

Schistosome N-glycans containing core α 3-fucose and core β 2-xylose epitopes are strong inducers of Th2 responses in mice

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During murine schistosomiasis, egg-derived glycoconjugates play a key role in skewing the immune response towards a Th2 phenotype. Among the candidates responsible for this effect, complex-type N-glycans containing the core α 3-fucose and core β 2-xylose determinants, two glycan epitopes found in some invertebrate- and plant-derived allergens, may be important. Here, we show that core α 3-fucose and core β 2-xylose determinants are expressed in the different developmental stages of *Schistosoma mansoni*, particularly in the excretory-secretory systems of schistosomula and adult worms and in eggs deposited in the liver. Glycosyltransferase assays confirmed the presence of core α 3-fucosyltransferase and core β 2-xylosyltransferase activities in egg extracts. Using a model of immunization with pulsed dendritic cells, we show that egg-derived glycoproteins containing the core α 3-fucose and core β 2-xylose determinants generate a strong Th2-biased cellular response in mice and that the glycan moieties of this extract are important in this effect. During murine infection, these complex-type N-glycans induce a glycan-specific Th2 cellular response and elicit T-dependent anti-core α 3-fucose and anti-core β 2-xylose IgG1 (a Th2-associated isotype), but not IgG2b (a Th1-associated isotype) Ab. Taken together, our results point out the importance of core fucosylated/xylosylated N-glycans in the Th2 immune response during murine schistosomiasis.

Key words: Core α 3-fucose/core β 2-xylose / *Schistosoma mansoni* / Th2 response

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1 Introduction

Murine schistosomiasis is characterized by a gradual switch from a predominant Th1 cytokine response (before the egg laying) to a Th2-dominated response, an event that favors the formation of granulomas around viable eggs in the liver [1, 2]. It is now clearly established that egg-derived glycan structures play a key role in this immunological phenomenon [3, 4]. Among them, glycoconjugates containing a fucose α 3-linked to GlcNAc (Gn) residues may be important. For instance, the lacto-N-fucopentaose III, which contains the Lewis X trisaccharide [Gal β 1–4(Fuc α 1–3)Gn], exerts immunomodulatory

function during infection [5–7], probably by activating cells of the innate immune system [8]. Moreover, although the Lewis X epitope is also expressed by host cells, it triggers a strong Ab response in mice and humans infected by *Schistosoma mansoni* [9, 10]. More recently, complex-type N-glycans possessing core α 3-fucose and core β 2-xylose determinants have been detected in *Schistosoma* [11–13]. The localization of these core fucosylated/xylosylated glycans within the parasite has, however, not been examined. Moreover, for the reasons explained below, these core α 3-fucosylated and core β 2-xylosylated N-glycans may be important in the outcome of the immune response during schistosomiasis infection.

The core α 3-fucose and core β 2-xylose determinants are found on many other invertebrate- and plant-derived complex-type N-glycans [Man₃(Xyl)FucGn₂]. In plants, the Man₃Gn₂ N-glycan core common to all eukaryotes is substituted by β 2-linked xylose and/or α 3-linked core

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Abbreviations: Gn: GlcNAc **PLA2:** Phospholipase A2 **MALDI-TOF MS:** Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry **SEA:** Soluble egg Ag **HRP:** Horseradish peroxidase

fucose residues rather than α 6-linked core fucose found on mammalian N-glycans (see Fig. 1) [14, 15]. Interestingly, core α 3-fucose and core β 2-xylose epitopes trigger high levels of IgE in atopic patients and may be involved in the pathophysiology of some food and respiratory allergies [16–21]. During schistosomiasis, the role of these glycan determinants in the outcome of the immune responses is currently unknown.

In the present study, our aims were (a) to define the localization of such glyco-epitopes in the different developmental stages of *S. mansoni* and (b) to study the cellular and humoral immune responses to these determinants during murine schistosomiasis. Our data show that both core α 3-fucose and core β 2-xylose epitopes are expressed in *S. mansoni* throughout its life cycle within the vertebrate host and elicit IgG1, but not IgG2b, Ab in infected C57BL/6 mice. Finally, we show that egg-derived complex-type N-glycans containing the core α 3-fucose and core β 2-xylose determinants induce a strong Th2 cytokine response in *S. mansoni*-infected mice as well as in mice immunized with sensitized dendritic cells (DC). Taken as a whole, our data suggest that schistosome core α 3-fucosylated and core β 2-xylosylated N-glycans are inducers of a specific Th2 immune response during schistosomiasis.

2 Results

2.1 Expression of core α 3-fucose and core β 2-xylose epitopes in *S. mansoni*

The expression of core α 3-fucose and core β 2-xylose epitopes in *S. mansoni* was analyzed using specific Ab. The anti-core α 3-fucose and anti-core β 2-xylose Ab were purified from an antiserum containing anti-horseradish peroxidase (HRP) Ab as described, [22] and their specificities were confirmed by ELISA using model glycoproteins. For this purpose, we used two model N-linked glycoproteins: honeybee venom phospholipase A2 (PLA2), an insect N-linked glycoprotein containing a core α 3-fucose [23] and *Helix pomatia* α -hemocyanin, a snail N-linked glycoprotein containing a core β 2-xylose [24] (Fig. 1). Moreover, we also used a mammalian glycoprotein either expressed in chicken (natural avidin) or in transgenic maize (recombinant avidin), the latter system of expression allowing the synthesis of glycoproteins bearing both the core α 3-fucose and core β 2-xylose determinants typical of plant N-glycans [25].

