
Universiteit
Leiden
The Netherlands

Helminth infections induce immunomodulation : consequences and mechanisms

Riet, P.H. van

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General discussion

Introduction

Immune responses induced by helminth infection can best be characterized by a combination of Th2 and regulatory properties, a so called “modified Th2” response. Immune responses generated in response to antigens were initially divided into Th1 and Th2 responses. Th1 responses are usually associated with strong immune activation (e.g. IL-2, TNF-α), while Th2 is more associated with regulatory responses (e.g. IL-10). However, the regulatory component was later defined by a separate cell type, called regulatory T cells (consisting of natural Treg, Th3 or Tr1) that could suppress responses of both type 1 and 2 effector cells. More recently a third type of effector T cells was identified and named Th17 after the cytokine IL-17 that these T cells characteristically produce. This cell type is thought to be mainly involved in immune responses to extracellular bacteria as well as in tissue pathology and pathogenesis of autoimmune diseases [247]. It has also been proposed that Th17 cells, instead of a third type of effector T-cell, represent a more general inflammatory response opposite to regulatory T cells, thus forming a “regulation-activation” balance that crosses the “Th1-Th2” framework [248]. However, independent of the models proposed, the balance of the sum of all different types of polarizing responses will determine the final outcome of an immune reaction.
For helminth infections this could mean that there may be a Th17 component present, that could for example play a role in tissue damage, but in general the dominant immune response is a type 2 response with an overlapping regulatory component, the latter arises during infection characterized by high helminth loads and chronic inflammation [40]. This combination of a dominant type 2 and regulatory response is thought to be the basis of the long-lived partnership between the parasite and its host: the helminth is not expelled and the host does not suffer from severe tissue damage.

Consequences

Spill-over suppression

The regulatory component induced by helminth infections was found not to exert its effects solely on helminth antigens, but could also affect responses to non-related antigens. Depending on the type of antigen this can be either beneficial or detrimental for the host. For example, in case of allergic and autoimmune diseases this spill-over effect could alleviate the symptoms. How this works and how it can be used for clinical applications is widely explored and already some clinical trials have been performed by giving *Trichuris suis* eggs to patients with ulcerative colitis and Crohn’s

disease [76, 77]. In addition, several trials are underway for treatment of allergic disorders with helminths [249, 250].
However, helminth induced immunomodulation could also be detrimental when one considers coinfections with other pathogens (bacteria, protozoa or viruses) or in the case of vaccination. For the latter it would be important to know whether or not the presence of helminths is preventing efficient responses as in this case it would be relatively easy to provide antihelminthic treatment before vaccination to enhance responses. Further, knowledge about the influence of helminth infections on vaccination outcome is important for the current development of many vaccines (malaria, HIV, tuberculosis) that will be mostly needed in areas where helminths are also highly endemic. To optimize vaccines, possibly different adjuvants would be needed for populations in areas endemic for helminths than those used in vaccines for the western world.

Helminth infection and vaccine efficacy
In general it is found that the presence of helminth infections induces a Th2 bias in immune responses mounted to different types of vaccines, even when the exposure to the helminths is *in utero* [29, 33]. This Th2 bias is mostly defined as an increased antigen specific IL-5/IFN-γ ratio produced upon *ex vivo* stimulation of the immune cells after vaccination. Usually a Th1 biased immune response to vaccination is regarded effective, however, the exact influence of T helper cell polarization on the protective effect of the vaccines is not known in most cases. For example, when Gabonese children from a rural and a semi-urban area were vaccinated with tetanus toxoid (TT), rural children with a stronger TT specific Th2 bias, unexpectedly showed higher levels of anti-TT IgG1 antibodies compared to the urban children that induce a higher TT specific IFN-γ and a lower IL-5 response (chapter four).
In contrast the same children were also vaccinated with a trivalent influenza vaccine. Again, an antigen-specific Th2 skewed immune response was found in the rural children, who for influenza showed lower levels of anti-influenza haemagglutinin antibodies (chapter three). However, this was only true for two strains, antibody responses to the third strain, H3N2, were slightly, but significantly higher in the rural schoolchildren two weeks after vaccination. Interestingly, a recent outbreak with an H3N2 strain had probably occurred, as antibody titres specific to the H3N2 strain were very high already before vaccination. Would rural children mount a stronger antibody response to a strain that caused a recent epidemic? When considering the responses to TT vaccination, to which there is continuous exposure, also antibody responses were higher in the rural cohort. However, the mechanism behind this is not clear. It is possible that the difference in T cell polarization and associated cytokines affects the activation of central or effector memory T cells [176].

