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Antigen Presentation by CD1d Contributes to the Amplification of Th2 Responses to *Schistosoma mansoni* Glycoconjugates in Mice

Christelle Faveeuw,* Véronique Angeli,* Josette Fontaine,* Charles Maliszewski,† André Capron,* Luc Van Kaer,‡ Muriel Moser,§ Monique Capron,* and François Trottein

During murine schistosomiasis, there is a gradual switch from a predominant Th1 cytokine response to a Th2-dominated response after egg laying, an event that favors the formation of granuloma around viable eggs. Egg-derived glycoconjugates, including glycolipids, may play a crucial role in this phenomenon. In this study, we used a model of dendritic cell sensitization to study the role of egg glycoconjugates in the induction of specific immune response to soluble egg Ag (SEA) and to investigate the possibility that CD1d, a molecule implicated in glycolipid presentation, may be involved in such a phenomenon. We show that, when captured, processed, and presented to naive T lymphocytes by dendritic cells, egg, but not larval, Ag skew the immune response toward a Th2 response. Periodate treatment reversed this effect, indicating that the sugar moiety of SEA is important in this phenomenon. Using DC treated ex vivo with a neutralizing anti-CD1d Ab or isolated from CD1d knockout mice, we show that CD1d is crucial in the priming of SEA-specific Th2 lymphocytes. We then evaluated the contribution of CD1d on the development of the SEA-specific immune response and on the formation of the egg-induced liver granuloma during murine schistosomiasis. We find that CD1d knockout mice have a reduced Th2 response after egg laying and develop a less marked fibrotic pathology compared with wild-type mice. Altogether, our results suggest that Ag presentation of parasite glycoconjugates to CD1d-restricted T cells may be important in the early events leading to the induction of Th2 responses and to egg-induced pathology during murine schistosomiasis. *The Journal of Immunology*, 2002, 169: 906–912.


Schistosomiasis is a chronic parasitic disease that affects over 200 million people worldwide. The pathology of the disease is associated with egg deposition in the liver and intestines and is characterized by the formation of granulomas developing around viable eggs. In humans, granulomatous disease may eventually lead to fibrosis, occlusion of the presinusoidal vascular beds, and fatal portal hypertension (for review, see Ref. 1). The host’s response to soluble secretions from mature eggs is characterized by an intense delayed-type hypersensitivity reaction that is principally mediated by egg Ag-specific MHC class II-restricted CD4+ T cells. Analysis of the immune response in *Schistosoma mansoni*-infected mice shows that the Th0/Th1 cytokine response shifts toward a strong Ag-specific as well as nonspecific Th2 response with the maturation of the granulomas (2, 3). It has been clearly demonstrated that soluble egg Ag (SEA) are the main inducers of this Th2-dominated response (3–5). Although numerous studies have been performed to understand the molecular and cellular events involved in granuloma formation, the early mechanisms leading to the switch toward the Th2 response after egg deposition are still unknown. Similarly, the nature of the egg Ag involved is poorly defined, although previous studies suggested that glycoconjugates expressed by the eggs may be crucial in such events (6). More recently, using a murine model of intranasal sensitization with SEA, Okano et al. (7) showed that carbohydrate determinants are required for induction of SEA-specific Th2 responses, but are not themselves epitopes of induced IgE responses. The molecular mechanisms leading to this adjuvancy remain unclear. Among the putative egg glycane structures implicated, lacto-N-fucopentaose III, which contains Lewis X (Le^a^) trisaccharide, may play a key role (8–11). This oligosaccharide determinant is expressed on schistosome glycoproteins and glycolipids (12, 13) and appears to polarize the immune response by activating cells of the innate immune system (14). In the present study, we investigated the possibility that, when presented by APC, egg glycoconjugates may impact the immune response. We also studied, for the reasons explained below, the role of CD1d in the induction of SEA-specific Th2 immune response. To this end, due to their key role in both innate and acquired immune responses (15, 16), we used dendritic cells (DC) as professional APC.

