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## Pathological Changes in the Spleens of Gamma Interferon Receptor-Deficient Mice Infected with Murine Gammaherpesvirus: a Role for CD8 T Cells

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**Murine gammaherpesvirus is a natural rodent pathogen which causes a primary infection in the lungs and establishes a persistent infection in B lymphocytes. During the primary infection, large amounts of gamma interferon (IFN- $\gamma$ ) are produced by spleen, mediastinal, and cervical lymph node cells. To investigate the role of IFN- $\gamma$  in control of the virus infection, mice lacking the cellular receptor for IFN- $\gamma$  (IFN- $\gamma$  R $^{-/-}$  mice) were infected with murine gammaherpesvirus 68 (MHV68). IFN- $\gamma$  R $^{-/-}$  mice showed no difference from wild-type mice in the titers of infectious virus in the lungs or in the rate of clearance of the lung infection. In the spleen, however, clear differences were observed. By 14 days postinfection, spleens from IFN- $\gamma$  R $^{-/-}$  mice were pale, shrunken, and fibrous. Histological examination showed that there was an early (day 10) infiltration of granulocytes followed by widespread destruction of splenic architecture (days 14 to 17). A marked decrease in the number of splenic B cells and CD4 $^{+}$  and CD8 $^{+}$  T cells occurred. These changes were accompanied by a 10- to 100-fold greater load of latently infected cells in IFN- $\gamma$  R $^{-/-}$  mice than in wild-type mice at 14 to 17 days postinfection, but this was reduced to the levels found in wild-type mice by 21 days postinfection. Treatment of the mice with the antiviral drug 2'-deoxy-5-ethyl-beta-4'-thiouridine from 6 days postinfection did not prevent the occurrence of these changes. The changes were, however, completely reversed by depletion of CD8 $^{+}$  T cells prior to and during the primary infection. Depletion of CD4 $^{+}$  T cells also reversed the major pathological and virological changes, although in this case there was evidence of some histological changes. Thus, the lack of IFN- $\gamma$  receptor had profound consequences in spleens of MHV68-infected mice. The possible mechanisms involved in these changes are discussed.**

Murine gammaherpesvirus 68 (MHV68) is a type 2 gamma-herpesvirus which was isolated from a free-living murid rodent (2) and which can be readily studied in laboratory mice. As such, it provides a useful model for the study of a gammaherpesvirus in its natural host. After intranasal inoculation, the virus establishes a productive infection in the lungs, which is cleared in 7 to 10 days. Replicating virus can be detected in other tissues, including the spleen, adrenal glands, the heart, and the kidney, for up to 15 days after infection, but thereafter no infectious virus can be detected (20). The virus causes a transient marked splenomegaly apparent 12 to 14 days after infection and establishes a latent infection in B lymphocytes (21). Latent virus can also be detected in lung tissue long after clearance of infectious virus (24).

Clearance of infectious virus from the lung is dependent on the presence of CD8 $^{+}$  T cells (8), and there is evidence for a role of CD8 $^{+}$  T cells in the control of latently infected B cells in the spleen (14). CD4 $^{+}$  T cells also play a pivotal role in control of infection. CD4 $^{+}$  T cell depleted animals show a slightly delayed clearance of lung infection (23). However, MHV68-infected major histocompatibility complex (MHC) class II  $^{-/-}$  mice which lack CD4 $^{+}$  T cells initially clear the lung infection, but it recurs at around 30 days postinfection and the animals eventually succumb to the virus infection (4). CD4 $^{+}$  T cells are essential for the development of splenomegaly and for the generation of high numbers of latently infected cells, which are found at this stage of infection (4, 23). In the

absence of CD4 $^{+}$  T cells, the total number of latently infected cells does not reach the levels found in immunocompetent mice; however, over a 130-day period CD4-deficient mice have higher numbers of latently infected cells in spleen, mediastinal lymph nodes, and cervical lymph nodes than wild-type infected mice (4).

Cells from the spleens and lymph nodes draining the lungs of MHV68-infected mice have been shown to produce high levels of interleukin 6 (IL-6) and gamma interferon (IFN- $\gamma$ ) and lower levels of IL-2 and IL-10 (17). CD4 $^{+}$  T-cell deficient, class II  $^{-/-}$  mice produce significantly lower levels of IFN- $\gamma$  than wild-type MHV68-infected mice, implicating IFN- $\gamma$  as a major player in the CD4 $^{+}$  T-cell-mediated control of MHV68 infection (4). Numerous reports indicate that IFN- $\gamma$  plays an important role in the control of other herpesvirus infections (3, 10, 12, 18, 19). In order to define the apparent role of IFN- $\gamma$  in the control of MHV68 infection, we have utilized the gene knockout IFN- $\gamma$  receptor-negative (IFN- $\gamma$  R $^{-/-}$ ) mouse strain (11). These mice lack the  $\alpha$  chain of the IFN- $\gamma$  receptor and hence the ability to respond to IFN- $\gamma$ , although they retain the ability to produce IFN- $\gamma$ . Our results indicate that IFN- $\gamma$  is not important in the clearance of the initial lung infection or in the long-term surveillance in the lungs, but the lack of IFN- $\gamma$  receptor has major implications for the maintenance of normal splenic architecture and plays a major role in the control of the infection in the spleen.

