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IgE expression on the surface of B1 and B2 lymphocytes in experimental murine schistosomiasis

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Abstract

In a previous study we monitored the distribution and phenotype expression of B1 cells during the evolution of experimental murine schistosomiasis mansoni and we proposed that the B1 cells were heterogeneous: a fraction which originated in the spleen and followed the migratory pathway to mesenteric ganglia, while the other was the resident peritoneal B1-cell pool. In the present study, we have addressed the question of whether these two B1-lymphocyte populations are involved in the production of the late Ig isotype IgE, which is present in high levels in schistosomal infection. Lymphocyte expression of surface markers and immunoglobulins were monitored by immunofluorescence flow cytometry. Both in the spleen and mesenteric ganglia, the B1 and B2 cells were induced to switch from IgM to IgE in the early Th2-dominated phase of the disease, with an increase of IgE in its later phases. Conversely, peritoneal B1-IgM$^+$ switched to the remaining IgE$^+$ present in high numbers in the peritoneal cavity throughout the disease. We correlated the efficient induction of the expression of late Ig isotypes by B1 cells with high levels of inflammatory cytokines due to the intense host response to the presence of worms and their eggs in the abdominal cavity. In conclusion, B1 cells have a different switch behavior from IgM to IgE indicating that these cell sub-populations depend on the microenvironment.

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- Schistosomiasis
- B lymphocytes
- Immunoglobulin switch

Introduction

B1 lymphocytes are defined as a subset of B cells that express the surface pan-T marker CD5 together with the B-cell surface markers B220^{lo}, IgM^{hi}, and IgD^{lo}. In contrast to standard B cells (B2 lymphocytes), they are CD23$^-$. When located in celomic cavities, which are the major site of their homing and proliferation, they are Mac1$^+$. In addition to their phenotypic differences, B1 cells are distinguished from the major B-lymphocyte population, the B2 cells, by their different origin, localization and immunoglobulin repertoires, presumably reflecting different functions in the immune system (reviewed in Refs. 1-3).

B1 cells develop early in ontogeny and

are the major if not the sole B-cell population in the fetal liver and omentum. In adult mice they predominate in the pleural and peritoneal cavities. They are frequent in the lamina propria, but represent only a small percentage of B cells in the spleen, and are essentially absent from lymph nodes and Peyer's patches. B1 cells are maintained by self-replenishment, i.e., by division of fully mature cells. In contrast, B2 cells are replenished throughout life by proliferation and differentiation of immature progenitors in bone marrow (1). In addition to these apparently separate life histories and characteristics of the B1 and B2 lineages, it has been shown that CD5 expression can be induced in mature B2 cells, and the alternative origin of B1 cells among the cells of the B2 lineage has been subsequently proposed (4,5). At present, heterogeneous properties of the B-cell compartment with a double origin have been suggested, depending on the quality of interaction with antigens and with other cells engaged in the immune response (6).

B1 cells produce antibodies that are frequently polyreactive and of low affinity. These "natural antibodies" produced spontaneously under low immunization conditions are often autoreactive, such as antibodies for phosphatidyl choline, DNA and immunoglobulins, or react with common bacterial carbohydrate antigens like phosphorylcholine. Most B1 cells produce IgM. *In vitro* studies have indicated the inability of B1 cells to produce more mature isotypes, but *in vivo* the B1 population as a whole can produce all Ig isotypes (1). The two subsets of B1 cells, the B1a and B1b cells, are distinguished by the presence of CD5 in B1a and its absence in B1b cells, but despite their similar origin and characteristics they switch to different Ig isotypes. In response to IL-5, B1a cells tend to produce IgG2a, IgG2b and IgG3, while B1b cells produce IgG1 and IgE in response to IL-9 (7,8).

