INFECTION AND IMMUNITY, Oct. 1995, p. 3980–3986 Vol. 63, No. 10
0019-9567/95/$04.00+0

T-Lymphocyte Responsiveness in Murine Schistosomiasis Mansoni Is Dependent upon the Adhesion Molecules Intercellular Adhesion Molecule-1, Lymphocyte Function-Associated Antigen-1, and Very Late Antigen-4

JANE G. LANGLEY* AND DOV L. BOROS

Department of Immunology and Microbiology, Wayne State University School of Medicine, Detroit, Michigan 48201

Received 22 March 1995/Returned for modification 16 May 1995/Accepted 7 July 1995

Granuloma formation in murine schistosomiasis is dependent on $CD4^+$ Th lymphocytes and requires recruitment and accumulation of inflammatory cells at the site of egg deposition. The present study examined the role of three adhesion molecules, intercellular adhesion molecule-1 (ICAM-1), lymphocyte function-associated antigen-1 (LFA-1), and very late antigen-4 (VLA-4), that participate in cellular recruitment, interaction, and lymphocyte activation during in vitro activation of acutely and chronically infected spleen and liver granuloma lymphocytes. Blockade of ICAM-1, LFA-1, or VLA-4 by rat monoclonal antibody inhibited spleen and granuloma lymphocyte interleukin-2 (IL-2) and IL-4 production as well as lymphoproliferative responses at similar levels (66 to 87%). The down-modulated cytokine and proliferative responses of chronically infected lymphocytes were inhibited to the same extent as their acutely infected counterparts. Cell sorting analysis demonstrated that acutely and chronically infected splenic and granuloma lymphocytes expressed similar levels of LFA-1, ICAM-1, and VLA-4 and that more ICAM-1 was expressed on infected than on uninfected mouse lymphocytes. By exposure of cells to paired monoclonal antibodies at suboptimal doses, it was determined that whereas all three adhesion molecules may participate, only ICAM-1 and LFA-1 showed synergistic interactions in determining lymphocyte responsiveness. These data suggest that spleen and liver granuloma lymphocytes are equally well armed with functional adhesion receptors. Thus, ICAM-1, LFA-1, and VLA-4 play an important accessory role in inflammatory cytokine production and lymphocyte proliferation, and therefore these adhesion molecules may participate in the initiation and maintenance of the granulomatous inflammation.

Schistosomiasis mansoni is a helminthic disease of humans which affects more than 100 million people throughout the tropics. In the infected host, disease is characterized by the presence of granulomas, the immunopathology resulting from cellular infiltration and residual fibrosis surrounding trapped eggs (4, 23, 30). Murine schistosomiasis has many of the characteristics of the human disease, including liver pathology and portal hypertension (4). Infected mice exhibit two distinct immunologic phases. The acute phase, seen 8 weeks following infection, is characterized by vigorous granulomatous and delayed-type hypersensitivity responses, with large amounts of interleukin-2 (IL-2) and IL-4 being produced. In the chronic phase, 16 to 20 weeks postinfection, there is a marked down-regulation of granulomatous inflammation, with lower lymphoproliferative responses and diminished cytokine production (3–5, 8, 15, 22, 24).

The granulomatous process in schistosomiasis is dependent on $CD4^+$ Th lymphocytes and requires the recruitment and migration of circulating inflammatory cells to the site of egg deposition (10, 21). The molecular mechanisms underlying the regulation of cellular immune interactions remain obscure, although it is known that accumulation of inflammatory cells and potentiation of the immunological response require several interdependent processes, such as cell-cell and cell-extracellular matrix (ECM) adhesion, cell signalling, and cytokine secretion (19, 31).

Among the molecules mediating adhesive cellular events are lymphocyte function-associated antigen-1 (LFA-1), found exclusively on leukocytes, and its ligand, intercellular adhesion molecule-1 (ICAM-1), which has a more general cellular distribution on a variety of hematopoietic and nonhematopoietic cells, including lymphocytes, granulocytes, and endothelia (19). ICAM-1 and LFA-1 play key roles in cell migratory events, and together are partially responsible for the adhesion of lymphocytes, monocytes, and neutrophils to endothelial cells (11, 19, 31). Recent work has demonstrated an association between ICAM-1 expression and granuloma formation in schistosome-infected mice (17, 25); it is likely that both ICAM-1 and LFA-1 are crucial in the maintenance of granulomatous cell interactions in schistosomiasis mansoni.

Recent studies in our laboratory demonstrated a costimulatory role for the ECM protein fibronectin in cytokine secretion by splenocytes and granuloma lymphocytes of *Schistosoma mansoni*-infected mice (36). Fibronectin is present in large amounts in the granulomatous liver (1, 13, 33, 34) and is a ligand for the β1 integrins very late antigen-4 (VLA-4) and VLA-5 (9, 27–29). Both VLA-4 and VLA-5 mediate cell-ECM binding; surface expression of these adhesion molecules is higher on activated and memory T cells (27–29). Unlike VLA-5, VLA-4 has an additional and unique role in adhesive cellular events, as it mediates, through different ligands, both cell-ECM and cell-cell binding (12).

* Corresponding author. Mailing address: Department of Immunology and Microbiology, Wayne State University School of Medicine, 540 E. Canfield, Detroit, MI 48201. Phone: (313) 577-1404. Fax: (313) 577-1155.