### MATERIALS AND METHODS

**Mice.** Wild-type 129/Sv/Ev mice and IFN- $\gamma$  R $^{-/-}$  129/Sv/Ev mice (11) were purchased from Bantin and Kingman and bred in-house.

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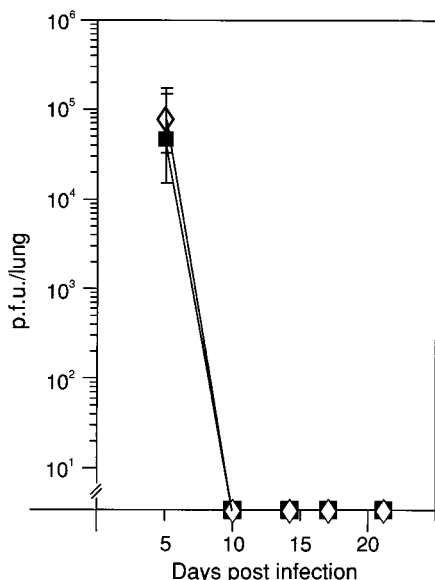


FIG. 1. MHV68 replication in the lungs of wild-type 129/Sv/Ev and IFN- $\gamma$  R $^{-/-}$  mice. Mice were inoculated with  $4 \times 10^5$  PFU of virus intranasally. Mean virus titer/lung  $\pm$  standard deviation for four mice/group is shown for each time point. ■, wild type; ◇, IFN- $\gamma$  R $^{-/-}$ . The limit of detection was 10 PFU/lung.

**Virus and cells.** Virus working stocks were prepared by infection of BHK-21 cells with MHV68 clone G2.4 (2, 7) at low multiplicity (0.1 PFU/cell) as previously described (20).

**Infection and sampling.** Age- and sex-matched mice were anesthetized with Halogen (Rhone Merieux Ltd., Harlow, Essex, United Kingdom) and inoculated intranasally with  $4 \times 10^5$  PFU of MHV68 in 40  $\mu$ l of sterile phosphate-buffered saline. At various times after infection, mice were killed by cervical dislocation and tissues were harvested for virus assays or histology.

**Virus assays.** Virus stocks were assayed on BHK-21 cell monolayers as previously described (20). For determination of virus titers in tissues, the tissues were frozen at  $-70^\circ\text{C}$ , thawed and homogenized, and then frozen again at  $-70^\circ\text{C}$ . After thawing, the homogenates were centrifuged to remove particulate matter and titers were determined on BHK-21 monolayers as described for virus stocks. For detection of latent virus, an infective center assay was carried out. Spleen cell suspensions were prepared by teasing cells out of the capsule, erythrocytes were lysed by brief resuspension in water, and the lymphocytes were cocultivated with BHK-21 cells for 5 days. Cell monolayers were fixed and stained with toluidine blue, and the numbers of splenocytes giving rise to plaques were determined. For detection of infectious virus in splenocytes, single-cell suspensions, prepared as for the infective-center assays, were either frozen at  $-70^\circ\text{C}$  or disrupted by sonication, clarified by centrifugation, and assayed on BHK-21 monolayers.

**Flow cytometry.** Splenic lymphocytes were stained with monoclonal antibodies directed against B220 (RA3-6B2; Pharmingen), CD4 (YTS177.9.6.1), or CD8 (YTS105.18.10) as previously described (8). Stained cells were analyzed on a FACScan flow cytometer (Becton Dickinson).

**In vivo inhibition of virus replication.** At six days postinfection, mice were injected with 1 mg of 2'-deoxyl-5-ethyl-beta-4'-thiouridine (4'-S-EtdU) (21a) in water and thereafter supplied ad libitum with drinking water containing 0.33  $\mu$ g of drug per ml.

**Depletion of CD4<sup>+</sup> and CD8<sup>+</sup> T cells.** Rat immunoglobulin G2b monoclonal antibodies YTS 191.1 (anti-CD4) and YTS 169.4 (anti-CD8) were used for depletion of lymphocyte subsets as previously described (6, 8). Briefly, the antibodies were injected intravenously 2 days prior to infection and 2 days postinfection and then at weekly intervals intraperitoneally.

