Antibodies to Glycans Dominate the Host Response to Schistosome Larvae and Eggs: Is Their Role Protective or Subversive?

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Multiple exposures of chimpanzees to the radiation-attenuated schistosome vaccine provoked a strong parasite-specific cellular and humoral immune response. Specific IgM and IgG were directed mainly against glycans on antigens released by cercariae; these were also cross-reactive with soluble antigens from larvae, adult worms, and eggs. Egg deposition was the major antigenic stimulus after challenge of vaccinated and control chimpanzees with normal parasites, eliciting strong antiglycan responses to egg secretions. Glycan epitopes recognized included LacdiNAc, fucosylated LacdiNAc, Lewis^x (weakly), and those on keyhole limpet hemocyanin. Antibodies to peptide epitopes became prominent only during the chronic phase of infection, as glycan-specific IgM and IgG decreased. Because of their intensity and cross-reactivity, the antiglycan responses resulting from infection could be a smoke screen to subvert the immune system away from more vulnerable larval peptide epitopes. Their occurrence in humans might explain the long time required for antischistosome immunity to build up after infection.

Epidemiologic studies show that schistosome infection intensities among persons living in endemic areas are not necessarily correlated with the pattern of contact with contaminated water [1, 2] but rather are influenced by the presence of natural resistance or acquired immunity [3–5]. Antibody production is a prominent feature of the immune response in patients with schistosomiasis, and particular isotypes correlate with resistance or susceptibility to infection [6–11]. A substantial proportion of the antibodies detected in patients with acute or chronic infections is directed against carbohydrate epitopes, many shared among larval, worm, and egg glycoproteins [12–14], and some cross-react with glycans of keyhole limpet hemocyanin (KLHg) [15, 16]. Levels of KLHg-reactive IgM and IgG antibody are high at the acute and low at the chronic stage of the infection, permitting diagnostic discrimination between the 2 patient groups [17, 18]. However, the overall importance of glycan-specific antibodies to protection or pathology is still not clear, and only a relatively modest amount of information is available [19, 20] about specific structures or expression of relevant glycan antigens. Thus, better analysis of the differential recognition of antigenic glycoconjugates and proteins is required for comprehension of the immune response in human schistosomiasis.

Studies of infected humans provide only cross-sectional data, because of the ethical requirement to give chemotherapy on diagnosis. This precludes a longitudinal examination of the immune response through acute to chronic infection, which can be done only in laboratory animals. In primate models, protective immunity against challenge infection with *Schistosoma mansoni* is at least partially dependent on an efficient humoral immune response. Thus, protection in baboons and vervets multiply exposed to the radiation-attenuated (RA) vaccine, the most effective schistosome vaccine available to date, correlates well with the levels of parasite-specific IgG antibodies [21–23]. These findings in primates are corroborated by experiments showing that a degree of protective immunity can be passively transferred to naive recipient mice by serum from multiply vaccinated donor mice [24]. Analysis of the reactivity to glycoconjugates in mice shows that the RA vaccine induces lower titers of anticarbohydrate antibodies than does a patent schistosome infection [13, 25]. Moreover, anti-KLHg antibodies from a rabbit do not confer passive protection on mice [26]. However, in primates no information is available about the recognition pattern of periodate-sensitive glycan epitopes and periodate-resistant peptide epitopes after vaccination or infection.

We recently investigated the efficacy of the RA vaccine in the chimpanzee, the host genetically and physiologically closest to humans. Multiply vaccinated chimpanzees developed a strong

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parasite-specific cellular and humoral immune response and were partially protected against subsequent challenge with *S. mansoni,* as judged by an amelioration of the acute disease and the overall morbidity and a 40% reduction in egg output compared with control chimpanzees (M.E., J.A.M.L., P. A. Frost, et al., unpublished data). Here we examined the reactivity of carbohydrate and peptide epitopes with antibodies from vaccinated and infected chimpanzees. Our findings provide a basis for understanding the development of the immune response to *S. mansoni* over the time from infection to chronic disease.

Materials and Methods

Hosts and parasites. This study was carried out in 6 unrelated, healthy 5–6-year-old male chimpanzees *(Pan troglodytes)* selected from the colony housed at the Biomedical Primate Research Centre (BPRC; Rijswijk, The Netherlands). The whole experimental system was validated at the BPRC by a successful vaccination/challenge study in C57Bl/6 mice (data not shown). In brief, 3 chimpanzees were exposed 3 times to 9000 attenuated *S. mansoni* cercariae (300 Gy γ-radiation from a ^{60}Co source at the BPRC) on shaved abdominal skin at 5-week intervals. Three weeks after the last exposure, the 3 vaccinated chimpanzees and 3 naive control chimpanzees were challenged percutaneously with 2000 normal (i.e., nonattenuated) cercariae. At the end of this study, at time points between postchallenge (PC) weeks 28 and 36, by clinical evaluation, all chimpanzees were cured with praziquantel (Droncit; Bayer). After being shown to be egg negative, each was retired within the BPRC facilities.