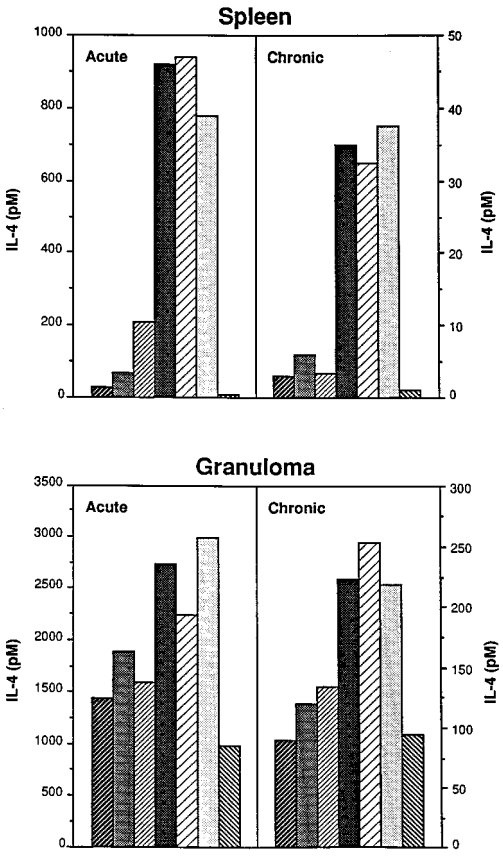

FIG. 1. Inhibition of IL-4 production by MAb to adhesion molecules. Supernatants were collected after 24 h of incubation of spleen and granuloma lymphoid cells from acutely and chronically infected mice with SEA in the presence of (left to right) anti-murine LFA-1, ICAM-1, or VLA-4 MAb and assayed for the presence of IL-4; rat IgG2a, IgG2b, SEA alone, and medium alone were included as controls. Data are representative of at least three experiments. In each experiment, spleen or granuloma lymphocytes were pooled from at least three mice.

In the study presented here, using monoclonal antibodies (MAb) directed against murine LFA-1, ICAM-1, and VLA-4, we measured three parameters, antigen-stimulated cytokine production, lymphoproliferative responses, and adhesion molecule expression, to examine in vitro the role that such molecules play in determining the immune responsiveness of spleen and liver granuloma T lymphocytes from *S. mansoni*-infected mice.

MATERIALS AND METHODS

Animals and parasites. Female CBA/J^k mice (Jackson Laboratories, Bar Harbor, Maine), 6 to 8 weeks old, were used throughout the study. Mice were infected subcutaneously with 25 cercariae of a Puerto Rican isolate of *S. mansoni.*

Antigen. Soluble egg antigen (SEA) was prepared from homogenized *S. mansoni* eggs harvested from mice infected subcutaneously with 200 cercariae, as previously described (6).

Antibodies. Rat MAb to murine adhesion molecules ICAM-1 (α-CD54: clone YN1/1.7.4), LFA-1 (α-CD11a: clone M17/5.2), and VLA-4 (α-CD49d: clone R1-2) were produced from hybridomas purchased from the American Type Culture Collection (Rockville, Md.). Hybridoma cells were injected intraperitoneally into Freund's incomplete adjuvant-primed CBA/J mice; ascites fluid was collected after 7 to 10 days, precipitated twice with 50% saturated ammonium sulfate, and dialyzed extensively against phosphate-buffered saline (PBS). The dissolved and dialyzed precipitate was purified over a protein G column (Pierce, Rockford, Ill.) and checked for purity by polyacrylamide gel electrophoresis (PAGE). In some in vitro studies, rat anti-ICAM-1 and LFA-1 MAb, purchased from Pharmingen (San Diego, Calif.), and rat anti-VLA-4, obtained from Endogen (Boston, Mass.), were used. Isotype-matched immunoglobulin G (IgG) antibodies (Serotec, Oxford, United Kingdom) were included as controls. Antibodies used for fluorescence-activated cell sorting (FACS) analysis were purchased from Pharmingen (anti-CD11a, anti-CD49d, and anti-CD54) or Gibco Life Technologies, Grand Island, N.Y. (anti-CD4).

Cell preparations. Granuloma and spleen cells were isolated from mice by established protocols (21, 24). Briefly, mice were killed by cervical dislocation, and the spleens and livers were removed under aseptic conditions. Spleen cell suspensions were washed, and erythrocytes were lysed before being resuspended in RPMI 1640 supplemented with 10% fetal calf serum (FCS), 100 U of penicillin per ml, 100 μg of streptomycin per ml, 2×10^{-5} M 2-β-mercaptoethanol, 20 mM HEPES (*N*-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid), 2 mM sodium pyruvate, and 1 mM glutamine. Liver granuloma cells were dispersed by incubation of granulomas in a shaker bath at 37°C in 0.2% collagenase type IV (Sigma, St. Louis, Mo.) and mechanical disruption. Liver granulomas from acutely infected mice were incubated for 35 min, whereas those of chronically infected mice were incubated for 50 min. Dispersed granuloma cells were filtered through gauze, washed, and poured over a glass wool column. Nonadherent cells were resuspended in supplemented RPMI. Macrophages were removed by adherence to plastic tissue culture plates during a 90-min incubation at 37°C. Macrophage numbers were reduced by 85 to 95% following incubation. Nonadherent cells, comprising predominantly eosinophils and ca. 15 to 20% lymphocytes, were washed and resuspended in fresh supplemented medium. For each experiment, single-cell spleen or granuloma suspensions were prepared from at least three mice.

Cytokine production and measurement. Supernatants for IL-2 and IL-4 cytokine determination were generated by 18 to 24 h of culture of 3×10^6 cells with 10 μg of SEA in a 1-ml volume of supplemented RPMI. In some cases, supernatants in which MAb to LFA-1 (at 1.6 μg/ml), ICAM-1 (at 12.5 μg/ml), or

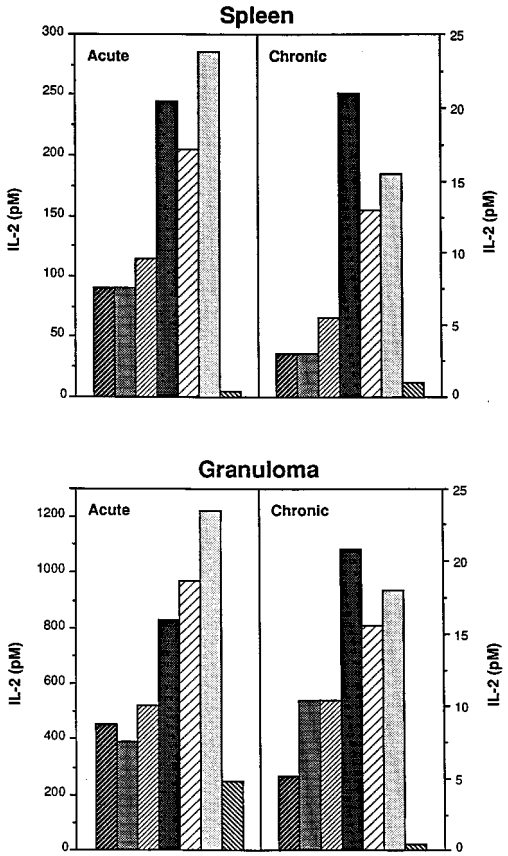

FIG. 2. Inhibition of IL-2 production by MAb to adhesion molecules. Supernatants were collected after 24 h of incubation of spleen and granuloma lymphoid cells from acutely and chronically infected mice with SEA in the presence of (left to right) anti-murine LFA-1, ICAM-1, or VLA-4 MAb and assayed for the presence of IL-2; rat IgG2a, IgG2b, SEA alone, and medium alone were included as controls. Data are representative of at least three experiments. In each experiment, spleen or granuloma lymphocytes were pooled from at least three mice.

