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# CR3-Dependent Resistance to Acute *Toxoplasma gondii* Infection in Mice

LAWRENCE L. JOHNSON,[1]* GEORGE W. GIBSON,[2] AND PETER C. SAYLES[1]

*Trudeau Institute, Inc., Saranac Lake, New York 12983,[1] and Procter and Gamble Pharmaceuticals, Norwich, New York 13815[2]*

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**Studies were performed to determine whether resistance to acute *Toxoplasma gondii* infection in mice depends on a mechanism involving CR3, the type 3 complement receptor. Nineteen of 22 mice (86%) given multiple injections of the anti-CR3 monoclonal antibody, 5C6, prior to and after intraperitoneal inoculation of cysts of the ordinarily mildly virulent ME49 strain of *T. gondii* died within 8 to 12 days, whereas control antibody-treated mice survived. All (five of five) anti-CR3-treated BALB/c mice infected via the natural peroral route died within 8 days of infection. Flow cytometric analysis of cells recovered from peritoneal lavages of anti-CR3-treated *T. gondii*-infected mice revealed that the percentage of Thy-1$^+$ CD4$^-$ CD8$^-$ cells was reduced to about 50% of that of control antibody-treated mice and to about 20% of the number of such cells in controls. The numbers of macrophages, polymorphonuclear leukocytes, and lymphocytes recovered from the peritoneal cavities of *T. gondii*-infected mice were all reduced in anti-CR3-treated mice to about 40% of those of controls. In addition, anti-CR3-treated mice had less than 25% of the induced NK cell activity of the controls, and gamma interferon was reduced to undetectable levels. Thus, the rapid death of anti-CR3-treated mice was probably caused by impaired preimmune defenses. Histological examination of anti-CR3-treated *T. gondii*-infected mice revealed extensive liver pathology compared with that of infected mice given a control antibody or uninfected mice given anti-CR3. The inflammation, degeneration, and necrosis in most of the anti-CR3-treated mice were severe enough to account for the observed mortalities.**

Mechanisms of resistance to the protozoan parasite *Toxoplasma gondii* have received considerable attention recently owing, in part, to recognition that the parasite is a significant cause of mortality and morbidity in chronically infected AIDS patients. In addition, experimental *T. gondii* infections have proven useful as a tool with which to study preimmune mechanisms of host defense, those called into play before T- and B-lymphocyte-dependent specific immunity is acquired and expressed.

Current knowledge regarding the early stages of a *T. gondii* infection indicates that soon after inoculation of *T. gondii*, host phagocytic cells produce interleukin-12, which in turn stimulates cells to make gamma interferon (IFN-γ), which is protective (4, 5, 7, 8, 12, 21). CD4$^+$ and CD8$^+$ T cells may also become activated to produce IFN-γ (22). Exactly how the IFN-γ is protective is not completely understood. IFN-γ, together with tumor necrosis factor alpha, is important in activating macrophages to limit the proliferation of *T. gondii* tachyzoites in vitro (13, 14, 23), suggesting that a major protective function of IFN-γ may be the activation of phagocytic cells in vivo. However, IFN-γ is not believed to be essential for the generation of *T. gondii*-specific acquired immunity (1).

IFN-γ neutralization studies in vivo leave no doubt that IFN-γ is an essential component of defense early in a *T. gondii* infection (11, 24) and must be produced within the first few days of infection to be protective (11, 22). Thy-1$^+$ CD4$^-$ CD8$^-$ cells accumulate in parallel with IFN-γ within a few days after an intraperitoneal (i.p.) inoculation of *T. gondii* cysts and are needed for the production of IFN-γ (11). Because Thy-1$^+$ CD4$^-$ CD8$^-$ cells are largely responsible for *T. gondii*-induced NK cell activity (11), it is likely that *T. gondii*-induced Thy-1$^+$ CD4$^-$ CD8$^-$ cells and NK cells producing IFN-γ are highly overlapping, perhaps identical, populations.

To analyze host defense against *T. gondii* further, we investigated whether there is a component dependent on the type 3 complement receptor (CR3), a β2 integrin. CR3 possesses several functions and is expressed on macrophages and monocytes, neutrophils, some B and T cells (reviewed in reference 20), including activated CD8$^+$ T cells (15). CR3 is also expressed on human NK cells (2), which may be especially relevant to preimmune defense. CR3 mediates extravasation of cells into inflammatory sites via an interaction with ICAM-1 expressed on endothelial cells (20). CR3 also binds iC3b-coated targets (20).

