Increased Gamma-Delta T-Lymphocyte Response to *Mycobacterium bovis* BCG in Major Histocompatibility Complex Class I-Deficient Mice

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Received 22 September 1994/Returned for modification 6 January 1995/Accepted 22 March 1995

Mice with a homologous deletion of the β_2 -microglobulin gene (β_2m^-) are deficient in class I major histocompatibility complex molecules (MHC) and consequently are deficient in $CD8^+$ T cells. These β_2m^- mutant mice control the intraperitoneal growth of an avirulent vaccine strain of mycobacteria, *Mycobacterium bovis* BCG, after intraperitoneal infection similarly to normal mice. We show that β_2m^- mice have an increased gamma-delta ($\gamma\delta$) T-cell response after infection with live avirulent mycobacteria. β_2m^- mice have an earlier and more sustained rise in the proportion of intraperitoneal $\gamma\delta$ T cells, averaging 17% of T cells, compared with 6% in normal mice, at 28 days after infection. Compared with the population in normal mice, $\gamma\delta$ T cells in the spleens of β_2m^- mice averaged a higher proportion of the total T-cell population of the spleen on days 5, 8, and 14 after intraperitoneal infection. These data document the kinetics of $\gamma\delta$ T cells reactive to mycobacterial antigens *in vivo* without class I MHC restriction and support a role for class I MHC and $CD8^+$ T cells in the *in vivo* regulation of $\gamma\delta$ T cells.

Gamma-delta ($\gamma\delta$) T lymphocytes have been postulated to play a role in controlling mycobacterial infections (22, 30). Some subsets of $\gamma\delta$ T cells from mice and humans appear to proliferate in response to mycobacterial antigens without prior exposure to the antigen (reviewed in reference 15). These $\gamma\delta$ T cells can be stimulated by peptides derived from highly evolutionarily conserved heat shock proteins that may bind to $\gamma\delta$ T-cell receptors in a manner analogous to a superantigen (13). Certain clonal $\gamma\delta$ T cells recognize heat shock proteins derived either from mycobacteria or from neoplastic cells in association with nonpolymorphic cell surface molecules (9, 15). Therefore, those subsets of $\gamma\delta$ T cells that proliferate in response to heat shock proteins may represent a population of T cells that appeared earlier in the evolution of the immune system (21). These $\gamma\delta$ T cells might provide the early immune responses to infections and neoplastic tissue that develop in the host prior to the onset of acquired cell-mediated immunity. Of note, other subsets of $\gamma\delta$ T cells recognize peptides in a major histocompatibility complex (MHC)-restricted fashion similarly to alpha-beta ($\alpha\beta$) T cells (15).

Macrophage activation by $\alpha\beta$ $CD4^+$ T cells is thought to play the major role in controlling virulent mycobacterial infections after specific immunity is acquired (11, 26, 32, 34). $CD4^+$ T cells release cytokines that activate macrophages to become competent at killing mycobacteria. However, some studies support a role for $\alpha\beta$ $CD8^+$ T cells in host resistance to virulent mycobacteria (19, 23, 28, 31, 33). $CD8^+$ cytotoxic T lymphocytes (CTL) may release mycobacteria from infected phagocytes, thus allowing further uptake by more highly activated phagocytes. Both $\gamma\delta$ T cells and some subsets of $\alpha\beta$ $CD4^+$ T cells demonstrate cytotoxic activity directed toward target cells infected with mycobacteria and could therefore play a role

similar to that of the $\alpha\beta$ $CD8^+$ CTL (29, 35). Alternatively, since $\alpha\beta$ $CD4^+$ and $CD8^+$ T cells, as well as $\gamma\delta$ T cells, can produce gamma interferon, all three of these cell types might contribute to the control of mycobacterial infections through the activation of macrophages (23, 29, 34). Therefore, $\gamma\delta$ T cells that recognize heat shock proteins may play an important role early during the course of mycobacterial infection, when acquired cell-mediated immunity has not yet arisen.