In the studies described in chapter three and four the aim was to compare vaccine efficacy in rural versus urban children. The presence of helminthes, determined just before vaccination, was the major difference between these populations. However, comparing antibody and cytokine production to current helminth infection status was difficult as all rural children were infected with at least one type of helminth. When comparing children with and without helminth infection within the urban cohort, helminth infection was found to significantly affect antibody production, but only for the influenza H1N1 strain. Different study designs are needed to specifically address the question of how helminth infections affect the responses to new vaccines.

It is often argued that not only current, but also early life exposure to infections may have a major influence on the immune system [251, 252]. Other characteristics of helminth infections can also influence the outcome, and these include the helminth species, the intensity of the infection, whether the infection is acute or chronic, and the history of infection. [40, 41, 253]. For example, the children in the rural area were all infected with at least one helminth species, and the chance that they were infected very early in life, or that they were even exposed *in utero* because their mothers were infected, is probably higher than for the semi-urban children. Also, the total time in life that they were infected is likely to be greater. In addition, schistosome infections might have a different influence on immune responses than intestinal helminths. These factors are difficult to study in a human population, as the populations studied need to be very large to distinguish amongst all these different characteristics, if possible at all. In animal models it has been shown that heavy and/or chronic infections can have a different effect on the immune system than when one considers acute or light infections [46]. In humans studies are needed to carefully examine these issues.

Read out of vaccine efficacy

The “ideal” responses to vaccines are mostly not known, even finding a good correlate of protection has proven to be difficult. What antibody levels are necessary? What kind of cellular responses are most effective? Which factors can improve the memory responses? Currently the correlates of protection that are used are mostly based on serum antibody titres. For example, vaccines to be developed for the yearly influenza vaccination are regarded to be effective when an haemagglutination inhibition (HI) titer of at least 40 can be induced, or alternatively a four times increase in HI titer compared to the prevaccination titer. However, when translating this to the titres found in Gabonese children, even the titres before vaccination would seem to be protective against influenza A viruses. It is possible that the viruses had been circulating very recently but it is also possible that in Gabonese population, exposed to many more pathogens than western

populations, the (persistence of) HI titres are influenced by unknown factors.

Also for TT, WHO describes protective levels as between 0.1 and 0.2 IU/ml, or 0.51-1.02 μg/ml found by ELISA (or 0.01 IU/ml in a neutralization assay). However, WHO emphasizes that often cases of tetanus occur in individuals with levels of antibodies in this range, or even above [185]. As for tetanus a fully standardized and readily used assay that correlates with toxin neutralization is not available, it is difficult to judge the situation. Consequently, antibody levels have not been validated against the relative risk of disease at a defined titer, something that has been done for e.g. pertussis, RSV, meningococci and pneumococci. Therefore, for tetanus, a "protective antibody concentration" may not be considered a guarantee of immunity under all circumstances.

