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Differential Requirements for an Intact Spleen in Induction and Expression of B-Cell-Dependent Immunity to *Plasmodium chabaudi* AS

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The requirement for an architecturally intact spleen in the afferent and efferent arms of immunity to the murine malaria parasite *Plasmodium chabaudi* AS was analyzed. C57BL/6 mice with intact spleens develop a single, patent parasitemia and resolve the infection. In contrast, surgically splenectomized mice experience persistent waves of patent parasitemia interrupted briefly by periods of parasitologic crises. Transfer of spleen cells from immune donors, but not transfer of spleen cells from normal mice, into splenectomized mice enabled the recipients to resolve the infection similar to mice with intact spleens. B-cell depletion, but not T-cell depletion, of spleen cells prior to transfer abrogated the ability of splenectomized recipients to resolve the infection. Compared with mice with intact spleens, splenectomized mice exhibited a delayed antibody response whereas all groups of immune cell recipients had an accelerated antibody response. Nevertheless, splenectomized mice and recipients of B-cell-depleted cells failed to resolve infections, despite the development of high-titer antibodies late during the course of infection. Analysis of immunoglobulin G isotype responses showed a lower representation of immunoglobulin G2a in mice which failed to resolve infections. The latter mice had characteristic histopathologic changes in the liver. These observations indicate a unique role of the splenic microenvironment for the induction and development of an effective B-cell-dependent response against malarial parasites.

That the spleen plays an important role in host defense against malarial infections is a well-established fact (27). This is perhaps best exemplified by the capacity of splenectomy to extend the host range of *Plasmodium* species. Furthermore, splenectomy of several rodent and primate hosts abrogates their capacity to control and clear the malarial parasites (15, 27). The precise mechanisms by which the spleen exerts its protective functions are less well understood. By virtue of its unique anatomical features and its cellular composition, three functions could be broadly ascribed to the spleen in the context of host responses to malarial infections. First, the spleen serves as a mechanical filter which surveys and sequesters rheologically or immunologically modified erythrocytes, including parasitized erythrocytes (28). Second, the spleen provides a microenvironment for the interaction of parasites, immune cells, and their products during the generation and execution of immune effector functions (23). Third, the spleen participates in the generation of new blood cells, especially in the erythropoietic response to hemolytic stress caused by malaria infection (29).

The immune response to the intraerythrocytic stages of malarial parasites has been best characterized in the rodent models of malaria. Evidence has accumulated suggesting that humoral and cell-mediated immunity may act in concert or sequentially to control and clear malaria parasites. The relative importance of either arm has been shown to depend upon the parasite species and specific phase of immunity in question. For instance, immunity to blood stage *Plasmodium yoelii* and *P. berghei* has been characterized as being antibody dependent whereas *P. chabaudi* and *P. vinckei* have been shown to be controlled primarily by cell-mediated immune mechanisms (11, 24). The cell-mediated immune responses to *P. chabaudi* are thought to be mediated by $CD4^+$ T-cell-dependent activation of an effector cell, generally regarded as the monocyte-macrophage, which mediates nonspecific killing or inactivation of the parasites. More recently, Meding and Langhorne (11) have provided evidence for the involvement of antibody-dependent mechanisms in the control and final clearance of the parasites. Thus, it is proposed that $CD4^+$ T cells may activate effector cells via elaboration of Th1 type cytokines in the acute phase, whereas Th2 type $CD4^+$ T cells help B cells to produce antibodies which ultimately clear the infection (7, 19, 21). The spleen is thought to be important both as a site of macrophage activation and nonspecific parasite killing and as a site of antibody synthesis, antigen focusing, and phagocytosis. Indeed, recent studies with *P. chabaudi adami* and *P. vinckei* demonstrate an absolute requirement for the presence and modification of an architecturally intact spleen for the expression of T-cell-dependent cell-mediated immunity (4, 6). This requirement has been attributed to the activation and development of barrier cell complexes which increase the clearance function of the spleen. However, efforts to induce barrier cell activation in the spleen nonspecifically by administering *Mycobacterium bovis* BCG, salmonellae, or vaccinia virus have not resulted in protection against *P. vinckei* (26).

In the present study, we investigated the requirements for an intact spleen in the development and expression of the immune response to *P. chabaudi* AS. Special attention was given to the early and late phases of the infection. Our results indicate that the splenic microenvironment is essential for development but is not required for expression of an effective immune response against this parasite. Furthermore, our results suggest that the spleen provides a unique tissue microenvironment for devel-

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opment of an effective B-cell-dependent immune response required to clear the parasites.

