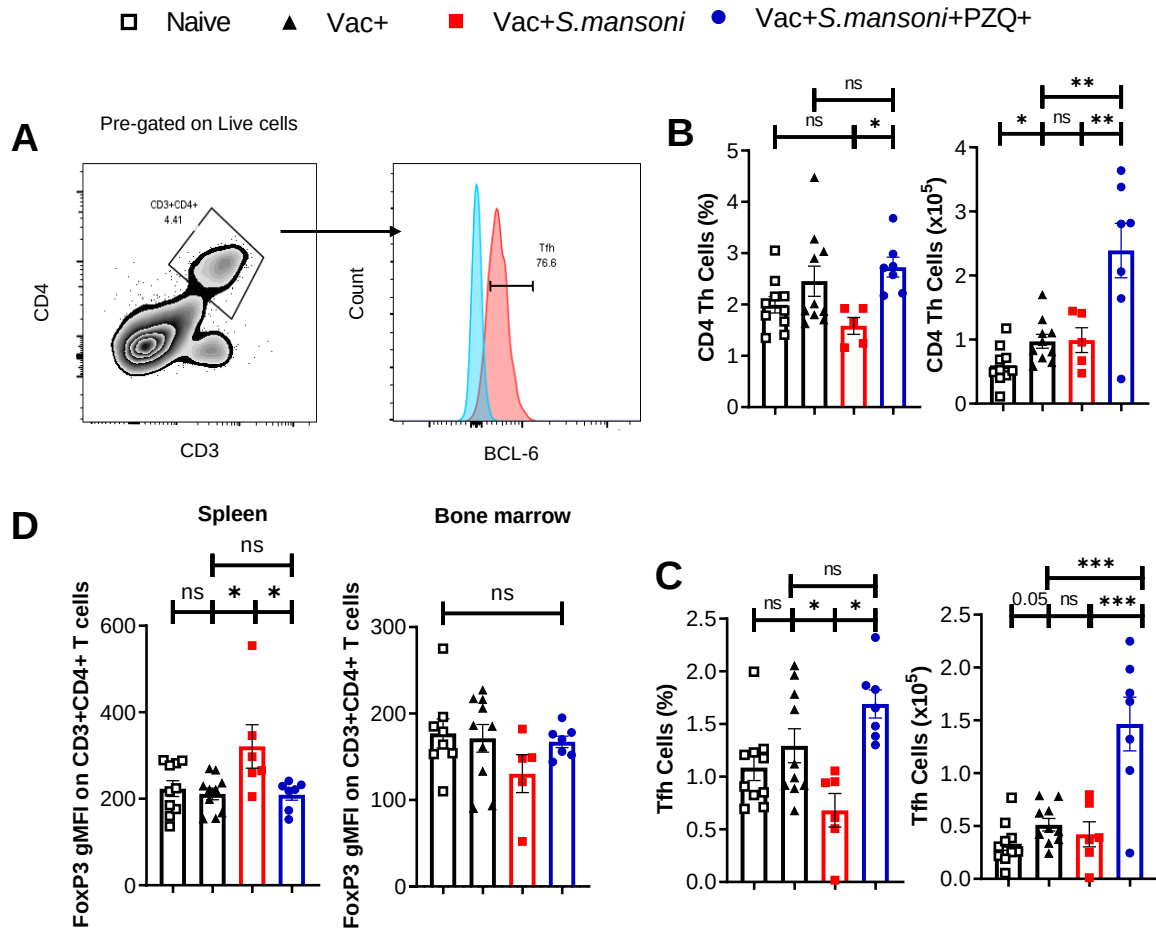


Figure 4. 13: Frequencies of CD4+ T cells, CD4 follicular T cells and Foxp3+ T cells.

A. Representative flow cytometry gating strategy for identifying CD3+CD4+ T helper cells and BCL-6+CD3+CD4+ follicular T helper cells. **B.** CD4+ T helper cell frequencies in the spleen. **C.** BCL-6+CD3+CD4+ follicular T cells frequencies in the spleen. **D.** Foxp3 gMFI expression on CD3+CD4+ T cells in the spleen and bone marrow. Data are expressed as mean $\pm$ S.E.M and representative of 2 independent experiments; $n = 6-10$ mice per group; Data was analysed by the Shapiro-Wilk test followed by either one way ANOVA with Bonferroni's multiple comparisons test or by Kruskal Wallis followed by the Dunn's multiple comparisons test; ns, $p > 0.05$; * $p < 0.05$, ** $p < 0.001$, *** $p < 0.0001$; Vac, DTPa-hepB-IPV-Hib; Sm, *S. mansoni*; PZQ, praziquantel; SSC, Side scatter; CD3, cluster of differentiation 3; CD4, cluster of differentiation 4; CD8, cluster of differentiation 8; BCL-6, IgG1, Immunoglobulin G1; IgD, Immunoglobulin D; B220, B cell isoform of 220 kDa; CD138, cluster of differentiation 138, BCL-6, B-cell lymphoma 6; Foxp3, forkhead box P3.

Our findings showed no significant differences in the frequencies nor counts of CD4+ Th cells in Vac+ mice in comparison to naïve mice ($p > 0.05$, respectively; Figure 4.13B). Schistosomiasis infected Vac+Sm+ mice had reduced CD4+ Th cells, although not statistically significant in comparison to Vac+ mice ($p > 0.05$). However,

in comparison to PZQ treated Vac+Sm+PZQ+ mice, Vac+Sm+ had significantly reduced CD4+ Th cell frequencies ($p<0.05$) and absolute counts ($p<0.001$), respectively (Figure 4.13B). Further, CD4+ Th cells in Vac+Sm+PZQ+ were higher than Vac+ frequencies but without statistical significance ($p>0.05$), yet significantly higher when Th cell absolute counts were evaluated ($p<0.001$) (Figure 4.13B).

BCL-6+CD4+ follicular T helper cells in naïve mice were comparable to Vac+ mice in both frequencies and absolute counts, although Vac+ mice had higher frequencies that were statistically non-significant ($p=0.05$; Figure 4.13C). Schistosome infection of vaccinated mice, Vac+Sm+, resulted in a statistically non-significant reduction of follicular helper T cell frequencies while no changes in absolute cell counts when compared to Vac+ mice ($p>0.05$, respectively; Figure 4.13C).

Moreover, previous studies including ours, have indicated that *Schistosoma* spp infections induce the expression of Foxp3, a marker for regulation, on CD4+ T cells (Abdel Aziz *et al.*, 2018). The expression of Foxp3 on CD4+ T cells is associated with the suppression of immunity (Kim, 2009). Hence, we questioned if these immunomodulatory regulatory CD4+ T cells (Tregs) were also expressed in vaccinated mice that were infected with schistosomiasis. To investigate, we measured the levels of Foxp3 gMFI expression on CD4+ T cells by flow cytometry (Figure 4.13A). Results showed no differences in Foxp3+ Treg cells were observed when naïve mice and Vac+ mice were compared. However, Foxp3 expression in CD4+ T cells was significantly elevated in schistosomiasis infected, Vac+Sm+, mice when compared to Vac+ mice ($p<0.05$; Figure 4.13D). Upon treatment of infection with PZQ, the expression of Foxp3 in Treg cells was significantly reduced in Vac+Sm+PZQ+ mice in comparison to their non-treated counterparts ($p<0.05$) to become similar to Vac+ mice ($p>0.05$; Figure 4.13D).

As such, we concluded that vaccination of mice with the DTPa-hepB-IPV-Hib vaccine is not associated with significantly increased CD4+ Th cells and BCL6+ follicular helper T cells at 18 weeks after vaccine administration. Additionally, chronic schistosomiasis did not significantly influence Th and follicular helper T cell population levels in vaccinated mice. However, PZQ treatment of vaccinated mice that were subsequently infected with schistosomiasis resulted in significantly raised Th and follicular helper T cell frequencies in comparison to non-treated mice.

Interestingly, PZQ treatment also resulted in Th and follicular helper T cell absolute cell counts that were higher than both vaccinated mice with and without schistosomiasis, thus suggesting PZQ might be independently influential in the increase of these cells. Further, we concluded that schistosomiasis is associated with increases in immunomodulatory Foxp3 expression in Tregs in vaccinated mice that were left untreated.

4.1.14 Chronic schistosomiasis reduces memory T cell populations in the spleen of DTPa-hepB-IPV-Hib vaccinated mice

Adaptive immunity to vaccination can induce long lived memory T cells that can be maintained in the secondary lymphoid organs (Gray *et al.*, 2018, Adam *et al.*, 2021). We wondered if DTPa-hepB-IPV-Hib vaccination of mice would induce splenic central memory T (T_{CM}) and effector memory T (T_{EM}) cells in mice, and if schistosomiasis and its treatment with PZQ would influence these cell populations. To investigate, we conducted flow cytometry analysis of T_{CM} and T_{EM} cells in the spleen of mice from our experimental strategy (Figure 4.3 & Figure 4.14A).

Our analyses revealed that $CD4+CD62+CD44+$ T_{CM} cells were significantly increased in Vac+ mice in comparison to naïve mice ($p<0.05$) (Figure 4.14B). Upon infection with schistosomiasis, Vac+Sm+ were found with significantly reduced T_{CM} cell frequencies ($p<0.05$) but not absolute cell counts ($p>0.05$) when compared to Vac+ mice (Figure 4.14B). Treatment of schistosomiasis with PZQ resulted in the restoration of T_{CM} cell frequencies to levels similar to Vac+ mice ($p>0.05$) but interestingly, absolute cell numbers were found significantly higher than Vac+ mice ($p<0.05$) (Figure 4.14B).

Effector memory T cell frequencies were similar in naïve mice and Vac+ mice ($p>0.05$) (Figure 4.14C). However, T_{EM} absolute cell numbers were significantly higher in vaccinated mice than naïve mice ($p<0.05$). In schistosomiasis infected Vac+Sm+ mice, T_{EM} cell frequencies, but not absolute cell counts, were significantly reduced in comparison with Vac+ mice ($p<0.05$, $p>0.05$, respectively; Figure 4.14C). Treatment of schistosomiasis with PZQ resulted in significantly increased T_{EM} cells in Vac+Sm+PZQ+ mice compared to untreated Vac+Sm+ mice ($p<0.05$; Figure

4.14C). No significant differences in T_{EM} cells between Vac+ and Vac+Sm+PZQ+ mice were observed ($p>0.05$).

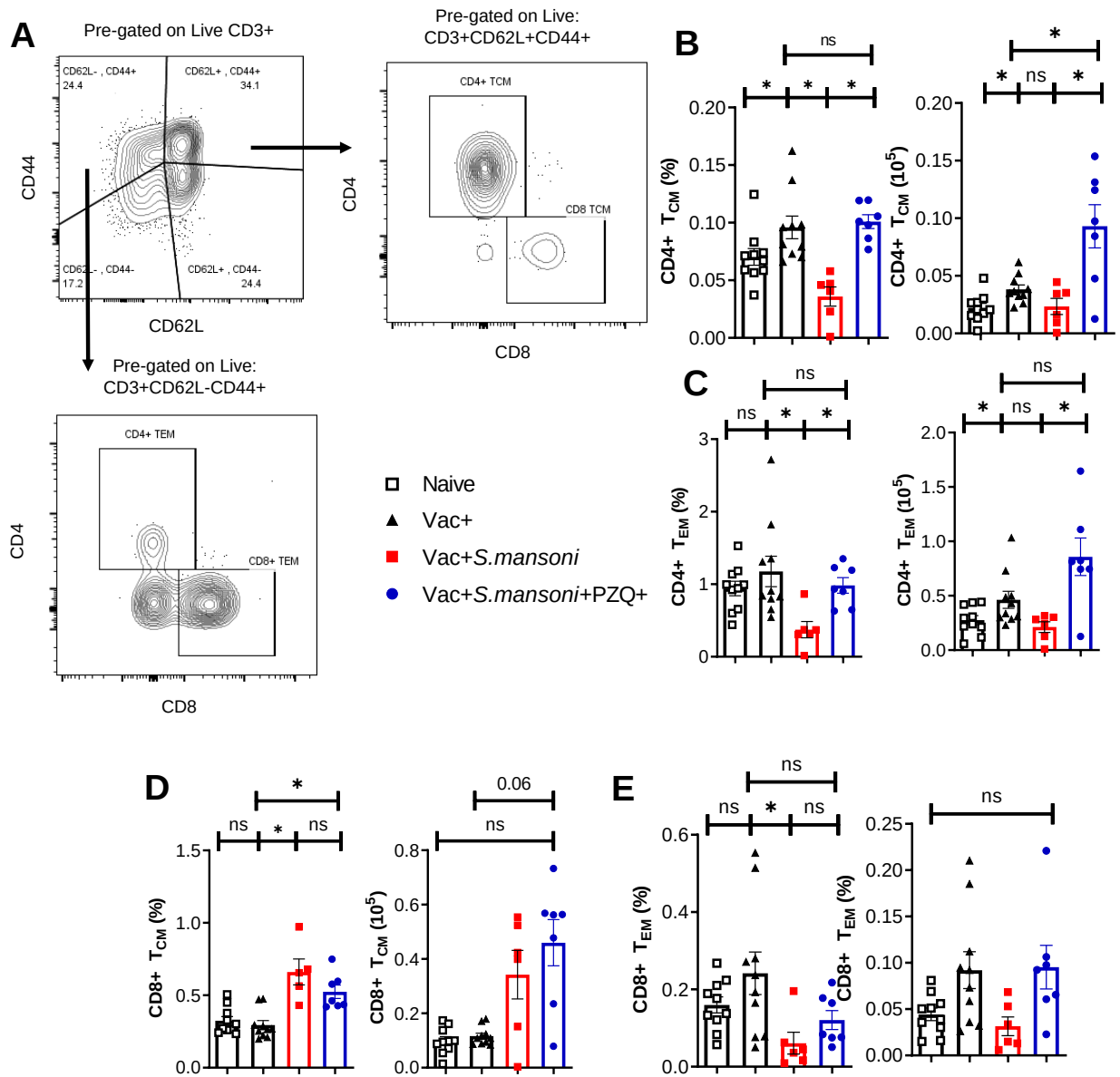


Figure 4. 14: Frequencies of splenic central memory and effector memory.

