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Interferon-γ is crucial for surviving a *Brucella abortus* infection in both resistant C57BL/6 and susceptible BALB/c mice

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SUMMARY

Brucella abortus is an intracellular bacterial pathogen that causes chronic infections in humans and a number of agriculturally important species of animals. It has been shown that BALB/c mice are more susceptible to infections with virulent strains of *Brucella abortus* than C57BL/6 or C57BL/10 strains. In experiments described here, gene knock-out mice were utilized to elucidate some of the salient components of resistance. Resistant C57BL/6 mice with gene deletions or disruptions in the interferon-γ (IFN-γ), perforin or β_2-microglobulin genes had decreased abilities to control intracellular infections with *B. abortus* strain 2308 during the first week after infection. However, only the IFN-γ knock-out mice had a sustained inability to control infections and this resulted in death of the mice at approximately 6 weeks post-infection. These mice had a continual increase in the number of bacterial colony-forming units (CFU) in their spleens until death. When BALB/c mice with the disrupted IFN-γ gene were infected they had more splenic CFU at one week post-infection than control mice but the increase was not statistically significant and by 3 weeks they did not have more CFU than control mice. Moreover, the number of splenic bacteria did not increase in the BALB/c IFN-γ knock-out mice between 6 and 10·5 weeks, although they died at 10·5 weeks, the time by which normal BALB/c mice were clearing the infection. Death in both strains of IFN-γ gene disrupted mice coincided with symptoms of cachexia and macrophages comprised $\geq 75\%$ of the splenic leucocytes.

INTRODUCTION

Brucella spp. are Gram-negative intracellular bacteria that cause serious chronic infections in humans and a number of agriculturally important species of animals. They are known to reside in professional phagocytes, i.e. macrophages, as well as non-professional phagocytes such as trophoblasts.[1,2] It is this property which is believed to be responsible for chronic infection of mammals. In humans, infection with brucella causes undulant fever that includes recurrent high fever, cachexia, lethargy, arthritis and splenomegaly. In ruminants, its association with trophoblasts and ability to use erythritol produced in the pregnant uterus as a carbohydrate source results in abortion and subsequent chronic shedding of the organism in milk and vaginal secretions (for review see 3). The latter are the source of bacteria that cause zoonotic infection of people.

As with other intracellular microbial pathogens, interferon-γ (IFN-γ) contributes to control of the infection. This has been demonstrated with *Brucella abortus* by showing that supplementing BALB/c mice with recombinant IFN-γ enhances resistance resulting in a 10-fold decrease in the number of bacteria at one week after infection.[4] Also, it has been shown that depleting IFN-γ by the administration of monoclonal antibodies which neutralize IFN-γ results in a 10-fold increase in the number of bacteria 1 week post-infection.[5,6] *In vitro* studies have suggested that the mechanism by which IFN-γ enhances resistance is through activation of macrophages for anti-brucella activity, largely by enhancing production of reactive oxygen intermediates.[7,8]

A number of studies have demonstrated a role for either CD4 or CD8 T cells in the control of brucellosis. A primary role for CD8 T cells in resistance has been shown for infections with the attenuated vaccine strain 19 of *B. abortus* when CD8 T cells were depleted from either CBA or C57BL/10 strains of mice.[9,10] In adoptive transfer studies CD8 T cells and CD4 T cells have been shown to be equally protective for resistance to infections with the virulent strain *B. abortus* 2308

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in BALB/c mice.[11] Since previous studies did not demonstrate a role for natural killer (NK) cells in control of brucellosis,[12] part of the role of T cells in immunity must be to produce the protective IFN-γ. While both subsets of T cells could produce IFN-γ, CD8 T cells have an additional potential role of lysing infected macrophages and thus either killing the intracellular bacteria through granulysin[13] or exposing them to IFN-γ-activated macrophages. In order to design effective methods to evaluate new vaccines for brucellosis, it is important to define the nature of a protective immune response to the virulent field strains.

To clarify this, we employed gene deleted or disrupted (knock-out, k/o) mice. C57BL/6 or C57BL/10 strains of mice have been defined as more resistant than BALB/c mice to infection caused by virulent field strains of *B. abortus*.[14] Thus, we reasoned that the most efficient mechanism of immune control would be revealed in these mice while BALB/c mice would have a compromised expression of those resistance mechanisms. The role of IFN-γ beyond the first week of infection has not been previously investigated and thus was done here. We also assessed the role of perforin-mediated cytotoxicity and the contribution of class I-restricted cells using C57BL/6 perforin or β_2-microglobulin-deficient mice, to determine the contribution by CD8 T cells, because the majority of studies suggested these were of primary importance for control of the attenuated strain 19.