MATERIALS AND METHODS

Parasites and experimental infection of mice. *P. chabaudi* AS, originally obtained from D. Walliker, University of Edinburgh, was preserved and maintained by passage in C57BL/6 mice as previously described (13). Experimental infection was initiated by intraperitoneal inoculation with 10^6 parasitized erythrocytes. Parasitemia was monitored by counting 200 to 10,000 erythrocytes per slide on Diff-Quik-stained blood smears.

Splenectomy. C57BL/6 mice obtained from our breeding colony at the Montreal General Hospital were used throughout. Splenectomy and sham splenectomy procedures were performed by using standard techniques previously detailed (20). Surgically manipulated mice were allowed to rest for at least 2 weeks before experimental infection.

In vivo cell transfer. As a source of immune spleen cells for transfer, sex-matched C57BL/6 mice were infected with 10^6 parasitized erythrocytes and allowed to resolve the infection. A secondary challenge with the same dose of parasites was administered 30 days after the first infection. Routinely, the mice were smear negative at 7 to 10 days postchallenge and were then used as donors. Spleen cells from immune and control animals were prepared by perfusion in complete medium (RPMI 1640 with 2% bovine serum albumin and antibiotics) and by washing twice. Erythrocytes were removed by hypotonic lysis. Spleen cells were either administered intravenously at a dose of 25×10^6 per mouse or subsequently depleted of T cells or B cells as outlined below.

T-cell and B-cell depletion. T cells were depleted by two cycles of treatment with a cocktail of anti-T-cell monoclonal antibodies (F5D5, GK 1.5, and 53-6.7.2, which are specific for Thy-1, CD4, and CD8 determinants, respectively) and rabbit complement (Cedarlane, Hornby, Ontario, Canada) (12). Viable cells were retrieved on a Lymphoprep gradient (Cedarlane). B cells were depleted by panning on petri dishes precoated with goat anti-mouse immunoglobulin M (IgM) and anti-mouse IgG (Caltag; Cedarlane). The extent of depletion was monitored by cell surface staining with an anti-Thy-1 monoclonal antibody and an anti-B220 antibody as outlined below. The resulting cell suspensions were injected at the same dose as undepleted cells.

Cell surface staining and flow cytometry. Spleen or liver cell suspensions (10^6 cells per sample) were stained with the appropriate antibodies for 20 min at 4°C by standard procedures. For T- and B-cell depletion experiments, monoclonal antibody 30H12 (rat IgG2b anti-Thy-1.2) and an anti-B220 antibody (Pharmingen, San Diego, Calif.) were used to monitor the extent of T- and B-cell depletion.

***P. chabaudi*-specific antibody enzyme-linked immunosorbent assay (ELISA).** *P. chabaudi* antigen was prepared and solubilized from saponin-lysed parasitized erythrocytes with a solution of 0.5% sodium dodecyl sulfate–1 mM EDTA in 50 mM Tris (pH 7) and used at an optical density at 280 nm (OD_{280}) of 0.05 as previously described (8). Immunolon II plates (Dynatech, Chantilly, Va.) were coated with antigen in bicarbonate buffer (pH 9.6) overnight and subsequently blocked with 5% skim milk in phosphate-buffered saline and washed with phosphate-buffered saline–Tween 20. Serial 10-fold dilutions of serum samples were incubated for 2 h at room temperature. After extensive washing, the plates were developed with either horseradish peroxidase-conjugated goat anti-mouse IgG for total parasite-specific IgG or horseradish peroxidase-conjugated anti-isotype antibodies (SBA, Birmingham, Ala.). The optimal dilutions of conjugated antibodies determined in pilot experiments were 1:3,000 for total IgG and 1:250 for isotype reagents. Reactivity was developed with an ABTS [2′,2′-azino-di-(3-ethyl-benzthiazoline-6-sulfonic acid)] substrate kit, and OD_{405} was read with an SLT Lab Instruments ELISA reader.

Histology. At the end of each experiment (day 60 or later), livers from splenectomized (SPLX) mice with or without transfer of various cells were excised and processed routinely for paraffin embedding, sectioned, and stained with hematoxylin and eosin. Two to four mice per group were examined.