A. Representative flow cytometry gating strategy for identifying central memory, effector memory and naive T cells. **B.** Frequency of CD3+ CD4+ CD62L+CD44+ central memory T cells. **C.** Frequency of CD3+ CD4+CD62L-CD44+ effector memory T cells. **D.** Frequency of CD3+ CD8+ CD62L+CD44+ central memory T cells. **E.** Frequency of CD3+ CD8+ CD62L-CD44+ effector memory T cells. Data are expressed as mean $\pm$ S.E.M and representative of 2 independent experiments; $n = 6-10$ mice per group; Data was analysed by the Shapiro-Wilk test followed by either one way ANOVA with Bonferroni's multiple comparisons test or by Kruskal Wallis followed by the Dunn's multiple comparisons test; ns, $p > 0.05$; * $p < 0.05$; Vac, DTPa-hepB-IPV-Hib; Sm, *S. mansoni*; PZQ, praziquantel; SSC, Side scatter; CD3, cluster of differentiation 3; CD4, cluster of differentiation 4; T_{CM}, central memory T cells; T_{EM}, effector memory T cells.

Looking at CD8+CD62+CD44+ T_{CM} cells, no differences were observed between Vac+ mice and naïve mice ($p>0.05$; Figure 4.14D). However, interestingly, upon infection with schistosomiasis, Vac+Sm+ had significantly increased T_{CM} cell frequencies ($p<0.05$) but not absolute cell counts despite visible higher numbers ($p>0.05$) when compared to Vac+ mice (Figure 4.14D). No differences in CD8+ T_{CM} between Vac+Sm+ and Vac+Sm+PZQ+ were observed following the administration of PZQ to infected mice ($p>0.05$; Figure 4.14D). Frequencies but not absolute counts of CD8+ T_{CM} were significantly higher in Vac+Sm+PZQ+ versus Vac+Sm+ mice ($p<0.05$, $p=0.06$, respectively; Figure 4.14D).

Further, assessments of CD8+ CD62L-CD44+ T_{EM} cells between Vac+ and naïve mice showed no differences. Infected Vac+Sm+ mice had reduced CD8+ T_{EM} cell frequencies, but not counts, in comparison to Vac+ mice ($p<0.05$, $p>0.05$, respectively; Figure 4.14E). No significant differences were observed between Vac+Sm+ and Vac+Sm+PZQ+ ($p>0.05$) despite the later having higher cell frequencies and counts. Additionally, no differences were observed between Vac+Sm+PZQ+ and Vac+ mice ($p>0.05$; Figure 4.14E).

Altogether, our findings suggest that schistosomiasis is associated with reduced frequencies of DTPa-hepB-IPV-Hib vaccine induced CD4+ T_{CM} and T_{EM} cells, and CD8+ T_{EM} cells. Conversely, schistosomiasis infection increases CD8+ T_{CM} frequencies. Additionally, the findings further suggest that PZQ significantly restores only the CD4+ T_{CM} and T_{EM} cells and not CD8+ T_{CM} and T_{EM} cells.

4.1.15 Chronic schistosomiasis is associated with the death of plasma cells and plasmablasts of DTPa-hepB-IPV-Hib vaccinated mice and PZQ selectively limits deaths of these populations

Our study's revelation that schistosomiasis may reduce specific B cells while PZQ may be selectively counteractive of the parasites' influence in the bone marrow and spleens of DTPa-hepB-IPV-Hib vaccinated mice prompted us to investigate further the potential mechanisms behind our findings. We wondered if schistosomiasis was driving the death of B cell populations of the vaccinated mice. To investigate, we quantified the deaths of specific B cells using our experimental strategy (Figure 4.3). Bone marrow and spleen cells were stained with an amine-based fixable viability dye to identify cell death by flow cytometry. We started off our investigations by assessing plasma cell and plasmablast death from the bone marrow and spleens (Figure 4.15A).

We observed that B220^{lo}CD138⁺ plasma cells in the bone marrow of Vac⁺ mice had significantly lower deaths in comparison to naïve mice ($p < 0.0001$; Figure 4.15B). Schistosomiasis infected Vac⁺Sm⁺ mice had significantly elevated bone marrow plasma cell death frequencies ($p < 0.0001$) and increased but non-significant dead plasma cell counts ($p = 0.057$) than Vac⁺ mice (Figure 4.15B). PZQ treatment of infected Vac⁺Sm⁺PZQ⁺ mice resulted in significantly reduced frequencies of plasma cell death in comparison to Vac⁺Sm⁺ ($p < 0.001$) although such a trend was not significant with absolute cell counts ($p = 0.076$; Figure 4.15B). Comparisons between Vac⁺ and Vac⁺Sm⁺PZQ⁺ mice showed significantly higher frequencies of bone marrow plasma cell death in Vac⁺Sm⁺PZQ⁺ ($p < 0.005$) but no significant differences in cell counts (Figure 4.15B).

Comparisons of B220^{hi}CD138⁺ bone marrow plasmablasts between naïve mice and Vac⁺ mice indicated reduced death frequencies and counts of these cells in vaccinated mice ($p < 0.05$, $p < 0.0001$; Figure 4.15C). When infected with schistosomiasis, increases in bone marrow plasmablast death frequencies and cell counts was noted in Vac⁺Sm⁺ mice versus Vac⁺ mice ($p < 0.05$). Treating infected mice with PZQ reduced bone marrow plasmablast death without statistical significance ($p > 0.05$, respectively, Figure 4.15C). Treating infected mice with PZQ

did not yield any significant changes on Vac+Sm+PZQ+ mice versus Vac+ mice ($p>0.05$, respectively, Figure 4.15C).

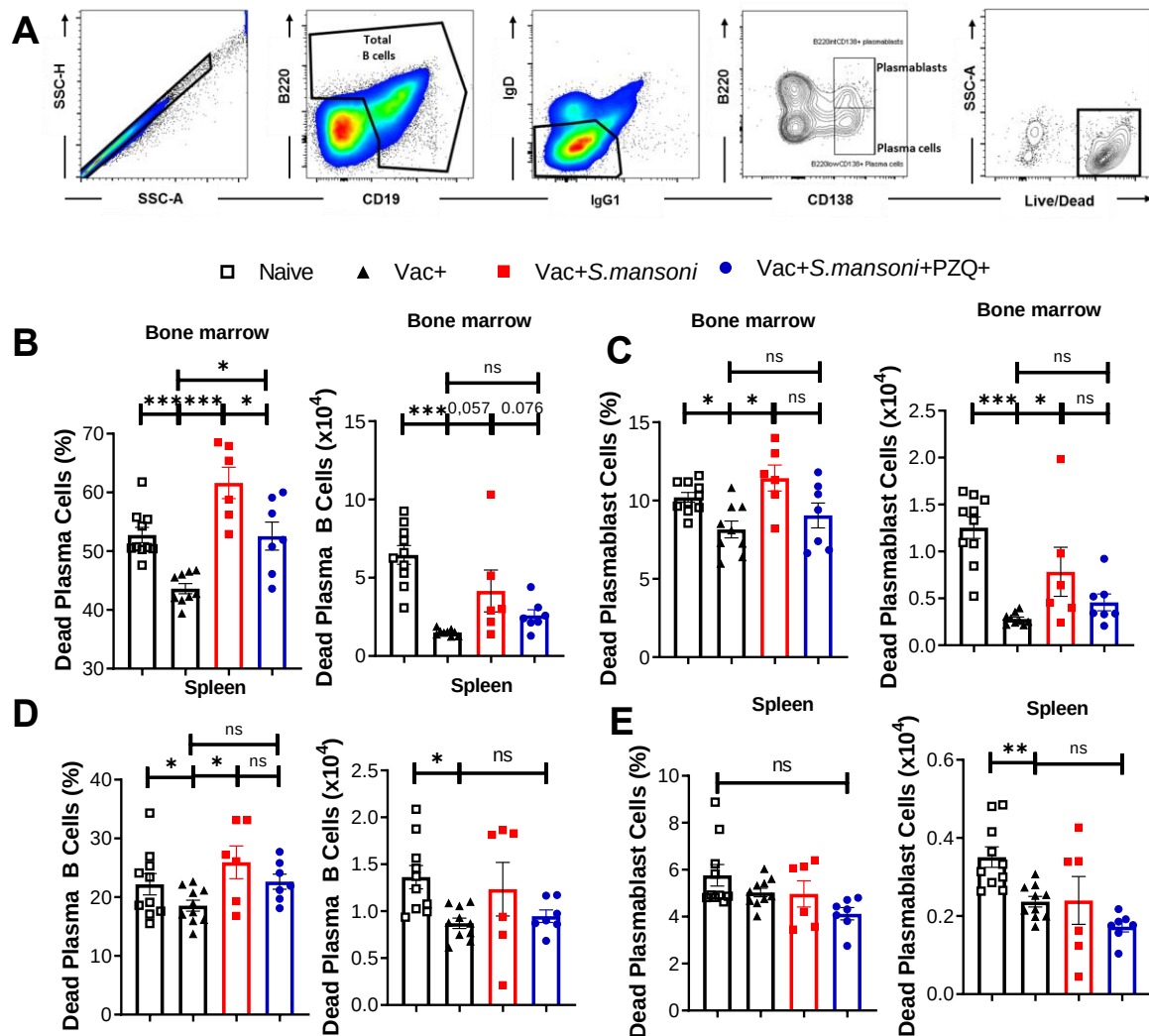


Figure 4. 15: Bone marrow and splenic plasma B cell and plasmablast death in vaccinated mice infected with schistosomiasis and after PZQ treatment.

A. Representative gating strategy of dead cells among plasmablasts and plasma cells. **B.** Dead bone marrow plasma cell frequencies and absolute numbers. **C.** Dead bone marrow plasmablast frequencies and absolute numbers. **D.** Dead spleen plasma cell frequencies and absolute numbers. **E.** Dead bone marrow plasmablast frequencies and absolute numbers. Data are expressed as mean $\pm$ S.E.M and representative of 2 independent experiments; $n = 6-10$ mice per group; Data analysed by Shapiro-Wilk test followed by either one way ANOVA with Bonferroni's multiple comparisons test or by Kruskal Wallis followed by the Dunn's multiple comparisons test; ns, $p > 0.05$; * $p < 0.05$, ** $p < 0.001$, *** $p < 0.0001$; Vac, DTPa-hepB-IPV-Hib; Sm, *S. mansoni*; PZQ, praziquantel; SSC, Side scatter; FSC, forward scatter; CD19, cluster of differentiation 19; IgG1, Immunoglobulin G1; IgD,

Immunoglobulin D; B220, B cell isoform of 220 kDa; CD138, cluster of differentiation 138.

In the spleen, plasma cell death frequencies and counts were lower in Vac+ mice in comparison to naïve mice ($p < 0.05$, respectively; Figure 4.15D). Schistosomiasis infected Vac+Sm+ mice were found with significantly higher plasma cell death frequencies, but not cell counts, than Vac+ mice ($p < 0.05$, $p > 0.05$, respectively; Figure 4.15D). Administration of PZQ in Vac+Sm+PZQ+ mice had no significant impact on plasma cell death in comparison to both Vac+Sm+ and Vac+ mice ($p > 0.05$, respectively; Figure 4.15D). Further, no significant differences were observed in the frequencies of spleen plasmablasts for all mice groups ($p > 0.05$; Figure 4.15E). However, cell counts of plasmablasts indicated significantly reduced deaths of these cells in Vac+ versus naïve mice ($p < 0.001$; Figure 4.15E). No differences were observed for spleen plasmablast death frequencies and counts for all comparisons among Vac+, Vac+Sm+ and Vac+Sm+PZQ+ mice ($p > 0.05$, respectively; Figure 4.15E).

Together, our results show that vaccination of mice the DTPa-hepB-IPV-Hib vaccine led to an increased survival or reduction of death of bone marrow plasma cells and plasmablasts, and for plasma cells but not plasmablasts in the spleen. In contrast, schistosomiasis increased the death or reduced the survival of bone marrow plasma cells and plasmablasts, and that of plasma cells in the spleen. Our findings also showed a reduction of plasma cell death frequencies in bone marrow following praziquantel treatment of infected mice. However, this PZQ conferred advantage was not observed for bone marrow plasmablasts and spleen plasma cells and plasmablasts.

