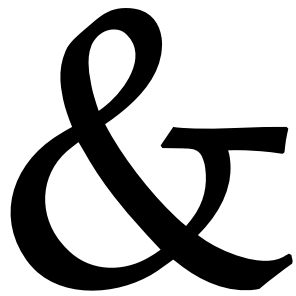
Addendum



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Summary

Schistosomes are parasitic helminths that cause chronical infections in over 200 million people in tropical and sub-tropical areas around the world. In the case of *Schistosoma mansoni*, one of the three major schistosome species infectious to humans, infection starts when cercariae penetrate the skin, after which the developing *S. mansoni* larvae travel to the mesenteric venules near the liver where they further mature. Adult male and female worms form pairs and start to produce eggs, which either leave the body to continue the life cycle, or get trapped in the liver and other organs. The host reacts to eggs and egg products by inducing Th2-mediated granulomatous immune responses which can lead to pathological tissue remodeling, fibrosis, severe organ damage and potentially death.

The majority of molecular and immunological *S. mansoni* egg-related studies have been performed using SEA (soluble egg antigens), an experimental preparation consisting of the soluble egg proteins and glycoproteins. Part of the immunological activity associated with *S. mansoni* eggs is mediated by protein glycosylation. An extensive overview of the immunological capacities of SEA, as well as the current knowledge on immunogenic SEA glycans and glycoproteins, is given in **chapter 1**. This chapter in addition introduces common mass spectrometry-based methods and their application to identify glycan structures on egg glycoproteins.

Three major, immunogenic SEA glycoproteins have been identified: omega-1, IPSE/α1 and kappa-5. The studies described in this thesis focus on the structural and molecular details of these three glycoproteins and their interactions with the human and murine immune systems. In **chapter 2 and 3**, the glycosylation of omega-1 and kappa-5 were studied by a mass spectrometry (MS)-based approach. Glycoproteins were digested with trypsine and analysed by nanoscale LC-MS and MALDI-TOF-MS to generate site-specific glycosylation information. Structural details were obtained by tandem mass analysis, exo-glycosidase treatments and anti-glycan antibodies We found that omega-1 contains two occupied glycosylation sites which for primarily carry diantennary N-glycans of the complex-type (**chapter 2**). The antennae are predominantly composed of Lewis X and *N*-acetyllactosamine structures, with a minority composed of (fucosylated) LDN structures. The innermost core *N*-acetylglucosamine is typically decorated with α3/α6 difucosylation. Interestingly, the glycosylation of IPSE/α1, which has previously been analyzed using a similar MS-based approach, is highly identical to that of omega-1. Kappa-5 on the other hand was found to express a completely different set of glycans (**chapter 3**). It mainly carries triantennary N-glycans that are substituted with LDN antennae, a minority of which carry an additional α3-fucose on the GlcNAc (LDN-F). Like omega-1 and IPSE/α1, most kappa-

5 glycans are α3/α6 core fucosylated, however kappa-5 glycans are in addition modified with a xylose attached to the β4-linked mannose. Notably, we found that kappa-5 is the major LDN-expressing glycoprotein within SEA.

SEA glycan motifs are primarily recognized by C type lectin receptors (CLRs) that are expressed by antigen-presenting cells, a type of innate immune cells that include the dendritic cells (DCs). SEA glycoproteins induce responses in DCs that lead to a characteristic Th2 skewing of the host immune system.The recognition and uptake of SEA glycoproteins by DCs is largely mediated through three CLRs; DC-specific ICAM-3-grabbing nonintegrin (DC-SIGN), mannose receptor (MR) and macrophage galactose-type lectin (MGL). **Chapter 4** investigates the binding of these receptors to native IPSE/α1 and kappa-5, in a plate-based assay as well as in a cellular context. To define the structural elements involved in binding to the three receptors, glycoprotein variants were created using exo-glycosidase digestions. In **Chapter 5**, a similar strategy was used to investigate binding of omega-1 to DC-SIGN and MR. We found that IPSE/α1 and omega-1 are primarily recognized by DCs via the interaction of LeX on the egg glycoproteins and MR on DCs. DC-SIGN and MGL are in addition involved in binding of DCs to IPSE/α1, albeit to a much lesser extent. Kappa-5 is recognized by DC-SIGN, MR and MGL without one of these being the dominant receptor, as well as via other, Ca^{2+}-independent receptor(s). Kappa-5 interacts with DC-SIGN via its LDN-F antennae, whereas binding of MR may involve either LDN-F antennae or the fucosylated and xylosylated chitobiose core. MGL binding of kappa-5 was mediated via LDN as well as LDN-F antennae. The binding studies in **chapter 4 and 5** provide a molecular basis for the CLR-mediated interaction of DCs with individual, native glycoproteins of schistosome eggs.

It has long been established that the SEA mixture has Th2 polarizing properties;, but the SEA molecules responsible for this effect have remained unknown. Recently, omega-1 was reported to be a major Th2-inducing component of SEA through functional modulation of DCs. In **Chapter 5**, we show using site-directed mutagenesis that two features of omega-1, its glycosylation and RNase activity, are involved in this process. Omega-1 glycosylation is necessary for the internalization of omega-1 by DCs. We show that this process is mediated via the interaction between LeX on omega-1 and MR on DCs. Within the cell, the RNase activity of omega-1 can then block protein production via cleavage of ribosomal RNA, leading to the conditioning of DCs for Th2 polarization. The immunological data as described in **chapter 5** provide new insights in the molecular processes that direct Th2 induction.

To study *S. mansoni* perioval granuloma formation, mouse models involving the injection of Sepharose beads into liver or lungs are commonly used tools. The beads can be coated with various schistosome egg preparations or schistosome-related molecules, thereby

enabling the identification of native granuloma-inducing and/or modulating molecules. Using such a model, SEA-induced granuloma formation has previously been demonstrated to be dependent on glycosylation. Interestingly, beads coated with LDN and *N*-acetyllactosamine-terminating glycoconjugates were able to induce granulomatous responses similar to SEA. As we found in **chapter 4** that kappa-5 is the major LDN-expressing glycoprotein in SEA, we hypothesized that kappa-5 might be an important granuloma-modulating molecule within eggs. Indeed, we showed in **chapter 6** that kappa-5 coated beads are able to induce granulomatous reactions in a pulmonary mouse model. Kappa-5-induced granulomas contain eosinophils, indicating a Th2-polarized nature that is also observed in egg- and SEA-induced granulomas. LDN is partly responsible for this effect, as enzymatic removal of LDN from kappa-5 coated beads results in significantly less and smaller granulomas as opposed to untreated kappa-5. **Chapter 6** describes kappa-5 as the first, native *S. mansoni* egg molecule with glycan-dependent, granuloma-inducing properties.

Various mechanisms exist via which interplay of pathogen-derived glycoconjugates with CLR on innate immune cells can mediate immunomodulation. In **Chapter 7,** we discuss the CLR-dependent mechanisms exploited by omega-1, IPSE/α1 and kappa-5, as well as related pathogenic molecules, in the light of the results presented in this thesis and previously published data. We conclude that pathogens make use of at least two separate CLR-dependent mechanisms to condition DCs for immune modulation of T cells: CLR-mediated internalization of bioactive molecules; and CLR-induced interference with TLR signalling. In **chapter 7** we additionally review the knowledge on other egg glycoconjugates and glycan motifs that might also be involved in the immunomodulatory activities of *S. mansoni* eggs.