VLA-4 (at 25.0 μg/ml) were cocultured with cells and SEA were generated. Supernatants were stored at −70°C until assayed for the presence of IL-2 and IL-4.

The levels of IL-2 secreted by spleen and granuloma cells were determined by using the IL-2-dependent cell line CTLL-20, originally provided by Frank Fitch (University of Chicago, Chicago, Ill.). Supernatants were serially diluted in triplicate with supplemented RPMI in round-bottomed plates (Corning Glass Works, Corning, N.Y.). Washed CTLL cells were added to serially diluted supernatant in each well, and the plates were incubated for 24 h. Cells were pulsed with [^{3}H]thymidine for the final 6 h of culture. The amount of IL-2 in each supernatant was determined by comparison with levels of thymidine incorporation detected in cells cultured with serial dilutions of recombinant IL-2 (kindly provided by Cetus Corporation, Emeryville, Calif.). The specificity of the CTLL cell response to IL-2 was confirmed by the almost complete abrogation (85 to 95%) of IL-2 proliferative responses by anti-IL-2 MAb (clone S4B6), kindly provided by DNAX Corporation, Palo Alto, Calif. Additionally, CTLL cells were shown to have negligible responses to extremely high levels of IL-4 (up to 5,000 pM).

IL-4 levels in supernatants were determined in similar fashion with the CT4S IL-4-dependent cell line (kindly provided by William Paul, National Institutes of Health, Bethesda, Md.), maintained in supplemented RPMI containing recombinant IL-4 (generously provided by Immunex Corporation, Seattle, Wash.). The proliferative responses of CT4S cells cultured with serially diluted supernatants were compared against a standard curve generated with serial dilutions of recombinant IL-4. CT4S cells were incubated for 48 h, with [^{3}H]thymidine incorporation being measured over the final 24 h of culture. An anti-IL-4 MAb (clone 11B11), provided by William Paul, confirmed that CT4S proliferative responses were absolutely dependent on the presence of IL-4.

Compared with isotype controls, antibodies to adhesion molecules had no effect on the ability of either CTLL or CT4S cells to respond to the presence of IL-2 or IL-4, respectively (results not shown).

Proliferation studies. The proliferative responses of spleen and collagenase-dispersed liver granuloma cells taken from mice with acute or chronic schistosomiasis were determined by culture of 10^6 cells per ml with 10 μg of SEA per ml in 200-μl volumes of supplemented RPMI. All assays were performed in triplicate. In some experiments, cells were also cocultured with MAb to adhesion molecules at antibody concentrations of 1.6 μg of anti-LFA-1, 12.5 μg of anti-ICAM-1, or 25.0 μg of anti-VLA-4 per ml, which had been determined previously as optimal. Isotype-matched rat IgG antibodies were used as controls. Proliferative responses were determined by uptake of 0.5 μCi of [^{3}H]thymidine during the final 6 h of a 96-h incubation.

FACS analysis. The distribution of ICAM-1, LFA-1, and VLA-4 on acutely and chronically infected spleen or granuloma CD4$^+$ lymphocytes was determined by two-color staining with MAb directed against adhesion molecules and against CD4. Briefly, cells were incubated on ice with biotin-conjugated anti-CD4 MAb, washed, and stained with either a streptavidin-fluorescein isothiocyanate (FITC) conjugate (Gibco Life Technologies) or a streptavidin-phycoerythrin (PE) conjugate. FITC-stained cells were washed and counterstained with PE-conjugated anti-ICAM-1 or anti-VLA-4 MAb, and PE-stained cells were counterstained with FITC-conjugated anti-LFA-1 MAb. Isotype-matched controls were used to confirm the specificity of each MAb. Stained cells were fixed with 1% paraformaldehyde and analyzed on a FACScan (Becton Dickinson, Mountain View, Calif.) with Lysis II software (Becton Dickinson). Cell preparations were gated around the lymphocyte-containing population based on forward scatter/side scatter characteristics.

Statistical analysis. Statistical analysis of our data was done with the unpaired Student's *t* test.