4.1.16 Schistosomiasis drives necrotic death of bone marrow plasma cells from DTPa-hepB-IPV-Hib vaccinated mice and PZQ treatment selectively reverses the parasite's impact

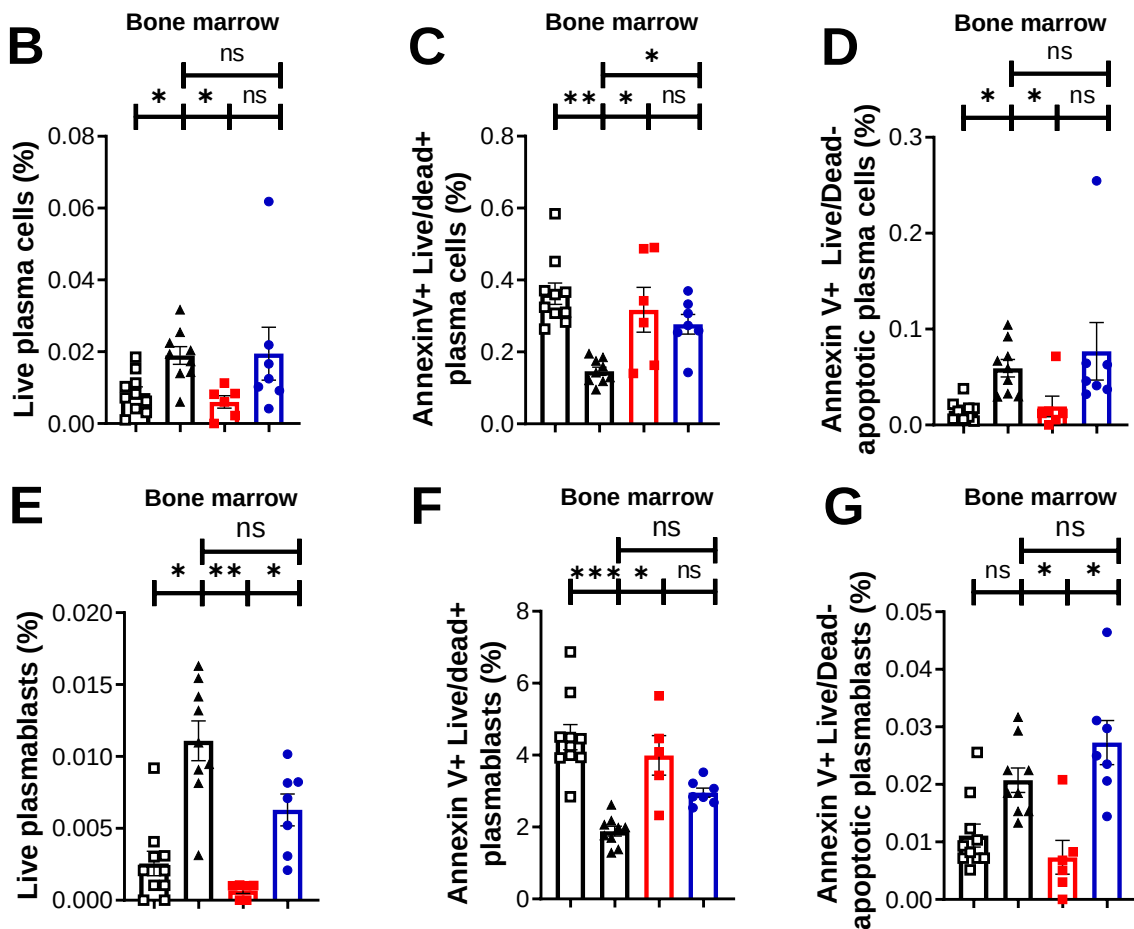
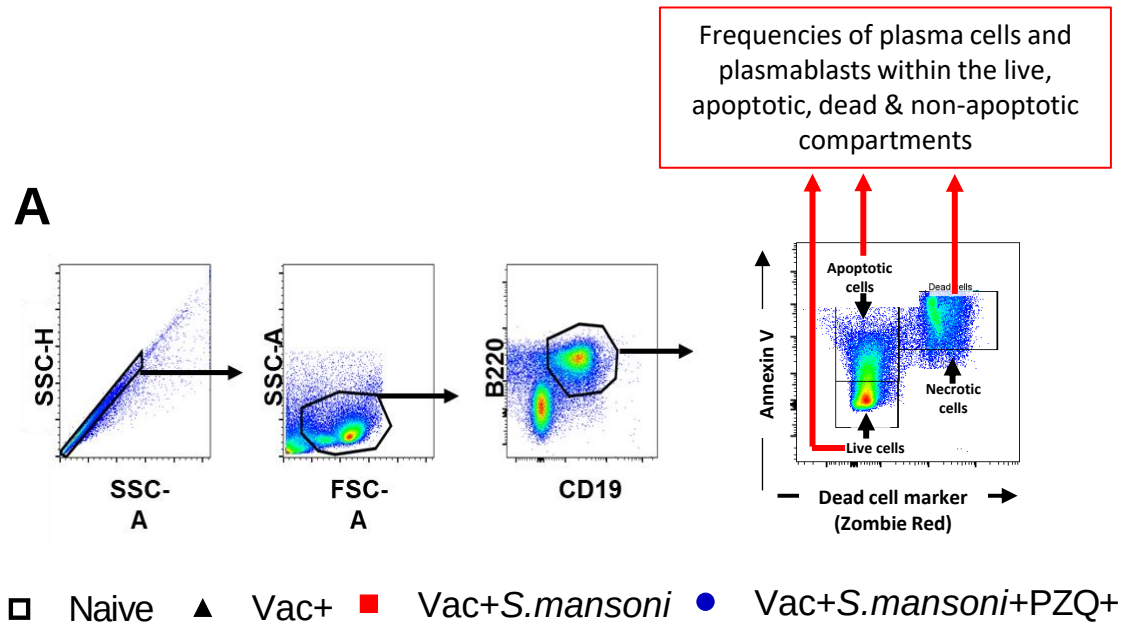


Figure 4. 16: Measuring apoptotic and necrotic cell death in plasma cells and plasmablasts from the bone marrow of DTPa-hepB-IPV-Hib vaccinated mice post *S. mansoni* infection and after PZQ treatment in mice.

A. Annexin V and FVD live/dead dual staining of cells before plasma cell and plasmablast identification. **B.** Annexin V-Live/Dead- plasma cells. **C.** Annexin V+ Live/Dead+ necrotic dead plasma cells. **D.** Annexin V+Live/Dead- apoptotic plasma cells. **E.** Annexin V-Live/Dead- live plasmablasts. **C.** Annexin V+ Live/Dead+ necrotic dead plasmablasts. **F.** Annexin V+Live/Dead- apoptotic plasmablasts. Data are expressed as mean $\pm$ S.E.M and representative of 2 independent experiments; n = 6-10 mice per group; Data analysed by Shapiro-Wilk test followed by either one way ANOVA with Bonferroni's multiple comparisons test or by Kruskal Wallis followed by the Dunn's multiple comparisons test; ns, $p > 0.05$; * $p < 0.05$, ** $p < 0.001$, *** $p < 0.0001$; Vac, DTPa-hepB-IPV-Hib; Sm, *S. mansoni*; PZQ, praziquantel; SSC, Side scatter; FSC, forward scatter; CD19, cluster of differentiation 19; B220, B cell isoform of 220 kDa;.

We investigated further into the mechanisms behind the observed death or rather the reduced survival of plasma cells and plasmablasts selectively in the bone marrow in a model similar to our experimental strategy (Figure 4.3). Bone marrow plasma cells and plasmablasts were stained with both FVD live/dead dye and Annexin V dye to assess apoptotic and necrotic cell deaths by flow cytometry (Figure 1.6A). As initially observed in prior experiments, viable Annexin V-Live/Dead- plasma cell frequencies were significantly higher in Vac+ mice in comparison to naïve and Vac+Sm+ mice ($p < 0.05$, respectively, Figure 4.16B). Treatment of schistosomiasis with PZQ in Vac+Sm+PZQ+ mice had no significant impact on viable plasma cell frequencies in comparison to both Vac+Sm+ and Vac+ mice ($p > 0.05$; respectively; Figure 4.16B).

Necrotic Annexin V+ Live/Dead+ plasma cells were found significantly higher in both naïve and Vac+Sm+ mice in comparison to Vac+ mice ($p < 0.001$, $p < 0.05$, respectively; Figure 4.16C). Further analysis showed no significant impact of PZQ treatment on necrotic plasma cell frequencies in Vac+Sm+PZQ+ mice versus Vac+Sm+ while a significant difference similar to Vac+Sm+ was observed in comparison to Vac+ ($p > 0.05$; $p < 0.05$; Figure 4.16C). Further analyses showed significantly increased Annexin V+Live/Dead- apoptotic plasma cell frequencies in Vac+ mice in comparison to naïve mice and Vac+Sm+ mice ($p < 0.05$, respectively; Figure 4.16D). PZQ treatment in Vac+Sm+PZQ+ mice showed a non-significant increase in apoptosis when compared to Vac+Sm+ mice ($p > 0.05$) while no differences were observed when the treated mice were compared to Vac+ mice ($p > 0.05$; Figure 4.16D).

Annexin V-Live/Dead- viable plasmablasts were found significantly elevated in Vac+ mice in comparison to both naïve and Vac+Sm+ mice ($p < 0.05$, $p < 0.001$, respectively; Figure 4.16E). Praziquantel treatment in Vac+Sm+PZQ+ mice significantly restored plasmablast cell frequencies in comparison to Vac+Sm+ mice ($p < 0.05$) and to similar levels with Vac+ mice ($p > 0.05$, Figure 4.16E). Annexin V+Live/Dead+ necrotic plasmablasts were found significantly elevated in both naïve mice and Vac+Sm+ mice when compared to Vac+ mice ($p < 0.0001$, $p < 0.05$, respectively; Figure 4.16F). PZQ treatment in Vac+Sm+PZQ+ had no significant impact on plasmablast cell frequencies when compared to both Vac+Sm+ and Vac+ mice ($p > 0.05$, respectively; Figure 4.16F). Apoptotic Annexin V+Live/Dead- plasmablasts were found higher in Vac+ mice, although with no statistical difference, in comparison naïve mice ($p > 0.05$; Figure 4.16G). Apoptotic bone marrow plasmablast frequencies were significantly reduced in schistosomiasis infected Vac+Sm+ mice in comparison to both Vac+ and Vac+Sm+PZQ+ mice ($p < 0.05$, respectively; Figure 4.16G). No differences were observed on the frequencies of apoptotic plasmablasts between Vac+ and Vac+Sm+PZQ+ mice ($p > 0.05$; Figure 4.16G).

Taken together, we concluded that the death of bone marrow plasma cells and plasmablasts, in the presence of schistosomiasis in DTPa-hepB-IPV-Hib vaccinated mice, may be driven by a non-apoptotic process that may involve necrosis. Treatment with PZQ, despite clearly showing improved survival of both plasma cells and plasmablasts, was selectively influential in reducing the death of these populations by a necrotic mechanism.

4.1.17 Schistosomiasis is associated with increased death of total B cells, IgG1 memory B cells, follicular B cells and marginal zone B cells in the spleens of the DTPa-hepB-IPV-Hib vaccinated mice and PZQ treatment selectively reverses this impact

Next, we investigated the death of B cell populations in the bone marrow and spleens of our experimental mice groups (Figure 4.3). We assessed, using flow cytometry, the expression of the Live/Dead marker on specific B cell populations to quantify their death frequencies (Figure 4.17A). In the bone marrow, Vac+ mice were observed with significantly lower B220+CD19+ total B cell death frequencies than naïve mice

($p < 0.0001$; Figure 4.17B). When compared to Vac+ mice, schistosomiasis infected Vac+Sm+ mice were found with significantly increased death frequencies of bone marrow total B cells ($p < 0.0001$; Figure 4.17B) whereas absolute cell count showed no differences ($p > 0.05$; Figure 4.17B). Praziquantel treated Vac+Sm+PZQ+ mice had reduced death frequencies, although non-significant, of bone marrow total B cells in comparison to non-treated Vac+Sm+ mice ($p > 0.05$; Figure 4.17B). Further, total B cell death frequencies, but not dead cell counts, remained significantly higher in Vac+Sm+PZQ+ than Vac+ mice ($p < 0.05$, $p > 0.05$ respectively; Figure 4.17B).

Splenic total B cell death frequencies and absolute counts were significantly lower in Vac+ mice versus naïve mice ($p < 0.001$, $p < 0.05$, respectively; Figure 4.17C). Upon *S. mansoni* infection, increased death frequencies, but not cell counts, of splenic total B cells in Vac+Sm+ mice were observed in comparison to Vac+ mice ($p < 0.05$, $p > 0.05$; Figure 4.17C). PZQ administration reduced splenic total B cell death frequencies and absolute cell counts in Vac+Sm+PZQ+ mice versus Vac+ mice ($p < 0.0001$, $p < 0.05$, respectively; Figure 4.17C). Additionally, total B cell death in the spleen of Vac+Sm+PZQ+ mice was significantly lower versus Vac+ mice ($p < 0.05$; Figure 4.17C).

Further analyses in the spleen indicated significantly lowered frequencies and cell counts of follicular B cell death in Vac+ mice versus naïve mice ($p < 0.0001$, $p < 0.01$, respectively; Figure 4.17D). Additionally, follicular B cell death frequencies, but not absolute counts, were lower in Vac+ mice in comparison to schistosomiasis infected Vac+Sm+ mice ($p < 0.05$, $p > 0.05$, respectively; Figure 4.17D). Praziquantel administration significantly reduced follicular B cell death frequencies and absolute counts in Vac+Sm+PZQ when compared to non-treated Vac+Sm+ mice ($p < 0.0001$, $p < 0.05$, respectively; Figure 4.17D). Notably, in comparison to Vac+ mice, Vac+Sm+PZQ+ mice had significantly reduced follicular B cell death frequencies and absolute cell counts ($p < 0.0001$, $p < 0.05$, respectively; Figure 4.17D).