In mice, peptide fragments are presented by MHC I and II molecules, whereas (glyco)lipids are preferentially presented via the CD1d molecule (17, 18). The later type of presentation is probably relevant during *S. mansoni* infection because glycosphingolipid...
structures are abundantly expressed by the eggs (12, 19, 20). Moreover, it has been recently shown that during human schistosomiasis, glycolipids from *Schistosoma* are the main targets for the IgE response, suggesting a role in the IgE-mediated host protective response (21). In this work, we show that DC selectively present glycosylated Ag from the egg stage of *S. mansoni* to polarize the immune response toward a specific Th2-biased response, and that the CD1d-restricted mode of Ag presentation plays a key role in this phenomenon. Finally, we provide evidence that CD1d is important for the generation of an optimal Th2 response and in the subsequent liver pathology that develops in infected mice.

**Materials and Methods**

**Animals**

Female BALB/c mice were purchased from Iffa-Credo (l’Arbresle, France). The generation of CD1d-deficient BALB/c mice has been described previously (22). CD1d knockout (KO) mice from the ninth backcross to BALB/c were used in this study.

**Reagents and Abs**

The purified anti-CD3 mAb and the biotin-conjugated anti-CD11c mAb were purchased from BD Biosciences (Le Pont de Claiix, France). Polyclonal rabbit serum directed against the *S. mansoni* 28-kDa protein (Sm28GST) has already been described (23). Mouse anti-CD1d mAb was purified from the 1B1 hybridoma supernatant (a gift from Dr. M. Kronenberg, La Jolla Institute for Allergy and Immunology, San Diego, CA) (24) using protein G-agarose columns (Life Technologies, Belgium). Human rFlt-3 ligand was provided by Immunex (Seattle, WA). Mouse rGM-CSF was purchased from Biosource (Nivelles, Belgium). Human rFli-3 ligand was provided by Immunex (Seattle, WA).

**Ag preparation and metaperiodate treatment**

*S. mansoni* (Puerto Rican strain) schistosomula were obtained by the skin infection of mice, as already described (26). Briefly, a small piece of liver from each mouse was fixed in acetic acid-90% ethanol (90%/90%) mixture, then 4% KOH, as previously described (28). Records of the organ weights allowed the calculation of total tissue eggs per organ. For the kinetic study, spleens were harvested at days 31, 49, and 63 postinfection, and cells were stimulated with anti-CD3 Ab (5 µg/ml), to allow the stimulation of all in vivo (pre)activated cells, for 3 days at 37°C. During the last 18 h, 0.5 µCi [3H]thymidine/well was added at 48 h time point, IFN-γ, IL-4, IL-5, and IL-10 production was measured in the culture supernatants by ELISA adapted from BD PharMingen (San Diego, CA) protocols (BD Biosciences). The supernatant was assayed for IFN-γ using an ELISA kit from R&D Systems (Abingdon, U.K.).

**Infection of mice and analysis of the parasitological and immunological parameters**

Eight-week-old wild-type (wt) and CD1d KO BALB/c mice were percutaneously infected with 60 cercariae, and worm burdens were measured by liver perfusion 49 days after infection (27). At the time of perfusion, the small intestines and the livers were also collected for measurement of egg numbers deposited in these organs. Tissues were digested in 4% KOH, as previously described (28). Records of the organ weights allowed the calculation of total tissue eggs per organ. For the kinetic study, spleens were harvested at days 31, 49, and 63 postinfection, and cells were stimulated with anti-CD3 Ab (5 µg/ml), to allow the stimulation of all in vivo (pre)activated cells, for 3 days at 37°C. During the last 18 h, 0.5 µCi [3H]thymidine/well was added at 48 h time point, IFN-γ, IL-4, IL-5, and IL-10 production was measured in the culture supernatants. To study the humoral response, mice were bled 49 days after the infection, and the anti-SEA IgG1 and IgG2a titrations were determined for each mouse by ELISA. Briefly, flat-bottom 96-well plates (Maxisorp; Nunc, Roskilde, Denmark) were coated overnight with 10 µg SEA in PBS at 4°C. After washing with PBS/0.1% Tween 20 (PBS/Tween), plates were incubated with PBS/0.5% gelatin in 1 h. Serum samples were serially diluted 1/2 (starting at 1/100 for IgG2a and 1/10,000 for IgG1 titrations) in PBS/Tween and incubated overnight at 4°C. After washing, HRP-conjugated anti-mouse IgG2a or anti-IgG1 was added to each well for another 2 h. Enzymatic activity was detected with 3,3’,5,5’-tetramethylbenzidine substrate and stopped with 2 N HCl. The absorbancies were read at 450 nm on a Titerite Multiskan. Serum titers are defined as the dilution that gives an OD reading at least 2-fold higher than the mean background of noninfected mouse sera, and results are expressed as IgG1-IgG2a serum titer ratio.