RESULTS

To better define the role of the spleen in *P. chabaudi* AS infection, we initially compared the course of infection in surgically SPLX mice with the course of infection in control, sham-operated mice. Figure 1A shows the course of infection in control C57BL/6 mice. As previously reported (13, 29), infection in this strain of mice is self-healing and nonlethal. Mean peak parasitemia of 25% occurred on days 10 to 12, after which parasitemia was rapidly reduced to subpatent levels. A recrudescence followed on days 28 to 30, which had a lower peak of parasitemia of approximately 1% or less. In sharp contrast, SPLX mice experienced a higher primary peak parasitemia of approximately 50% or higher and had chronically patent parasitemia (Fig. 1B). The patent parasitemia was persistent, interrupted only by short episodes of parasitologic crises and severe anemia. These results indicate that SPLX mice were able to decrease parasitemia during the several parasitological crises observed but were unable to suppress the remaining parasitemia from resurgence into patency. The inability of the SPLX mice to suppress infections from becoming patent may be due to either their inability to generate effector cells or the lack of a proper tissue microenvironment (that is, the splenic reticular network) required for the effector function had the relevant cells been present. To distinguish between these possibilities, we transferred normal and immune spleen cells into the SPLX mice prior to infection. As previously reported for SPLX mice infected with *P. chabaudi adami*, transfer of normal spleen cells did not enable the SPLX recipients to clear the patent parasitemia as animals with intact spleens would have (data not shown). However, transfer of immune cells totally suppressed the secondary and subsequent parasitemias without affecting the timing and peak of the primary parasitemia (Fig. 1C). This indicates that the spleen is required for development of the immune cells which directly or indirectly effect the suppression of secondary patent parasitemia. However, an architecturally intact spleen was not required for the effector function of the protective immune cells transferred.

To gain insight into the cellular requirement for the transfer of immunity to SPLX recipients, the immune spleen cells were depleted of T or B cells prior to transfer. Figure 1D (closed symbols) shows that depletion of T cells (from 30 to 3% $Thy\text{-}1^+$ cells) failed to abrogate the protective effect. In contrast, B-cell depletion (from 44 to 3% $B220^+$ cells) of the cells transferred did not allow the mice to clear the infection (Fig. 1D, open symbols). However, in recipients of B-cell-depleted immune cells, the T cells, null cells, and possibly the remaining B cells were able to prevent the infections from becoming patent. Thus, recipients of B-cell-depleted transfers experienced only low-grade parasitemias not exceeding 1%. In a subsequent transfer of B-cell-depleted immune cells (0.5% $B220^+$ cells), persistent, subpatent parasitemia was similarly

