# Helminth infections induce immunomodulation : consequences and mechanisms

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# Summary

## Chapter 1

Chapter 1 provides a general introduction to immune responses evoked by helminths. It describes how the helminth-induced Th2 and immunomodulatory responses affect immune responses to other infections (bacterial, viral, parastic), to vaccines and to allergens as well as possible self antigens. In addition, the molecular mechanisms involved in immune modulation are reviewed, with a particular focus on the modulation of dendritic cell (DC) function. The effect of DC maturation as well as the role of Toll-like receptors and downstream MAP kinase activation in shaping the dendritic cell function have been described.

## Chapter 2

In chapter 2 the presence of specific antibodies against *Ascaris lumbricoides* derived glycolipids in patients infected with this helminth were studied. The highest IgG reactivity to glycolipids was found in children carrying heavy infections, compared to lightly-infected or uninfected children. Substantial IgG antibody reactivity to (glyco)proteins was found to be directed to the phosphorylcholine moiety. For glycolipids this was even more pronounced, as removal of the phosphorylcholine moieties by hydrofluoric acid treatment abrogated IgG antibody reactivity. For helminth infections, IgG4 has been associated with susceptibility to reinfection after treatment, whereas IgE is thought to be protective against reinfection. Measurement of IgG4 and IgE isotypes showed no IgG4 reactivity to *Ascaris* glycolipids, but indicated increased IgE responses in subjects with light or no *Ascaris* infections, suggesting that IgE responses to glycolipids may play a role in controlling parasite burden.

## Chapter 3

In chapter 3 the difference in efficacy of vaccination with a trivalent influenza vaccine in children living in a rural area, with a high prevalence of helminth infections, compared to a semi-urban area, with a lower prevalence of helminth infections, was compared. First it is described that, unexpectedly, anti-influenza antibody levels before vaccination were present in all children, indicating that influenza viruses had been circulating previously. Cytokine responses were induced within a week after vaccination and showed a Th2 bias in rural children; more IL-5 and less IFN-γ. Antibody levels were significantly increased in both rural and semi-urban children after vaccination and reached significantly higher

levels for the semi-urban children compared to the rural children one month after vaccination for 2 of the strains. However, post vaccination responses to the third strain (H3N2) were higher in the rural cohort compared to semi-urban group. The extremely high pre-vaccination levels specific for this strain meant that it had caused a very recent epidemic, and although purely speculation, this might indicate that recently memory is boosted more strongly in rural areas. The exact role of parasite infections in the differential response between rural and semi-urban children needs to be investigated in an appropriately powered study. However, for the H1N1 strain helminth infection in semi-urban children reduced the anti-H1N1 antibody levels compared to non-infected semi-urban children. Malaria infection in rural children suppressed antibody responses to the H1N1 strain. Overall, influenza vaccination induced weaker responses in rural compared to a semi-urban population of Gabonese schoolchildren.

## Chapter 4

In chapter 4 the immune responses induced upon vaccination with tetanus vaccine in children from rural and semi-urban areas of Gabon is described. The same population as described in chapter 3 was studied and similarly, also in response to tetanus toxoid, more IL-5 and less IFN-γ, indicating a Th2 biased immune response, was found in the rural children. However, to this vaccine higher antibody levels were found also in the rural group. Total IgG as well as antigen specific subclasses of the IgG1, IgG2, IgG3 and IgG4 isotype and the avidity of the dominating IgG1 subclass were determined. For these, differences between rural and semi-urban children were found one month after vaccination for tetanus-specific IgG1 and IgG3; both were higher in the rural children. This might be in line with the finding in Chapter 3, that vaccine induced responses to the recently circulating influenza H3N2, were also higher in rural children. Subjects with plasmodium infections showed higher levels of IgG3, but multivariate linear regression analysis showed that this could not account for the difference in anti-TT IgG3 between the rural and the semi-urban children. Furthermore, current helminth infections could also not explain the difference between rural and semi-urban responses. This indicates that other environmental influences and/or the history of helminth infection might be important factors that could explain the Th2 and stronger antibody responses upon a tetanus booster vaccination in rural compared to semi-urban children.

## Chapter 5

To link immune responses to lipids derived from helminths and *in vivo* vaccination responses in areas where helminth infections are highly

prevalent, dendritic cells, which are central to the immune system, were studied. In chapter 5 the pathways within dendritic cells that are activated by helminth derived lipids are described. The lipid fractions of both *Schistosoma mansoni* and *Ascaris lumbricoides* activate Toll Like Receptor 2. Activation of this receptor is an important step in the initiation of an appropriate adaptive immune response. However, several ligands activate TLR2 and these can have different effects on immune polarization. Dendritic cell activation by both helminth derived (Th2 inducing) and bacterial (Th1 inducing) TLR2 ligands was studied and the molecular profile of these DCs was determined. It was found that the MAP kinase activation correlated with the T cell polarizing effects. Thus, the bacterial ligands showed a low *p*-ERK/*p*-p38 ratio, whereas this was high for the helminth derived lipids. However, unlike a schistosomal egg extract (SEA) that increases this ratio by increasing the amount of activated ERK, the lipids specifically reduced the phosphorylation of p38. In addition, mRNA expression profiles were very different. Most clearly, notch ligand delta-4 was associated with a Th1 polarization and transcription factor c-fos showed a strong correlation with Th2 responses. The overall profile of the two Th1 inducing bacterial TLR2 ligands was very similar, whereas the Th2 promoting lipid extracts showed a profile closer to that of SEA. Thus, the activation of TLRs within different antigenic mixtures can lead to very different polarization of the immune system, which can be explained by involvement of additional receptors. More importantly, the molecular signature of the DCs upon activation by antigenic mixtures can be used to predict the polarizing capacity of those compounds.