Investigation of the Role of CD8$^+$ T Cells in Bovine Tuberculosis In Vivo

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Received 25 October 2002/Returned for modification 22 January 2003/Accepted 15 April 2003

Mycobacterium bovis is the causative agent of bovine tuberculosis (TB), and it has the potential to induce disease in humans. CD8$^+$ T cells (CD8 cells) have been shown to respond to mycobacterial antigens in humans, cattle, and mice. In mice, CD8 cells have been shown to play a role in protection against mycobacterial infection. To determine the role of CD8 cells in bovine TB in vivo, two groups of calves were infected with the virulent M. bovis strain AF2122/97. After infection, one group was injected with a CD8 cell-depleting monoclonal antibody (MAb), and the other group was injected with an isotype control MAb. Immune responses to mycobacterial antigens were measured weekly in vitro. After 8 weeks, the animals were killed, and postmortem examinations were carried out. In vitro proliferation responses were similar in both calf groups, but in vitro gamma interferon (IFN-\(\gamma\)) production in 24-h whole-blood cultures was significantly higher in control cattle than in CD8 cell-depleted calves. Postmortem examination showed that calves in both groups had developed comparable TB lesions in the lower respiratory tract and associated lymph nodes. Head lymph node lesion scores, on the other hand, were higher in control calves than in CD8 cell-depleted calves. Furthermore, there was significant correlation between the level of IFN-\(\gamma\) and the head lymph node lesion score. These experiments indicate that CD8 cells play a role in the immune response to M. bovis in cattle by contributing to the IFN-\(\gamma\) response. However, CD8 cells may also play a deleterious role by contributing to the immunopathology of bovine TB.

MATERIALS AND METHODS

Cattle. Ten male Friesian calves 2 to 4 weeks of age obtained from a TB-free farm were divided into two groups of five. The calves were housed in appropriate containment category III facilities for 4 weeks prior to any experimentation. This

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α, that are central to macrophage activation and granuloma formation (8, 19). T cells are also able to kill mycobacterium-infected cells (32, 33, 37, 48). The killing of infected cells can result in either the release of intracellular bacteria or killing of both the infected cell and the infecting bacteria. It has been shown that ATP can induce apoptosis of macrophages infected with mycobacteria, as well as inducing the killing of the infecting pathogen (25, 50), and it is postulated that secretion of extracellular ATP directed to the infected macrophage could be a mechanism by which T cells stimulate the killing of intracellular mycobacteria (50). More recently, CD8$^+$ T cells have been shown to release granulysin into infected macrophages following the delivery of perforin, which would kill the host cell and then kill the infecting mycobacteria; granulysin has been shown to be capable of killing free-living mycobacteria (48). Thus, CD4$^+$ and CD8$^+$ cells contribute to the formation of the TB granuloma and the arrest of mycobacterial growth mainly by the expression of a T helper type 1 (Th1) response.

Although a role for T cells in immunity to mycobacteria has been shown directly (in vivo) in mice and indirectly (in vitro) in mice, humans, and cattle, there are few studies documenting the direct (in vivo) involvement of T cells in immunity to mycobacteria in other species. Here, we report that in vivo depletion of CD8$^+$ T cells in cattle in the early stages of infection with M. bovis did not affect the ability of peripheral blood cells to proliferate in response to mycobacterial antigens but did reduce their ability to produce IFN-\(\gamma\). Depletion also resulted in a lower level of pathology in the head lymph nodes than for nondepleted control calves. These results suggest that CD8$^+$ cells play a role in the immunopathology of TB.
period was used to observe the calves and to ensure their freedom from preexis-
ting respiratory infections. All experiments conformed to local and national
guidelines on the use of experimental animals and category III infectious
organisms.

**Inoculation of cattle with M. bovis and depletion of CD8⁺ T cells.** The cattle
were inoculated intratracheally with 10⁵ CFU of *M. bovis* strain AF2122/97. Pilot
experiments indicated that this strain at this dose and by this route induced TB
in calves 6 to 8 weeks of age. The inoculum titer was confirmed by colony counts
on 7H10 agar plates. Ten days after inoculation with *M. bovis*, the calves were
injected intravenously with 10 mg of the CD8-depleting MAb CC63 (52) (CD8-
depleted calves) or 10 mg of the isotype control, MAb AV55 directed against a
chicken leukocyte antigen (F. Davison, personal communication) (control
calves) for seven consecutive days. In pilot experiments, this protocol proved to
reduce the number of CD8⁺ T cells in peripheral blood (data not shown).
Additionally, MAb CC63 has been shown to affect the course of respiratory
syncytial virus infection, indicating that it is capable of depleting CD8⁺ T cells in
the respiratory tract (52). Prior to the inoculation of MAb, the calves were
injected with flumixin meglumine (Scherer and Plough, Uxbridge, Middlesex,
United Kingdom) to prevent the occurrence of an anaphylaxis-like reaction due
to the depletion of CD8⁺ T cells. The depletion of CD8⁺ T cells was monitored
in peripheral blood cells by flow cytometry as described below.