MATERIALS AND METHODS

Mice

The following strains of mice were purchased (Jackson Labs, Bar Harbor, ME) either as individuals for immediate use or as breeding pairs: BALB/cByJ, C57BL/10 and C57BL/6J as control mice, BALB/c-*Ifg*$^{tml@}$ and C57BL/6J-*Ifg*$^{tml@}$ for IFN-γ gene disruption, C57BL/6J-*B2m*^{tmlUnc} for β_2-microglobulin disruption, and C57BL/6J-*Pfp*$^{tml@}$ for perforin disruption. All mice were housed in a biohazard level 3 facility in micro-isolator cages and handled under sterile conditions including sterile food, water, cages, breeding and personnel handling of animals. Mice for experiments were infected when 6–10 weeks of age.

Infection of mice and post-infection analyses

Mice were infected with 5×10^3 colony-forming units (CFU) of *B. abortus* strain 2308 as described.[12] At the time indicated for the experiment, spleens were weighed and CFU/spleen was determined by disrupting the spleens and plating serially diluted aliquots onto *Brucella* agar plates (Difco, Detroit, MI). The number of total leucocytes per spleen was determined, an aliquot of splenocytes was used to prepare cytospin slides stained with Leukostat (Fisher Scientific, Pittsburgh, PA) for performing differential cell analysis based on morphology to distinguish polymorphonuclear neutrophils (PMN), and another aliquot was stained by either direct or indirect immunofluorescence by standard techniques with monoclonal antibodies (mAb) reactive with murine CD4 (L3T4), CD8 (Ly-2), Mac1 (M1/70), NK cells (DX5) and γδ T cells (GL3) (all mAb from PharMingen, San Diego, CA) and analysed by flow cytometry using a FACSCalibur (Becton Dickinson, San Jose, CA) and Cell Quest software (Becton Dickinson). To determine the percentage of macrophages, the percentage of PMN was subtracted from the percentage of Mac1$^+$ cells. Additional aliquots of splenocytes (10^6/well in 48-well plates) were cultured *in vitro* in complete culture medium (RPMI-1640 with 10% heat-inactivated fetal bovine serum, 50 μM 2-mercaptoethanol and 100 μg/ml gentamicin) with or without heat-killed *B. abortus* strain 2308 (5×10^7 bacteria/well) and with recombinant murine IL-2 (PharMingen, San Diego, CA) in a total volume of 0·5 ml for 3 days. After 72 hr, the supernatants were collected, filtered through 0·45 μm filters and stored at -70° until assessment for cytokine production by enzyme-linked immunosorbent assay (ELISA).

Cytokine measurements

Interleukin (IL)-12 and IFN-γ were measured in culture supernatants using standard sandwich ELISA. To measure total IL-12, anti-IL-12 mAb clone C15.6 (kindly provided by Dr G. Trinchieri) was used as the capture mAb and biotinylated anti-IL-12 mAb clone C17.8 was used as the revealing mAb. The revealing mAb was detected by avidin conjugated with horseradish peroxidase (HRP) and addition of 2,2′-azinobis (3-ethylbenzthiazoline-sulphonic acid) (ABTS) as substrate. For IFN-γ detection in supernatants, anti-IFN-γ mAb (clone HB170; American Type Culture Collection (ATCC), Rockville, MD) was used as the capture antibody and biotinylated anti-IFN-γ mAb (clone XMG 1.2, Pharmingen) as the revealing antibody, which was detected as described for the IL-12 ELISA. Standard curves for assessing cytokine concentrations were generated using recombinant cytokines (Pharmingen).

For assessing IL-12 biological activity of culture supernatants, they were added to splenocytes cultures from naïve mice and IFN-γ production by the naïve cells assessed 3 days later as above. Recombinant murine IL-12 (Pharmingen) was used as a control. To assess the ability of the supernatants to inhibit IL-12 bioactivity, the supernatnats were diluted 1 : 2 and 1 : 4 and spiked with rMuIL-12 which was then assessed for its ability to induce naïve splenocytes to make IFN-γ.