In vivo studies with the anti-CR3 monoclonal antibody (MAb) 5C6 (17) have shown that a CR3-dependent mechanism is very important in combatting disseminated listeriosis (18) and is involved in the mechanism by which macrophages kill *Listeria monocytogenes* (3). However, certain in vivo responses to *Candida albicans*, *Plasmodium yoelii*, and *Mycobacterium bovis* BCG were apparently not CR3 dependent (unpublished observations cited in reference 16). Furthermore, treatment of mice with anti-CR3 did not impair their ability to resolve *Pneumocystis carinii* pulmonary infection (6). Thus it appears that resistance to various pathogens may or may not involve CR3. The present study investigates whether CR3 is involved in defense against *T. gondii.*

## MATERIALS AND METHODS

**Mice.** Female C57BL/6J and BALB/cByJ mice obtained from the Animal Breeding Facility of the Trudeau Institute were used at ages of between 8 and 12 weeks. Mice were reared under barrier-sustained conditions and were free of the common viral pathogens of mice as judged by periodic serological screening.

***T. gondii*.** Cysts of mildly virulent *T. gondii* ME49 were produced and prepared for inoculation as previously described (9).

**MAb treatments.** Anti-IFN-γ (R4-6A2, HB 170), anti-CR3 (5C6 [17]), or a control MAb (LTF6.1, anti-KLH) produced in this laboratory was administered

* Corresponding author. Mailing address: Trudeau Institute, Inc., Saranac Lake, NY 12983. Phone: (518) 891-3080. Fax: (518) 891-5126.

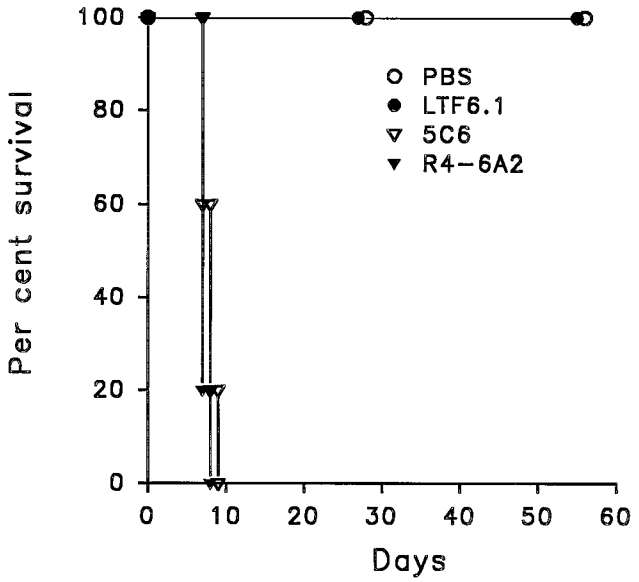


FIG. 1. Survival of B6 mice treated with PBS, anti-CR3 (5C6, see the text), an isotype matched control antibody (LTF6.1), or anti-IFN-γ (R4-6A2, $2 \times 10^4$ neutralizing units) and inoculated i.p. with 20 ME49 cysts (five mice per treatment group).

to mice, at the times and in the amounts indicated below, by i.p. injection. Each of these antibodies was partially purified from ascite fluids by DEAE fractionation.

**Flow cytometry.** Cells were obtained from peritoneal lavages (3 ml per mouse) as described elsewhere (11). Unfractionated populations were stained with FITC-conjugated $F(ab')_2$ antibodies specific for CD4 (GK1.5, ATCC TIB 207), CD8 (2.43, ATCC TIB 210), or Thy-1.2 (30H12, TIB 107). Antibodies were conjugated with FITC at this institute. Staining of $NK1.1^+$ cells was performed with a commercially obtained phycoerythrin-conjugated MAb (PK136; Pharmingen). Cytofluorometric analyses were conducted as described previously (11). The number of cells staining positively for each marker was determined by multiplying the percentages of positively stained cells in a sample by the total number of nucleated cells in the peritoneal lavage fluids from each mouse. Analysis of cells expressing NK1.1 and Thy-1 was performed with populations gated to exclude autofluorescent cells.

**Cytocentrifuge preparations.** Cells recovered from the peritoneal lavages were cytocentrifuged (Shandon, Inc.) onto slides and stained with Diff-Quik (Baxter) to determine the percentages of macrophages, polymorphonuclear leukocytes, and lymphocytes.