**Histopathological studies.** Portions of spleen were fixed in buffered Formol saline, processed routinely to 5- $\mu$ m paraffin wax-embedded sections, stained with hematoxylin and eosin, and examined by light microscopy.

## RESULTS

**Replication of MHV68 in the lungs of IFN- $\gamma$  R $^{-/-}$  mice.** Wild-type 129/Sv/Ev mice and IFN- $\gamma$  R $^{-/-}$  mice were infected intranasally with  $4 \times 10^5$  PFU of MHV68. The lungs were examined for the presence of infectious virus at various times after infection. Figure 1 shows the titers of infectious virus in

the lungs of wild-type and IFN- $\gamma$  R $^{-/-}$  mice. No difference in the rates of clearance of virus from the lungs of the two groups of animals was observed.

**Effect of MHV68 infection on spleen cell populations and splenic architecture.** Early after MHV68 infection, it was clear that there were major differences in the effects of virus infection on the spleens of wild-type and IFN- $\gamma$  R $^{-/-}$  mice. On day 14 postinfection, it was noted that the spleens of the IFN- $\gamma$  R $^{-/-}$  mice were pale and shrunken, and these changes became more pronounced as the infection progressed (Fig. 2). The effect of MHV68 infection on the splenic architecture in wild-type and IFN- $\gamma$  R $^{-/-}$  mice was examined histologically. From day 10 postinfection, spleens from wild-type mice showed moderate white pulp hyperplasia. Follicles, periarteriolar areas, and marginal zones showed increased cellularity and expansion. Lymphoblasts and mitotic figures were apparent in these regions, and there were also scattered foci of apoptotic bodies and nuclear debris. Red pulp areas were largely unaffected except for some mild hyperplasia of splenic cords (Fig. 3a). By day 21 postinfection, white pulp hyperplasia was less pronounced, and thereafter there were decreasing signs of activation in the spleen (Fig. 3b). The IFN- $\gamma$  R $^{-/-}$  spleens showed some white pulp hyperplasia at day 10 postinfection, but by day 14 and beyond, changes were distinctly different. There was a marked, diffuse and focal infiltrate of granulocytes into the white pulp areas, especially the periarteriolar sheath (Fig. 3c). By day 17 postinfection, early fibroplasia with plump fibroblasts and collagenous matrix deposition in peripheral white pulp and all red pulp areas was obvious. By day 21 postinfection, lesions consisted of substantial areas of more mature fibrosis admixed with fewer granulocytes and splenic cord cells. These lesions dominated the splenic tissue and surrounded multiple, small focal aggregates of surviving B-cell areas of white pulp. In one sample at day 31 there were focal areas of bony metaplasia within the fibrotic areas (Fig. 3d). The virus-induced histological changes progressed no further after day 21 but, unlike the findings for the wild-type spleens, the changes were not reversible.

The decreased cellularity in the infected IFN- $\gamma$  R $^{-/-}$  spleens was reflected in the numbers of cells recovered from these spleens. Figure 4 shows the total numbers of splenocytes (panel a) and the numbers of cells in each of the three major lymphocyte subsets (panel b) in the spleens of wild-type and IFN- $\gamma$  R $^{-/-}$  mice at various times after infection. Whereas the spleens of wild-type mice developed splenomegaly and showed a twofold increase in cell numbers characteristic of MHV68 infection (14), there was a rapid fall in the number of cells in the spleens of IFN- $\gamma$  R $^{-/-}$  mice such that by 21 days postinfection there were more than 10-fold fewer cells in the

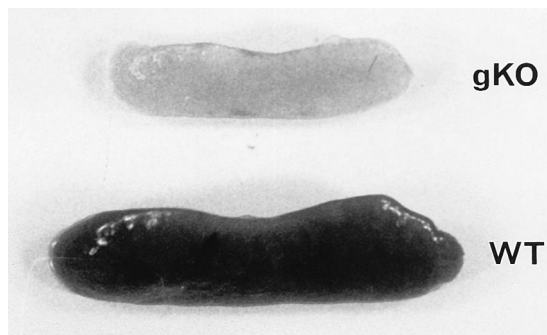


FIG. 2. Spleens from MHV68-infected wild-type and IFN- $\gamma$  R $^{-/-}$  mice 17 days postinfection. WT, wild type; gKO, IFN- $\gamma$  R $^{-/-}$ .



