Cross-reactivity of *Schistosoma mansoni* antigens and Timothy grass pollen allergens Phl p 1 and Phl p 5: Blocking activity of rabbit Sm-SEA-induced IgG anti TGP antibodies

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GENERAL ABSTRACT

Western immunoblotting has been used to investigate IgG cross-reactivity between *Schistosoma mansoni* soluble egg antigens and crude extracts of the allergen Timothy grass pollen. Cross-reactivity was observed, with antibodies binding to allergen extracts also recognising epitopes present on the major immunogenic egg glycoproteins, namely IPSE/alpha-1, kappa-5 and omega-1. The blocking activity of cross-reactive rabbit IgG antibodies was tested using the RS-ATL8 basophil cell line, in which the cells were sensitised with human sera from patients allergic to Timothy grass pollen and activated with the pollen extract. Although minimal, some blocking ability of cross-reactive rabbit IgG antibodies was detected. The results are discussed in the context of hygiene hypothesis.

ACKNOWLEDGEMENTS

This project was part of the ongoing research to investigate cross-reactivity between common allergens and *Schistosoma mansoni* antigens. I would like to express my gratitude to the following people because without them the project would never have succeeded. I wish to thank my project supervisor Professor Mike Doenhoff for his expertise, support and guidance throughout my year in research. Thanks also to Prof Jan Bradley for agreeing to supervise me. In particular thanks to Dr. Franco Falcone and Eman Ali who offer help to me generously. Thank you to the post-doctorate workers and PhD students, Marwa El-faham and Joseph Igetei, for demonstrating the procedures necessary to carry out the laboratory work and for consistently answering my questions. Thanks to all the technical staff that provided information and worked tirelessly behind the scenes to ensure the laboratories run smoothly. Finally, I would like to thank my dear family and friends who supported me unconditionally.

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Abbreviations

BSA	Bovine serum albumin
CCD(s)	Carbohydrate determinant(s)
CH	Cercariae homogenate
FcεRI	high-affinity receptor Fc epsilon RI for IgE
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IL	Interleukin
IPSE/alpha-1	interleukin-4-inducing factor from schistosome eggs
NCP	Nitrocellulose
NFAT	Nuclear factor of activated T-cells
SDS	sodium dodecyl sulphate
SEA	schistosome egg antigens
SmSEA/SEA	*Schistosoma mansoni* soluble egg antigen

TGP	Timothy grass pollen
Th1	T helper 1
Th2	T helper 2
Treg	Regulatory T
WH	Worm homogenate

Chapter 1 INTRODUCTION

1.1 General introduction – the hygiene hypothesis

The hygiene hypothesis has often been seen as one of the explanations for rising allergic diseases. It is speculated that human populations that have had lower exposure to parasitic and microbial infections due to living in environments and prevention of diseases by vaccinations, both brought about by modern civilisation, have immune systems which have undergone maturation in a different manner when compared to the pre-hygienic olden days (Rook, 2007).

The immuno-pathogenesis of allergy and asthma is mediated by immunoglobulin E (IgE) antibody. Exposure to allergen molecules in sensitised individuals, results in IgE antibodies, which are located on the surface of tissue mast cells or blood basophils, cross-linking cell surface IgE receptors (FcεRI). The cells are stimulated to produce inflammatory mediators such as prostaglandins and histamine (Platts-Mills, 2001; Gould and Sutton, 2008). Th2 cells produce cytokines, namely interleukin (IL)-4, IL-5 and IL-13, to modulate B lymphocytes to produce IgE. This is different from Th1 cells which produce interferon gamma (IFNγ) as their main cytokine product (Coffman, 1989).

Allergy and asthma are seen as the consequences of immune response disorders due to the occurrence of hypersensitive reactivity against allergens, involving Th2 cells and specific IgE antibody production.

Advanced health care systems and improved hygiene, the outcomes of modern civilisation, have lessened contact with pathogens and contributed to reduced infections among us. However, decreased exposure to microorganisms might result in a lack of stimulation of the immune system. Such insufficiency might lead to an altered programming of the immune system and promote uncontrolled expression of inflammatory molecules, resulting in increasing inflammatory diseases especially in developed countries.

1.2 Helminth infection and protection against allergy

Over the years, evidence has shown that although helminths are strong inducers of Th2-biased immune responses, helminth infections can protect the host against Th2-mediated allergic pathologies. Studies on murine model systems have shown that alleviation of allergy is related to expansion of

regulatory T (Treg) cell activity instead of immune deviation to Th1. However, helminth infection does not prevent allergen sensitisation. In fact, helminth infection restricts the Th2 effector phase which is responsible for inflammation. Treg cells can transfer suppression of allergic inflammation from an infected, allergen-naive animal to an uninfected, sensitised recipient (Okano et al., 1999; Maizels, 2005; Kitagaki et al., 2006a; Anthony et al., 2007). A similar regulatory network may be controlling immunopathologic disease in man, as allergy in humans may also be modulated by helminth infections (Maizels, 2005).

Although Th2 immune responses triggered by allergens or helminths share many common features, there are some differences between them. Allergen-specific IgE can be detected in atopic patients most of the time. The hallmark of helminth infections is high IgE levels, both of specific antibodies and of apparently non-specific IgE. Apart from that, anaphylaxis mostly occurs in atopy but only uncommonly in helminth infections, possibly due to concomitant high IgG levels in the latter or to the activity of Treg cells which are induced by the helminths. These mechanisms have been

thought to be associated with helminth-induced protection from allergic responses (Erb, 2007).

1.3 *Schistosoma mansoni* (*S. mansoni*)

Parasitic helminths, including *Schistosoma mansoni*, have been widely studied as one explanation of the hygiene hypothesis suggests they are a key immune modulator that can promote proper development of the immune response.

1.3.1 Schistosomiasis

Schistosoma mansoni (*S. mansoni*) is responsible for a neglected tropical disease (NTD) called human schistosomiasis. Schistosomiasis, also known as bilharzia, is caused by infection with blood flukes (trematode worms) of the genus *Schistosoma* (CDC, 2012). Schistosomiasis is a disease which has the highest rates of incidence in Africa, but is also wide-spread in other tropical and sub-tropical areas. It is an acute and chronic parasitic disease that affects over 200 million people worldwide (Chitsulo et al. 2004) and results in hepato-intestinal or urinary tract pathology. Infection results from exposure to contaminated water.

In 2014, at least 258 million people were estimated to receive preventive treatment. In order to reduce and prevent morbidity, the World Health Organisation has suggested preventive treatment need to be repeated over a number of years (WHO, 2016).

1.3.2 The *S. mansoni* life cycle

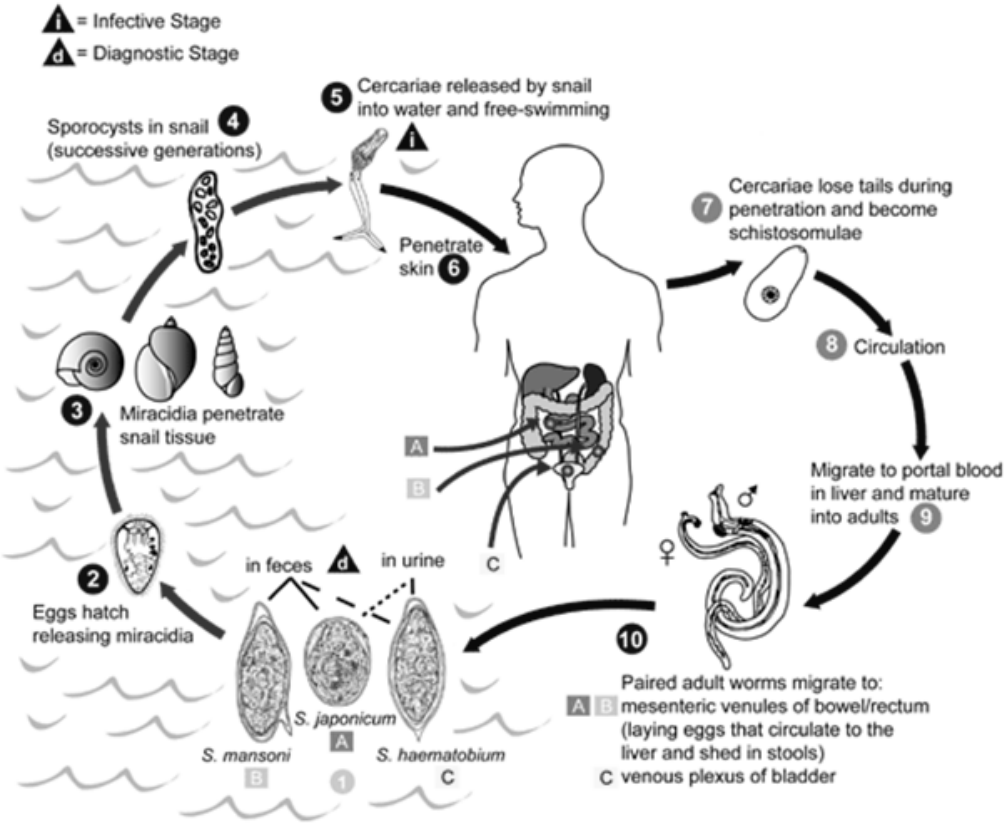


Figure 1-1 Different stages of *S. mansoni* life cycle (CDC, 2012)

Different stages of *S. mansoni* life cycle are illustrated in Figure 1-3. Two hosts, a vertebrate and a molluscan invertebrate, are required for completion of the lifecycle of *S. mansoni* and of all other schistosomes. Sexual reproduction and egg-laying occur in the vertebrate as the definite host whereas asexual reproduction occurs in the molluscan invertebrate host which serves as the intermediate host.

1.3.2.1 Cercariae

During daylight, cercariae emerge from the infected snails into their surrounding water. With the aid of their bifurcated tail, cercariae swim rapidly to scout for a susceptible host. The forked-tailed larvae are stimulated upon sensing lipid on the surface of human skin to make contact therewith, get attached and begin penetration. The carbohydrate-rich surface glycocalyx, a potential activator of complement, is shed by the cercariae after the penetration is complete (Tavares et al., 1978). At the same time, the cercaria loses its tail while the head transforms into an endoparasitic larvae called the schistosomulum (Al-Adhami et al., 2005).

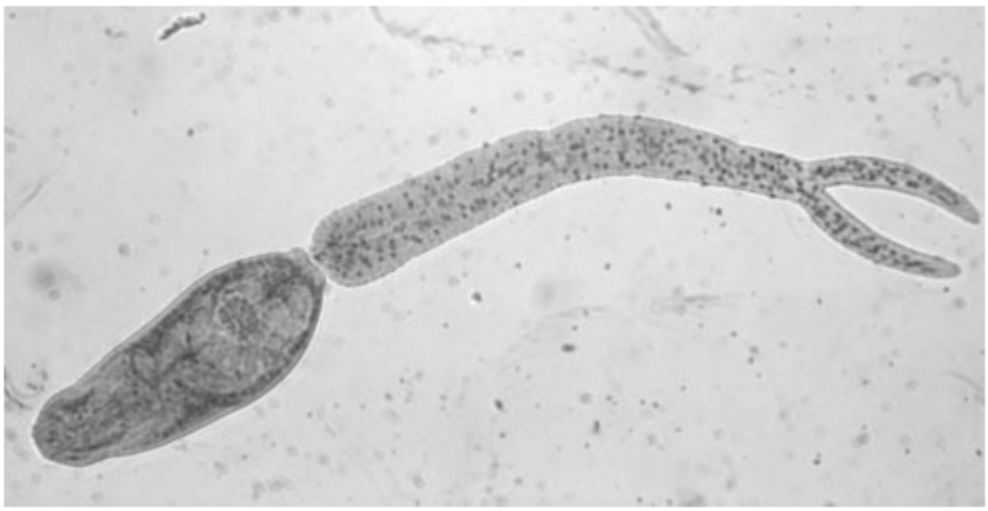


Figure 1-2 *S. mansoni* cercariae

Figure 1-2 from Emery (2011) shows a cercaria of *S. mansoni.*

1.3.2.2 Schistosomula

Schistosomula are the last larval stage in the life cycle of schistosome development. After spending 2 to 3 days in the host's skin (Kusel et al., 2007), the schistosomulum penetrates a blood or lymphatic vessel and is carried in the circulation throughout the host's body. Five to six days post-penetration, a large numbers of larvae ae found in the lungs (of infected mice) on their migrate route throughout the body's blood circulation. Schistosomula or young worms are found in the hepato-portal circulation from 7 to 10 days after cercarial infection. From around 10 days after skin penetration, they begin to feed on red blood cells and regurgitate

indigestible haem as heamozin pigment (Oliveira et al., 2000). When suitable partners of the opposite sex come into contact, a monogamous pair is formed and the worms further develop into sexually mature adults while migrating to the mesenteric veins together (Beltran and Boissier, 2008).

1.3.2.3 Adult Worms

Adult schistosomes are sexually dimorphic and live as pairs: the female is thinner, more elongated and is held within the gynaecophoric canal of the male. The adult worm pair travels against the blood flow to a mesenteric capillary, the final niche for *S. mansoni* worms, to start the process of egg-laying 4 to 5 weeks after infection (of mice). Females of *S. mansoni* are capable of releasing approximately 300 eggs per day (1 egg in every 4.8 minutes) inside the blood vessel (Erasmus, 1987). Depending on species, some of the eggs are passed out along with the host's urine or excreta while others are carried by blood flow to become trapped in the liver (*S. mansoni* and *S. japonicum*) or in the urino-genital tissues (*S. haematobium*), which leads to pathology.

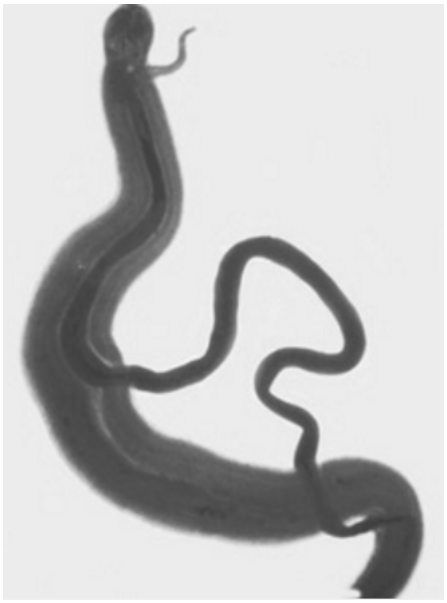


Figure 1-3 Adult worms of *S. mansoni*

Figure 1-3 from CDC (2012) shows the adult worms of *S. mansoni* in which the thinner female resides in the gynaecophoric canal of the thicker male.

1.3.2.4 Egg production

Females of *S. mansoni* devote a huge portion of their body mass to the reproductive system (Cheever et al., 1994). Approximately the dry weight of the female's body is converted each day to eggs production inside the blood vessel. Eggs become extravasated and enter the liver, intestinal or bladder tissue. The excretion of eggs from host tissues in vertebrates is said to be facilitated by granulomas that form

around them, allowing the eggs to penetrate into the gut lumen where they leave the body in stool (Doenhoff et al., 1986). When an egg reaches fresh water, it hatches into a miracidium, a ciliated, free-swimming larva. Egg hatching takes place in response to favourable environmental factors (for instance, light and temperature), osmotic changes caused by water entry (Kusel, 1970; Kassim and Gibertson, 1976), molecular and physiological activities such as calcium ion fluxes and some enzymatic activities such as leucine amino-peptidases (Ashton et al., 2001; Rinaldi et al., 2009).

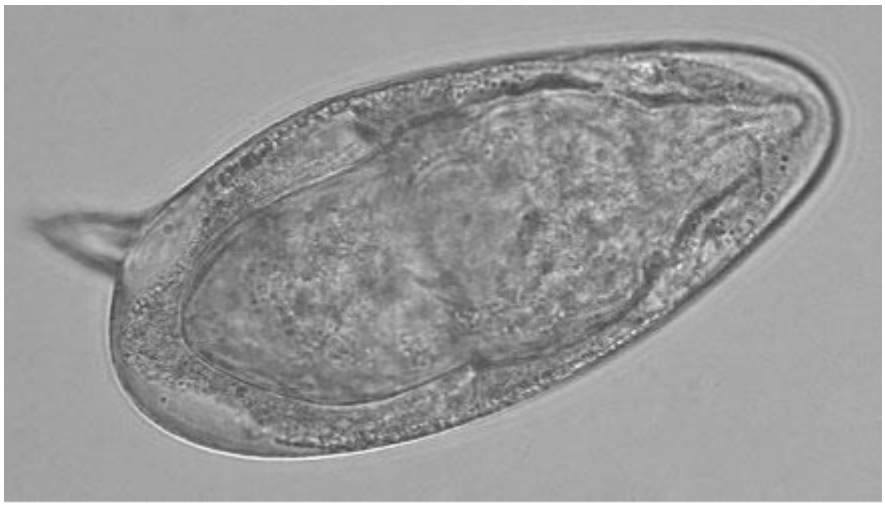


Figure 1-4 Egg of *S. mansoni*

Figure 1-4 from Emery (2011) shows the image of an egg of *S. mansoni*.

1.3.2.5 Miracidium

The miracidium is free-living and has a short life span. In order to survive, it must find a suitable host to undergo further development. Studies suggested a miracidium that hatches from an egg will survive for five to six hours in optimum conditions (Haas et al., 1995; Haberl et al., 1995; Roberts and Janovy, 2010). Miracidia of schistosomes are positively phototrophic even though they do not possess photoreceptors (Brooker et al., 1972). Haas (2003) stated that a miracidium is stimulated to swim more rapidly and change direction much more frequently when it enters the vicinity of a snail host, thus increasing its chances of host-encounter (Haas, 2003). Miracidia of *S. mansoni* search for susceptible fresh water snails of the genus *Biomphalaria*, with species varying between regions, for example, *B. alexandrina* in North Africa, Yemen and Saudi Arabia; *B. glabrata* in the Western Hemisphere and *B. rupellii*, *B. sudanica*, *B. pfeifferi* and others of the same genus in other areas of Africa (Kalbe et al., 1996) Several hours post-penetration of a miracidium, it undergoes a few asexual reproductive stages of differentiations and develops via two generations called mother-sporocyst and daughter sporocyst, into the cercaria. One consequences of asexual reproduction within the snail is

the numerical multiplication of the parasite as a single miracidium can give rise to thousands of viable cercariae. Each cercaria has the potential to transmit infection to the next stage.

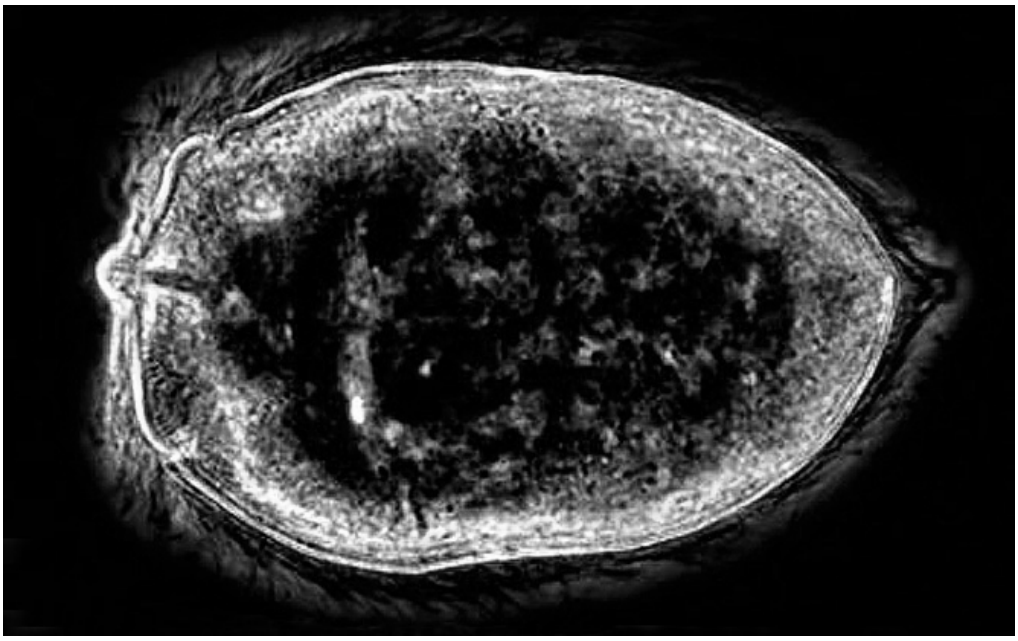


Figure 1-5 Miracidium of *S. mansoni*

Figure 1-5 from Emery (2011) shows the miracidium of *S. mansoni*.

1.3.3 Transmission of schistosomiasis and epidemiology

Human schistosomiasis is a human disease caused mainly by *S. mansoni*, *S. haemoatobium* or *S. japonicum*. Infection is transmitted by cercariae, which are present in fresh water bodies in subtropical and tropical regions (NHS, 2016b). As such, schistosomiasis transmission takes place when

freshwater sources are contaminated with the excreta of schistosomiasis patients as their excreta contain parasite eggs from which miracidia hatch and penetrate and undergo asexual reproduction within the snail host. When an individual comes into contact with the infested water, they will be infected as cercariae, the larval forms of the parasite which are released by freshwater snails, penetrate their skin. These larvae will further develop into adult schistosomes in the body in which they reside in the blood vessels where the female worms release eggs. Some of the eggs are expelled from the body in urine or faeces as a continuation of parasite's life-cycle whereas others that are trapped in body tissues will further cause immune reactions, damaging organs progressively.

Schistosomiasis is prevalent in tropical and subtropical areas, particularly in poor communities with inadequate sanitation and limited or no access to safe drinking water. It is estimated that at least 90% of the people suffering from schistosomiasis who require treatment live in Africa (WHO, 2016).

Hepato-intestinal and urogenital schistosomiasis are the two major forms of the disease, caused by five main species of blood fluke, namely *S. mansoni*, *S. japonicum*, *S. mekongi*, *S. guineensis* and *S. haematobium*. According to the statistics provided by (WHO, 2016), intestinal schistosomiasis is mainly caused by *S. mansoni* (Africa, Middle Easy, Caribbean, Brazil, Venezuela and Suriname).

1.3.4 Causes and symptoms of schistosomiasis

Schistosomiasis mostly affects poor and rural communities, especially among agricultural and fishing populations. Carrying out domestic chores in infested water will put people at risk. Inadequate hygiene and exposure to infested water will make children especially vulnerable to infection (NHS, 2016b).

The disease has been introduced to new areas when migration to urban areas and population movements take place. Increased population size, hydrological development schemes and environmental modifications to supply basic needs are some of the factors that facilitate transmission. In addition, the increasing numbers of tourists or travellers to these areas

has heightened the risk of those not living in endemic areas contracting schistosomiasis.

Symptoms of schistosomiasis are caused by the body's reaction to the worms' eggs. Depending on the exact area that's infected, people affected with chronic schistosomiasis can suffer from a range of symptoms and problems. Generally, affected organs can suffer long-term damage when the patients fail to receive treatment (NHS, 2016b).

Intestinal schistosomiasis can lead to anaemia, abdominal pain and swelling, diarrhoea and hematochezia (blood in the stool) in patients. Symptoms commonly seen in advanced cases include liver enlargement, fluid accumulation in the peritoneal cavity. Hypertension of the abdominal blood vessels and enlargement of spleen may also occur (WHO, 2016). Schistosomiasis can also cause anaemia, stunted growth and a reduced ability to learn in children, though the effects are usually reversible with treatment.

Chronic schistosomiasis may disrupt people's working ability and in some cases may result in death (van der Werf et al.,

2003). Schistosomiasis is not the type of disease that causes death directly or instantly but causes more discomfort or disability which eventually leads to death in the long run by other pathologies or complications. Estimation of the number of deaths due directly to schistosomiasis is difficult because of hidden pathologies such as liver failure, kidney failure and bladder cancer and the presence of other infections. Therefore, estimates range widely between 20 000 and 200 000 deaths per annum (WHO, 2016).

1.3.5 Diagnosis of schistosomiasis

Diagnosis of schistosomiasis is through the detection of parasite eggs in urine or stool samples from the patients (WHO, 2016). Another indication of infection is the presence of antibodies and or antigens in urine or blood specimens.

The Kato-Katz technique is used to detect the eggs of intestinal schistosomiasis in faecal specimens through use of methylene blue-stained cellophane soaked in glycerine placed on samples of stool on glass slides (WHO, 2016).

Serological and immunological tests are performed, to check exposure to infection and the need for thorough examination, treatment and follow-up, especially for people residing in non-endemic or low-transmission areas.

1.3.6 Control and treatment of schistosomiasis

Schistosomiasis is being controlled by implementing large-scale treatment targeted at affected population groups, particularly school children, hygiene education, better sanitation, access to safe water as well as snail control.

Based on recommendations of the World Health Organisation (WHO, 2016), the strategy is generally to focus on achieving a reduction in disease levels through periodic, regular and targeted treatment with praziquantel through the large-scale drug administration in at-risk population groups. Elimination of the disease is aimed for in a few low transmission countries.

Praziquantel is an effective, safe and low-cost treatment that has been recommended to treat all forms of schistosomiasis. Although re-infection often occurs after treatment has been given, the risk of developing severe late-stage disease is

reduced and even reversed, particularly in children, when treatment is initiated and repeated early on (WHO, 2016).

1.4 Immune-pathogenesis of *S. mansoni* infections

S. mansoni infection can be further categorised into acute infection and chronic infection. The immune-pathogenesis of both types of infection is different from each other. Most information regarding immunopathology of *S. mansoni* infection is derived from murine models in various studies (Pearce et al., 1991; Pearce and MacDonald 2002; Araújo et al., 2004; Wilson et al., 2007).

Immune responses induced by *S. mansoni* egg antigens are highly Th2-biased. As the mechanisms by which *S. mansoni* eggs traverse the endothelium, intervening tissues and mucosal epithelium are dependent on host immune responsiveness, inflammation is required for the eggs to exit the body successfully. Hence, these eggs are highly visible to the host immune system and are vital for inflammatory lesion development as they pass across the intestinal or bladder wall (Pearce, 2005).

