Learn from the worm!

Parasitic worms could reveal much about our immune responses to disease. **Drs Gabriele Schramm** and **Helmut Haas** of the Research Center Borstel, Germany outline their studies in this engaging line of research

Could you begin by outlining your primary research interests and aims?

Parasitic worms are masters of manipulation: to ensure reproduction and avoid rejection, they have developed potent strategies and molecules for controlling the immune response of their hosts. Following our motto, 'Learn from the worm!', our major interest focuses on understanding key strategies and molecules from parasitic worms and applying them in helping patients overcome diseases.

Depending on the situation, worms are able to both efficiently activate or dampen defence reactions. While the immune response has to be enhanced in diseases such as cancer, it should be turned down when it is exaggerated in asthma/allergy and autoimmune disorders.

Your team has already succeeded in sequencing and characterising three of the major immunogens produced by *Schistosoma mansoni* eggs. Could you provide an insight into the process through which you achieved this?

Following our previous observation that extracts of eggs from *S. mansoni* trigger basophils from non-sensitised donors to produce interleukin-4 (IL-4) and IL-13 (the key messenger molecules of a Th2 response), we have identified, cloned and recombinantly expressed the active principle which we called the IL-4-inducing principle of *S. mansoni* eggs (IPSE). Our collaborator Professor Mike J Doenhoff, a pioneer in many aspects of schistosome research, suggested to us that IPSE might be identical to alpha-1, one of three major egg antigens described by his group many years ago. Since this turned out to be true, the molecule was called IPSE/alpha-1. Mike also made us aware of the other two major egg antigens called omega-1 and kappa-5, and anticipated that they were likely to fulfil important biological functions. This was the beginning of a very efficient and lasting collaboration with Mike.

Are you collaborating with any other groups?

We are working alongside Professor Maria Yazdanbakhsh and her team at Leiden University Medical Center, investigating the immunomodulatory effects of IPSE/alpha-1 and especially omega-1. We have important collaborations in Germany with Professor Christoph G Grevelding in Giessen on the *in vitro* culture and with Professor Michael Sattler, solving the structure of IPSE/alpha-1, which we hope to publish soon.

What is your research methodology for studying the mechanism and nature of Th2 induction via parasitic worms and allergens?

In our studies of Th2 induction we are working with differently conditioned dendritic cells, which are co-cultured with naive T helper cells. The kind of stimulus determines dendritic cell conditioning and subsequent T helper cell phenotype. The polarisation of the T cells is analysed by measuring cytokine production intra-cellularly or in the supernatant. Through this approach we found that dendritic cells conditioned with omega-1 drive naive T helper cells towards the Th2 phenotype.

The other important innate immune cell type in our lab is the basophil. This is the major source of the Th2 key messenger molecule IL-4 in parasite infections. IPSE/alpha-1 triggers the release of IL-4 and IL-13 from basophils, thereby amplifying the Th2 response.

What is the potential impact of these discoveries?

Recently accumulated evidence points to IL-4 and IL-13 having an anti-inflammatory role. Thus, these cytokines inhibit the release of pro-inflammatory cytokines from activated immune cells and calm down aggressive, classically activated macrophages by an alternative mode of activation. Importantly, animals unable to produce or react to these interleukins die rapidly upon schistosome infection due to massive inflammation of the gut. We expect that a more detailed understanding of the immunological mechanisms will support the development of novel approaches to protect against chronic inflammatory diseases such as asthma/allergy and autoimmunity.

Parasitology in the fight against allergies

We seldom think of parasites with anything other than disgust. But as a team of researchers in Germany turn to parasitic worms in their search for treatments of allergic and autoimmune diseases, this may be about to change

IN THE DEVELOPED world, we tend to think of getting parasitic worms (or helminths) as uncommon, unpalatable and, frankly, a relic medical condition akin to smallpox. However, as industrialised nations have banished hookworms and the like, incidences of allergies, asthma and autoimmune conditions such as Crohn's disease and multiple sclerosis (MS) have begun to rise. These conditions remain comparatively rare in developing countries, where helminth infection is common.