Antigen preparations. Soluble adult worm antigen preparation (SWAP), soluble lung-stage antigen preparation (SLAP), freshly transformed schistosomula (known as 0–3-h released antigen preparation [0–3-hRAP]), soluble egg antigens (SEAs), and egg-secreted proteins (ESPs) were formulated, as described elsewhere [27, 28]. Recombinant SmE16 [29] was purified by using Talon affinity matrix (Clontech Laboratories) [30]. Bovine serum albumin (BSA)–conjugated lacto-N-neotetraose (LNnT-BSA), LacdiNAc tetraose (LDNT-BSA), and LacdiNAc fucopentaose (LDNFP-BSA) were synthesized as before [31]. BSA-conjugated lacto-N-fucopentaose III (LNFPIII-BSA) was purchased from V-Labs and KLH from Sigma.

Antibody levels. Serum samples were prepared from aliquots of peripheral blood taken from each chimpanzee at 1–3-week intervals and were stored at −80°C until further use. IgG and IgM antibodies against various schistosome antigen preparations were determined by ELISA, and the following coating concentrations and serum dilutions were determined to be optimum for the antigen preparations used (data not shown): SWAP and SLAP, 2.5 μg/mL (1:1500); 0–3-hRAP, 0.2 μg/mL (1:20,000); SEAs, 0.5 μg/mL (1:20,000); ESPs, 0.1 μg/mL (1:20,000); SmE16, 0.2 μg/mL (1:500); KLH, 0.25 μg/mL (1:1500); LNnT-BSA, LDNT-BSA, and LDNFP-BSA, 2.5 μg/mL (1:1500); LNFPIII-BSA, 2.5 μg/mL (1:500). The plates were probed with alkaline phosphatase–labeled goat anti-human IgG or IgM (Biosource), both diluted 1:5000, and developed with BluePhos Microwell substrate solution (Kirkegaard & Perry Laboratories). Absorbance was quantified at 630 nm with an ELISA reader (Dynatech MR500); data shown in the figures represent raw optical density (OD) values.

To remove periodate-resistant carbohydrate epitopes [32], the plates were coated overnight with antigens as described above, washed twice with 0.1 *M* sodium acetate (pH 4.5), and incubated at 37°C for 1 h with 20 m*M* sodium m-periodate (Sigma) dissolved in 0.1 *M* sodium acetate or with 0.1 *M* sodium acetate only as a control. After 2 washes with sodium acetate and one with PBS, the plates were treated with 50 m*M* sodium borohydride (Sigma) in PBS. The reaction was stopped after 30 min at room temperature by 5 washes with 10 m*M* Tris/HCl (pH 8.0), 150 m*M* NaCl, and 0.05% Tween-20. Subsequently, the routine ELISA protocol was applied.

Results

Antibody response to stage specifically released antigens. Antigens released by invading schistosome larvae or by eggs deposited in the host tissue are likely to interact directly with the immune system and provoke a detectable response. Therefore, we investigated the reactivity of serum samples from vaccinated and challenge control chimpanzees against antigen either released by freshly transformed schistosomula (0–3-hRAP) or secreted by cultured eggs (ESPs). For comparison, we tested the commonly used crude antigen preparations, SLAP, SWAP, and SEAs.

In the vaccinated group, SLAP-specific IgM was present at the first sampling time point 3.5 weeks after the first immunization. The level increased with each vaccination and peaked at the time of challenge infection, then dropped rapidly (figure 1*A*). A small secondary peak at about PC week 6 represented a memory response triggered by worm maturation and the onset of egg laying, as it coincided with the development not only of high SLAP- but also of high SWAP- and SEA-specific IgM levels in the challenge control group (data not shown). In the controls, the antibody response to SLAP was not evident before PC week 5 and peaked at PC weeks 8–10, with maximum levels identical to those in the vaccinated animals. In both groups, the IgM levels gradually returned to near baseline by PC week 36. The initial IgG response followed the time course observed for IgM but with a slight delay (figure 1*B*). In the vaccinated group, repeated exposure to attenuated parasites boosted the IgG titers, which peaked by PC week 1 and had a secondary increase from week PC 6 onward. Control animals produced detectable SLAP-specific IgG, starting at PC week 6, until they reached a stable maximum. During the chronic disease phase, the IgG levels in the 2 groups were indistinguishable.