1.4.1 A comparison between acute and chronic *S. mansoni* infection

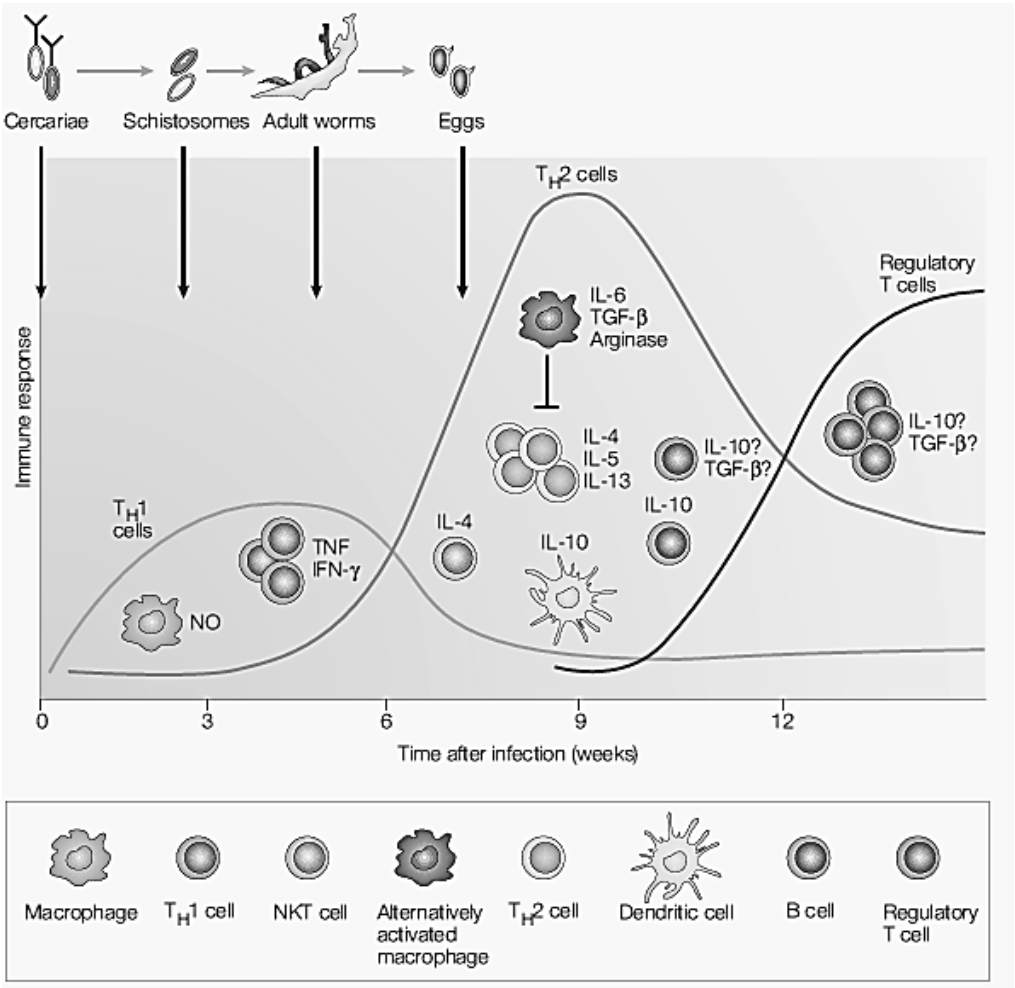


Figure 1-6 Induction of Th1 and Th2 cell responses and development of Treg cells after *S. mansoni* infection (Dunne and Cooke, 2005)

Figure 1-6 from Dunne and Cooke (2005) illustrates the development of the immune response triggered by *S. mansoni*

infection in vertebrate hosts. After infection with *S. mansoni*, a Th1 cell response is the first to be initiated and developed. As the parasitic worm develops and eggs are released and deposited, natural killer T (NKT) cells are activated. This is followed by increased production of interleukin-10 (IL-10) and less production of IL-12 by the dendritic cells, and a Th2 cell response also develops.

Acute *S. mansoni* infection occurs in individuals who encounter the parasite for the first time in an endemic area, with no previous exposure. This type of infection is characterised by leucocytosis (abnormally high leukocyte counts in blood), eosinophilia (increased number of eosinophils in blood) and a high cellular immune response to schistosome antigens particularly those from the parasite's eggs (Caldas et al., 2008). However, a mixed T helper type 1 (Th1) and T helper type 2 (Th2) cytokines profile can be observed during acute infection.

A previous study by Grzych et al. (1991) mentioned that during the first 5 weeks of infection, Th1 cell immune response develops. However, as the infection progresses and

eggs start being release by the worm, a Th2 immune response arises while the Th1 response diminishes.

A recent review by Schramm and Haas (2010) that when acute infection takes place, schistosome eggs trigger an inflammatory Th2 response that is characterised by high immunoglobulin E (IgE) levels and increased numbers of eosinophils in the blood. Subsequently, anti-inflammatory mechanisms help to down-regulate the Th2 response in order to reduce host tissue damage (Schramm and Haas, 2010). In view of this, the cytokine interleukin 4 (IL-4) plays a central role, acting as the key cytokine to induce a Th2 response as well as a key factor to limit inflammation from wide-spreading (Schramm and Haas, 2010).

According to Okano et al. (2001), induction of Th2 responses by *S. mansoni* egg antigen is largely due to carbohydrates on the antigen that function as Th2 immune response-promoting adjuvants. A schistosome-egg glycoprotein has also been identified that induces the release of IL-4 and IL-13 from basophils, by non-specifically binding and possibly crosslinking cell-surface IgE (Schramm et al., 2003). In addition, egg glycoproteins can also bind macrophage mannose receptors,

and this might be important in enabling alternatively activated macrophages to dampen inflammatory responses to schistosome eggs, thereby preventing organ damage. These macrophages require IL-4 for their development, and mice that have macrophages deficient in the IL-4 receptor alpha-chain are extremely susceptible to acute morbidity after infection with *S. mansoni*. This morbidity occurs concomitantly with increased levels of Th1 cell cytokines (Herbert et al., 2004).

In contrast, chronic disease takes place when an *S. mansoni* infected individuals, often those who reside in endemic areas whom appeared to be asymptomatic, have not been treated with specific chemotherapy (Caldas et al., 2008). Immune responses of the chronic disease patients to *S. mansoni* egg antigens are modulated. Most chronic disease patients presented a Th2-biased immune response with low production of interferon-gamma (IFN-γ), favouring the production of IL-10. The key feature of chronic helminth infections is the production of regulatory cytokines, such as IL-10, and Treg cells. As portrayed in the study by Grogan et al. (1998b), *in vitro* cultures of peripheral-blood mononuclear cells of helminth-infected patients that has abundant presence of IL-

10-secreting cells has shown an increase in the cytokine response to parasite-derived antigens.

There is evidence to indicate that helminth infections can down-modulate Th2 response. As described by Grogan et al. (1998a), an increase of the *in vitro* parasite-specific Th2 cell response of peripheral-blood mononuclear cells has been observed after clearance of the parasite from humans who received treatment.

The induction of Treg cells by these mechanisms might be an important way of controlling over-vigorous immune responses during chronic *S. mansoni* infection. Evidence has shown that such regulation can be seen in both Th1 and Th2 immune responses in helminth-infected individuals and reduced allergic responses has been observed among these infected individuals (van den Biggelaar et al., 2000b). Relatedness can be seen in the immune response towards house dust mite-derived antigens as reduced IL-5 production and elevated IL-10 production by peripheral-blood mononuclear cells has been reported.

During chronic phase of schistosomes infection, antibodies to peptide epitopes became significant as glycan-specific IgM and IgG lessened (Eberl et al., 2001).

Chapter 1.5, 1.6 & 1.9 have been re-organised

1.5 What is cross-reactivity?

Cross-reactivity refers to the similarity among different antigens of various sources from the perspective of the immune system. The nature of antigenic variation as well as the selective processes leading to immune cell populations' distribution is said to be defined by the molecular determinants of specificity and cross-reactivity (Frank, 2002).

In allergic reations, cross-reactivity takes place when similar proteins are found on two or more different substances, in which pollens such as Timothy grass pollens (TGPs) are typically known to cross-react with other substances. One widely accepted explanation for allergen cross-reactivity is due to the recognition of epitopes with similar structures on different proteins, which may be closely related phylogenetically or possess evolutionarily conserved structures, by IgE antibodies (Wagner and Breiteneder, 2002).

There are several explanation for cross-reacivity, one of which is peptide homology, even though it does not represent all the plant allergens cross-reactivity (Wicklein et al., 2004). Alternatively, existence of cross-reactive carbohydrate determinants (CCDs), which are the shared carbohydrate structures between allergens, is said to play a role in cross-reactivity between plants (Van Ree et al., 2000; Wicklein et al., 2004).

Various allergens in plants and invertebrates are glycoproteins with shared glycans, or CCDs, are known to be the major targets of IgE antibodies and are induced upon insect sting or exposure to plant pollens or food (Tretter et al. 1993; Kwaasi et al. 2002; Aalberse 1998; Wicklein et al. 2004). Major IgE targets such as N-glycans containing fucose and xylose are accounted for cross-reactivity between plants and invertebrates, for instance, insect venom phospholipase A in honeybee and some helminths antigens (Van Ree et al. 2000; Yazdanbakhsh et al. 2002; Paschinger et al. 2009; Bencurova 2004). In general, shared glycan epitopes or CCDs between plant and invertebrates contributed to most of the cross-reactivity.

Both innate and adaptive immune responses can be initiated by allergens. Allergens are foreign proteins that induce type I hypersensitivity reactions and further provoke Th2 immune responses, resulting in increased IgE production and allergic responses (Al-Ghouleh et al. 2012). Allergens are being recognised by epithelial and dendritic cells (DCs) at the point of entry, in which both epithelial and DCs proceed with activation of innate inflammatory responses and results in inducing Th2 immune responses.

1.6 Three egg antigens of *S. mansoni*

The major pathology consequences of *S. mansoni* infection, due to Th2 immune responses and granuloma formation that eventually results in fibrotic tissue damage, are mainly caused by the egg stage of the parasite's life cycle. IPSE/alpha-1, omega-1 and kappa-5 are the three termed antigens of *S. mansoni* eggs which have been determined to be the primary targets of the egg-induced antibody response of the host (Schramm et al., 2009).

These glycoproteins from *S. mansoni* egg contain glycans which are responsible for various immune responses of the

human host, such as T-cell modulation and granuloma formation. These glycans are also the target of glycan-specific antibodies (Meevissen et al. 2011). The glycosylation of these *S. mansoni* egg antigens are crucial aspect because of their potential relevance to antigenic cross-reactivity between schistosomes and allergens.

Basophils are well known to produce interleukin 4 (IL-4) and interleukin 13 (IL-13) in which both interleukins play an important role in the immune response towards *S. mansoni*, and most likely also towards other parasites (Schramm and Haas, 2010).

As mentioned earlier, the eggs or egg extracts of the parasitic trematode *S. mansoni* are powerful inducers of Th2 immune responses as well as production of IgE. When basophils encountered egg of *S. mansoni,* large amounts of IL-4 are released rapidly, promoting Th2 immune response.

1.6.1 IPSE/alpha-1

IPSE/alpha-1 is one of the secretory glycoprotein which is produced exclusively by *S. mansoni* eggs. Previous studies by

Schramm et al. (2003) were performed involving human basophils with the use of *S. mansoni* egg antigen fractions IPSE/alpha-1, previously termed as IPSE. IPSE/alpha-1 is produced in the subshell area of the egg and is not detectable in the miracidium, the parasitic larval stage present in mature eggs, as either protein or mRNA (Schramm et al., 2006). After secretion, IPSE/alpha-1 comes into close contact with inflammatory cells recruited to the vicinity of the egg surface (Schramm et al., 2006).

Sequence analyses revealed a potential nuclear localization signal (NLS) at the C terminus of IPSE/alpha-1 (Kaur et al., 2011). Native IPSE/alpha-1 released by schistosome eggs is a dimer as a consequence of an interchain disulfide bond involving the C-terminal cysteine residue C132 (Wuhrer et al. 2006). On top of that, Kaur et al. (2011) has demonstrated that the C terminus of IPSE appears to have multiple functions, as an NLS and in basophil activation.

It was previously shown that IPSE/alpha-1 has immunoglobulin-binding properties and activates basophils of immunologically naïve donors, resulting in histamine release and Th2-type cytokine production (Schramm et al., 2003).

IPSE/alpha-1 also induces IL-4 secretion from murine basophils *in vivo* (Schramm et al., 2007). IPSE/alpha-1 activates human basophils via an IgE-dependent but non-antigen-specific mechanism. On top of that, neutralisation and immune-depletion studies using antibodies against IPSE revealed that IPSE is indeed the bioactive component in *S. mansoni* egg antigen which causes basophils activation, leading to further expression of IL-4 and IL-13. The blot analysis carried out by Schramm et al. (2003) also suggested the mechanism of action is due to IPSE acting as an IgE-binding factor, in which it turns effective by cross-linking receptor-bound IgE on basophils. Because it activated basophils to release IL-4, IPSE was thought to be a component derived from the parasite which is responsible for inducing Th2 immune responses. Generally, IPSE/alpha-1 is considered as IL-4 inducing factor from schistosome eggs.

Various studies suggested that activation of basophils by IPSE/alpha-1 was found to rely on the existence of IgE on the surface of the basophils regardless of IgE's antigen specificity (Schramm et al., 2003; Falcone et al., 1996)

It is well established (Patella et al., 2000) that IgE-dependent, antigen-independent basophil activation is due to multivalent lectins or B cell super-antigens when cross-linking of IgE takes place via binding to its carbohydrate side chains or directly to its immunoglobulin backbone, one way or the other.

Interestingly, a more recent *in vivo* study by Schramm et al. (2007) using murine model suggested that IPSE/alpha-1 is capable of activating basophils in the absence of antigen-specific IgE to produce antigen-independent IL-4. Schramm et al. (2007) also showed that IPSE/alpha-1 triggers the basophils to release IL-4 when introduced to non-sensitised healthy mice whereas intravenous injection of IPSE/alpha-1 into mice results in basophil-IL-4 production in the liver, which is one of the major sites of egg deposition during *S. mansoni* infection.

Meyer et al. (2015) found that IPSE/alpha-1 is the new member of the βγ-crystallin superfamily which has been revealed to have an immunomodulatory function, involving the interaction of a crystallin fold with IgE.

MS analysis by Wuhrer et al. (2006) revealed that IPSE/alpha-1 express two N-glycosylation sites, which are each occupied for a large proportion with core-difucosylated diantennary glycans that carry one or more Lewis X motifs. Lewis X, the major terminal glycan motif in the antennae of N-glycans on IPSE/alpha-1, in combination with α1-3- and/or α1-6-fucosylation of the chitobiose core, is found to be the major immunogenic glycan element of schistosomes.

1.6.2 Omega-1

The soluble egg antigens (SmSEA) of the parasitic helminth *S. mansoni* are known to trigger strong Th2 responses both *in vitro* and *in vivo*. Researchers strived to identify the specific molecules that promote the development of Th2 responses. Everts et al. (2009) reported that similarly to whole SmSEA, omega-1, a glycoprotein which is secreted from *S. mansoni* eggs and a constituent of SmSEA, is involved in conditioning human monocyte-derived dendritic cells *in vitro* and to induce them to further develop towards a Th2 polarisation. The study also showed that in the absence of IL-4R signalling, both natural and recombinant omega-1 is sufficient to generate Th2 responses *in vivo* on their own. Interestingly, omega-1–

depleted SmSEA behaves differently *in vitro* and *in vivo* in which its capacity for Th2 priming in vitro appeared to be impaired and thus different from its action *in vivo*. This finding suggested additional factors within SmSEA may be present to compensate for the omega-1–mediated effects. The results nevertheless collectively indicate that omega-1, a single component of SmSEA, can act as a potent inducer of Th2-biased immune responses (Everts et al., 2009).

Previous studies had proved that *S. mansoni* eggs are capable of inducing Th2 differentiation during infection in which both schistosome eggs alone, or soluble *S. mansoni* egg antigens (SmSEA), are sufficient to drive Th2 polarisation even in the absence of infection (Vella & Pearce 1992; Jankovic et al., 2004).

Th2-polarising properties of SmSEA have been shown to be determined by the glycans on proteins from *S. mansoni* eggs (Okano et al., 1999; Thomas et al., 2003).

Structural wise, both IPSE/alpha-1 and omega-1 are said to express diantennary N-glycans with a difucosylated core and

one or two Galβ1– 4(Fucα1–3) GlcNAc (Lewis X) antennae (Wuhrer et al., 2006).

1.6.3 Kappa-5

Kappa-5 is one of the major *S. mansoni* egg antigens that have been found to be expressed in the parasites' miracidia and eggs at both mRNA and protein levels. This feature of kappa-5 is different than the other two major *S. mansoni* egg antigens, namely IPSE/alpha-1 and omega-1, which can only be found in eggs and its excretory or secretory products (Meevissen et al. 2011).

Kappa-5 is also known to be a glycoprotein that has a specific sequence which is shared with other similar molecules found in other schistosome species. As mentioned earlier, kappa-5 is known to have the ability to elicit IgG antibodies, apart from acting as a target of a pronounced IgE antibody response in human host. The biological function of kappa-5 is still uncertain at this stage, but its high levels of expression as well as strong immunogenicity has been suggested to be responsible for causing immune-pathology in human host (Schramm et al., 2009).

1.7 Immunomodulated helminth infections

The egg stage of *S. mansoni* is responsible for inducing Th2 immune responsiveness in this particular parasite infection. Secretory egg products including glycoproteins, the most abundant secreted components namely IPSE/alpha-1 and omega-1, and glycans play a major role in triggering such immune response in host.

As mentioned earlier, both IPSE/alpha-1 and omega-1 from *S. mansoni* egg antigens are capable of triggering basophils to release IL-4 and IL-3 and priming dendritic cells to drive Th2 polarisation, respectively. As such, it is easy to tell that these two molecules, with omega-1 taking part in the initiation and IPSE/alpha-1 in charge of amplification, are responsible of evoking the host's Th2 immune response during *S. mansoni* infection.

During the chronic phase of schistosome infection, the host Th2 immune response is modulated with an anti-inflammatory approach involving regulatory T cells (Tregs) and the release of IL-10 and TGF-beta and involvement of alternatively

activated macrophages (AAMs), in order to limit further parasite-induced damage.

1.7.1 Regulatory T cells and B cells

Various studies involving in human and murine models have reported that an elevation of IL-10 during helminth infections has been detected, even though the source and role of IL-10 is said to vary in response to different helminth species. The high levels of IL-10 were related to adaptive regulatory T (Treg) cells in experimental models described by Wilson et al. (2005); Kitagaki et al. (2006); Taylor et al. (2006); Hesse et al. (2004) and Smits et al. (2010), whereas studies by Mangan et al. (2006); Baumgart et al. (2006) and Sadler et al. (2003) reported that IL-10 has been linked to non-Treg populations.

Chronic helminth infection has been said to have the ability to drive immune responses to act protectively against allergic disorders in host. Study by van den Biggelaar et al. (2000a) has also shown that IL-10, the anti-inflammatory cytokine which is associated with chronic schistosome infection, is able to suppress atopy in African children. This hypothesis suggested that immune-regulatory processes are triggered

during the chronic stage of the disease in which the exposure of certain unique immunomodulatory molecules might result in development of regulatory T (Treg) and B cells through dendritic cell (DCs) priming or as a result of release of innate signals from epithelial cells (Smits et al., 2010).

Regulatory B (Breg) cells, when induced by different helminths, are said to offer protection against allergic diseases. With the use of both anaphylaxis and allergic airway inflammation models, Amu et al. (2010) proved that B cells induced by *S. mansoni* infection provided protection against allergic reactions in an IL-10-dependent manner.

In general, Breg cells are involved in different stages of natural *S. mansoni* infections with active regulatory mechanisms in action during chronic infection of the parasite.

Previous studies revealed the active roles for both IL-10-producing B cells and $CD4^+$ T cells as IL-10 protected chronically infected mice against

Previous study by Mangan et al. (2006) found that *S. mansoni* worms are capable of inducing immunomodulatory mechanism which is dependent on IL-10-producing B cells

and such populations are able to protect against allergic hypersensitivity. This finding portrayed the role of helminth immunomodulation in the hygiene hypothesis, describing the fine balance between protective regulatory IL-10 responses and detrimental IL-4 allergic responses, both induced by helminth (Mangan et al., 2006).

1.7.2 IgE and IgG

Immunoglobulin E (IgE) has often been regarded as a component that evolved into the first line of defence in mammals against pathogens as intact IgE present in amniotic fluid might have bound to $CD23^{+}$ cells within the lymphoid follicles of the fetal gastrointestinal tract. Such interactions in the womb might aim to educate the immune system for parasite and helminth exposure at birth through IgE-mediated antigen targeting or to prepare the developing immune system about the prevailing extrauterine environment (Thornton et al., 2003). This mechanism may still be functional in present days, even though helminth infection is not as significant now due to progress in improving health and hygiene, and so might be a case of atopic disease development (Thornton et al., 2003). The increase in numbers of people affected by allergies may be the

consequences of the above-mentioned mechanism no longer playing the same role (Finkelman & Vercelli 2007).

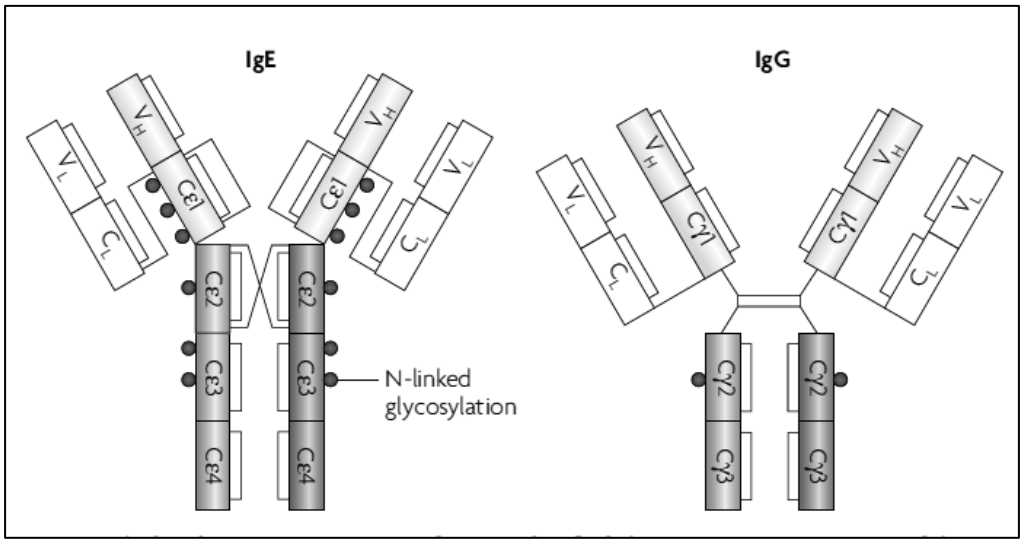


Figure 1-7 The domain structures of IgE and IgG (Gould and Sutton, 2008)**.**

Figure 1-1 from Gould and Sutton (2008) illustrates the polypeptide and domain structures of human IgE and IgG1, showing the intra- and inter-domain disulphide bridges, and the sites of N-linked glycosylation.

The biological and clinical consequences that take place within the first hour in which IgE crosslinks with the high-affinity Fc receptor for IgE (FcεRI) at the surface of mast cells and or basophils by allergens is known to be the early phase of an allergic reaction (Gould & Sutton 2008).

Both mast cells and basophils are involved in immediate hypersensitivity. As mast cells and IgE can be found mostly in the mucosal tissues, IgE is part of the first line defence that reacts against an invading pathogen. The formation of an IgE-FcεRI complex on mast cells and basophils mediates an allergic response; i.e., results in an immediate hypersensitivity reaction, in which histamine, prostaglandins and other factors released by the activated granulocytes play a part.

Survival of mast cells can be promoted via the binding of monomeric IgE to FcεRI which suggested that IgE-mediated allergic reaction is accelerated when there is an increase in the FcεRIα-chain on the cell surface (Asai et al., 2001; Kalesnikoff et al., 2001). In general, the FcεRI receptor initiates allergic response when allergen-bound IgE molecules brought two or more FcεRI receptors together, resulting in the release of mediators responsible for allergy symptoms such as histamine. (Limb et al., 2005; Burrows et al., 1989; Liao et al., 2015).

Allergens are known to act in pump priming of the allergic response. B cells synthesise and secrete IgE as a result of switching of synthesis of heavy chains from IgM to ε heavy chains of IgE.

On the other hand, allergens that binds to APCs results in allergen peptides being presented to T helper 2 (Th2) cells. Th2 cells that are activated by these allergens will release interleukin-4 (IL-4) in order to maintain the Th2 cell lineage and recruit more Th cells into this lineage. Apart from IL-4, IL-13 and CD40 ligand (CD40L) are also produced and expressed by Th2 cells in which all three trigger heavy-chain class switching to IgE. IL-4 and IL-13 production and CD40L expression may also be done by allergen-activated mast cells, further promoting IgE heavy-chain class switching (Punnonen et al., 1993; Gould and Sutton, 2008) On top of that, expression of CD23 and the release of soluble CD23 can also be stimulated by IL-4, IL-13 and CD40L. IgE synthesis and secretion in human is upregulated by soluble trimeric CD23 via interaction with CD21 (Gould and Sutton, 2008)

1.8 Allergy, atopy, allergic rhinitis and asthma

Allergy is one of the most prevalent diseases in developed countries. As technology and hygiene systems advance, particularly in developed countries such as United Kingdom, United States and Australia, parasitic infections become less prevalent. In contrast, developing countries are still haunted by parasitic infection. Interestingly, allergy is less prevalent in developing countries as compare with developed countries. Over the years, researchers have been trying to come up with explanations for this scenario, one of which is the “hygiene hypothesis” (Romagnani, 2004).

Allergy is characterised with high serum levels of allergen-specific immunoglobulin E and eosinophilia which are induced by different Th2 type cytokines such as interleukin (IL)-4, IL-13 and IL-5 (Plopper et al., 2008)

These Th2 type cytokines, including IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13, are produced by Th2 cells. Th2 cells not only induce strong antibody responses (including those of the IgE class) and eosinophil accumulation, but also inhibit several functions of phagocytic cells including phagocyte-independent inflammation. Generally, promotion of IgE is associated with IL-4 and IL-13 whereas IL-5 is associated with increased

number of eosinophils in atopy. Th2 responses tend to counteract the Th1 mediated microbial-cidal action when it is in excess (Del Prete et al. 1988; Punnonen et al. 1993; Walker et al. 1991).

In contrast, Th1 cells, which promote production of interferon gamma (IFN-γ), interleukin 2 (IL-2) and tumour necrosis factor (TNF-β), induce cell-mediated immunity and phagocyte-dependent inflammation. These Th1-type cytokines produce pro-inflammatory responses aimed to kill intracellular parasites and perpetuate autoimmune responses. On the other hand, immunity to allergens which are acting as allergens results in allergy, characterised by IgE, activation of mast cells and basophils as well as accumulation of eosinophils. However, when the “allergen” is not being responded to as an allergen, IgGs such as the IgG4 class may predominate. This seems to be the situation after desensitisation or after immunotherapy. For instance, some of those working with bees who get stung often seemingly develop higher IgG4 than IgE levels (Aalberse et al., 1983).

1.8.1 Atopy

Atopy, also known as atopic syndrome, is defined by the tendency to produce immunoglobulin E (IgE) antibodies in response to the exposure of natural occurring allergens in the environment, further developing allergic diseases such as allergic rhinitis and/or asthma (Wortmann 1979).

1.8.2 Allergic rhinitis

Allergic rhinitis (AR), commonly known as hay fever, is an inflammatory, IgE-mediated allergic reaction caused by allergens such as pollen, dust, mould and certain animal danders (NHS 2016b). It is one of the most common diseases affecting adults and is characterised by itchy eyes, mouth or skin, stuffy or runny nose, sneezing and fatigue due to poor quality of sleep (Seidman, et al., 2015). The socioeconomic impact of allergic rhinitis is considerable as it may impair quality of life resulting in loss of work and reduced school attendance, lowering productivity, posing a huge burden to the individual and society.

1.8.3 Asthma

Asthma is a common chronic disease with its sufferers number approximately 200 million worldwide, affecting both

adults and children (Seidman, et al., 2015). Asthma is caused by inflammation or swelling of the airways, breathing tubes that carry air in and out of the lungs, which tightens when exposure to allergens or irritants. This can lead to chest tightness and wheezing, causing breathing difficulty. Airway of a person with asthma is generally sensitive and can get inflamed easily and causes narrowing of the air passage. Common allergens that trigger asthma include pollens, house dust mites and animal dander. Other irritants such as cigarette smoke, cold air or chest infections can also act as asthma triggers (NHS, 2016a).

1.8.4 Immunoglobulin E

As immunoglobulin E (IgE) plays a central role in the widespread epidemic of asthma and allergies, it is only natural for researchers to have their attention drawn towards IgE. In allergic responses, IgE is associated with a network of proteins including the principal receptors, such as Fc epsilon receptor I (FcεRI) and CD23, and co-receptors, such as CD21, and several integrins (Gould and Sutton, 2008). FcεRI is a high-affinity receptor for IgE.

1.9 Timothy grass pollen

Evidence has shown that the geographic distribution of the prevalence of most atopic diseases and *S. mansoni* infection does not coincide.