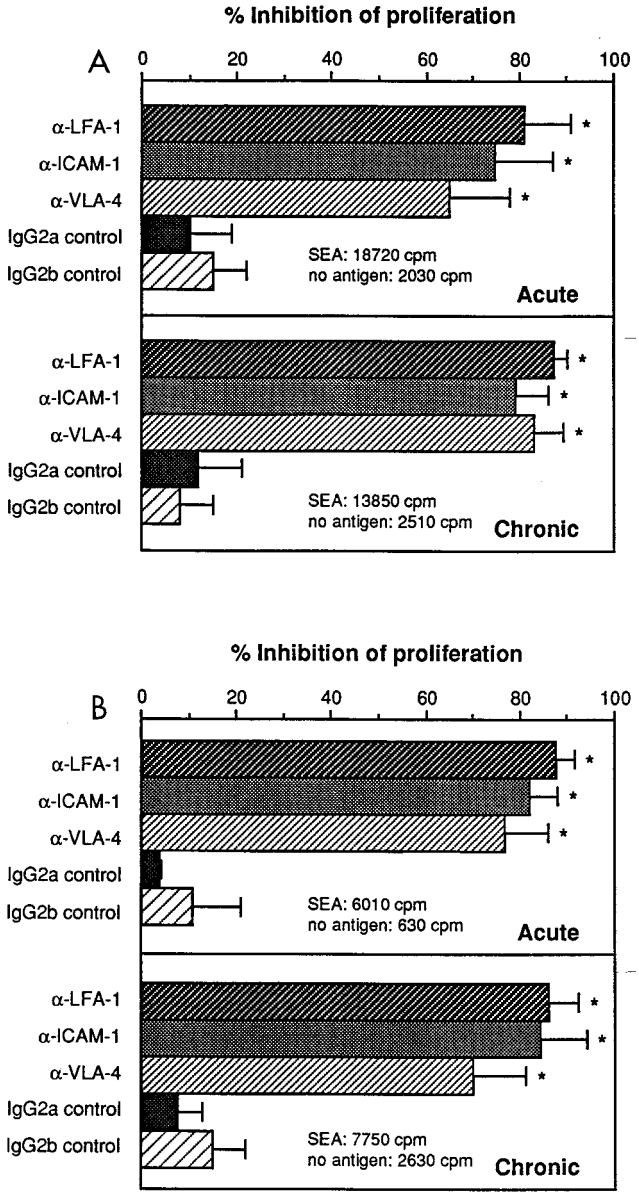

FIG. 3. Inhibition of lymphoproliferation by MAb to adhesion molecules. Spleen (A) and granuloma (B) lymphoid cells from acutely and chronically *S. mansoni*-infected mice were cultured with 10 μg of SEA per ml in the presence or absence of anti-murine VLA-4, LFA-1, or ICAM-1 MAb. Results are expressed as the mean + SEM of three experiments. In each experiment, spleen or granuloma lymphocytes were pooled from at least three mice.

RESULTS

Cytokine production is inhibited by blocking adhesion molecule interactions. To examine whether adhesion molecules were necessary for antigen-dependent cytokine production, spleen and granuloma lymphoid cells were stimulated with SEA in the presence or absence of MAb to murine adhesion molecules ICAM-1, LFA-1, and VLA-4 and assayed for IL-4 and IL-2 activity. Figure 1 shows the effect of antiadhesion MAb on IL-4 secretion by spleen and granuloma cells. As can be seen for acutely infected spleen cell cultures, all three MAb were effective ($P < 0.05$), although the anti-LFA-1 MAb achieved the best result in diminishing IL-4 production. In cultures of chronically infected cells, all three MAb were equally effective in reducing IL-4 production. Unlike splenic lymphocytes, unstimulated granuloma lymphocytes spontaneously secreted substantial levels of IL-4. This spontaneous production could not be reduced by blockade with any of the anti-adhesion molecule MAb (results not shown). Granuloma cells stimulated with SEA also secreted substantially more cytokine than splenic lymphocytes. Antiadhesion MAb equally and significantly ($P < 0.05$) inhibited IL-4 production in both acutely and chronically infected granuloma lymphocyte cultures, although the degree of inhibition appeared to be less than that seen in spleen cell cultures.

Figure 2 shows the effect of antiadhesion MAb on production of IL-2 by splenic and granuloma lymphocytes. All three MAb equally and significantly ($P < 0.05$) diminished IL-2 cytokine production by acutely and chronically infected splenic and granuloma lymphocytes. Unlike chronically infected cells, unstimulated acutely infected granuloma lymphocytes from mice also spontaneously secreted IL-2. While lymphoid cells

from chronically infected mice showed 10- to 50-fold less IL-4 and IL-2 production than their acutely infected counterparts, the percent inhibition attained by the three anti-adhesion molecule MAb was virtually identical.

Adhesion molecules are necessary for antigen-specific proliferation of spleen and granuloma lymphocytes. As cytokine production is an early event in lymphocyte activation, it was of interest to ascertain whether soluble anti-adhesion molecule MAb would also act to inhibit the ensuing proliferative responses. Splenic lymphocytes from acutely and chronically infected mice cocultured with SEA and MAb to ICAM-1, LFA-1, or VLA-4 showed substantial and significant ($P < 0.05$) reductions in proliferative responsiveness compared with cells cultured with isotype control MAb (Fig. 3). The IgG2a and IgG2b control MAb caused minimal inhibition of proliferative responses. Acutely and chronically infected granuloma cell proliferation was inhibited by the three MAb at levels similar to spleen lymphocyte inhibition. No difference was seen in the level of inhibition provided by the three different anti-adhesion molecule MAb. The immunoreactivity of spleen and granuloma cells from chronically infected animals was found to be generally considerably less than that of acutely infected cells, as has been shown in previous work (5, 8, 22, 24, 35). The reduced proliferative reactivity of chronically infected cells was not concomitant with reduced susceptibility to anti-ICAM-1, -LFA-1, and -VLA-4 MAb-mediated inhibition of SEA-induced responsiveness, as these cells showed levels of inhibition very similar to those of the more vigorously responding acutely infected cells.

Synergistic interactions between blocking anti-adhesion molecule antibodies. As adhesion molecules require a receptor for effect and do not usually interact in isolation of other receptors and counterreceptors, it was of interest to determine whether a combination was more effective in mediating activation events than any single molecule. Dilutions of anti-LFA-1, ICAM-1, and VLA-4 MAb, starting at the optimal concentrations (1.6, 12.5, and 25 μg/ml, respectively) and then at 1, 2, 5, and 10% of these concentrations, were cultured with acutely infected splenic lymphocytes (Fig. 4A). Antibodies were added either separately or in combination at each concentration. Apart from anti-ICAM-1, which at suboptimal concentrations caused a slight diminution of the stimulation index, anti-LFA-1 and anti-VLA-4 MAb mixed with isotype control globulins did not inhibit SEA-induced proliferation. Blockade of LFA-1 and ICAM-1 by a mixture of antiadhesion MAb (1 and 5% of the optimal concentrations, respectively) significantly enhanced inhibition ($P < 0.05$) of the proliferative responses (Fig. 4B); blockade of ICAM-1 and VLA-4 or LFA-1 and VLA-4 by mixed antiadhesion MAb at suboptimal concentrations was never found to have a significant inhibitory effect on spleen lymphocyte proliferation. Even when antibody pairs were used at maximal inhibitory concentrations, we were not able to completely block proliferative responses (results not shown).