In view of their early appearance and broad reactivity, as well as their strategic submucosal location, B1 cells are considered to be part of the natural resistance to the intestinal flora (9). They can also participate in defense against viral infections (10), and in protection or recovery from experimental infections with large parasites such as *Brugia* and *Schistosoma* (11,12). Murine infection with *Schistosoma mansoni* is relevant for *in vivo* studies of B1 lymphocytes for several reasons. Schistosomiasis is an abdominal disease involving both the luminal and the peritoneal sides of the gut. Adult worms live in the mesenteric venous system, depositing their eggs in small submucosal veins of the intestine. Some of the eggs elicit small abscesses and are expelled into the lumen, but some are trapped in the mesenteric vessels or washed through the portal blood flow into the liver, where they cause granulomatous inflammatory reactions. Besides the typical cellular reaction to the parasites and their eggs, B-cell hyperplasia is a hallmark of schistosomiasis (13). The acute phase of the disease is characterized by a typical Th2 response with high IL-4 and IL-5 levels. The evolution towards the chronic phase is associated with a down-regulation of several aspects of the immune response to parasites, and a modified cytokine pattern (14). However, splenomegaly progressively increases throughout the disease, with activation of germinative centers and enhancement of antibody production with polyclonal B-cell activation (13,15,16). A substantial immunoglobulin response is initiated after 40 days of infection, reaching highest levels in the chronic phase of the disease. IgE levels follow the production of IL-4. They are high immediately after the beginning of oviposition, reaching a peak from the 8th to the 12th week of infection, and progressively decreasing thereafter. Specific antibodies to schistosome egg antigens represent a minor part of the total immunoglobulins (17,18). In addition to the splenic reactivity, the peritoneal cavity is the site of extensive myeloid and lymphoid cell proliferation. Intense

perivascular inflammation of the mesenteric venous system gives rise to plasmacytogenic foci, where immature plasmacytes are observed close to the blood vessel walls and mature ones are located on the surface of the mesenterium, predominantly secreting IgG and IgM (19,20).

In a previous study, we monitored the distribution and phenotype expression of B1 cells during the evolution of experimental murine schistosomiasis mansoni (21). We found no evidence of an increase of B1 cells in the peritoneum, nor a mobilization of B1 cells expressing the peritoneal phenotype ($CD5^{lo}$, IgM^{hi}) in the tissues involved by the infection, despite the systemic increase of the B-cell response and overall hyperplasia of peritoneal cells. Conversely, we detected in the spleen a subpopulation of B lymphocytes characterized as $CD5^{hi}$ and IgM^{lo}, which appeared subsequently in Peyer's patches and mesenteric ganglia, with a progressive acquisition of Mac-1 expression. We proposed that the B1-cell population may be heterogeneous in schistosomiasis: a fraction is originated in the spleen and follows the migratory pathway to mesenteric ganglia, while the other is the resident peritoneal pool that is apparently not increased *in situ*. In the present study, we have addressed the question of whether these two lymphocyte populations (the splenic and the peritoneal pools) are involved in the production of IgE antibodies, which are produced in high levels in schistosomal infection.

Material and Methods

Schistosomal infection

Inbred C3H/HeN mice of both sexes were obtained from the colony bred at the Federal University of Rio de Janeiro and infected by transcutaneous penetration of 40 *S. mansoni* cercariae (BH strain, Oswaldo Cruz Institute, Rio de Janeiro, RJ, Brazil). Mice were studied after 45-50 and 90-95 days of infection, corresponding to the acute phase and the beginning of the chronic phase of the disease, respectively (20). Uninfected age- and sex-matched mice were used as controls.

Preparation of cell suspensions

Cell suspensions from normal and infected mice were obtained *ex vivo* by standard mechanical dissociation of spleen, mesenteric ganglia and Peyer's patches. The peritoneal cavity was washed with phosphate-buffered saline (PBS) and red blood cells were lysed with Gey's solution. After lysis, the cell suspensions were washed twice with PBS, pH 7.2, containing 3% fetal bovine serum, and cell concentration was adjusted to 1×10^6 cells/ml. In order to saturate the Fc receptors, the cells were incubated with normal mouse serum for 10 min before adding the specific monoclonal antibodies.

Immunofluorescence

The presence of cell surface markers on lymphoid cells was monitored with the following monoclonal antibodies for three-color analysis: fluorescein isothiocyanate-conjugated anti-IgM (Sigma, St. Louis, MO, USA) and anti-IgE (Pharmingen, San Diego, CA, USA); phycoerythrin-conjugated anti-B220 (Pharmingen), and quantum red-conjugated anti-CD5 (Sigma). Staining was done separately for each monoclonal antibody for 30 min on ice, followed by washing with cold PBS.