ESP-reactive IgM and IgG were already detectable after vaccination and reached maximum levels about the time of challenge (similar to the SLAP-driven response). This suggests that previous exposure to the RA vaccine induced antibodies cross-reactive with various parasite stages, including eggs, before challenge (figure 1*C,* 1*D*). However, unlike the response to SLAP, a stronger IgM response to ESPs was seen in the controls

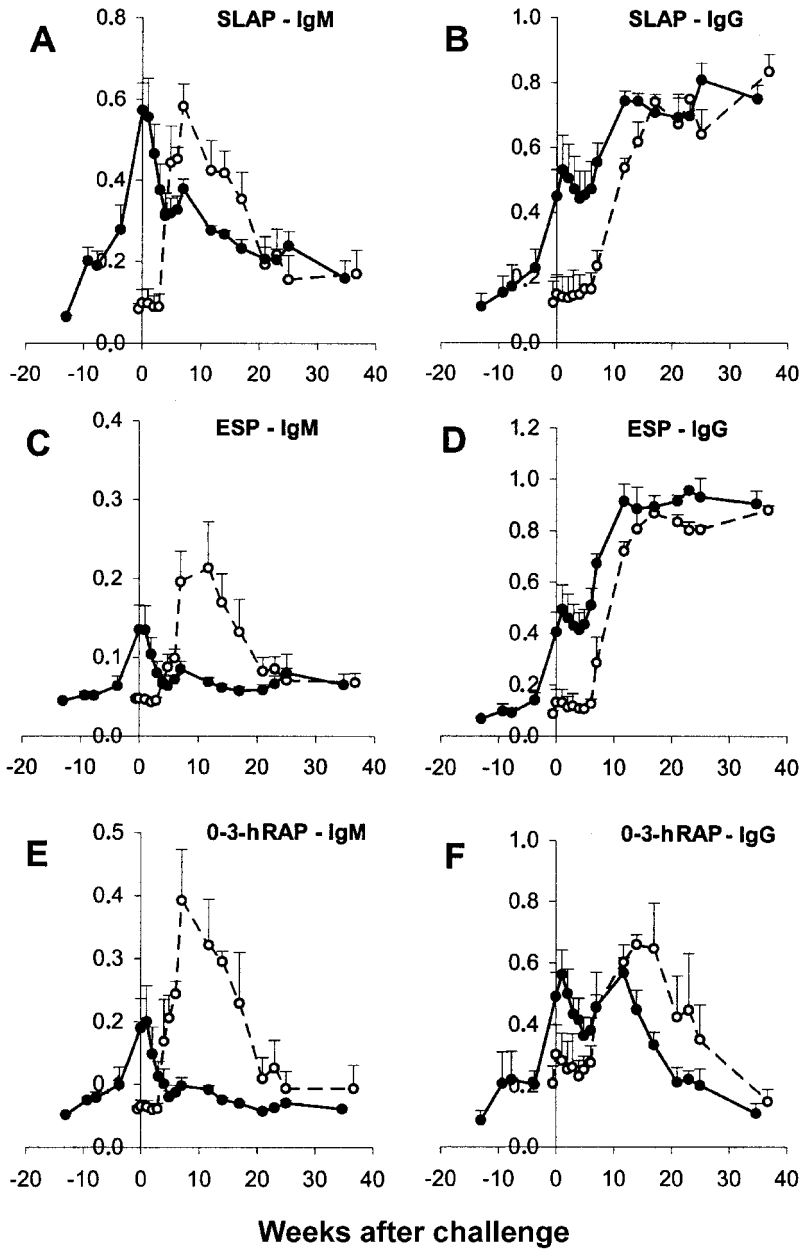

Figure 1. Levels of schistosome-specific antibodies, shown as mean optical densities (ODs) and SEM of 3 chimpanzees for IgM *(A, C,* and *E)* and IgG *(B, D,* and *F)* antibodies against soluble lung-stage antigen preparation (SLAP) *(A* and *B),* egg-secreted proteins (ESPs) *(C* and *D),* and 0–3-h released antigen preparation (0–3-hRAP) *(E* and *F).* ○, Control group; ●, vaccinated group.

than in the vaccinated chimpanzees. Moreover, both groups produced much higher amounts of IgG (OD, 0.8–1.0) than of IgM (OD, 0.1–0.2) specific for ESPs than for SLAP (OD, 0.6–0.7 vs. 0.6).

Analysis of larval secretions revealed that the vaccinated chimpanzees exhibited lower 0–3-hRAP–specific IgM levels than did the controls, similar to findings for ESPs (figure 1*E*). Also, the initial pattern of primary and secondary IgG responses against 0–3-hRAP in the vaccinated group was the same as for SLAP and ESPs (figure 1*F*). However, in striking contrast to all other antigens tested, which showed stable IgG levels throughout the chronic disease (figure 1*B,* 1*D;* data not shown), the 0–3-hRAP–specific IgG levels dropped after PC weeks 15–20 and were at baseline levels by PC week 36. Similarly, 0–3-hRAP–specific IgG in the control group developed analogously to SLAP and ESPs but declined rapidly after reaching a maximum level at PC week 12.