Mice genetically engineered to be deficient in β_2 -microglobulin (β_2m^-) are deficient in class I MHC and therefore fail to develop functional $CD8^+$ CTL. This is due to the requirement for class I MHC for positive selection of most $CD8^+$ T cells in the thymus (24, 45). Experiments using β_2m^- mice have shown that some subsets of $\gamma\delta$ T cells that display a transgenic T-cell receptor known to be restricted by class I MHC also require class I MHC for positive selection (36, 43). Another group, using nontransgenic β_2m^- mice, has found that other subsets of $\gamma\delta$ T cells do not require class I MHC for positive selection, and the overall numbers of $\gamma\delta$ T cells are not decreased (5). $\gamma\delta$ T cells can recognize peptides bound to polymorphic class Ia MHC or to the nonpolymorphic class Ib MHC (15). However, certain $\gamma\delta$ T cells can recognize antigens on cells that are defective in antigen processing and hence may recognize antigens directly in a manner more similar to immunoglobulin recognition (39).

β_2m^- mice have been used as a model of $CD8^+$ T-cell deficiency to show that $CD8^+$ T cells either are required or are not required for protection against several infectious agents (37). Several studies have demonstrated that in β_2m^- mice, $CD4^+$ T cells can assume the role previously held predominantly by $CD8^+$ CTL in clearing viral infections or in mediating immunopathology after viral infection (2, 8, 18, 27, 40). During infection with the intracellular bacterial pathogen *Listeria monocytogenes*, $\gamma\delta$ T cells can partially compensate for the lack of $CD8^+$ T cells in β_2m^- mice (16, 38). However, unlike the case of avirulent mycobacterial infection, in which wild-type and β_2m^- mice control the infection similarly, β_2m^- mice infected with virulent mycobacteria succumb to infection (12).

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FIG. 1. Numbers of mycobacteria in the peritoneal cavities (per milliliter) (A) and spleens (per organ) (B) of β_2m^+ (open triangles), β_2m^- (closed triangles), and SCID (open circles) mice infected with 3×10^5 CFU of BCG intraperitoneally. Shown are the means $\pm$ standard errors of the means of four animals, except for SCID mice, for which three animals per point were analyzed. Error bars for SCID mice at day 8 are smaller than the symbol.

Here we demonstrate equivalent and possibly increased early control of avirulent mycobacteria in β_2m^- mice compared with β_2 -microglobulin-sufficient (β_2m^+) mice and an increase in the number and percentage of $\gamma\delta$ T cells during infection.

C57BL/6 ($H-2^b$) mice were purchased from Harlan Sprague-Dawley (Madison, Wis.). Flora-defined C.B-17 SCID ($H-2^d$) mice (3) were purchased from the Gnotobiotic Laboratory at the University of Wisconsin—Madison. Original breeding pairs of mice homozygous for disruption of the β_2 -microglobulin gene (β_2m^-) were a kind gift from Beverly H. Koller (University of North Carolina, Chapel Hill) (24); they are a recombinant inbred strain of C57BL/6 $\times$ 129/Ola ($H-2^b$) and were used at F_4 to F_6 . β_2m^- and (C57BL/6 $\times$ β_2m^-) F_1 mice were bred in our colony at the University of Wisconsin under barrier conditions. β_2m^- mice have remained healthy for 3 years in our facility. (C57BL/6 $\times$ β_2m^-) F_1 mice, designated β_2m^+ mice, in preliminary experiments showed essentially the same results as C57BL/6 mice in the replication of *Mycobacterium bovis* BCG and the $\gamma\delta$ T-cell response to infection. Male mice were used at 8 to 16 weeks of age. All procedures were reviewed by the UW-Institutional Animal Care Committee.