As shown in Fig. 2A, anti-core $\alpha 3$ -fucose Ab reacted against PLA2 and against recombinant, plant-derived, avidin. On the other hand, no reactivity was detected against hemocyanin and natural, chicken-derived, avi-

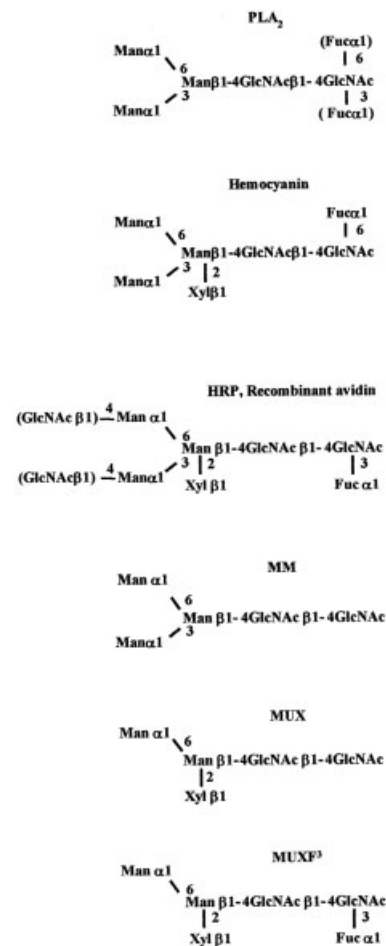


Fig. 1. Structures of glycans N-linked to honeybee venom PLA2, to snail (*H. pomatia*) hemocyanin, to HRP, to recombinant avidin and to the neoglycoconjugates used in this study (BSA-MM, BSA-MUX and BSA-MUXF³). Note that the core $\alpha 3$ -fucosylation occurs on the N,N'-diacetylchitobiose core (Gn $\beta 1$ –4Gn), whereas core $\beta 2$ -xylosylation is a modification of the internal mannose residue.

din. Moreover, the binding of anti-core $\alpha 3$ -fucose Ab to PLA2 was inhibited in a dose-dependent manner by BSA-MUXF³, having a core $\alpha 3$ -fucose epitope, but by neither BSA-MUX nor BSA-MM, used as negative controls (see Fig. 1 for structures). As represented in Fig. 2B, anti-core $\beta 2$ -xylose Ab reacted against hemocyanin and against recombinant avidin but against neither PLA2 nor natural avidin. Moreover, BSA-MUXF³ and BSA-MUX, both harboring a core $\beta 2$ -xylose epitope, but not BSA-MM, dose-dependently inhibited the binding of anti-core $\beta 2$ -xylose Ab on hemocyanin. Taken as a whole, these results confirmed the high specificity of the Ab used.

As a second step, we analyzed the expression of core α 3-fucose and core β 2-xylose epitopes in the different

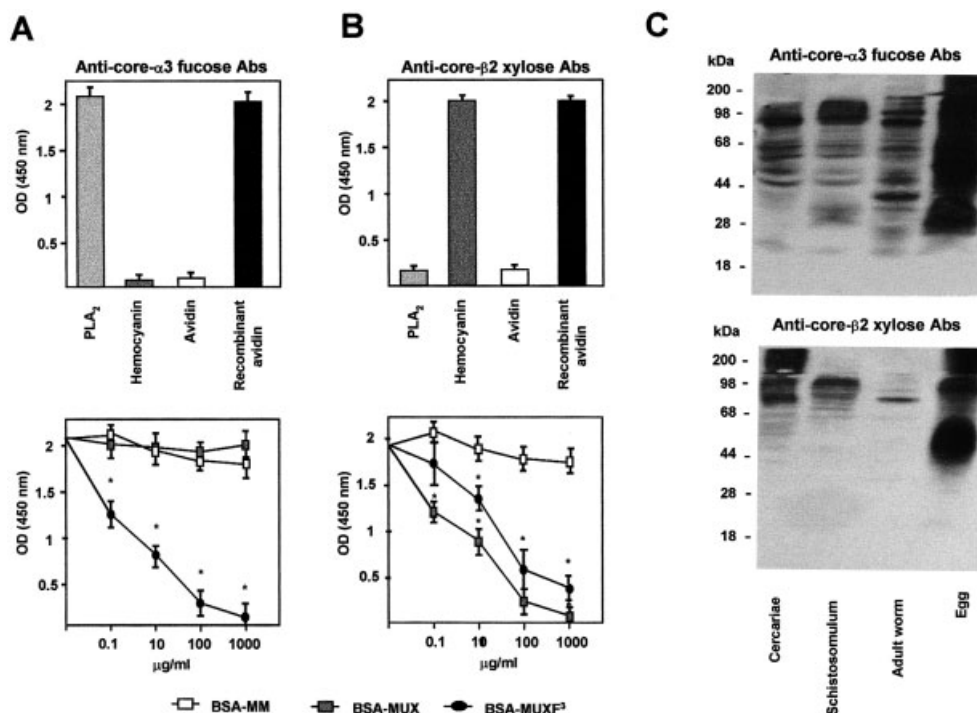


Fig. 2. Specificities of anti-core α 3-fucose and anti-core β 2-xylose Ab, and expression of core α 3-fucose and anti-core β 2-xylose in the different developmental stages of *S. mansoni*. ELISA plates were coated with PLA₂, hemocyanin, avidin or recombinant avidin in order to confirm the specificities of anti-core α 3-fucose (A) and anti-core β 2-xylose (B) Ab. Affinity-purified rabbit anti-core α 3-fucose and anti-core β 2-xylose Ab were pre-incubated, or not, with increasing amounts of BSA-MM, BSA-MUX or BSA-MUXF³ before being applied to PLA₂- (A) or hemocyanin- (B) coated plates. * $p < 0.01$ compared to BSA-MM. (C) Equal quantities of protein (10 μ g/lane) extracted from the different stages of *S. mansoni* were separated by SDS-PAGE and transferred to nitrocellulose membranes. Western blots were probed with anti-core α 3-fucose and anti-core β 2-xylose Ab. The apparent molecular masses are indicated in kDa.