Dependence of antibody cross-reactivity after vaccination on

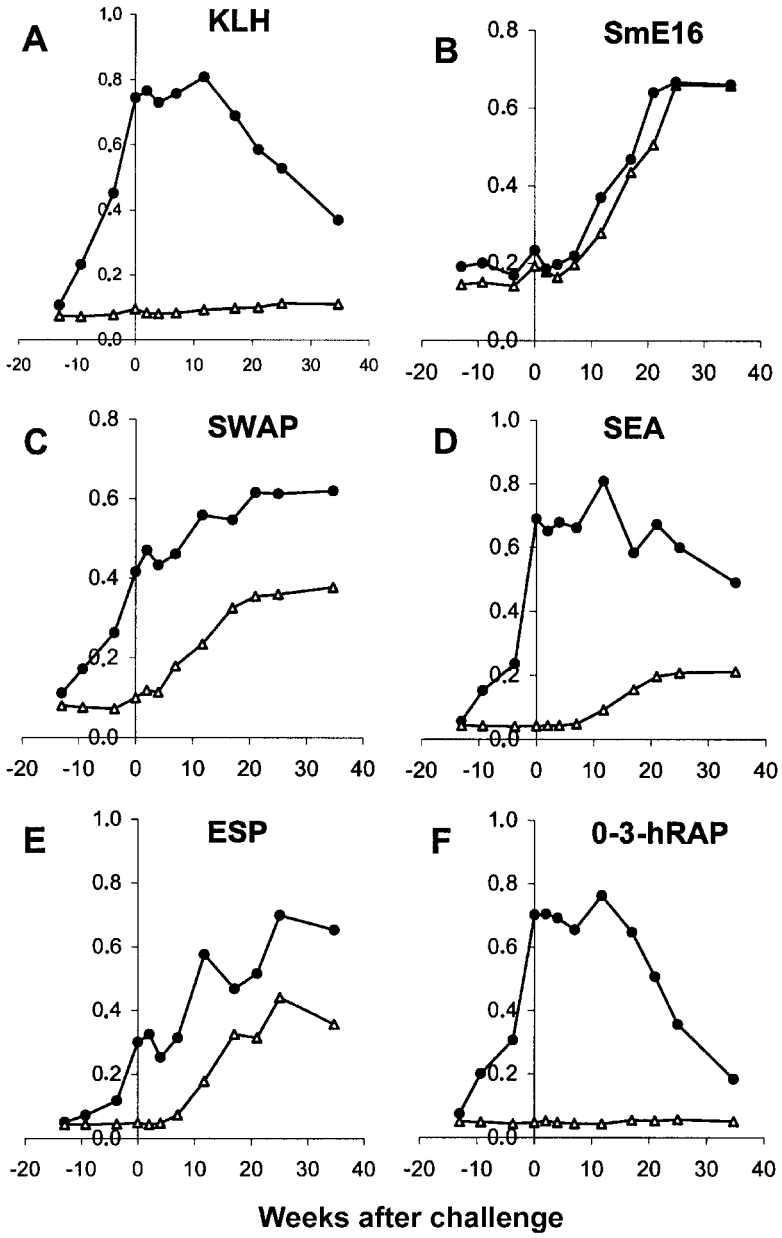

Figure 2. Levels of schistosome-specific antibodies before and after removal of carbohydrate epitopes. Data are representative optical densities from 1 vaccinated chimpanzee (V2) for IgG antibodies against periodate-treated keyhole limpet hemocyanin (KLH) *(A), Schistosoma mansoni* egg–specific calcium-binding protein (SmE16) *(B),* soluble adult worm antigen preparation (SWAP) *(C),* soluble egg antigens (SEAs) *(D),* egg-secreted proteins (ESPs) *(E),* and 0–3-h released antigen preparation (0–3-hRAP) *(F).* Results from other vaccinated animals were similar (data not shown). ●, Antigens incubated in buffer only; △, antigens treated with periodate.

antigen glycosylation. Because of the cross-reactivity of antibodies with egg and larval antigens, we examined in more detail the importance of protein glycosylation in the development of the immune response after vaccination and subsequent infection. This was done with serum from only 1 vaccinated chimpanzee (V2) due to limitations of material, but data from representative time points for the other vaccinated animals were similar (data not shown). IgG antibodies reactive with KLHg were induced shortly after exposure to attenuated cercariae (figure 2*A*) but declined after PC week 12. Not surprisingly, the anti-KLHg IgG reactivity was abrogated by previous treatment of the antigen with sodium periodate. In contrast, recognition of the egg-specific calcium-binding protein SmE16 was not influenced by periodate oxidation of the antigen (figure 2*B;* i.e.,