Freeze-dried stocks of BCG vaccine (lot no. 9aW105; Glaxo Laboratories, Greenford, England) containing 1.2×10^7 CFU were a kind gift from Donald W. Smith (University of Wisconsin—Madison). Additional stocks were grown in Middlebrook 7H9 broth (Difco, Detroit, Mich.) and frozen at mid-log phase at -75°C . Animals were injected with 3×10^5 CFU of BCG intraperitoneally. There is no difference between the laboratory-grown BCG and the stock vaccine BCG in (i) replication in the peritoneal cavities or the spleens of C57BL/6, β_2m^- , or SCID mice or (ii) elicitation of $\alpha\beta$ or $\gamma\delta$, CD3 $^+$ T cells in the peritoneal cavity or spleen. Mice were killed by cervical dislocation. The peritoneal cavity was irrigated with 4 ml of phosphate-buffered saline containing 5% fetal calf serum. Spleens were removed, placed in RPMI 1640 supplemented with 5% fetal calf serum, and macerated into single-cell suspensions, using the frosted ends of glass slides. Half of each sample was used for flow cytometry; the other half was ground in glass-Teflon homogenizers for bacterial enumeration. Undiluted homogenates and 10-fold dilutions in Middlebrook 7H9 broth were plated on Middlebrook 7H10 agar (Difco) supplemented with OADC (Difco) and containing 0.01% cycloheximide (Upjohn, Kalamazoo, Mich.). Plates were incubated for 3 to 6 weeks at 37°C in 10% CO_2 in polyethylene bags. Data were

analyzed as the mean $\log_{10}$ number of bacteria from tissues of three to four mice. Analysis of variance was used to determine significant differences within each experiment, followed by a comparison of mean differences by using a modified *t* test (InStat statistical analysis; GraphPad, San Diego, Calif.).

Course of BCG infection in β_2m^- and control mice. The growth of BCG over time was determined for β_2m^- , β_2m^+ , and SCID mice. To establish an initial dose of recoverable bacteria, designated in the figures as the value at time zero, an intraperitoneal injection of 3×10^5 CFU of BCG was followed 1 h later by peritoneal lavage. We were able to recover approximately 2 logs fewer bacteria from the peritoneal cavity after 1 h (Fig. 1A). Within this period of time, a portion of mycobacteria was seeded to other organs, as seen by the number of organisms cultured from the spleen (Fig. 1B). We then determined the number of mycobacteria at 5, 8, 14, and 28 days after intraperitoneal infection. SCID mice served as a control for nonacquired immune mechanisms controlling mycobacterial growth. β_2m^- mice controlled the growth of avirulent mycobacteria in the peritoneal cavity similarly to β_2m^+ mice, whereas SCID mice had significantly higher numbers of avirulent mycobacteria in the peritoneal cavity on day 8 after infection ($P < 0.001$) (Fig. 1A).

BCG organisms were detectable in the spleen within 1 h after intraperitoneal infection (Fig. 1B). SCID, β_2m^+ , and β_2m^- mice all showed similar levels of avirulent mycobacteria in the spleen at 5 days after infection. β_2m^- mice showed slightly lower numbers of BCG in the spleen on day 8 after infection compared with SCID or β_2m^+ mice ($P < 0.05$). However, mycobacterial counts continued to rise in spleens of SCID mice, whereas β_2m^+ mice started to control mycobacterial growth in the spleen by day 14 after infection. Similar amounts of mycobacteria were recovered from β_2m^+ and β_2m^- mice at 28 days after infection.

Flow cytometry of peritoneal and splenic T cells in β_2m^- and control mice. Single-cell suspensions of peritoneal and spleen cells were labeled with a fluorescein-labeled monoclonal antibody to CD3 (145-2C11; PharMingen, San Diego, Calif.) and then subjected to a second label with a biotinylated monoclonal antibody (anti- $\alpha\beta$ T-cell receptor [H57-597], anti- $\gamma\delta$ T-cell receptor [GL3], anti-CD8 [53-6.7], or anti-CD4 [RM4-5]; all from PharMingen) followed by streptavidin-phycoerythrin to measure T-cell subsets. Cells were analyzed on a FACScan flow cytometer (Becton Dickinson, San Jose, Calif.).