**NK cell assay.** The NK cell activity present in peritoneal lavage cell populations was assayed against $^{51}Cr$-labelled YAC-1 target cells in a 4-h assay as described previously (11), except that $5 \times 10^3$ labelled target cells were used in each well rather than $10^4$. In order to compare the total lytic activity per animal among various groups, recovered peritoneal lavage cells were suspended in a fixed volume of medium prior to progressive fourfold dilution and addition to the assay wells. Thus, the percentages of $^{51}Cr$ release are estimates of the relative cytolytic capacity on a per host basis. In addition, calculations were made with the three point titration values to obtain the number of lytic units of cytolytic activity both per cell and per host. For these calculations, 1 lytic unit was defined as the number of peritoneal lavage cells need to achieve a 10% specific $^{51}Cr$ release from $5 \times 10^3$ YAC-1 targets. These values are reported and discussed below.

**IFN-γ assay.** IFN-γ in peritoneal lavage fluids was quantified by enzyme-linked immunosorbent assay as described previously (11). A laboratory standard preparation of IFN-γ, used to produce a reference absorbance curve in each assay, has been calibrated in a bioassay against National Institutes of Health murine IFN-γ research standard. Thus, the amounts of IFN-γ are reported as units of antiviral activity, with 20 U of antiviral activity equivalent to 1 ng of IFN-γ. The number of units per host was calculated by multiplying the number of units per milliliter in lavage fluids by the volume of fluid used to lavage each mouse. The limit of detection in this assay is approximately 0.5 U per ml.

**Histology.** Groups of five B6 mice were administered 0.5 mg of MAb (anti-CR3 or control) 6 h before and 3 and 6 days after i.p. injection of 20 ME49 cysts. Tissues (lung, liver, heart, kidney, and brain) taken 8 days after inoculation of cysts were fixed in neutral-buffered formalin. Fixed tissues were embedded in paraffin and 4-μm sections were stained with hematoxylin and eosin by routine procedures. Slides were examined by one of us (G.W.G.) without knowledge of the treatment groups to which they belonged. Slides were scored on an ordinal scale with regard to the degree of inflammation (0, no inflammation; 1, trace; 2, slight; 3, mild; 4, moderate; and 5, severe) as well as for extent of tissue degeneration and cellular necrosis.

**Statistics.** Group means (number of cells, and amounts of IFN-γ) were compared by a *t* test. Variability is expressed as standard deviations. The durations of survival were compared by the Mann-Whitney test.

## RESULTS

**Early death of mice treated with anti-CR3 prior to and during infection with *T. gondii*.** Groups of five B6 mice were treated with 1 mg of anti-CR3 or a control MAb or phosphate-buffered saline (sham infected) (PBS) 1 day before inoculation of 20 ME49 cysts i.p. Antibody treatments (0.5 mg per mouse) were repeated on day 3 and day 5 postinoculation. An additional control group of mice received a single injection of $2 \times 10^4$ neutralizing units of anti-IFN-γ 1 day before cyst inoculation. The cumulative survival of the mice in each group is shown in Fig. 1, which is representative of four similar experiments. The compiled results from these experiments showed that only 3 of 22 anti-CR3-treated mice survived beyond day 10 of infection whereas only 3 of 18 controls died before day 35. Thus, anti-CR3 treatment significantly ($P < 0.01$) shortened the duration of survival of *T. gondii*-infected mice to a degree similar to that of mice given an IFN-γ-neutralizing antibody. It should be noted that the effect of anti-CR3 was to exacerbate the *T. gondii* infection rather than simply to render the mice more susceptible to death from another cause, because none of the nine uninfected mice treated with anti-CR3 died during the course of the experiments.

Because we were interested in sampling cells and cytokines at the site at which infections were initiated, most of the experiments described herein were conducted with mice inoculated with cysts i.p. However, it was deemed important to investigate the effect of anti-CR3 treatment on mice infected by the natural peroral route. All (5 of 5) BALB/c mice inoculated perorally with *T. gondii* cysts and treated with anti-CR3 died within 8 days, whereas 10 of 10 infected mice given either PBS or an irrelevant control MAb survived in apparently good health to the end of the experiment on day 38. In an additional experiment, two or five anti-CR3-treated B6 mice inoculated perorally with 10 ME49 cysts died within 1 week, whereas all (five of five) control MAb-treated mice survived until the ex-