Surface expression of adhesion molecules on CD4$^+$ T cells during the course of infection with *S. mansoni*. Because blockade by MAb was equally effective in inhibiting the proliferative responses of acutely and chronically infected lymphocytes, it was of interest to determine whether changes in the surface expression of LFA-1, VLA-4, and ICAM-1 occur during the course of infection. All CD4$^+$ spleen cells from uninfected and infected mice expressed LFA-1 at equally high levels. Within the lymphocyte-containing population, there was a marked increase in expression of ICAM-1, from 8 to 22%, on spleen CD4$^+$ T lymphocytes from infected animals versus uninfected ones (Fig. 5). The percentage of gated cells displaying ICAM-1

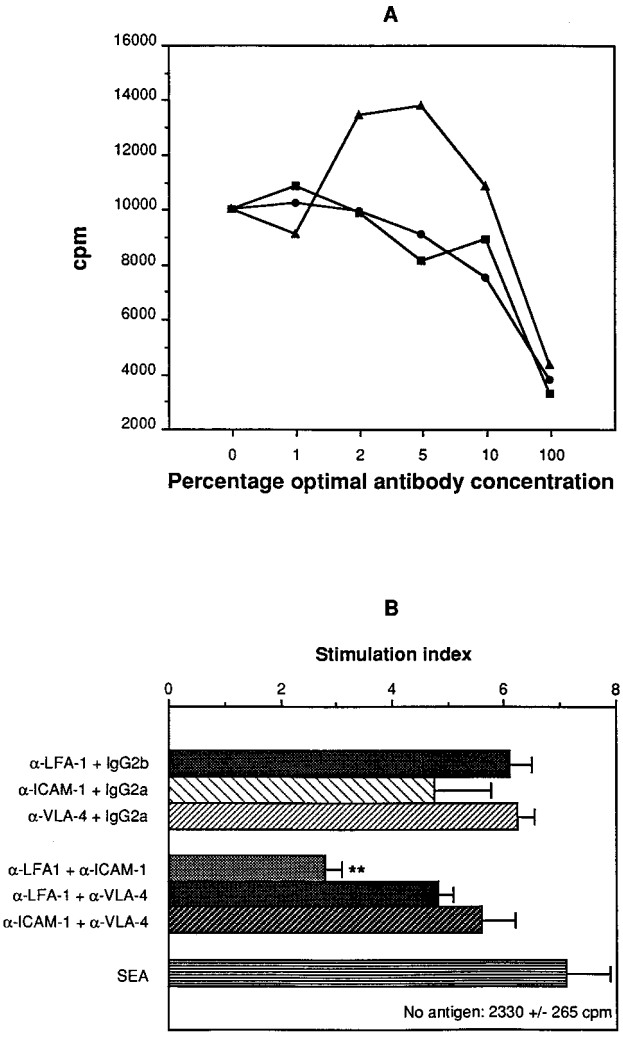

FIG. 4. (A) Titration of anti-adhesion molecule antibody inhibition of lymphoproliferative responses. Splenocytes from acutely infected mice were cultured with SEA (10 μg/ml) and dilutions of anti-LFA-1 (●), ICAM-1 (■), and VLA-4 (▲) MAb, starting at 1.6, 12.5, and 25 μg/ml, respectively. Data are representative of three experiments. In each experiment, spleen lymphocytes were pooled from at least three mice. (B) Synergism between antiadhesion MAb in the inhibition of lymphoproliferative responses. Splenic lymphoid cells from acutely infected mice were cultured with SEA (10 μg/ml) and combinations of suboptimal levels of anti-murine LFA-1 (0.016 μg/ml), ICAM-1 (0.63 μg/ml), and VLA-4 (2.5 μg/ml) MAb. Results are expressed as the mean stimulation index + SEM of three experiments. In each experiment, spleen lymphocytes were pooled from at least three mice. **, $P < 0.05$.

and CD4 showed no difference between acutely and chronically infected animals. There was little change in the expression of VLA-4 on CD4$^+$ spleen cells between infected and uninfected mice. A population of cells lacking both CD4 and VLA-4 receptors was present in the lymphocyte-containing population of chronically infected spleen cells which was absent in spleens from acutely infected animals.

Whereas the T-lymphocyte-containing population of acutely infected spleen cells contained significant proportion of CD4$^-$ cells (66%), most of the nonadherent cells in acutely infected granulomata (58%) were found to be CD4$^+$ T cells (Fig. 6). Unlike acute-infection lesions, chronic-infection granulomata did contain a clearly identifiable, albeit small, CD4$^-$ population (22%). Expression of ICAM-1 on granuloma-derived