**Preparation and sensitization of DC**

DC were isolated from spleens obtained from F13 ligand-treated BALB/c mice, as already described (26). Briefly, spleens were digested with collagenase for 30 min at 37°C and then dissociated in HBSS containing 10 mM EDTA. The cell suspension was separated into low and high density fractions on a Nycodenz gradient (Nycomed Pharma, Oslo, Norway) (1700 × g for 15 min). Splenic DC were collected at the interface, washed twice with RPMI, and purified by positive selection over a MACS column by using microbead-conjugated anti-CD11c mAb. After purification, the cells were cultured in 100-mm petri dishes for 2 h at 37°C in RPMI supplemented with 2% ultrorser (Life Technologies), a serum-free medium supplement. Nonadherent cells were removed by vigorous pipetting, and the adherent cells (immature DC) were allowed to mature for 18 h in RPMI supplemented with 2% ultrorser and 10 ng/ml GM-CSF in the absence (unpulsed DC) or in the presence (pulsed DC) of 50 µg/ml Ag. In general, nonadherent mature DC represented at least 99% of the total cell population, as assessed by FACS analysis with anti-CD11c mAb.

**Immunization protocol and analysis of the immune response**

After overnight culture, nonadherent unpulsed or Ag-pulsed DC were collected, washed, and administered at a dose of 3 × 10^5 cells into the hind footpads of syngeneic mice. In some cases, SEA-pulsed DC were treated for 1 h at 37°C with 50 µg/ml anti-CD1d mAb (B1) or isotype control mAb before their transfer into recipient mice. Five days later, popliteal lymph node (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 µCi [3H]thymidine/well was added. At the 72-h time point, IFN-γ, IL-4, IL-5, and IL-10 production was measured in the culture supernatants from LN cells by ELISA. IL-4, IL-5, and IL-10 were quantified by two-site ELISA adapted from BD Phamingen (San Diego, CA) protocols (BD Biosciences). The supernatant was assayed for IFN-γ using an ELISA kit from R&D Systems (Abingdon, U.K.).

**Analysis of the egg-associated liver pathology in infected mice**

The egg-associated pathology was analyzed on the livers of mice 49 days after the infection (20–23 mice per group) in a double blind fashion. Briefly, a small piece of liver from each mouse was fixed in acetic acid-based fixative for 2–3 days and embedded in paraffin. Then, 4-µm liver sections were stained with either H&E for granuloma diameter measurement or May Grunwald Giemsa for eosinophilia examination. The diameters and eosinophil contents of granulomas (10/mouse) surrounding sinusoids or bile ducts were visualized on H&E-stained sections, was quantified and expressed as the mean number of granulomas. The granuloma was calculated assuming a spherical shape. The degree of necrosis, inflammation, and tissue eosinophil contents of granulomas (10/mouse) surrounding sinusoids or bile ducts were visualized on H&E-stained sections, was quantified and expressed as the mean number of granulomas.