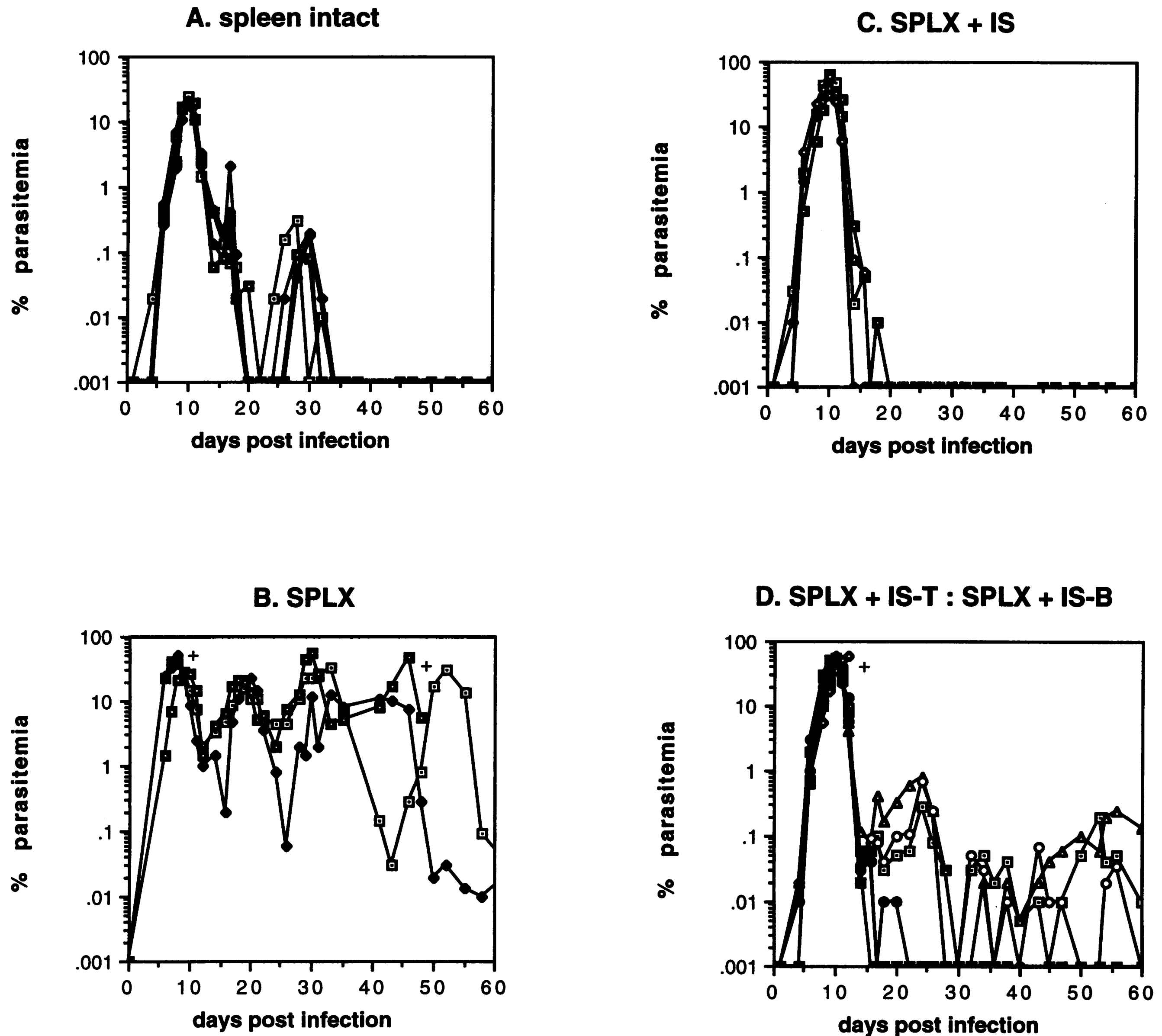

FIG. 1. Course of blood stage infection of *P. chabaudi* AS in control mice with spleens intact (A), SPLX mice, (B), SPLX mice given immune spleen cells (SPLX + IS) (C), and SPLX mice given immune spleen cells depleted of T cells (SPLX + IS-T; closed symbols) or depleted of B cells (SPLX + IS-B; open symbols) (D). Each symbol represents an individual mouse. Four mice (C57BL/6 strain) per group were infected by intraperitoneal injection with 10^6 parasitized erythrocytes. The results shown are representative of three independent experiments.

observed. We therefore conclude that B cells, but not T cells, are required for transfer of the capacity to resolve parasitemia. However, in the absence of transferred immune B cells, T cells and other cells from immune mice are able to confer the ability to control the primary parasitemia to subpatent levels. Nonetheless, immune B-cell transfer was absolutely required to effect complete resolution of infections in SPLX mice.

Since B cells function primarily as antibody-producing cells, we compared the antibody responses of SPLX mice with or without various cell transfers at early and later time points during the infection. To compare the results of individual determinations done on separate days, normal mouse serum and immune mouse serum (obtained from immune spleen donors) were included as negative and positive reference controls, respectively. As shown in Fig. 2, on day 17, parasite-reactive IgG was undetectable (i.e., OD not significantly higher than that of normal mouse serum) in SPLX mice without cell transfer. However, these mice developed higher levels of IgG later during the infection. Transfer of untreated or T-cell-depleted immune cells led to an accelerated IgG response relative to that of control, intact mice. Depletion of B cells prior to transfer did not result in abrogation of the early antibody response compared with B-cell-sufficient transfers. This indicates that the T cells transferred were able to cooperate with endogenous B cells to generate an accelerated antibody response. However, these antibodies were apparently not effective in the resolution of parasitemia (Fig. 1D). Consistent with this notion is the observation that IgG levels

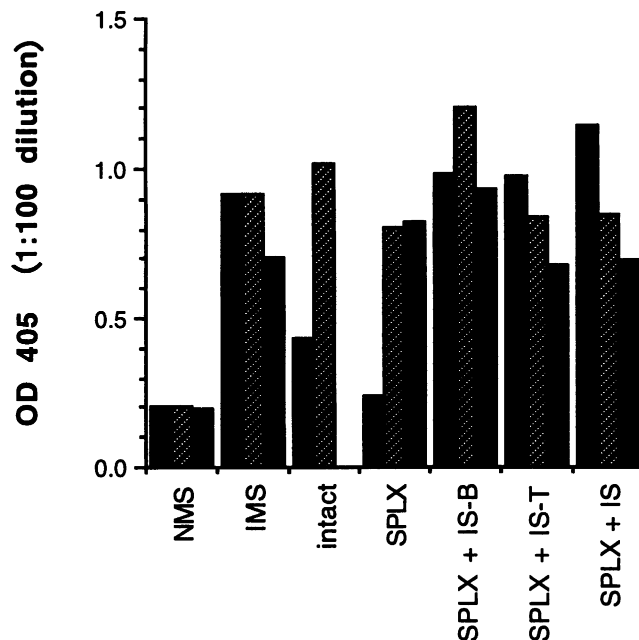

FIG. 2. Anti-*P. chabaudi* AS IgG responses in intact and SPLX mice with or without various cell transfers. OD values are shown for a serum dilution of 1:100 on days 17 (■), 43 (▨), and 56 (▩). OD values for normal mouse serum (NMS) and immune mouse serum (IMS) are also shown for reference. Each value represents the mean of two to four serum samples from different groups of mice. For definitions of abbreviations, see the legend to Fig. 1.