TABLE 1. Cellular composition of peritoneal lavages of B6 mice treated with anti-CR3 and infected with *T. gondii* cysts

| Treatment[a] | No. of cells, $10^5$ (%) | | | |
|---|---|---|---|---|
| | Total | $CD4^+$ | $CD8^+$ | $Thy\text{-}1^+$ $CD4^-$ $CD8^-$ |
| PBS | 50 ± 12 | 3.4 ± 1.2 (6.8 ± 0.10) | 2.5 ± 1.4 (4.8 ± 1.6) | 6.2 ± 1.9 (12.4 ± 1.0) |
| Control MAb | 48 ± 5 | 2.7 ± 0.2 (5.7 ± 0.66) | 1.5 ± 0.1 (3.2 ± 0.47) | 4.6 ± 1.1 (9.5 ± 0.8) |
| Anti-CR3 | 16 ± 4[b] | 0.5 ± 0.2[b] (3.1 ± 0.69) | 0.5 ± 0.2[b] (2.9 ± 0.50) | 0.8 ± 1.4[b] (4.8 ± 1.4)[b] |

[a] MAb treatments of 1.0 mg per mouse on day −1 and 0.5 mg per mouse on day +3. Peritoneal lavages performed on day 4 after inoculation of 20 ME49 cysts i.p. N = 4 mice per group.

[b] Significantly ($P < 0.05$) lower than the corresponding value for control MAb-treated mice.

TABLE 2. Effect of anti-CR3 on the number of leukocytes in peritoneal lavages of *T. gondii*-infected B6 mice

| Treatment[a] | No. of cells, $10^6$ (%) | | |
|---|---|---|---|
| | Macrophages | PMN[b] | Lymphocytes |
| Control MAb | 3.66 ± 1.21 (47.3 ± 3.2) | 0.60 ± 0.32 (7.67 ± 2.31) | 3.57 ± 1.54 (44.7 ± 4.16) |
| Anti-CR3 | 1.54 ± 0.46 (47.3 ± 16.6) | 0.21 ± 0.18 (6.00 ± 5.29) | 1.53 ± 0.46 (46.0 ± 11.0) |

[a] Treatments of 1.0 mg of MAb per mouse and 0.5 mg of MAb per mouse were administered 1 day before and 3 days after infection with *T. gondii*, respectively. Peritoneal lavages were performed on day 4 after i.p. inoculation of 20 ME49 cysts (three mice per group).
[b] PMN, polymorphonuclear leukocytes.

periment was terminated 5 weeks later. Moreover, the three surviving anti-CR3-treated mice weighed significantly ($P < 0.05$) less than did control MAb-treated mice at day 7 of infection (14.7 ± 0.29 g versus 16.7 ± 1.7 g, respectively). The body weights of the anti-CR3-treated mice remained about 10% less than the weights of control MAb-treated mice throughout the experiment. Also, the mean number of cysts per brain in the anti-CR3-treated B6 survivors was about 50% more than that in controls (3,000 ± 800 versus 2,040 ± 754), although this difference was not statistically significant by *t* test.

**Effect of anti-CR3 on the composition of cell populations in peritoneal lavages of *T. gondii*-infected mice.** The survival data shown in Fig. 1 suggest that the effect of anti-CR3 may be to impair a preimmune resistance mechanism. Studies in this laboratory have shown that Thy-$1^+$ $CD4^-$ $CD8^-$ cells associated with the production of IFN-γ and with NK cell activity are induced in the peritoneal cavities of mice infected i.p. with *T. gondii*. Moreover, depletion of Thy-$1^+$ $CD4^-$ $CD8^-$ cells, or asialo-$GM1^+$ cells, or neutralization of IFN-γ results in very early death (11) (Fig. 1), whereas depletion of $CD4^+$ and $CD8^+$ T lymphocytes does not cause death until later (11). Thus, it was of interest to determine whether anti-CR3 treatment of *T. gondii*-infected mice impaired the production of cells or a cytokine known to be essential for the survival of mice beyond the first week or so of infection.

Table 1 summarizes the percentages and numbers of cells in peritoneal lavage fluids at 4 days postinoculation of 20 ME49 cysts i.p. in B6 mice treated with anti-CR3 or given control MAb injections 1 day prior to and 3 days after infection. Four days corresponds to a peak period of induction of preimmune cells and cytokines in B6 mice inoculated i.p. with ME49 cysts (11). It is clear that anti-CR3 treatment reduced the percentage of Thy-$1^+$ cells bearing neither the CD4 nor CD8 marker (i.e., Thy-$1^+$ $CD4^-$ $CD8^-$ cells) by about 50% and the absolute number of such cells by about 80%. Comparable results were obtained in four similar experiments, each with four or five B6 mice per treatment group.