**Statistical analysis**

The statistical significance of differences between experimental groups was calculated using the Student’s t test. Values of *p* < 0.05 were considered as significant.
DC pulsed ex vivo with egg Ag skew the immune response toward a Th2 response in recipient mice

Using DC as APC, we first compared the nature of the T cell response induced by larval (SSA) or egg (SEA) Ag. To this end, mice were immunized in the hind footpads with $3 \times 10^5$ SSA- or SEA-pulsed DC (or unpulsed DC as a control), after which popliteal LNs were harvested 5 days later and restimulated in vitro with SSA or SEA, respectively. As shown in Fig. 1, administration of DC sensitized ex vivo with SSA or with SEA resulted in identical levels of T cell priming, as determined by Ag-dependent proliferation in culture. We next analyzed the cytokines released by LN cells primed with the different DC preparations. As depicted in Fig. 1, SSA-pulsed DC induced the activation of cells secreting higher amounts of IL-4, IL-5, and IL-10 following restimulation with the same Ag in vitro. SEA-pulsed DC sensitized cells to produce less IFN-γ, IL-4, IL-5, and IL-10, and less IFN-γ. This experiment shows that, when captured, processed, and presented to T lymphocytes by DC, both schistosome larval and egg Ag induce the development of a specific mixed response, with the latter skewing the cytokine profile toward a more Th2-like response.

Alteration of carbohydrate structures of egg Ag affects the ability of SEA-pulsed DC to induce a Th2 response

Because glycoconjugates from SEA are important targets for Th2 responses in vivo (7), we determined whether, when presented by DC, carbohydrates on SEA are the main inducers of Th2 responses in our model of immunization. For this purpose, mild periodate oxidation was performed, and the ability of the periodate-treated SEA preparation to induce Th2 responses was analyzed. We first validated the method of periodate treatment by analyzing the antigenic preparations by Western blotting using specific Abs directed against schistosome carbohydrate (anti-Le^a^ Ab) or peptide (anti-Sm28GST Ab) epitopes. As seen in Fig. 2A, the anti-Le^a^ Ab was reactive with untreated and mock SEA, but not with periodate-treated SEA. Conversely, the monospecific anti-protein Ab revealed a unique band in all preparations (with an identical intensity). This experiment thus confirmed that the periodate treatment altered the glycan moiety and appeared to preserve the antigenic integrity of SEA.

Mice were immunized in the hind footpads with DC pulsed with mock- or periodate-treated SEA and, 5 days later, LN cells were restimulated in vitro with SEA. As Fig. 2B shows, LN cells from mice injected with DC pulsed with either mock- or periodate-treated SEA proliferate in a similar manner and produce equal quantities of IFN-γ in response to SEA. In contrast, the production of Th2 cytokines was dramatically reduced (6- to 10-fold) in mice that received periodate-treated SEA-pulsed DC. This indicates that, in our model of immunization, the sugar moiety of SEA is particularly important in generating specific Th2 immune response.

CD1d is involved in Th2 immune responses induced by SEA-pulsed DC

Because glycolipid structures are abundant in *S. mansoni* eggs (12, 19, 20), we evaluated the contribution of CD1d, a molecule known to capture and present microbial and self (glyco)lipid Ag to T cells (17, 18), to Th2-biased response induced by SEA-sensitized DC. To this end, SEA-pulsed DC were treated with a neutralizing anti-CD1d mAb before their transfer into recipient mice and, 5 days later, LN cells were restimulated in vitro with SEA. As Fig. 3A shows, LN cells from mice injected with DC treated with either the anti-CD1d or isotype control Ab proliferate in a similar manner and produce equal quantities of IFN-γ in response to SEA. In contrast, the production of Th2 cytokines was dramatically reduced in mice that received DC treated with the anti-CD1d Ab. This indicates that the availability of CD1d on SEA-pulsed DC is particularly important in generating specific Th2 immune response. To confirm this, spleen DC were purified from wt or CD1d KO mice, pulsed with SEA, and then injected into wt mice. Fig. 3B shows that administration of wt or CD1d KO-pulsed DC resulted in comparable T cell priming, as assessed by SEA-dependent proliferation of LN cells in culture. However, cytokine released into the supernatants of these cultures differed dramatically because CD1d KO-pulsed DC induced the activation of cells that secrete IFN-γ, but little IL-4, IL-5, and IL-10. These results suggest that the CD1d-restricted Ag presentation is crucial in the priming of SEA-specific Th2 lymphocytes.