Data acquisition and analysis

Data acquisition and analysis by flow cytometry were performed with a FACS calibur apparatus, using the Cell Quest Software (Becton Dickinson, Mount View, CA, USA). The fluorescence was detected by photomultiplier tubes and adjusted on scatter-gated unstained lymphocytes, and com-

pensation was performed using single-color-stained cells from each organ separately. Cell debris and dead cells were excluded according to the light scatter parameters, and the gate including the lymphocyte population was named R1. Data are reported as dot plots and histograms.

Statistical analysis

Data were analyzed statistically by the Tukey multiple comparison test, with the level of significance set at $P < 0.05$.

Results

Expression of IgE on the surface of B cells was monitored in the mesenteric ganglia, Peyer's patches, spleen, and peritoneal cavity of normal and schistosome-infected mice during the evolution of the disease from the acute to the chronic phase (Figures 1 to 4). B1, B2 and T lymphocytes were defined according to differential expression of B220 and CD5 (Figures 1-5A).

The labeling pattern of the cells that did not produce IgE was recorded in all experiments (Figures 1B to 4B) and pooled, and compared to the pattern of cells that reacted with anti-IgE antibodies. In agreement with reported levels of Th2 cytokines (14), mesenteric ganglia showed increased IgE$^+$ cell numbers from the acute to the chronic phase of the disease (Figure 1B). The median val-