To determine directly whether the Thy-$1^+$ $CD4^-$ $CD8^-$ cells that were reduced in number by anti-CR3 treatment were NK cells, groups of three *T. gondii*-infected B6 mice were treated with anti-CR3 antibodies or the control antibody as described above, and the cells recovered from their peritoneal cavities on day 4 of infection were stained with antibodies to detect Thy-$1^+$ and $NK1.1^+$ cells. The absolute number of Thy-$1^+$ $NK1.1^+$ cells in anti-CR3-treated mice was reduced by about 70%, from $18.7 \times 10^4 \pm 6.5 \times 10^4$ to $5.73 \times 10^4 \pm 0.45 \times 10^4$. Virtually none of the Thy-$1^+$ $NK1.1^+$ cells stained positively for either $CD4^+$ or $CD8^+$ T-cell markers (data not shown).

Cytocentrifuge preparations were also made from cells from these mice. Table 2 shows the percentages and absolute numbers of leukocytes for the two groups. Whereas the relative proportions of leukocyte subpopulations were nearly identical in the anti-CR3-treated and control groups, the absolute numbers of cells in each subpopulation were reduced by about 60% in the anti-CR3-treated group.

**Anti-CR3 treatment reduces induced NK cell cytolytic activity and IFN-γ levels in *T. gondii*-infected mice.** Additional correlates of preimmune resistance to *T. gondii* are the induction of NK cell cytolytic activity and IFN-γ. The results of experiments to determine the effects of anti-CR3 on these parameters are shown in Table 3. B6 mice treated with anti-CR3 1 day before and 3 days after inoculation of ME49 cysts possessed cytolytic activity against NK-sensitive YAC-1 targets which was reduced to a level equivalent to that of control mice cells diluted between 4- and 16-fold (Table 3, group A versus group C); i.e., the levels of cytolytic activities in anti-CR3-treated mice were reduced to between 6 and 25% of those in the controls. Comparable reductions were observed in three additional experiments. However, it should be noted that anti-CR3 treatment did not completely eradicate anti-YAC activity (Table 3, group C versus group B or D).

As described in Materials and Methods, the number of lytic units of cytolytic activity was also calculated. From the data shown in Table 3 for groups A and C, anti-CR3 treatment reduced the geometric mean of lytic units per $10^6$ cells to about 50% of that for the controls (75.9 versus 40.5 for groups A and C, respectively). Also, in agreement with that which was found by direct comparison of the percentages of $^{51}Cr$ release for groups A and C, the geometric mean of lytic units per anti-CR3-treated host was calculated to be less than 25% of that of the controls (565 and 133 for groups A and C, respectively). Thus, anti-CR3 treatment reduced the cytolytic capacity of *T. gondii*-infected mice on both a per cell and per host basis.

The levels of IFN-γ in peritoneal lavage fluids of MAb-treated, *T. gondii*-infected B6 mice were also quantitated. Anti-CR3 reduced IFN-γ to below detectable levels whereas the levels in *T. gondii*-infected mice given PBS or a control MAb were 34.0 ± 20 or 24.8 ± 8 U per host, respectively. No IFN-γ was found in anti-CR3-treated infected B6 mice in three additional experiments. Thus anti-CR3-treatment severely re-

TABLE 3. NK cell activity in peritoneal lavage fluids of anti-CR3-treated B6 mice infected with ME49 cysts

| Group | Treatment | % $^{51}Cr$ release at E-T ratio of[a]: | | |
|---|---|---|---|---|
| | | 20:1 | 5:1 | 1.25:1 |
| A | Control MAb, ME49 | 48.1 ± 9.3 | 23.0 ± 9.7 | 8.3 ± 4.7 |
| B | Control MAb (sham infection) | 3.5 ± 2.9 | 1.5 ± 0.9 | 0.9 ± 1.0 |
| C | Anti-CR3, ME49 | 14.6 ± 8.3[b] | 7.6 ± 4.9[b] | 1.5 ± 0.5[b] |
| D | Anti-CR3 (sham-infection) | 4.0 ± 0.4 | 2.8 ± 0.5 | 0.8 ± 0.5 |

[a] An effector/target (E-T) ratio of 20:1 was calculated for group A. Samples for the remaining groups were diluted to same volume as that for group A. The assays were performed on day 4 after i.p. inoculation of 20 ME49 cysts (three mice per group).
[b] Significantly ($P < 0.05$) lower than the corresponding value for group A.