The pollen is the source for allergens that causes inhalant allergies which can be found broadly across the globe, affecting temperate regions of northern and central Europe, Northern America, Africa and Asia (Wüthrich et al., 1995; D'Amato et al., 1998; White and Bernstein, 2003; Sibanda, 2003; Kaneko et al., 2005; Boral et al., 2004). During the pollen season when pollens are produced in abundance from grasses, trees and weeds, sensitised populations will suffer from allergic symptoms such as hay fever, conjunctivitis, rhinorrhea and asthma (Radauer & Breiteneder, 2006).

Grass pollen has been reported to be a potent source of allergen that causes IgE sensitisation in 40% of all allergic patients (Freidhoff et al., 1986; Stumvoll et al., 2002).

Pollen allergens have been categorised into 29 protein families, with major protein families consisting of profilins, calcium-

binding proteins and expansins, out of a total of 2615 seed plant proteins (Radauer & Breiteneder, 2006).

Studies by Valenta et al. (1992) and Laffer et al. (1994) have shown that in grass-allergic individuals, members of group 1 pollen allergens, the most significant cause of allergic reaction, are recognised by 95% of IgE antibodies in their sera.

Group 12 pollen allergens constitutes the most cross-reactive group belongs to the major family of profilins with highly conserved peptides (Valenta et al. 1991; Valenta et al. 1994). Profilins are good inducers of IgE and are commonly found in most multicellular organism with 20% of cross-reactivity among pollens and plant related food being accounted for (Valenta et al. 1994; Valenta et al. 1991).

Phleum pratense, commonly known as Timothy grass, is one of the most important pollen producers. 13 allergens, numbered 1 to 13, have been identified in this species of pollen, with molecular weights ranging from 6 to 55 kDa.

1.10 Immunotherapy

In the effort of treating allergy, immunotherapy, also known as desensitisation, has been implemented. There are several well-established treatments for some allergies which involve the sublingual or intravenous administration of allergen extracts to the patients, in gradually increasing dosage over the years (Walker et al. 2011; Chivato et al. 2012; Larsen et al. 2016)

The basis of immunotherapy can be demonstrated by study by Marichal et al. (2013). Marichal et al. (2013) reported that mice injected with one or two bee stings worth of amounts of honeybee venom aids to develop a specific Th2 immune response which increased the mice resistance to subsequent challenge with potentially lethal amounts of the venom. Thus, suggesting that IgE, which also contributes to allergic disorders, has an important function in protection of the host against harmful substances such as bee venom.

1.11 What is the hygiene hypothesis? The 'mainstream' explanation: regulatory T cells and B cells, IL-10 and TGF-β

According to Bach (2002), an observed decrease in incidence of various infectious diseases due to improved hygiene and socioeconomic conditions in developed countries might be the reason behind the increase of incidence and prevalence of allergic diseases, such as asthma and allergic rhinitis. In contrast, developing countries suffered from infectious diseases more than allergic diseases.

There is strong evidence to indicate that genetic background, lifestyle and environment influences the development of allergies and auto-immunity (Araújo et al., 2004; Herz et al., 2000; Weinstock et al., 2002).

Poor socio-economic status and the occurrence of infections are two of the environmental factors (Patterson et al. 1996; von Mutius et al. 1994; Staines et al. 1997; Blanchard et al. 2001) that are said to provide protection against allergy (Lynch et al. 1993; Araujo et al. 2000) and autoimmune diseases (Cooke et al. 1999; Kurtzke 2000; La Flamme et al. 2003).

Various studies have shown that production of IL-10 in Th2 immune response is heightened in the case of chronic *S. mansoni* infection. Study by Araújo et al. (2004) revealed that chronic *S. mansoni* infection aids in down regulating Th1 immune responsiveness which prevents the onset of Th1-mediated diseases such as diabetes mellitus and multiple sclerosis. On top of that, the production of IL-10 has the ability to down regulate Th2-mediated diseases including atopic diseases. An inverse relationship has been observed between skin prick test reactivity and *S. mansoni* infection, and the severity of asthma is being reduced in people residing in *S. mansoni* endemic areas (Araújo et al. 2004). As such, regulatory mechanisms in immune response during helminth infections, such as increased IL-10, are capable of modulating inflammation involved in immuno-pathogenesis of allergy and autoimmune diseases.

Inherent in the hygiene hypothesis is the idea that maturation of the immune response is promoted by microbial signals in early life but that lack of this signalling due to modern civilisation results in the rising prevalence of allergy and other diseases (Romagnani, 2004; Von Hertzen et al., 2004; Plopper et al., 2008). However, the effect of helminth

infections is enigmatic as exposure to helminth infection not only stimulates the production of Th2 cytokines and IgE, but also aid in initiating regulatory T cell immune responses and reducing allergic responses. This is supported by evidence in several developing countries where an inverse relation between helminth infections and the prevalence of allergy and atopic diseases has been observed (Yazdanbakhsh et al. 2002; van den Biggelaar et al. 2000a; Nyan et al. 2001; Huang et al. 2002; Cooper, Chico, Bland, et al. 2003).

Helminth infection and allergy induce similar immune responses, both characterised by amplified activity of Th2 cells, which produce IL-4, IL5 and IL-13. The effects of these cytokines include increased eosinophil numbers and heightened IgE production. Yazdanbakhsh et al. (2001) stated that the geographical distribution of allergy and helminth infection is complementary and not coincident.

1.12 An alternative explanation -blocking antibodies- IgG 4

Chronic helminth infections, including schistosomiasis, have been repeatedly suggested to provide protection against

immune mediated pathologies such as allergic diseases. It is the apparent ability of helminth infections to induce 'immunomodulated' or Th2 modified immune responses that is assumed to be responsible for the protection against allergies and other immunological disorders (Maizels & Yazdanbakhsh 2003). Maizels (2005) suggested that the immune-modulated stage of *S. mansoni* infection is mostly characterised by a polarised Th2 immune response that is similar to most allergic diseases.

A possible explanation for hygiene hypothesis has been suggested by researchers stating that IgG anti-schistosome antibodies which cross-react with allergens may be able to block allergic responses induced by IgE (Doenhoff et al., 2016).

There are several possibilities as to how some immunoglobulins or antibodies may be capable of blocking allergic reactivity. One of them is that high levels of helminth-induced specific or non-specific IgE may occupy FcεRI on mast cells and thus somehow prevent access of allergen-specific IgE to these receptors (Flicker et al., 2013). IgE-dependent inhibiting activity has yet to be determined as there is still a

lack in evidence. However, Mitre et al. (2005) suggested that in helminth infections, particularly in filarial infections, the ratios of polyclonal to allergen-specific IgE rarely reach those levels required for the inhibition of allergen-specific IgE- FcεRI binding as well as suppression of allergen-induced degranulation of mast cells and basophils.

Furthermore, Dodev et al. (2015) demonstrated that specificity of an antibody towards an allergen determines its ability to block allergen-dependent IgE responses; in particular, all IgGs and IgA are capable of inhibiting the IgE activity in an allergen-specific manner. Non-IgE antibodies, such as IgG antibodies, might carry out blocking activity as they compete with IgE for binding sites hence masking the epitopes on the allergen during allergic reaction (Flicker et al., 2013; Dodev et al., 2015). These “blocking antibodies” are yet to be tested for potential usefulness in passive allergen immunotherapy, and it is very unlikely that human immunoglobulins of any sort would ever be tested as immunotherapeutics as it is far too much risk such as transfer of viruses and many more.

Holm et al. (2011) emphasised that these “blocking antibodies” come into action based on epitope specificity, concentration and affinity for antigen. “Blocking antibodies” will need to possess similar or higher affinity than IgE for the allergen to prevent IgE binding (Christensen et al., 2008; Dodev et al., 2015). As such, the affinity of the antibody to the allergen is a deciding factor which will affect whether or not it will be selected as a blocking antibody for passive immunotherapy. Shamji et al. (2012) found out that B cells that expressed IgG-4 play a major role in producing the most efficacious “blocking antibodies” *in vivo* after specific allergen immunotherapy. Other studies also supported the suggestion that IgG-4 is a “blocking antibody” because of its unique feature such as exchanging one heavy or light-chain pair with an IgG-4 antibody of a different specificity to generate a bispecific antibody unable to form immune complexes, as well as its inability to bind complement (Rispens et al., 2010). Interestingly, allergen specificity in IgE-derived allergen-specific single chain variable fragments (ScFvs) is determined mainly by the heavy chains and which different pairings of heavy and light chains lead to differences in affinity and epitope specificity of allergen recognition (Gadermaier et al., 2013).

1.13 Objectives of this study

Prophylaxis of chronic inflammatory responses such as allergy and asthma as well as development of new strategies for *S. mansoni* infection treatment is widely needed. In order to do so, identification and better understanding of schistosomes molecules that plays the vital role in host immune response is essential. The relationship between Timothy grass pollen (TGP), one of the major allergens, and *S. mansoni* antigens served as the backbone of this study. This study also focused in finding a possible explanation for the blocking antibodies hypothesis. The objectives of this study are summarised as shown:

1. To identify cross-reactivity between *S. mansoni* antigens and Timothy grass pollen.
2. To purify cross-reactive Timothy grass pollen antigens by repeated PAGE/elution from gel films.
3. To perform mass spectrometric analysis of Timothy grass pollen antigens.
4. To acid-elute rabbit anti-*S. mansoni* antibodies from blot of cross-reactive Timothy grass pollen molecules.

5. To test the ability of anti-SmSEA derived anti-TGP rabbit IgG antibodies to prevent or hinder activation of basophils that have been sensitised with IgE antibodies.

1.14 Humanised rat basophil leukaemia reporter system

In this study, Timothy grass pollen (TGP) allergens were purified by isolation from SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) gels and IgG antibodies were eluted from Western immunoblots of the purified allergens probed with rabbit anti-SmSEA sera. The allergens and antibodies were then used in a humanised rat basophil leukaemia (RBL) reporter system, as illustrated in figure below, adapted from Siraganian (2003).

The reporter system in this study used humanised rat basophils and which were then sensitised with IgE from human sera from patients allergic to TGP. The cell line had been developed using the FCεRI-mediated signalling pathways in mast cells as described by Siraganian (2003). The IgE antibodies were cross-linked with FCεRI on the cell membranes. When antigen specific to the IgE is present and becomes bounded to the cell, an activation process in the form of an inositol triphosphate and calcium cascade is initiated. The cells have been developed in such a way so that a calcium influx through the cell membrane would allow nuclear factor of activated T-cells (NFAT) transcription factors

to enter the nucleus and initiate mRNA for luciferase. Luciferase protein was then made in the endoplasmic reticulum (ER) and a luminescence measurement of cells can be recorded.

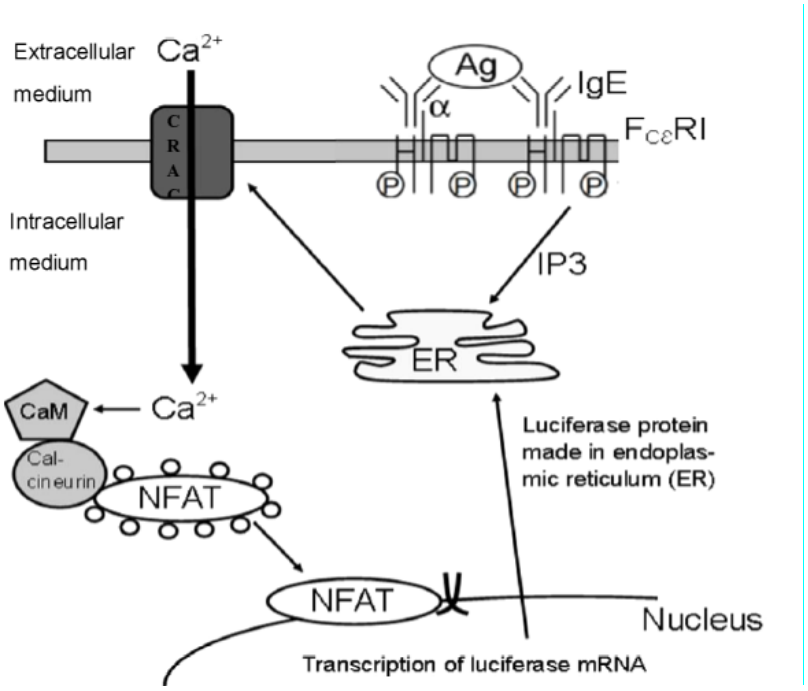


Figure 2-8 Humanised rat basophil leukaemia reporter system (Nakamura et al., 2010)

The system was adapted by incubating the purified TGP allergens with eluted antibodies before introduction to the sensitised cells. This was to check if the IgG antibodies bound to the allergens and therefore prevented the activation of the cells (Nakamura et al., 2010).

Chapter 2 MATERIALS AND METHODS

2.1 Preparation of *S. mansoni* antigens

A Puerto-Rican isolate of *S. mansoni* was maintained by continuous passages through *Biomphalaria glabrata* (freshwater snails) and random-bred CD1 strain mice. The parasite life cycle was maintained and performed with strict adherence to the regulations set out in the UK Animals (Scientific Procedures) Act, 1986, (project licence numbers PPL 40/3024 and 40/3595). A lethal dose of pentobarbitone anaesthetic was administered to euthanise the animals. Preparation of *S. mansoni* soluble egg antigens (SmSEA) extracted from the livers and intestines of infected mice bearing adult worms was as described in (Doenhoff et al., 1988). SmSEA was lyophilised in 1 mg aliquots and stored at -80°C until use.

2.2 Preparation of polyspecific rabbit anti-*S. mansoni* egg, anti-cercariae and anti-whole adult worm antisera and rabbit antisera monospecific for *S. mansoni* egg antigens

Polyspecific anti-*S. mansoni* antisera were raised against homogenates of *S. mansoni* eggs (anti-SmSEA), cercariae (anti-Ch) and adult worm (anti-Wh) by immunisation of rabbits with the respective homogenates as described by Modha et al., (1988) and Dunne et al. (1986).

Preparation of rabbit antisera against three *S. mansoni* egg antigens, namely IPSE/alpha-1, kappa-5 and omega-1 were prepared as described in Dunne et al. (1986). Through immunoelectrophoresis, replicates of immunoprecipitin arcs against the egg antigen were produced respectively with the suitable rabbit antisera. Non-percipitated serum and schistosome constituents were removed by washing the excised precipitin arcs extensively in isototonic saline. The excised precipitin arcs were then homogenised in 3 times the volume of isotonic saline. Finally, the homogenates were emulsified with Freund's adjuvant and were used to immunise rabbits.

The rabbit antisera that were used in this study were listed in the Table 2.1.

Table 2.1 List of rabbit antisera

Rabbit antisera	Abbreviation
Anti-*S.mansoni* IPSE/alpha-1	BR114
Anti-*S.mansoni* IPSE/alpha-1	562H
Anti-*S.mansoni* omega-1	BR16
Anti-*S.mansoni* kappa-5	BR15
Anti-*S.mansoni* cercaria	1025O
Anti-S.mansoni egg	1025Z

2.3 Preparation of a crude aqueous extract of Timothy grass pollen (TGP)

Timothy grass pollen (TGP) powder was kindly donated by Dr Gabi Schramm of Forschungszentrum Borstel, Germany and was kept at 4°C until preparation of the crude aqueous extract. Preparation of the crude aqueous extract of TGP was adapted from Schenk et al. (2011).

Timothy grass pollen (TGP) powder was diluted to a concentration of 50 mg/mL with 0.05M Tris-HCl buffer, pH 7.4, in a 15 mL Falcon tube. The tube was inverted for several

times to achieve an even suspension and was placed on the rocker for 1 hour at room temperature. The suspension was then centrifuged at 10,000 x g at 25°C for 5 minutes. The pellet and supernatant were homogenized into finer paste. The resulting homogenate was placed on the rocker for 1 hour at room temperature. The homogenate was centrifuged at 1000 xg at 25°C for 5 minutes. The resulting supernatant was the crude aqueous extract of TGP. It was stored at -20°C.

2.4 Estimation of protein concentration

The protein concentration of the crude extract of Timothy grass pollen (TGP), purified Phl p 1 TGP allergen, purified Phl p 5 TGP allergen, and purified anti-SmSEA derived anti-TGP rabbit IgG antibodies were estimated using the BioRad DC Protein Assay (BioRad Laboratories, Inc., Hercules, California USA), using bovine serum albumin (BSA) as the reference standard.

2.5 Preparation of buffer, reagents and solutions

Buffers, reagents and solutions used in the study are listed in the Appendix.

2.6 Immunological and protein purification techniques

2.6.1 One dimensional electrophoresis in sodium dodecyl polyacrylamide gel (SDS-PAGE)

This procedure was performed to distribute proteins according to their relative molecular sizes through an acrylamide gel film after treatment with sodium dodecyl sulphate (SDS). One-dimensional SDS-PAGE in 10% acrylamide gels was performed as described by Studier (1973) and adapted from Laemmli (1970) in a BioRad Minigel SDS system (Bio-Rad Laboratories, Inc., Hercules, California USA).

2.6.1.1 Equipment set-up

The equipment needed for the assembly of the BIORAD Minigel SDS system included casting apparatus, spacer plates, short plates, and combs to form wells in the gel. All these were cleaned by spraying and wiping down with 70% ethanol. The gel rig was unscrewed to allow room for inserting the spacer plate first and followed by placing the short plate over it. The set-up was then adjusted to ensure that both plates were properly aligned with all edges to prevent leakage of gel, before screwing the plates firmly together. The set-up was then fixed onto the gel casting unit and ready for the addition

of the resolving gel and stacking gel provided there had been no leakage.

2.6.1.2 Preparation of resolving gel and stacking gel

The preparation of resolving gel and stacking gel were as described in Table 2.2 and Table 2.3 respectively. The resolving gel acted as a matrix which allowed the movement and separation, by size of the individual proteins or peptides, in any mixture that is driven down the gel by electrophoresis.

Table 2.2 Volumes of reagents and solutions required for two 0.8mm thick, 10% resolving gels in the BIORAD minigel system

Stock solution	Volume, mL
1.5 M Tris HCL, pH 8.8	2.5
10 % SDS	0.1
30 % Acrylamide	3.3
Deionised Water	4.1
10% APS	0.1
TEMED	0.005
Final Volume	10.0

A 10% stock solution of the resolving gel was prepared subsequently in a Falcon tube as shown in the Table 2.2. Firstly, 1.5 M Tris HCL, 10% SDS, 30 % acrylamide and deionised water were added and mixed in a 15 mL Falcon tube. After that, 10 % APS and TEMED were added into the solution and mixed well. The solution was then poured into the gel sandwich until the desired level up the glass plates was reached, leaving enough room for addition of a stacking gel. The solution was then overlaid with saturated butanol to even out the surface of the resolving gel and the resolving gel was allowed to polymerise for 30 to 60 minutes at room temperature. Once the resolving gel was polymerised, the butanol was removed and washed off with deionised water.

Table 2.3 Volumes of reagents and solution for preparation of two 0.8mm-thick stacking gels for the BIORAD minigel system

Reagents/Solutions	Volume, mL
0.5 M Tris, pH 6.8	1.25
10 % SDS	0.05
30 % Acrylamide	0.75
Deionised water (dH2O)	2.95

10 % APS	0.05
TEMED	0.0025
Final Volume	5.0

A stacking gel was used to create wells or lanes above the resolving gel, in which the protein or peptide mixtures can be added. The wells were prepared using an appropriate well comb. The reagents required for the preparation of stacking gels are listed in Table 2.3. The stock solution was mixed well by inverting the Falcon tube before pouring it on top of the polymerised resolving gel to the top edge of the short plate. An appropriate comb, either a broad well comb or a 10-well comb, was immediately inserted into the stacking solution leaving approximately 1 cm between the lower tip ends of the well comb and the top of the resolving gel. The stacking gel was then allowed to be polymerised for 30 to 60 minutes at room temperature.

Once the stacking gel had polymerised, the gel was clamped into the electrode assembly unit and placed into a buffer tank. The inner chamber of the unit was filled up with 1X running buffer. The well comb was removed carefully, revealing the opening of each well in the stacking gel. The bubbles in the

wells were removed by pipetting 1X running buffer into the well using SDS gel loading pipetting tips.

2.6.1.3 Preparation of protein sample

Protein samples such as the crude aqueous extract of Timothy grass pollen (TGP) and *S. mansoni* soluble egg antigen (SmSEA), were thawed to room temperature. Protein sample were treated with ¼ volume of reducing loading buffer, which contained β-mercaptoethanol, and boiled for 5 minutes.

2.6.1.4 Loading of protein samples into gel wells

3 µL to 5 µL of ready-to-use prestained protein ladder (BlueRay Prestained Protein Marker, Jena Bioscience) was added into the first well to serve as a reference. Protein samples were then loaded into the wells in the gel accordingly. Loading buffer of the same volume and concentration was loaded into any empty or extra well that was not being used.

2.6.1.5 Running of polyacrylamide gel

The buffer tank was half-filled with 1X running buffer after the loading was done. The apparatus was then connected to a power pack with corresponding electrodes and set to a

voltage of 80 V to 120 V. Electrophoresis was allowed to run for around 1.5 to 2 hours with regular checking at intervals until the blue-stained line of the loading dye (marker) reached the lower edge of the gel.

2.6.1.6 Staining and visualization of protein bands

The equipment was then switched off to stop the electrophoresis. The power cables were disconnected and the gel was removed from the unit. The gel would either be stained to reveal the protein bands or gel contents electro-transferred onto nitrocellulose paper (NCP) for western immunoblotting.

The gel was removed from the glass plates and rinsed with deionised water. The gel was then placed in Coomassie staining solution, which is made up of 0.25 g Coomassie Brilliant Blue R250; 10 ml of Glacial acetic acid and 90 ml of Methanol: deionised water (1: 1 v/v), for at least 1 hour with gentle agitation on a rocker (He, 2011). The staining solution was removed and the gel was rinsed with deionised water to remove excess stain. The gel was then destained in deionised water during gentle agitation until protein bands became clearly visible.

2.6.2 Excision and elution of electrophoresed proteins in SDS-PAGE

Excision of protein bands of interest was done by placing a de-stained gel containing the electrophoresed proteins on a clean glass plate. A sterile scalpel was used to cut out narrow horizontal slices of each protein band with the cut as close to the protein band as possible. The excised gel bands were then each placed into an individual 1 ml micro-centrifuge tube and covered with minimal volume of elution buffer (0.06 M Tris-HCL with 10% SDS, pH 7.4). The tube containing the excised electrophoresed protein was incubated at 37ºC overnight and thereafter was centrifuged at 14,000x g for 30 minutes at 37°C. The eluate was removed, concentrated and stored at -20 º C until use or re-electrophoresised in a second PAGE, as described by Beyer et al. (2008).

2.7 Western immunoblotting

Western immunoblotting was done as described by Towbin et al. (1979) and adapted as in Dunne et al. (1986) and Doenhoff et al. (2016).

2.7.1 Gel casting and protein electro-transfer

SDS-PAGE gels were run as described in 2.6.1 without staining the gel. After electrophoresis, the gel was equilibrated in pre-chilled 1X transfer buffer for 15 minutes. The membrane and filter paper (Whatman qualitative filter paper, Grade 1) were cut according to the dimension of the gel. 2 fibre pads, 2 sheets of filter paper and the 0.2 μm nitrocellulose membrane (NCP) membrane were soaked in 1x transfer buffer.

A “sandwich” was prepared by placing the cassette, 1 pre-wetted fibre pad, a sheet of pre-wetted filter paper, the equilibrated gel, pre-wetted NCP membrane, a sheet of pre-wetted filter paper and lastly a pre-wetted fibre pad in that sequential order. Throughout the assembly, any air bubbles were removed. The “sandwich” was locked and closed firmly with the latch of the cassette. The cassette was then fitted into the module and placed into the buffer tank. The buffer tank was filled with cool 1x transfer buffer and placed in a cooling unit. A magnetic stir bar was placed into the tank to ensure even buffer temperature as well as even ion distribution. The set -up was connected to a power pack and

subjected to a potential difference of 30 V to 50 V for around 2 hours at 4°C.

2.7.2 Blocking and incubation of electroblotted nitrocellulose membrane

The NCP membrane carrying the electroblotted proteins was removed from the “sandwich” once the protein had been subjected to the transfer process. The NCP membrane was placed in 50mL of blocking buffer for 2 hours at room temperature or at 4°C overnight with gentle agitation. After blocking, the NCP membrane was rinsed with 1x TBST solution three times at 5 minutes interval.

Rabbit antisera used as the sources of primary antibodies were diluted 1:100 to the working concentration in 1XTBS solution. NCP membrane was incubated with 5mL of the desired primary antibodies for two hours at room temperature or overnight at 4°C with gentle agitation.

After incubation in primary antibodies, the NCP membrane was rinsed in 1XTBST solution three times at 5 minutes interval.

Peroxidase-conjugated goat anti-rabbit IgG (Sigma, UK), acting as secondary antibodies, were diluted 1:1000 to the working concentration in 1XTBS solution. The NCP was incubated in the secondary antibodies for 1.5 hour at room temperature.

After incubation in secondary antibodies, the NCP membrane underwent a final wash by rinsing in 1XTBST solution three times at 5 minutes interval.

2.7.3 Visualisation of immunoblotted proteins

Immunoblotted proteins were developed and visualized by incubating the NCP membrane in developing solution. Developing solution contained the chromogenic substrate 4-chloro-1-naphthol dissolved in 4 ml ethanol, warm 20 ml TBS solution and 10 µL of hydrogen peroxide. The developing solution was mixed well and was poured evenly over the NCP membrane. The NCP membrane was incubated on a rocker for 5 minutes until clear bands of protein appeared. The reaction was halted by rinsing the NCP membrane twice with deionised water.

2.8 Acid-elution of cross-reactive antibodies

Specific anti-SmSEA antibodies that cross-reacted with the two TGP molecules were purified by acid-elution, a method modified as in Doenhoff et al. (2016).

Acid elution was performed to purify rabbit anti-SmSEA antibodies that were cross-reactive with the 31 kDa and 35 kda timothy grass pollen molecules. Extracts of Timothy grass pollen containing the antigens of interest (31 kDa TGP molecule and 35 kDa TGP molecules) were electrophoresed in SDS-PAGE gel, electroblotted onto a NCP membrane and incubated with primary antibody (1025Z).

A thin strip was cut out from each end of the NCP membrane. The two thin strips were incubated in secondary antibody (peroxidase-conjugated goat anti-rabbit IgG) and developed to visualize the molecules that were cross-reactive. Both strips showing immunoblot reactivity were placed on either edge of the NCP membrane (mid portion). A strip of NCP membrane in line with areas of the edge strips that had reacted with secondary antibody was excised horizontally. The

narrow horizontal strips of NCP membrane, carrying specific antigen with the bound cross-reactive primary antibody of interest, were incubated in primary antibody for 2 hours. After incubation, the horizontal strips were rinsed with 1x PBS solution.

Acid-elution of cross-reactive antibodies was carried out by incubating the horizontal NCP strip in 1 mL of 0.1 M glycine buffer solution (elution buffer) and agitated on a rocker for 10 minutes. The elution buffer was removed and neutralised with neutralisation buffer (1 M Tris, pH 8.0). This solution contained the eluted antibodies. The eluted antibodies were stored at -20ºC until use. The horizontal NCP strip was rinsed with 1x PBS solution and could be re-incubated in the same primary antibody. The process of incubation and antibody acid elution was repeated up to 4 times. Solutions of the eluted antibodies were pooled and concentrated to 25% of the initial volume using Amikon ultra centrifugal filters (Millipore, Corrigtwohill, Co. Cork, Ireland). The final solution containing the concentrated eluted antibodies was stored at -20ºC.

The protocol of acid elution is illustrated in Figure 2-1.