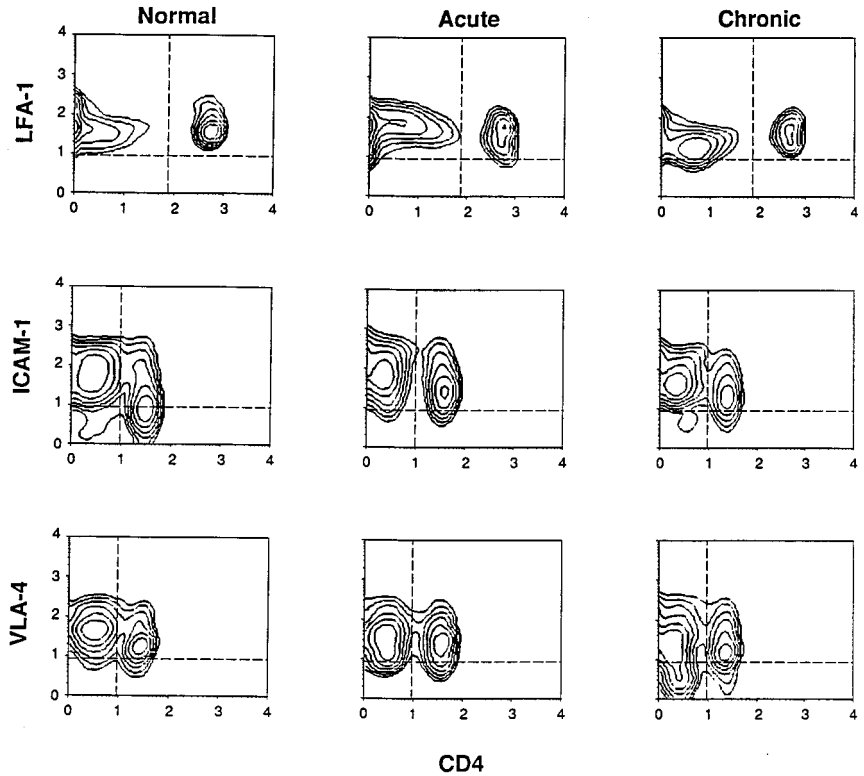

FIG. 5. LFA-1, ICAM-1, and VLA-4 expression on $CD4^+$ splenocytes from normal mice and mice with acute or chronic schistosomiasis. Cells were stained with anti-CD4–PE and anti-LFA-1–FITC or with anti-CD4–FITC and either anti-ICAM-1–PE or anti-VLA-4–PE, and the lymphocyte-containing population was analyzed by flow cytometry. The scale is $\log_{10}$ fluorescence intensity.

$CD4^+$ cells, like $CD4^+$ splenocytes, was elevated compared with that on normal spleen cells. There were no apparent differences in the levels of expression of LFA-1, ICAM-1, or VLA-4 between acute- and chronic-infection granuloma-derived cells.

DISCUSSION

In recent years it has become apparent that cellular adhesive interactions are obligatory for the evolution of an immunologic response (19, 31). Modulation of adhesive events becomes important in determining the extent of diseases, such as schistosomiasis, in which the immune granulomatous inflammatory response itself causes pathology (4, 23, 30). Initiation and maintenance of the granulomatous reactions characteristic of *S. mansoni* infections require recruitment and accumulation of inflammatory cells around parasite eggs trapped in the liver and intestines. The inflammatory cells are not only in close apposition within the granuloma (1, 13), but are also embedded in the ECM. Consequently, substantial quantities of newly deposited ECM proteins such as collagen, elastin, and fibronectin are present in the diseased liver. Granulomatous immunopathology in schistosomiasis mansoni will therefore be defined by interactions among infiltrating cells and among these cells and the ECM.

Consistent with previous data (8, 16, 35), we report here that SEA-stimulated splenocytes and liver granuloma lymphocytes from mice with acute schistosomiasis exhibited vigorous proliferative responses and secreted substantial amounts of IL-2 and IL-4, whereas the downmodulated chronically infected spleen and granuloma cells showed diminished levels of lymphocyte activity. Recently, anti-ICAM-1 MAb has been shown to decrease SEA-specific proliferation of splenocytes from egg-sensitized mice (17). We confirm and extend those observations by showing that regardless of the immunologic state of the animal, blockade by MAb directed against LFA-1, ICAM-1, or VLA-4 of either spleen or granuloma cells substantially reduced antigen-specific cytokine production and proliferative responses. In agreement with other studies (14, 18, 20), we attribute diminished responsiveness to a blockade of LFA-1, ICAM-1, and VLA-4 binding, which is essential for lymphocyte-antigen-presenting cell interactions, lymphocyte traffic, and activation. Although the lymphocyte responsiveness of chronically infected cells was substantially lower than that of acutely infected lymphocytes, the percent reduction in antigen-specific responsiveness following blockade by anti-LFA-1, -ICAM-1, and -VLA-4 MAb was almost identical. Judging from the high percentage of inhibition achieved and the lack of difference in inhibitory effects on spleen and granuloma lymphocytes, it would appear that cells from both spleen and liver granulomas were equally well armed with functional adhesion receptors, even though one is a peripheral lymphoid organ and the other is inflammatory tissue derived.

The lack of difference in the inhibition by anti-adhesion molecule MAb between acute- and chronic-infection lymphocyte responses was in agreement with the FACS observations (Fig. 5 and 6). The two-color display examining adhesion molecule expression on $CD4^+$ T cells confirmed similar levels of LFA-1, ICAM-1, and VLA-4 adhesion molecule expression on acute- and chronic-infection Th lymphocytes. Increased ICAM-1 membrane expression on $CD4^+$ and other granuloma cells is consonant with the recently observed increased steady-state expression of ICAM-1 mRNA

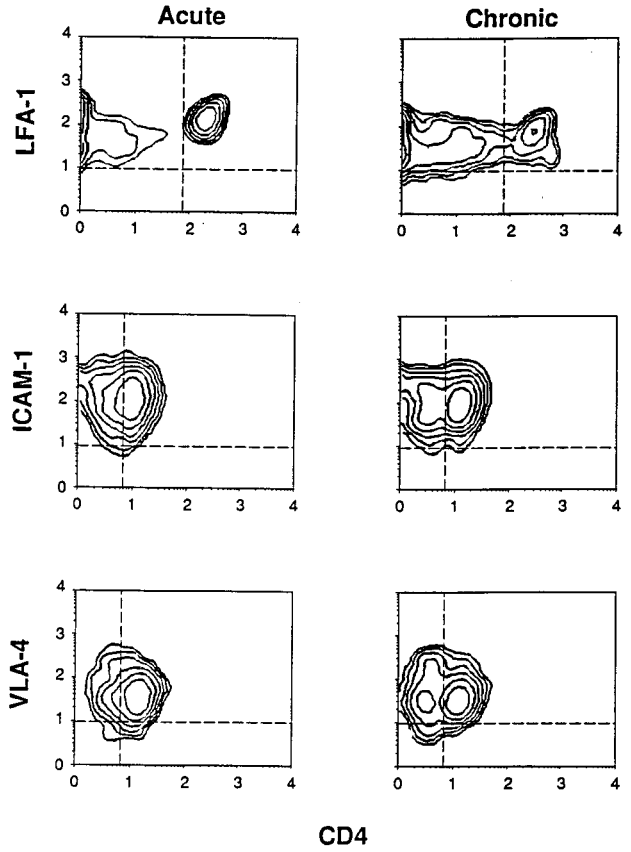

FIG. 6. LFA-1, ICAM-1, and VLA-4 expression on $CD4^+$ granuloma cells from mice with acute or chronic schistosomiasis. Cells were stained with anti-CD4–PE and anti-LFA-1–FITC or with anti-CD4–FITC and either anti-ICAM-1–PE or anti-VLA-4–PE, and the lymphocyte-containing population was analyzed by flow cytometry. The scale is $\log_{10}$ fluorescence intensity.

in cells of the primary lung granuloma (17). Although the level of membrane adhesion molecule display appears to be similar, it remains to be seen whether receptors on chronic-infection cells exhibit less avidity than their acute-infection counterparts.