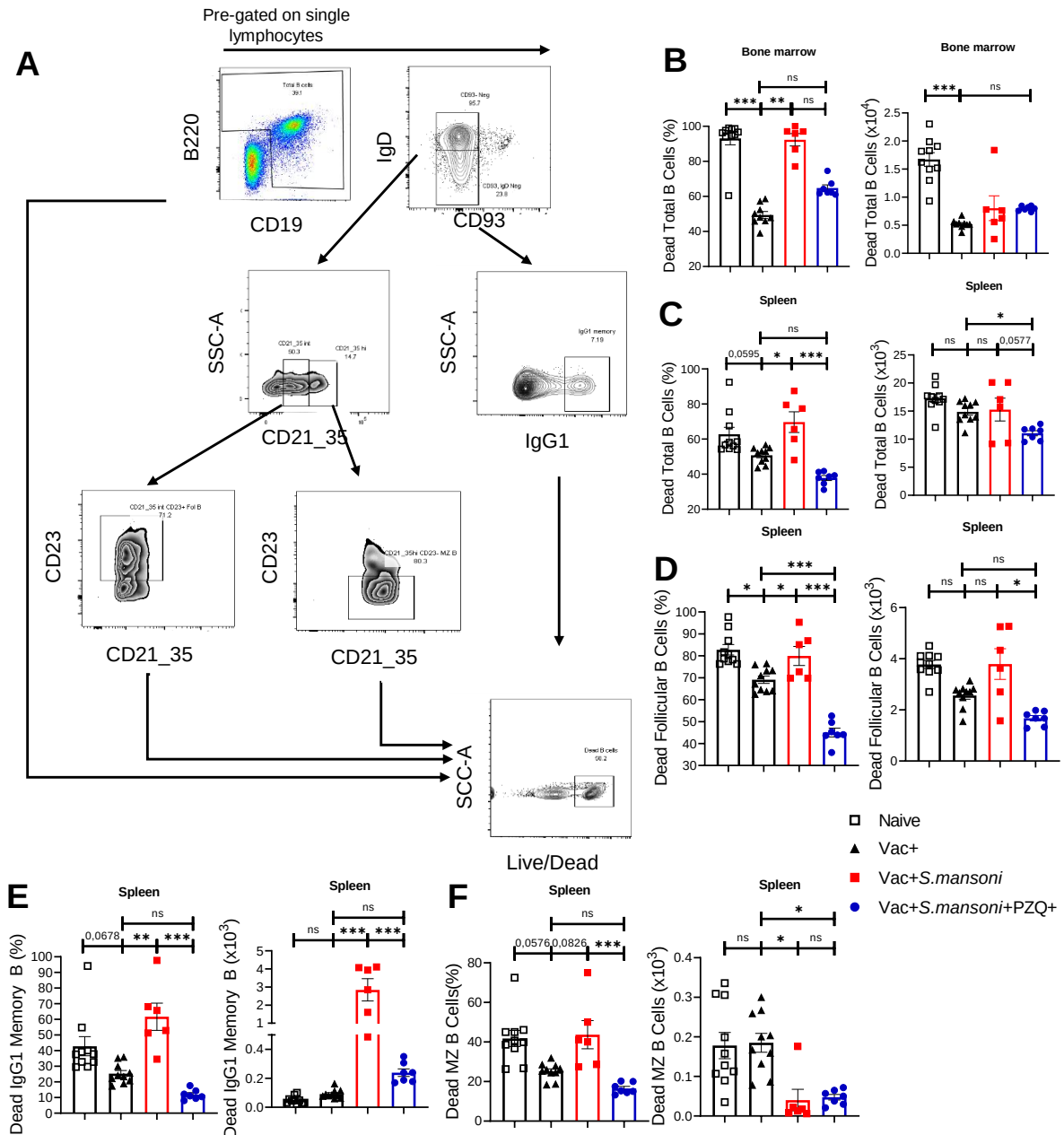


Figure 4. 17: Frequencies of dead Total B cells, IgG1 memory B cells, Follicular B cells and Marginal zone B cells.

A. Representative flow cytometry analysis of dead total B cells from bone marrow and spleen, and dead IgG1 memory B cells, follicular B cells and MZ B cells from the spleen. **B.** Dead total B cell frequencies in the bone marrow. **C.** Dead total B cell frequencies in the spleen. **D.** Dead IgG1 memory B cells frequencies in the spleen. **E.** Dead follicular B cell frequencies in the spleen. **F.** Dead MZ B cell frequencies in the spleen. Data are expressed as mean $\pm$ S.E.M and representative of 2 independent experiments; n = 6-10 mice per group; Data was analysed by the Shapiro-Wilk test followed by either one way ANOVA with Bonferroni's multiple comparisons test or by Kruskal Wallis followed by the Dunn's multiple comparisons test; ns, $p > 0.05$; * $p < 0.05$, ** $p < 0.001$, *** $p < 0.0001$; Vac, DTPa-hepB-IPV-Hib; Sm, *S. mansoni*; PZQ, praziquantel; SSC, Side scatter; FSC, forward scatter; CD19, cluster of differentiation 19; IgG1, Immunoglobulin G1; IgD, Immunoglobulin D;

B220, B cell isoform of 220 kDa; CD93, cluster of differentiation 93; CD21/35, cluster of differentiation 21 and 35; CD23, cluster of differentiation 23.

Splenic IgG1 memory B cell death frequencies and counts in Vac+ were significantly lower in Vac+ mice in comparison to naïve mice ($p<0.001$, $p<0.05$; Figure 4.17E). Infection with schistosomiasis drove a significant increase in death frequencies and counts of IgG1 memory B cells in Vac+Sm+ mice versus Vac+ mice ($p<0.0001$, respectively; Figure 4.17E). Praziquantel treated Vac+Sm+PZQ+ mice had significantly reduced these IgG1 memory B cell deaths than Vac+Sm+ mice ($p<0.0001$; Figure 4.17E). Furthermore, IgG1 memory B cell death was similar between Vac+ and Vac+Sm+PZQ+ mice ($p>0.05$; Figure 4.17E).

Assessing MZ B cell death, Vac+ had significantly lower MZ B cell death frequencies, and not cell counts, in comparison to naïve cells ($p<0.001$, $p>0.05$, respectively; Figure 4.17F). Observations in Vac+Sm+ mice showed increased death frequencies of MZ B cells versus Vac+ mice ($p<0.05$; Figure 4.17F). Intriguingly, MZ B cell death counts were significantly lower in Vac+Sm+ mice in comparison to Vac+ mice ($p<0.05$; Figure 4.17F). Praziquantel treated Vac+Sm+PZQ+ mice were found with notably lower MZ B cell death frequencies but not cell counts in comparison to Vac+Sm+ mice ($p<0.001$, $p>0.05$, respectively; Figure 4.17F). Further, there were no differences in MZ B cell death frequencies between Vac+Sm+PZQ+ and Vac+ mice ($p>0.05$) but intriguingly once more, MZ B cell count death in Vac+ mice was significantly higher than Vac+Sm+PZQ+ mice ($p<0.05$; Figure 4.17F).

Taken together, we concluded that DTPa-hepB-IPV-Hib vaccination was associated with increased survival or lower death frequencies of bone marrow and splenic total B cells, as well as splenic frequencies of follicular B cells, IgG1 memory B cells and MZ B cells. Additionally, our results show that while schistosomiasis was associated with the increased death of these B cell subsets, PZQ treatment of infected mice reduced the death frequencies of these cells.

4.1.18 Schistosomiasis is associated with increased death of specific splenic T cell populations in DTPa-hepB-IPV-Hib vaccinated mice and PZQ treatment selectively reverses the parasite's impact

Having observed that schistosomiasis drives B cell death, we wondered if T cells in in DTPa-hepB-IPV-Hib vaccinated mice also succumbed in the presence of the parasite infection, and how PZQ may influence these dynamics. Hence, using our experimental strategy (Figure 4.3), CD4⁺ Th cell and follicular T cell death was investigated using the live/dead stain measured by flow cytometry (Figure 4.18A).

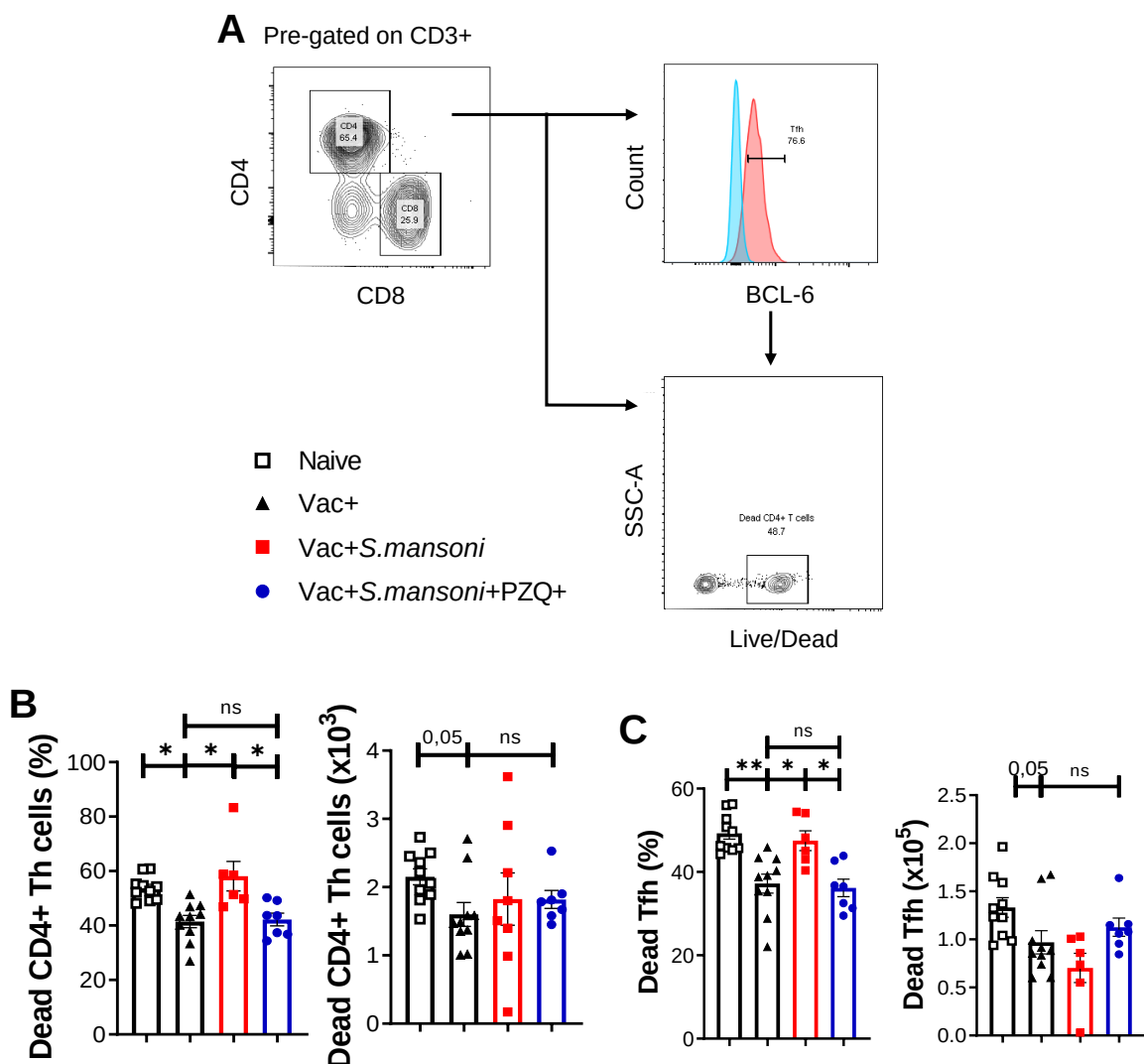


Figure 4. 18: Death of CD4⁺ Th cells and follicular T cells in DTPa-hepB-IPV-Hib vaccinated mice infected with schistosomiasis and treated with PZQ.

A. Representative flow cytometry gating strategy for identifying dead CD3⁺CD4⁺ T helper cells and BCL-6⁺CD3⁺CD4⁺ follicular T cells in the spleen. **B.** Frequencies of

dead splenic CD3+CD4+ T helper cells. **C.** Frequencies of dead splenic BCL-6+CD3+CD4+follicular T helper cells in the spleen. Data are expressed as mean $\pm$ S.E.M and representative of 2 independent experiments; n = 6-10 mice per group; Data was analysed by the Shapiro-Wilk test followed by either one way ANOVA with Bonferroni's multiple comparisons test or by Kruskal Wallis followed by the Dunn's multiple comparisons test; ns, $p > 0.05$; * $p < 0.05$, ** $p < 0.001$; Vac, HDTPa-hepB-IPV-Hib); Sm, *S. mansoni*; PZQ, praziquantel; SSC, Side scatter; CD3, cluster of differentiation 3; CD4, cluster of differentiation 4; CD8, cluster of differentiation 8; BCL-6, B-cell lymphoma 6; Foxp3, forkhead box P3.

Evaluation of CD4+ Th cells showed significantly reduced death frequencies in Vac+ mice when compared to naïve mice ($p < 0.05$; Figure 4.18B). A similar but non-significant trend was observed between the two mice groups when dead CD4+ Th cell counts were assessed ($p = 0.05$; Figure 4.18B). The Th cell death frequencies in Vac+ mice were significantly reduced when compared to Vac+Sm+ mice whereas no differences were noted based on cell counts ($p < 0.05$, $p > 0.05$, respectively; Figure 4.18B). Praziquantel treatment in Vac+Sm+PZQ+ mice, when compared to Vac+Sm+ mice, resulted in a significant reduction in Th cell death frequencies but not cell counts ($p < 0.05$, $p > 0.05$, respectively; Figure 4.18B). In addition, we found no notable differences between Th cell death frequencies and counts between Vac+ and Vac+Sm+PZQ+ mice ($p > 0.05$, respectively; Figure 4.18B)

Showing a similar trend to observations made for Th cells, follicular helper T cell death frequencies, but not counts although also reduced, were significantly lower in Vac+ versus naïve mice ($p < 0.05$, $p = 0.05$, respectively; Figure 4.18C). The follicular helper T cell death frequencies, but not counts, of Vac+ were also significantly lower than that of infected Vac+Sm+ mice ($p < 0.05$, $p > 0.05$, respectively; Figure 4.18C). Comparisons between follicular helper T cells in Vac+Sm+ mice versus Vac+Sm+PZQ+ mice showed significantly reduced cell death frequencies, but not counts, in the PZQ treated mice ($p < 0.05$, $p > 0.05$, respectively; Figure 4.18C). No differences in death frequencies and counts of the follicular helper T cells were observed between Vac+ and Vac+Sm+PZQ+ ($p > 0.05$, respectively; Figure 4.18C).