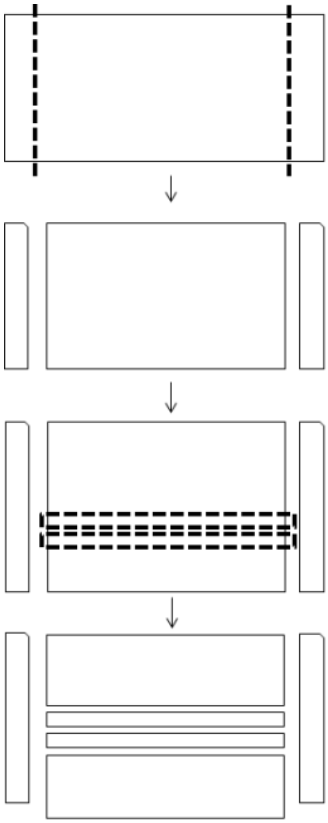


Figure 2-1 Illustration of protocol used to purify rabbit anti-SmSEA antibodies that are cross-reactive with 31 kDa and 35 kda Timothy grass pollen molecules by acid elution from electroblot of Timothy grass pollen extract.

2.9 Treatment of nitrocellulose membrane with sodium metaperiodate

In order to destroy carbohydrate determinants, with minimal alteration of protein or lipid epitopes, NCP membrane carrying electroblotted antigens were treated with sodium meta-periodate. Controls were treated in the sodium acetate buffer under similar conditions, without the sodium meta-periodate. This procedure was adapted from Hamilton et al. (1999) and Eberl et al. (2001), performed as described by Doenhoff et al. (2016).

The equilibrated NCP membrane containing the protein was cut into 2 halves. One half of the NCP membrane was treated in a cold solution of 10 mM sodium meta-periodate dissolved in 0.05 M sodium acetate buffer, pH 4.5, in the dark on a rocker for 30 minutes at room temperature. The other half of the NCP membrane was incubated in the same amount of buffer under the same conditions, without the sodium meta-periodate, to act as control. Each of the 2 halves was washed twice in 0.05 M sodium acetate buffer, pH 4.5, and incubated at room temperature in a sodium borohydride solution for 30 minutes. Each membrane was then washed twice in 1xPBS solution and three times in 1xTBST solution. After blocking

the membranes (Section 2.7.2), the process continued for western immunoblotting (Section 2.7.3).

2.10 Mass spectrometry (MS) analyses of protein samples

Mass spectrometry involved the ionisation of proteins to generate charged molecules or molecule fragments, the mass-to-charge ratios of which were measured. Tandem mass spectrometry (MS) was performed on a Waters Corporation Q-TOF 2 instrument (Papayannopoulos, 1995) according to methods described in Doenhoff et al. (2016).

Potential glycosylation sites on amino acid sequences of a MS-identified allergen and two parasite antigens were predicted using the GlycoEP software (Chauhan et al., 2013) and the ExPASy Bioinformatics Resource Portal (GlycoMod) tool (http://web.expasy.org/glycomod/). The amino acid sequence of the protein were pasted into the software and the prediction based on binary profile of patterns (BPP) was highlighted while leaving other settings as default and set to run, thereby revealing the number(s) of potential N-linked or

O-linked glycosylation site(s) on the amino acid sequence of the protein.

2.11 Mammalian Cell Culture

The cell line used in this study was RS-ATL8 cell line. The frozen stock of this cell line was kindly donated by Dr. Franco Harold Falcone of the Division of Molecular and Cellular Science, University of Nottingham, United Kingdom.

Nakamura et al. (2010) reported the RS-ATL8 cell line is one of the the highest luciferase responder subclone after stimulation with 10 nM phorbol myristate acetate and 10 μM ionomycin, was established by limiting dilution and was grown in minimum essential medium (MEM) (Invitrogen) supplemented with 10% heat-inactivated fetal calf serum (FCS; Nichirei Biosciences, Tokyo, Japan), penicillin/streptomycin, GlutaMAX-I, 1.2 mg/ml geneticin, and 200 μg/ml hygromycin (Invitrogen).

The cells were grown in tissue culture-treated flasks and MEM medium was used as the cell culture medium. Cell culture was

performed as described by Wan et al. (2014) with slight modifications.

2.11.1 Defrosting of frozen cells

RBL medium consisted of minimum essential medium (MEM), 10% v/v heat-inactivated fetal bovine serum (FBS), 100 U/mL penicillin; 100 μg/mL streptomycin and 2 mM L-glutamine. RBL medium was warmed up in water bath which was set to 37°C. The vial containing the frozen cells was partially defrosted by warming in hand.

1 mL of the pre-warmed RBL medium was added into the vial to fully defrost the cells. The cells were transferred to a 25 cm^2 flask that contained 8 mL of the pre-warmed RBL medium. The flask was then placed in a cell culture incubator at 37°C and 5 % CO_2 overnight. The cells grew in a monolayer at the bottom of the flask. A 1/5 split was performed for the cells to be confluent in 3 days, 1/10 split for 4 days and 1/20 split for 5 days.

2.11.2 Passaging or splitting of the cells

When the cells were confluent, they were passaged to prevent the cells from dying. First, the old medium in the flask was discarded. The adherent cells were washed once with 10 mL of Dulbecco's phosphate-buffered saline (DPBS) that is calcium and magnesium free (Thermo Fisher Scientific, Renfrew, UK). The DPBS was then discarded. 2mL of trypsin-EDTA was added to the flask and the flask was placed in an incubator for 15 minutes at 37°C until clumps of detached cells were visible.

10 mL of pre-warmed MEM (containing fetal bovine serum which helped to stop the trypsin-EDTA reaction) was added to the flask. The cells were aspirated up and down using a pipette controller to remove aggregates and to create a single cell suspension. After detachment of the cells, 9mL of the contents in the flask were discarded and 9 mL of fresh MEM added to the flask. The cells thus underwent a 1/10 passage.

2.11.3 Sensitisation of the cells with human serum

In this study, two human sera from patients allergic to Timothy grass pollen, namely serum DG and serum KH, were

used. The human sera were kindly donated by Dr. Gabriele Schramm, Borstel.

MEM medium was pre-warmed in a water bath set at 37°C. The old medium from 75 cm^2 flask containing the confluent cells was removed. 10 mL of DPBS without Ca^{2+} or Mg^{2+} was added to the flask. The flask was tilted to wash the cells with DPBS. DPBS was then discarded. 2 mL of trypsin-EDTA was added to the flask and the flask was tilted to ensure the cells were exposed to the trypsin-EDTA solution. The flask was incubated for 15 minutes at 37°C in a cell culture incubator until the clumps of detached cells were visible.

8 mL of pre-warmed RBL medium was added to the flask and the cell suspension was re-suspended. The cells were then transferred to a 50 mL tube with some cells left behind in the flask for passaging purposes (see 2.12.2).

A sample of cells was counted in a hemocytometer and the total cell number was calculated. The cells were pelleted by centrifuging at 300 x g for 5 minutes. The cells were then re-suspended using RBL medium to a concentration of 1×10^6

cells/mL. The cells were sensitised using the desired optimum dilution of serum (As described in 2.12.6). 50 µL cells/well were pipetted into a 96-well plate. The 96-well plate was incubated for 16 to 20 hours at 37°C with 5% CO_2 in the cell culture incubator.

2.11.4 Stimulation of cells

The 96-well plate was checked under a light microscope to ensure the cells were in a healthy state. Optimum dilutions of the allergens and other stimuli were prepared in RBL medium. The optimal concentration for each allergen was determined experimentally. 2 µg/mL was the optimum concentration of anti-IgE which was used as a positive control. The old medium from the cells in the 96-well plate was removed. 50 µL stimuli were added to the appropriate wells of the plate. This step was done quickly to prevent the cells from drying out when the medium was removed. The cells were then incubated in the cell culture incubator for 4 hours.

2.11.5 Measurement of activation using a luciferase assay

The luciferase assay reagent and cells were brought to room temperature before mixing. 50 µL of ONE-Glo Luciferase

Assay Substrate (Promega) was added directly to the stimulated cells. The luminescence was measured in a plate reader.

2.11.6 Optimal concentration of human sera and allergens

Purified Phl p 1 and Phl p 5 allergens from Timothy grass pollen (TGP) and their corresponding eluted antibodies were used in the blocking experiment.

The cells were sensitised with human sera at a ratio of 50:1 and 50 µL of cell sera mix was added to each well. The plate was then incubated for 16-20 hours so cell sensitisation could take place.

After sensitisation, cells were checked under a light microscope to ensure they were in a healthy state. To ensure the allergen activated the cells, a series of dilution of the allergens were prepared from 100 µg to 1 pg to find an optimal concentration.

When testing the blocking ability of the rabbit IgG antibody, the purified allergen solutions were mixed with eluted antibodies at five ratios, namely 5:1, 1:1, 1:5, 1:10 and 1:20, and incubated for 16 hours. This was to allow the antibody to bind to the allergen before they were added to the sensitised cells. 1ml of medium was used as the negative control and 1 µL of anti-human IgE was used as the positive control. Because anti-human IgE was able to bind with any IgE on the surface of the cell line, it gave the highest values indicative of activation. The medium was removed from the cells in the 96-well plate and rapidly replaced with 50 µL of the stimulating solution so the cells did not dry out.

The cells were incubated for a further four hours in a 5 % CO2 incubator. After incubation the cells were washed once with 50 µL DPBS. The DPBS was removed and 100 µL 1 % Triton X-100 in DPBS was added to lyse the cells.

The ONE-Glo Luciferase Assay Substrate (Promega, Wisconsin, US) was thawed to room temperature before adding it to the cells as the assay was temperature-sensitive. This method allowed more reproducible results. Each well had 50 µL of luciferase assay reagent added directly to stimulate the cells.

The mixture in each well was then transferred to a white 96-well plate. Luminescence was measured by using icontrolTM software on a TECAN® Infinite 200 (Tecan Group Ltd., Männedorf, Switzerland).

Chapter 3 RESULTS

3.1 Cross-reactivity with molecules in a crude extract of Timothy grass pollen

Two protein bands in a Coomassie blue-stained SDS-PAGE gel (see Figure 3-1) were present in the positions that corresponded with the immuno-reactive 31 kDa and 35 kDa molecules in the Western immunoblots. The proteins were purified by excising the respective bands from replicate gels, eluting proteins from the excised gel strips into Tris-HCl buffer, pH 7.4, concentrating the eluate and re-electrophoresing the product, as shown in Figure 3-2.

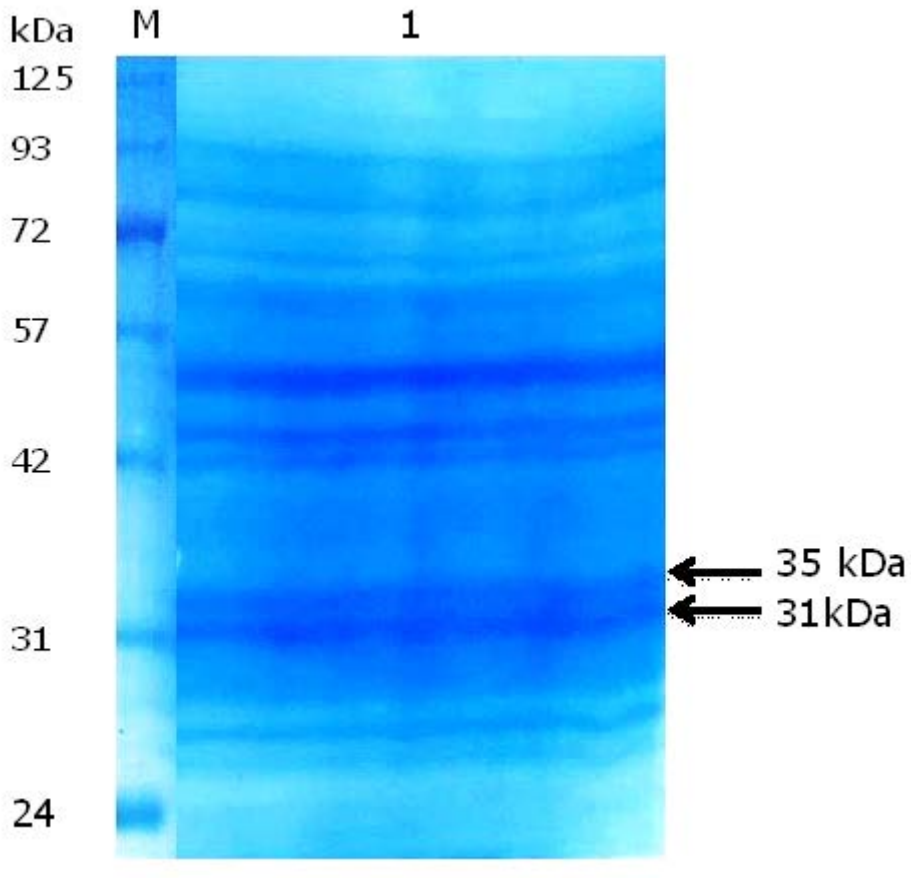


Figure 3-1 Coomassie—stained SDS-PAGE containing electrophoresed aqueous crude extract of Timothy grass pollen. Figure legend: M = molecular weight marker; 1 = aqueous crude extract of Timothy grass pollen.

Figure 3-1 shows one of the Coomassie blue-stained SDS-PAGE replicate gels that contained electrophoresed aqueous crude TGP extract with two protein bands present at 31 kDa and 35 kDa, as indicated by the arrows.

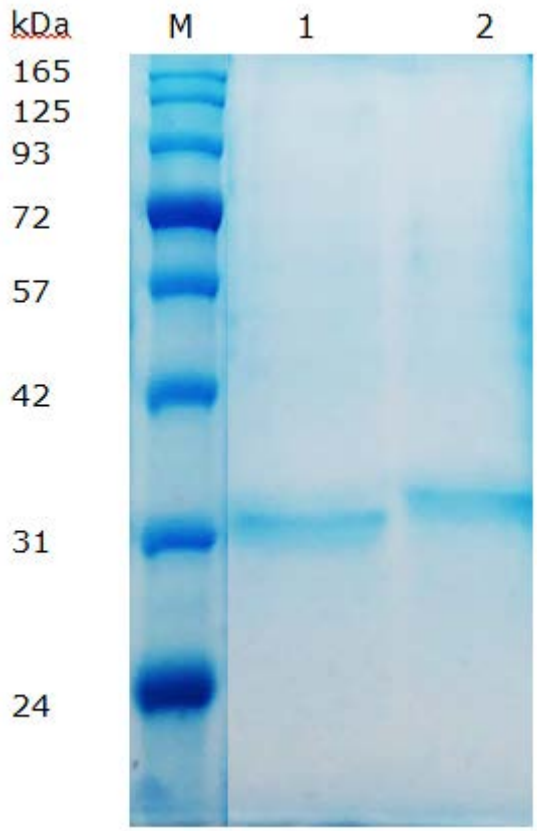


Figure 3-2 Coomassie—stained SDS-PAGE containing a 31 kDa protein (lane 1) and a 35 kDa protein (lane 2) purified from Timothy grass pollen

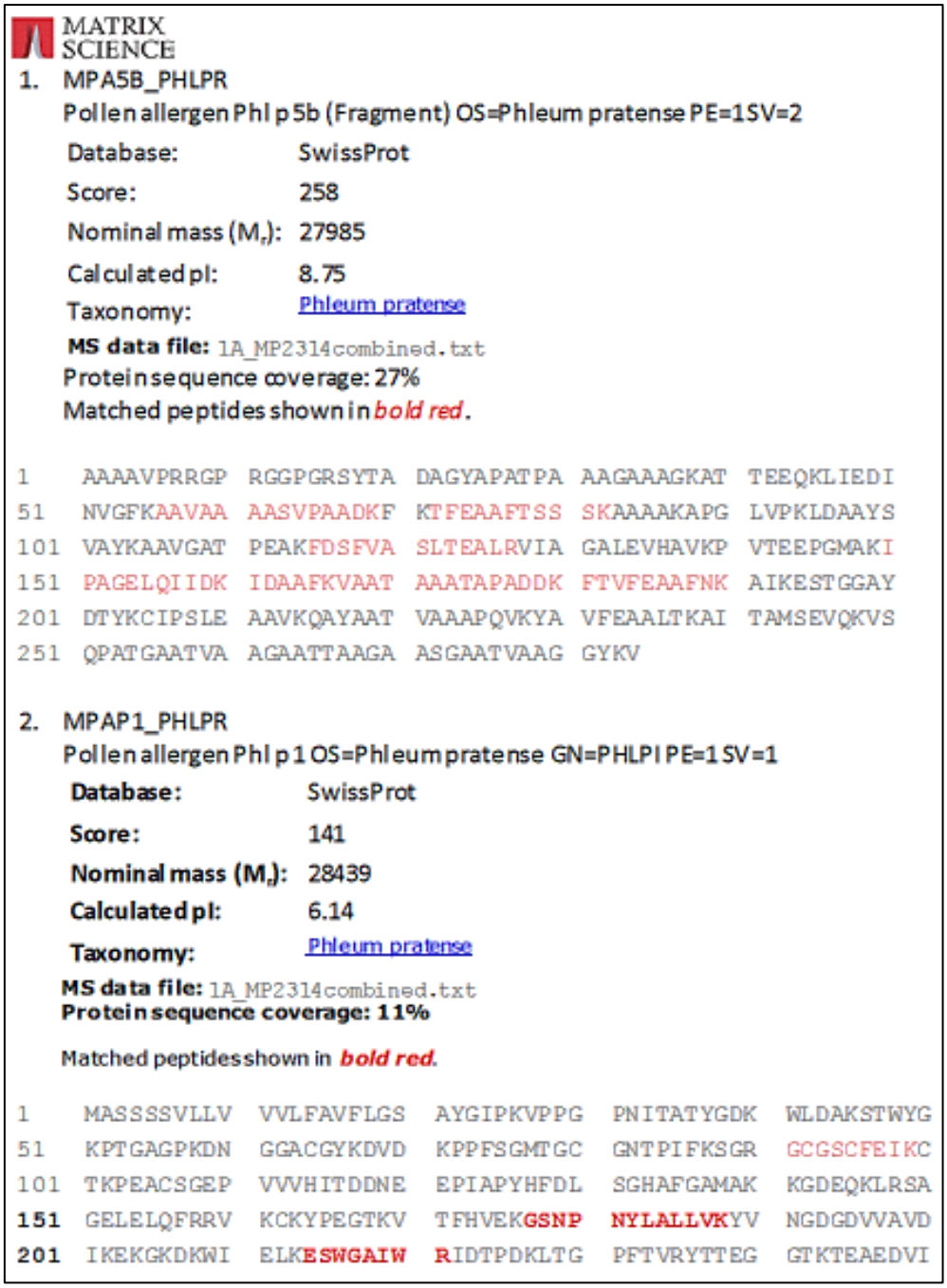
Figure 3-2 shows the purified 31 kDa (Figure 3-2, Lane 1) and 35 kDa (Figure 3-2, Lane 2) TGP molecules which were obtained from multiple eluates of excised gel strips from gel replicates containing electrophoresed crude TGP.

3.2 Identification of the 31 kDa and 35 kDa molecule as Phl p 5 and Phl p 1

Previous mass analysis (Igetei, 2015) revealed that the two purified 31 kDa TGP and 35 kDa TGP protein bands were Phl p 5 and Phl p 1 respectively. Both purified bands have been sent to mass spectrometric (MS) analysis separately, data from each band was being searched against the NCBInr database while peptide matching and protein identification were performed using Mascot “MSMS ions search” tool.

The purified bands in Figure 3-2 were excised and digested using trypsin in separate sterile tubes. Mass spectrometric analysis and a Mascot search of derived data from the purified 31 kDa band showed a significant peptide match for the *Phleum pratense* (TGP) allergen Phl p 5b (Swiss-prot, MPA5B_PHLPR) (Table 3.1), while MS derived peptides from the purified 35 kDa band were significant for the TGP Phl p 1 (NCBInr, gi|3901094) (Table 3.2). However, as indicated in the lower halves of two tables, some peptides derived from the purified 35 kDa band also showed some peptide matches with the sequence of Phl p 5b and vice versa, some peptides from the purified 31 kDa band showed some peptide matches with the sequence of Phl p 1 as well.

Table 3.1 MASCOT search output of tandem MS data from the purified 31 kDa TGP molecule



MATRIX SCIENCE

1. MPA5B_PHLPR

Pollen allergen Phl p 5b (Fragment) OS=Phleum pratense PE=1SV=2

Database:	SwissProt
Score:	258
Nominal mass (M_r):	27985
Calculated pI:	8.75
Taxonomy:	Phleum pratense

MS data file: 1A_MP2314combined.txt

Protein sequence coverage: 27%

Matched peptides shown in ***bold red***.

```
1    AAAAVPRRGP  RGGPGRSYTA  DAGYAPATPA  AAGAAAGKAT  TEEQKLIEDI
51   NVGFKAAVAA  AASVPAADKF  KTFEAAFTSS  SKAAAAKAPG  LVPKLDAAYS
101  VAYKAAVGAT  PEAKFDSFVA  SLTEALRVIA  GALEVHAVKP  VTEEPGMAKI
151  PAGELQIIDK  IDAAFKVAAT  AAATAPADDK  FTVFEAAFNK  AIKESTGGAY
201  DTYKCIPSLE  AAVKQAYAAT  VAAAPQVKYA  VFEAALTKAI  TAMSEVQKVS
251  QPATGAATVA  AGAATTAAGA  ASGAATVAAG  GYKV
```

2. MPAP1_PHLPR

Pollen allergen Phl p 1 OS=Phleum pratense GN=PHLP1 PE=1 SV=1

Database:	SwissProt
Score:	141
Nominal mass (M_r):	28439
Calculated pI:	6.14
Taxonomy:	Phleum pratense

MS data file: 1A_MP2314combined.txt

Protein sequence coverage: 11%

Matched peptides shown in ***bold red***.

```
1    MASSSSVLLV  VVLFAVFLGS  AYGIPKVPPG  PNITATYGDK  WLDAKSTWYG
51   KPTGAGPKDN  GGACGYKDVD  KPPFSGMTGC  GNTPIFKSGR  GCGSCFEIKC
101  TKPEACSGEP  VVVHITDDNE  EPIAPYHFDL  SGHAFGAMAK  KGDEQKLRSA
151  GELELQFRRV  KCKYPEGTKV  TFHVEKGSNP  NYLALLVKYV  NGDGDVVAVD
201  IKEKGKDKWI  ELKESWGAIW  RIDTPDKLTG  PFTVRYTTEG  GTKTEAEDVI
```

Table 3.2 MASCOT search output of tandem MS data from the purified 31 kDa TGP molecule

MATRIX SCIENCE

1) gi|3901094
pollen allergen Phl p1 [Phleum pratense]

Database: NCBInr
Score: 453
Nominal mass (M_r): 28185
Calculated pI: 7.53
Taxonomy: Phleum pratense
MS data file: 1A_MP2314combined.txt

Protein sequence coverage: 27%
Matched peptides shown in ***bold red***.

```
1    MASSSSVLLV VALFAVFLGS AHGIPKVPPG PNITATYGDK WLDAKSTWYG
51   KPTAAGPKDN GGACGYKDVD KPPFSGMTGC GNTPIFKSGR GCGSCFEIKC
101  TKPEACSGEP VVVHITDDNE EPIAAYHFDL SGIAFGSMAK KGDEQKLRSA
151  GEVEIQFRRV KCKYPEGTKV TFHVEKGSNP NYLALLVKFV AGDGDVVAVD
201  IKEKGKDKWI ALKESWGAIW RIDTPEVLKG PFTVRYTTEG GTKGEAKDVI
251  PEGWKADTAY ESK
```

2) gi|2851457
RecName: Full=Pollen allergen Phl p 5b; AltName: Full=Allergen Phl p Vb; AltName: Allergen=Phl p 5b; Flags: Precursor

Database: NCBInr
Score: 247
Nominal mass (M_r): 27985
Calculated pI: 8.75
Taxonomy: Phleum pratense
MS data file: 1A_MP2314combined.txt

Protein sequence coverage: 25%
Matched peptides shown in ***bold red***.

```
1    AAAAVPRRGP RGGPGRSYTA DAGYAPATPA AAGAAAGKAT TEEQKLIEDI
51   NVGFKAAVAA AASVPAADKF KTFEAAFTSS SKAAAAKAPG LVPKLDAAYS
101  VAYKAAVGAT PEAKFDSFVA SLTEALRVIA GALEVHAVKP VTEEPGMAKI
151  PAGELQIIDK IDAAFKVAAT AAATAPADDK FTVFEAAFNK AIKESTGGAY
201  DTYKCIPSLE AAVKQAYAAT VAAAPQVKYA VFEAALTKAI TAMSEVQKVS
251  QPATGAATVA AGAATTAAGA ASGAATVAAG GYKV
```

Further analysis of Phl p 1 and Phl p 5b was carried out to predict the number of potential glycosylation sites on their respective sequences. The amino acid sequence of each TGP allergen derived from the National Centre for Bioinformatics information (NCBI) was blasted against GlycoEP software for the prediction of potential N- and O-linked glycosylation sites (Chauhan et al., 2013). Predictions revealed that Phl p 5b has 2 N-glycosylated sites (Table 3.3) and 17-O-linked sites (Table 3.4) whereas Phl p 1 has 1 N-linked (Table 3.5) and 13-O-linked (Table 3.6) potential glycosylation sites.