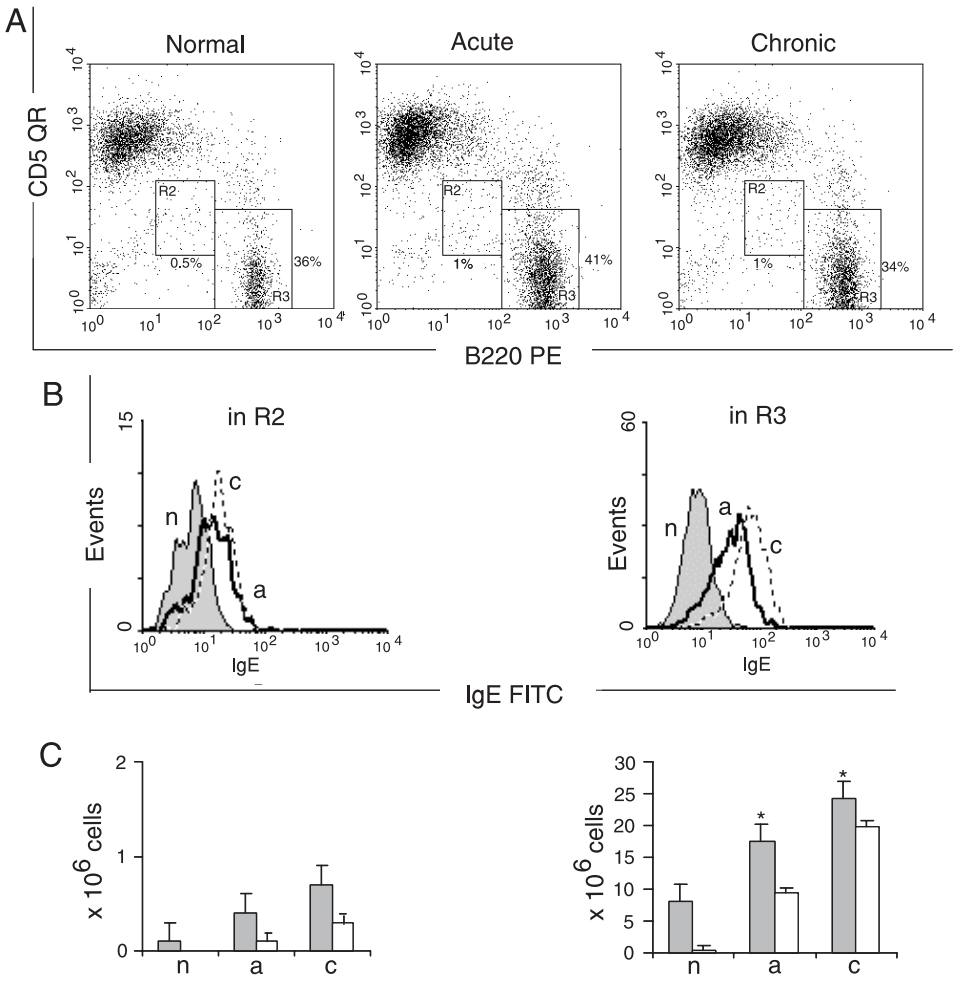

Figure 1. Surface expression of B220, CD5 and IgE on cells from mesenteric ganglia. *A*, Dot plot of cells in the lymphocyte gate. The R2 and R3 regions represent B1 and B2 cells, respectively. *B*, Intensity of IgE on the B-cell surface. *C*, Total cell numbers (filled columns) and IgE+ cells (open columns). Data are reported as means ± SEM and are representative of five independent experiments, each carried out in 2 normal mice (n), 5 mice with acute infection (a) and 5 with chronic infection (c). *P < 0.05 for B-IgE$^+$ cells compared to total B cells (Tukey multiple comparison test).

ues were moderately increased in diseased animals, suggesting that rare cells presented low to intermediate IgE fluorescence intensity on their surface. This low or intermediate reactivity is not suggestive of immunoglobulin secretion, but indicates that this B-cell subpopulation has the genetic machinery able to perform all the gene recombinations necessary to switch from IgM to IgE. Due to the low number of events, the difference between the groups was only suggestive (P = 0.08). Conversely, in Peyer's patches, only B2 lymphocytes were involved in the switching from IgM to IgE isotype (Figure 2B and C), with a progressive increase from the acute to the chronic phase indicative of switching from the IgM isotype.

In schistosomiasis, the spleen receives a permanent stimulation of the immune system, evolving to splenomegaly associated with an increase in B-cell numbers and their polyclonal activation (13). In normal spleens, IgE$^+$ cells were very rare in all the B-cell subsets studied (Figure 3B), but their number increased in the acute and chronic phases of the disease. The number of B1-IgE$^+$ cells remained stable in infected mice (Figure 3C). In contrast, the total B2 and B2-IgE$^+$ cells increased during the evolution of the disease (Figure 3B and C). In agreement with the results shown for mesenteric ganglia, B1-IgE$^+$ cells were found in the spleen, participating with B2 lymphocytes in the production of IgE, present in high concentrations in serum of mice during both the acute and chronic phases of the disease.

The peritoneal cavity is the major site of maintenance and production of B1 cells. In