Thus, the current correlates of protection are based on a single characteristic that mostly will not suffice to define efficacy of the complex immune responses induced by vaccines. Therefore, next to the humoral component, for many vaccines a T cell component should be included in the definition of protection. To this end, the quality of the antigen specific T cell populations should be defined and currently the role of the so-called poly- or multi-functional T cells is gaining more and more importance. The presence of T cells that produce more than one cytokine at the same time, have been found to be related to better protection in different infectious disease models (inclucing HIV, *Leishmania major* and *Mycobacterium tuberculosis*) than the presence of T cells producing only one cytokine as reviewed by Seder *et al* [254]. It was hypothesized that measurement of a relatively simple set of cytokines, IL-2, TNF and IFN-γ, in the same T cells can be used to define a vaccine-elicited response; TNF and IFN-γ are involved in clearance of the pathogen and IL-2 promotes expansion of $CD4^+$ as well as $CD8^+$ T cells, thereby having an indirect effect. The superior protective effects of multifunctional T cells was ascribed to increased efficacy at both the effector and the memory T cell level: multifunctional T cells produce more cytokines on a per cell basis and induce more efficient killing, but they were also found to serve as a reservoir of memory $CD4^+$ T cells with effector potential [254].
However, in order to be able to translate these multifunctional T cell responses to a correlate of protection for vaccination, a definition based on the magnitude as well as the quality of the multifunctional and memory T cells would be needed. Moreover, it could be necessary to include also cytokines produced by Th2, Th17 and Treg cells into the analysis, as the balance between the different T cells will define the final outcome of the immune response.

Mechanisms

Lipid molecules are immuno-active moieties

The helminth derived molecules studied in this thesis are predominantly of lipid nature. Traditionally, lipids were not thought to have an immunological role, as for lipid molecules, if involved, often the immunoactive group was found to be the headgroup and not the lipid moiety itself. However, the influence of these headgroups could differ between protein and lipid molecules, as for example shown in chapter two where antibody profiles to *A. lumbricoides* derived glycolipids and (glyco)proteins were described to be very different. The influence of the phosphorylcholine moiety was found to be stronger for lipids compared to proteins, which indicates that these different classes of compounds may have distinct roles in interacting with, and shaping of humoral immune responses.

Recently, also the immunoactivity of the lipids themselves is receiving more attention, especially since it was found that the lipid moieties present in products of pathogens can have an essential role in receptor recognition. In this respect, TLR2 is an interesting receptor that can be activated by several types of lipids, with or without forming heterodimers with TLR1 or 6 (for recognition of tri- and di-acylated lipids, respectively). Moreover, TLR2 signalling showed to be very diverse as it has been reported to be capable of inducing Th1 [239], Th2 [108, 237] as well as regulatory responses [96, 238, 255]. Understanding the pathways involved in downstream signalling upon TLR activation by different ligands is a topic of current research, as TLR ligands are thought to be interesting candidates for use as adjuvants in vaccines. In order to use them in clinical applications, it should be clear which parts of the pathways should or should not be targeted to cure for example an allergic disease but prevent adverse effects.

TLR related signalling

Starting close to the receptor itself, two main adaptor molecules can be involved in TLR signalling, MyD88 and TRIF. The latter is used by TLR3 and is optional for TLR4. TLR ligands activating both the MyD88 and the TRIF pathway were found to act synergistically [256]. This could also be true for other receptors, as still much is to be discovered about how signalling pathways of different TLRs interfere with pathways activated by other receptor families, including CLRs, NLRs, RLRs and scavenger receptors [194, 246].

All signals from activation of receptors or combinations thereof need to be integrated to mount a final immune polarization profile that should be

communicated to T cells. This signal integration is performed by antigen presenting cells, and of these the dendritic cells (DCs) are thought to be central to immune activation and silencing [88]. Phosphorylation of the MAP kinases is an early event in the signal integration after receptor ligation and differential activation of the MAP kinases p38 and ERK in DCs has been associated with specific T cell polarization. p38 is thought to be important in mediating DC maturation and pro-inflammatory and Th1 responses, whereas ERK activation has more often been associated with anti-inflammatory and Th2 responses. Thus, helminth derived antigens would induce an increased *p*-ERK/*p*-p38 ratio and this is described in chapter five as well as by others [93, 108, 235].