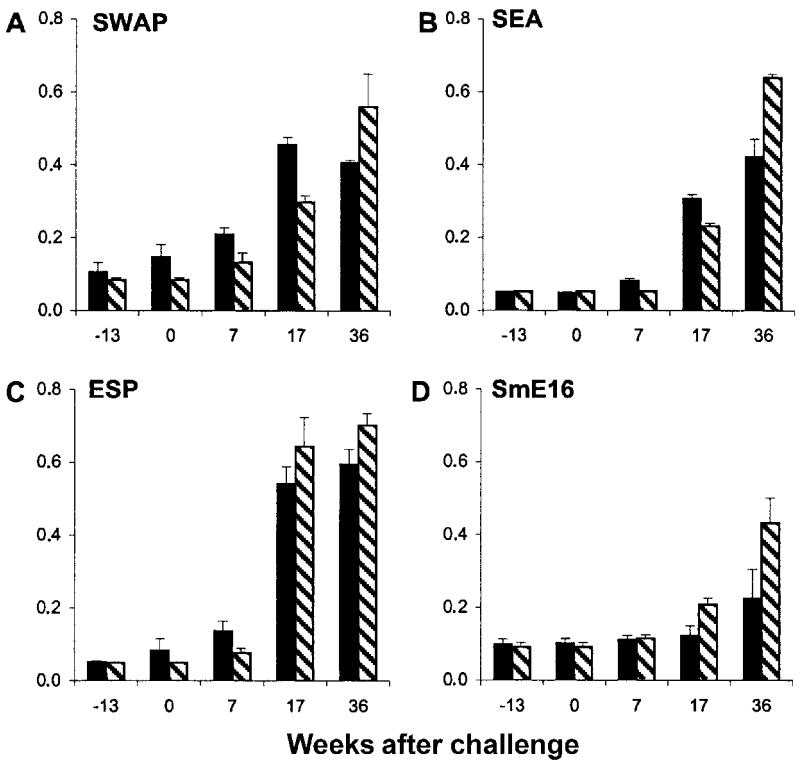

Figure 3. Levels (optical densities + SEM) of antibodies recognizing periodate-resistant peptide epitopes during chronic infection of 3 individual chimpanzees, namely, IgG antibodies against soluble adult worm antigen preparation (SWAP) *(A),* soluble egg antigens (SEAs) *(B),* egg-secreted proteins (ESPs) *(C),* and *Schistosoma mansoni* egg–specific calcium-binding protein (SmE16) *(D).* For better comparisons between groups, naive values for controls are shown at week −13 (before vaccination) and at postchallenge week 0. *Hatched bars,* control group; *filled bars,* vaccinated group.

treatment did not modify peptide epitopes). IgG antibodies against SWAP, SEAs, and ESPs produced before challenge largely bound to periodate-sensitive epitopes, whereas reactivity against periodate-resistant epitopes built up later in the course of the infection (figure 2*C*–2*E*). Of importance, treatment with periodate abrogated the reaction with 0–3-hRAP (figure 2*F*), thus revealing a close parallel with KLHg. The discrepancy between intact and periodate-treated antigen preparations revealed a gradation in the reactivity attributable to glycans (0–3-hRAP >SEAs >SWAP >ESPs). For all antigens tested, levels of protein-specific IgM antibodies were very low under the assay conditions used (data not shown).

Antibody response to periodate-resistant peptide epitopes during chronic infection. For investigation of the protein-specific antibody response after challenge, all antigens were treated with periodate (after doubling the coating concentrations to increase the signal in this particular experiment). A significant periodate-resistant antibody response to various antigen preparations developed between PC weeks 7 and 17, somewhat earlier and slower for SWAP (figure 3*A*), and later but steeper for SEAs (figure 3*B*) and ESPs (figure 3*C*). In contrast, in all 6 chimpanzees, recognition of KLHg and 0–3-hRAP was completely abrogated by prior treatment with periodate (data not shown; figure 2). At the chronic stage of the infection, SEA-specific antibody levels and, less noticeably, SWAP- and ESP-specific levels were higher in the control chimpanzees; this was mirrored by a similar difference in the SmE16-specific response between the groups (figure 3*D*).

Antibody response to defined carbohydrate epitopes. Since the antibody response after vaccination was mainly directed against periodate-sensitive structures, we further analyzed the involvement of several known glycan epitopes and compared the data with the reaction against the heterogeneous glycans of KLH. Multiple vaccinations induced a stronger anti-KLHg IgG response than that induced by a normal schistosome infection (data not shown) but a weaker IgM response (figure 4*A*). In both cases, the antibody titers did not remain stable over the course of the disease and gradually declined from maximum levels. In both groups, we detected IgM antibodies specific for LDN (figure 4*B*) and LDNF (figure 4*C*) and, to a lesser extent, LNFPIII, which contains the Lewis X (Le^X) epitope (figure 4*D*). A control glycan (LNnT) was not recognized by any serum (data not shown). In