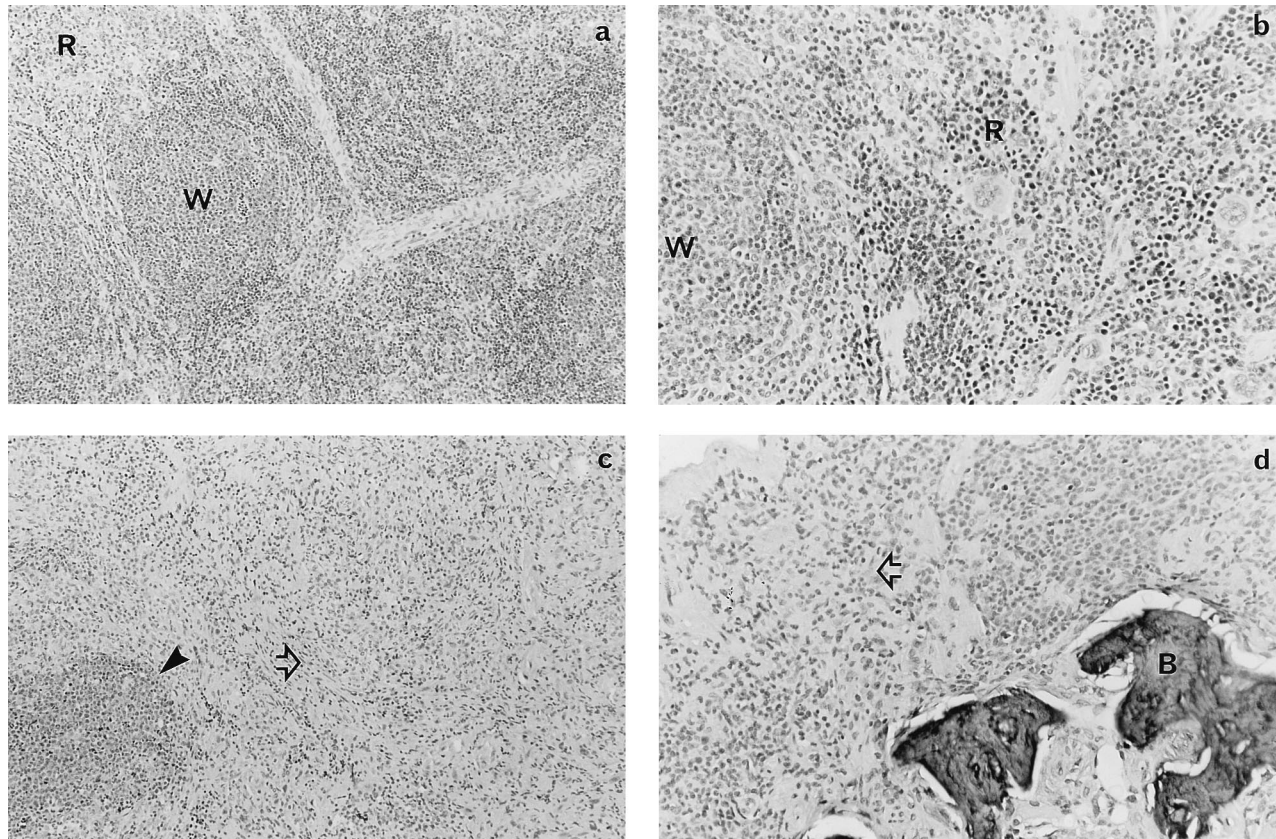


FIG. 3. Histopathology of wild-type 129/SvEv and IFN- $\gamma$  R $^{-/-}$  spleens after MHV68 infection. Hematoxylin and eosin stain; objective magnifications are in parentheses. (a) Wild-type spleen 14 days postinfection showing white pulp hyperplasia and nonreactive red pulp ( $\times 7$ ); (b) wild-type spleen 31 days postinfection showing less-prominent white pulp hyperplasia with nonreactive red pulp ( $\times 14$ ); (c) IFN- $\gamma$  R $^{-/-}$  spleen 14 days postinfection showing follicular lymphoid area surrounded by sheets of mononuclear cells and infiltrating neutrophil polymorphs and fibrotic areas; red pulp areas are involved in the reaction ( $\times 7$ ); (d) IFN- $\gamma$  R $^{-/-}$  spleen, day 31 postinfection; splenic parenchyma is dominated by areas of mature fibrosis with mononuclear inflammatory cells; there are also focal areas of bony metaplasia in this sample ( $\times 14$ ). R, red pulp; W, white pulp; B, bony metaplasia; closed arrow, follicular lymphoid area; open arrow, area containing infiltrating polymorphs and fibrosis.

spleens of these mice. Figure 4b shows that the decrease in cell number was not confined to a particular cell subset but occurred in B-cell and CD4 $^{+}$  and CD8 $^{+}$  T-cell subsets.