developmental stages of *S. mansoni* by Western blotting. Fig. 2C clearly indicates that core α 3-fucose is expressed in all the stages tested, particularly in eggs. Comparatively, the expression of core β 2-xylose in *S. mansoni* appears to be lower, especially in adult worms. As observed with the core α 3-fucose epitope, eggs appear to synthesize higher amounts of core β 2-xylose than the other stages. This shows that, in its mammalian hosts, the expression of these glycoepitopes is developmentally regulated in *S. mansoni* and that the egg stage represents the major source of their synthesis.

2.2 Analysis of the core α 3-fucosyltransferase and core β 2-xylosyltransferase activities in egg extracts

In order to determine whether schistosome eggs are capable of biosynthesizing core α 3-fucosylated and core β 2-xylosylated glycan structures, relevant glycosyltransferase assays were performed. A dabsyl glycopeptide

carrying a core N-linked oligosaccharide terminated with two Gn residues (dabsyl GnGn) was used as the acceptor substrate for determinations of core α 3-fucosyl-, core α 6-fucosyl- and core β 2-xylosyltransferases in schistosome eggs. After 2 days of incubation, as assessed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) analysis, around 30% of dabsyl GnGn was converted to the xylosylated form in the presence of UDP-Xyl (Fig. 3B), whereas around 50% of dabsyl GnGn was converted to a monofucosylated form and further 20% to a difucosylated form in the presence of GDP-Fuc (Fig. 3C). The difucosylation was assumed, according to previous data with insect cells [26], to be due to core α 6-fucosylation followed by core α 3-fucosylation. This conclusion was corroborated by data showing that, after pre-incubation of dabsyl GnGn with chicken heart extract, containing core α 6-fucosyltransferase activity, and GDP-Fuc, it was possible to detect the addition of a second fucose upon incubation with the schistosome extract and GDP-Fuc (Fig. 3D).

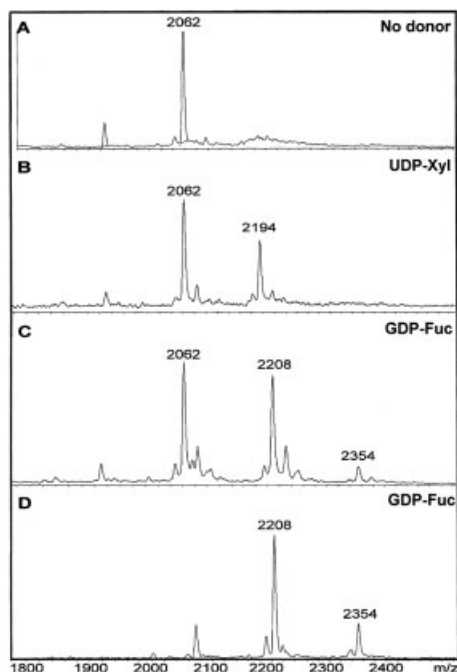


Fig. 3. Xylosyl- and fucosyltransferase activities of schistosome eggs. Dabsyl GnGn glycopeptide was incubated for 48 h at 37°C with schistosome extract in the absence of donor (A), the presence of UDP-Xyl (B) or the presence of GDP-Fuc (C). In addition, dabsyl GnGn glycopeptide was pre-incubated with GDP-Fuc and chicken heart extract for 48 h prior to incubation with schistosome extract for 48 h (D). Species with *m/z* ratios of 2062, 2194, 2208 and 2354 correspond, respectively, to the unmodified, xylosylated, monofucosylated and difucosylated forms of dabsyl GnGn.

2.3 Localization of core α 3-fucose and core β 2-xylose in *S. mansoni*

We then proceeded to determine the localization of core α 3-fucose and core β 2-xylose epitopes in *S. mansoni*. Indirect immunofluorescence studies showed that both epitopes principally localized in the post-acetabular glands of schistosomula (Fig. 4A, B). In adult worms, core α 3-fucose and core β 2-xylose were principally located in the cecal epithelium and in cellular connections that cross the parenchyma to reach the dorsal tubercles (Fig. 4C, D). In male, sexual organs were also labeled with the anti-core α 3-fucose (Fig. 4C) and anti-core β 2-xylose (not shown) Ab, whereas in female, labeling was detected in forming eggs (not shown). Immunohistochemical analysis also revealed the expression of core α 3-fucose and core β 2-xylose determinants in eggs deposited in the liver of *S. mansoni*-infected mice (Fig. 4E, F). Taken together, these findings indicate that core α 3-fucose and core β 2-xylose determinants are principally expressed in the excretory-secretory system