TABLE 1. Anti-*P. chabaudi* AS isotypic responses in intact and SPLX mice with or without immune spleen cell transfers

Time and mouse	OD U[a]			
	IgG1	IgG2a	IgG2b	IgG3
Day 17				
Intact	0.04	0.15	0.07	0.11
SPLX	0	0	0	0
SPLX + IS-B	0.22	0.33	0.39	0.42
SPLX + IS-T	0.17	0.48	0.42	0.38
SPLX + IS	0.17	0.52	0.43	0.39
Day 43				
Intact	0.31	0.39	0.73	0.60
SPLX	0.26	0.22	0.24	0.21
SPLX + IS-B	0.42	0.43	1.77	0.52
SPLX + IS-T	0.09	0.57	0.77	0.18
SPLX + IS	0.15	0.38	0.52	0.19
Day 56				
Intact	ND[b]	ND	ND	ND
SPLX	0.29	0.26	0.45	0.83
SPLX + IS-B	0.12	0.44	0.90	0.72
SPLX + IS-T	0.03	0.40	0.45	0.07
SPLX + IS	0	0.30	0.42	0.04

[a] The values represent net readings determined by ELISA. Wells of a 96-well assay plate were coated with *P. chabaudi* AS antigen, and 1:10 dilutions of serum samples were incubated in coated, washed wells. Peroxidase anti-isotype reagents were added to wells before development with ABTS substrate. Each value represents the mean of two to four samples of serum from different groups of mice. The OD value for preimmune mouse serum has been subtracted from each isotype determination. For abbreviations, see the legend to Fig. 1.

[b] ND, not done.

continued to rise at later time points in B-cell-depleted transfer recipients, whereas antibody levels declined in B-cell-replete transfer recipients. The antibodies produced in SPLX mice given B-cell-depleted cells were ineffective at resolving the parasitemia but may have contributed to the control of patency (compare SPLX mice [Fig. 1B] and SPLX mice given B-cell-depleted immune spleen cells [Fig. 1D, open symbols]). More importantly, these results strongly suggest that in the absence of an intact spleen, an effective B-cell-dependent response fails to develop.

Since increased levels of antibodies are eventually produced in SPLX mice with or without B-cell transfer, it is reasonable to posit that a qualitative difference in the antibody repertoire may exist between these groups of mice. The difference may be related to either the antigen recognition or isotype distribution of the antibodies or both. To investigate potential differences in antigen recognition, Western blot (immunoblot) analysis of *P. chabaudi* antigens were probed by using sera from SPLX mice with or without B-cell transfer. The same banding patterns were obtained regardless of the serum used (data not shown). It appeared that there was no gross difference in antigen recognition between the antibodies. We cannot discount the possibility that the antibodies differentially recognize different epitopes on the same antigen as well as different antigens with similar molecular weights.

A more likely difference may exist in the isotype distribution of antibodies in the different groups which may relate to the functional capacity of antibodies to effect resolution. Table 1 shows the relative amounts of parasite-reactive IgG isotypes in the different groups of mice at early and later stages of infection. SPLX mice exhibited a delayed antibody response with respect to all isotypes compared with control intact mice. Control mice had low levels of each isotype on day 17 but subsequently developed higher levels on day 43. In contrast, SPLX mice did not have detectable antibodies on day 17 and still had low levels of each isotype on day 43. Higher levels of IgG2b and IgG3, but not IgG2a and IgG1, were detected on day 56. As expected, SPLX mice which received cell transfers had an accelerated response compared with mice without cell transfer. On day 17, the isotype distribution among the three groups of cell recipients did not differ markedly. Subsequently, in SPLX mice which received immune B cells and were able to clear the infection, the levels of IgG1 and IgG3 decreased progressively, while IgG2a and IgG2b remained elevated. In contrast, in recipients of B-cell-depleted transfers, which were unable to resolve subpatent parasitemia, IgG2b and, to a lesser extent, IgG3 were markedly increased on days 43 and 56. IgG2a levels did not increase substantially in the recipients of B-cell-depleted transfers.