4.1.19 Chronic schistosomiasis reduces bone marrow total B cells, plasmablasts and plasma B cells in the bone marrow of non-vaccinated mice

Having made our observations on the impact of schistosomiasis, and its treatment with PZQ, on IPV serologic memory and specific B and T cells in DTPa-hepB-IPV-Hib vaccinated mice, we wondered if schistosomiasis would affect specific B cell populations in a comparable manner in non-vaccinated mice. We therefore sought to conclusively determine the influence of schistosomiasis on B cell populations directly involved in antibody production, the bone marrow plasma cells and plasmablasts, in the absence of vaccination.

An experiment was designed to assess if schistosomiasis bone marrow B cell populations without the DTPa-hepB-IPV-Hib vaccination in mice (Figure 4.19A-B). Briefly, age and gender matched BALB/c mice were placed into groups. The first group of mice were mock infected (naïve) while the second group were infected with a low dose of *S. mansoni* cercariae (35 larvae) to induce a chronic model of schistosomiasis. Mice from both groups were euthanised at week 4 and week 10 post *S. mansoni* infection (Sm+) and their left femur bone marrow were collected for single cell isolation, followed by flow cytometric evaluation to assess the influence of schistosomiasis on bone marrow plasma cells, plasmablasts and total B cells.

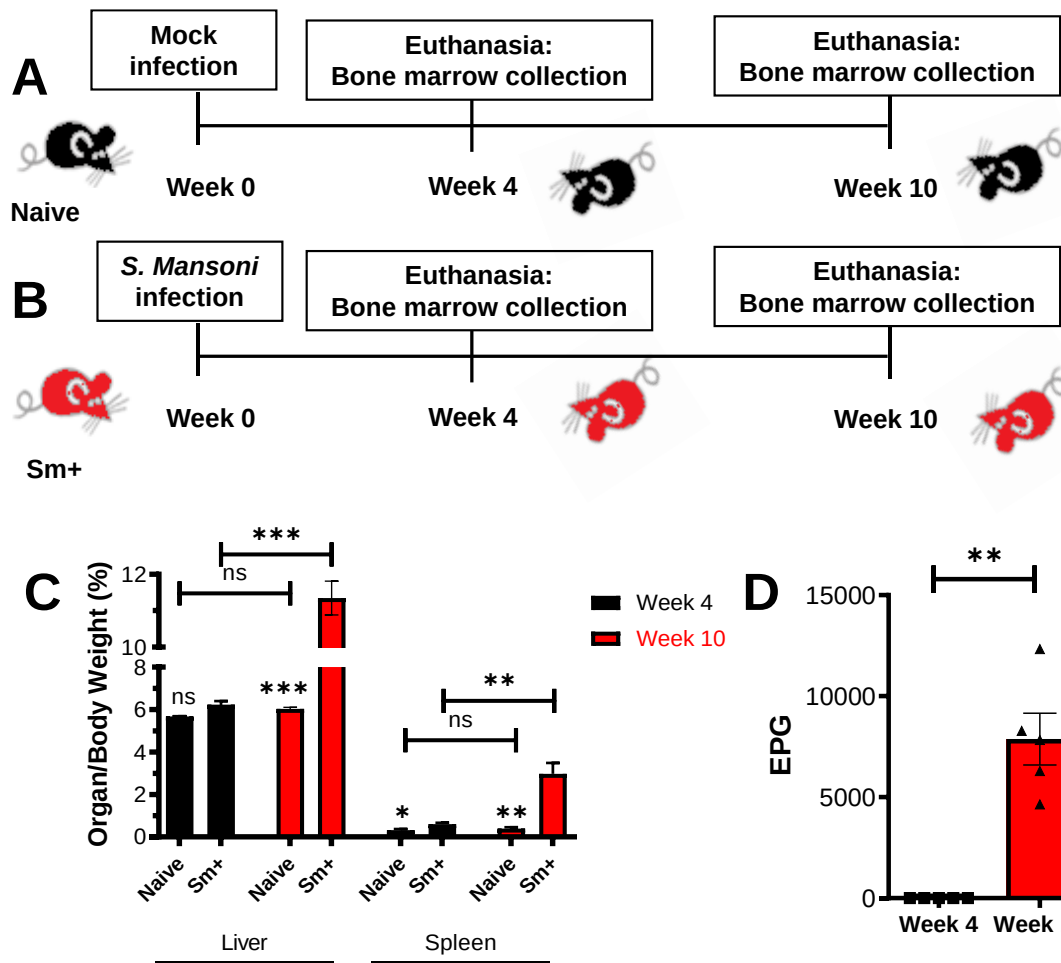


Figure 4. 19: Experimental design to assess the influence of schistosomiasis on bone marrow B cells.

BALB/c mice (6-8 weeks old) were separated into 2 experimental groups. **A.** Mice were mock infected with PBS. **B.** Mice were percutaneously infected with a low dose of *S. mansoni* (35 cercariae) to establish a chronic disease course. At week 4 and 10 post infection, representative mice from each group were euthanised and their bone marrows were collected for B cell analyses using flow cytometry. **D.** Number of eggs per gram of liver. Data are expressed as mean $\pm$ S.E.M and representative of 2 independent experiments; n = 4 - 5 mice per group; Data was analysed by one way ANOVA followed by the Bonferroni's multiple comparisons test (**C**) and two tailed Student's t test (**D**); ns, $p > 0.05$; * $p < 0.05$, ** $p < 0.001$, *** $p < 0.0001$; Sm, *S. mansoni*

First, we assessed the impact of schistosomiasis on the liver and the spleen at different time points. At week 4 post infection, no difference between naïve mice and Sm+ mice were observed on liver pathology which was measured as a ratio of liver weight to body weight ($p > 0.05$; Figure 4.19C). However, the spleens of Sm+ mice had significantly increased weight ratios indicating significantly inflamed spleens ($p < 0.05$; Figure 4.19C). At week 10 post infection, Sm+ mice had significantly increased liver and spleen weight indexes in comparison to the naïve mice ($p < 0.001$,

$p < 0.05$, respectively). Additional analyses revealed that the liver and spleen weights of Sm+ mice at week 10 were significantly higher than those of Sm+ mice at week 4 ($p < 0.0001$, $p < 0.05$, respectively; Figure 4.19C). Furthermore, Sm+ mice at week 10 post infection had a significantly higher liver egg burden than Sm+ mice at week 4 post infection ($p < 0.001$; Figure 4.19D).

Having confirmed early-stage infection as indicated by spleen inflammation of Sm+ mice at week 4, and chronic infection as indicated by egg recovery in the livers of Sm+ at week 10, we assessed the bone marrow of these two groups of mice for B cell populations.

Results showed that at week 4 post infection, although Sm+ mice had increased frequencies of bone marrow plasma cells, plasmablasts and total b cells than naïve mice, there were no statistically significant differences between the two mice groups ($p > 0.05$, respectively; Figures 4.20A-C). Additionally, no significant differences between plasma cell and plasmablast counts were observed in the bone marrow ($p > 0.05$, respectively; Figure 4.20A-B). In contrast, absolute bone marrow total B cell counts of Sm+ mice were significantly higher than those of naïve mice at week 4 ($p < 0.05$; Figure 4.20C).

At week 10 post infection, when compared to naïve mice, Sm+ mice were found with significantly reduced bone marrow frequencies of plasma cells, plasmablasts and total B cells ($p < 0.005$, $p < 0.005$, $p < 0.001$, respectively) (Figure 3.24D-E). In addition, a trend of reduced cell counts was observed for plasma cells, plasmablasts and total B cells absolute counts of Sm+ versus naïve mice at week 10, although the reduction was significant only for total B cells ($p > 0.05$, $p > 0.05$, $p < 0.001$, respectively; Figure 4.20D-E).

Taken together, a conclusion was reached that chronic schistosomiasis is associated with the depletion of bone marrow plasma cells, plasmablasts and total B cells at week 10 but this was not observed for acute schistosomiasis at week 4 post infection in mice.

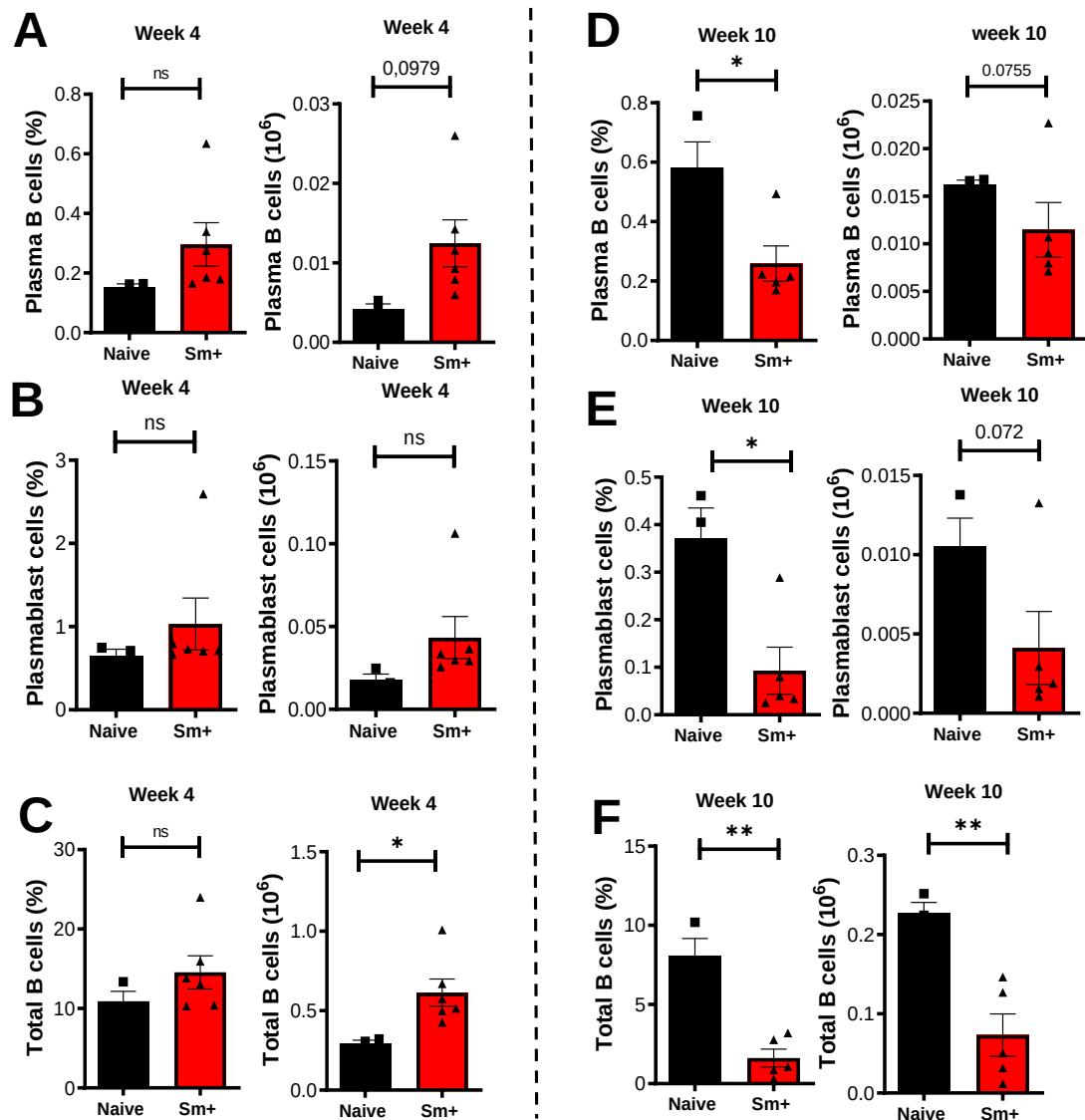


Figure 4. 20: Chronic schistosomiasis reduces bone marrow plasma cells by week 10 post infection in mice. Week 4 post *S. mansoni* infection.

A. Plasma cell frequencies and cell numbers; **B.** Plasmablast frequencies and cell numbers; **C.** Total B cell frequencies and cell numbers; **Week 10 post *S. mansoni* infection, D.** Plasma cell frequencies and cell numbers; **E.** Plasmablast frequencies and cell numbers; **F.** Total B cell frequencies and cell numbers; Data are expressed as mean $\pm$ S.E.M and are representative of 2 experiments; $n = 4 - 5$ mice; Data analysed Shapiro-Wilk test followed by either One way ANOVA and Bonferroni's multiple comparisons test or by Kruskal wallis followed by Dunn's multiple comparisons test; ns,, $p > 0.05$; * $p < 0.05$, ** $p < 0.001$; Sm, *S. mansoni*.

4.2 Discussion: Influence of schistosomiasis and its treatment with PZQ on polio vaccination in mice

Current strategies in measuring vaccine threshold levels of protection against disease are in many instances dependent on vaccine-specific antibodies (Plotkin, 2010). Under ideal circumstances, vaccine-specific serological memory in humans and animals can be maintained for months and years (Xiao and Daniell, 2017, Lightman *et al.*, 2019). This study demonstrates that DTPa-hepB-IPV-Hib vaccinated mice can produce long term polio specific serological memory in mice. Our findings corroborate with a previous study where IPV was shown to induce long term polio specific immunity for over 1 year (Xiao and Daniell, 2017). Combined with our laboratory's established mouse model of chronic schistosomiasis that is designed to mimic human infection in the field (Nono *et al.*, 2017), our study demonstrated that polio specific antibodies in DTPa-hepB-IPV-Hib vaccinated mice are depleted during chronic *S. mansoni* infection (Figure 4.21).

Similar findings where schistosomiasis drove impaired vaccine immune responses have been observed elsewhere in mice vaccinated with the diphtheria toxoid vaccine (Haseeb and Craig, 1997), bacille Calmette-Guérin (BCG) vaccine (Elias *et al.*, 2005), and HIV vaccines (Dzhivhuho *et al.*, 2018). In another study, Chen and colleagues reported a chronic *S. japonicum* driven impairment of immune responses to the hepatitis B vaccine in mice (Chen *et al.*, 2012). Experiments involving Olive baboons showed similar findings where the depletory influence of schistosomiasis on serological antibody memory immunity mounted by the Human Papillomavirus vaccine was observed (Gent *et al.*, 2019). This evidence taken together with our currently reported findings indicate that *Schistosoma* spp. impairs hosts' ability to maintain robust vaccine-induced long-term immunity that would otherwise be maintained without the parasite infections.