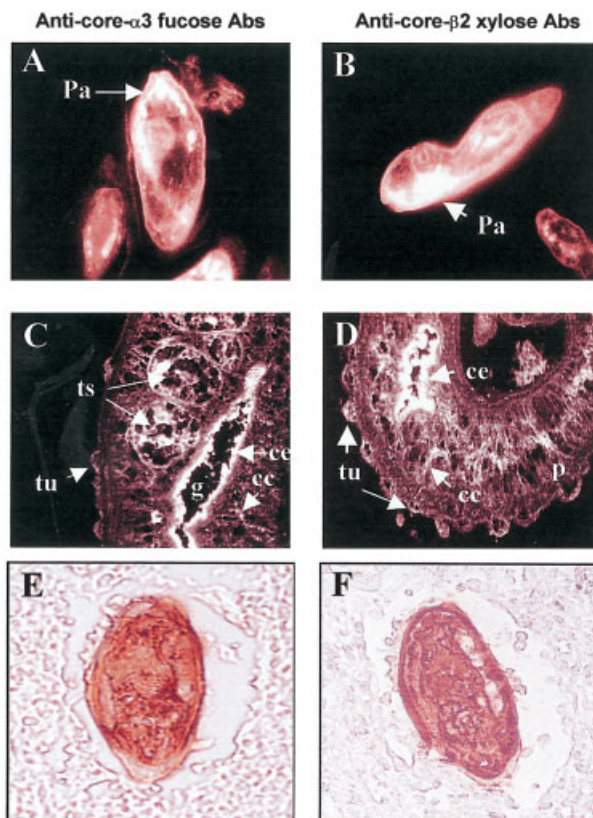


Fig. 4. (A–D) Immunofluorescence staining of schistosomula (A, B) and adult worms (C, D) with anti-core α 3-fucose and core β 2-xylose Ab. Pa, post-acetabular glands; tu, dorsal tubercles; ts, testis; g, gut; ce, cecal epithelium; cc, cellular connections; p, parenchyma. (E, F) Immunolabeling of eggs within liver granulomas of *S. mansoni*-infected mice, 49 days post-infection, with either anti-core α 3-fucose and core β 2-xylose Ab. In each stage, the immunoaffinity-purified rabbit Ab directed against an irrelevant Ag (control Ab) gave no labeling (not shown). Magnification $\times 400$.

of schistosomula and adult worms as well as in eggs during granuloma formation, thus suggesting that these glyco-epitopes may be accessible to immune cells, including APC, during infection.

2.4 Analysis of the cellular immune response to egg-derived anti-HRP-binding N-glycoproteins in mice injected with sensitized DC

Using DC as APC, we next analyzed the nature of the T cell response induced by egg-derived N-linked glycoproteins harboring the core α 3-fucose and/or core β 2-xylose determinants. To this end, a fraction containing N-linked glycoproteins was prepared from soluble egg Ag (SEA) by passage through a column containing affinity-

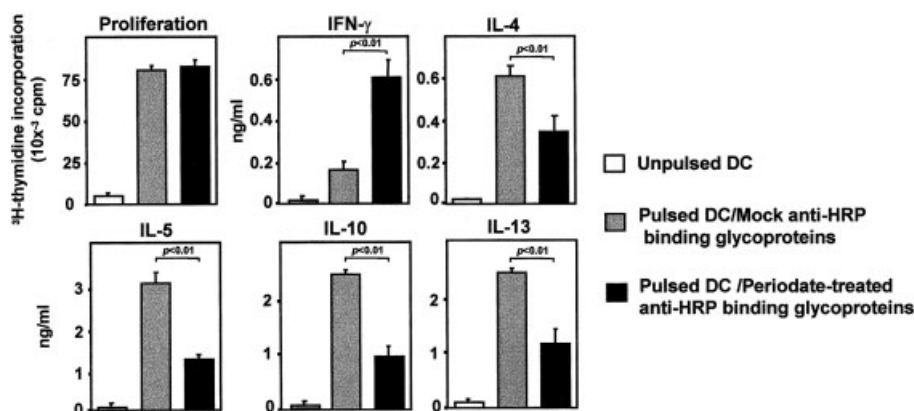


Fig. 5. Proliferative response and cytokine production of LN cells from mice injected with DC pulsed with egg-derived anti-HRP-binding glycoproteins. LN cells were restimulated with increasing doses of mock or periodate-treated anti-HRP-binding glycoproteins (shown is 5 μ g/ml). Cytokine production and proliferation were measured after 3 and 4 days of culture, respectively. Results represent the mean of triplicate cultures $\pm$ SD ($n=6$). One representative experiment out of four performed is shown.

purified anti-HRP Ab; and C57BL/6 mice were immunized in the hind footpads with DC pulsed with this glycoprotein extract (termed as anti-HRP-binding glycoproteins). Five days after immunization, popliteal LN were harvested and restimulated *in vitro* with either the mock or the periodate-treated fraction. As shown in Fig. 5, administration of DC sensitized *ex vivo* with anti-HRP-binding glycoproteins resulted in a T cell priming as shown by the Ag-specific proliferation of LN cells. Restimulation with the periodate-treated Ag resulted in an identical T cell proliferation compared to that obtained after restimulation with the mock preparation.

The cytokines released by LN cells primed with sensitized DC were then assayed. As depicted in Fig. 5, LN cells from mice injected with DC pulsed with anti-HRP-binding glycoproteins produced high levels of Th2-type cytokines (IL-4, IL-5, IL-10 and IL-13), and less IFN- γ , following restimulation with the same Ag *in vitro*. In sharp contrast, cells restimulated with the periodate-treated Ag produced higher amounts of IFN- γ (3.6-fold increase) and reduced concentrations of IL-4, IL-5, IL-10, and IL-13 (45–65% decrease). This experiment shows that, when captured, processed and presented to T lymphocytes by DC, egg-derived glycoproteins having core α 3-fucose and/or core β 2-xylose determinants induce the development of a strong Th2 cytokine response. This also suggests that, in this model of immunization, T cells specific for the sugar moiety of the anti-HRP-binding glycoprotein fraction are strong producers of Th2 cytokines.