Table 3.3 Potential non-glycosylated sites for Phl p 5b

```
>Phlp5b Length = 284

AAAAVPRRGPRGGPGRSYTADAGYAPATPAAAGAAAGKATTEEQKLIEDINVGFKAAVAAAASVPAAD
KFKTFEAAFTSSSKAAAAKAPGLVPKLDAAYSVAYKAAVGATPEAKFDSFVASLTEALRVIAGALEVH
AVKPVTEEPGMAKIPAGELQIIDKIDAAFKVAATAAATAPADDKFTVFEAAFNKAIKESTGGAYDTYK
CIPSLEAAVKQAYAATVAAAPQVKYAVFEAALTKAITAMSEVQKVSQPATGAATVAAGAATTAAGAAS
GAATVAAGGYKV

-------------------------------------------------

Position    Residue      Score          Prediction

-------------------------------------------------

51           NVG        -1.2671764      Non-glycosylated
189          NKA        -0.76057737     Non-glycosylated

**********************************
```

Table 3.4 Potential O-linked glycosylated sites for Phl p 5b

>Phlp5b Length = 284

AAAAVPRRGPRGGPGRSYTADAGYAPATPAAAGAAAGKATTEEQKLIEDINVGFKAAVAAAASVPAAD
KFKTFEAAFTSSSKAAAAKAPGLVPKLDAAYSVAYKAAVGATPEAKFDSFVASLTEALRVIAGALEVH
AVKPVTEEPGMAKIPAGELQIIDKIDAAFKVAATAAATAPADDKFTVFEAAFNKAIKESTGGAYDTYK
CIPSLEAAVKQAYAATVAAAPQVKYAVFEAALTKAITAMSEVQKVSQPATGAATVAAGAATTAAGAASS
GAATVAAGGYKV

Position	Residue	Score	Prediction
17	S	-0.037219921	Non-glycosylated
19	**T**	**0.24243257**	**Potential Glycosylated**
28	**T**	**0.30599874**	**Potential Glycosylated**
40	**T**	**0.018684405**	**Potential Glycosylated**
41	**T**	**0.036401006**	**Potential Glycosylated**
63	S	-0.003169364	Non-glycosylated
72	T	-0.080874397	Non-glycosylated
78	T	-0.047933681	Non-glycosylated
79	S	-0.23103798	Non-glycosylated
80	S	-0.081418022	Non-glycosylated
81	S	-0.088031568	Non-glycosylated
100	S	-0.23905449	Non-glycosylated
110	**T**	**0.0088164932**	**Potential Glycosylated**
117	S	-0.23487916	Non-glycosylated
121	S	-0.36093834	Non-glycosylated
123	**T**	**0.058422284**	**Potential Glycosylated**
142	**T**	**0.21134668**	**Potential Glycosylated**
170	**T**	**0.16090342**	**Potential Glycosylated**
174	**T**	**0.16183771**	**Potential Glycosylated**
182	T	-0.044324336	Non-glycosylated
195	S	-0.1446803	Non-glycosylated
196	**T**	**0.057675723**	**Potential Glycosylated**
202	T	-0.17220422	Non-glycosylated
208	S	-0.31585482	Non-glycosylated
220	**T**	**0.012210485**	**Potential Glycosylated**
237	T	-0.028408959	Non-glycosylated
241	T	-0.17600358	Non-glycosylated
244	S	-0.08593959	Non-glycosylated
250	S	-0.10705108	Non-glycosylated
254	**T**	**0.1392236**	**Potential Glycosylated**
258	**T**	**0.41428013**	**Potential Glycosylated**
265	**T**	**0.56073719**	**Potential Glycosylated**
266	**T**	**0.45153701**	**Potential Glycosylated**
272	**S**	**0.30832147**	**Potential Glycosylated**
276	**T**	**0.34431126**	**Potential Glycosylated**

Table 3.5 Potential N-linked glycosylated sites for Phl p 1

>Phlp1 Length = 263

MASSSSVLLVVVLFAVFLGSAYGIPKVPPGPNITATYGDKWLDAKSTWYGKPTGAGPKDNGGACGYKD
VDKPPFSGMTGCGNTPIFKSGRGCGSCFEIKCTKPEACSGEPVVVHITDDNEEPIAPYHFDLSGHAFG
AMAKKGDEQKLRSAGELELQFRRVKCKYPEGTKVTFHVEKGSNPNYLALLVKYVNGDGDVVAVDIKEK
GKDKWIELKESWGAIWRIDTPDKLTGPFTVRYTTEGGTKTEAEDVIPEGWKADTSYESK

Position	Residue	Score	Prediction
32	**NIT**	**0.7670472**	**Potential Glycosylated**
60	NGG	-0.97708469	Non-glycosylated
82	NTP	-1.3149035	Non-glycosylated
119	NEE	-1.4222115	Non-glycosylated
179	NPN	-1.3878037	Non-glycosylated
181	NYL	-0.98800826	Non-glycosylated
191	NGD	-1.2761456	Non-glycosylated

Table 3.6 Potential O-linked glycosylated sites for Phl p 1

>Phlp1 Length = 263

MASSSSVLLVVVLFAVFLGSAYGIPKVPPGPNITATYGDKWLDAKSTWYGKPTGAGPKDNGGACGYKD
VDKPPFSGMTGCGNTPIFKSGRGCGSCFEIKCTKPEACSGEPVVVHITDDNEEPIAPYHFDLSGHAFG
AMAKKGDEQKLRSAGELELQFRRVKCKYPEGTKVTFHVEKGSNPNYLALLVKYVNGDGDVVAVDIKEK
GKDKWIELKESWGAIWRIDTPDKLTGPFTVRYTTEGGTKTEAEDVIPEGWKADTSYESK

Position	Residue	Score	Prediction
3	S	-0.00095546551	Non-glycosylated
4	S	-0.067242144	Non-glycosylated
5	S	**0.11158392**	**Potential Glycosylated**
6	S	**0.09579778**	**Potential Glycosylated**
20	S	-0.32331112	Non-glycosylated
34	T	**0.064133352**	**Potential Glycosylated**
36	T	-0.10302248	Non-glycosylated
46	S	-0.093445547	Non-glycosylated
47	T	**0.011143839**	**Potential Glycosylated**
53	T	**0.22606906**	**Potential Glycosylated**
75	S	-0.094465604	Non-glycosylated
78	T	**0.046254624**	**Potential Glycosylated**
83	T	**0.11919246**	**Potential Glycosylated**
88	S	-0.31269445	Non-glycosylated
94	S	-0.32203716	Non-glycosylated
101	T	-0.0030757463	Non-glycosylated
107	T	-0.063710994	Non-glycosylated
116	T	-0.038581421	Non-glycosylated
131	S	-0.40961583	Non-glycosylated
149	S	-0.47592039	Non-glycosylated
224	T	**0.077373158**	**Potential Glycosylated**
229	T	**0.14841502**	**Potential Glycosylated**
233	T	**0.1507103**	**Potential Glycosylated**
237	T	**0.20143532**	**Potential Glycosylated**
238	T	**0.2743446**	**Potential Glycosylated**
242	T	-0.065456417	Non-glycosylated
244	T	-0.011155067	Non-glycosylated
258	T	-0.016934199	Non-glycosylated
259	S	**0.093261094**	**Potential Glycosylated**
262	S	-0.13262934	Non-glycosylated

3.3 Immunoreactivity of purified anti-SmSEA derived anti-TGP rabbit IgG antibodies against Timothy grass pollen crude extract.

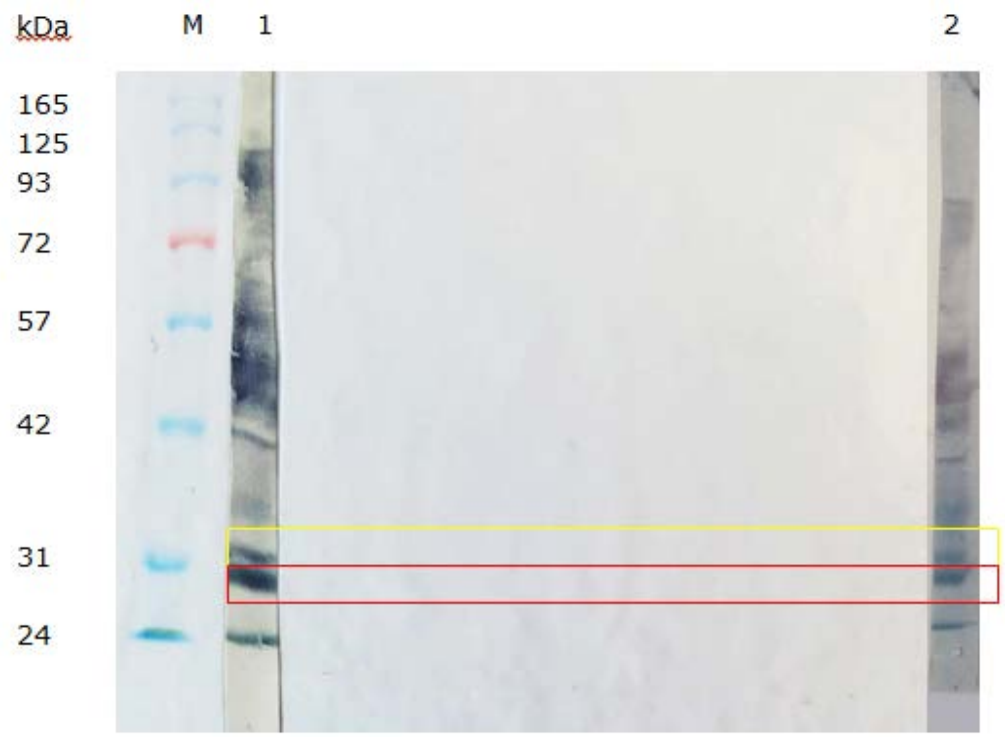


Figure 3-3 Purification of rabbit anti-SmSEA antibodies which were cross-reactive with the 31 kDa and 35 kDa TGP molecule by acid elution from western immunoblot of crude Timothy grass pollen extract.

Figure 3-3 illustrates the purification of rabbit anti-*S. mansoni* egg antibodies specific for the 31 kDa and 35 kDa Timothy grass pollen (TGP) molecules. 200 µL of crude Timothy grass pollen extract were electrophoresed and blotted. The areas of

the NCP outlined by the horizontally-marked rectangles, which are the result obtained after completion of the immunoblotting on the 2 strips at both edges (Figure 3-3, lane 1 and 2) were carrying immune complexes of rabbit antibodies and the 31 kDa and 35 kDa molecule. These horizontal strips of NCP were excised and incubated briefly in low pH buffer to elute the antibodies. Both eluates were kept separately as each eluate contained rabbit anti-*S. mansoni* egg antibodies specific for the 31 kDa and 35 kDa TGP molecules respectively. These antibodies were abbreviated as anti-SmSEA-anti-31 kDa-TGP and anti-SmSEA-anti-35 kDa-TGP. 1:100 dilutions of rabbit serum used for purification of cross-reactive rabbit IgG was added and incubated at room temperature for 2 hours. Concentrations of eluted antibodies were determined as shown in 3.6.2.

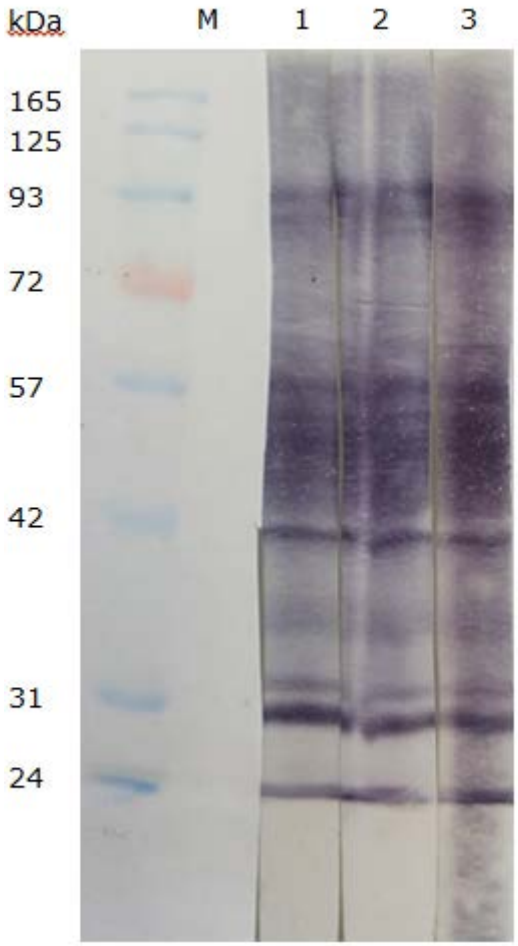


Figure 3-4 Western immunoblot showing reactivity of anti-SmSEA derived anti-TGP rabbit IgG antibodies eluted from 31 kDa & 35 kDa TGP molecule against crude TGP extract

Reactivity of anti-SmSEA-anti-31 kDa-TGP and anti-SmSEA-anti-35 kDa-TGP antibodies with crude TGP against crude TGP were tested, with polyspecific anti-*S. mansoni* antisera raised against homogenates of *S. mansoni* egg (1025Z) acting as a control. Figure 3-4 showed both eluted antibodies: anti-SmSEA-anti-31 kDa-TGP (Figure 3-4, lane 1) and anti-

SmSEA-anti-35 kDa-TGP (Figure 3-4, lane 2), were reactive with 31 kDa and 35 kDa TGP antigen as well as many other antigens in the crude TGP extract. The results also showed 1025Z (Figure 3-4, lane 3) was reactive with the TGP antigens in crude TGP extract. Lane M contained molecular weight marker.

Similar binding pattern of purified and non-purified rabbit IgG was observed. This may be caused by unsuccessful purification or unspecific binding. As such, a control of the reactivity of the residual rabbit antiserum after depletion should be tested in near future. This should be performed in order to determine the binding pattern of depleted serum. A dilution row should also be performed to show if there is a difference between purified and non-purified antiserum visible at lower concentrations. Also, reactivity of non-purified rabbit antiserum with SmSEA should be showed as controls in all blots.

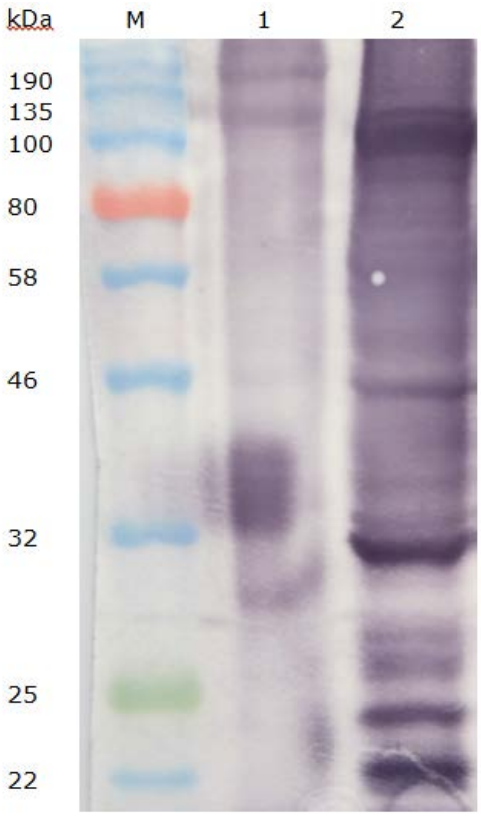


Figure 3-5 Western immunoblot showing reactivity of anti-SmSEA-derived anti-31 kDa TGP rabbit IgG antibodies against SEA extract and crude TGP extract

Figure 3-5 showed the reactivity of anti-SmSEA-derived anti-31 kDa TGP rabbit IgG against SmSEA extract (Figure 3-5, Lane 1) and crude TGP extract (Figure 3-5, Lane 2). Lane M contained molecular weight marker.

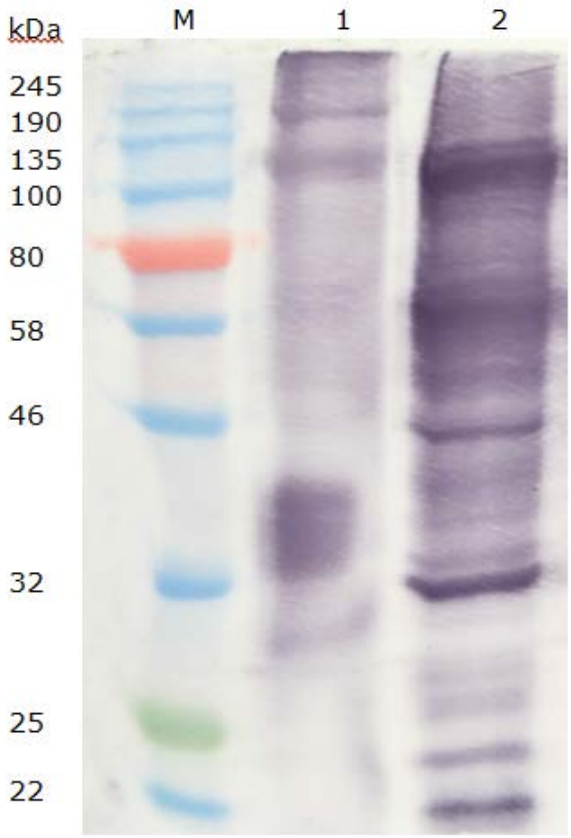


Figure 3-6 Western immunoblot showing reactivity of anti-SmSEA-derived anti-35 kDa TGP rabbit IgG antibodies against SEA extract and crude TGP extract. Lane M contained molecular weight marker.

Results showed that both anti-SmSEA rabbit IgG antibodies cross-reacted with a broad band at ~36 to 41 kDa, a band at >100 kDa and a band with low intensity at ~31 kDa in the SmSEA extract.

Based on previous experience of western immunoblot reactivity of the constituents of SmSEA, the three antigens that induced reactivity of the eluted antibodies were most likely to be IPSE/alpha-1 (broad band at ~36 to 41 kDa), kappa-5 (~100 kDa) and omega-1 (~31 kDa) respectively (Schramm et al., 2006; Schramm et al., 2009b; Everts et al., 2009). However, some reactivity smeared throughout the rest of the strip has been observed in which the purified antibodies seemed to recognise more or less most of the antigens in SmSEA.

As expected, both eluted antibodies cross-reacted with a band at 31 kDa, a band at 35 kDa and some other antigens in crude TGP extract. 31 kDa and 35 kDa in crude TGP extract were found to be TGP allergen Phl p 5 and Phl p 1 respectively.

3.4 Tentative identification of *S. mansoni* egg antigens that induced the rabbit IgG antibodies in anti-SmSEA that cross-reacted with Phl p 1, Phl p 5 and other Timothy grass pollen antigens

The rabbit anti-SmSEA IgG antibodies that were cross-reactive with Phl p 1 (35 kDa) and Phl p 5 (31 kDa) and that

had been purified from western immunoblots by acid-elution were tested for reactivity on the crude extracts of SmSEA.

To identify the *S. mansoni* soluble egg antigens (SmSEA) that potentially induced the cross-reactive IgG antibodies in anti-SmSEA, the antibodies eluted from the 31 kDa and 35 kDa TGP molecules were used to probe NCP carrying electro-transferred SmSEA in a western immunoblot. On top of that, the reactivities of the acid-eluted antibodies with those of rabbit antisera rose against whole SmSEA and of rabbit antisera monospecific and polyspecific for different S. *mansoni* egg antigens were also compared in Figure 3-7.

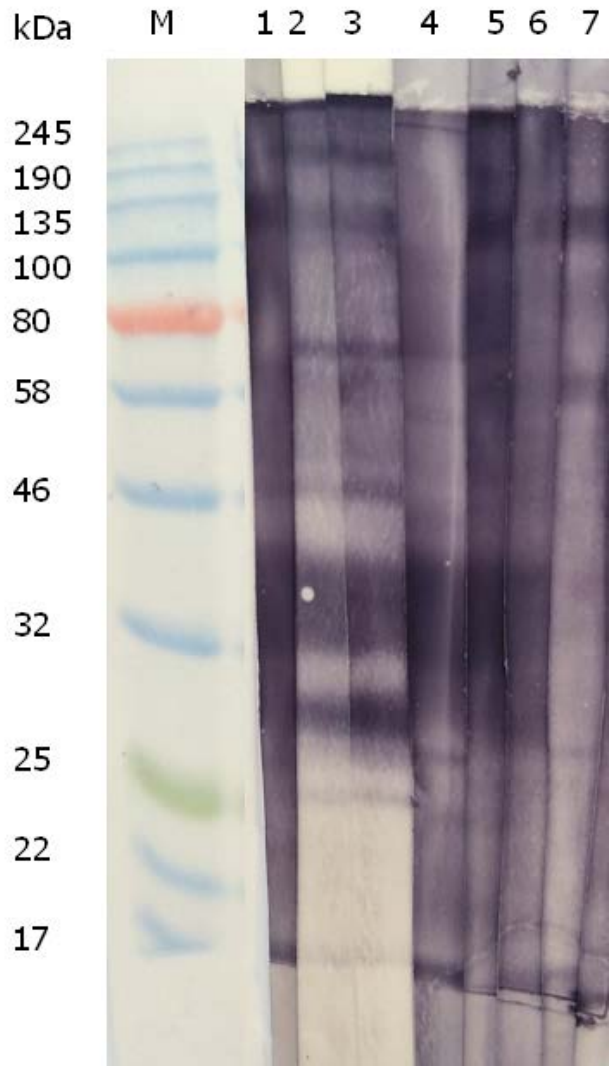


Figure 3-7 Western immunoblot showing reactivity of anti-SmSEA-derived anti-TGP rabbit IgG(s) and various rabbit antisera against SmSEA extract. Lane M contained molecular weight marker.

Based on previous experience of western immunoblot reactivity of the constituents of SmSEA, these three antigens were deemed likely to be IPSE/alpha-1 (Schramm et al., 2006), kappa-5 (Schramm et al., 2009b) and omega-1

(Everts et al., 2009) respectively. Support for this conclusion was obtained by showing that the purified antibodies reacted with the same three antigens in SmSEA (Figure 3-7, lane 1) as well as four different sera from rabbits immunised with IPSE/alpha-1 and (Figure 3-7, lanes 4 and 5); kappa-5 (Figure 3-7, lane 7) as well as omega-1 (Figure 3-7, lane 6) respectively. These monospecific sera were chosen as it had shown promising results in previous experiments (Igetei 2015). However, smearing activities have been observed throughout the rest of the strips, suggesting that the monospecific sera were not as monospecific as expected.

3.5 Possible involvement of carbohydrate determinants in the cross-reactivity of the eluted anti-SmSEA rabbit IgG antibodies with TGP molecules

Replicates of NCP containing TGP antigens were treated with 20mM sodium metaperiodate prior to incubation with the eluted antibodies.

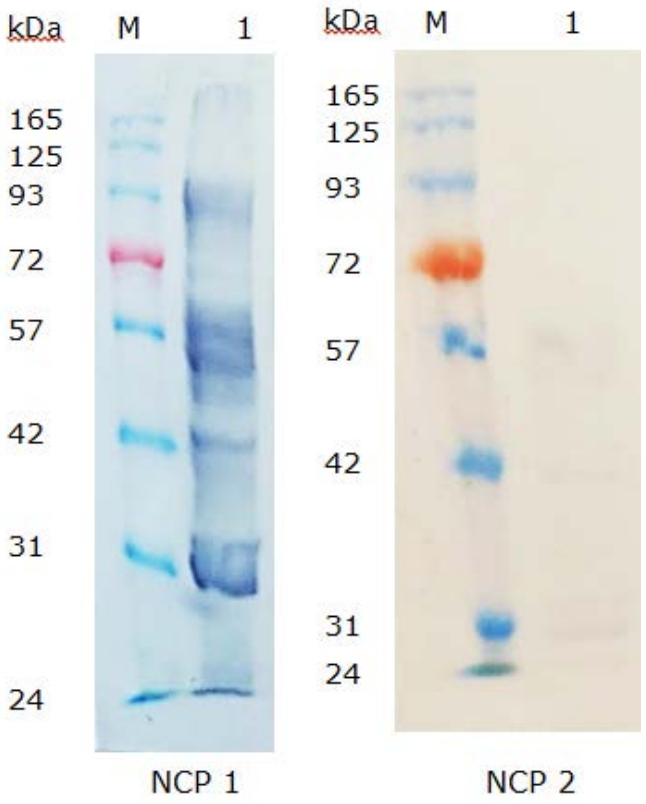


Figure 3-8 Western immunoblot, with and without sodium metaperiodate treatment, showing reactivity of anti-SmSEA anti-31 kDa-TGP rabbit IgG antibodies against crude TGP extract.

Figure 3-8 featured two NCP replicates carrying electroblotted crude TGP extract that were probed with anti-SmSEA anti-31 kDa-TGP rabbit IgG antibodies, in which one of the lanes (Figure 3-8, NCP 2) was treated with sodium meta-periodate prior to incubation with the eluted antibodies whereas the other NCP (Figure 3-8, NCP 1) was treated as usual. The

results showed that there was little to no reactivity after sodium meta-periodate treatment.

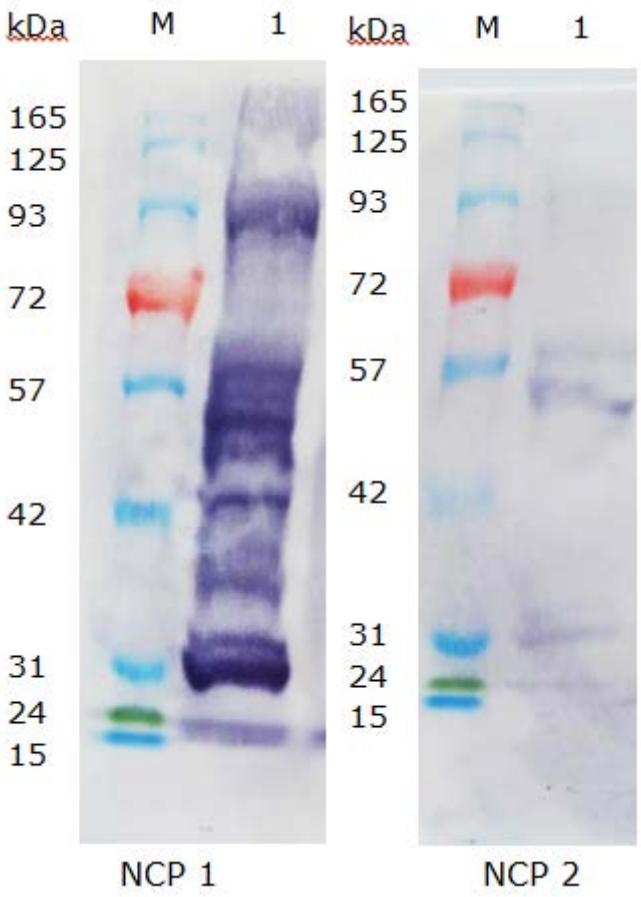


Figure 3-9 Western immunoblot, with and without sodium metaperiodate treatment, showing reactivity of anti-SmSEA anti-35 kDa-TGP rabbit IgG against crude TGP extract.

Figure 3-9 featured two NCP replicates containing crude TGP extract that were probed with anti-SmSEA anti-31 kDA-TGP rabbit IgG, in which one of the NCP (Figure 3-9, NCP 2) was

treated with sodium meta-periodate prior incubation of eluted antibodies whereas the other NCP (Figure 3-9, NCP 1) was blotted as usual. The results showed that most reactivity of the eluted antibodies was diminished after sodium meta-periodate treatment.

3.6 Protein concentration of crude TGP and eluted antibodies

In order to determine the concentration of crude TGP and the eluted antibodies, Bradford protein assay was used by comparing the assay response of these samples to bovine serum albumin (BSA), which concentration is known.

Crude TGP, eluted antibodies and BSA were processed in the same manner by mixing them with assay reagent and using a spectrophotometer to measure the absorbance. The protein concentration was quantified using the absorbance obtained.

3.6.1 Protein concentration of crude TGP

Concentration of crude TGP was calculated using a standard curve as shown in Figure 3-10.

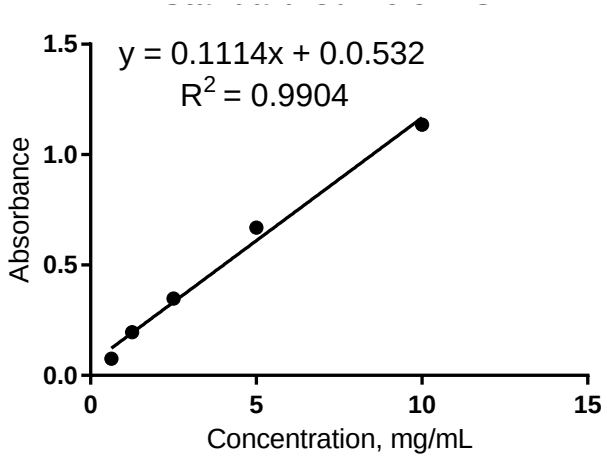


Figure 3-10 Graph of standard curve for measurement of crude TGP concentration

Concentration of crude TGP was found to be 4.74 mg/mL using BSA as standard reference as shown in Figure 3-10. The thick straight line in Figure 3-10 is the linear regression that best describes the entire set of BSA standard points (R^2=0.9904). The equation for this line is y = 0.1114x + 0.0532. As such, solving for x gives x = (y-0.0532)/0.1114. Interpolation for crude TGP was found to have an absorbance of 0.582. Hence, when y = 0.582, x = 4.74.

3.6.2 Protein concentration of eluted antibodies

Concentration of both eluted antibodies was calculated using a standard curve as shown in Figure 3-11.