FIG. 2. Flow cytometric analysis of peritoneal cavity and spleen cells from a representative β_2m^- mouse on day 8 after intraperitoneal infection with BCG. Similar profiles with similar relative mean fluorescence were seen on all days and for all strains, differing only in relative percentages of doubly stained cells. A gate was set to include all live mononuclear peritoneal cells; spleen cells are shown as the entire, ungated, mononuclear cell population. In the upper left corner is shown the percentage of cells double staining for CD3 and $\gamma\delta$ or $\alpha\beta$ T-cell receptor of the total mononuclear cell population.

Representative flow cytometry data showing characteristic cell populations derived from the peritoneal cavities and spleens from β_2m^- mice 8 days after BCG infection are shown in Fig. 2. Similar flow cytometry profiles were seen for peritoneal and spleen populations from β_2m^- and β_2m^+ mice. We controlled for the nonspecific staining of peritoneal cells by the anti- $\gamma\delta$ monoclonal antibody by double staining with anti-CD3 (Fig. 2, upper left quadrant). All CD3⁺ cells were stained by either the anti- $\alpha\beta$ monoclonal antibody or anti- $\gamma\delta$ monoclonal antibody, as shown by reciprocal plots in Fig. 2. SCID mice did not have significant levels of CD3⁺ populations, and there was no development of these populations after infection (data not shown).

Kinetics of changes in peritoneal $\gamma\delta$ T lymphocytes after BCG infection in β_2m^- and β_2m^+ mice. The peritoneal cavity is reported to be a site for preferential accumulation of $\gamma\delta$ T cells after intraperitoneal infection with avirulent mycobacteria (20). Total numbers of mononuclear cells in the peritoneal cavity remained stable at 5 days after infection for both mouse strains ($P > 0.05$) (Fig. 3A). The peak of cellular increase occurred at about 8 days after infection and returned to baseline levels by 28 days after infection. Total numbers of $\gamma\delta$ T cells roughly followed the rise of mononuclear cells in the peritoneal cavity for β_2m^+ mice (Fig. 3B). β_2m^- mice had higher numbers of baseline total mononuclear cells and $\gamma\delta$ T cells compared with β_2m^+ mice ($P < 0.02$). β_2m^- mice had higher numbers of peritoneal mononuclear cells at day 28 after infection ($P < 0.01$) and higher numbers of $\gamma\delta$ T cells at day 14 after infection ($P < 0.05$) compared with β_2m^+ mice. Total numbers of $\gamma\delta$ T cells averaged fourfold higher in the peritoneal cavities of β_2m^- mice than in those of β_2m^+ mice on days 0, 5, 14, and 28.

Most striking was the ratio of $\gamma\delta$ T cells to total T cells (CD3⁺) in the peritoneal cavities of β_2m^- mice compared with those of β_2m^+ mice (Fig. 3C). Similar percentages were present at baseline, but the β_2m^- mice had already begun to show an increase in the percentage $\gamma\delta$ T cells by day 5 after infection, whereas β_2m^+ mice had not yet manifested an increase ($P < 0.02$). The two strains had similar peaks at about 8 days after infection. Compared with β_2m^- mice, β_2m^+ mice had a more rapid decrease in $\gamma\delta$ T cells, with a return to baseline percentages by day 28 after infection ($P < 0.02$). The increased percentage of $\gamma\delta$ T cells in the peritoneal cavities of β_2m^- mice at day 5 after infection (Fig. 3C) was at least partially due to a decrease in $\alpha\beta$ T cells, since the absolute number of $\gamma\delta$ T cells did not change significantly between days 0 and 5 ($P > 0.05$) (Fig. 3B). β_2m^+ mice had a parallel increase in absolute numbers and percentages of $\gamma\delta$ T cells (Fig. 3B and C). Overall, for both mouse strains, the percentage of $\gamma\delta$ T cells rose after infection and peaked on the same day, averaging 17% of the total T cells in the peritoneal cavity. However, β_2m^- mice had higher numbers of $\gamma\delta$ T cells in the peritoneal cavity prior to infection, an earlier rise in percentage of $\gamma\delta$ T cells within the T-cell population after infection, and a more sustained increase in total numbers of $\gamma\delta$ T cells. Most evident was a higher percentage of $\gamma\delta$ T cells remaining at 28 days after infection, averaging 17% of the total