TABLE 4. Effect of a single injection of anti-CR3 on day 0 or day 4 of infection on parameters of preimmune resistance

| Treatment and day[a] | No. of Thy-1$^+$ CD4$^-$ CD8$^-$ cells ($10^4$) | IFN-γ (U) | % $^{51}$Cr release of E-T ratio of: | | |
|---|---|---|---|---|---|
| | | | 20:1 | 5:1 | 1.25:1 |
| Control MAb, day 0 | 49.6 ± 3.7 | 12.5 ± 7.7 | 38.5 ± 6.4 | 15.0 ± 2.9 | 5.3 ± 1.7 |
| Anti-CR3 | | | | | |
| Day 0 | 21.4 ± 4.2 | 7.4 ± 0.8 | 23.0 ± 3.0 | 7.3 ± 3.1 | 1.5 ± 1.5 |
| Day 4 | 11.4 ± 3.5 | <1 | 15.5 ± 5.0 | 4.5 ± 2.6 | 1.8 ± 1.3 |
| Day 0, uninfected mice | 7.4 ± 4.0 | <1 | 1.9 ± 1.6 | 0.5 ± 0.4 | 0.6 ± 0.7 |

[a] Assays were performed on day 5 after infection of 20 ME49 cysts i.p. (four mice per group). Single injections of 1.0 mg of MAb were administered on the indicated days postinfection. All tabulated values for each anti-CR3-treated group are significantly ($P < 0.05$) lower than the corresponding values for the control MAb-treated group.

duces the ability of mice to produce IFN-γ, a cytokine essential for survival, in response to *T. gondii* inoculated into the peritoneal cavities.

**Effect of administering anti-CR3 to *T. gondii*-infected mice at a time when components of preimmune resistance have already been induced.** In *T. gondii*-infected mice, if CR3 functions only to allow cells to extravasate and enter the peritoneal cavity, then the treatment of mice with anti-CR3 at a time when agents of preimmune resistance (Thy-1$^+$ CD4$^-$ CD8$^-$ cells and IFN-γ) have already accumulated in the peritoneal cavity (day 4 postinoculation) would be expected to produce little effect on the number of relevant cells, cytolytic activity, or IFN-γ levels, assuming that the rate of turnover of cells or cytokine is relatively low. (It should be remembered that the anti-CR3 antibody used in these studies is not believed to be cell depleting [17].) Alternatively, if CR3 functions in the cytolytic pathway of NK cells, perhaps by promoting binding of appropriately activated NK cells to sensitive targets (see the discussion in reference 20), or if CR3 transduces a signal needed for upregulation of IFN-γ in NK cells, then anti-CR3 treatment of mice already infected would impair NK-mediated cytolysis and perhaps reduce IFN-γ levels.

To investigate these possibilities, B6 mice were inoculated with 20 ME49 cysts i.p. on day 0 and given a single injection of 1 mg of anti-CR3 6 h before or 4 days after cyst inoculation. On day 5, peritoneal cavities were lavaged, and the recovered cells and fluids were analyzed for Thy-1$^+$ CD4$^-$ CD8$^-$ cells, NK cell activity, and IFN-γ. The results (Table 4) demonstrate that an injection of anti-CR3 on day 4 postinoculation of cysts reduced the numbers of Thy-1$^+$ CD4$^-$ CD8$^-$ cells and the NK cytolytic activity per host by about 80% compared with those of controls that received a control antibody and reduced IFN-γ to undetectable levels.

**Histopathology.** Tissues from *T. gondii*-infected B6 mice treated with anti-CR3 as described in Materials and Methods were compared histologically with those from controls to characterize the effects of anti-CR3 further. Uninfected control mice given only anti-CR3 were essentially histologically indistinguishable from untreated mice (data not shown). *T. gondii*-infected mice given an isotype-matched control antibody exhibited mild inflammation in all tissues examined, except liver tissues, that did not differ markedly from the inflammation in anti-CR3-treated infected mice. The most dramatic difference between the anti-CR3-treated infected group (group A) and the control MAb-treated infected group (group B) was in the liver. Both groups had prominent inflammatory infiltrates consisting of multifocal aggregates of mononuclear cells. However, the affected areas were less localized in group A mice, and bridging between the aggregates of inflammatory cells was more common, as was the Kupffer cell hypertrophy. Four of the five group A mice had hepatocellular degenerative changes, including cell swelling and vacuolization. Only one animal in the control group had these changes. The mice in group A had a disrupted hepatic cord architecture, with frequent mitoses indicative of regenerative activity. In addition, three of the five group A animals had patchy areas of frank hepatocellular necrosis. A tabular summary of the histopathological findings for the liver tissues is given in Table 5, and illustrative photomicrographs are shown in Fig. 2. Together, these changes were deemed sufficient to account for the difference in mortality rates between the two groups.