However, in chapter five we describe a difference between protein and lipid molecules derived from schistosomes; both SEA and the phospholipid preparation induced an increase in the *p*-ERK/*p*-p38 ratio, but SEA did so by increasing levels of activated ERK, whereas the phospholipid preparation reduced levels of activated p38. Other differences between the protein and lipid preparations derived from schistosomes include the strength of the T cell polarization, which was somewhat stronger for SEA and more moderate for the lipid preparation, and the levels of mRNA in the DCs 16 hours after stimulation. The mRNA profiles were very similar for the protein and the lipid preparation, thus the same type of mRNA molecules were usually up- (e.g. c-fos) or down-regulated (e.g. delta4), however, the expression levels were generally lower in the DCs pulsed with the lipid preparation. Together, the T cell polarization and levels of mRNA indicate that SEA has a stronger stimulatory component compared to the phospholipid preparations.
Relating this to the MAP kinase responses, the *p*-ERK/*p*-p38 ratio, which was similar for SEA and the lipid preparation, is indicative for the Th2/Th1 inducing potential which is in agreement with what others have found. But it is tempting to speculate that, in addition to the effect of the *p*-ERK/*p*-p38 ratio on the polarization, the absolute levels of phosphorylation of ERK and p38 influence the activation / regulation balance, with higher levels leading to more immune activation.

Involvement of additional TLR or non-TLR receptors
Concerning involvement of other receptors in addition to TLR2, the Th1 inducing bacteria described in chapter five, *E. coli* an *L. monocytogenes* were reported to involve additional receptors. *E. coli* has been shown to activate TLR4 and NOD1 [243, 244], whereas resistance to *Listeria* infection was related to the presence of functional NOD2 [245]. Relatively little is known about Th2 skewing by the helminth derived compounds, but in a previous study for schistosomal lipids it was shown that TLR2 activation was not needed for Th2, but rather for regulatory responses [96]. Our own preliminary results with stimulation of peritoneal macrophages from

MyD88, TIRAP, TLR1, TLR2 and TLR6 deficient mice, indicate that the phospholipid fractions activate MyD88 as well as TIRAP, of which the latter is specifically involved in TLR2 signalling. Further, when the preparation was separated into several subfractions, using HPLC, two of these fractions seemed to contain TLR2 activating molecules, of which one, undefined, subfraction involved TLR1, whereas another subfraction, containing lysophosphatidylserine molecules, as defined by mass spectrometry, was found to activate TLR2 in the presence of TLR6. However, further research is needed to confirm these results and to identify the molecules responsible for this activation.

Clinical implementations of helminth-derived molecules

Clinical implementations of helminth derived antigens could be manifold: many inflammatory diseases may be the possible targets of treatment with modulatory helminth based compounds. For example the filarial ES-62, at concentrations equivalent to those found in the bloodstream of filaria-infected humans, was recently shown to prevent degranulation of mast cells, thereby reducing allergic responses. Interestingly, also for this function TLR4 ligation of the ES-62 molecule was shown to be necessary [257].
Moreover, as helminth infections clearly influence the immune response to non-related antigens, also this more general effect of helminth derived antigens could be used in a clinical setting. In this respect, the treatment of patients with inflammatory bowel disease or asthma with live (porcine) helminth eggs or larvae respectively [77, 79, 249] is encouraging. However, to use this in a more controlled setting, more studies are needed to investigate the effects of single molecules, as these will be easier to administer, especially for use in larger populations. On the other hand, it is very likely that a mixture of at least a few molecules is needed to induce a controlled immune response. Moreover, research will be necessary to study long-term effects, as induction of a regulatory response might be interesting for curing allergies or autoimmune diseases, but when this response is not antigen specific, it might have adverse effects on protection from infectious diseases and cancer. Thus, influencing immune responses in a controlled and antigen specific manner is our future challenge.