CD1d is involved in the induction of Th2 responses to egg Ag during murine schistosomiasis

We then studied the involvement of CD1d in the induction of Th2 responses to egg Ag in *S. mansoni*-infected mice. We first analyzed the parasitological parameters in wt and CD1d KO-infected mice, 49 days after infection. As shown in Table I, worm burdens as well as tissue (liver and intestine) egg numbers were similar in the two animal groups, indicating that the lack of CD1d does not influence parasite survival and fecundity in infected mice. We then determined the titers of SEA-specific IgG1 (a marker of a Th2 response) and IgG2a (a marker of a Th1 response) in infected mice, 49 days postinfection. Although the overall Ig levels were identical in both groups (not shown), the average IgG1-IgG2a ratio was dramatically reduced in CD1d KO mice (22 ± 10) compared with that of wt mice (76 ± 20) (66% reduction) (Fig. 4). This suggests that CD1d is important in the Th2 bias of SEA-reactive T
cells and in the subsequent generation of egg Ag-specific Th2-associated Ab isotypes during infection.

**Infected CD1d KO mice have reduced capacity to up-regulate Th2 cytokines after egg laying**

As mentioned in the introduction, the immune response in infected mice switches from a Th1 to a Th2 response after egg laying. To know whether infected CD1d KO mice failed to switch the immune response after egg deposition, spleens were removed on day 31 (before egg laying when the Th1 response prevails) and on days 49 and 63 (when egg-driven Th2 responses predominate), and cells were restimulated with anti-CD3. As represented in Fig. 5, IFN-γ production gradually declined in CD3-stimulated cells from wt, as well as from CD1d KO-infected mice. In contrast, IL-4 and IL-5 production increased with the time of infection, but in a less marked manner in CD1d KO-infected mice compared with wt mice. Similarly, the synthesis of IL-13, a cytokine known to be important in fibrosis, was dramatically reduced in CD1d KO mice compared with wt mice. This indicates that CD1d deficiency does not appear to alter the capacity of mice to down-regulate Th1 responses during infection, but affects, in a significant manner, their ability to up-regulate the production of Th2 cytokines.

**CD1d influences the formation of liver granulomas in S. mansoni-infected mice**

Because the development of the Th2 response is important in the egg-induced pathology in mice (29), we then proceeded to evaluate the influence of CD1d on the formation of liver granuloma during murine experimental schistosomiasis. To this end, livers were examined histologically 49 days after the infection. In both animal groups, the size of the granulomas surrounding eggs was not statistically different (Table II). Compared with wt mice (Fig. 6, A and C), granulomas from CD1d KO-infected mice appeared less cellular (Fig. 6B) and contained a reduced number of eosinophils (Fig. 6D) (30% reduction, Table II). Because fibrosis is a sequel to egg granuloma formation during infection, we then visualized collagen deposition within the liver. Sirius red staining demonstrated a dramatic decrease in collagenous material (red stain) in the granulomas of CD1d KO animals (Fig. 6F) compared with control mice (Fig. 6E). Quantitative studies were conducted by scoring the sections using well-defined criteria for degree of collagen deposition and necrosis. As Table II shows, CD1d deficiency resulted in a reduction of collagen deposition in granulomas (40%) and of necrosis (60%) in the livers of *S. mansoni*-infected mice.

**Discussion**

Murine schistosomiasis is characterized by a gradual switch from a predominant Th1 cytokine response during the first 4 wk of infection to a Th2 cytokine-polarized response after egg laying (2, 3). This phenomenon is induced by egg Ag and favors the development of granuloma around them. Recently, Okano et al. (7) provided evidence that carbohydrate residues on SEA are important in the induction of a specific Th2 response, and we suggest that the CD1d mode of Ag presentation may be crucial in this event. Finally, we show that during *S. mansoni* infection, CD1d is important for the generation of an optimal Th2 response and for the subsequent granulomatous response in mice.