2.5 Analysis of the cellular immune response to egg-derived anti-HRP-binding N-glycoproteins in *S. mansoni*-infected mice

We next investigated whether *in vivo* sensitization of APC with schistosome N-glycans containing core α 3-fucose and core β 2-xylose epitopes could result in a specific T cell response during infection. To this end, spleens from *S. mansoni*-infected mice were recovered 49 days post-infection, the peak Th2 phase, and cells were restimulated *in vitro* with mock or periodate-treated egg-derived HRP-binding N-glycoproteins. The proliferative response and the cytokine production were then measured. As shown in Fig. 6, the proliferative responses of spleen cells from *S. mansoni*-infected mice to the mock Ag and to the periodate-treated Ag were similar. Analysis of cytokine production in the supernatants revealed that when restimulated with the mock Ag, cells produced IFN- γ and Th2 cytokines. In sharp contrast, the production of Th2 cytokines was dramatically decreased (50–90%) when cells were restimulated with periodate-treated Ag whereas that of IFN- γ was unaffected. These data show that in infected mice, egg-derived glycoproteins having core α 3-fucose and/or core β 2-xylose determinants induce the development of a Th2-biased cytokine response.

2.6 Immunogenicity of core α 3-fucose and core β 2-xylose in *S. mansoni*-infected mice

The presence of anti-core α 3-fucose and anti-core β 2-xylose Ab in the sera of *S. mansoni*-infected C57BL/6 mice was next investigated during the course of infection by ELISA using PLA2 and hemocyanin as target Ag. IgM

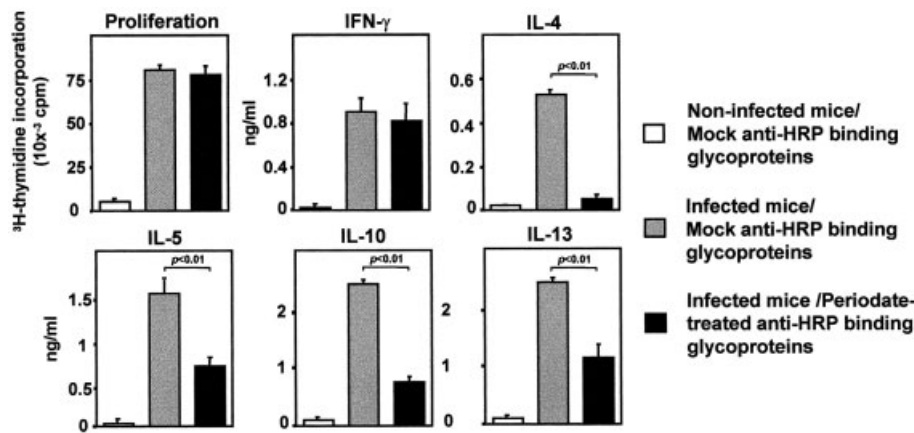


Fig. 6. Proliferative response and cytokine production of restimulated spleen cells recovered from *S. mansoni*-infected mice, 49 days after infection. Cells were cultured with mock or periodate-treated anti-HRP-binding glycoproteins (shown is 20 μ g/ml). Cytokine production and proliferation were measured after 3 and 4 days of culture, respectively. Results represent the mean of triplicate cultures $\pm$ SD (four mice/group). Unstimulated spleen cells showed no cytokine production (not shown). One representative experiment out of three performed is shown.

and total IgG Ab reactive to PLA2 and hemocyanin were detected in the sera of infected mice (titers comprised between 10^1 and 10^2 , 42–98 days post-infection) whereas sera from uninfected mice were negative for PLA2 and hemocyanin binding (not shown). Interestingly, although we found relatively high titers (approx 10^3 , not shown) of anti-SEA IgG1 and IgG2b Ab (Fig. 7A), assessment of the subclasses of Ab revealed the presence of IgG1 (a marker of a Th2 response), but not IgG2b (a marker of a Th1 response), reactive to PLA2 (Fig. 7B) and hemocyanin (Fig. 7C). This suggests that during infection, C57BL/6 mice mount a Th2- but not Th1-associated IgG Ab response against the core α 3-fucose and core β 2-xylose determinants.

3 Discussion

During murine infection, schistosome glycans are crucial in biasing the immune response towards a Th2 phenotype [3]. They also elicit the production of anti-carbohydrate Th2-associated Ab (including IgE) that are believed, at least in humans, to be important in limiting reinfections [27, 28]. Among the candidates, and for the reasons exposed below, complex-type N-glycans carrying core α 3-fucose and/or core β 2-xylose residues may be important. These oligosaccharide structures are found in many plants and some invertebrates and elicit, in allergic patients, high levels of IgE directed against the core α 3-fucose and core β 2-xylose epitopes. Although the mechanisms underlying this specific Th2-biased response are still unknown, it is likely that APC present such structures to naive T cells, which in turn differenti-

ate into Th2 cytokine-producing cells. The aim of the present study was to establish the expression and the localization of core α 3-fucose and core β 2-xylose epitopes in *S. mansoni*, the major schistosome species for humans, and to investigate the possibility that such *Schistosoma* complex-type N-glycans carrying these glyco-epitopes may induce specific glycan-restricted Th2 response in mice.

In this study, using specific fractions of an anti-HRP antiserum, we show that core α 3-fucose and core β 2-xylose epitopes are expressed in the different developmental stages of *S. mansoni*, particularly in eggs. Immunofluorescence analysis showed that these glyco-epitopes are principally located in the excretory-secretory system of schistosomula and adult worms suggesting that, early after infection, these structures are released by the parasites and may be accessible to immune cells, including APC. For instance, core α 3-fucose and core β 2-xylose epitopes are expressed in the gut of the adult worm, an organ known to contain high amounts of immunogenic carbohydrates able to prime egg-induced hepatic granuloma formation [10].