RESULTS

Infection of resistant C57BL/6 mice

A panel of knockout strains of mice on a C57BL/6 background were compared for their abilities to control infections with the virulent strain *B. abortus* 2308. The absence of perforin, IFN-γ, and class I major histocompatibility complex (MHC) molecules due to the deletion of the β_2-microglobulin gene all resulted in a significant increase in the number of splenic CFU recovered compared to control mice at one week post-infection. After this point, the β_2-microglobulin-deficient and perforin-deficient mice began clearing the infection as efficiently as, or more so than, the control mice (Fig. 1a). In contrast, the IFN-γ-deficient mice continued to have an increase in splenic CFU as the infection progressed. These mice eventually succumbed to the infection at 6·5 weeks post-infection with more than a 100-fold increase in brucellae per spleen since the 3 week time point. At the time of death, the IFN-γ deficient mice were emaciated, lethargic, exhibited hair loss, had pale spleens and livers and multiple internal abscesses in their body cavities and livers. Normal C57BL/6

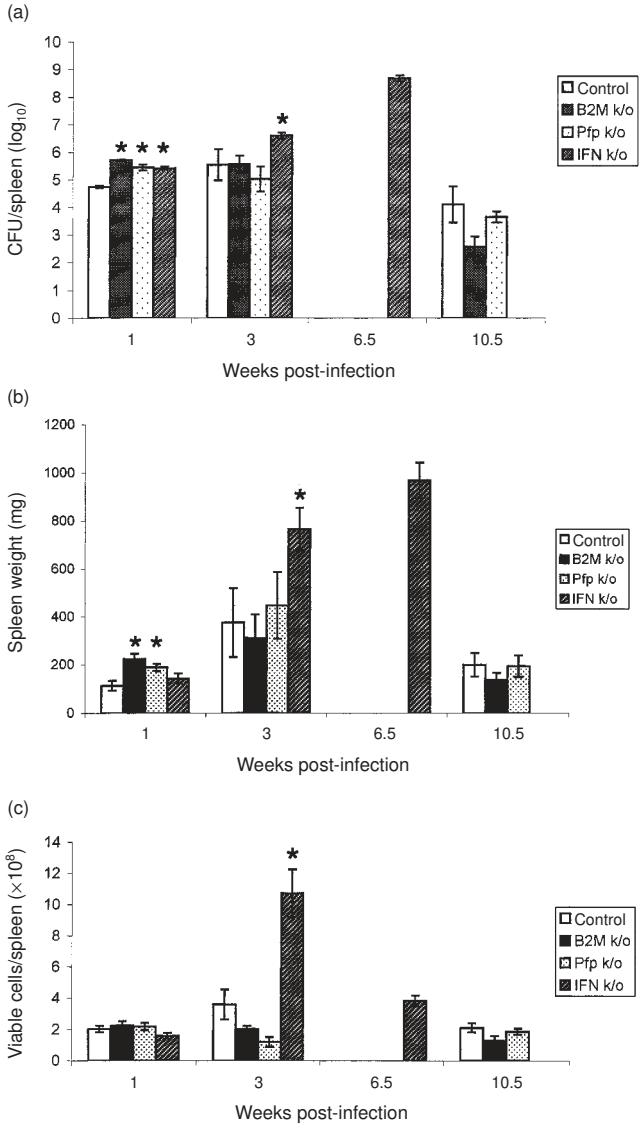

Figure 1. *Brucella abortus* strain 2308 infection in C57BL/6J control, β_2-microglobulin$^{-/-}$ (B2M), perforin$^{-/-}$ (Pfp), and IFN-$\gamma^{-/-}$ mice (k/o, knock-out). Data represent the mean $\pm$ SEM for groups of five mice. (a) CFU of *Brucella abortus* per spleen: CFU in all three groups of gene-disrupted mice were significantly higher than the control group using a one-tailed test at 1 week post-infection ($P \leq 0{\cdot}01$); at 3 weeks the IFN-γ-deficient group was significantly higher than the control group ($P \leq 0{\cdot}05$); at 10·5 weeks neither group was significantly different from the control group. (b) Spleen weights: spleen weights were significantly higher than in control mice at one-week for the β_2-microglobulin and perforin gene-disrupted groups and for the IFN-γ-deficient mice at 3 weeks ($P \leq 0{\cdot}04$). (c) Total number of splenic leucocytes was determined and were significantly higher only for the IFN-γ deficient mice at 3 week ($P \leq 0{\cdot}05$). Significant differences relative to control mice are indicated with an asterisk.

mice were not included in the 6 week time point because death was not expected and thus unplanned for. In a second experiment, the mean CFU in IFN-γ-deficient mice was $\log_{10}$ $6{\cdot}2 \pm 0{\cdot}1$ at 4 weeks (control mice had CFU $\log_{10}$ $4{\cdot}9 \pm 0{\cdot}8$) and $\log_{10}$ $7{\cdot}3 \pm 0{\cdot}1$ at 5·5 weeks (control mice had CFU $\log_{10}$ $5{\cdot}8 \pm 0{\cdot}2$), while in the third group the time of death, number of CFU/spleen and clinical symptoms at the time of death were similar to the group reported in Fig. 1(a) while CFU in control mice did not increase (data not shown). Because the β_2-microglobulin-deficient and perforin-deficient mice did not have a sustained CFU compared to control mice, they were not evaluated further.