We first compared the nature of the T cell response induced by larval or egg Ag-pulsed DC in recipient mice. Our results show that when captured and presented to naive T cells by DC, larval Ag induce a mixed response, whereas, in agreement with a recent report (30), SEA-pulsed DC skew the immune response toward a Th2 response, although significant levels of IFN-γ are still produced. This correlation between immune response profile and the infectious stage of the pathogen has already been reported in the
case of the fungus *Candida albicans* (31), and is a likely consequence of the high antigenic diversity between *S. mansoni* schistosomula and eggs. We then investigated the role of egg glycan structures in this phenomenon. We show that modification of the structures of SEA carbohydrates sensitive to mild periodate oxidation abolished the ability of pulsed DC to induce a specific Th2 response. Numerous studies have revealed that eggs contain large amounts of highly antigenic N- and O-linked proteins as well as lipid-associated glycoconjugates (12, 19, 20). Consequently, as a next step, we studied the impact of CD1d neutralization or CD1d deficiency on the immune response after immunization with SEA-pulsed DC. In this model, we found that ex vivo blockade of CD1d availability (Fig. 3A) or CD1d deficiency on DC (Fig. 3B) affects the priming of SEA-specific Th2 lymphocytes in recipient mice. At present, the presentation of microbial glycolipids to T cells by the CD1d molecule is still controversial. However, our findings suggest that the CD1d mode of Ag presentation may be crucial in amplifying egg Ag-specific Th2 responses. It is therefore possible that SEA, or processed forms of these Ag, directly bind with CD1d molecules and activate CD1d-restricted T cells, particularly NK T cells. In this regard, it is noteworthy that SEA contain a majority of saline soluble Ag and no, or few, lipophilic Ag. This suggests that the structures in SEA presented by CD1d probably possess amphiphatic properties. Preliminary results, based on the fractionation of SEA according to lipophilic properties, suggest that this is indeed the case. Attempts to identify the Ag candidate(s) are underway at the moment. An alternative explanation for our findings is that SEA may induce the expression of endogenous glycolipid Ag that, in turn, bind with CD1d molecules and activate NKT cells. In this regard, Table 1. Analysis of the worm burden and of the total tissue eggs in wt and CD1d KO-infected mice 49 days postinfection

<table>
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<th>wt</th>
<th>CD1d KO</th>
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<tbody>
<tr>
<td>Worm burden</td>
<td>28.2 ± 7.3</td>
<td>28 ± 6.8</td>
</tr>
<tr>
<td>Eggs/gram of liver</td>
<td>7,209 ± 2,391</td>
<td>7,967 ± 2,675</td>
</tr>
<tr>
<td>Eggs/gram of intestine</td>
<td>13,426 ± 3,817</td>
<td>15,957 ± 3,512</td>
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* Results represent the mean number ± SD (11 mice per group). One representative experiment of three is represented.
cells. There is significant evidence that NKT cells recognize self Ag (17, 32–35), which may be induced by stress and/or pathogen-derived products. Future experiments will be aimed at deciphering the precise role of the CD1d Ag presentation pathway in the ability of SEA to promote Th2 responses.

We then investigated the impact of CD1d deficiency on the immune response and on granuloma formation during infection. Analysis of the humoral response revealed that the production of the Th2-associated Ab isotypes IgG1 and IgE (analyzed at day 63; not shown) was dramatically reduced in infected CD1d KO mice compared with wt mice. Moreover, but in a less marked fashion, the Th2 cellular response (as assessed by IL-4, IL-5, and IL-13 production) was also diminished, but not abrogated, in CD1d KO mice. This suggests that CD1d plays a role in the priming of Th2 cells specific for SEA in vivo. Because the Th1 to Th2 switch is important in the formation of granulomas in infected mice, we studied the effect of CD1d deficiency on egg-induced pathology in the liver. The size of granulomas surrounding eggs of CD1d KO mice was identical with those of granulomas in control infected animals. However, compared with wt mice, the intrgranuloma cellularity was reduced in CD1d KO mice. Interestingly, the number of eosinophils, a cell population that is driven by Th2 cytokines and that is important in the immunopathology during murine schistosomiasis (36), was significantly diminished in CD1d KO mice. In these mice, we also observed a strong reduction in collagen deposition in the livers, a marker of liver fibrosis, and also a decrease in intra- and extragranuloma necrosis. Taken together, these findings suggest that CD1d deficiency results in a less marked hepatic pathology in S. mansoni-infected mice.