Intriguingly, in our findings, *S. mansoni* induced serum SEA specific antibodies increased exponentially over the course of the infection. Contrasting sharply, vaccine induced serum anti-polio antibodies were consistently reduced over time in the same mice. These juxtaposed findings in schistosomiasis burdened hosts indicated the impaired host's ability to produce anti-polio IgG antibodies in an environment where SEA antibody production remained efficient. These findings point towards the direct

impact of schistosomiasis in disrupting, specifically, unrelated antigen antibody memory but not that of schistosomiasis in the host.

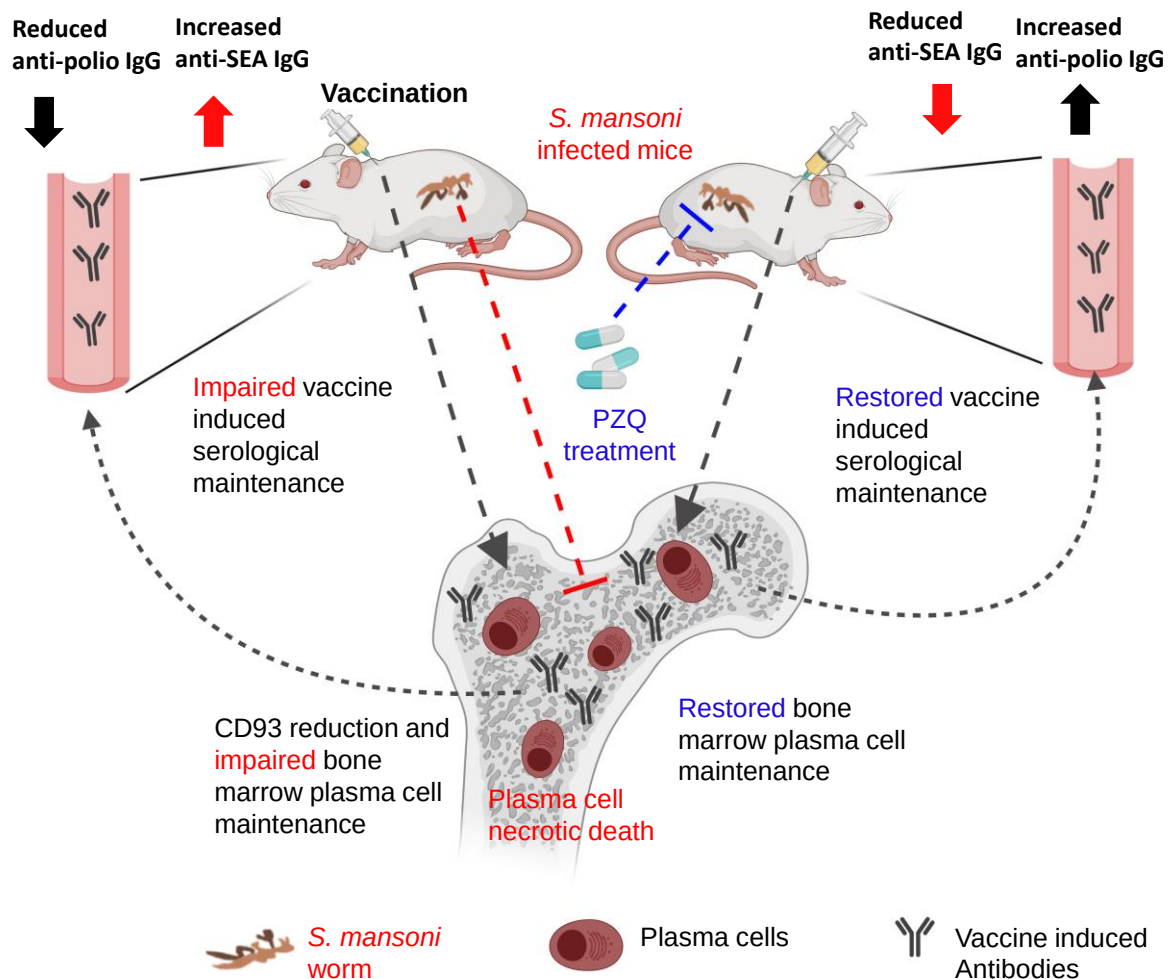


Figure 4. 21: Impact of schistosomiasis, and its treatment with PZQ, on vaccine induced serological immunity and bone marrow plasma cellular immunity.

Schistosomiasis reduces IPV induced anti-polio IgG antibodies in the serum of mice. The parasite also depletes bone marrow plasma cells through reduction of survival signal CD93 and increased necrotic driven death of plasma cells and plasmablasts in DTPa-hepB-IPV-Hib vaccinated mice. Treatment of infected mice with PZQ ameliorates the impact of schistosomiasis on serum anti-polio IgG antibodies and reduces the death of bone marrow plasma cells and plasmablasts to improve plasma cell survival. Figure made using Biorender.com

Plasma cells are critical in maintaining antibody production after vaccination (Slifka *et al.*, 1998, Khodadadi *et al.*, 2019, Lightman *et al.*, 2019). Our study showed the sustained influence of the DTPa-hepB-IPV-Hib vaccine in mice as indicated by

robustly elevated bone marrow plasma cells of vaccinated mice at week 18 post vaccination versus unvaccinated mice. Long lived mature antibody producing plasma cells reside in the bone marrow and some are found in secondary lymphoid organs such as the spleen (Slifka *et al.*, 1995, Ellyard *et al.*, 2004, Lemke *et al.*, 2016, Lightman *et al.*, 2019). Our evaluations of plasma cells in the bone marrow indicated a substantial impairment in the maintenance of these cells in vaccinated mice that were infected with schistosomiasis. This necrotic death driven impairment can be associated with the onset of the egg producing phase of *S. mansoni* infection as highlighted by the reduction of plasma cells at week 10 in non-vaccinated mice and at week 18 post infection in vaccinated mice. Combined, it is within reason to suggest that the depletory impact of schistosomiasis on plasma cells could be concentrated in the bone marrow. Hence, further experiments to uncover the impact of schistosomiasis on bone marrow health and its ability to sustain plasma cell homing factors in a schistosomiasis diseased environment are critical

Moreover, schistosomiasis has recently been demonstrated to promote bone loss in mice (Li *et al.*, 2021). We speculate that bone loss could be one of the drivers behind the bone marrow's reduced ability to sustain a conducive homeostasis in the presence of schistosomiasis. Indeed, it is a plausible speculation that the pathological changes induced by schistosomiasis within the bone marrow could have negatively impacted its ability to sustain the continual supply of survival signals to the bone marrow resident long-lived plasma cells. Supporting our speculation, our findings demonstrated a substantial reduction of the bone marrow associated plasma cell survival marker, CD93, a critical marker in long term plasma cell homing and antibody production persistence (Chevrier *et al.*, 2009, Haralambieva *et al.*, 2016, Lightman *et al.*, 2019). However, whether chronic schistosomiasis deleteriously influences the bone marrow hematopoietic processes during infection progression (Dessie *et al.*, 2020), and if these would interfere with the optimal maintenance of important survival signals for plasma cells in the bone marrow, for example, CD93 expression, requires further experimental evaluations.

Strikingly, correlation analyses showed that bone marrow plasma cells, while positively correlating with anti-SEA IgG titres, correlated negatively with anti-polio IgG titres. These findings strongly suggest a specific mechanism of the depletory nature of schistosomiasis towards plasma cells induced by unrelated antigens but

not those induced by the parasite. Perhaps the host limits the immunity against other non-threatening antigens in its bid to drive out the increasingly intense schistosomiasis pathology over time. However, this remains to be investigated. Baffled, we wondered if the negative impact of schistosomiasis on bone marrow plasma cells would be maintained in the absence of the vaccine. Indeed, our findings showed that in unvaccinated mice, schistosomiasis is associated with depleted bone marrow plasma cells, plasmablasts and total B cells by week 10 and not week 4 post infection, corresponding to the onset of chronic disease. This further suggests that chronic schistosomiasis was the major driver behind reductions in plasma cells. Combined, these results imply that although the body loses plasma cells during chronic schistosomiasis, immunity against the parasite is maintained while immunity to unrelated antigens is significantly lowered in a bid to selectively protect host from the currently pressing parasitic infection.

In the spleen, a similar trend to bone marrow findings was observed where vaccinated mice burdened with schistosomiasis had lower plasma cell frequencies than their non-infected counterparts. However, although the plasma cells were depleted in the spleen, our findings suggest the depletion was general and not specific for vaccinated animals because no plasma cell differences were observed between the spleens of naïve and vaccinated only mice. A possible reason behind our failure to identify heightened plasma cells in the spleen despite vaccination may be a case answered by the previously reported migration and homing of long-term plasma cells from lymphoid organs to the bone marrow niches (Szyszko *et al.*, 2011, Nutt *et al.*, 2015, Lightman *et al.*, 2019). Coupled with our earlier described findings of heightened plasma cell responses in the bone marrow of vaccinated mice, the migration and homing suggestion appears logical and highly probable.

Further reinforcing the success of our vaccination model, IgG1 memory B cells in the spleens of vaccinated mice were found at higher frequencies than those of naïve mice. In vaccinated mice, long lived IgG1 memory B cells have, in other studies, been identified as long as 60 days to 8 months post vaccination (Dogan *et al.*, 2009, Aiba *et al.*, 2010). Further, class switched IgG1 B cells are predisposed to differentiate into antibody producing plasma cells (Dogan *et al.*, 2009, Aiba *et al.*, 2010, Zuccarino-Catania *et al.*, 2014). Despite successful vaccination, our results showed notably increased IgG1 memory B cells in vaccinated mice infected with

schistosomiasis. These results show that the parasite also induces IgG1 memory B cells. Indeed, Th2 cell phenotype domination during schistosomiasis could be instrumental in driving B cell class switching to the IgG1 isotype (Fairfax *et al.*, 2012). Treatment against the parasite resulted in lowered IgG1 memory B cells, although this change was not significant. These findings suggest that the majority of these cells were products of schistosomiasis, although the IgG1 memory B cells induced by vaccination alone cannot be set aside, thus highlighting this study's resource driven limitation where antigen specificity could not be accounted for in our cellular analyses.

Yet another marker of successful vaccination, CD4⁺ T_{CM} cells (Esser *et al.*, 2003) were found significantly increased in vaccinated mice in opposition to non-vaccinated mice. However, CD4⁺ T_{EM} cells were not significantly elevated. Literature highlights that T_{CM} cells are generally found in lymphoid organs whereas T_{EM} cells are more common in peripheral tissue (Esser *et al.*, 2003). As such, it is possible that T_{EM} cells were lower in the spleen, a lymphoid organ, due to their migration to surrounding tissues. However, this was not investigated further in our study in which we did not assess other non-lymphoid tissue immunity. Intriguingly, the frequencies of both CD4⁺ T_{CM} and CD4⁺ T_{EM} cells were notably reduced in vaccinated mice that were infected with schistosomiasis. This implies that schistosomiasis also impairs the memory T cell machinery in vaccinated mice. A study by Xu and colleagues showed that schistosomiasis (*S. japonicum*) can drive Th1 and Th2 cell death by apoptosis to maintain a Th1/Th2 balance that hallmarks chronicity of the infection (Xu *et al.*, 2010). Likewise in our study, although we observed no significant differences in the frequencies of CD4⁺ Th cells among naïve mice, vaccinated only mice and vaccinated mice infected with schistosomiasis, we noted significantly higher deaths of CD4⁺ Th and follicular helper T cells during the infection. *In vitro* culturing has shown that SEA induces CD4⁺ T cell death by apoptosis (Lundy *et al.*, 2001). In our study, however, apoptosis was not measured for CD4⁺ T cells, but we do speculate that CD4⁺ T cell death could potentially have been driven by similar death mechanisms *in vivo*.

Furthermore, the substantial presence of regulatory Foxp3 expressing CD4⁺ T cells in the spleen of vaccinated mice exposed to schistosomiasis could suppress immune responses to third party antigens in both mice and humans, in line with previous

reports (Pacifico *et al.*, 2009, Layland *et al.*, 2013, Schmiedel *et al.*, 2015). The notable reduction of follicular B cells, follicular helper T cells and CD4⁺ T_{CM} in the spleen could negatively impact GC reactions that facilitate faster and more robust secondary immunity should vaccinated hosts encounter antigen from pathogen targeted for by vaccination. Hence our study strongly suggests that schistosomiasis could impair cellular responses that are essential in mounting vaccine immunity responses as well as vaccine immunity maintenance in the spleen. Along with the parasite's impact on the bone marrow, the infection could be immunosuppressive with severity associated with progressive disease in untreated hosts.

Assessing the impact of disrupting the progression of schistosomiasis, administration of PZQ treatment to vaccinated then schistosomiasis diseased mice positively countered the depletory impact of the parasitic disease on humoral responses and specific B and T cell responses. In the serum, anti-polio specific antibodies were partially restored in schistosomiasis infected mice that were treated with PZQ. In the bone marrow, plasma cells depleted through parasite association were restored. In the spleen, PZQ failed to restore plasma cell frequencies, nor did it manage to reduce the death of these cells and their precursors, the plasmablasts. However, notably, the drug restored frequencies of schistosomiasis reduced splenic total B cells, follicular B cells, MZ B cells, follicular helper T cells, CD4⁺ T_{CM} and T_{EM} cells. As such PZQ improved the sustenance of vaccination critical cells that could facilitate the replenishment of bone marrow plasma cells. Further, bone marrow plasma cell expression of survival marker CD93 was replenished, although non-significantly mathematically, post PZQ treatment. Together these findings suggest that PZQ treatment of schistosomiasis infected host is beneficial to vaccine specific humoral immune responses, and more generally, cellular immune responses. At the same time, the restoration of humoral and cellular immunity post successful treatment against the parasite as indicated by reduced schistosomiasis associate liver and gut pathology post treatment clearly demonstrates that the parasite is causative in the observed immunosuppression in untreated mice. Intriguingly, however, our study unravelled the complexity of the immunological machinery behind the restoration of antipolio IgG antibodies in mice treated with PZQ. As the impact of schistosomiasis was not strongly noted in the spleen, unlike in the bone marrow, it remains possible that perhaps traces of vaccine memory immunity remained within the splenic

compartment with the ability to partially restore vaccine specific immunity, thus representing a limitation of our study in which, due to technicalities and difficulties associated with polio tetramer acquisitions in our settings, we could not measure polio specific cellular immune responses in this compartment. However, considering the compromised state of the bone marrow, the threat to the survival of plasma in schistosomiasis infected mice indicates a dire outcome in the absence of praziquantel treatment.