There were no striking differences in spleen weights (Fig. 1b) amongst the control, β_2-microglobulin-deficient, and perforin-deficient mice. Although at 1 week post-infection, the β_2-microglobulin- and perforin-deficient mice had slightly higher spleen weights these differences were not sustained throughout the infection and were not supported by higher numbers of splenocytes (Fig. 1c). Cellular composition of the spleens was also very similar amongst these three groups of mice (Fig. 2).

In contrast to the other three C57BL/6 mouse lines evaluated, the IFN-γ-deficient mice had twice the spleen weights of control mice at 3 week post-infection (Fig. 1b) and a threefold increase in the total number of splenic leucocytes (Fig. 1c). This increase was reflected by a similar proportional increase in CD4 and CD8 T cells and macrophages and a 10-fold increase in neutrophils in the spleen (Fig. 2). At the time of death the spleen weights were still high although the number of viable splenocytes was much lower. Population analyses showed that the number of CD4 and CD8 T cells and neutrophils had declined by this time but the number of macrophages remained high, comprising 90% of the splenocytes.

Infection of susceptible BALB/c mice

Because IFN-γ was clearly an essential component of the immune control of brucellosis in the more resistant C57BL/6 mice and necessary for their survival, we evaluated the course of infection in IFN-γ-deficient BALB/c mice. While there was a slightly higher number of CFU in these mice at 1 week post-infection compared to control mice, similar to the results with the C57BL/6 strain, it was not statistically significant (Fig. 3). Similarly, at 3 weeks the CFU were not significantly higher in the IFN-γ K/O mice compared to the control mice. This was repeated a second time and again no significant increase in CFU at 3 weeks occurred (data not shown). The number of CFU in the spleens of the BALB/c IFN-γ deficient mice did not increase between 6 weeks and their death at 10·5 weeks although there were signficantly more CFU than in the control mice by 10·5 weeks. It is notable that the BALB/c IFN-γ k/o mice had 100-fold fewer CFU at 6 week than the C57BL/6 IFN-γ k/o (Fig. 3) mice and survived for almost twice as long although the BALB/c mice also eventually succumbed to the infection in the absence of a functional IFN-γ gene. The spleen weights and total leucocyte numbers in the spleens of the BALB/c IFN-γ-deficient mice were very similar to the control BALB/c mice at 1, 3 and 6 weeks post-infection (Fig. 4). They diverged at 10·5 weeks post-infection when the control mice began to resolve the infection and the IFN-γ-deficient mice became moribund. At this time, the IFN-γ-deficient mice maintained their high spleen weights and higher splenocyte counts. They had three times as many CD8 T cells and macrophages. The number of macrophages had increased sixfold between 3 and 6 weeks post-infection in both strains of mice. The number remained high in the BALB/c

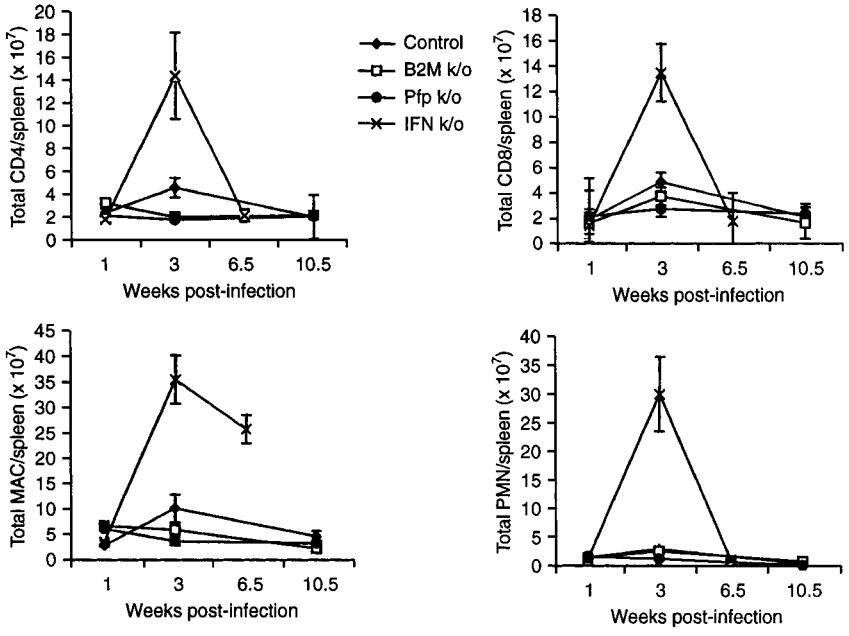

Figure 2. Spleen cell populations during *Brucella abortus* strain 2308 infection in C57BL/6J control, β2-microglobulin$^{-/-}$, perforin$^{-/-}$, and IFN-γ$^{-/-}$ mice. The total number of CD4 T cells, CD8 T cells, PMN and macrophages (MAC) per spleen was determined by multiplying the percentage of positive cells obtained by differential morphology on cytospins and flow cytometry analysis by the total leucocyte count. Data represent the mean ± SEM for groups of five mice.