**MHV68 latent infection in the spleens of IFN- $\gamma$  R $^{-/-}$  mice.** The number of latently infected cells in the spleens of MHV68-infected wild-type and IFN- $\gamma$  R $^{-/-}$  mice was investigated by an infective-center assay. Spleens were also assayed for the presence of infectious virus. In no case did we detect infectious virus in wild-type spleens. In the case of IFN- $\gamma$  R $^{-/-}$  spleens, low levels of infectious virus were detected ( $<50$  PFU/ $10^7$  cells). Similar titers of virus were obtained when the splenocytes were lysed by freeze-thawing or by sonication (data not shown). Figure 5 shows the number of infective centers per  $10^7$  cells in wild-type and IFN- $\gamma$  R $^{-/-}$  spleens corrected for the infectious virus titers present in the IFN- $\gamma$  R $^{-/-}$  spleens. Although the total number of B cells in the IFN- $\gamma$  R $^{-/-}$  spleens decreased, the latency load (number of latently infected cells/ $10^7$  splenocytes) was clearly higher in the spleens of IFN- $\gamma$  R $^{-/-}$  mice than in wild-type mice. As has been shown for other mouse strains (14), the number of latently infected spleen cells in wild-type mice reached a peak around day 14 but in the third week declined to a relatively stable level of around  $1$  in  $10^6$  spleen leukocytes. The number of infective centers per  $10^7$  cells in IFN- $\gamma$  R $^{-/-}$  spleens reached 10- to 100-fold higher levels than were seen in wild-type spleens. However, the infective center load in IFN- $\gamma$  R $^{-/-}$  spleens did not remain high

but declined to around  $1$  in  $10^6$  splenocytes by the end of the fourth week after infection.

**Role of infectious virus in pathological changes.** The presence of low levels of MHV68 in the spleens of IFN- $\gamma$  R $^{-/-}$  mice suggested that the pathological changes were unlikely to be attributed to virus-mediated cell destruction or damage. To investigate this more fully, we treated wild-type and IFN- $\gamma$  R $^{-/-}$  mice with the antiviral drug 4'-S-EtdU from 6 days postinfection. Treatment with this drug completely inhibits virus replication within 24 h (21a). Day 6 was chosen because previous work has shown that treatment from this time does not alter the time course of spleen infection. Peak levels of latently infected cells and splenomegaly occur at the same time as in untreated mice (1). At 15 days postinfection, spleen cell numbers were determined and splenocytes were assayed for the presence of infective centers and infectious virus. No infectious virus could be detected in either IFN- $\gamma$  R $^{-/-}$  or wild-type-spleens. Table 1 shows that treatment with the drug did not prevent the decrease in spleen cell numbers or the development of an increased load of latently infected cells in MHV68-infected IFN- $\gamma$  R $^{-/-}$  mice. 4'-S-EtdU-treated mice show levels of infective centers similar to those found in untreated mice. These levels were fivefold higher than those found in wild-type mice. The cellular destruction and increased latency load could not therefore be attributed to virus infection of splenocytes followed by lysis of infected cells but is more

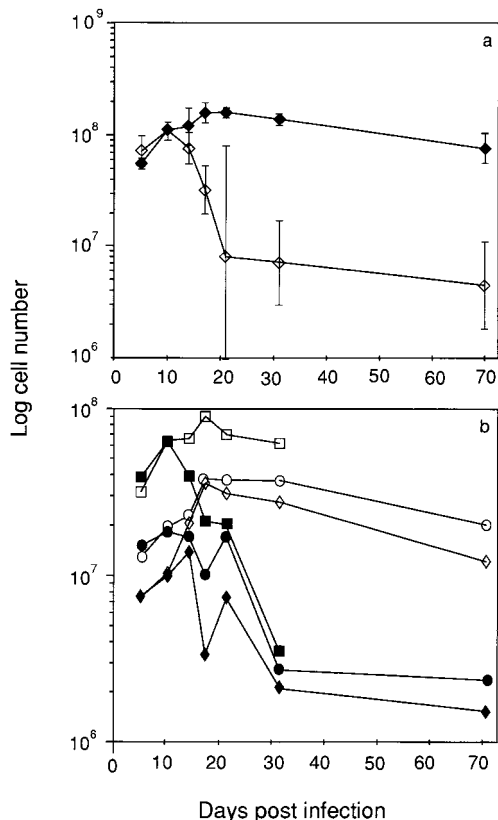


FIG. 4. Spleen cell numbers following MHV68 infection of wild-type 129/Sv/Ev and IFN- $\gamma$  R $^{-/-}$  mice. Mice were inoculated with  $4 \times 10^5$  PFU of virus intranasally. (a) Total number of splenocytes. Mean cell number  $\pm$  standard deviation for four mice/group is shown for each time point.  $\diamond$ , wild type;  $\blacklozenge$ , IFN- $\gamma$  R $^{-/-}$ . (b) Total number of B cells (B220 $^{+}$ ), CD4 $^{+}$  T cells, and CD8 $^{+}$  T cells. Numbers for four mice were determined for each time point.  $\square$ , wild-type B cells;  $\circ$ , wild-type CD4 $^{+}$  T cells;  $\diamond$ , wild-type CD8 $^{+}$  T cells;  $\blacksquare$ , IFN- $\gamma$  R $^{-/-}$  B cells;  $\bullet$ , IFN- $\gamma$  R $^{-/-}$  CD4 $^{+}$  T cells;  $\blacklozenge$ , IFN- $\gamma$  R $^{-/-}$  CD8 $^{+}$  T cells.

likely to be due to perturbation of the mechanisms controlling latency.