Since core α 3-fucose and core β 2-xylose determinants may represent important components involved in the egg-mediated immunomodulation during murine schistosomiasis, we next studied by immunohistochemistry their expression in liver granulomas from *S. mansoni*-infected mice, 49 days post-infection. We found that core α 3-fucose and core β 2-xylose determinants are also expressed in liver-deposited eggs during granuloma formation. Importantly, enzymatic assays revealed core

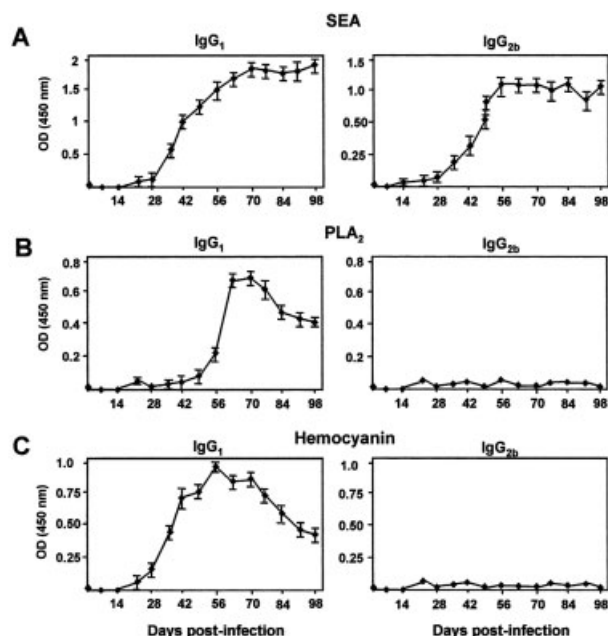


Fig. 7. Core α 3-fucose- and core β 2-xylose-specific Ab iso-type responses in *S. mansoni*-infected mice. Sera from *S. mansoni*-infected mice were diluted 1:1,000 (A) or 1:25 (B, C) and applied to wells coated with SEA (A), PLA2 (B) or hemocyanin (C). The subclasses of bound IgG Ab (IgG1 and IgG2b) were detected as described in Sect. 4. Results are presented as the averages of optical density values $\pm$ SD (six mice). One representative experiment out of three performed is shown.

α 3-fucosyltransferase and core β 2-xylosyltransferase activities in egg homogenates. At present, attempts are on the way to study more fully the enzymatic characteristics of these glycosyltransferases, particularly the putative *S. mansoni* core α 3-fucosyltransferase we recently cloned [29]. Taken together, our data confirm the recent finding of Khoo and colleagues [13] who provided structural evidence that core α 3-fucosylation and core β 2-xylosylation occur on schistosomal egg complex-type N-glycans.

We next investigated the nature of the specific, glycan-restricted, T cell response induced by egg-derived N-linked glycoproteins having core α 3-fucose and/or core β 2-xylose epitopes. First, a glycoprotein fraction was prepared by immunoaffinity to anti-HRP Ab; the resulting preparation contained core α 3-fucose and/or core β 2-xylose epitopes as assessed by ELISA and Western blotting (not shown). Subsequently, we used a model of immunization based on the transfer of sensitized DC into recipient mice. As a positive control, we used SEA-pulsed DC, since SEA has previously been shown to induce the development of a specific Th2-biased response [4, 30]. We found that T cells primed with anti-

HRP-binding N-glycoprotein-sensitized DC proliferate in a similar manner and produce equal quantities of IFN- γ and Th2 cytokines as LN cells from mice previously injected with SEA-pulsed DC (not shown). This indicates that anti-HRP-binding N-glycoproteins from the eggs are strong Th2 inducers. On the other hand, when the periodate-treated sample was used to restimulate LN cells *in vitro*, the production of Th2 cytokines was dramatically reduced whereas that of IFN- γ was increased. This indicates that, in our model of immunization, the sugar moieties are particularly important in generating the specific, Th2-biased, immune response.

It is noteworthy that no specific T cell response was detected after restimulation with core α 3-fucose- or core β 2-xylose-containing glycoproteins (such as PLA2 or hemocyanin, respectively), thus indicating that the peptide backbone of the schistosome N-glycans is essential for the T cell response. In *S. mansoni*-infected mice, spleen cells restimulated *in vitro* with egg-derived anti-HRP-binding N-glycoproteins secrete IFN- γ and Th2 cytokines. Here too, restimulation with the periodate-treated sample strongly abrogated the production of Th2 cytokines thus indicating that during infection the sugar moieties of anti-HRP-binding N-glycoproteins are important in generating the specific Th2 response. As in the aforementioned immunization model, no detectable response was observed after restimulation with core α 3-fucose- or core β 2-xylose-containing glycoproteins from other origins (not shown).

Finally, we determined the immunogenicity of the core α 3-fucose and core β 2-xylose determinants in *S. mansoni*-infected mice. We show that core α 3-fucose and core β 2-xylose determinants elicited a weak, but detectable, production of the Th2- (IgG1) but not Th1-associated (IgG2b) isotype IgG during infection in C57BL/6 mice, whereas IgE was barely detected (not shown). The lack of specific IgE is probably due to the fact that C57BL/6 mice are poor IgE producers compared for instance to BALB/c mice. In sharp contrast, and in accordance with a recent study that aimed at defining the immunogenicity of these glyco-epitopes [25], no specific Ab was detected in infected BALB/c mice (not shown). Indeed, using HRP, a plant glycoprotein with up to eight complex-type N-glycans carrying core α 3-fucose and core β 2-xylose epitopes, we showed that these determinants are immunogenic in immunized C57BL/6 (H2b) but not BALB/c (H2d) mice [25]. Taken together, these findings suggest that in infected or immunized mice, genetic factors are important in generating specific Ab against these glyco-epitopes. In contrast, we found relatively high titers of IgG1, and to a lesser extent of IgG2 and IgG3, against core α 3-fucose and core β 2-xylose epitopes in *S. mansoni*-infected

humans (C. Faveeuw, manuscript in preparation). Whether or not these Ab take part in limiting reinfection and/or contribute to the low level of allergic diseases observed in humans living in areas endemic for helminthiasis [31, 32], including schistosomiasis *mansoni*, is an open question that deserves further studies.