Upon noticing this correlation, the medical community has recently begun to investigate the potential link between our bodily parasites and immune response.

AUTOIMMUNE ILLNESS AND SOME UNLIKELY SAVIOURS

Early studies in which MS patients in Argentina were treated for worms which worsened their symptoms, and Ethiopian children who were treated developed dust mite allergies, seemed to confirm the link between sanitation and autoimmune response. This prompted some patients to acquire worm eggs on the black market and treat themselves in an unregulated and dangerous fashion.

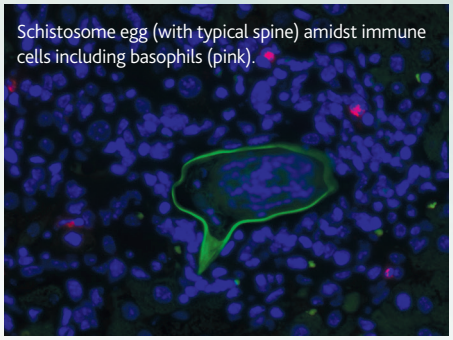
Thankfully, in the past decade controlled clinical trials with suspensions of worm eggs or larvae have delivered promising results for some diseases. However, treatment with a worm infection may be compromised by undesired side effects and lack of patient compliance. This calls for identifying the underlying active molecules.

Now, rather than administering live worm larvae to test subjects, a group of scientists in Germany are trying to exploit the parasites by alternative means to discover potential treatments for autoimmune conditions. This is the case at Research Center Borstel, where Drs Gabriele Schramm and Helmut Haas and their team are using *in vitro* culture techniques to isolate which molecules affect immune response and understand how the molecules work.

A NEW DIRECTION

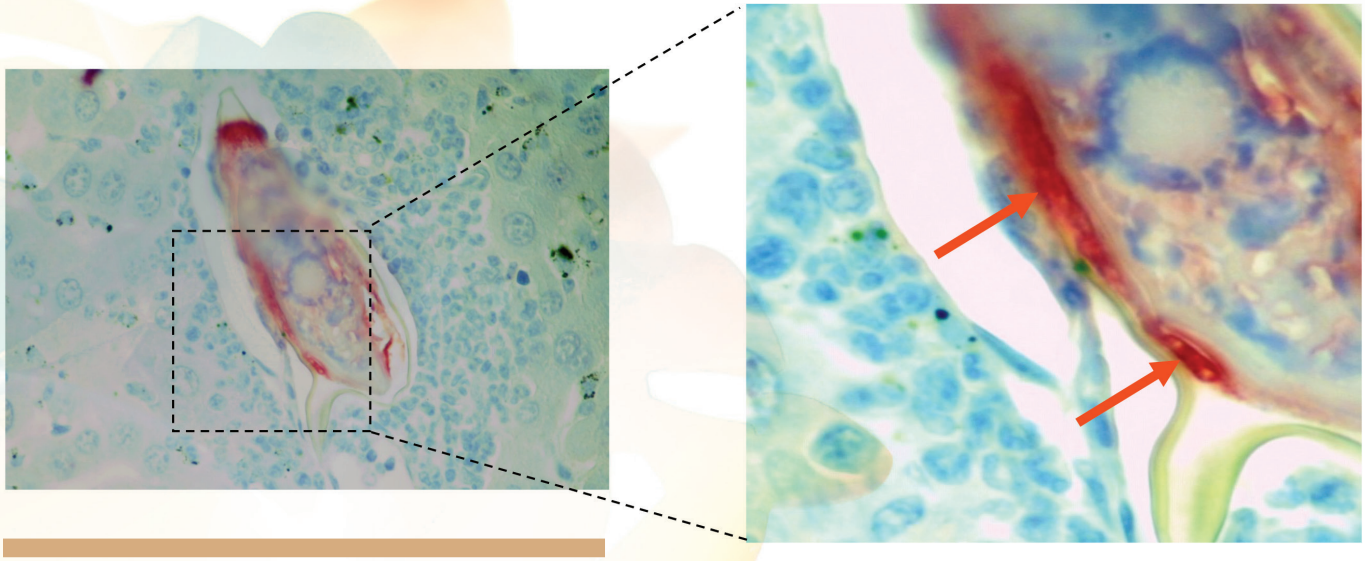
It is hoped that unlike present steroid treatment, future anti-inflammatory drugs will be very specific. The key lies in obtaining immunomodulatory molecules from the parasites.