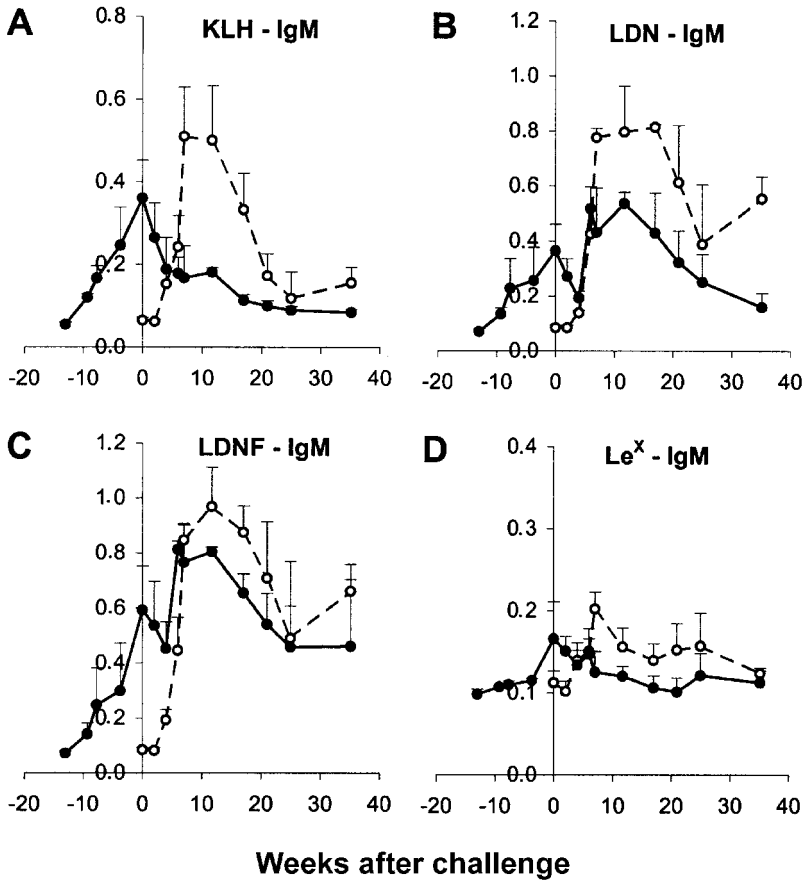

Figure 4. Carbohydrate-specific IgM levels (mean optical densities + SEM) of 3 individual chimpanzees for antibodies against keyhole limpet hemocyanin (KLH) *(A),* LacdiNAc (LDN) *(B),* fucosylated LacdiNAc (LDNF) *(C),* and Lewis X (Le^X) *(D).* ○, Control group; ●, vaccinated group.

the vaccinated chimpanzees, LDN- and LDNF-driven IgM peaked at the time of challenge, rose after PC week 4 to a secondary maximum, and slowly decayed. However, in 1 vaccinated chimpanzee (V3), IgM levels stayed elevated until PC week 36 (data not shown). In the control group, LDN- and LDNF-specific IgM increased at the same time as the secondary response in the vaccinated group and peaked between PC weeks 7 and 17. The antibody titers were elevated longer than was observed for anti-KLHg IgM and did not wane as rapidly as with KLHg. By PC week 36, a substantial reactivity against LDN and LDNF was still present in 2 control serum samples (C2, C3; data not shown). Of importance, for all 3 glycans tested (LDN, LDNF, and Le^X), peak antibody levels in the control group were higher than those in the vaccinated chimpanzees.

Surprisingly, the IgG response against these glycans was much more variable within the 2 groups than that for IgM (figure 5). Only some chimpanzees mounted significant responses of LDN- (V2; C1, C2) and LDNF-specific IgG (V1, V2; C2, C3), respectively, despite similar IgG levels against KLHg. Levels of Le^X-specific IgG were generally insignificant, with a marginal increase in C1 (data not shown). In all cases, the IgG levels increased after PC week 6 and were negligible in the vaccinated chimpanzees before challenge infection.

Discussion

Our longitudinal study of more than 1 year's duration of chimpanzees exposed to schistosomes is unique in charting the evolution of antibody responses through acute and chronic phases of the disease. We examined the effects both of vaccination with attenuated cercariae and of challenge infection with normal cercariae by using the conventional soluble antigenic preparations, SLAP, SWAP, and SEAs. In addition, we exploited 2 further preparations, both produced by parasite stages during normal biologic processes. The antigens in 0–3-hRAP are released by cercariae, primarily from acetabular glands, during penetration of the host's skin [33]; ESP antigens are released by viable mature eggs and are thought to be involved