## DISCUSSION

The results presented here establish an important role for CR3 in resistance to a primary i.p. or peroral infection with *T. gondii* in B6 mice. Moreover, BALB/c mice, a strain which is innately more resistant to chronic ME49 infection than is B6, died within 8 days after peroral inoculation of ME49 cysts when treated with anti-CR3. Thus, the results presented here very likely apply to other strains of mice infected via the peroral or i.p. route.

Ongoing studies involve the examination of whether CR3-dependent mechanisms function protectively in already established chronic infections or in resistance to a vaccine strain of *T. gondii* (ts-4) which is avirulent for immunocompetent mice but lethal for lymphocyte-deficient mice. To date, there is little evidence that CR3 figures significantly in either of these models; however, additional studies are needed before this conclusion can be stated confidently.

The effect of anti-CR3 on CD4$^+$ and CD8$^+$ T cells (Table 1)

TABLE 5. Summary of liver histopathologies

| Group and animal no.[a] | Inflammation score[b] | Hepatocellular pathology[c] | | Kupffer cell hypertrophy[c] |
|---|---|---|---|---|
| | | Degeneration | Necrosis | |
| A | | | | |
| 1 | 5 | + | +++ | + |
| 2 | 5 | + | ++ | + |
| 3 | 3 | − | − | − |
| 4 | 5 | + | ++ | + |
| 5 | 4 | + | − | − |
| B | | | | |
| 1 | 3 | − | − | − |
| 2 | 3 | − | − | − |
| 3 | 3 | − | − | − |
| 4 | 3 | + | − | − |
| 5 | 4 | − | − | + |

[a] The animals in group A were treated with anti-CR3 and infected with ME49 cysts as described in Materials and Methods. The animals in group B were treated with the control MAb and infected with ME49 cysts.

[b] Degree of inflammation: 5, severe; 4, moderate; 3, mild.

[c] −, none; +, slight; ++, moderate; +++, severe.