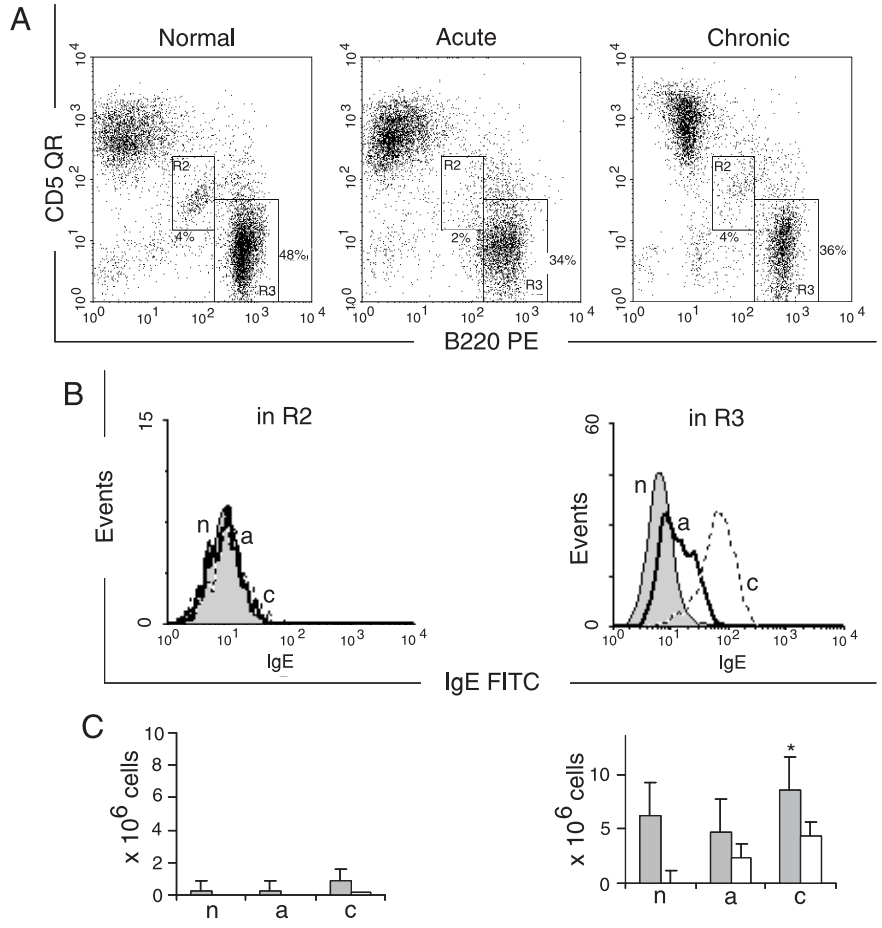

Figure 2. Surface expression of B220, CD5 and IgE on cells from Peyer's patches. *A*, Dot plot of cells in the lymphocyte gate. The R2 and R3 regions represent the B1 and B2 cells, respectively. *B*, Intensity of IgE on the B-cell surface. *C*, Total cell numbers (filled columns) and IgE+ cells (open columns). Data are reported as means ± SEM and are representative of five independent experiments, each carried out in 2 normal mice (n), 5 mice with acute infection (a) and 5 with chronic infection (c). *P < 0.05 for B-IgE$^+$ cells compared to total B cells (Tukey multiple comparison test).

normal adult mice B1 lymphocytes represent up to 30% of peritoneal cells. B1a cells were increased during the acute phase of the disease, and in the chronic phase their relative number decreased compared to normal mice. According with previously published data (21), the total number of peritoneal B1 cells remained constant throughout the disease as a consequence of the increased number of peritoneal cells. B1a-IgE$^+$ cells were negligible in normal mice (Figure 4B and C). Mice with acute infection had an increased B1a-IgE$^+$ cell fraction, which remained at the same level during chronic infection. B1b cells (Figure 4B) were negative in normal mice and in mice with acute infection, but in the chronic phase, they were split into two clearly distinct IgE$^+$ and IgE$^-$ subsets (Figure 4B and C). The conventional B2 lymphocytes present in the peritoneal cavity were not involved in IgE production in the studied groups (Figure 4B and C). The increase of IgE$^+$ cells was thus due to the appearance of a new increasing IgE$^+$ cell population in the peritoneal cavity of infected mice, which did not exist in normal mice.

We have addressed the question of the presence of IgM$^+$ cells in this cavity in order to monitor the kinetics of the switch of IgM$^+$ cells to IgE$^+$ cells (Figure 5A and B). IgM$^+$ cells were the vast majority in the entire peritoneal B-cell population of normal mice (Figure 5B). The evolution of the disease was concomitant with a progressive decrease of IgM$^+$ cells in all B-cell populations. Monitoring IgM expression identified two subpopulations among IgM$^+$ cells: a very bright