Adhesion molecules not only promote intercellular contact through interactions with their natural ligands, but also provide accessory signals for T-cell proliferation (31). Working with suboptimal concentrations, we were able to demonstrate synergism between the action of MAb directed against LFA-1 and ICAM-1 in the inhibition of antigen-induced lymphoproliferative responses, whereas combinations of MAb specific for LFA-1 and VLA-4 or ICAM-1 and VLA-4 failed to affect proliferation. As we used whole antibodies, it was not possible to conclude whether the inhibitory effect observed was the result of direct blocking of cell-cell interaction or whether antibody-triggered receptor signalling contributed to lymphocyte unresponsiveness. The blocking experiments do demonstrate, however, that the most important adhesion molecule synergism occurs between the LFA-1 and ICAM-1 membrane receptors. Interestingly, even at maximal inhibitory concentrations, simultaneous blockade of LFA-1 and ICAM-1 failed to completely inhibit proliferation, which may be evidence for the involvement of other receptors in lymphoproliferative responses.

The results of the present study are in agreement with previous observations on the role of adhesion molecules in antigen-specific lymphocyte activation in various models (2, 7, 14, 18, 20, 28). They underscore the importance of these receptors in the granuloma inflammatory response of schistosome-infected animals. Compared with normal splenic cells, significantly more cells from the spleens of infected mice displayed ICAM-1, indicating their state of activation. It is noteworthy that blockade of VLA-4 very effectively inhibited SEA-driven T-cell activation and cytokine production, albeit at higher antibody concentrations. Thus, VLA-4 not only plays an important role in cell-ECM but, along with ICAM-1 and LFA-1, also has a role in T-cell–antigen-presenting cell interactions. In the present study, three different adhesion molecules were found to play an important accessory role in inflammatory IL-2 and IL-4 production as well as in lymphocyte proliferation. Therefore, regardless of the stage of the disease (acute or chronic), these adhesion molecules appear to participate in the initiation and maintenance of the granulomatous inflammation. The molecules should promote transit of cells from blood vessels to tissues, cell-cell adhesion, cell-ECM interaction, and intracellular signalling of lymphocytes. The equal display of adhesion molecules on acute and chronic cells presently observed does not account for the downmodulated proliferative and cytokine production responses of chronically infected mice. However, the inability of lymphocytes to increase adhesion molecule avidity after antigenic stimulus may account for a diminished responsiveness (31). Moreover, shed soluble adhesion molecules, such as soluble ICAM-1, capable of modulating antigenic responsiveness, possibly by blockade of costimulatory activity (26), have been found to circulate in the blood of patients with hepatosplenic schistosomiasis. Further experiments to explore the significance of adhesion molecules in alleviating egg-induced granulomatous response and pathology are under way.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant AI-12913 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Md.

REFERENCES

1. **Al Adnani, M. S.** 1985. Concomitant immunohistochemical localization of fibronectin and collagen in schistosome granulomata. J. Pathol. **147:**77–85.
2. **Böhmig, G. A., J. Kovarik, W. Holter, E. Pohanka, and G. J. Zlabinger.** 1994. Specific down-regulation of proliferative T cell alloresponsiveness by interferences with CD2/LFA-3 and LFA-1/ICAM-1 in vitro. J. Immunol. **152:** 3720–3728.
3. **Boros, D. L.** 1986. Immunoregulation of granuloma formation in murine schistosomiasis mansoni. Ann. N. Y. Acad. Sci. **465:**313–323.
4. **Boros, D. L.** 1989. Immunopathology of *Schistosoma mansoni* infection. Clin. Microbiol. Rev. **2:**250–269.
5. **Boros, D. L., R. P. Pelley, and K. S. Warren.** 1975. Spontaneous modulation of granulomatous hypersensitivity in schistosomiasis mansoni. J. Immunol. **114:**1437–1441.
6. **Boros, D. L., and K. S. Warren.** 1970. Delayed hypersensitivity granuloma formation and dermal reaction induced and elicited by a soluble factor isolated from *Schistosoma mansoni* eggs. J. Exp. Med. **132:**448–507.
7. **Carrera, A. C., M. Rincón, F. Sánchez-Madrid, M. López-Botet, and M. O. de Landázuri.** 1988. Triggering of co-mitogenic signals in T cell proliferation by anti-LFA-1 (CD24, CD11a). LFA-3 and CD7 monoclonal antibodies. J. Immunol. **141:**1919–1924.
8. **Colley, D. G.** 1975. Immune responses to a soluble egg antigen preparation during chronic primary infection with *Schistosoma mansoni*. J. Immunol. **115:**150–156.
9. **Davis, L. S., J. L. Oppenheimer-Marks, B. Bednarczyk, W. McIntyre, and P. E. Lipsky.** 1990. Fibronectin promotes proliferation of naive and memory T cells by signaling through both the VLA-4 and VLA-5 integrin molecules. J. Immunol. **145:**785–793.
10. **Doughty, B. L., and S. M. Phillips.** 1982. Delayed hypersensitivity granuloma formation around *Schistosoma mansoni* eggs in vitro. I. Definition of the model. J. Immunol. **128:**30–36.
11. **Dustin, M. L., and T. A. Springer.** 1988. Lymphocyte function associated antigen-1 (LFA-1) interactions with intercellular adhesion molecules-1