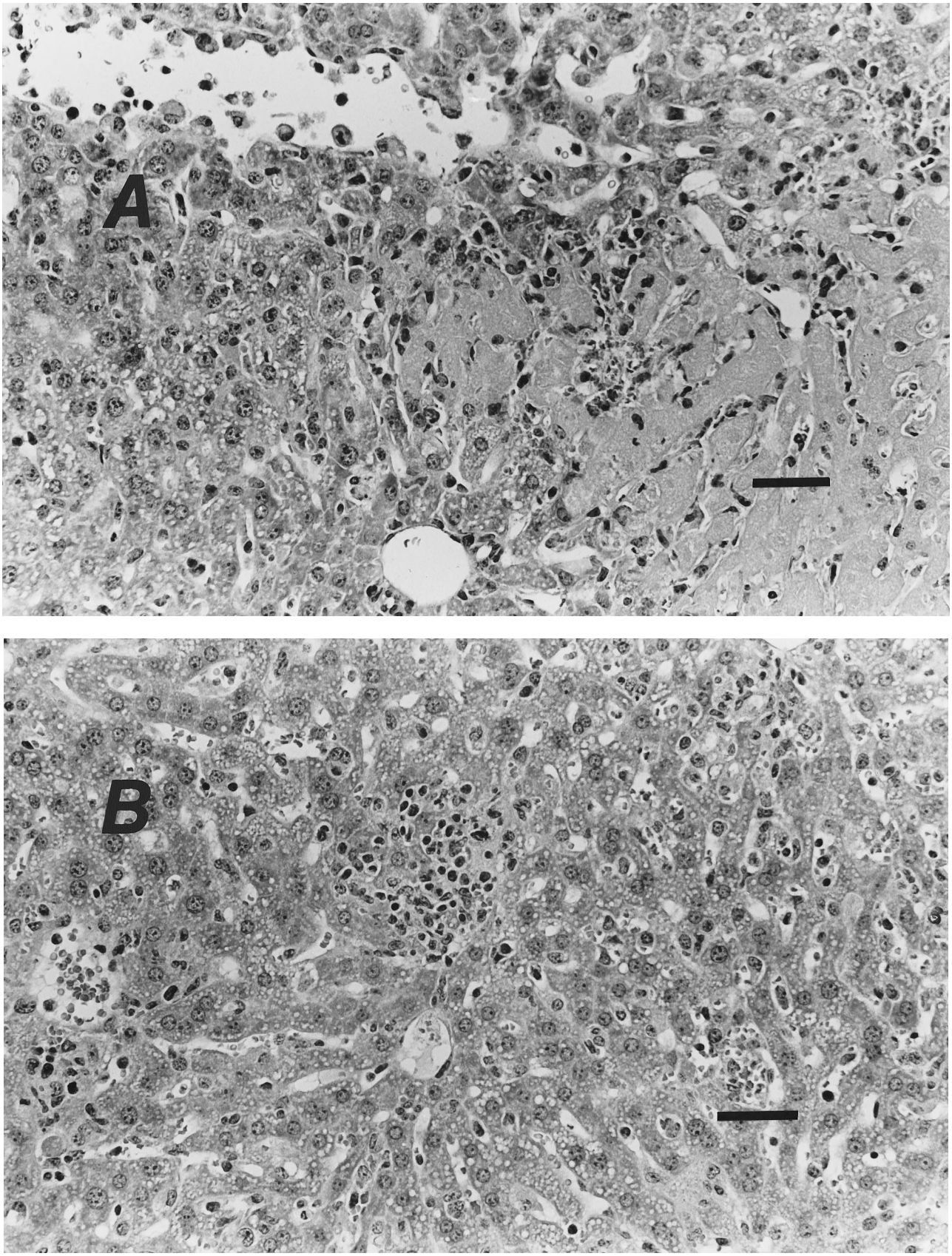


FIG. 2. Representative sections of liver tissues from a *T. gondii*-infected mice treated with anti-CR3 (A) and a control antibody (B). In panel A, hepatic damage is extensive. There is a midzonal area of coagulation necrosis. Adjacent hepatocytes show degenerative vacuolar change with disorganized hepatic cords. There is a diffuse inflammatory infiltrate focally more severe at the margin of the area of necrosis. Note the margination of leukocytes within the vascular space. In panel B, hepatic damage is mild. Inflammatory cells are confined in multifocal areas, and the hepatic cord architecture is largely preserved. Bars, 50 μm.

suggests that treatment with this antibody may also impair the generation or expression of T-cell-dependent acquired immunity. Although this possibility remains to be determined, in a single preliminary experiment groups of five B6 mice vaccinated with ts-4 tachyzoites and treated with three injections of anti-CR3 5 weeks later were fully resistant to a challenge with highly virulent tachyzoites (RH strain), a strain which kills all unimmunized mice within 7 to 10 days but to which ts-4-immunized mice are resistant. Thus we found little in this experiment to suggest that CR3 is involved in acquired resistance to *T. gondii.*

Although it is clear that CR3 is involved in host defense against acute *T. gondii* infection, the basis for its protective effect has not been identified. The experiment for which the results are summarized in Table 4 begins to address this issue. The results indicate that anti-CR3 adversely affects preimmune resistance components even after NK cells and IFN-γ have accumulated at the site of inoculation (Table 2 and the text). This effect could be due to the direct elimination of induced NK cells and therefore the IFN-γ that they produce. However, the investigators who studied the 5C6 MAb reported no evidence of cell death in the bone marrow, livers, or spleens of 5C6-treated mice, although NK cells were not specifically examined in that study (17).

There is compelling evidence that the 5C6 MAb inhibits inflammatory cell recruitment in vivo (17, 19), and the results reported in Table 2 are consistent with this finding. Thus, it is possible that the numbers of cells could be reduced after anti-CR3 treatment solely from an effect on recruitment, provided that the rate of turnover of relevant cells is high. Indirect evidence that this may be the case is provided by the finding that a single injection of anti-CR3 prior to the inoculation of cysts is insufficient to cause the death of *T. gondii*-infected mice (10) and does not result in reduction of NK cell or IFN-γ activity (Table 4) as extensive as that with repeated injections (Table 2 and the text). Yet a single injection is sufficient to reduce these components extensively, provided that they are assayed soon after the antibody is administered (Table 4). This finding suggests that a single injection of anti-CR3 is ineffective not because it contains an insufficient amount of antibody but instead because there is rapid cell replenishment by the host. This argument is speculative, however, and will require experiments that determine the rate of cell turnover directly to establish its validity.

An indirect effect of anti-CR3 on NK function may occur via an effect on macrophages. The observation that the absolute number of macrophages in the peritoneal cavities of anti-CR3-treated mice is reduced suggests that a smaller amount of interleukin-12 may be produced in these mice. If this is so, it may result in reduced production of IFN-γ by NK cells and a corresponding increase in susceptibility to *T. gondii.*

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