The liver has been shown to take over some of the splenic functions in SPLX mice. We therefore analyzed the histological features of the liver tissue from different groups and correlated the histopathological reactions with persistence of parasitemia. Examination of liver tissues demonstrated a good correlation between the levels and persistence of recrudescent parasitemia and two pathologic changes, namely, leukocytic infiltrates surrounding portal veins and ischemic necrosis of hepatic parenchyma. In mice which had cleared the infection (that is, SPLX mice given undepleted or T-cell-depleted transfers), the liver histology was normal except for residual pigment deposition in Kupffer cells and occasional lymphoid aggregates in hepatic sinuses (Fig. 3a). In contrast, SPLX mice which had not received any cell transfer had massive leukocytic infiltrates and ischemic necrosis (Fig. 3b). The midzonal necrosis of the parenchymal tissue most likely resulted from

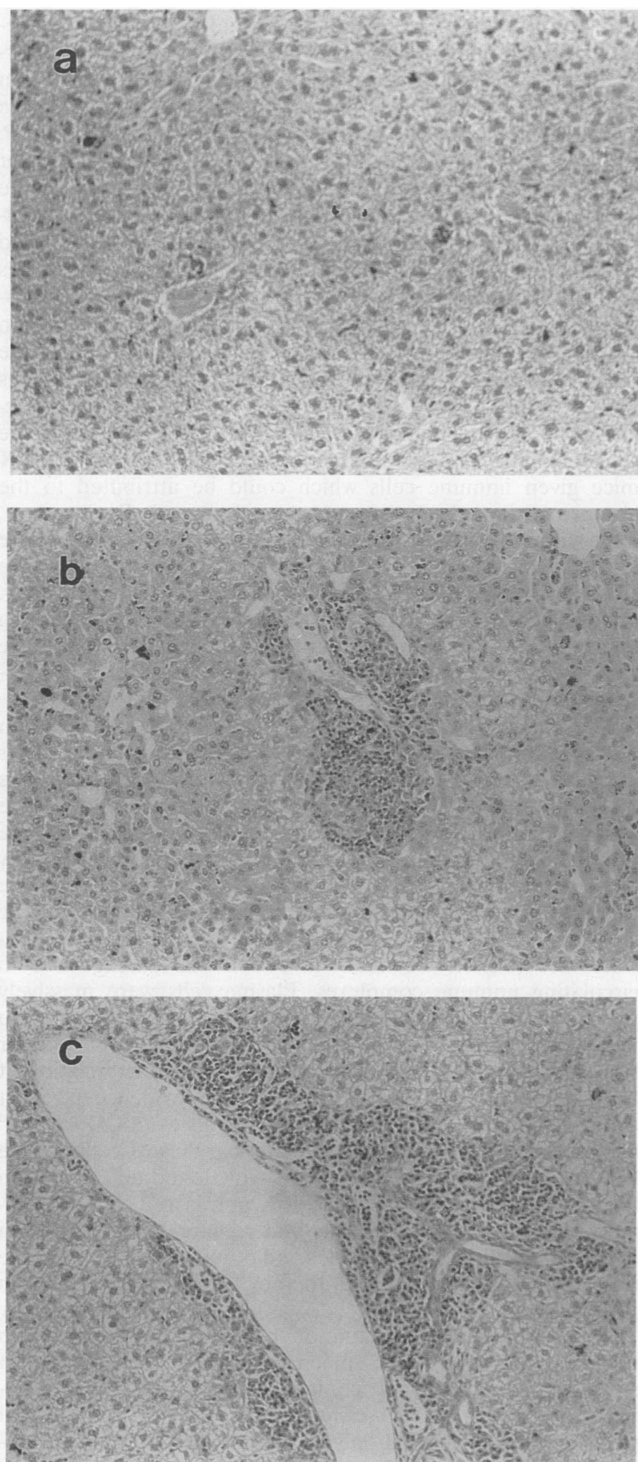

FIG. 3. (a) Liver tissue of an SPLX mouse given whole immune cells, showing normal parenchyma with pigment deposition in Kupffer cells and occasional leukocytes. (b) Hepatocyte necrosis (left and right sides) and leukocytic infiltrate surrounding a vessel (middle) in the liver of an SPLX mouse not given immune cells. (c) Liver tissue of an SPLX mouse given B-cell-depleted immune cells, showing massive leukocytic infiltration in the portal vein without hepatocyte necrosis. Magnification, × 120.

anoxia and perfusion deficits accompanying the severe episodes of anemia. Interestingly, livers of B-cell-depleted transfer recipients, which experienced low-grade, persistent parasitemia, exhibited a similar degree of leukocytic infiltrates in the portal veins but showed no evidence of necrotic lesions (Fig. 3c). These results indicate that low levels of parasitemia are sufficient to induce the leukocytic aggregates. The cellular infiltrates were composed predominantly of plasmacytes and macrophages and, occasionally, granulocytes and small lymphocytes.