**The role of CD8 $^{+}$  and CD4 $^{+}$  T cells in MHV68 infection of IFN- $\gamma$  R $^{-/-}$  mice.** In order to investigate whether immunopathology was contributing to the splenic destruction observed in IFN- $\gamma$  R $^{-/-}$  mice infected with MHV68, we removed the CD8 $^{+}$  and CD4 $^{+}$  T-cell subsets by treating the mice anti-CD4 and anti-CD8 monoclonal antibodies. Mice were injected with anti-CD8 or -CD4 antibodies 2 days prior to infection and 2 and 9 days postinfection. At 16 days postinfection, spleens were harvested and weighed. Half of each spleen was fixed and stained for histological examination, and the other half was used to determine cell number, infective-center load, and infectious virus titer. CD8 $^{+}$  and CD4 $^{+}$  T-cell depletions were monitored by staining with anti-CD4 and anti-CD8 antibodies and analyzing by FACSscan. In all cases, the antibody treatment removed >95% of the relevant T-cell subset.

Table 2 shows the results of infection of CD8 $^{+}$  T-cell-depleted and nondepleted wild-type and IFN- $\gamma$  R $^{-/-}$  mice. Removal of CD8 $^{+}$  cells from the IFN- $\gamma$  R $^{-/-}$  mice substantially abrogated the changes seen in the nondepleted mice. Spleens from the IFN- $\gamma$  R $^{-/-}$  CD8 $^{+}$  T-cell-depleted mice were similar in gross appearance and weight to wild-type CD8 $^{+}$  T-cell-depleted or untreated spleens. Histological examination confirmed that these spleens, like the wild-type spleens, showed mild white pulp hyperplasia with no obvious fibrosis and only a

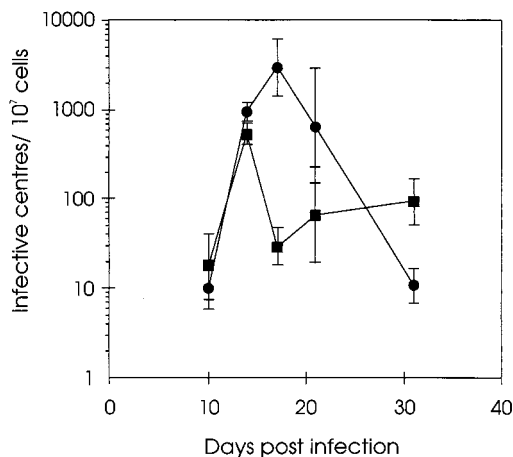


FIG. 5. Infective centers in the spleens of wild-type 129/Sv/Ev and IFN- $\gamma$  R $^{-/-}$  mice. Mice were inoculated with  $4 \times 10^5$  PFU of virus intranasally. Mean infective-center numbers  $\pm$  standard deviations for four mice/group are shown for each time point. Results are expressed as infective centers/ $10^7$  splenic leukocytes. Infectious virus titers (50 PFU/spleen) have been subtracted from the infective-center numbers.  $\blacksquare$ , wild type;  $\bullet$ , IFN- $\gamma$  R $^{-/-}$ .

few infiltrating granulocytes. CD8 $^{+}$  T-cell-depleted, MHV68-infected IFN- $\gamma$  R $^{-/-}$  spleens developed splenomegaly, and the infective-center load was not significantly different from that seen in wild-type mice (Table 2). These results showed quite clearly that the pathological and histological changes seen in MHV68-infected IFN- $\gamma$  R $^{-/-}$  mice were mediated by CD8 $^{+}$  T cells.

Table 2 also shows the results of a similar experiment in which CD4 $^{+}$  T cells had been depleted. Removal of CD4 $^{+}$  T cells reversed the changes in spleen appearance and the weight and cell loss seen in nondepleted IFN- $\gamma$  R $^{-/-}$  mice infected with MHV68 and reduced the number of infective centers to levels found in untreated wild-type mice. As previously observed, the development of splenomegaly was dependent on the presence of CD4 $^{+}$  T cells, and lower numbers of infective centers were found in CD4 $^{+}$  T-cell-depleted wild-type mice (4, 23). However, histological examination showed that a mild to moderate granulocytic infiltration and fibroplasia had occurred in the interfollicular areas of the white pulp. Thus, some pathological changes were occurring in the absence of CD4 $^{+}$  T cells.