In conclusion, our data show that schistosome N-glycoproteins carrying core α 3-fucose and core β 2-xylose determinants induce a strong Th2 cytokine response in both, mice immunized with Ag-pulsed DC and infected with *S. mansoni*. Moreover, during infection, specific anti-core α 3-fucose and anti-core β 2-xylose Th2-associated IgG Ab are generated and may play important functions in the host-parasite interactions. The mechanism of this Th2-driving capacity, potentially important also for other Ag of infectious agents or allergens, is still unknown. It has been shown that specific receptors for N-glycans (for instance C-type lectins) affect DC-mediated immune responses by enhancing the Ag capture/uptake/presentation processes and by transducing signals into DC [33]. Whether or not these properties affect the polarization of the immune response clearly requires further investigations.

4 Materials and methods

4.1 Materials

The following model glycoproteins used in this study were purchased from Sigma (St. Quentin-Fallavier, France), with the exception of α -hemocyanin (Serva, Heidelberg, Germany): HRP (type VI), a plant glycoprotein with eight N-glycans predominantly substituted with both core α 3-fucose and core β 2-xylose moieties; honeybee venom PLA2, a glycoprotein sharing a glycan epitope in common with HRP (the core α 3-fucose epitope); α -hemocyanin from *H. pomatia*, a glycoprotein possessing only the core β 2-xylose epitope in common with HRP; avidin from chicken (natural avidin carrying oligomannose and hybrid structure); and avidin produced in maize (recombinant avidin). The latter carries the core α 3-fucose and core β 2-xylose determinants [25]. Examples of N-glycan structures from these glycoproteins are shown in Fig. 1, the information being taken from previously published studies [23, 24, 34, 35].

4.2 Preparation of anti-core α 3-fucose and anti-core β 2-xylose Ab

Rabbit polyclonal anti-core α 3-fucose and anti-core β 2-xylose Ab were obtained as follows. New Zealand rabbits were immunized by subcutaneous injection of 200 μ g HRP in CFA, followed by two boosts with the same dose of immunogen in IFA 2 and 6 weeks after the first immunization. Sera

were collected 1 week after the last injection. Pre-immune sera were used as negative controls. Anti-HRP Ab were then fractionated by affinity chromatography into a bee venom PLA2-binding fraction (anti-core α 3-fucose Ab) and a remaining non-binding fraction (assumed to contain anti-core β 2-xylose Ab) as described [22]. For Ab binding inhibition studies, fucosylated and chemically defucosylated bromelain glycopeptides cross-linked to BSA (BSA-MUXF³ and BSA-MUX, respectively) were prepared as described [36]. BSA-MM was used as a negative control (see Fig. 1).

These neoglycoconjugates were incubated for 2 h at room temperature with affinity-purified anti-core α 3-fucose Ab or with anti-core β 2-xylose Ab prior to the use of these primary Ab in ELISA. Briefly, flat-bottom 96-well plates (Maxisorp, Nunc) were coated overnight with 1 μ g glycoprotein in PBS at 4°C. After washing with PBS/0.1% Tween-20, plates were incubated with PBS/0.5% gelatin for 2 h. Fractionated Ab (1:1,000 in PBS/1% BSA) were incubated overnight at 4°C. After washing, HRP-labeled goat anti-rabbit Ab (Sanofi Diagnostics Pasteur, Marnes, France) were added to each well for another 2 h. Enzyme activity was detected with 3,3',5,5'-tetramethyl-benzidine substrate and stopped with 2N HCl. The absorbencies were read at 450 nm on a Titertek Multiskan.

4.3 Ag preparation and Western blotting

S. mansoni (Puerto Rican strain) schistosomula were obtained by the skin penetration procedure from cercariae shed from infected *Biomphalaria glabrata* snails [37]. Adult schistosomes and eggs were obtained from infected golden hamsters by portal vein perfusion and from the livers, respectively. Soluble skin schistosomulum, adult worm and egg Ag were prepared as follows. Parasites or eggs were resuspended in cold PBS plus protease inhibitors (1 mM PMSF, 1 μ g/ml leupeptin) and sonicated (Misonix, Vasse, Templemars, France) 4×15 s at 4°C. The homogenates were then centrifuged twice at 13,000×g for 20 min, dialyzed for 12 h against cold PBS and filtered sterile. Protein concentration was determined using the Sigma modified Lowry kit. For Western blot analysis, Ag were resolved on SDS-polyacrylamide gels (10%) and electroblotted onto nitrocellulose membranes. Membranes were incubated overnight at 4°C with primary anti-core α 3-fucose or anti-core β 2-xylose Ab diluted in PBS supplemented with 0.1% (vol/vol) Tween-20 and 5% (wt/vol) nonfat dried milk. After washing, membranes were incubated with HRP-labeled anti-rabbit Ab and processed for enhanced chemiluminescence detection (Amersham).