FIG. 3. Kinetics of the accumulation of mononuclear cells (A), total $\gamma\delta$ T cells (B), and $\gamma\delta$ T cells as the percentage of total CD3⁺ T cells (C) in the peritoneal cavities of β_2m^+ (open triangles) and β_2m^- (closed triangles) mice after intraperitoneal infection with BCG. Total $\gamma\delta$ T cells are calculated by percentage of positive $\gamma\delta$ T cells (doubly stained with anti- $\gamma\delta$ and anti-CD3) multiplied by the number of total mononuclear cells. $\gamma\delta$ T cells as a percentage of total T cells is calculated by dividing the percentage of positive $\gamma\delta$ T cells by the percentage of positive $\alpha\beta$ T cells (doubly stained with anti- $\alpha\beta$ and anti-CD3) and multiplying by 100. Shown are the means $\pm$ standard errors of the means of four animals.

FIG. 4. Kinetics of the accumulation of mononuclear cells (A), total $\gamma\delta$ T cells (B), and $\gamma\delta$ T cells as the percentage of total $CD3^+$ T cells (C) in the spleens of β_2m^+ (open triangles) and β_2m^- (closed triangles) mice after intraperitoneal infection with BCG. Calculations were as for Fig. 3. Shown are the means $\pm$ standard errors of the means of four animals.

T-cell population for β_2m^- mice, compared with 6% for β_2m^+ mice.

Kinetics of changes in spleen $\gamma\delta$ T lymphocytes after BCG infection in β_2m^- and β_2m^+ mice. Mycobacteria are detectable in the spleen within 1 h after intraperitoneal infection (Fig. 1B). Although mycobacteria steadily decrease in the peritoneal cavity, they increase and then plateau in the spleen. We and others have found that BCG can be cultured from the spleens of β_2m^+ and β_2m^- mice as late as 3 months after intravenous or intraperitoneal infection (11, 12) and in individual mice for as long as 1 year after infection (data not shown). Therefore, we examined changes in the mononuclear cell population of the spleen, an organ responsible for controlling a chronic infection. In the β_2m^+ mice, the total numbers of mononuclear cells decrease at 5 days after BCG infection ($P < 0.001$) and then rise to normal levels by day 8 (Fig. 4A). In the β_2m^- mice total numbers of spleen mononuclear cells decrease slightly and then increase between days 5 and 8 after infection ($P < 0.05$). Total spleen mononuclear cells return to normal levels in β_2m^+ mice but remain slightly elevated in β_2m^- mice at 28 days after infection ($P < 0.05$).

In both strains of mice, the total numbers of $\gamma\delta$ T cells fall slightly in the spleen at 5 days after infection and then rise on day 8 for β_2m^- mice ($P < 0.001$) and day 14 for β_2m^+ mice ($P < 0.02$) (Fig. 4B), following the total mononuclear cell count (Fig. 4A). As was seen in the peritoneal cavity, β_2m^- mice have higher numbers of $\gamma\delta$ T cells than β_2m^+ mice, twice as high on day 8 ($P < 0.01$) and three times as high on day 28 ($P < 0.02$). At all time points examined, $\gamma\delta$ T cells averaged a higher proportion of the total splenic T-cell population of β_2m^- mice compared with β_2m^+ mice but reached significance only at days 5 ($P < 0.05$), 8 ($P < 0.002$), and 14 ($P < 0.05$) after infection (Fig. 4C). However, the maximum average percentage of $\gamma\delta$ T cells in spleens of β_2m^- mice was only 8% of T cells, compared with 17% of T cells in the peritoneal cavity.