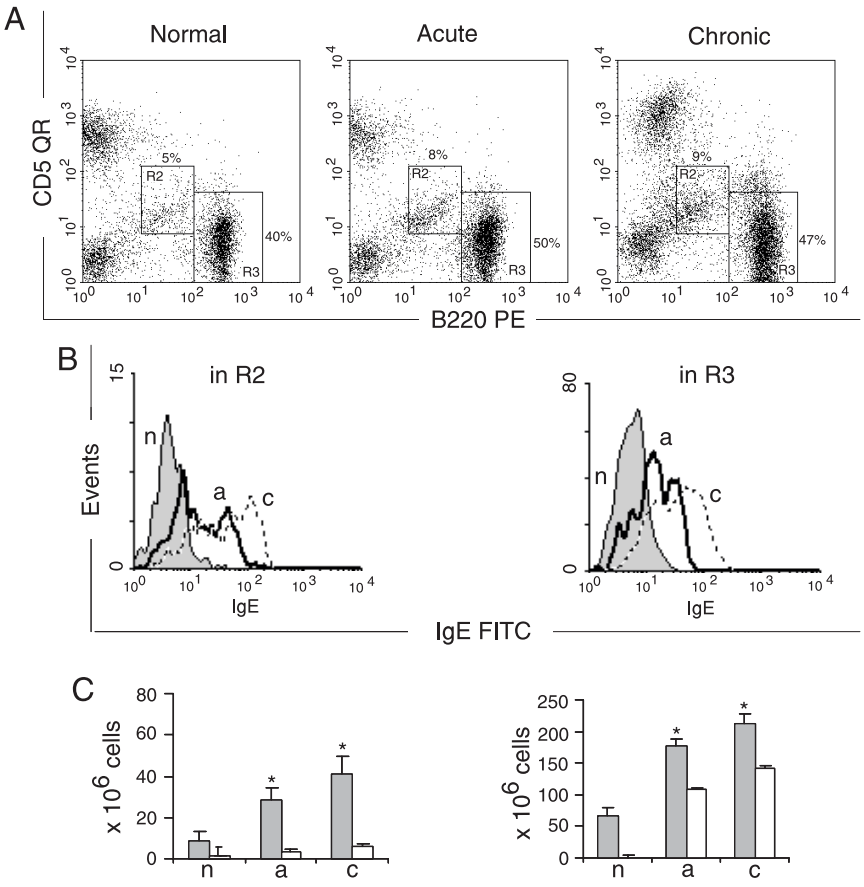

Figure 3. Surface expression of B220, CD5 and IgE on cells from spleen. *A*, Dot plot of cells in the lymphocyte gate. The R2 and R3 regions represent the B1 and B2 cells, respectively. *B*, Intensity of IgE on the B-cell surface. *C*, Total cell numbers (filled columns) and IgE+ cells (open columns). Data are reported as means ± SEM and are representative of five independent experiments, each carried out in 2 normal mice (n), 5 mice with acute infection (a) and 5 with chronic infection (c). *P < 0.05 for B-IgE$^+$ cells compared to total B cells (Tukey multiple comparison test).

and another one with slightly lower IgM expression (Figure 5B). We understand that the latter population corresponds to the IgM cells switching to the expression of IgE. In the acute phase, an increase of IgM^{lo} cells was concomitant with the appearance of IgE^+ cells (Figures 4B and 5B) indicating the isotype switching. In the chronic phase, a larger part of B1 cells were IgM^- (Figure 5B), indicating a full switch to more mature Ig isotypes, as demonstrated by the presence of B1a and B1b-IgE^+ cells (Figure 4B). A smaller IgM^{lo} population potentially corresponds to the cells switching to other isotypes. It is notable that no IgM^{hi} cells were found in this phase of infection, indicating that self-replenishment of IgM-producing cells did not occur. Low quantities of IgM^+ cells in this phase (Figure 5C) were apparently engaged in switching to more mature isotypes, while the constant presence of IgE-producing cells in the peritoneal cavity may correlate with the permanent granulomatous and diffuse inflammation of the viscera in response to the long-lasting presence of worms and their eggs in the tributaries of the mesenteric venous system. In conclusion, we understand that IgE^+ cell kinetics in the peritoneal cavity has different patterns and is controlled by distinct molecular mechanisms.

Discussion

B1 cells can produce IgM and IgG3 spontaneously in the absence of T cells. This production is considered to be part of the natural resistance to exogenous antigens,