4.4 Fucosyl- and xylosyltransferase assays

Schistosome eggs were resuspended in 50 mM 2(N-morpholino)ethanesulfonic acid, pH 7.0, in the presence of protease inhibitors and sonicated 4×15 s at 4°C. Triton

X-100 was added at a final concentration of 0.5%, the mixture was kept for 30 min on ice and debris were removed by centrifugation [38]. Fucosyl- and xylosyltransferase assays were performed basically as previously described [39, 40]. In brief, 1 nmol dabsyl GnGn (a glycotetrapeptide derived from fibrin carrying a biantennary N-glycan with two non-reducing terminal Gn residues) was used as acceptor and 2 μ l schistosome extract (1.5 mg protein/ml) was used as the enzyme source. The assays (final volume 10 μ l) were performed at 37°C with final concentrations of 20 mM 2(N-morpholino)ethanesulfonic acid, pH 7.0, 10 mM MnCl₂, 2 mM AMP, 100 mM Gn and either 0.5 mM GDP-Fuc or UDP-Xyl (CarboSource Services, University of Georgia, GA). For the pre-incubation experiment, chicken heart extract (1 μ l) prepared by Dr. E. Staudacher was used. The incubations were analyzed at the indicated time-points by means of MALDI-TOF MS: 0.1 μ l sample was mixed with 0.9 μ l water and 1 μ l 1% (wt/vol) α -cyanohydroxycinnamic acid (dissolved in 70% acetonitrile) as matrix. After drying on the platen, spectra were recorded using a ThermoBioanalysis Dynamo instrument in delayed extraction mode [40].

4.5 Immunolabeling studies

S. mansoni schistosomula, adult worms and eggs were fixed in ImmunoHistoFix as detailed by the manufacturer (Interstiles, Brussels, Belgium) for 5 days at 4°C. After dehydration in graded alcohol baths, embedding was performed with ImmunoHistoWax at 37°C for 12 h. Sections (4 μ m) were mounted on glass slides, dewaxed in acetone for 5 min and transferred to PBS supplemented with 1% (wt/vol) ovalbumin (PBS/OVA) for 1 h at room temperature. After 2-h incubation with fractionated Ab (1:100 in PBS/OVA), sections were extensively rinsed and incubated with FITC- or HRP-conjugated goat anti-rabbit IgG (Sigma; 1:200 in PBS/OVA) for 2 h. Controls were performed using pre-immune rabbit Ab. In the final step, sections were developed with 3-amino 9-ethyl carbazol, washed three times in PBS and mounted onto glass slides in Immumount (Shandon, Pittsburgh, PA) for immunohistochemical analysis.

4.6 Preparation and metaperiodate treatment of anti-HRP-binding N-glycoproteins

Anti-HRP-binding N-glycoproteins were purified from SEA by chromatography on affinity-purified anti-HRP Ab covalently coupled to NHS-activated agarose (Bio-Rad Laboratories, Hercules, CA). The anti-HRP-binding N-glycoprotein preparation was then extensively dialyzed and filtered sterile. ELISA confirmed that this fraction contained core α 3-fucose or core β 2-xylose epitopes (not shown). To oxidize the carbohydrate structures, anti-HRP-binding N-glycoproteins (2 mg/ml in a final concentration of 50 mM sodium acetate buffer, pH 4.6) was added to 10 mM sodium metaperiodate for 15 min, after which oxidation was stopped by adding a final concentration of 50 mM of sodium

borohydride. Mock anti-HRP-binding N-glycoproteins were obtained by the same procedure, except that sodium metaperiodate was omitted. After treatment, mock or periodate-treated anti-HRP-binding N-glycoproteins were extensively dialyzed against cold Click's medium. The effectiveness of the periodate treatment was validated by Western blot analysis using anti-core α 3-fucose or anti-core β 2-xylose Ab as described [4].

4.7 Sensitization of DC, immunization protocol and analysis of the immune response

Splenic DC were isolated from Flt-3 ligand-treated C57BL/6 mice (l'Arbesle, France) and purified by positive selection over a MACS column using microbeads-conjugated anti-CD11c mAb (Miltenyi Biotec, Paris, France) exactly as described [4, 41]. After purification, splenic DC were cultured in RPMI supplemented with 2% ultrosor (Life Technologies, Cergy Pontoise, France), a serum-free medium supplement, and 10 ng/ml of GM-CSF in the absence (unpulsed DC) or in the presence (pulsed DC) of 50 μ g/ml Ag. After overnight culture, nonadherent DC were collected, washed and administered at a dose of 3×10^5 cells into the hind footpads of syngenic mice. Five days later, popliteal LN cells (5×10^5 cells/well in flat-bottom 96-well plate) were cultured in Click's medium supplemented with 0.5% normal mouse serum and various concentrations of Ag for 4 days at 37°C. During the last 18 h, 0.5 μ Ci of [³H]thymidine per well was added. At 72 h of culture, IFN- γ , IL-4, IL-5, IL-10 and IL-13 production was measured in the culture supernatants from cells by ELISA (R&D Systems, Abingdon, GB, and Becton Dickinson, Pont de Claix, France).

4.8 Infection of mice and analysis of the humoral and cellular immune response

Female 8-week-old C57BL/6 mice were percutaneously infected with 40 cercariae and animals were bled weekly from 7 to 98 days post-infection. Humoral response was studied by ELISA using SEA, PLA2 or hemocyanin. HRP-conjugated anti-mouse IgG1 or IgG2b were used as secondary Ab. To analyze the cellular response during infection, spleens were removed 49 days post-infection and cells (5×10^5 cells/well in flat-bottom 96-well plate) were cultured in RPMI supplemented with 5% fetal calf serum and various concentrations of mock or periodate-treated Ag for 4 days at 37°C. Proliferation and cytokine production were measured as described above.

4.9 Statistical analysis

The statistical significance of differences between experimental groups was calculated using the Student's *t*-test. *p* < 0.01 was considered as significant.

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