(ICAM-1) is one of at least three mechanisms for T lymphocyte adhesion to cultured endothelial cells. J. Cell Biol. **107:**321–331.
12. **Elices, M. J., L. Osborn, Y. Takeda, C. Crouse, S. Luhowskl, M. E. Hemler, and R. R. Lobb.** 1990. VCAM-1 on activated endothelium interacts with leukocyte integrin VLA-4 at a site distinct from the VLA-4/fibronectin binding site. Cell **60:**577–584.
13. **Grimaud, J. A., D. L. Boros, C. Takiya, R. C. Mathew, and H. Emonard.** 1987. Collagen isotypes, laminin and fibronectin in granuloma of the liver and intestines of *Schistosoma mansoni* infected mice. Am. J. Trop. Med. Hyg. **37:**335–344.
14. **Gronberg, A., E. Halpi, M. Ferm, M. Peterson, and M. Patarroyo.** 1993. Regulation of lymphocytic aggregation and proliferation through adhesion molecule CD54 (ICAM-1). Cell. Immunol. **147:**12–24.
15. **Grzych, J.-M., E. Pearce, A. Cheever, Z. A. Caulder, P. Caspar, S. Heiny, F. Lewis, and A. Sher.** 1991. Egg deposition is the major stimulus for the production of Th2 cytokines in murine schistosomiasis mansoni. J. Immunol. **146:**1322–1327.
16. **Lukacs, N. W., and D. L. Boros.** 1992. Utilization of fractionated soluble egg antigens reveals selectively modulated granulomatous and lymphokine responses during murine schistosomiasis mansoni. Infect. Immun. **60:**3209–3216.
17. **Lukacs, N. W., S. W. Chensue, R. M. Strieter, K. Warmington, and S. L. Kunkel.** 1994. Inflammatory granuloma formation is mediated by TNF-α-inducible intercellular adhesion molecule-1. J. Immunol. **152:**5883–5889.
18. **Lukacs, N. W., S. L. Kunkel, M. D. Burdick, and R. M. Strieter.** 1993. The production of chemotactic cytokines in allogeneic responses: the role of intercellular adhesion molecule-1 and lymphocyte function-associated antigen-3. Am. J. Pathol. **143:**1179–1188.
19. **Makgoba, M. W., M. E. Sanders, and S. Shaw.** 1989. The CD2-LFA-3 and LFA-1-ICAM-1 pathways: relevance to T cell recognition. Immunol. Today **10:**417–422.
20. **Marakovsky, E., A. B. Troutt, and A. Kelso.** 1992. Co-engagement of CD3 with LFA-1 or ICAM-1 adhesion molecules enhances the frequency of activation of single murine $CD4^+$ and $CD8^+$ T cells and induces synthesis of IL-3 and IFNγ but not IL-4 or IL-6. Int. Immunol. **4:**475–485.
21. **Mathew, R. C., and D. L. Boros.** 1986. Anti-L3T4 antibody treatment suppresses hepatic granuloma formation and abrogates antigen-induced interleukin-2 production in *Schistosoma mansoni* infection. Infect. Immun. **54:**820–826.
22. **Mathew, R. C., S. Ragheb, and D. L. Boros.** 1990. Recombinant interleukin 2 therapy reverses diminished granulomatous responsiveness in anti-L3T4 treated, *S. mansoni*-infected mice. J. Immunol. **144:**4356–4361.
23. **Phillips, S. M., and P. Lammie.** 1986. Immunopathology of granuloma formation and fibrosis in schistosomiasis mansoni. Parasitol. Today **2:**296–302.
24. **Ragheb, S., and D. L. Boros.** 1989. Characterization of granuloma T lymphocyte function from *Schistosoma mansoni*-infected mice. J. Immunol. **142:**3239–3246.
25. **Ritter, D. M., S. Rosen, and J. H. McKerrow.** 1994. Induction of ICAM-1 expression by products of the deposited egg of *Schistosoma mansoni*, abstr. 1342, p. A232. Abstr. Exp. Biol. '94.
26. **Secor, W. E., M. G. dos Reis, E. A. G. Ramos, E. P. Matos, E. A. G. Reis, T. M. A. do Carmo, and D. A. Harn, Jr.** 1994. Soluble intercellular adhesion molecules in human schistosomiasis: correlations with disease severity and decreased responsiveness to egg antigens. Infect. Immun. **62:**2695–2701.
27. **Shimizu, Y., and S. Shaw.** 1991. Lymphocyte interactions with extracellular matrix. FASEB J. **5:**2292–2299.
28. **Shimizu, Y., G. A. van Seventer, K. J. Horgan, and S. Shaw.** 1990. Regulated expression and binding of the three VLA (β1) integrins on T cells. Nature (London) **345:**250–253.
29. **Shimizu, Y., G. A. van Seventer, K. J. Horgan, and S. Shaw.** 1990. Costimulation of proliferative responses of resting $CD4^+$ T cells by the interaction of VLA-4 and VLA-5 with fibronectin or VLA-6 with laminin. J. Immunol. **145:**59–67.
30. **Smithers, S. R., and M. J. Doenhoff.** 1982. Schistosomiasis, p. 527. *In* S. Cohn and K. S. Warren (ed.), Immunology of parasitic diseases. Blackwell Scientific, Oxford.
31. **Springer, T. A.** 1990. Adhesion receptors of the immune system. Nature (London) **346:**425–434.
32. **van Seventer, G. A., Y. Shimizu, K. J. Horgan, and S. Shaw.** 1991. The LFA-1 ligand ICAM-1 provides an important co-stimulatory signal for T cell receptor-mediated activation of resting T cells. J. Immunol. **144:**4579–4586.
33. **Wyler, D. J., H. P. Erlich, A. E. Postlethwaite, R. Raghow, and M. M. Murphy.** 1987. Fibroblast stimulation in schistosomiasis. VII. Egg granulomas secrete factors that stimulate collagen and fibronectin synthesis. J. Immunol. **138:**1581–1586.
34. **Wyler, D. J., and A. E. Postlethwaite.** 1983. Fibroblast stimulation in schistosomiasis. IV. Isolated egg granulomas elaborate a fibroblast chemoattractant in vitro. J. Immunol. **130:**1371–1375.
35. **Yamashita, T., and D. L. Boros.** 1992. IL-4 influences IL-2 production and granulomatous inflammation in murine schistosomiasis mansoni. J. Immunol. **149:**3659–3664.
36. **Zhu, Y., and D. L. Boros.** Unpublished data.