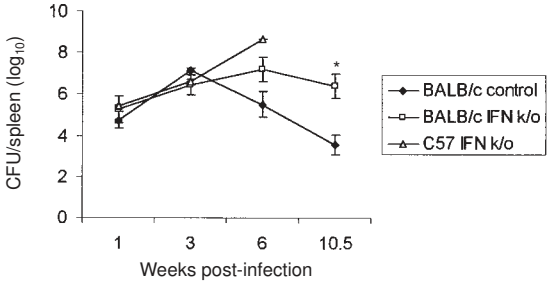

Figure 3. *Brucella abortus* strain 2308 infection in BALB/c control and IFN-γ$^{-/-}$ mice. CFU/spleen were determined at 1, 3, 6, and 10·5 weeks post-infection. The CFU were significantly higher in the IFN-γ-deficient group than the control group at 6 weeks post-infection ($P \leq 0{\cdot}04$) and at 10·5 weeks ($P \leq 0{\cdot}005$), indicated by an asterisk. For comparison the course of *Brucella abortus* strain 2308 infection in C57BL/6J IFN-γ$^{-/-}$ mice from Fig. 1 is also indicated. Data represent the mean ± SEM for groups of five mice.

IFN-γ-deficient mice while returning to baseline levels in the controls (Fig. 5). At the time of death, the mice died of symptoms similar to those seen in the C57BL/6 mice. At the time of death, they were emaciated, lethargic, and exhibited hair loss and pale spleens and livers, and multiple abscesses were noted.

IFN-γ and IL-12 production in infected mice

The above results indicated that IFN-γ did not contribute to control of the infection in BALB/c mice infected with *B. abortus* strain 2308 at the 3 week time point. *In vitro* analyses indicated that secretion of IFN-γ by splenocytes from infected BALB/c mice stimulated with heat-killed brucella ceased by 3 weeks post-infection although production of IL-12,

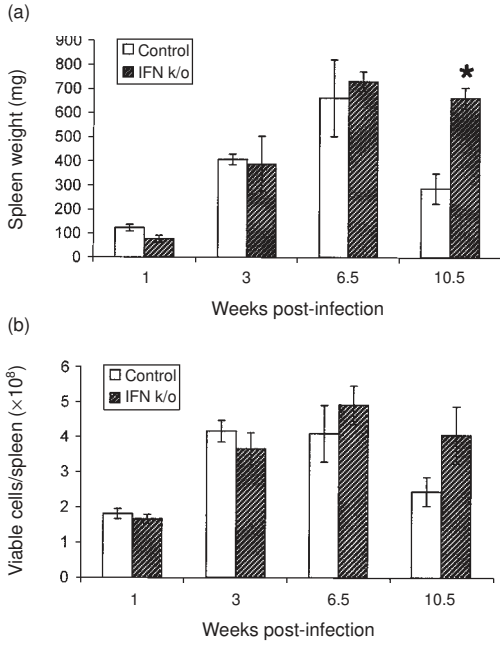

Figure 4. BALB/c control and IFN-γ$^{-/-}$ mice were infected with *Brucella abortus* strain 2308 and (a) spleen weights and (b) total leucocytes in the spleen determined. Data represent the mean ± SEM for groups of five mice. The spleen weights were significantly greater for the IFN-γ$^{-/-}$ mice at 10·5 weeks only ($P \leq 0{\cdot}005$), indicated by an asterisk.

important in stimulating IFN-γ production, did not. This is in contrast to 1 week post-infection when BALB/c splenocytes produced as much IFN-γ and IL-12 as the splenocytes from control C57BL/10 mice (Fig. 6). The 3 week time point was