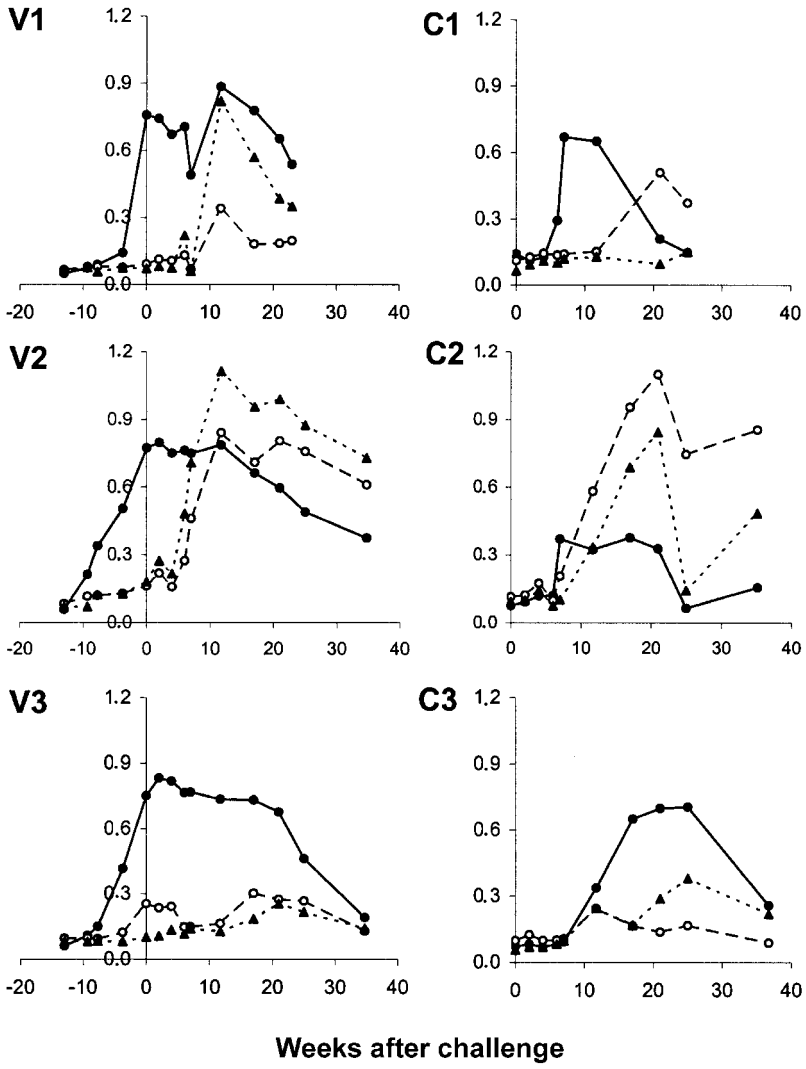

Figure 5. Levels of carbohydrate-specific IgG, shown as optical densities for antibodies against keyhole limpet hemocyanin (●), LacdiNAc (○), and fucosylated LacdiNAc (▲) in 6 individual chimpanzees. V1, V2, and V3, vaccinated group; C1, C2, and C3, control group.

in their interactions with gut and liver tissues [28]. Larval secretions should provide more meaningful provocations of the immune response with respect to protection than do the somatic antigens released when attenuated larvae die (i.e., SLAP); similarly, ESPs may be more relevant to the induction of granulomatous pathology than are the somatic constituents of the egg (e.g., SEAs). It must be emphasized that the mammalian host will be exposed to much smaller amounts of secreted larval antigens during the infection process than of secreted egg antigens after patency. Indeed, it is possible to dissect the antibody responses to larval secretions only by giving the host a significant dose of attenuated larvae that cannot mature.

Multiple exposures of the 3 test chimpanzees to irradiated cercariae (cumulatively 27,000) elicited potent IgM and IgG responses to both 0–3-hRAP and SLAP that peaked at the time of challenge. Not surprisingly, in view of the small antigenic input, 2000 normal parasites provoked little primary antibody in the challenge controls or even a secondary response in vaccinated animals. The start of egg deposition was the major antigenic event that induced and sustained antibody production in both groups of animals. Probing of antibody responses after vaccination with the various antigens revealed considerable cross-reactivity between the larval preparations and SWAP, SEAs, and ESPs. It is very unlikely that this was due to breakthrough of vaccinating parasites, since eggs were not detected in the feces before PC weeks 5–6, nor were IgG antibodies detected against the stage-specific egg antigen SmE16 in serum before PC weeks 6–7. Moreover, levels of circulating anodic antigen were not elevated before challenge (G. J. van Dam, personal communication).

Virtually the entire vaccine-induced antibody was directed at periodate-sensitive glycan epitopes, regardless of the preparation, suggesting common glycosylation motifs shared between disparate proteins in the various life cycle stages. Cross-reactivity of egg glycan epitopes and the surface of newly transformed schistosomula with antibodies from chronically infected mice has been reported [12]. The early antibodies elicited by egg deposition in the chimpanzees were also predominantly directed against glycan epitopes, and levels of antibodies recognizing periodate-resistant and presumably peptide-specific epitopes became marked only as the infection progressed from the acute to the chronic phase. The significance to protective immunity of the strong antiglycan responses to larvae, the sharing of epitopes between larvae and eggs, and the boosting of those responses by egg deposition is unclear. Indeed, the antiglycan responses might collectively be interpreted as a smoke screen to divert the immune system; this would mask vulnerable peptide epitopes or larva-specific glycan epitopes that could mediate a protective response. Support for the immune subversion hypothesis is provided by the lack of evidence from animal experiments that schistosome eggs, used as immunogens, can protect rodents or primates against challenge [34–36]. Also, if the very slow buildup of antipeptide responses in chimpanzees also occurs in humans, it could partly explain the long time required for protective immunity to develop after a natural schistosome infection [37, 38]. Clearly, further studies are required to establish the importance of antiglycan versus antipeptide responses to protective immunity.