Summarised, our study demonstrates that chronic *S. mansoni* infection is strongly associated with reduced maintenance of serological memory immunity in mice. The parasite also reduces vaccine cellular immunity induction and maintenance that is critical for effective vaccine immunity. The parasite reduces specific B cells in the bone marrow and spleen, particularly, plasma cells, and specific B and T cells in the spleen. In the bone marrow of *S. mansoni* infected mice, plasma cells are depleted by non-apoptotic necrotic cell deaths in addition to reduced survival through the reduction of plasma cell survival marker CD93. Importantly, PZQ treatment of schistosomiasis infected mice can reverse the negative influence of schistosomiasis vaccine specific immunity through the restoration of both humoral immunity and specific B and T cellular immunity.

Chapter 5: Concluding remarks

5.1 Investigating the influence of schistosomiasis on serological antibody memory from polio vaccination in schoolchildren from a schistosomiasis endemic area of Cameroon

Our study has demonstrated that schistosomiasis lowers poliovirus vaccine-specific immunity in children. Intriguingly, however, the parasite's impact on anti-polio serological antibody memory was dependent on the area of sampling and for reasons currently unknown. Building on these findings, there is a clear need for further exploratory research in children from areas where the *Schistosoma* spp. parasites are endemic. Examples of schistosomiasis critical research areas would be the evaluation of metabolomics and genetic studies, among others, to investigate the possible intrinsic host determinant factors that may render the parasite selectively influential on vaccine immunity in children from separate locations within the same parasite endemic region. However, the mechanisms behind such findings in our case are unknown, partly due to limitations on conducting potentially invasive studies in humans. For further investigations, we suggest the consideration of carefully controlled and ethically validated experiments involving human challenge models. These models would offer valuable evidence to decisively elucidate the parasite's impact on human immunity, in addition to offering effective potential insight for the much-needed alternative schistosomiasis treatment development (Langenberg *et al.*, 2020). Furthermore, based on our findings indicating the reduction of polio vaccine responses in older schoolchildren infected with schistosomiasis whereas the WHO recommended age of polio vaccination is below the age of 5 years (WHO, 2022), we suggest the consideration of catch up mass vaccinations for polioviruses and potentially other viruses such as measles (Nono *et al.*, 2018, Tweyongyere *et al.*, 2019), for older children in schistosomiasis endemic areas.

5.2 Assessing the impact of treating schistosomiasis by using PZQ, the sole commercially available drug against the disease, on serological antibody memory from polio vaccination in schoolchildren from a schistosomiasis endemic area of Cameroon

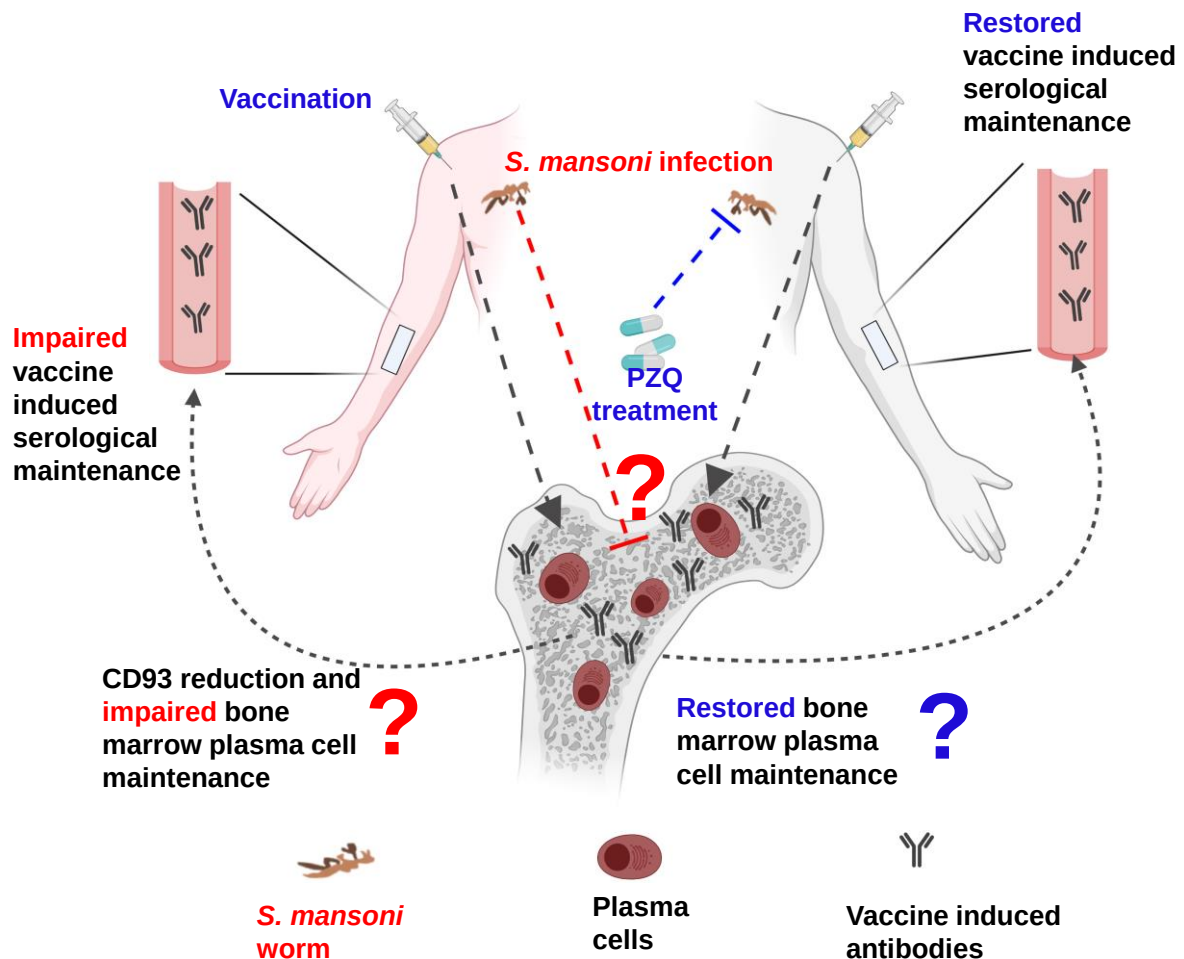


Figure 5. 1: Does schistosomiasis compromise the human bone marrow and consequently plasma cell Responses?

Figure made using Biorender.com

In the field, schistosomiasis is controlled primarily by PZQ treatment. In areas where the *Schistosoma* parasites are endemic, annual MDA campaigns are critical strategic interventions that control the parasitic disease. However, in endemic areas, people are often reinfected with schistosomiasis despite these annual mass PZQ treatments (Mutsaka-Makuvaza *et al.*, 2018), although new infections are notably reduced over time owing to the successes from continued PZQ treatment in both humans and mice (Mutsaka-Makuvaza *et al.*, 2018, Nono *et al.*, 2021). In our study, PZQ treatment of

schistosomiasis infected children who clear the disease, as shown by clearance of *Schistosoma* spp. eggs in stools, was instrumental in the restoration of polio specific humoral immunity (Figure 5.1). Hence, a conclusion is made that PZQ mediated clearance of the disease in children is a suitable strategy in curbing the immunomodulatory nature of *Schistosoma* spp. in vaccinated children. Additionally, we recommend the consideration of repeat mass PZQ treatment regimens, a strategy which has been demonstrated to dramatically improve cure rates, for example, a double dose of PZQ with over 90% cure rates in comparison to 63% from a single dose (Munisi *et al.*, 2017). However, although our study is robust statistically, where public health policymaking is concerned, we acknowledge the potential sample size limitations regarding the influence of PZQ on anti-polio immunity in children in our study. As such, we suggest the conduction of robust studies with larger sample sizes for confirmation and decision making where public health policy is concerned. Despite these limitations, our case highlights the importance of PZQ, whose impact not only controls morbidity against schistosomiasis but could well be indirectly relevant to impactful vaccination efforts in schistosomiasis endemic regions. Noteworthy, the potential resistance to PZQ remains concerning (Aruleba *et al.*, 2019, Spangenberg, 2021), especially considering the potentially far-reaching impact, as far as vaccination is concerned, of this drug in schistosomiasis endemic regions. Hence, as previously suggested, we additionally raise attention to the critical need for alternative treatment against schistosomiasis as the consequences of a diminished PZQ impact could be more far-reaching than previously envisaged.

5.3 Establishing an IPV vaccinated moused model, confirm anti-polio IgG specific antibodies maintenance following the subcutaneous injection of laboratory mice in our setting, and investigating the influence of *S. mansoni* infection on the serum titres of IPV-elicited polio-specific IgGs in the established IPV vaccinated laboratory mice.

To circumvent human-associated study limitations to mechanistically investigate the impact of *S. mansoni* in our study, we used mouse models for detailed insights. Confirming our earlier findings in humans, *S. mansoni* infection was associated with impaired serological anti-polio antibody memory in mice. Additionally, anti-polio antibody maintenance kinetic evaluations highlighted that the parasite's detrimental

impact occurs steadily over time, with a notable impact in the chronic stage of the infection. The detrimental capacity of schistosomiasis on anti-polio vaccine immunology was also demonstrably influenced by disease burden, as measured by organ (gut and liver) pathology, *S. mansoni* egg burden and SEA IgG antibody titres in comparison to anti-polio vaccine serological antibody memory.

5.4 Investigating if PZQ treatment against *S. mansoni* influences the IPV vaccination responses in *S. mansoni* diseased laboratory mice.

Similar to our findings in children, PZQ treatment in mice restored antipolio vaccine induced serological antibody immunity. Notably, similar to findings in humans, a recent study by our research group indicated that repeated rounds of PZQ treatment renders mice less susceptible to *S. mansoni* reinfections (Mutsaka-Makuvaza *et al.*, 2018, Nono *et al.*, 2021). Hence, in addition to reinforcing the importance of continued PZQ treatment campaigns despite reinfections, our combined mouse studies highlight the potential need for continued regular PZQ treatment consideration to ensure vaccination program success and effective parasite associated morbidity control in *Schistosoma* spp. endemic areas.

5.5 Assessing the influence of schistosomiasis and PZQ treatment on spleen and bone marrow B and T cells with particular interest on antibody producing plasma B cells.

Mechanistically, the lowered expression of plasma cell bone marrow survival markers in schistosomiasis infected mice, the reduction of plasma cell frequencies, and their increased death in both the bone marrow and spleens indicates, generally, that schistosomiasis affects systemic immunity in addition to the specific impact on vaccine serologic immunity.

Reinforcing the negative impact of *S. mansoni* infection in mice, splenic follicular B cells, follicular helper T cells, T_{CM} and T_{EM} cell reductions in anti-polio vaccinated/ *S. mansoni* infected mice demonstrates the parasite's ability to disrupt cell populations which are critical in adaptive immunity. These findings suggest that *S. mansoni* infection can impair, through cellular death, the adaptive-immunity-mediated

vaccine-specific immunity induction and maintenance. In humans, it has been reported that schistosomiasis downregulates the proliferation of B cells in *S. haematobium* infected children (Labuda *et al.*, 2013). Combined with our findings, it is reasonable to believe that *Schistosoma* spp. may lower the survival and production of specific B cell populations that are critical to the development of plasma cells in both humans and mice. However, as our cellular observations in our study were conducted only in mice, it still remains to be explored if *S. mansoni* infection impairs plasma cell maintenance in the spleens and bone marrow of humans (Figure 5.1).

5.6 Overall conclusion

In conclusion, this study demonstrated that *S. mansoni* infection can induce impaired vaccine immune responses in both children and mice. The parasite suppresses poliovirus vaccine induced antibody-mediated serological memory in both children and mice. The parasite also lowers the survival of plasma B cells in mice by reducing their survival as evidenced by reduced CD93 expression on the plasma cells, and their increased death by necrosis. The parasite also impairs plasma cells, follicular B cells, Th, follicular helper T cells and memory T cells in the mouse spleen, thereby essentially impairing cellular machinery critical to vaccine immunity mounting and maintenance within the lymphoid organ. These findings strongly suggest that schistosomiasis may even impair priming vaccine immunity in already infected mice, and potentially humans. However, this remains to be investigated. Additionally, anti-schistosomiasis treatment, PZQ, was demonstrated to restore humoral vaccine-specific immunity in both schistosomiasis infected children and mice, in addition to improving the general bone marrow and splenic adaptive cellular immunity in mice. Altogether, this study indicates that while schistosomiasis can be detrimental to viral vaccine immunity in children and mice, PZQ treatment provides an accessible counteractive strategic and critical control measure with the potential to improve the maintenance and possibly the induction of viral vaccine immunity in schistosomiasis endemic areas.