## DISCUSSION

Clearance of MHV68 from the lungs of infected mice is dependent on the presence of CD8 $^{+}$  T cells and to some extent on that of CD4 $^{+}$  T cells (8, 14). Both these cell types are potent producers of IFN- $\gamma$ . IFN- $\gamma$  has a dual role in interfering with

TABLE 1. Effect of inhibition of virus replication on MHV68-induced changes in the spleens of IFN- $\gamma$  R $^{-/-}$  mice<sup>a</sup>

Mouse phenotype	$10^7$ spleen cell count (SD)	No. of infective centers/ $10^7$ cells (SD)
Wild type	14.5 (2.4)	200 (146)
Wild type, 4'-S-EtdU treated	18.8 (2.6)	355 (105)
IFN- $\gamma$ R $^{-/-}$	5.6 (1.1)	1,613 (333)
IFN- $\gamma$ R $^{-/-}$ , 4'-S-EtdU treated	8.0 (2.3)	1,200 (492)

<sup>a</sup> Mice were injected intraperitoneally with 1 mg of the antiviral drug 4'-S-EtdU 6 days postinfection and thereafter supplied ad libitum with drinking water containing 0.33  $\mu$ g of drug per ml. Spleens were harvested 15 days postinfection.



TABLE 2. Effect of T-cell subset depletion on MHV68-induced events in the spleens of IFN- $\gamma$  R $^{-/-}$  mice<sup>a</sup>

Depletion expt and phenotype	Spleen appearance	Spleen wt (mg) (SD)	10 <sup>7</sup> spleen cell count (SD)	No. of infective centers/10 <sup>7</sup> cells (SD)
<b>CD8<sup>+</sup></b>				
Wild type	Splenomegaly	195 (64)	8.9 (2.8)	794 (184)
Wild type, CD8 depleted	Splenomegaly	191 (84)	11.2 (3.0)	340 (309)
IFN- $\gamma$ R $^{-/-}$	Pale, fibrous	91 (8)	1.7 (0.2)	11,292 (7,039)
IFN- $\gamma$ R $^{-/-}$ , CD8 depleted	Splenomegaly	145 (80)	8.8 (2.9)	351 (238)
<b>CD4<sup>+</sup></b>				
Wild type	Splenomegaly	229 (20)	13.9 (0.1)	486 (112)
Wild type, CD4 depleted	Normal	82 (21)	3.81 (1.0)	47 (45)
IFN- $\gamma$ R $^{-/-}$	Pale, fibrous	88 (16)	3.03 (1.5)	9,657 (4,621)
IFN- $\gamma$ R $^{-/-}$ , CD4 depleted	Normal	82 (28)	3.47 (1.6)	264 (133)

<sup>a</sup> Mice were depleted of specific T-cell subsets by intravenous injection of depleting anti-CD8 or anti-CD4 monoclonal antibodies 2 days prior to infection and 2 days postinfection and by intraperitoneal injection 9 days postinfection. Spleens were harvested 17 days postinfection.

virus infections: it acts directly to induce an antiviral state within the infected cell, and it acts indirectly by upregulating the activity of the immune system (reviewed in reference 1a). The data presented here show that there is no difference in the amount of replication of MHV68 as measured by virus titer or in the rate of clearance of infectious virus from the lungs between wild-type and IFN- $\gamma$  R $^{-/-}$  mice. This suggests that IFN- $\gamma$  does not have a direct effect on virus replication and is not involved in the process by which CD8<sup>+</sup> T cells clear MHV68 from the lungs (8).

In contrast to the apparent lack of a role for IFN- $\gamma$  in the lungs of IFN- $\gamma$  R $^{-/-}$  MHV68-infected mice, there are profound effects on splenic architecture and on the process of establishment of latency in the spleens of these mice. Early in the second week after infection, large numbers of granulocytes are observed in the spleen. It is not clear what causes this infiltration, and the role of granulocytes in the subsequent pathological changes in the spleen is at present unknown. The disruption of red pulp and white pulp associated with fibrosis, which occurs around day 14, is reflected in the decrease in the numbers of B cells and CD4<sup>+</sup> and CD8<sup>+</sup> T cells. There is a marked increase in the proportion of cells that are latently infected, although these appear to be cleared as normal. Both these events are mediated by CD8<sup>+</sup> T cells, as the changes are completely reversed in CD8<sup>+</sup> T-cell-depleted mice. Depletion of CD4<sup>+</sup> T cells also reversed the gross pathological changes and the virological changes, although minor changes could be observed histologically. These results suggest that CD8<sup>+</sup> T cells are the major mediators of the damage, but they may require help in some form from CD4<sup>+</sup> T cells.