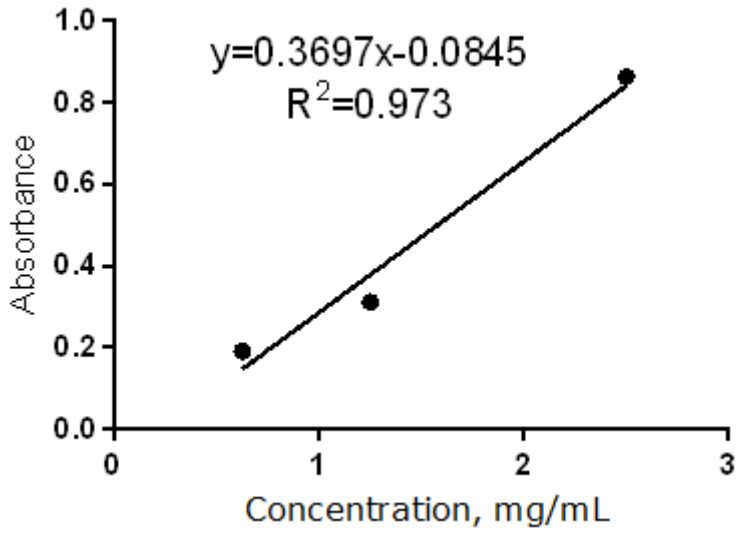


Figure 3-11 Graph of standard curve to measure the concentration of eluted antibodies

Concentration of eluted rabbit anti-SmSEA anti-31 kDa TGP IgG and anti-SmSEA anti-35kDa TGP IgG antibodies were found to be 0.3 mg/ml and 0.2 mg/ml respectively, using BSA as standard reference, as shown in Figure 3-11.

The thick straight line in Figure 3-11 is the linear regression that best describes the entire set of BSA standard points (R^2=0.973). The equation for this line is y = 0.3697x - 0.0845. As such, solving for x gives x = (y+0.0845)/0.3697.

Interpolation for eluted rabbit kDa TGP IgG antibodies was found to have an absorbance of 0.12. Hence, when y = 0.12, x = 0.39. On the other hand, interpolation for eluted rabbit anti-SmSEA anti-35 kDa TGP IgG antibodies was found to have an absorbance of 0.11. Hence, when y = 0.11, x = 0.239.

3.7 Cell culture

RS-ATL8 cell line was used in the following experiments. Purified Phl p 1 and Phl p 5 allergens from Timothy grass pollen (TGP) as well as their corresponding eluted antibodies, anti-SmSEA derived anti-Phl p 1 rabbit IgG antibodies and anti-SmSEA derived anti-Phl p 5 rabbit IgG antibodies, were used in the blocking experiment. In this study, two human sera from patients allergic to Timothy grass pollen, namely serum DG and serum KH were used. The human sera were kindly donated by Dr. Gabriele Schramm, Borstel.

Optimum dilutions of the stimuli, including purified allergens and eluted antibodies, were prepared in RBL medium. Basophil activation was measured using a luciferase assay in the following experiments.

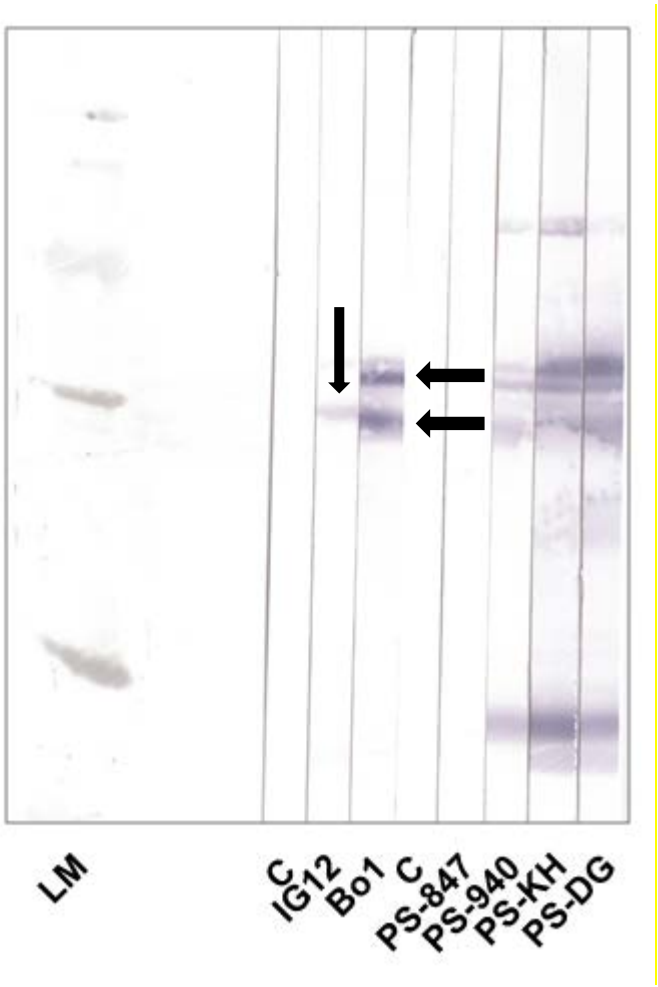


Figure 3-12 Western immunoblot showing reactivity of IgE antibodies in the human sera against TGP

Results shown in Figure 3-12 was obtained and kindly provided by Dr. Gabriele Schramm, Borstel. Western immunoblot in Figure 3-12 shows the reactivity of IgE antibodies in human sera against TGP. PS-KH and PS-DG were two of the human sera from patients allergic to Timothy grass pollen, termed as serum KH and serum DG in this study. Reactivity to TGP has been observed in both sera. Positions of

Phl p 1 and Phl p 5b identified by the respective specific monoclonal antibodies, IG12 and Bo1, were marked as shown in Figure 3-12. In this study, both sera which were kindly donated by Dr. Gabriele Schramm, Borstel, were used in cell culture experiment aimed to determine the blocking activity of eluted antibodies.

3.7.1 Optimisation of dilution of antisera

In order to sensitise the cells with human sera allergic to TGP, optimal serum dilution for each serum was determined experimentally. Several sera dilutions were prepared with a serum to basophil in culture medium (serum: basophil) ratio ranging from 1:10 to 1:100. The serum is diluted in the culture medium containing also the basophils.

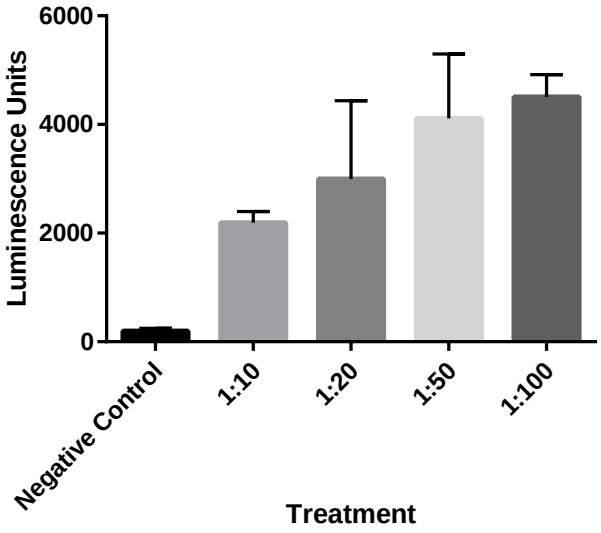


Figure 3-13 Activation of basophils sensitised with Timothy grass pollen allergic serum DG after incubation with 1 ug/ml anti-IgE

Figure 3-12 showed the activation of basophils sensitised with TGP allergic human serum DG after incubation with 1 ug/ml anti-IgE. A range of dilutions of serum DG to basophils were used, ratios were shown as serum:basophil, ranging from 1:10, 1:20, 1:50 to 1:100. Optimum dilution of serum DG was found to be 1:50 dilution.

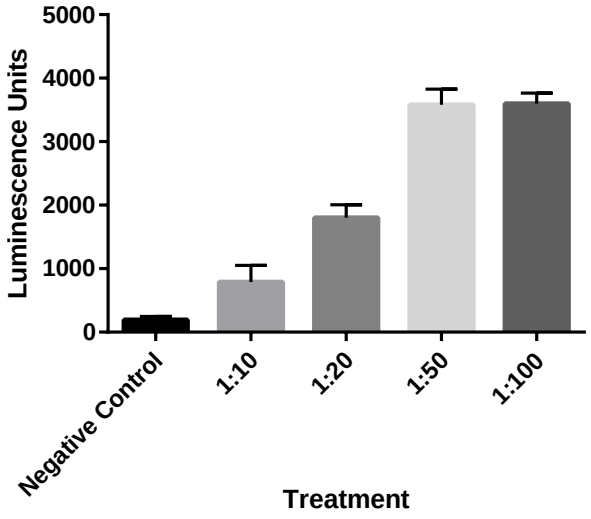


Figure 3-14 Activation of basophils sensitised with Timothy grass pollen allergic serum KH after incubation with 1 ug/ml anti-IgE

Figure 3-13 showed the activation of basophils sensitised with TGP allergic human serum KH after incubation with 1 ug/ml anti-IgE. A range of dilutions of serum KH to basophils were used, ratios were shown as serum:basophil. The optimum dilution of serum KH was found to be 1:50 dilution.

3.7.2 Optimisation of allergen concentrations

Results from 3.7.1 showed that the optimum human serum dilution to sensitise the cells is at a serum: basophil ratio of 1:50. Hence, the cells were sensitised with human sera with

the optimum sera dilution. After cell sensitisation, the cells were stimulated with the purified allergens. In order to determine the optimum allergen concentration, a series of dilution of the allergens, ranging from 100 µg/mL to 1 pg/mL, were tested. The luminescence was measured to determine the basophil activation after stimulation of cells with the purified allergens.

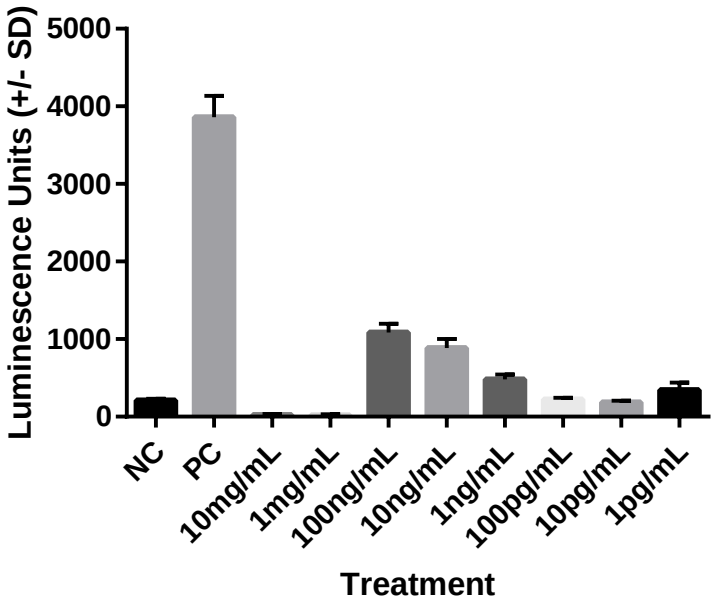


Figure 3-15 Activation of basophils sensitised with Timothy grass pollen allergic serum DG after incubation with a range of concentrations of allergen Phl p 5

Figure 3-14 shows the activation of basophils sensitised with TGP allergic human serum DG after incubation with a range of

concentrations of allergen 31 kDa Phl p 5. Concentrations of allergen Phl p 5 used were as shown, ranging from 10 mg/mL to 1 pg/mL. NC in Figure 3-14 represented a negative control which consisted of cells alone, i.e., without sensitisation by human allergic serum or addition of anti-human IgE; PC in Figure 3-14 represents a positive control consisting of cells incubated with 1 ug/mL anti-IgE. The optimum concentration of Phl p 5 was found to be 100ng/mL.

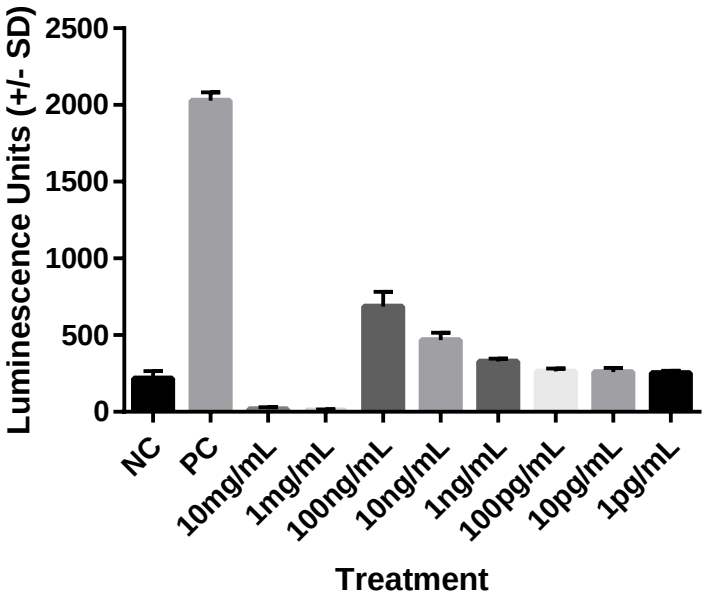


Figure 3-16 Activation of basophils sensitised with Timothy grass pollen allergic serum KH after incubation with a range of concentrations of allergen Phl p 5

Figure 3-15 shows the activation of basophils sensitised with Timothy grass pollen allergic serum KH after incubation with a range of concentrations of allergen 31 kDa Phl p 5. Concentrations of allergen Phl p 5 used were as shown, ranging from 10 mg/mL to 1 pg/mL. NC in Figure 3-15 represents a negative control which consisted of cells alone, i.e., without sensitisation by human allergic serum or addition of anti-human IgE; PC in Figure 3-15 represents a positive control consisting of cells incubated with 1 ug/mL anti-IgE. The optimum concentration of Phl p 5 was found to be 100ng/mL.

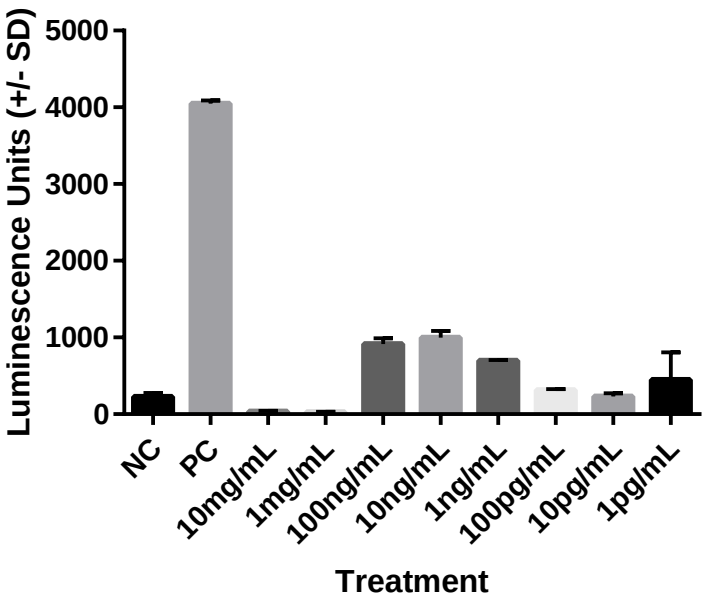


Figure 3-17 Activation of basophils sensitised with Timothy grass pollen allergic serum DG after incubation with a range of concentrations of allergen Phl p 1

Figure 3-16 shows the activation of basophils sensitised with Timothy grass pollen allergic serum DG after incubation with a range of concentrations of allergen 35 kDa Phl p 1. Concentrations of allergen Phl p 1 used were as shown, ranging from 10 mg/mL to 1 pg/mL. NC in Figure 3-16 represents a negative control consisting of cells alone, i.e., without sensitisation by human allergic serum or addition of anti-human IgE; PC in Figure 3-16 represents a positive control consisting of cells incubated with 1 ug/mL anti-IgE.

The optimum concentration of allergen Phl p 1 was found to be 100ng/mL.

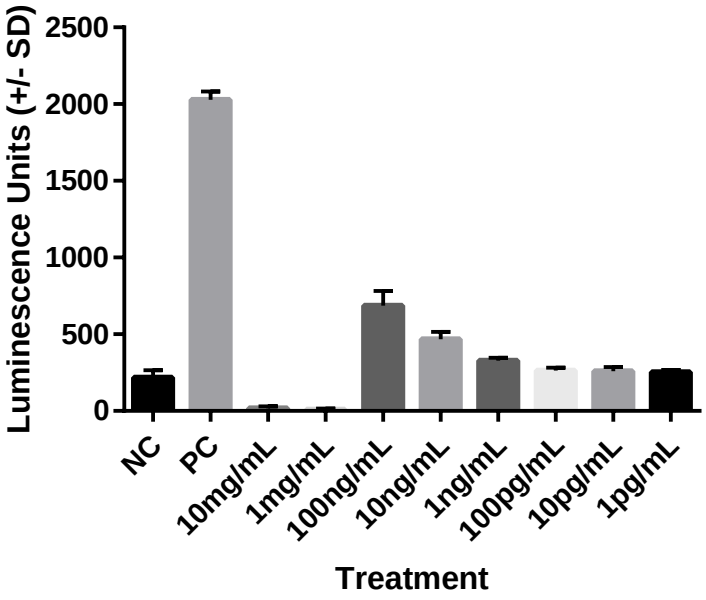


Figure 3-18 Activation of basophils sensitised with Timothy grass pollen allergic serum KH after incubation with a range of concentrations of allergen Phl p 1

Figure 3-17 shows the activation of basophils sensitised with Timothy grass pollen allergic serum KH after incubation with a range of concentrations of allergen 35 kDa Phl p 1. Concentrations of allergen Phl p 1 used were as shown, ranging from 10 mg/mL to 1 pg/mL. NC in Figure 3-17 represents a negative control consisting of cells alone, i.e., without sensitisation by human allergic serum or addition of

anti-human IgE; PC represents a positive control consisting cells incubated with 1 ug/mL anti-IgE. The optimum concentration of allergen Phl p 1 was found to be 100ng/mL.

3.8 Anti-SmSEA anti-TGP rabbit IgG Blocking Effect

Blocking ability of the eluted anti-SmSEA anti-TGP rabbit IgG antibody was tested. The purified allergen solutions were mixed with eluted antibodies at five ratios, namely 5:1, 1:1, 1:5, 1:10 and 1:20, and incubated for 16 hours to allow the antibody to bind to the allergen before they were added to the sensitised cells. The luminescence was measured to determine the basophil activation after stimulation of cells with the eluted antibodies.

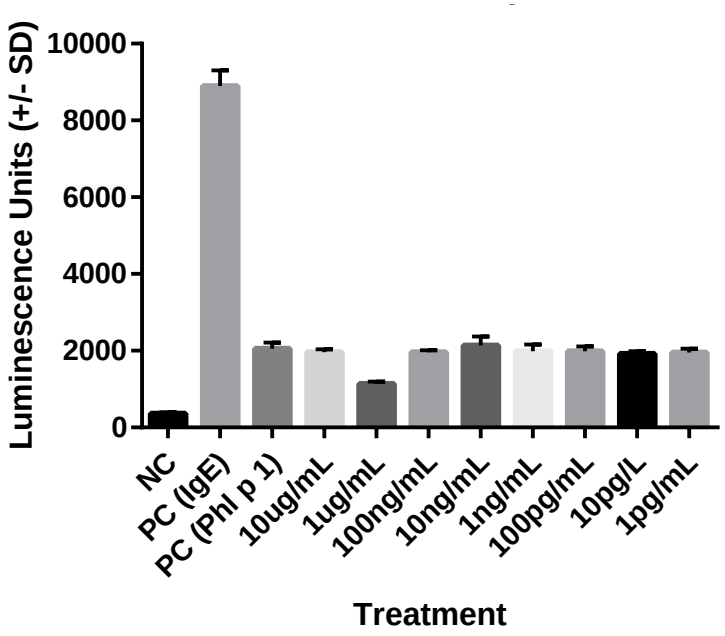


Figure 3-19 Activation of basophils sensitised with Timothy grass pollen allergic serum DG after incubation with anti-SmSEA anti-Phl p 1 rabbit IgG.

Ability of anti-SmSEA derived anti-Phl p 1 rabbit IgG antibodies to cross-react with allergen Phl p 1 was tested. NC represented negative control containing cells alone. PC (IgE) represents positive control containing cells with human anti-IgE whereas PC (Phl p 1) represents positive control containing cells that were stimulated with Phl p 1 alone. Both positive controls were not sensitised with human sera. Cells were sensitised using the optimum dilution of serum DG: 1/50 (as obtained in Figure 3-12). Anti-SmSEA anti-Phl p 1 rabbit IgG was serial diluted and then mixed with the allergen Phl p

1 to achieve the optimum concentration of the allergen, which is 100 ng/mL (as obtained in Figure 3-14), and different concentrations of the rabbit IgG, ranging from 10 ug/mL to 1 pg/mL, as seen on the X-axis of the paragraph. A negative control (cells only) and two positive controls, one with anti-IgE and one with the allergen only, are included. The whole treatments were mixed overnight using a sample mixer and then used to stimulate the cells. Luminescence was measured 4 hours after stimulation.

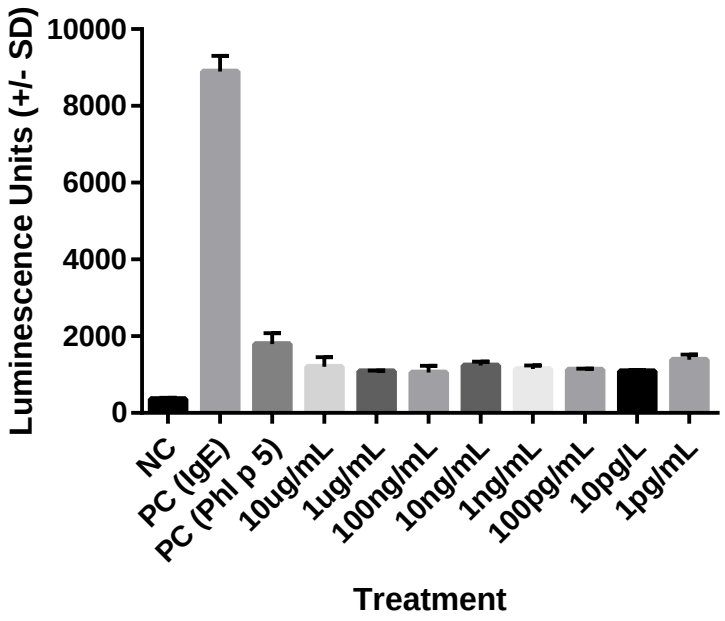


Figure 3-20 Activation of basophils sensitised with Timothy grass pollen allergic serum DG after incubation with anti-SmSEA anti-Phl p 5 rabbit IgG.

Ability of anti-SmSEA derived anti-Phl p 5 rabbit IgG to cross-react with allergen Phl p 5 was tested. NC represented negative control containing cells alone. PC (IgE) represents positive control containing cells with human anti-IgE whereas PC (Phl p 5) represents positive control containing cells that were stimulated with Phl p 5 alone. Both positive controls were not sensitised with human sera. Cells were sensitised using the optimum dilution of each serum, DG: 1/50 (as obtained in Figure 3-12). Anti-SmSEA anti-Phl p 5 rabbit IgG was serial diluted and then mixed with the allergen Phl p 1 to achieve the optimum concentration of the allergen , which is 100ng/mL (as obtained in Figure 3-14), and different concentrations of the rabbit IgG, ranging from 10 ug/mL to 1 pg/mL, as seen on the X-axis of the paragraph. A negative control (cells only) and two positive controls, one with anti-IgE and one with the allergen only, are included. The whole treatments were mixed overnight using a sample mixer and then used to stimulate the cells. Luminescence was measured 4 hours after stimulation.

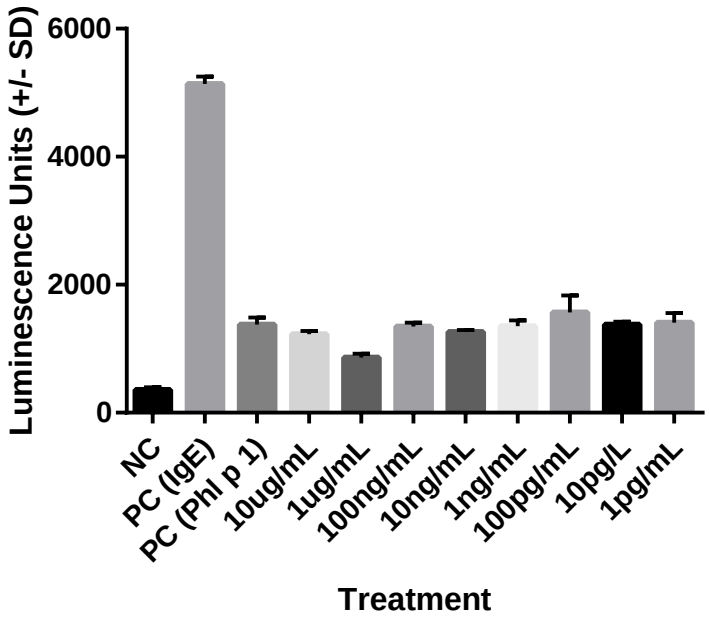


Figure 3-21 Activation of basophils sensitised with Timothy grass pollen allergic serum KH after incubation with anti-SmSEA anti-Phl p 1 rabbit IgG.

Ability of anti-SmSEA derived anti-Phl p 1 rabbit IgG to cross-react with allergen Phl p 1 was tested. NC represented negative control containing cells alone. PC (IgE) represents positive control containing cells with human anti-IgE whereas PC (Phl p 1) represents positive control containing cells that were stimulated with Phl p 1 alone. Cells were sensitised using the optimum dilution of serum KH: 1/50 (as obtained in Figure 3-13). Anti-SmSEA anti-Phl p 1 rabbit IgG was serial diluted and then mixed with the allergen Phl p 1 to achieve the optimum concentration of the allergen, which is 100

ng/mL (as obtained in Figure 3-17), and different concentrations of the rabbit IgG, ranging from 10 ug/mL to 1 pg/mL, as seen on the X-axis of the paragraph. A negative control (cells only) and two positive controls, one with anti-IgE and one with the allergen only, are included. The whole treatments were mixed overnight using a sample mixer and then used to stimulate the cells. Luminescence was measured 4 hours after stimulation.

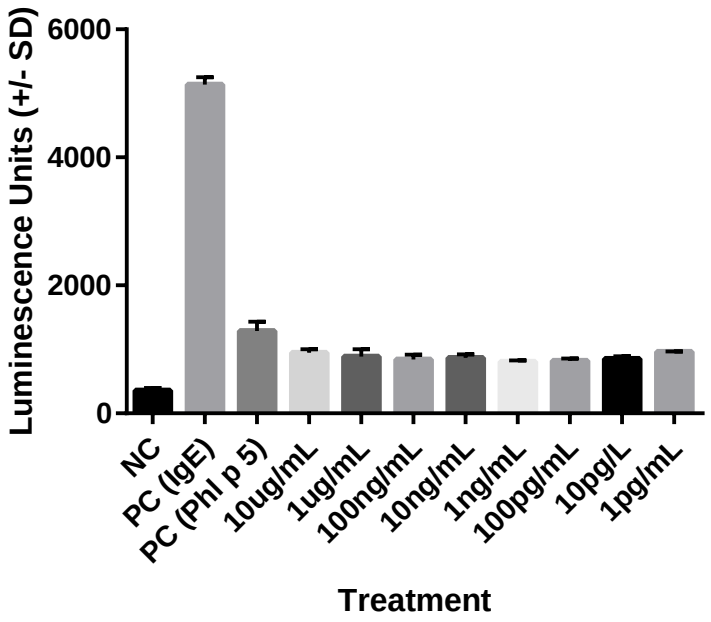


Figure 3-22 Activation of basophils sensitised with Timothy grass pollen allergic serum KH after incubation with anti-SmSEA anti-Phl p 5 rabbit IgG

Ability of anti-SmSEA derived anti-Phl p 5 rabbit IgG to cross-react with allergen Phl p 1 was tested. NC represented negative control containing cells alone. PC (IgE) represents positive control containing cells with human anti-IgE whereas PC (Phl p 5) represents positive control containing cells that were stimulated with Phl p 5 alone. Cells were sensitised using the optimum dilution of serum KH: 1/50 (as obtained in Figure 3-13). Anti-SmSEA anti-Phl p 5 rabbit IgG was serial diluted and then mixed with the allergen Phl p 5 to achieve the optimum concentration of the allergen, which is 100 ng/mL (as obtained in Figure 3-15), and different concentrations of the rabbit IgG, ranging from 10 ug/mL to 1 pg/mL, as seen on the X-axis of the paragraph. A negative control (cells only) and two positive controls, one with anti-IgE and one with the allergen only, are included. The whole treatments were mixed overnight using a sample mixer and then used to stimulate the cells. Luminescence was measured 4 hours after stimulation.