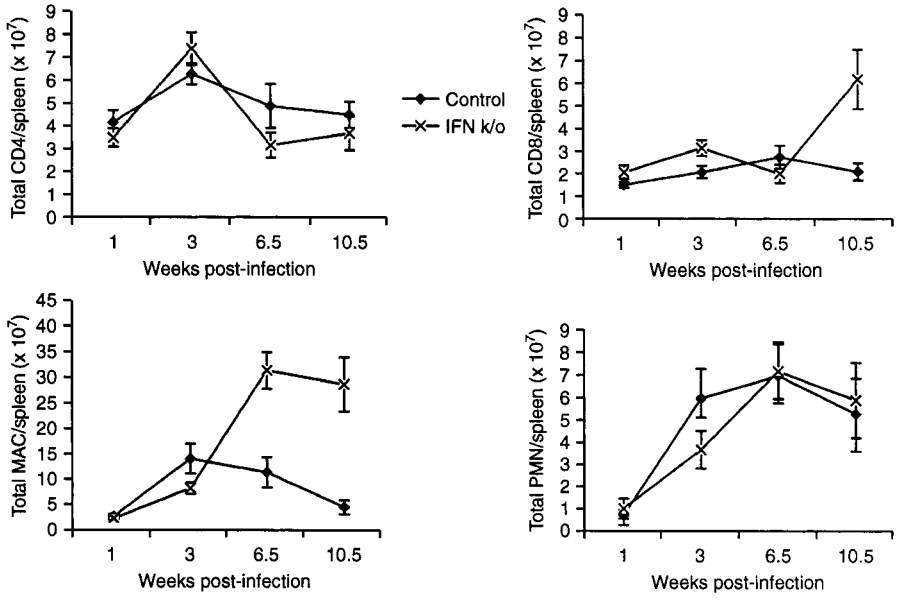

Figure 5. Spleen cell populations during *Brucella abortus* strain 2308 infection in BALB/c control and IFN-$\gamma^{-/-}$ mice. The total number of CD4 T cells, CD8 T cells, PMN and macrophages (MAC) per spleen was determined by multiplying the percentage of positive cells obtained by differential morphology on cytospins and flow cytometry analysis by the total leucocyte count. Data represent the mean ± SEM for groups of five mice.

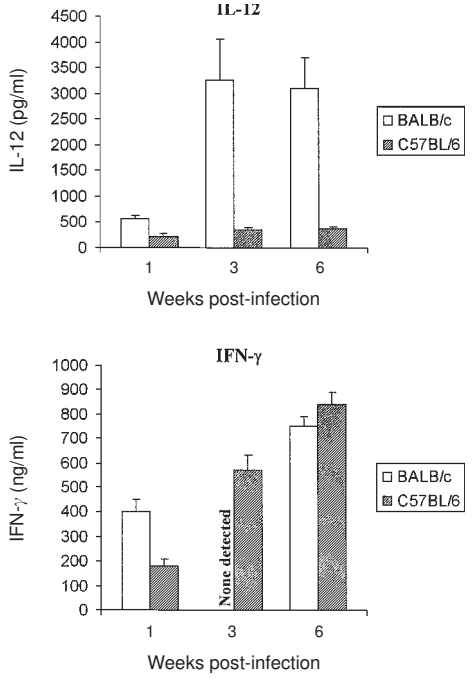

Figure 6. *In vitro* cytokine production by splenocytes from infected mice at 1 week, 3 weeks and 6 weeks after infection with *Brucella abortus* 2308. Splenocytes from BALB/c and C57BL/10 mice were cultured with heat-killed (HK) *B. abortus* 2308. Culture supernatants were tested for IFN-γ and IL-12 content by ELISA and the mean ± SEM for groups of five mice is shown here. The level of sensitivity for IFN-γ was > 2 ng/ml. The amount of cytokine made in control cultures without heat-killed brucellae was always substantially lower than that with antigen (not shown).

repeated three times with a total of 15 BALB/c mice tested. Except for those from one mouse, none of the splenocyte cultures produced detectable IFN-γ (data not shown). By 6 weeks post-infection, the BALB/c splenocytes again produced IFN-γ in response to antigen (Fig. 6). While the splenocyte culture supernatants from BALB/c mice that had been infected for 3 weeks had high levels of IL-12 as detected by an ELISA that measures both homodimeric and heterodimeric IL-12 (Fig. 6), they showed very little bioactivity with regard to stimulating IFN-γ production (Fig. 7a). The amount was equivalent to about 10 pg/ml of rMuIL-12 p70. The supernatants also blocked the ability of rMuIL-12 to stimulate IFN-γ production, suggesting much of the enormous amount of IL-12 measured was of the homodimeric p40 type (Fig. 7b).