DISCUSSION

We have examined the requirement for an intact spleen in the induction and expression of the immune response to nonlethal *P. chabaudi* AS in C57BL/6 mice. Our results demonstrate that an intact spleen is required for development of an effective immune response to this parasite. However, the immune cells do not require intact splenic tissue for their effector function to be expressed. Earlier studies by Grun and Weidanz (4) showed that SPLX mice given dispersed normal spleen cells did resolve rescrudescent *P. chabaudi* subsp. *adami* parasitemia. However, as we have shown in the present study, transfer of dispersed immune spleen cells allowed SPLX recipients to clear their infection similarly to mice with intact spleens. This indicates that immune cells (present in immune spleens but absent in normal spleens) capable of suppressing recrudescent parasitemia fail to develop in the absence of an intact spleen. The present results are reminiscent of earlier studies by Playfair and colleagues (2) on the protective immunity against lethal *P. yoelii* induced by vaccination. In this study, mice vaccinated with fixed parasitized erythrocytes and *Bordetella pertussis* were protected from an otherwise lethal infection. Splenectomy prior to but not after the vaccination protocol abolished the protective effect. An intact spleen is therefore required for the induction but not the effector phase of protective immunity to these intraerythrocytic malarial parasites.

Transfer of protective immunity to SPLX recipients is abrogated by depletion of B cells, but not T cells, in the immune cells transferred. Only those SPLX mice which received immune B cells were capable of suppressing and clearing parasitemia. Our findings are consistent with the previous observations that anti-IgM-treated mice, although capable of suppressing patent parasitemia, fail to resolve *P. chabaudi adami* infections completely (5). More recently, it has been shown that both T cells and B cells must be transferred into *scid* mice for them to control and clear *P. chabaudi* AS infections (11). Since in these studies a clear presence or absence of parasite-reactive IgG corresponded to the ability or inability to resolve infections, it was concluded that anti-*P. chabaudi* IgG mediated the resolution. The inability of SPLX mice to clear parasitemia cannot be explained by the mere absence of parasite-reactive IgG. SPLX mice without a transfer or with a B-cell-depleted transfer eventually develop high titers of IgG but fail to resolve parasitemia. This implies that subtle qualitative differences exist between the antibody repertoire that develops in the presence or absence of the spleen. By Western blot analysis, no obvious difference in the antigen recognition patterns of sera from different groups of mice was detected. This is consistent with findings of a recent study which investigated the difference between protective and nonprotective antibodies to *P. yoelii* (25). It was found that when hyperimmune sera were fractionated according to isotypes, the protective fraction resided in the IgG2a fraction, which is the major complement-fixing and cytophilic IgG subclass in mice.

However, analysis of the antigen recognition patterns of protective and nonprotective isotypes by immunoprecipitation revealed identical patterns. A recent study of human populations also indicates that clinical immunity correlates with isotype distribution rather than antigen recognition patterns (1). Cytophilic IgG1 and IgG3 were associated with clinical immunity. Nonprotected subjects have either low levels of all antibody isotypes or a disproportionate representation of noncytophilic IgG2 and IgM. Furthermore, IgG from nonprotected individuals blocked the efficacy of IgG from protected individuals in an in vitro antibody-dependent cellular inhibition assay.

The significance of the differences in kinetics and distribution of IgG isotypes that we observed among different groups of mice remains conjectural. In all likelihood, the reasons for the inability of SPLX mice and SPLX mice with B-cell-depleted transfers to control and resolve infections are different. In SPLX mice, there is a generalized delay in the IgG response and protective IgG2a is not well represented. This could be due to lack of a splenic microenvironment for the appropriate cognate cellular interactions to occur. Given that the IgG response to *P. chabaudi* is absolutely T cell dependent (11, 21), a paucity of competent T-helper cells may explain the low levels of antibodies formed. It is also possible that the splenic microenvironment provides a more conducive milieu for the activation and maturation of parasite-reactive B cells. Similar to B-cell-sufficient transfer recipients, SPLX mice which received B-cell-depleted transfers develop high levels of IgG and are able to prevent the parasitemia from becoming patent. However, they are unable to clear the parasitemia. Interestingly, there is a higher representation of IgG2a in B-cell-sufficient than in B-cell-deficient transfer recipients. Since activated macrophages preferentially upregulate Fc receptors for IgG2a (3), it could be argued that IgG2a protects the mice by opsonizing parasites, while the nonprotective isotypes block the efficacy of IgG2a in the B-cell deficient transfers. However, it is equally probable that these patterns evolved as a result of the divergence in the levels of parasitemia. Further studies using in vivo depletion of specific isotypes (21) and functional characterization of these isotypes are required to resolve this question.