The involvement of CD1d in the induction of the SEA-specific Th2 response in DC injected as well as in S. mansoni-infected mice strongly suggests a role for CD1d-restricted NKT cells in this process. These cells are believed to play important functions in innate and adaptive immune responses during infection (33, 35, 37), including parasite infection (38), and also have been shown to be important players in some Th2-associated responses (32, 34, 39). Recently, Brown et al. (40), using a model of synchronous formation of pulmonary granulomas elicited by injected S. mansoni eggs, suggested that β2-microglobulin-dependent T cells, including NKT cells, play a minor role in the Th2 granulomatous response in C57BL/6 mice. This apparently conflicting result can be explained: 1) by the fact that in β2-microglobulin-deficient mice a residual population of CD1d-restricted T cells remains, and 2) by differences in the mouse strain (C57BL/6 vs BALB/c) or in the protocol used to induce and to study granuloma formation (synchronous lung granulomas vs nonsynchronous liver granulomas). Taken together, we speculate that, in our model of immunization as well as during infection, CD1d-restricted NKT cells may be important cellular components in the early events, leading to the activation of MHC class II-restricted SEA-specific Th2 cells, for instance by rapidly secreting large amounts of IL-4 after their activation. Interestingly, a recent study suggests that certain glycolipid structures are indeed capable of inducing a predominant production of IL-4 (but not IFN-γ) by CD1d-restricted NKT (41). At the moment, we are studying the kinetics of NKT cell activation in vivo after DC injection as well as during murine infection, particularly in the liver, an organ known to be rich in NKT cells.

To conclude, our data suggest that the CD1d-restricted presentation of egg glycoconjugates plays an important role in the induction of Th2 responses during murine schistosomiasis, and underlie the possible involvement of CD1d-restricted T cells in the early immunological events leading to the egg-associated pathology in infected mice.

Acknowledgments

We gratefully acknowledge Dr. M. Kronenberg for kindly providing the 1B1 hybridoma; Dr. C. A. Maurage (Center Hospitalier Régional Universitaire, Lille, France) for his expertise on histological analysis; and P. Verme (Université Libre de Bruxelles) for maintaining CD1d KO mice. We greatly thank Drs. M. Leite de Moraes and R. Maldonado-Lopez for helpful discussions.

References


Table II. Pathological parameters in S. mansoni-infected wt and CD1d KO mice

<table>
<thead>
<tr>
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<th>wt</th>
<th>CD1d KO</th>
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<tbody>
<tr>
<td>Granuloma volume (× 10⁶ mm³)</td>
<td>55.4 ± 15.3</td>
<td>51.1 ± 10.8</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>36.4 ± 7.1</td>
<td>25.4 ± 5.7*</td>
</tr>
<tr>
<td>Collagen deposition (arbitrary unit)</td>
<td>2.4 ± 0.4</td>
<td>1.4 ± 0.4*</td>
</tr>
<tr>
<td>Necrosis (arbitrary unit)</td>
<td>1 ± 0.3</td>
<td>0.4 ± 0.2**</td>
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* Data are presented as mean ± SD and are representative of results obtained from three different experiments (20–23 mice per group). Pathological parameters were quantified as described in Materials and Methods.

†, p < 0.001; ‡, p < 0.01.
ROLE OF CD1d IN MURINE SCHISTOSOMIASIS