DISCUSSION

The purpose of this study was to further define the immune components involved in resistance to a virulent strain of *Brucella abortus* by taking advantage of gene k/o mice. Previous *in vivo* and *in vitro* studies supported a role for IFN-γ in resistance to both the attenuated strain 19 and the virulent strain 2308 during the first week after infection.[4–8] The studies reported here showed that IFN-γ was indeed crucial for the control of the virulent stain 2308 in the more resistant C57BL/6 mice throughout the infection and ultimately for survival of the mice. Death of mice following infections with *B. abortus* has not been reported previously, including in experiments using much higher infection doses in BALB/c or C57BL/10 strains[12] or in experiments using CByB6 nude mice.[15] (NK cells may have produced IFN-γ in the nude mice even though NK cells do not contribute to control in normal

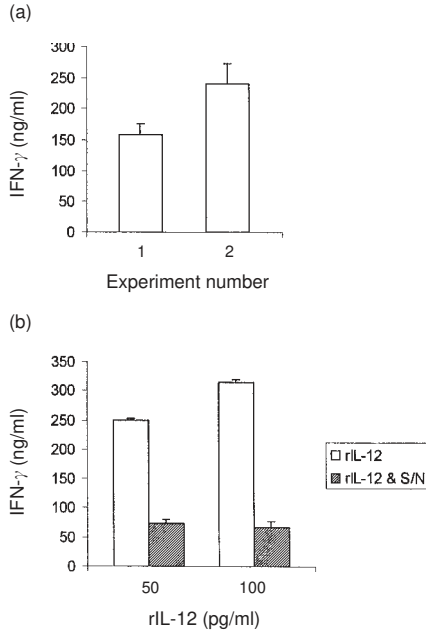

Figure 7. Supernatants from cultures of splenocytes from five BALB/c mice infected for 3 week were tested for their (a) biological activity by culturing with naïve BALB/c splenoctyes for 3 days and assessing those supernatants for IFN-γ by ELISA. The amount of IFN-γ from two experiments are shown as the mean ± SEM. (b) The ability of a 1 : 4 dilution of the supernatants (S/N) to inhibit either 50 or 100 pg/ml of rMuIL-12 from inducing IFN-γ production by naïve BALB/c splenocytes is shown. The control is the rIL-12 alone shown as open bars and rIL-12 spiked supernatants are shown as hatched bars. This is an example of one of two experiments performed shown as the mean ± SEM.

BALB/c and C57BL/10.[12]) The number of CFU in the spleens of the IFN-γ-deficient mice increased at a regular exponential rate from the time of infection until death indicating that the plateau in number of bacterial CFU in the spleens of normal C57BL/6 mice between weeks 1 and 6 is a result of immunological mechanisms and not a result of the bacteria entering a stasis phase as has been shown for some bacteria when they are in adverse conditions.[16] The significance of the CD4 and CD8 T-cell expansion in the C57BL/6 IFN-γ gene-disrupted mice at 3 week post-infection is unresolved as we did not determine whether they were antigen-specific. However, one of the most dramatic changes was the large influx of macrophages, which others have shown does not correlate with to control of brucellosis.[17] These large increases in macrophages as well as PMN may actually have contributed to the death of the mice because of production of cytokines and reactive oxygen intermediates.

The absence of β_2-microglobulin and perforin also decreased control in C57BL/6 mice at 1 week. We presume the role of perforin in immunity during this time was not attributable to mechanisms involving NK cells because elsewhere we found no role for these cells in immunity during the first week of infection of either C57BL/10 or BALB/c mice.[12] Perforin may have been needed by CD8 T cells to lyse infected macrophges, thereby releasing bacteria to neutrophils or activated macrophages, which are efficient at killing brucellae.[18] However, neither perforin-mediated cytotoxicity nor MHC class I-restricted responses were ultimately needed for control. Later in the infection the mechanism(s) may become redundant or less crucial if all macrophages are activated as a result of the efficient IFN-γ production that apparently occurs in the C57BL/6 strains of mice. Our results differed substantially from studies using the less virulent strain 19 in which CD8 T cells played a major role.[9,10] It is possible that antigens are more effectively displayed with MHC class I on cells infected with the attenuated strain and thus the role of MHC class I-restricted T cells more apparent or prolonged throughout the infection. However, such an explanation would not account for the protective response against strain 2308 infections that Winter and colleagues found for CD8 T cells in adoptive transfer studies in BALB/c mice.[11] We also have been able to demonstrate a minor protective role for CD8 T cells in BALB/c mice during the first 3 weeks of infection (E. A. Murphy, J. Sathiyaseclan, M. Parent, X. Jiangi and C. L. Baldwin, submitted for publication). Thus both the strain of mice and strain of *B. abortus* may influence the relative contribution of CD8 T cells to control.