The differences in isotype profiles between protective and nonprotective antibody responses probably reflects T-accessory cell cytokine regulation of Ig isotype switching and selection in the B-cell compartment (18). It is known that different cytokines may skew IgG isotype responses. For instance, gamma interferon is known to induce IgG2a while interleukin 4 induces IgG1 and IgE and transforming growth factor β induces IgG2b. Thus, the persistence of IgG2a and IgG2b in protected mice probably reflects the presence of gamma interferon and transforming growth factor β, respectively. Conversely, the decrease in IgG1 may indicate that interleukin 4 production does not persist. The remarkable increase in IgG2b in B-cell-deficient transfers may indicate overproduction of transforming growth factor β. The cytokine profile of the T-cell response could have been altered because of the redistribution of immune responses to other lymphoid organs. It is known that different lymphoid organs show characteristic bias in the Th1-Th2 profiles (14). The influence of accessory cell heterogeneity in different lymphoid organs on the isotype profile of nascent and established antibody responses needs to be investigated further (17, 22).

In the absence of the spleen, certain arms of the immune response continue to be effectively expressed. The primary and subsequent peaks of parasitemia were rapidly, albeit temporarily, decreased in SPLX mice. These rapid decreases in parasitemia are thought to be induced by T-cell-dependent macrophage activation. It has been suggested that T cells of the Th1 type secrete gamma interferon and other macrophage-activating lymphokines which induce macrophages into a parasiticidal state. Consistent with this notion is the observation that these rapid decreases in recrudescent patent parasitemia are not observed in T-cell-deficient mice infected with *P. chabaudi* (11, 13, 21). It appears that this putative effector mechanism remains essentially intact in the absence of the spleen. Also, in SPLX mice which had been given B-cell-sufficient transfers, the antibody response effectively resolved the infection. It has been suggested previously that antibodies against plasmodia act by opsonization of parasitized erythrocytes and free merozoites rather than by simple neutralization (1, 7). The spleen is considered to be a major site for immune phagocytosis and parasite killing. It appears from our studies that other reticuloendothelial organs, such as the liver, effectively subsume the phagocytic and parasiticidal functions of the spleen. All this being said, there are functional deficits in SPLX mice given immune cells which could be attributed to the absence of the splenic tissue microenvironment. For instance, mice with intact spleens given immune spleen cells experience low-level parasitemia, whereas similarly treated SPLX mice suffer from higher levels of primary parasitemia. Conceivably, the transferred cells home and differentiate more efficiently in the splenic tissue. Furthermore, rechallenge of SPLX mice given immune cells results in parasitemia, which is not observed in mice with intact spleens (our unpublished data).

The liver has been shown to be a site of immune activation during malaria, especially in SPLX mice (2). Previously, Sayles et al. (16) observed mononuclear cell infiltrates in liver tissue of SPLX mice infected with *P. yoelii*. These infiltrates were observed both in mouse strains which survived and in those that died. Our findings that these aggregates were chronically present only in livers of unprotected mice indicate that they are induced by the persistence of the parasites, regardless of the level of parasitemia. It is possible that these periportal infiltrates are formed secondary to endothelial damage due to circulating immune complexes. Plasma cells were massively prominent in these infiltrates. It would be interesting to investigate whether they secrete antibodies against parasite or host determinants. Preliminary phenotypic characterization of hepatic $CD4^+$ T cells from SPLX mice show evidence of recent activation; that is, 60 to 90% of $CD4^+$ cells were CD 45 RB^{lo} (our unpublished data). We speculate that these T cells behave like Th2 type cells, secreting B-cell growth and differentiation factors which induce plasma cell hyperplasia (10). Furthermore, the chronic inflammatory and regenerative processes in the liver may induce hepatocytes and other cell types to secrete transforming growth factor β, thereby favoring the increase in IgG2b.

A seemingly parallel situation may exist in humans with respect to the role of the spleen in malaria. In a case report study of nonimmune and clinically immune, SPLX Thai patients infected with *P. falciparum* and *P. vivax*, clearance of parasitemia was delayed only in nonimmune patients. Therefore, the processes leading to parasite clearance in partially immune SPLX patients did not require the presence of an intact spleen (9).

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