The mechanism by which CD8<sup>+</sup> T cells mediate the effects is at present unknown. In the absence of the IFN- $\gamma$  receptor, CD8<sup>+</sup> T cells may produce a factor or factors which are responsible for the splenic damage and the high latency load. The production of these factors may be controlled in wild-type mice by ligation of the IFN- $\gamma$  receptor. Thus, IFN- $\gamma$  may have a direct effect on CD8<sup>+</sup> T-cell activity in the spleen. Alternatively, the IFN- $\gamma$  R may act as a receptor for another cytokine molecule, either a cellular cytokine, the synthesis of which is upregulated by the virus, or a virus-encoded cytokine. It is clear

that IL-15 utilizes the IL-2 receptor molecule (5), and such dual usage may occur for other receptors. No MHV68-encoded cytokine has yet been detected, but cytokine analogs have been found to be encoded by other gammaherpesviruses, including Epstein-Barr virus, herpesvirus saimiri, equine herpesvirus 2, and equine herpesvirus 5 (13, 22, 25). Such a cytokine might downregulate the virus latency and regulate pathological mechanisms following MHV68 infection.

Production of damage-mediating factors by CD8<sup>+</sup> T cells might be regulated by cytokines or factors produced by other cell types. Clearly CD4<sup>+</sup> T cells are involved in such a process. A second IFN- $\gamma$  receptor has been reported on human macrophages adhered to polystyrene in vitro (9). This is a low-affinity receptor but might be active if high levels of IFN- $\gamma$  are present. The role of macrophages in the changes in IFN- $\gamma$  R $^{-/-}$  spleens remains to be investigated.

The identity of the factor or factors which are responsible for the splenic destruction is at present unknown. Changes in splenic architecture have been observed in normal mice treated with high doses of recombinant IL-12. These mice exhibited a poorly discernible white pulp and also changes in the red pulp. Infection with lymphocytic choriomeningitis virus, but not murine cytomegalovirus, led to the appearance of necrotic lesions in the white pulp of IL-12-treated mice, although in these cases the splenic architecture was maintained (15). The mechanism of the IL-12-mediated toxicity involves induction of tumor necrosis factor alpha (TNF- $\alpha$ ) and results from an increased sensitivity of splenocytes to TNF- $\alpha$ -mediated killing. Thus, T-cell populations undergoing activation and proliferation in response to viral infection were targeted for death (16). The operation of this pathway in MHV68-infected IFN- $\gamma$  R $^{-/-}$  mice would require a mechanism for inducing large amounts of IL-12 in the absence of induction of IL-12 by IFN- $\gamma$ . Such induction could, however, occur if an alternative IFN- $\gamma$  receptor was present on macrophages. It is also possible that TNF- $\alpha$  levels are elevated independently of IL-12 in the MHV68-infected IFN- $\gamma$  R $^{-/-}$  mice. These mice do not, however, exhibit other signs of TNF- $\alpha$ -mediated damage (thymic atrophy and wasting), and a different mechanism may be operating in these mice.

Little is known about the mechanisms which control the proportion of latently infected B cells in MHV68-infected mice. The IFN- $\gamma$  R $^{-/-}$  mice clearly have a higher transient load of latently infected cells than wild-type mice despite the decreased number of B cells. CD4<sup>+</sup> T-cell-depleted mice do not show the transient high levels of latently infected cells that are present in immunocompetent mice (4, 23) and CD8<sup>+</sup> T-cell-depleted mice develop a higher latency load by day 12 than wild-type mice (8). Interestingly, the CD8<sup>+</sup> T-cell-depleted IFN- $\gamma$  R $^{-/-}$  mice have a latency load similar to that of wild-type mice. At present it is not known whether cells become latently infected in the spleen or in peripheral tissues. It is conceivable that latently infected cells traffic to the spleen and proliferate there, and in wild-type mice the proliferation is controlled by direct anti-proliferative action of IFN- $\gamma$ . In the IFN- $\gamma$  R $^{-/-}$  mice there may be an uncontrolled proliferation which precedes the downregulation of latently infected cells by other IFN- $\gamma$ -independent mechanisms. It is also clear that there is a higher level of virus replication in the spleens of IFN- $\gamma$  R $^{-/-}$  mice, and this may be in some way related to the high latency load.

The data presented here show that there are drastic consequences of the lack of the IFN- $\gamma$  receptor in MHV68-infected mice. While it is possible that the pathological and virological observations are due simply to a lack of functional IFN- $\gamma$ , it is also possible that the mechanisms are more complex and that

it is the lack of the receptor itself which is responsible. These mechanisms are currently under investigation.

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