Normal BALB/c mice typically have 10-fold more CFU in their spleens than in either C57BL/10 or C57BL/6 mice, suggesting they have a less efficient immune control. However, it was striking that IFN-γ did not contribute at all to control of bacterial numbers in the more susceptible BALB/c mice during the interval represented by 3 weeks post-infection. The spleen is the principal site of infection for murine brucellosis and this was true for the IFN-γ-deficient mice as well as many fewer CFU were found in liver and lymph nodes than in the spleen (M. Parent and C. Baldwin, unpublished observations). Thus, it is not likely that large numbers of bacteria were sequestered elsewhere and thus uncounted. Moreover, previously published information showed that depletion of IFN-γ by mAb results in a 10-fold increase at 1 week post-infection.[6] While there were more CFU in the IFN-γ knock-out mice at 1 week it was not significantly different from the number of CFU in control mice. Similar differences in cytokine effects have been seen with IL-4-gene-disrupted mice compared to those in which the IL-4 had been neutralized with anti-IL-4 antibody. It has been suggested by others that the difference is due to the development of compensatory mechanisms in the gene-disrupted mice.[19]

We previously demonstrated that IFN-γ production by BALB/c splenocytes in response to brucella antigens *in vitro* was measurable by 1 week post-infection but that the amount produced was reduced by 3 weeks post-infection compared to that by C57BL/10 splenocytes,[20] supporting the *in vitro* and *in vivo* observations made in this study. The previous study showed the IFN-γ was made by CD4 T cells at 1 week post-infection and found no measurable IL-4 produced by splenocytes during the infection.[20] Thus, there is no supporting evidence that the turn-off of IFN-γ production is caused by a turn-off of production by NK cells or by an increase in IL-4 production at 3 weeks. Decreased IFN-γ production in BALB/c mice could be partially a result of increased IL-10, a cytokine we have noted elsewhere to have an adverse effect in BALB/c mice.[6] IL-10 can affect both production of IFN-γ through altering the antigen-presenting capacity of macrophages[21] as well as decreasing IL-12 production by macrophages.[22] While data presented here suggests that IL-12 is

still produced, it did not distinguish between homodimeric and heterodimeric IL-12. The data suggests that there is little IL-12 bioactivity in the supernatants from cultures of splenocytes from 3 week infected BALB/c mice. It was comparable to about 40 pg/ml of rMu p70 IL-12. Thus, a significant proportion of the IL-12 measured by the ELISA may be of the blocking p40 homodimeric type as suggested by its ability to block rMuIL-12 bioactivity reported here. The loss of production of IFN-γ in BALB/c mice may also be partially related to a loss of the high affinity IL-12 receptor β2. This has recently been described as a mechanism that contributes to the greater tendency of BALB/c mice to make T helper 2 immune responses.[23] Experiments are underway to test this.

Ultimately, IFN-γ was necessary for clearance of *Brucella abortus* strain 2308 and survival of BALB/c mice, indicated by their demise at 10·5 weeks post-infection in its absence. Even at the time of death, however, the BALB/c IFN-γ-deficient mice never had the large increase in splenic bacterial numbers that were found in the C57BL/6 IFN-γ-deficient mice. Either other mechanisms of immune control that are not operative in the C57BL/6 mice are operative in BALB/c mice or the microenvironment in the BALB/c spleens signals the bacteria to enter a state of stasis. Additional candidates for control in BALB/c mice include CD8 T cells, because there was a large increase in this cell population at 10·5 weeks in the k/o mice, and tumour necrosis factor-α, which has been shown by Cheers and colleagues to have a role in controlling murine brucellosis.[24] Like the C57BL/6 mice, the IFN-γ deficient BALB/c mice are likely to have died as a consequence of chronic inflammation as suggested by the enormous number of macrophages in their spleens after 6 week. The number was similar to that found in the spleens of IFN-γ-deficient C57BL/6 mice at the time of death.

In summary, our results demonstrated that IFN-γ is crucial for control of brucellosis in resistant C57BL/6 mice throughout the infection while the more susceptible BALB/c mice controlled brucellosis for a longer duration in its absence. It is curious why BALB/c mice have a hiatus for IFN-γ involvement as IFN-γ is so effective at controlling the infection. We speculate that it may actually be because these mice make an overzealous type 1 cytokine response initially following brucella infection that threatens their survival. We have shown that these mice produce large amounts of IFN-γ *in vivo* since higher levels are measured in their serum during the first week after infection than in the serum of C57BL/10 mice (Fernandes and Baldwin, unpublished observations). Thus, the shut-down of response to IFN-γ may be necessary for survival in the short term.

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