Chapter 4 DISCUSSION

In an effort to identify cross-reactivity between *S. mansoni* antigens and Timothy grass pollen, western immunoblotting and SDS-PAGE have been performed. In addition, the blocking ability of IgG antibodies against cross-reactive epitopes and thus the possibility of their inhibiting allergic reactions have also been tested with sera from patients allergic to TGP in RS-ATL8 cell line.

4.1 Cross-reactivity between Timothy grass pollen allergens and *S. mansoni* antigens

In this study, cross-reactivity has been demonstrated between *S. mansoni* antigens and the constituents of crude extracts of Timothy grass pollen, which is one of the major sources of allergic rhinitis and asthma (Radauer & Breiteneder, 2006). In this study, Timothy grass pollen (TGP) extracts were probed for antigenic reactivity with IgG antibodies obtained from rabbits that had been immunised with the egg stages of *S. mansoni*.

Two TGP molecules that were antigenically cross-reactive with parasite molecular weights of 35 kDa and 31 kDa were

identified and subjected to further analysis. The two TGP proteins that cross-reacted with the rabbit anti-SmSEA antibodies were purified and MS analysis confirmed them to be the TGP allergens, Phl p 1 (35 kDa) and Phl p 5 (31 kDa) respectively. Phl p 1 is a glycoprotein usually found to have an approximate molecular mass of 35 kDa in SDS-PAGE and immunoblots whereas Phl p 5 is a glycoprotein which is usually found to have an approximate molecular weight of 31 kDa in SDS-PAGE and immunoblots. Phl p 1 and Phl p 5 are two major grass pollen allergens which are known to have epitopes reactive with IgE (Maglio et al., 2002; Ball et al., 2004).

IgG antibodies from a rabbit #1025Z that had been immunised with *S. mansoni* egg was found to cross-react strongly with 31 kDa and 35 kDa TGP molecules. Rabbit anti-*S. mansoni* egg antibodies specific for the 31kDa and 35kDa TGP molecules were purified and eluted separately. These antibodies were abbreviated as anti-SmSEA-anti-31 kDa-TGP and anti-SmSEA-anti-35 kDa-TGP.

In this study, the validity of both antibodies (anti-SmSEA-anti-31 kDa-TGP and anti-SmSEA-anti-35 kDa-TGP) has been

tested. Results obtained from western immunoblots showed that both antibodies were reactive against TGP. Furthermore, both antibodies reacted with a broad band ~36 to 41 kDa, a band at >100 kDa and a band with low intensity at ~31 kDa in the SmSEA extract, as shown in Figure 3-5 and Figure 3-6.

The allergen cross-reactive antibodies were possibly induced by the three SmSEA antigens. Based on Schramm et al. (2006), Schramm et al. (2009) and Everts et al. (2009), the identity of the three SmSEA antigens were most likely to be IPSE/alpha-1 (broad band at ~36 to 41 kDa), kappa-5 (~100 kDa) and omega-1 (~31 kDa) respectively. Previously, IPSE/alpha-1, one of the main *S. mansoni* egg antigens, has been shown to be a heterodimer with peptides of approximate molecular weight of 41 kDa and 36 kDa. In addition, omega-1 has been identified to be a monomeric glycoprotein with an approximate molecular weight of 31 kDa (Dunne et al., 1991).

The smearing reactivity throughout the rest of the strip might be due to non-specific binding of the eluted antibodies. To overcome this, the blocking process during western immunoblotting can be prolonged to allow sufficient blocking to take place.

From the results obtained from western immunoblotting, cross-reactivity has been observed, showing that antibodies involved in helminth infections have the ability to cross-react with allergenic extracts. Such cross-reactivity gives the impression that *S. mansoni* antigens might be structurally similar to antigens present in TGP extracts.

When treated with sodium metaperiodate, the reactivity of the antibodies diminished, indicating the antigenicity of the TGP molecules that are cross-reactive with *S. mansoni* antigen is lost, as shown in Figure 3-8 and Figure 3-9. In other words, sodium metaperiodate was able to remove or destroy the formation of immune complexes. This suggested periodate-sensitive epitopes are involved in the cross-reactivity between the purified Phl p 1 and Phl p 5 TGP allergens and 1025Z antiserum.

A study by Al-Ghouleh et al. (2012) suggested that carbohydrate moieties on allergens are responsible for their recognition by innate immune cells, thus resulting in

downstream pathological Th2 cell activation as well as IgE production.

Cross-reacting carbohydrate determinants (CCDs) are antigenic structures shared by allergenic components from taxonomically distant sources (Mari et al. 1999). A study by Mari et al. (1999) indicated that periodate treatment led to variable degrees of reduction of IgE binding to the different antigens thus suggesting that different CCDs may played distinct roles in allergy.

Phl p 1 and Phl p 5 are two important allergenic molecules in TGP as they are the main targets of IgE antibodies in the sera of TGP-allergic patients, as discussed in various studies (Laffer et al. 1994; Stumvoll et al. 2002; Ball et al. 2004; Sekerkova et al. 2012; Levin et al. 2013; Schenk et al. 1995; Röschmann et al. 2012; Maglio et al. 2002). As shown in Table 3.3, Table 3.4, Table 3.5 and Table 3.6, Phl p 1 has 1 N-linked and 13-O-linked potential glycosylation sites whereas Phl p 5 has 2 N-linked sites and 17-O-linked sites. The presence of CCDs on these potential glycosylation sites might provide an explanation for the occurrence of cross-reactivity between the schistosome antigens and TGP allergens. Due to

time constraints and resource limitations, characterisation of the glycanic residues which may have accounted for the cross-reactivity observed here has not been carried out. Further investigations are therefore needed in order to study the structures of both TGP molecules and the schistosome antigens in more detail to identify and characterise the particular epitopes that are involved in this cross-reactivity. Further work could be performed through deglycosylation experiments, as described by Al-Ghouleh et al. (2012).

4.2 Blocking antibodies hypothesis: Ability of cross-reactive IgG antibodies to block allergen binding to IgE

The other question raised in this study is whether IgG anti-schistosome antibodies which cross-react with TGP allergens are able to block allergic responses induced by IgE.

Although blocking activity was not substantial, anti-SmSEA derived anti-TGP rabbit IgG antibodies were able to hinder the activation of basophils that have been sensitised with IgE antibodies from sera of patients allergic to TGP, as seen in Figure 3-19, Figure 3-20, Figure 3-21 and Figure 3-22.

Interestingly, anti-SmSEA derived anti-Phl p 5 rabbit IgG antibodies appeared to be slightly more inhibitory than anti-SmSEA derived anti-Phl p 1 rabbit IgG antibodies. On a side note, basophil activation levels achieved by anti-human IgE was a lot higher than activation by the reaction of IgE in sera from allergic human subjects on the cell-bound TGP molecules.

In the early stage of this experiment, optimal concentrations of the purified TGP allergens and human sera allergic to TGP were determined. Such information is practically useful for the continuation of this study. This experiment has been repeated with different parameters altered, such as purifying and using anti-SmSEA derived anti-TGP rabbit IgG antibodies with a higher concentration, prolonged incubation to allow sufficient time for antigen and antibody to complex, in order to try and obtain stronger inhibition. The final outcome is as discussed above.

There are some alternatives approaches that may help to improve the experimental outcome. The results for both purified anti-SmSEA derived anti-Phl p 1 rabbit IgG antibodies

and anti-SmSEA derived anti-Phl p 5 rabbit IgG antibodies are in fact quite similar, and the same applies to the 2 purified allergens Phl p 1 and Phl p 5. One test could be to pre-mix the 2 purified antibodies as well as the 2 purified allergens, prior to testing out the blocking ability of the antibodies on IgE-induced basophil activation.

Another reason for the rabbit IgG antibodies not being able to inhibit the reactivity of the allergen completely could be the use of rabbit antibodies itself. IgE/IgG immune complexes can simultaneously engage the activating high affinity IgE receptor (FcεRI) and an inhibitory IgG receptor (FcγRIIB). The net effect of this simultaneous receptor engagement is inhibition of cellular activation. This however may not work with the mixture of rabbit and human antibodies in the RS-ATL8 cell line sustem that was used in this study, even though RS-ATL8 has been suggested as a useful *in vitro* IgE test for the assessment of biological activity of IgE and allergen binding (Nakamura et al. 2010).

One way to improve this matter in future work will be to create a new transgenic rat cell line which expresses both the human FcεRI and human FcγRIIB. On top of that, IgG

antibodies from humans immune to *S. mansoni* may work better than rabbit IgG antibodies.

Apart from that, instead of sensitising the reporter cell line over night with the allergic donor serum and stimulating it with an IgG and allergen mixture on the next day, another reporter cell line NRAT-DsRed can be sensitised overnight with a mixture of IgG, IgE and allergen and basophil activation can be measured after 24 hours (Wan et al., 2014).

In addition, in order to see if there is any difference between rabbit IgG antibodies and human IgG antibodies, basophils freshly isolated from peripheral blood, either from an allergic individual or by stripping the IgE from a healthy individual, might be used, the latter after resensitisation with allergic sera. This could be done as rabbit IgG is incapable of engaging the inhibitory FcγRIIB. Although feasible, the difficulty level and cost of such experiments will likely be a lot greater.

Previous studies on house dust mite suggested helminth reactions are commonly associated with reduced allergic

reactions to allergens, despite the presence of allergen-specific antibodies present in the sera of infected individuals (Yazdanbakhsh et al. 2001; Cooper, Chico, Rodrigues, et al. 2003; Araújo et al. 2004). In addition, glycan-specific antibodies, including IgG and IgM which are present during chronic schistosome infection, are capable of blocking the interaction of allergens with the host's IgE-biased immunity.

In short, based on the results obtained, IgG anti-schistosome antibodies that cross-react with TGP allergens were able to block IgE-induced allergeic reactions to a small extent and these results thus may help provide a possible explanation for the hygiene hypothesis. Also, a thorough understanding of the interaction between IgE antibodies and allergen during the onset of an allergic reaction is vital for the purpose of decoding the mechanisms of disease development and thus, aid in tackling allergic diseases.

Chapter 5 CONCLUSION

Cross-reactivity between Timothy grass pollen and *S. mansoni* egg antigens, especially IPSE/alpha-1, has here been indicated by western immunoblotting results. Structural similarity of antigens, most likely their carbohydrate residues, may be responsible for the cross-reactivity between *S. mansoni* and Timothy grass pollen. By challenging anti-SmSEA derived anti-TGP rabbit IgG antibodies on TGP-sensitised rat basophils, the basophil activation, representing IgE-biased Th1 immune response and cross-linking with FcεRI, were inhibited to some extent. This study provided preliminary evidence for one possible explanation of hygiene hypothesis concerning blocking antibodies, which may serve as a basis for novel forms of allergy treatment. Our immune system has been shaped over time by exposure to infectious agents, some of which harness the host immune response to facilitate their own life cycles. A key element in this co-evolution is the need to dampen down the immune response such that host pathology is minimised. A better insight of parasites' abilities to undergo immuno-modulation in their hosts is very much needed for the pharmacological intervention in the future. As the molecular mechanisms by which infectious agents interface with the immune system become clearer, a range of parasite-derived immunogens that

can elicit anti-inflammatory responses could be identified, and these could be of considerable therapeutic value.

Chapter 6 REFERENCES

Aalberse, R., van der Gaag, R. & van Leeuwen, J., 1983. Serologic aspects of IgG4 antibodies. I. Prolonged immunization results in an IgG4-restricted response. *Journal of Immunology*, 130(2), pp.722–726.

Aalberse, R.C., 1998. Clinical relevance of carbohydrate allergen epitopes. *Allergy*, 53, pp.54–57. Available at: http://doi.wiley.com/10.1111/j.1398-9995.1998.tb04940.x.

Akdis, C.A. & Akdis, M., 2011. Mechanisms of allergen-specific immunotherapy. *Journal of Allergy and Clinical Immunology*, 127(1), pp.18–27.

Al-Adhami, B.H. et al., 2005. The role of acidic organelles in the development of schistosomula of Schistosoma mansoni and their response to signalling molecules. *Parasitology*, 130(Pt 3), pp.309–322. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15796014.

Al-Ghouleh, A. et al., 2012. The Glycosylation Pattern of Common Allergens: The Recognition and Uptake of Der p 1 by Epithelial and Dendritic Cells Is Carbohydrate Dependent L. Chatenoud, ed. *PLoS ONE*, 7(3), p.e33929. Available at:

http://dx.plos.org/10.1371/journal.pone.0033929.

Amu, S. et al., 2010. Regulatory B cells prevent and reverse allergic airway inflammation via FoxP3-positive T regulatory cells in a murine model. *Journal of Allergy and Clinical Immunology*, 125(5), p.1114–1124.e8. Available at: http://dx.doi.org/10.1016/j.jaci.2010.01.018.

Anthony, R.M. et al., 2007. Protective immune mechanisms in helminth infection. *Nature reviews. Immunology*, 7(12), pp.975–87. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2258092&tool=pmcentrez&rendertype=abstract.

Araujo, M.I. et al., 2000. Inverse association between skin response to aeroallergens and Schistosoma mansoni infection. *International archives of allergy and immunology*, 123(2), pp.145–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11060486.

Araújo, M.I. et al., 2004. Schistosoma mansoni infection modulates the immune response against allergic and auto-immune diseases. *Memorias do Instituto Oswaldo Cruz*, 99(5 Suppl 1), pp.27–32. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15486631.

Asai, K. et al., 2001. Regulation of mast cell survival by IgE.

Immunity, 14(6), pp.791–800. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11420048.

Ashton, P.D. et al., 2001. The schistosome egg: development and secretions. *Parasitology*, 122, pp.329–338.

Bach, J., 2002. S Usceptibility To a Utoimmune and a Llergic D Iseases. *New England Journal of Medicine*, 347(12), pp.911–920.

Ball, T. et al., 2004. Gain of structure and IgE epitopes by eukaryotic expression of the major Timothy grass pollen allergen, Phl p 1. *FEBS Journal*, 272(1), pp.217–227. Available at: http://doi.wiley.com/10.1111/j.1432-1033.2004.04403.x.

Baumgart, M. et al., 2006. Naturally Occurring CD4+Foxp3+ Regulatory T Cells Are an Essential, IL-10-Independent Part of the Immunoregulatory Network in Schistosoma mansoni Egg-Induced Inflammation. *The Journal of Immunology*, 176(9), pp.5374–5387. Available at: http://www.jimmunol.org/content/176/9/5374.full.

Beltran, S. & Boissier, J., 2008. Schistosome monogamy: who, how, and why? *Trends in Parasitology*, 24(9), pp.386–391.

Bencurova, M., 2004. Specificity of IgG and IgE antibodies against plant and insect glycoprotein glycans determined

with artificial glycoforms of human transferrin. *Glycobiology*, 14(5), pp.457–466. Available at: http://www.glycob.oupjournals.org/cgi/doi/10.1093/glycob/cwh058.

van den Biggelaar, A.H. et al., 2000a. Decreased atopy in children infected with Schistosoma haematobium: a role for parasite-induced interleukin-10. *The Lancet*, 356(9243), pp.1723–1727. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0140673600032062.

van den Biggelaar, A.H. et al., 2000b. Decreased atopy in children infected with Schistosoma haematobium: a role for parasite-induced interleukin-10. *Lancet (London, England)*, 356(9243), pp.1723–7. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0140673600032062.

Blanchard, J.F. et al., 2001. Small-area variations and sociodemographic correlates for the incidence of Crohn's disease and ulcerative colitis. *American journal of epidemiology*, 154(4), pp.328–35. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11495856.

Boral, D., Chatterjee, S. & Bhattacharya, K., 2004. The occurrence and allergising potential of airborne pollen in

West Bengal, India. *Annals of agricultural and environmental medicine : AAEM*, 11(1), pp.45–52. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15236497.

Brooker, B.E., Canning, E.U. & Wright, C.A., 1972. *Behavioural aspects of parasite transmission*, Linnean Society of London by Academic Press.

Burrows, B. et al., 1989. Association of Asthma with Serum IgE Levels and Skin-Test Reactivity to Allergens. *New England Journal of Medicine*, 320(5), pp.271–277. Available at: http://www.nejm.org/doi/abs/10.1056/NEJM198902023200502.

Caldas, I.R. et al., 2008. Human schistosomiasis mansoni: Immune responses during acute and chronic phases of the infection. *Acta Tropica*, 108(2–3), pp.109–117. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0001706X08001733.

CDC, 2012. Parasites - Schistosomiasis. Available at: http://www.cdc.gov/parasites/schistosomiasis/biology.html [Accessed September 21, 2016].

Chauhan, J.S., Rao, A. & Raghava, G.P.S., 2013. In silico Platform for Prediction of N-, O- and C-Glycosites in Eukaryotic Protein Sequences. *PLoS ONE*, 8(6), pp.1–10.

Cheever, A.W. et al., 1994. Kinetics of egg production and egg excretion by Schistosoma mansoni and S. japonicum in mice infected with a single pair of worms. *The American journal of tropical medicine and hygiene*, 50(3), pp.281–95. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8147487.

Chitsulo, L., Loverde, P. & Engels, D., 2004. Focus: Schistosomiasis. *Nature Reviews Microbiology*, 2(1), pp.12–13. Available at: http://www.nature.com/doifinder/10.1038/nrmicro801.

Chivato, T. et al., 2012. Allergy, living and learning: diagnosis and treatment of allergic respiratory diseases in Europe. *Journal of investigational allergology & clinical immunology*, 22(3), pp.168–79. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22697006.

Christensen, L.H. et al., 2008. Several distinct properties of the IgE repertoire determine effector cell degranulation in response to allergen challenge. *Journal of Allergy and Clinical Immunology*, 122(2), pp.298–304.

Coffman, T.R.M. osmann and R.L., 1989. THI AND TH2 CELLS : Different Patterns of Lymphokine Functional Properties. *Ann. Rev. Immunol*, 7, pp.145–173.

Cooke, A. et al., 1999. Infection with Schistosoma mansoni prevents insulin dependent diabetes mellitus in non-obese diabetic mice. *Parasite immunology*, 21(4), pp.169–76. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10320614.

Cooper, P.J., Chico, M.E., Bland, M., et al., 2003. Allergic Symptoms, Atopy, and Geohelminth Infections in a Rural Area of Ecuador. *American Journal of Respiratory and Critical Care Medicine*, 168(3), pp.313–317. Available at: http://www.atsjournals.org/doi/abs/10.1164/rccm.200211-1320OC.

Cooper, P.J., Chico, M.E., Rodrigues, L.C., et al., 2003. Reduced risk of atopy among school-age children infected with geohelminth parasites in a rural area of the tropics. *Journal of Allergy and Clinical Immunology*, 111(5), pp.995–1000. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0091674903801264.

D'Amato, G. et al., 1998. Pollen-related allergy in Europe. *Allergy*, 53(6), pp.567–78. Available at:

http://www.ncbi.nlm.nih.gov/pubmed/9689338.

Dodev, T.S. et al., 2015. Inhibition of allergen-dependent IgE activity by antibodies of the same specificity but different class. *Allergy: European Journal of Allergy and Clinical Immunology*.

Doenhoff, M.J. et al., 2016. Cross-Reactivity between Schistosoma mansoni Antigens and the Latex Allergen Hev b 7: Putative Implication of Cross-Reactive Carbohydrate Determinants (CCDs). *PLoS Neglected Tropical Diseases*, 11(7).

Doenhoff, M.J. et al., 1988. Immunological identification of Schistosoma mansoni peptidases. *Molecular and Biochemical Parasitology*, 31(3), pp.233–240.

Doenhoff, M.J. et al., 1986. The schistosome egg granuloma: Immunopathology in the cause of host protection or parasite survival? *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 80(4), pp.503–514.

Dunne, D.W. et al., 1986. Schistosoma mansoni egg antigens: Preparation of rabbit antisera with monospecific immunoprecipitating activity, and their use in antigen characterization. *Parasite Immunology*, 8(6), pp.575–586.

Dunne, D.W. & Cooke, A., 2005. A worm's eye view of the

immune system: consequences for evolution of human autoimmune disease. *Nature reviews. Immunology*, 5(5), pp.420–426. Available at: http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=15864275&retmode=ref&cmd=prlinks\npapers2://publication/doi/10.1038/nri1601.

Dunne, D.W., Jones, F.M. & Doenhoff, M.J., 1991. The purification, characterization, serological activity and hepatotoxic properties of two cationic glycoproteins (alpha 1 and omega 1) from Schistosoma mansoni eggs. *Parasitology*, 103 Pt 2, pp.225–36. Available at: http://www.ncbi.nlm.nih.gov/pubmed/1745548.

Eberl, M. et al., 2001. Antibodies to glycans dominate the host response to schistosome larvae and eggs: is their role protective or subversive? *The Journal of infectious diseases*, 183(8), pp.1238–47. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11262206.

Emery, A., 2011. Population genetics of schistosomiasis. Available at: http://www.nhm.ac.uk/our-science/our-work/sustainability/population-genetics-schistosomiasis.html [Accessed August 12, 2016].

Erasmus, D.A., 1987. The adult schistosome: structure and reproductive biology. In A. J. G. Simpson & D. Rollinson,

eds. *The Biology of Schistosomes*. London: Academic Press, pp. 51–82.

Erb, K.J., 2007. Helminths, allergic disorders and IgE-mediated immune responses: Where do we stand? *European Journal of Immunology*, 37(5), pp.1170–1173. Available at: http://doi.wiley.com/10.1002/eji.200737314.

Everts, B. et al., 2009. Omega-1, a glycoprotein secreted by Schistosoma mansoni eggs, drives Th2 responses. *The Journal of experimental medicine*, 206(8), pp.1673–1680.

Falcone, F.H. et al., 1996. Human basophils release interleukin-4 after stimulation with Schistosoma mansoni egg antigen. *European Journal of Immunology*, 26(5), pp.1147–1155.

Finkelman, F.D. & Vercelli, D., 2007. Advances in asthma, allergy mechanisms, and genetics in 2006. *Journal of Allergy and Clinical Immunology*, 120(3), pp.544–550.

La Flamme, A.C., Ruddenklau, K. & Bäckström, B.T., 2003. Schistosomiasis decreases central nervous system inflammation and alters the progression of experimental autoimmune encephalomyelitis. *Infection and immunity*, 71(9), pp.4996–5004. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12933842.

Flicker, S. et al., 2013. Passive immunization with allergen-specific IgG antibodies for treatment and prevention of allergy. *Immunobiology*, 218(6), pp.884–891. Available at: http://dx.doi.org/10.1016/j.imbio.2012.10.008.

Frank, S. a, 2002. Immunology and Evolution of Infectious Disease. *Health San Francisco*, 300(5617), p.358. Available at: http://books.google.com/books?hl=en&lr=&id=8IoePM19llYC&oi=fnd&pg=PR11&dq=Immunology+and+Evolution+of+Infectious+Disease&ots=aampvJHYAW&sig=FmvGQ4Enka1aF0SWMq6wUK7dIg8.

Freidhoff, L.R. et al., 1986. A study of the human immune response to Lolium perenne (rye) pollen and its components, Lol p I and Lol p II (rye I and rye II). I. Prevalence of reactivity to the allergens and correlations among skin test, IgE antibody, and IgG antibody data. *The Journal of allergy and clinical immunology*, 78(6), pp.1190–201. Available at: http://www.ncbi.nlm.nih.gov/pubmed/3782681.

Gadermaier, E. et al., 2013. Determination of allergen specificity by heavy chains in grass pollen allergen-specific IgE antibodies. *Journal of Allergy and Clinical Immunology*,

131(4), p.1185–1193.e6. Available at: http://dx.doi.org/10.1016/j.jaci.2012.10.010.

Gould, H.J. & Sutton, B.J., 2008. IgE in allergy and asthma today. *Nature reviews. Immunology*, 8(3), pp.205–17. Available at: http://dx.doi.org/10.1038/nri2273.

Grogan, J.L. et al., 1998a. Antigen-specific proliferation and interferon-gamma and interleukin-5 production are down-regulated during Schistosoma haematobium infection. *The Journal of infectious diseases*, 177(5), pp.1433–7. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9593042.

Grogan, J.L. et al., 1998b. The effect of anti-IL-10 on proliferation and cytokine production in human schistosomiasis: fresh versus cryopreserved cells. *Parasite immunology*, 20(7), pp.345–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9717196.

Grzych, J.M. et al., 1991. Egg deposition is the major stimulus for the production of Th2 cytokines in murine schistosomiasis mansoni. *Journal of immunology (Baltimore, Md. : 1950)*, 146(4), pp.1322–7. Available at: http://www.ncbi.nlm.nih.gov/pubmed/1825109.

Haas, W. et al., 1995. Finding and recognition of the snail

intermediate hosts by 3 species of echinostome cercariae. *Parasitology*, 110 (Pt 2, pp.133–42. Available at: http://www.ncbi.nlm.nih.gov/pubmed/7885732.

Haas, W., 2003. Parasitic worms: strategies of host finding, recognition and invasion. *Zoology (Jena, Germany)*, 106(4), pp.349–364.

Haberl, B. et al., 1995. Schistosoma mansoni and S. haematobium: Miracidial host-finding behaviour is stimulated by macromolecules. *International Journal for Parasitology*, 25(5), pp.551–560.

Hamilton, J. V et al., 1999. Periodate-sensitive immunological cross-reactivity between keyhole limpet haemocyanin (KLH) and serodiagnostic Schistosoma mansoni egg antigens. *Parasitology*, 118 (Pt 1(October 2016), pp.83–89. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10070665.

He, F., 2011. Coomassie Blue Staining. *BIO-PROTOCOL*, 1(11). Available at: http://www.bio-protocol.org/e78.

Herbert, D.R. et al., 2004. Alternative macrophage activation is essential for survival during schistosomiasis and downmodulates T helper 1 responses and immunopathology. *Immunity*, 20(5), pp.623–635.

Von Hertzen, L.C. & Haahtela, T., 2004. Asthma and atopy - the price of affluence? *Allergy*, 59(2), pp.124–37. Available at: http://www.ncbi.nlm.nih.gov/pubmed/14763924.

Herz, U. et al., 2000. The influence of infections on the development and severity of allergic disorders. *Current opinion in immunology*, 12(6), pp.632–40. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11102765.

Hesse, M. et al., 2004. The pathogenesis of schistosomiasis is controlled by cooperating IL-10-producing innate effector and regulatory T cells. *Journal of immunology (Baltimore, Md.: 1950)*, 172(5), pp.3157–66. Available at: http://www.ncbi.nlm.nih.gov/pubmed/14978122.

Holm, J. et al., 2011. Facilitated antigen presentation and its inhibition by blocking IgG antibodies depends on IgE repertoire complexity. *Journal of Allergy and Clinical Immunology*, 127(4), pp.1029–1037.

Huang, S.-L., Tsai, P.-F. & Yeh, Y.-F., 2002. Negative association of Enterobius infestation with asthma and rhinitis in primary school children in Taipei. *Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology*, 32(7), pp.1029–32. Available at:

http://www.ncbi.nlm.nih.gov/pubmed/12100049.

Igetei, J., 2015. Purification and characterisation of Schistosoma mansoni antigens.