The theory at work in this study is that parasitic worm eggs can expel effective molecules which calm aggressive inflammatory immune cells by reverting them back to harmless well-regulated cells. Although schistosome eggs contain several hundred different proteins, the Borstel team succeeded in identifying and purifying a few proteins that polarise T helper cells, resulting in the anti-inflammatory Th2 response.



Schistosome egg (with typical spine) amidst immune cells including basophils (pink).

When it came to choosing which worm to investigate, there was no contest. "We focused on *Schistosoma mansoni,* because this parasite is a grandmaster in both aspects of manipulation: enhancing and dampening immune responses," says Schramm. In schistosomes it is eggs rather than adult helminths that have been found to secrete molecules that dampen the body's immune response, a survival technique that enables the parasites to reside inside us undetected. "Since humans are its natural hosts, this parasite – and its immunomodulatory mechanisms – have co-evolved with humans, and thus are optimally adapted to the human immune system." So far, the team has succeeded in sequencing and characterising three molecules which contribute towards the anti-inflammatory effect of worm eggs.



IPSE/alpha-1 (stained red) is produced underneath the schistosome egg shell (immunohistology of infected mouse liver). Schramm G. et al., *Mol Biochem Parasitol* 2006.

Following the rehabilitation of leeches as a medical aid, we might yet end up being very grateful to the humble parasitic worm too

INTELLIGENCE

ANTI-INFLAMMATORY FACTORS FROM PARASITIC WORMS

OBJECTIVES

Some parasitic worms, namely *Schistosoma mansoni*, effectively dampen inflammation by releasing powerful anti-inflammatory products. The objective of this study is to identify such products as lead compounds for developing new drugs against asthma, allergies and autoimmune diseases.

KEY COLLABORATORS

Professor Maria Yazdanbakhsh; Dr C H Hokke; Dr Hermelijn H Smits, Leiden University Medical Center, The Netherlands

Professor Michael J Doenhoff, University of Nottingham, UK

Professor Christoph G Grevelding, University of Giessen, Germany

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DR GABRIELE SCHRAMM studied Biochemistry in Tübingen and Bayreuth. She undertook her PhD and postdoc studies at the Research Center Borstel (RCB) on the characterisation and engineering of allergens (Herxheimer Prize 1999). Since 2000 Schramm has been senior scientist at RCB.

DR HELMUT HAAS, MD worked on the characterisation of grass pollen allergens and the regulation of the IgE synthesis from 1981. Since 1990 he and his coworkers used parasitic worms *in vitro* as model organisms. His scientific focus has been on immunomodulatory factors in *S. mansoni* eggs.

Leibniz Gemeinschaft
Research Center Borstel

MARVELLOUS MOLECULES

Working in collaboration with Professor Maria Yazdanbakhsh at Leiden University Medical Center (LUMC) and other groups, the team has isolated three key molecules from schistosome eggs that may help to develop anti-inflammatory drugs in the future: omega-1, IL-4-inducing principle of *Schistosoma mansoni* eggs (IPSE)/alpha-1 and kappa-5. Omega-1 initiates the Th2 immune response and IPSE/alpha-1 seems to amplify it when tested against samples of basophil cells in the laboratory.