The 40% protection elicited in chimpanzees by the RA vaccine deserves comment. The nature of the protective mechanism appears to differ in rodents and primates, the former being protected by a single vaccination whereas the latter require $\geqslant 3$ exposures [39]. There is a much higher parasite load relative to body mass in the rodent host, and there may also be differences in immune priming due to variations in the migration pattern of larvae [39, 40]. The outcome in the mouse is a strong type 1 cell-mediated immunity that is responsible for the bulk of protection and is associated with low antibody levels largely reactive with peptide epitopes [13]. In the chimpanzee, type 2 cell-mediated responses are prominent at the time of challenge, implicating antibody in the effector mechanism (M.E., J.A.M.L., P. A. Frost, et al., unpublished data). Thus, protection may be mediated either by the low levels of antipeptide antibody achieved by 3 vaccinations or by larva-specific non–cross-reacting glycan responses. Indeed, one effect of irradiation might be to modify glycan expression by attenuated larvae [41], thereby diminishing the smoke screen and facilitating antipeptide immunity. However, this hypothesis needs to be validated by using known larval or worm recombinant proteins; the higher early antipeptide response to SWAP in vaccinated chimpanzees is promising in this context. We are currently testing the smoke screen hypothesis in mice and baboons exposed to the RA vaccine.

Peripheral blood mononuclear cells (PBMC) from patients with acute disease are strongly responsive to schistosome antigens, whereas PBMC from persons with the chronic intestinal form are invariably less reactive [42]. Whether the pattern of reduced cellular reactivity is repeated for antibody responses is less clear. In the current study, specific chimpanzee IgM levels to all antigens declined during PC weeks 10–20, regardless of the vaccinated or control status of the animals. The IgG responses to 0–3-hRAP and KLHg also declined in parallel with IgM, whereas IgG responses to SLAP, SWAP, SEAs, and ESPs remained elevated until the end of the study. This drop in specific IgM levels could reflect the progression from a primary to a secondary immune response and involve isotype switching. The absence of restimulation by incoming larvae might be another factor influencing the decline in IgM responses to both larval preparations after challenge, but the cross-reactivity seen with egg antigens makes this unlikely. Finally, since much of the IgM is directed against glycan epitopes, decreasing levels might simply indicate a failure to establish memory for carbohydrate antigens. However, the production of IgG to glycan epitopes reveals some degree of isotype switching, implying major histocompatibility complex type II restriction and T cell help. Conversely, the production in some chimpanzees of IgM but not IgG to the defined glycan epitopes LDN and LDNF [31, 43, 44] suggests a T cell–independent response in those animals. Presumably, certain antigens released by eggs or larvae behave in this way because they are largely polysaccharide or bear repeating glycan epitopes on a peptide carrier backbone that lacks T cell epitopes. The fact that IgM against LDN and LDNF was induced by vaccination, but that IgG did not appear before patency, argues for the presence of these carbohydrate structures on both larval and egg antigens, but on different protein backbones. The low overall response to Le^x in chimpanzees is consistent with earlier observations in human patients, unlike rhesus monkeys, mice, and hamsters [45–47]. Nevertheless, glycan epitopes including Le^x may play an important role in down-modulating the cellular immune response [48, 49].

IgG antibodies directed against the protein backbone of larval, adult worm, and egg antigens rose steadily during the chronic phase. Unfortunately, we could not analyze IgG isotype responses in the chimpanzees and correlate them with data from human patients [20], because monoclonal antibodies against human IgG2 and IgG4 react with the same chimpanzee immunoglobulin fraction [50] (authors' unpublished data), which suggests that chimpanzees have only 3 IgG subclasses. Higher amounts of peptide-specific IgG against SEAs and SmE16 were present in the controls than in the vaccinated chimpanzees, which could be due to the difference in egg production between groups. Of note, IgE and IgA antibodies have been postulated to participate in protective immunity in humans [7–9, 51]. Unfortunately, owing to limitations of material, we could not do assays for parasite-specific IgA or IgE.

Many of the data presented here may have direct implications

for human patients, for whom there is negligible longitudinal information on the development of antibody responses during the acute and chronic stages of a schistosome infection. Our results in control chimpanzees most likely mirror the events that occur after schistosome infection of persons living in endemic areas (in the absence of chemotherapeutic intervention) and therefore provide a vital and detailed reference for the interpretation of data from epidemiologic field studies. There is a need to expand our knowledge of the structure and diversity of schistosome larval and egg glycans if we are to understand fully the complex interactions of the schistosome parasite with the host's immune system and its ability to subvert protective mechanisms.

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