$\gamma\delta$ T cells may defend against bacterial, viral, and parasitic intracellular pathogens (15). $\gamma\delta$ T cells also may play a role in immune-mediated pathology in such diseases as multiple sclerosis and rheumatoid arthritis (17, 44). However, the relative contribution of $\gamma\delta$ T cells to protection or to immune-mediated pathology may be difficult to delineate because of overlapping compensatory immune responses (38, 41). The role of $\gamma\delta$ T cells in protection against mycobacteria is also unproved. Janis and colleagues (22) showed that the accumulation of $\gamma\delta$ T cells averaged 10% of $CD3^+$ cells in the draining lymph nodes of

mice infected with avirulent *Mycobacterium tuberculosis*. In the peritoneal cavity of mice infected with avirulent mycobacteria (BCG), Inoue and colleagues (20) documented an increase of $\gamma\delta$ T cells of 26% of $CD3^+$ cells by day 7 after intraperitoneal infection. However, Griffin and colleagues (14) found only a 10 to 15% increase in the draining lymph nodes after subcutaneous infection with BCG and no significant increase in the peritoneal cavity at day 7 after intraperitoneal infection. The $\gamma\delta$ T-cell response of mice to infection with live avirulent mycobacteria clearly varies according to the model used. One important contribution to the differences in $\gamma\delta$ T-cell responses between different strains of mice are genes linked to the MHC (25).

The etiology of the increased quantity of $\gamma\delta$ T cells in β_2m^- mice is not clear. Others have shown that uninfected β_2m^- mice, not transgenic for a $\gamma\delta$ T-cell receptor, showed normal or even increased numbers of $\gamma\delta$ T cells in the spleen, thymus, lymph node, and intestines (5). We confirm a higher number of $\gamma\delta$ T cells in the peritoneal cavities and spleens of newly infected (Fig. 4) and uninfected (data not shown) β_2m^- mice. We also show that the increase in $\gamma\delta$ T cells in β_2m^- mice infected with avirulent mycobacteria is similar to the increase that we see in normal β_2m^+ mice, in that a similar peak occurs temporally. However, higher numbers of $\gamma\delta$ T cells are seen in β_2m^- mice, and the increase is sustained. We do not know what factors normally regulate the number or percentage of $\gamma\delta$ T cells. Competition for, and stimulation by, cytokines or antigen and the direct regulation by other immune cells are all possible mechanisms of control. There is a deficiency of $CD8^+$ T cells in β_2m^- mice. This deficiency might support an increased number of $\gamma\delta$ T cells through increased availability of cytokines which would otherwise have been used by the $CD8^+$ T-cell compartment. Alternatively, in the normal mouse, the $CD8^+$ T cells might regulate $\gamma\delta$ T-cell numbers through direct cytotoxic activity or through the production of suppressive cytokines. Cytotoxic activity is decreased in β_2m^- mice coincident with the deficiency in $CD8^+$ T cells (7, 24, 27, 45). Additionally, the major target for cytotoxic T cells, class I MHC, is expressed at an insufficient level. Class II MHC is normally expressed only on antigen-presenting cells and not on mouse T cells (4). Therefore, both the deficiency of $CD8^+$ T cells and the deficiency of class I MHC may alter some of the normal control mechanisms regulating $\gamma\delta$ T cells.

$\gamma\delta$ T cells could increase as a result of the increased level of accessible antigen resulting from the absence of competing