The release of the Th2 interleukins IL-4 and IL-13 is essential for protection in schistosome infection. An animal suffering from schistosomiasis (the potentially serious disease that results from the infection), but unable to produce or react to these interleukins will quickly die of massive inflammation. Thus, IL-4 and IL-13 appear to have anti-inflammatory properties. Not only do they provoke alternatively activated macrophages (the anti-inflammatory, wound-healing version of the classically activated aggressive macrophages), but they reduce the production of pro-inflammatory cytokines such as IL-1β and IL-6. The abilities of omega-1 and IPSE/alpha-1 to induce considerable amounts of IL-4 and IL-13 has been earmarked for future potential in pharmaceutical development. The combination of all these molecules appears to be central to the egg's effect on inflammation control.

After the rehabilitation of leeches as a medical aid, we might yet end up being very grateful to the humble parasitic worm too.

A NOVEL APPROACH

Supported by the Federal Ministry of Education and Research (BMBF), the German team has developed a technique wholly unique to their study. This involves replicating the schistosomes' natural habitats. By keeping the worms with human blood constituents which effectively mimic the worms existence in human blood vessels, the researchers can examine the development of the helminths from larvae to adulthood, including the pairing and deposition of the eggs.

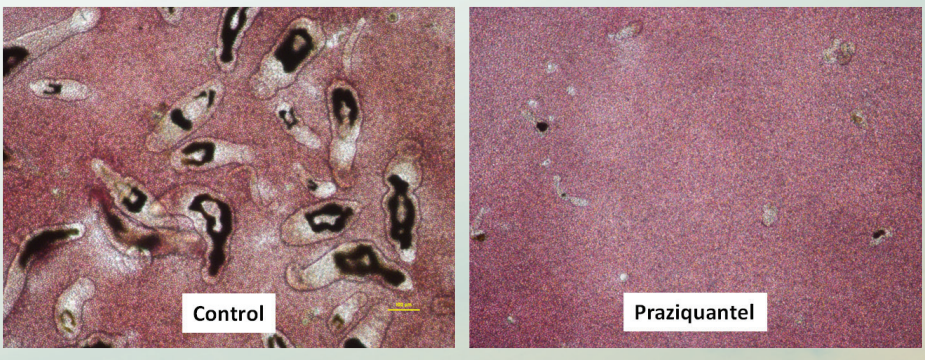
In contrast to animal experiments, the *in vitro* culture allows the group to continuously monitor growth and development of the parasite, as well as deviations thereof following addition of reagents. This practice is not only more cost-effective, but it also promotes the 3R's concept (Refine, Reduce, Replace) as fewer animals will be needed as test models.

The group has found the technique to be successfully applicable for high throughput screening for schistosomiasis. Having obtained schistosome larvae from certain water snails (the obligate intermediate hosts of schistosomes), and cultured these larvae for 14 days, a great number of compounds have been able to be tested in parallel, with any deviations from the normal morphological development of larvae easily spotted in a sensitive, reliable and easily reproducible method.

FUTURE PLANS

Regardless of the health benefits of helminths in the developed world, the effects of schistosomiasis are still wreaking havoc across the developing world. The World Health Organization estimates that approximately 240 million people are infected worldwide and that 700 million are at risk. The effects can cause failure of the intestines and renal system, and incidences of bladder cancer appear to rise in affected geographical areas. The novel *in vitro* technique has been adopted by researchers seeking a vaccine for schistosomiasis, in case the parasite becomes resistant to the existing treatment, praziquantel.

In light of the success of using *in vitro* techniques, Haas is currently setting up a company called *helmin*Guard in conjunction with Hamburg-based screening specialists European ScreeningPort. The company hopes to use *in vitro* culture systems to engage in high-throughput screenings of new drugs against all manner of helminth infections, but against schistosomiasis in particular.



The *in vitro* culture of *Schistosoma mansoni* is as close as we can get to being able to monitor the parasite behaviour in humans (left). Treating with the current generation of medicines, praziquantel at 10 µM (right) is effective, but resistance is increasing and new molecules are needed. This assay platform is highly disease relevant and will enable the team to identify new drugs against this parasite.