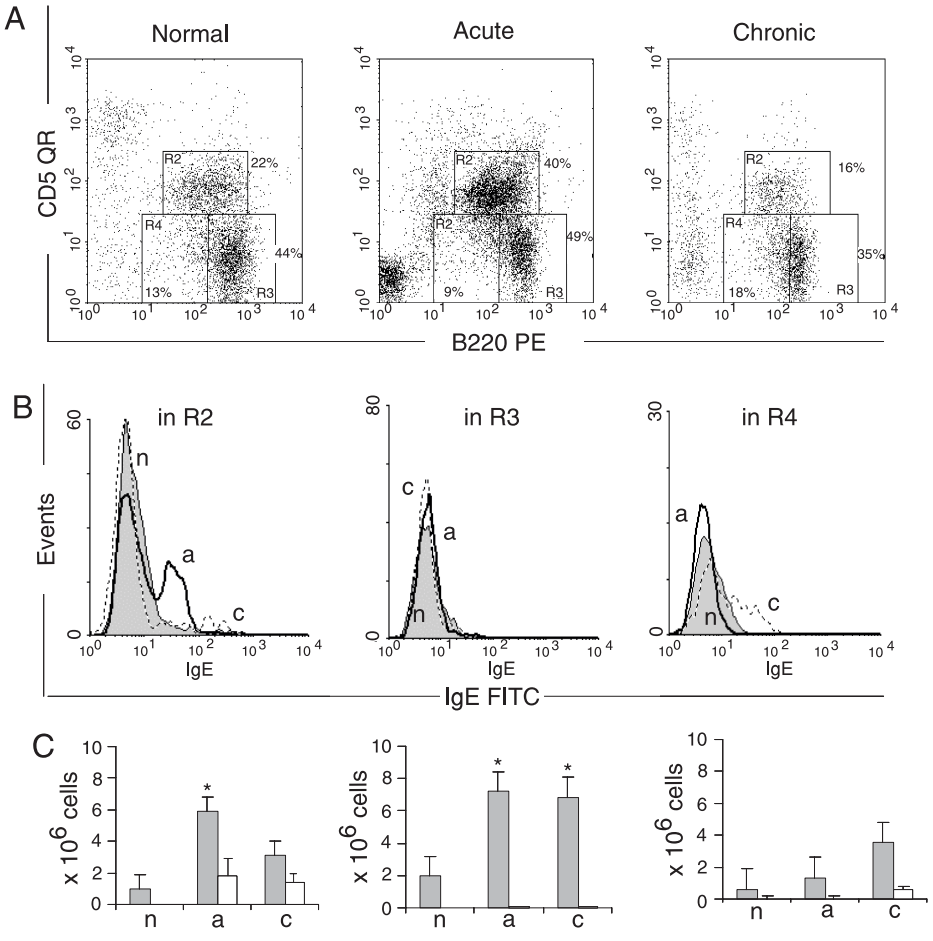

Figure 4. Surface expression of B220, CD5 and IgE molecules on cells from the peritoneal cavity. *A*, Dot plot of cells in the lymphocyte gate. The R2, R3 and R4 regions represent the B1a, B2 and B1b cells, respectively. *B*, Intensity of IgE on the B-cell surface. *C*, Total cell numbers (filled columns) and IgE+ cells (open columns). Data are reported as means ± SEM and are representative of five independent experiments, each carried out in 2 normal mice (n), 5 mice with acute infection (a) and 5 with chronic infection (c). *P < 0.05 for B-IgE^+ cells compared to total B cells (Tukey multiple comparison test).

and can participate in a short-term response of the immune system to infectious agents (22-24). T cells can increase the production of these isotypes, presumably in an antigen-dependent manner, as part of the adaptive immune response. The production of IgG1 by B1 cells is totally dependent on T cells.

Production of other immunoglobulins by B1 cells can be either T-independent or T-dependent, and these antibodies strongly contribute to the IgA-secreting plasma cells of the intestinal lamina propria (25,26). A significant difference between the response of splenic and peritoneal B cells to IL-5 has been reported (27-29). We observed simultaneous generation of B1-IgE$^+$ cells in the spleen and in the peritoneal cavity during the acute phase of the disease when the overall pattern of the immune response is Th2-dominated. While the great majority of splenic cells that switched to IgE$^+$ were B2, in the peritoneal cavity IgE$^+$ cells apparently originated in the B1-IgM$^+$-cell population, suggesting the generation and expansion of a new cell population in response to the infection. Schistosome soluble egg antigens are known to elicit a very strong Th2 response, with high levels of IL-4 and IL-5 (15,30).

In vitro, the induction of IgE switch in B1 cells has been shown to be difficult, requiring very high levels of cytokines (29), in studies based on the production of IgM, IgG1, IgE, and IgA by B1 (CD23$^-$) and B2 lymphocytes (CD23$^+$). Both subpopulations were obtained by sorting from the spleen and peritoneal cavity and cultured with lipopolysaccharide and IL-4. Only the B1 and B2 cells purified from spleen were capable