CD8⁺ T cells. This antigen could still be presented by certain class I MHC molecules. Although little class Ia MHC is expressed, some class Ib MHC molecules are expressed despite the absence of β_2 -microglobulin (1). Alternatively, mycobacterial antigens might be presented independently of class I MHC (10), and these antigens could have superantigen-like activity (13). A third explanation implicates the dependence of $\gamma\delta$ T cells upon CD4⁺ T cells for expansion, with the higher numbers of CD4⁺ T cells in β_2 m⁻ mice resulting in the observed expansion of $\gamma\delta$ T cells (42). A recent report describes an inherited human TAP peptide transporter deficiency with lower class I MHC expression, decreased numbers of CD8⁺ cells, and higher numbers of $\gamma\delta$ T cells (6). We have not examined the subset specificity of the $\gamma\delta$ T-cell receptors on the cells of infected β_2 m⁻ mice. Correa and colleagues have reported similar patterns of $\gamma\delta$ T-cell receptor subsets in β_2 m⁻ and β_2 m⁺ mice (5). A final explanation for the higher numbers of $\gamma\delta$ T cells in β_2 m⁻ mice will require studies with multiple models of cytotoxic and MHC deficiencies. Nonetheless, the data document a subset of $\gamma\delta$ T cells that are responsive in vivo to avirulent mycobacteria independent of class I MHC restriction.

SCID, β_2 m⁺, and β_2 m⁻ mice all showed similar levels of avirulent mycobacteria in the peritoneum and spleen prior to day 8 after infection. Therefore, the early clearance of avirulent mycobacteria up to this time is probably dependent on innate rather than acquired immune mechanisms. Compared with the β_2 m⁺ or SCID mice, the β_2 m⁻ mice have fewer mycobacteria in the spleen by day 8. Therefore, 8 days after infection, a transition period occurs in which acquired mechanisms of immunity become relevant for controlling avirulent mycobacterial growth in some strains of mice. We noted slightly increased numbers of BCG in spleens of β_2 m⁺ mice compared with β_2 m⁻ mice at 8 days after infection; this was also documented previously by Flynn and colleagues (12). This transient increased control of mycobacteria in the spleen by β_2 m⁻ mice at day 8 after infection could be due to higher levels of innate immunity, an earlier onset of acquired immunity, or both. Therefore, we can only speculate that the initial high levels of $\gamma\delta$ T cells in the spleens of β_2 m⁻ mice may have contributed to an earlier control of BCG growth in the spleen. Of note, this slightly increased control is likely not to be relevant to infection with virulent mycobacteria, as others have shown that β_2 m⁻ mice fail to show any early control of virulent mycobacteria compared with β_2 m⁺ mice (12). β_2 m⁻ mice also have increased numbers of CD4⁺ T cells, but these cells are less likely to respond to mycobacterial antigens without prior sensitization. Similar kinetics were seen in a mouse model of *Listeria* infection, in which β_2 m⁻ mice controlled the early growth of bacteria better than β_2 m⁺ mice (38).

In summary, we confirm that β_2 m⁻ mice control infection with avirulent mycobacteria BCG despite a deficiency of class I MHC and CD8⁺ T cells (12). The onset of control of mycobacterial growth in the spleen may be slightly earlier in β_2 m⁻ than in β_2 m⁺ mice and seems to correlate with an earlier and more sustained rise in $\gamma\delta$ T cells in this compartment. However, we can only speculate that the slightly lower number of bacilli is due to the increased numbers of $\gamma\delta$ T cells. Clearly, the deficiencies in β_2 -microglobulin, CD8⁺ T cells, and class I MHC lead to altered regulation of $\gamma\delta$ T cells and the increased in vivo proliferation in response to avirulent mycobacteria. Since β_2 m⁻ mice are deficient in functional cell surface class I MHC, we conclude that some subsets of $\gamma\delta$ T cells are reactive to mycobacterial antigens in vivo without MHC class I restriction.

We thank Donald W. Smith for the gift of BCG stocks, Beverly H. Koller for the original breeding pairs of β_2 m⁻ mice, and Julia Jernberg, Charles Czaprynski, and David Hildeman for critical reading of the manuscript.

This investigation received financial support to D.M. from the WHO/UNDP Programme for Vaccine Development, the American Cancer Society (institutional grant IN-35-34), the National Institutes of Health (grant AI 33601), and the Department of Medicine, the Medical School, and the Graduate School at UW—Madison and to V.M. from the National Institutes of Health (grant CA 55623).

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