Jankovic, D. et al., 2004. Parasite-induced Th2 polarization is associated with down-regulated dendritic cell responsiveness to Th1 stimuli and a transient delay in T lymphocyte cycling. *Journal of immunology (Baltimore, Md.: 1950)*, 173(4), pp.2419–27. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15294955.

Kalbe, M., Haberl, B. & Haas, W., 1996. Schistosoma mansoni miracidial host-finding: Species specificity of an Egyptian strain. *Parasitology Research*, 82(1), pp.8–13.

Kalesnikoff, J. et al., 2001. Monomeric IgE stimulates signaling pathways in mast cells that lead to cytokine production and cell survival. *Immunity*, 14(6), pp.801–811.

Kaneko, Y. et al., 2005. Increasing Prevalence of Japanese Cedar Pollinosis: A Meta-Regression Analysis. *International Archives of Allergy and Immunology*, 136(4), pp.365–371. Available at: http://www.karger.com/?doi=10.1159/000084256.

Kassim, O. & Gibertson, D.E., 1976. Hatching of Schistosoma

mansoni eggs and observations on motility of miracidia. *The Journal of parasitology*, 62(5), pp.715–720.

Kaur, I. et al., 2011. Interleukin-4-inducing principle from Schistosoma mansoni eggs contains a functional C-terminal nuclear localization signal necessary for nuclear translocation in mammalian cells but not for its uptake. *Infection and Immunity*, 79(4), pp.1779–1788.

Kitagaki, K. et al., 2006a. Intestinal helminths protect in a murine model of asthma. *Journal of immunology (Baltimore, Md. : 1950)*, 177(3), pp.1628–35. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16849471.

Kitagaki, K. et al., 2006b. Intestinal helminths protect in a murine model of asthma. *Journal of immunology (Baltimore, Md. : 1950)*, 177(3), pp.1628–35. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16849471.

Kurtzke, J.F., 2000. Multiple sclerosis in time and space--geographic clues to cause. *Journal of neurovirology*, 6 Suppl 2, pp.S134-40. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10871801.

Kusel, J.R., 1970. Studies on the structure and hatching of the eggs of Schistosoma mansoni. *Parasitology*, 60(1), p.79.

Kusel, J.R., Al-Adhami, B.H. & Doenhoff, M.J., 2007. The schistosome in the mammalian host: understanding the mechanisms of adaptation. *Parasitology*, 134(Pt 11), pp.1477–1526.

Kwaasi, A.A.A. et al., 2002. Cross-reactivities between date palm (Phoenix dactylifera L.) polypeptides and foods implicated in the oral allergy syndrome. *Allergy*, 57(6), pp.508–18. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12028116.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227(5259), pp.680–685.

Laffer, S. et al., 1994. Complementary DNA cloning of the major allergen Phl p I from timothy grass (Phleum pratense); recombinant Phl p I inhibits IgE binding to group I allergens from eight different grass species. *The Journal of allergy and clinical immunology*, 94(4), pp.689–98. Available at: http://www.ncbi.nlm.nih.gov/pubmed/7930302.

Larsen, J.N., Broge, L. & Jacobi, H., 2016. Allergy immunotherapy: the future of allergy treatment. *Drug Discovery Today*, 21(1), pp.26–37. Available at: http://linkinghub.elsevier.com/retrieve/pii/S13596446150

02810.

Levin, M. et al., 2013. Phl p 1-Specific Human Monoclonal IgE and Design of a Hypoallergenic Group 1 Grass Pollen Allergen Fragment. *The Journal of Immunology*, 191(2), pp.551–560. Available at: http://www.jimmunol.org/cgi/doi/10.4049/jimmunol.1202051.

Liao, E.-C. et al., 2015. An Exploratory Pilot Study of Genetic Marker for IgE-Mediated Allergic Diseases with Expressions of FcεR1α and Cε. *International Journal of Molecular Sciences*, 16(5), pp.9504–9519. Available at: http://www.mdpi.com/1422-0067/16/5/9504/.

Limb, S.L. et al., 2005. Adult asthma severity in individuals with a history of childhood asthma. *Journal of Allergy and Clinical Immunology*, 115(1), pp.61–66.

Lynch, N.R. et al., 1993. Effect of anthelmintic treatment on the allergic reactivity of children in a tropical slum. *The Journal of allergy and clinical immunology*, 92(3), pp.404–11. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8360391.

Maglio, O. et al., 2002. A major IgE epitope-containing grass pollen allergen domain from Phl p 5 folds as a four-helix

bundle. *Protein Engineering Design and Selection*, 15(8), pp.635–642. Available at: https://academic.oup.com/peds/article-lookup/doi/10.1093/protein/15.8.635.

Maizels, R.M., 2005. Infections and allergy - Helminths, hygiene and host immune regulation. *Current Opinion in Immunology*, 17(6), pp.656–661.

Maizels, R.M. & Yazdanbakhsh, M., 2003. Immune Regulation by helminth parasites: cellular and molecular mechanisms. *Nature Reviews Immunology*, 3(9), pp.733–744. Available at: http://www.nature.com/doifinder/10.1038/nri1183.

Mangan, N.E. et al., 2006. Helminth-Modified Pulmonary Immune Response Protects Mice from Allergen-Induced Airway Hyperresponsiveness. *The Journal of Immunology*, 176(1), pp.138–147. Available at: http://www.jimmunol.org/cgi/doi/10.4049/jimmunol.176.1.138.

Mangan, N.E. et al., 2006. Helminth-modified pulmonary immune response protects mice from allergen-induced airway hyperresponsiveness. *Journal of immunology (Baltimore, Md. : 1950)*, 176(1), pp.138–47. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16365404.

Mari, A. et al., 1999. Specific IgE to cross-reactive carbohydrate determinants strongly affect the in vitro diagnosis of allergic diseases. *The Journal of allergy and clinical immunology*, 103(6), pp.1005–11. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10359878.

Marichal, T. et al., 2013. A Beneficial Role for Immunoglobulin E in Host Defense against Honeybee Venom. *Immunity*, 39(5), pp.963–975. Available at: http://linkinghub.elsevier.com/retrieve/pii/S1074761313004391.

Meevissen, M.H. et al., 2011. Targeted glycoproteomic analysis reveals that kappa-5 is a major, uniquely glycosylated component of Schistosoma mansoni egg antigens. *Mol Cell Proteomics*, 10(5), p.M110.005710. Available at: http://www.mcponline.org/content/10/5/M110.005710.full.pdf.

Meyer, N.H. et al., 2015. A crystallin fold in the interleukin-4-inducing principle of schistosoma mansoni eggs (IPSE/??-1) mediates IgE binding for antigen-independent basophil activation. *Journal of Biological Chemistry*, 290(36), pp.22111–22126.

Mitre, E., Norwood, S. & Nutman, T.B., 2005. Saturation of

Immunoglobulin E (IgE) Binding Sites by Polyclonal IgE Does Not Explain the Protective Effect of Helminth Infections against Atopy Saturation of Immunoglobulin E (IgE) Binding Sites by Polyclonal IgE Does Not Explain the Protective Effec. *Infection and Immunity*, 73(7), pp.4106–4111.

Modha, J. et al., 1988. An association between schistosomes and contrapsin, a mouse serine protease inhibitor (serpin). *Parasitology*, 96 (Pt 1), pp.99–109. Available at: http://www.ncbi.nlm.nih.gov/pubmed/3129693.

von Mutius, E. et al., 1994. Prevalence of asthma and atopy in two areas of West and East Germany. *American Journal of Respiratory and Critical Care Medicine*, 149(2), pp.358–364. Available at: http://www.atsjournals.org/doi/abs/10.1164/ajrccm.149.2.8306030.

Nakamura, R. et al., 2010. A convenient and sensitive allergy test: IgE crosslinking-induced luciferase expression in cultured mast cells. *Allergy: European Journal of Allergy and Clinical Immunology*.

NHS, 2016a. Asthma. Available at: http://www.nhs.uk/conditions/asthma/Pages/Introduction.aspx.

NHS, 2016b. Hay fever - causes. Available at: http://www.nhs.uk/Conditions/Hay-fever/Pages/Causes.aspx [Accessed August 14, 2016].

NHS, 2016c. Schistosomiasis (bilharzia). Available at: http://www.nhs.uk/conditions/schistosomiasis/pages/introduction.aspx [Accessed June 13, 2016].

Nyan, O.A. et al., 2001. Atopy, intestinal helminth infection and total serum IgE in rural and urban adult Gambian communities. *Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology*, 31(11), pp.1672–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11696042.

Okano, M. et al., 1999. Induction of Th2 responses and IgE is largely due to carbohydrates functioning as adjuvants on Schistosoma mansoni egg antigens. *Journal of immunology (Baltimore, Md. : 1950)*, 163(12), pp.6712–7. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10586068.

Okano, M. et al., 2001. Lacto-N-fucopentaose III found on Schistosoma mansoni egg antigens functions as adjuvant for proteins by inducing Th2-type response. *Journal of immunology (Baltimore, Md. : 1950)*, 167(1), pp.442–50. Available at:

http://www.ncbi.nlm.nih.gov/pubmed/11418681.

Oliveira, M.F. et al., 2000. Haemozoin in Schistosoma mansoni. *Molecular and Biochemical Parasitology*, 111(1), pp.217–221.

Papayannopoulos, I. a, 1995. The interpretation of collision-induced dissociation tandem mass spectra of peptides. *Mass Spectrometry Reviews*, 14(1), pp.49–73. Available at: http://doi.wiley.com/10.1002/mas.1280140104.

Paschinger, K., Rendić, D. & Wilson, I.B.H., 2009. Revealing the anti-HRP epitope in Drosophila and Caenorhabditis. *Glycoconjugate Journal*, 26(3), pp.385–395. Available at: http://link.springer.com/10.1007/s10719-008-9155-3.

Patella, V. et al., 2000. HIV-1 gp120 Induces IL-4 and IL-13 Release from Human Fc RI+ Cells Through Interaction with the VH3 Region of IgE. *The Journal of Immunology*, 164(2), pp.589–595. Available at: http://www.researchgate.net/publication/12690091_HIV-1_gp120_induces_IL-4_and_IL-13_release_from_human_FcRI_cells_through_interaction_with_the_V(H)3_region_of_IgE.

Patterson, C.C., Carson, D.J. & Hadden, D.R., 1996. Epidemiology of childhood IDDM in Northern Ireland

1989?1994: Low incidence in areas with highest population density and most household crowding. *Diabetologia*, 39(9), pp.1063–1069. Available at: http://link.springer.com/10.1007/BF00400655.

Pearce, E.J. et al., 1991. Downregulation of Th1 cytokine production accompanies induction of Th2 responses by a parasitic helminth, Schistosoma mansoni. *J Exp Med*, 173(1), pp.159–166. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2118762&tool=pmcentrez&rendertype=abstract\nhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2118762{&}tool=pmcentrez{&}rendertype=abstract.

Pearce, E.J., 2005. Priming of the immune response by schistosome eggs. *Parasite Immunology*, 27(7–8), pp.265–270. Available at: http://doi.wiley.com/10.1111/j.1365-3024.2005.00765.x.

Pearce, E.J. & MacDonald, A.S., 2002. The immunobiology of schistosomiasis. *Nature reviews. Immunology*, 2(7), pp.499–511. Available at: http://dx.doi.org/10.1038/nri843.

Platts-Mills, T.A.E., 2001. The role of immunoglobulin E in allergy and asthma. *American Journal of Respiratory and Critical Care Medicine*, 164(8 II).

Plopper, C.G. et al., 2008. *Allergy and Allergic Diseases*, Available at: http://www.scopus.com/inward/record.url?eid=2-s2.0-84889386853&partnerID=tZOtx3y1.

Del Prete, G. et al., 1988. IL-4 is an essential factor for the IgE synthesis induced in vitro by human T cell clones and their supernatants. *Journal of Immunology*, 140, pp.4193–4198.

Punnonen, J. et al., 1993. Interleukin 13 induces interleukin 4-independent IgG4 and IgE synthesis and CD23 expression by human B cells (B-cell differentiation/isotype switching/allergy). *Immunology*, 90(April), pp.3730–3734.

RADAUER, C. & BREITENEDER, H., 2006. Pollen allergens are restricted to few protein families and show distinct patterns of species distribution. *Journal of Allergy and Clinical Immunology*, 117(1), pp.141–147. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0091674905020580.

Van Ree, R. et al., 2000. ??(1,2)-xylose and??(1,3)-fucose residues have a strong contribution in IgE binding to plant glycoallergens. *Journal of Biological Chemistry*, 275(15), pp.11451–11458.

Rinaldi, G. et al., 2009. RNA interference targeting leucine aminopeptidase blocks hatching of Schistosoma mansoni eggs. *Molecular and Biochemical Parasitology*, 167(2), pp.118–126.

Rispens, T., den Bleker, T.H. & Aalberse, R.C., 2010. Hybrid IgG4/IgG4 Fc antibodies form upon “Fab-arm” exchange as demonstrated by SDS-PAGE or size-exclusion chromatography. *Molecular Immunology*, 47(7–8), pp.1592–1594. Available at: http://dx.doi.org/10.1016/j.molimm.2010.02.021.

Roberts, L.S. Janovy, J., 2010. *Foundations of Parasitology* 8th ed., McGraw-Hill International.

Romagnani, S., 2004. The increased prevalence of allergy and the hygiene hypothesis: missing immune deviation, reduced immune suppression, or both? *Immunology*, 112(3), pp.352–363. Available at: http://doi.wiley.com/10.1111/j.1365-2567.2004.01925.x.

Rook, G.A.W., 2007. The hygiene hypothesis and the increasing prevalence of chronic inflammatory disorders. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 101(11), pp.1072–1074.

Röschmann, K.I.L. et al., 2012. Purified Timothy grass pollen

major allergen Phl p 1 may contribute to the modulation of allergic responses through a pleiotropic induction of cytokines and chemokines from airway epithelial cells. *Clinical & Experimental Immunology*, 167(3), pp.413–421. Available at: http://doi.wiley.com/10.1111/j.1365-2249.2011.04522.x.

Sadler, C.H. et al., 2003. IL-10 is crucial for the transition from acute to chronic disease state during infection of mice with Schistosoma mansoni. *European Journal of Immunology*, 33(4), pp.880–888. Available at: http://doi.wiley.com/10.1002/eji.200323501.

Schenk, M.F. et al., 2011. Proteomic analysis of the major birch allergen Bet v 1 predicts allergenicity for 15 birch species. *Journal of Proteomics*, 74(8), pp.1290–1300.

Schenk, S. et al., 1995. T-cell epitopes of Phl p 1, major pollen allergen of timothy grass (Phleum pratense): Evidence for cross-reacting and non-cross-reacting T-cell epitopes within grass group I allergens. *Journal of Allergy and Clinical Immunology*, 96(6), pp.986–996. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0091674995702377.

Schramm, G. et al., 2007. Cutting edge: IPSE/alpha-1, a glycoprotein from Schistosoma mansoni eggs, induces

IgE-dependent, antigen-independent IL-4 production by murine basophils in vivo. *Journal of immunology*, 178(10), pp.6023–6027.

Schramm, G. et al., 2006. IPSE/alpha-1: A major immunogenic component secreted from Schistosoma mansoni eggs. *Molecular and Biochemical Parasitology*, 147(1), pp.9–19.

Schramm, G. et al., 2009a. Molecular characterisation of kappa-5, a major antigenic glycoprotein from Schistosoma mansoni eggs. *Molecular and Biochemical Parasitology*, 166(1), pp.4–14.

Schramm, G. et al., 2009b. Molecular characterisation of kappa-5, a major antigenic glycoprotein from Schistosoma mansoni eggs. *Molecular and Biochemical Parasitology*, 166(1), pp.4–14.

Schramm, G., Falcone, F.H., Gronow, A., Haisch, K., Mamat, U., Doenhoff, M.J., Oliveira, G., Galle, J., et al., 2003. Molecular characterization of an interleukin-4-inducing factor from Schistosoma mansoni eggs. *Journal of Biological Chemistry*, 278(20), pp.18384–18392.

Schramm, G., Falcone, F.H., Gronow, A., Haisch, K., Mamat, U., Doenhoff, M.J., Oliveira, G., Galle, J., et al., 2003.

Molecular characterization of an interleukin-4-inducing factor from Schistosoma mansoni eggs. *Journal of Biological Chemistry*, 278(20), pp.18384–18392.

Schramm, G. & Haas, H., 2010. Th2 immune response against Schistosoma mansoni infection. *Microbes and Infection*, 12(12–13), pp.881–888. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20542135.

Seidman, M.D., Gurgel, R.K., Lin, S.Y., Schwartz, S.R., Baroody, F.M., Bonner, J.R., Dawson, D.E., Dykewicz, M.S., Hackell, J.M., Han, J.K., Ishman, S.L., Krouse, H.J., Malekzadeh, S., Mims, J.W., Omole, F.S., Reddy, W.D., Wallace, D.V., Walsh, S.A., Warr, L.C., 2015. Clinical Practice Guideline Allergic Rhinitis. *Otolaryngology -- Head and Neck Surgery*, 152(1 Suppl), pp.S1–S43.

Sekerkova, A., Polackova, M. & Striz, I., 2012. Detection of Phl p 1, Phl p 5, Phl p 7 and Phl p 12 Specific IgE Antibodies in the Sera of Children and Adult Patients Allergic to Phleum Pollen. *Allergology International*, 61(2), pp.339–346. Available at: http://linkinghub.elsevier.com/retrieve/pii/S1323893015302288.

Shamji, M.H. et al., 2012. Functional rather than immunoreactive levels of IgG4 correlate closely with

clinical response to grass pollen immunotherapy. *Allergy: European Journal of Allergy and Clinical Immunology*, 67(2), pp.217–226.

Sibanda, E.N., 2003. Inhalant allergies in Zimbabwe: a common problem. *International archives of allergy and immunology*, 130(1), pp.2–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12576728.

Siraganian, R.P., 2003. Mast cell signal transduction from the high-affinity IgE receptor. *Current Opinion in Immunology*, 15(6), pp.639–646.

Smits, H.H. et al., 2010. Chronic helminth infections protect against allergic diseases by active regulatory processes. *Current Allergy and Asthma Reports*, 10(1), pp.3–12.

Staines, A. et al., 1997. Small area variation in the incidence of childhood insulin-dependent diabetes mellitus in Yorkshire, UK: links with overcrowding and population density. *International journal of epidemiology*, 26(6), pp.1307–13. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9447411.

Studier, F.W., 1973. Analysis of bacteriophage T7 early RNAs and proteins on slab gels. *Journal of Molecular Biology*, 79(2).

Stumvoll, S. et al., 2002. Purification, Structural and Immunological Characterization of a Timothy Grass (Phleum pratense) Pollen Allergen, Phl p 4, with Cross-Reactive Potential. *Biological Chemistry*, 383(9). Available at: http://www.degruyter.com/view/j/bchm.2002.383.issue-9/bc.2002.157/bc.2002.157.xml.

Tavares, C.A.P. et al., 1978. Schistosoma mansoni: Evidence for a role of serum factors in protecting artificially transformed schistosomula against antibody-mediated killing in vitro. *Parasitology*, 77(2), p.225.

Taylor, J.J., Mohrs, M. & Pearce, E.J., 2006. Regulatory T cell responses develop in parallel to Th responses and control the magnitude and phenotype of the Th effector population. *Journal of immunology (Baltimore, Md.: 1950)*, 176(10), pp.5839–47. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16670290.

Thomas, P.G. et al., 2003. Maturation of dendritic cell 2 phenotype by a helminth glycan uses a Toll-like receptor 4-dependent mechanism. *Journal of immunology (Baltimore, Md.: 1950)*, 171(11), pp.5837–41. Available at: http://www.ncbi.nlm.nih.gov/pubmed/14634093.

Thornton, C.A. et al., 2003. Fetal exposure to intact

immunoglobulin E occurs via the gastrointestinal tract. *Clinical and Experimental Allergy*, 33(3), pp.306–311.

Towbin, H., Staehelin, T. & Gordon, J., 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proceedings of the National Academy of Sciences of the United States of America*, 76(9), pp.4350–4. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=411572&tool=pmcentrez&rendertype=abstract.

Tretter, V. et al., 1993. Fucose alpha 1,3-linked to the core region of glycoprotein N-glycans creates an important epitope for IgE from honeybee venom allergic individuals. *International archives of allergy and immunology*, 102(3), pp.259–66. Available at: http://www.ncbi.nlm.nih.gov/pubmed/7693094.

Valenta, R. et al., 1994. cDNA Cloning and Expression of Timothy Grass (Phleum pratense) Pollen Profilin in Escherichia coli: Comparison with Birch Pollen Profilin. *Biochemical and Biophysical Research Communications*, 199(1), pp.106–118. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0006291X84712010.

Valenta, R. et al., 1992. Diagnosis of grass pollen allergy with

recombinant timothy grass (Phleum pratense) pollen allergens. *International archives of allergy and immunology*, 97(4), pp.287–94. Available at: http://www.ncbi.nlm.nih.gov/pubmed/1597349.

Valenta, R. et al., 1991. Identification of profilin as a novel pollen allergen; IgE autoreactivity in sensitized individuals. *Science (New York, N.Y.)*, 253(5019), pp.557–60. Available at: http://www.ncbi.nlm.nih.gov/pubmed/1857985.

Vella, A.T. & Pearce, E.J., 1992. CD4+ Th2 response induced by Schistosoma mansoni eggs develops rapidly, through an early, transient, Th0-like stage. *Journal of immunology (Baltimore, Md.: 1950)*, 148(7), pp.2283–90. Available at: http://www.ncbi.nlm.nih.gov/pubmed/1347553.

Wagner, S. & Breiteneder, H., 2002. The latex-fruit syndrome. *Biochemical Society transactions*, 30(Pt 6), pp.935–40. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12440950.

Walker, C. et al., 1991. T cell subsets and their soluble products regulate eosinophilia in allergic and nonallergic asthma. *Journal of Immunology*, 146(6), pp.1829–1835.

Walker, S.M. et al., 2011. Immunotherapy for allergic rhinitis.

Clinical & Experimental Allergy, 41(9), pp.1177–1200. Available at: http://doi.wiley.com/10.1111/j.1365-2222.2011.03794.x.

Wan, D. et al., 2014. Use of Humanised Rat Basophilic Leukaemia Cell Line RS-ATL8 for the Assessment of Allergenicity of Schistosoma mansoni Proteins. *PLoS Neglected Tropical Diseases*, 8(9).

Watanabe, T. et al., 1999. Lipid A directly inhibits IL-4 production by murine Th2 cells but does not inhibit IFN-gamma production by Th1 cells. *European journal of immunology*, 29(2), pp.413–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10064056.

Weinstock, J. V et al., 2002. The possible link between de-worming and the emergence of immunological disease. *The Journal of laboratory and clinical medicine*, 139(6), pp.334–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12066130.

van der Werf, M.J. et al., 2003. Quantification of clinical morbidity associated with schistosome infection in sub-Saharan Africa. *Acta tropica*, 86(2–3), pp.125–39. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12745133.

White, J.F. & Bernstein, D.I., 2003. Key pollen allergens in North America. *Annals of Allergy, Asthma & Immunology*, 91(5), pp.425–435. Available at: http://linkinghub.elsevier.com/retrieve/pii/S1081120610615098.

WHO, 2016. Schistosomiasis. Available at: http://www.who.int/mediacentre/factsheets/fs115/en/ [Accessed June 18, 2016].

Wicklein, D. et al., 2004. Carbohydrate moieties can induce mediator release: a detailed characterization of two major timothy grass pollen allergens. *Biological Chemistry*, 385(5). Available at: http://www.degruyter.com/view/j/bchm.2004.385.issue-5/bc.2004.044/bc.2004.044.xml.

Wilson, M.S. et al., 2007. Immunopathology of schistosomiasis. *Immunology and Cell Biology*, 85(2), pp.148–154. Available at: http://www.nature.com/doifinder/10.1038/sj.icb.7100014.

Wilson, M.S. et al., 2005. Suppression of allergic airway inflammation by helminth-induced regulatory T cells. *The Journal of Experimental Medicine*, 202(9), pp.1199–1212. Available at: http://www.jem.org/lookup/doi/10.1084/jem.20042572.

Wortmann, F., 1979. [The atopy syndrome]. *Schweizerische medizinische Wochenschrift*, 109(33), pp.1205–12. Available at: http://www.ncbi.nlm.nih.gov/pubmed/227050.

Wuhrer, M. et al., 2006. IPSE/alpha-1, a major secretory glycoprotein antigen from schistosome eggs, expresses the Lewis X motif on core-difucosylated N-glycans. *FEBS Journal*, 273(10), pp.2276–2292.

Wüthrich, B. et al., 1995. Prevalence of atopy and pollinosis in the adult population of Switzerland (SAPALDIA study). Swiss Study on Air Pollution and Lung Diseases in Adults. *International archives of allergy and immunology*, 106(2), pp.149–56. Available at: http://www.ncbi.nlm.nih.gov/pubmed/7819743.

Yazdanbakhsh, M., van den Biggelaar, A. & Maizels, R.M., 2001. Th2 responses without atopy: immunoregulation in chronic helminth infections and reduced allergic disease. *Trends in immunology*, 22(7), pp.372–7. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11429321.

Yazdanbakhsh, M., Kremsner, P.G. & Van Ree, R., 2002. Parasites and the hygiene hypothesis. *Science*, 296(April), pp.490–494. Available at: http://dx.doi.org/10.1385/CRIAI:26:1:15.

Appendix

Table 6.1 Buffers, reagents and solutions

Buffers, reagents and solutions	Recipe
10X SDS Running Buffer	30g Tris Ultrapure (Invitrogen), 144g Glycine (Sigma), 10g SDS (Sigma), topped up with 1L deionised water
1X SDS Running Buffer	100mL 10X SDS running buffer, 900mL deionised water
10X Transfer Buffer	250mM Tris base, 1.92mM glycine, topped up with deionised water to 1L
1X Transfer Buffer	100mL 10X transfer buffer, 900mL deionised water
Blocking Buffer	50mL TBST, 2.5g skimmed milk powder

10X PBS	80g NaCL, 2.0g KCL, 14.4g Na_2HPO_4, 2.4g KH_2PO_4, adjusted pH to 7.4, topped up with deionised water to 1L
1X PBS	100mL of 10X PBS, 900mL deionised water
10X TBS	500mM Tris base, 1.5M NaCL, topped up with deionised water to 1L, adjusted to pH 7.5
1X TBST	100mL 10X TBS, Tween20 500 µL, top up with deionised water to 1L
10% SDS	10g SDS (Sigma), 50mL deionised water
1.5 M Tris HCL, pH 8.8	18.17g Tris Ultrapure (Invitrogen), 100mL deionised water, adjusted to

	pH 8.8
1.0 M Tris HCL, pH 6.8	12.11g Tris Ultrapure (Invitrogen), 100mL deionised water, adjusted to pH 6.8 with concentrated HCL
Elution buffer	0.06 M Tris-HCl, 10% SDS pH 7.4
Non-reducing Laemli Buffer (Loading buffer)	250mM Tris pH 6.8, 8% SDS, 40% glycerol, 8.2 mg bromophenol blue, 2.5 mL deionised water 1mL of β-mercaptoethanol was added to obtain reducing buffer
10% APS	0.1g Ammonium persulfate, 1mL deionised water
Acid elution buffer	0.1M glycine, adjusted to pH 2.8
RBL medium	Minimum essential medium

	(MEM), 10% v/v heat-inactivated fetal bovine serum, 100 U/mL penicillin, 100 µg/.mL streptomycin, 2mM L-glutamine
Dulbecco's Phosphate Buffered Saline (DPBS)	200 mg/L potassium chloride, 200 mg/L potassium phosphate monobasic, 8000 mg/L sodium chloride, 2160 mg/L sodium phosphate dibasic