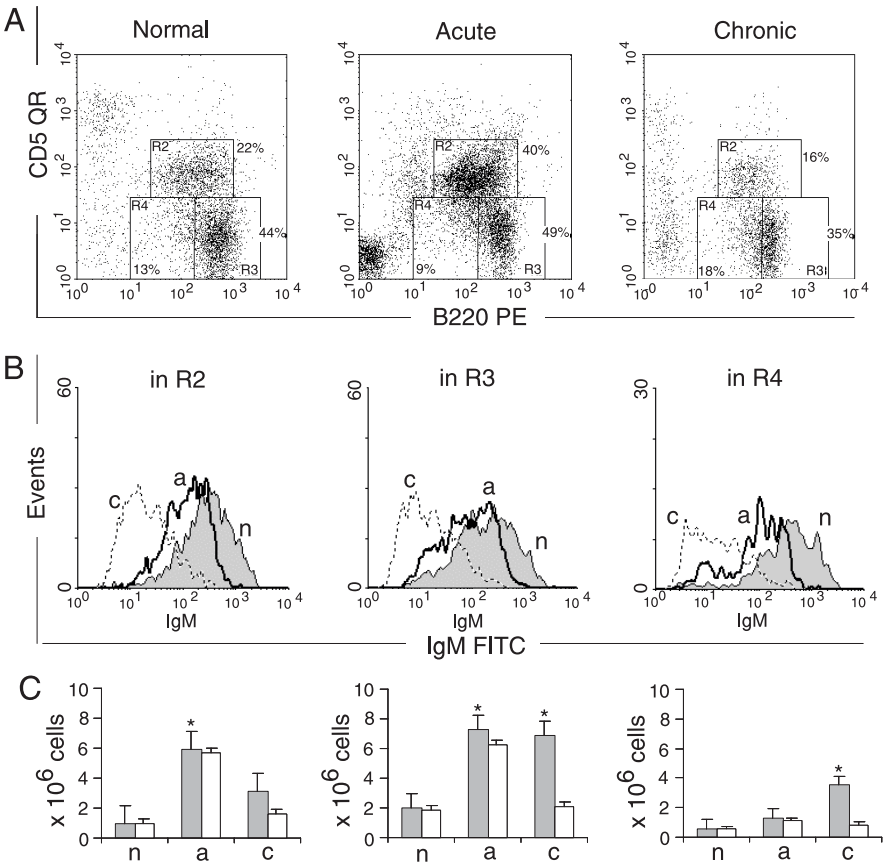

Figure 5. Surface expression of B220, CD5 and IgM on cells from the peritoneal cavity. *A*, Dot plot of cells in the lymphocyte gate. The R2, R3 and R4 regions represent the B1a, B2 and B1b cells, respectively. *B*, Intensity of IgM on the B-cell surface. *C*, Total cell numbers (filled columns) and IgM$^+$ cells (open columns). Data are reported as means ± SEM and are representative of five independent experiments, each carried out in 2 normal mice (n), 5 mice with acute infection (a) and 5 with chronic infection (c). *P < 0.05 for B-IgM$^+$ cells compared to total B cells (Tukey multiple comparison test).

of producing all the tested isotypes, including high levels of IgE. Here we demonstrated *in vivo* the presence of B1-IgE$^+$ in infected mice. Although B1-IgE$^+$ were relatively rare compared to B2-IgE$^+$ cells, they showed intermediate to high fluorescence intensity, suggesting that both cell types participated in the production of IgE, known to be high in this disease (31). *In vitro*, peritoneal B1a cells were able to produce IgG1, but only rarely switched further to the IgE isotype, indicating that this second step requires additional stimuli (32). Moreover, the rare B1a cells that were reactive for IgE on the surface did not produce the antibody to the supernatant. In our model, the peritoneal environment was able to fulfill these requirements, generating a considerable quantity of IgE-producing B1 cells, particularly so during the acute phase of the disease, consistently with the high level of Th2 cytokines in this phase.

Our observation of the relatively large number of B1 cells switching to IgE$^+$ cells *in vivo* potentially corresponds to the association between high cytokine levels and appropriate presentation of antigens in the granulomatous and diffuse inflammatory reactions in the abdominal cavity, and in the downstream sites of the antigen circulation in the spleen. The presence of B-IgE$^+$ cells in all the compartments studied agrees with the high concentration of IgE in the serum of infected mice in the late chronic phase of the disease (normal mice = 1.07 ± 1.01 µg/ml, versus infected mice = 69.4 ± 43.3 µg/ml; El-Cheikh MC, unpublished data).

In the experimental model employed here, the peritoneal B1-IgE$^+$ cells did not decrease during the evolution of the disease, indicating that their migration and homing are different from those observed for IgA$^+$ cells (33). Although only a fraction of IgE has been shown to be specific for the assayed schistosome antigens (31), the IgE response is apparently a part of the specific response to the infection, since its patterns follow the overall kinetics of the immune relationship between the host and the parasite.

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