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Appendix 1: Voluntary questionnaires used for voluntary sample collection

FICHE D'ENQUETE		Projet : TMA2016CDF-1571																	
Site : _____																			
Ecole : _____ ; Classe : _____																			
A- PARTICIPANT																			
I- DONNEES SOCIODEMOGRAPHIQUES PARTICIPANT 1. Noms et prénoms _____ 2. Code _____ 3. Age _____ (ans) 4. Sexe _____ 5. Temps de résidence _____ ; Moyenne en classe (/20): _____ II- EXPOSITION 6. Contact avec l'eau 7. Nombre de fois / jour <table style="width: 100%;"> <tr> <td>- Bains ☐</td> <td>- Matin ☐</td> </tr> <tr> <td>- Lessive ☐</td> <td>- Midi ☐</td> </tr> <tr> <td>- Jeux ☐</td> <td>- Soir ☐</td> </tr> <tr> <td>- WC ☐</td> <td></td> </tr> </table> III- TRAITEMENT 8. Traitements reçus les 3 derniers mois PZQ OUI ☐ NON ☐ Autres OUI ☐ NON ☐ Lesquels ? _____ Date dernier traitement avec Praziquantel _____ Nombre de fois Praziquantel reçu _____ IV- SYMPTOMES 9. Avez-vous les symptômes de la Bilharziose ? Diarrhée OUI ☐ NON ☐ Fréquence selles / jour 1 ☐ 2 ☐ 3 ☐ 4 ☐ 4+ ☐ Brûlures/douleurs anales OUI ☐ NON ☐ Douleurs Abdominales OUI ☐ NON ☐ Nausées OUI ☐ NON ☐ Vomissement OUI ☐ NON ☐ Sang dans selles OUI ☐ NON ☐ Vertiges OUI ☐ NON ☐ Fièvre OUI ☐ NON ☐ Dyspnée OUI ☐ NON ☐ Céphalées OUI ☐ NON ☐ Courbatures OUI ☐ NON ☐ V- VACCINS Avez-vous vos vaccins à jour ? - Polio ☐ - Rougeole ☐ - Tuberculose ☐ - Hépatite B ☐ - Autres _____ VI- ANTECEDENTS 10. Avez-vous déjà eu la Bilharziose ? OUI ☐ NON ☐ 11. Combien de fois ? _____	- Bains ☐	- Matin ☐	- Lessive ☐	- Midi ☐	- Jeux ☐	- Soir ☐	- WC ☐		VI- EFFETS SECONDAIRES DU PRAZIQUANTEL 11. Parmi les effets suivants, lesquels avez-vous ressenti après traitement au Praziquantel ? <table style="width: 100%;"> <tr> <td>- Maux de tête ☐</td> <td>- Douleurs musculaires ☐</td> </tr> <tr> <td>- Etourdissement ☐</td> <td>- Perte d'appétit ☐</td> </tr> <tr> <td>- Maux d'estomac / crampes ☐</td> <td>- Sudation ☐</td> </tr> <tr> <td>- Nausées / vomissements ☐</td> <td>- Aucun ☐</td> </tr> <tr> <td>- Fatigue ☐</td> <td>- Autres _____ ☐</td> </tr> </table> 12. Si effet ressenti, avez-vous des lors peur de prendre le Praziquantel lors des campagnes de traitement ? OUI ☐ NON ☐ VII- LOCALISATION VIS-A-VIS DE LA RIVIERE 13. Quelle distance sépare la maison de la rivière ? _____ 14. Combien de temps mettez vous pour aller de la maison a la rivière ? _____ Données collectées par _____ Date: ____/____/____ Signature Superviseur: _____ Date: ____/____/____ <u>Investigateur Principal:</u> Dr Justin Komguep Nono (PhD), Chercheur, MinRESI <u>Co-Investigateur:</u> Mr Donald Severin Kamdem (MSc), PhD Fellow, Division Immunology, UCT, South Africa	- Maux de tête ☐	- Douleurs musculaires ☐	- Etourdissement ☐	- Perte d'appétit ☐	- Maux d'estomac / crampes ☐	- Sudation ☐	- Nausées / vomissements ☐	- Aucun ☐	- Fatigue ☐	- Autres _____ ☐
- Bains ☐	- Matin ☐																		
- Lessive ☐	- Midi ☐																		
- Jeux ☐	- Soir ☐																		
- WC ☐																			
- Maux de tête ☐	- Douleurs musculaires ☐																		
- Etourdissement ☐	- Perte d'appétit ☐																		
- Maux d'estomac / crampes ☐	- Sudation ☐																		
- Nausées / vomissements ☐	- Aucun ☐																		
- Fatigue ☐	- Autres _____ ☐																		

Code _____

FICHE D'ENQUETE

Projet : TMA2016CDF-1571

B- PARENT**I- NIVEAU D'EDUCATION**

Aucun ☐ Primaire ☐ Secondaire ☐
 Supérieur ☐ Inconnu ☐

II- CONNAISSANCES SUR LA BILHARZIOSE

1. Niveau de connaissance sur la Bilharziose

Aucun ☐ Faible ☐ Modérée ☐ Approfondie ☐2. Avez-vous déjà entendu parler de la Bilharziose ? OUI ☐ NON ☐

3. Ou avez-vous entendu parler de la Bilharziose ?

- Famille/ Amis/Voisins ☐ - Professionnel de la santé ☐- Réunion de quartier ☐ - Radio / TV ☐4. Etes vous concerné par la Bilharziose ? OUI ☐ NON ☐5. Savez-vous que la Bilharziose peut à long terme entraîner des dommages graves dans vos vies ? OUI ☐ NON ☐**III- CONNAISSANCES SUR LES COMPORTEMENTS A RISQUE**6. Savez-vous comment on contracte la Bilharziose ? OUI ☐ NON ☐

7. Parmi les propositions suivantes lesquelles pensez-vous être des comportements à risque pour attraper la Bilharziose ?

- Rapports sexuels ☐ - Agriculture ☐- Mauvaise hygiène sanitaire ☐ - Alimentation ☐- Contact avec l'eau (pêche ; bain...) ☐ - Alcool ☐- Consommation eau contaminée ☐ - Héritaire ☐- Echange de plats ☐ - Autres _____

Comment est votre système d'évacuation des selles ?

- Cours d'eau ☐- Fosse septique ☐- Toilettes modernes ☐**IV- CONNAISSANCES SUR LES MODES DE TRANSMISSION**8. Savez-vous comment la Bilharziose peut être transmise ? OUI ☐ NON ☐

9. Parmi les propositions suivantes lesquelles pensez-vous être des moyens de transmission de la Bilharziose d'une personne à l'autre ?

- Rapports sexuels avec une personne infectée ☐- Personne infectée qui urine ou fait les selles dans l'eau ☐**V- CONNAISSANCES SUR LA PREVENTION**

10. Comment pensez vous que l'on peut éviter la Bilharziose ?

- En traitant les personnes malades ☐- En traitant tout le monde ☐- En construisant des toilettes modernes ☐- En observant une meilleure hygiène de vie ☐- En traitant les sources d'eau ☐- Abstinence sexuelle/Rapports sexuels protégés ☐- Aucune idée ☐**VI- CONNAISSANCES SUR LES SYMPTOMES/CAT**11. Connaissez-vous les symptômes de la Bilharziose ? OUI ☐ NON ☐12. Citez _____

 _____**VI- CONNAISSANCES SUR LES SYMPTOMES/CAT (suite)**

13. Quand vos enfants ont les symptômes de la Bilharziose. Que faites-vous ?

- Attendre que ça finisse seul (pas d'argent) ☐- Aller à l'hôpital consulter ☐- Aller chez un guérisseur traditionnel ☐- Aller acheter les médicaments en pharmacie ☐- Prier ☐- Rien ☐**VII- CONNAISSANCES SUR LE TRAITEMENT**14. Existe-t-il un traitement contre la Bilharziose OUI ☐ NO ☐15. Connaissez-vous le nom ? OUI ☐ NO ☐

Si oui donnez le nom _____

16. Vos enfants ont déjà reçu le traitement ? OUI ☐ NO ☐17. Voulez-vous qu'ils continuent de recevoir OUI ☐ NO ☐**VIII- CONNAISSANCES SUR LA PROTECTION**18. Protégez-vous contre la Bilharziose ? OUI ☐ NO ☐

Comment ?

- En évitant les rapports non protégés ☐- En évitant les contacts avec l'eau ☐- En respectant une bonne hygiène de vie ☐- En traitant l'eau à boire ☐- En Prier ☐- Rien ☐**VIII- STATUT FAMILIAL**19. Combien de personnes vivent en permanence chez vous ? _____

Données collectées par _____

Date: ____/____/____

Signature Superviseur: _____

Date: ____/____/____

Investigateur Principal:**Dr Justin Komguep Nono (PhD),
Chercheur, MinRESI****Co-Investigateur:****Mr Donald Severin Kamdem (MSc), PhD Fellow,
Division Immunology, UCT, South Africa**

FICHE D'ENQUETE

Project : TMA2016CDF-1571

Site : _____

C- EXAMINATION et LABORATOIRE

1. Code _____

2. Echantillons reçus

- Selles ☐ Couleur des selles : _____
- Sang ☐
- Echo ☐
- Salive ☐

3. Examen

- Taille (cm) : _____
- Poids (kg) : _____

- | | Oui | Non |
|--|--------------------------|--------------------------|
| - Signes d'anémie ☐ | ☐ | ☐ |
| - Hépatomégalie ☐ | ☐ | ☐ |
| - Splénomégalie ☐ | ☐ | ☐ |

4. Kato Katz

- *S. mansoni* Négatif ☐ Positif ☐
- Charge parasitaire _____
- Autres parasites Négatif ☐ Positif ☐
- Espèces _____
- Charge parasitaire _____

5. TDRs

- | | | |
|------------------|----------------------------------|----------------------------------|
| - TDR HIV | Négatif ☐ | Positif ☐ |
| - TDR Hépatite B | Négatif ☐ | Positif ☐ |
| - TDR Hépatite C | Négatif ☐ | Positif ☐ |
| - TDR Paludisme | Négatif ☐ | Positif ☐ |
| Espèces _____ | | |

6. Biochimie

- ASAT _____
- ALAT _____
- Gamma GT _____
- Alkaline phosphatase _____
- Billirubine _____
- Albumine _____
- Ostéopontine _____

7. ELISA

- IgG Polio _____
- IgG Rougeole _____
- IgG HepB _____
- IgG BCG _____
- Multiplex Oui ☐ Non ☐

Investigateur Principal :

Dr Justin Komguep Nono (PhD),
Chercheur, Ministère Recherche Scientifique (MinRESI), Yaounde, Cameroun / Group Leader
Immunobiology of Helminth infection group,
Division Immunology, UCT, South Africa

Co-Investigateur:

Mr Donald Severin Kamdem (MSc),
PhD Fellow, Division Immunology, UCT, South Africa

Appendix 2: Representative childhood vaccination schedule in Cameroon

(N.B.: Population ciblée 0-11 mois
N.B.: Target population 0-11 months)

CALENDRIER DE VACCINATION INFANTILE CHILD VACCINATION SCHEDULE

AGE	VACCINS	MALADIES	FAIT LE/DONE ON	RENDEZ-VOUS	SIGNATURE
A la naissance At birth	BCG	Tuberculose / Tuberculosis			
	VPO 0	Poliomyélite / Poliomyelitis			
A 1 mois 1/2 At 1 st months	VPO 1	Poliomyélite / Poliomyelitis			
	DTC-Hep-Hib 1	Diphtérie - Tétanos - Coqueluche / Hépatite - Hemophilus Int*			
	Pneumo 13-1	Infections à Pneumocoque / Pneumococcal Infections			
	Rotarix 1	Diarrhée / Diarrhea			
A 2 mois 1/2 At 2 nd months	VPO 2	Poliomyélite / Poliomyelitis			
	DTC-Hep-Hib 2	Diphtérie - Tétanos - Coqueluche / Hépatite - Hemophilus Int*			
	Pneumo 13-2	Infections à Pneumocoque / Pneumococcal Infections			
	Rotarix 2	Diarrhée / Diarrhea			
A 3 mois 1/2 At 3 rd months	VPO 3	Poliomyélite / Poliomyelitis			
	DTC-Hep-Hib 3	Diphtérie - Tétanos - Coqueluche / Hépatite - Hemophilus Int*			
	Pneumo 13-3	Infections à Pneumocoque / Pneumococcal Infections			
	Polio injectable-VPI	Poliomyélite / Poliomyelitis			
A 6 mois At 6 months	Vitamine A	Avitaminose A / A Deficiency			
A 9 mois At 9 months	VRR	Rougeole / Measles			
	VAA	Fièvre Jaune / Yellow Fever			

* Diphtérie - Tétanos - Whooping cough - Hépatite B - Hemophilus influenza

AUTRES VACCINATIONS ET VIT. A - RAPPELS / Others vaccinations Booster

Date	Vaccin	Rendez-vous	Signature	Date	Vaccin	Rendez-vous	Signature
27/11/21	RR2	18/12/21					

Appendix 3: List of antibodies used for flow cytometry analysis of lymphocyte populations

Dye	Marker	Company	Lot number
APC	CD21/35	BD Bioscience	558658
APC	Foxp3	BD Bioscience	560401
PE-CY7	CD23	BD PharMingen	562825
PE-CY7	CXCR5	BD Bioscience	560617
PerCP-Cy5.5	CD93	Biolegend	136511
PerCP-Cy5.5	CD4	BD Bioscience	550954
PE	Annexin V	BD Bioscience	51-65875X
FITC	IgD	BD PharMingen	05064D
FITC	CD44	BD Biosciences	553133
V450	IgG1	BD Bioscience	562580
V450	CD62L	BD Biosciences	560507
APC-R700	CD138	BD Bioscience	565176
A700	CD3	BD Bioscience	557984
APC-CY7	CD19	BD Bioscience	557655
APC-CY7	BCL6	BD Bioscience	563581
BV510	B220	Biolegend	103248
V500	CD8	BD Biosciences	560776
PE-TR	IgM	BD Biosciences	553515
BV605	Live/Dead	BD Biosciences	565694