**Measurement of immunological responses to *M. bovis* antigens.** Blood was
collected into heparin (final concentration, 10 IU/ml) to monitor immune re-
 sponses. IFN-γ was measured in plasma taken from blood incubated with RPMI
1640 medium or purified protein derivative (PPD) from *Mycobacterium avium*
(PPD-A) or *M. bovis* (PPD-B) (VLA, Surrey, United Kingdom) at 20 μg/ml final
concentration at 37°C in an atmosphere of 5% CO₂ and 95% humidity for 24 h.
This protocol is similar to that used to determine IFN-γ production against
mycobacterial antigens for diagnostic purposes (57). The blood was centrifuged
at 800 × g for 10 min, and the plasma was harvested and stored at −20°C until
it was assayed. The concentration of IFN-γ was determined by standard capture
enzyme-linked immunosorbent assay as described previously (22) using MAb
CC330 as the capture antibody and MAb CC302 as the detection antibody. To
determine proliferative responses, 200 μl of blood/well diluted 1:10 with RPMI
1640 containing glutamax-II (Gibco, Paysley, United Kingdom) was incubated in
triplicate in 96-well round-bottom plates for 6 days with medium alone or with 10 μg of PPD-A or PPD-B/ml (final concentration). For the last 18 h, 37 MBq of
dom) was added, and the plates were frozen. The plates were thawed,
parafomaldehyde was added to a final concentration of 1%, and the plates were
incubated for 1 h at room temperature to eliminate the risk of mycobacteria
being present in the sample. The cells were harvested with a Skatron semi-auto-
mated cell harvester onto glass fiber filter mats (Walac, Tuulu, Finland). The
incorporated radioactivity was determined in a scintillation counter (Pharmacia,
Uppsala, Sweden) as counts per minute. The results are expressed as stimulation
index (SI), which is the counts incorporated by the cells cultured in the presence
of antigen divided by the counts incorporated by the cells cultured in medium
alone.

**Flow cytometry.** To monitor peripheral blood cell populations, blood was
placed in tubes with 5 volumes of Gey’s solution (29) containing 1% parafor-
maldehyde. After 1 h of incubation at room temperature, the peripheral blood
leukocytes (PBL) were pelleted at 500 × g for 10 min and washed three times
with phosphate-buffered saline (PBS) by centrifugation at 400 × g for 5 min.
Phenotypic analysis of the cells was carried out as described elsewhere (17)
with slight modifications. In brief, 10⁶ cells were incubated with MAb directed against
bovine leukocyte antigens for 10 min at room temperature. The cells were
washed three times in PBS, incubated with goat antibodies specific for mouse
isotype immunoglobulin G1 (IgG1), IgG2a, or IgG2b (Southern Biotechnology
Associates) for 10 min, washed as described above, and analyzed in a FACScal-
bur (Becton Dickinson). Staining was performed using MAb to bovine CD4
(CD4), CD8 (CC63), WC1 (CC15), CD14 (CCG33), major histocompatibility
complex class II (IL-A88), and CD25 (IL-A111), together with control MAb
AV20, AV29 (42, 43), and AV37 (F. Davison, personal communication) directed
against chicken antigens. The data are expressed as percentages of target cells in
the total PBL.

**Postmortem examinations.** The following tissues were examined at postmor-
tem: head lymph nodes (parotid, submandibular, and retropharyngeal), lower
respiratory tract-associated lymph nodes (mediastinal and up to four bronchial
nodes), tonsils, and lungs. The tissues were sliced at 0.5- to 1-cm intervals and
examined macroscopically. To determine the magnitude of the lesion, the fol-
lowing grading system was used (55). Lymph nodes were assigned 0 for no visible
lesion, 1 for a small focus 1 to 2 mm in diameter, 2 for several small foci 1 to 2
mm in diameter or a necrotic area of 5 by 5 mm, and 3 for extensive necrosis.

Lungs were assigned a value of 0 for no visible lesions, 1 for no gross lesion but
lesions apparent upon slicing, 2 for up to five lesions <10 mm in diameter, 3 for
more than six lesions <10 mm in diameter, 4 for one distinct lesion >10 mm in
diameter, and 5 for gross coalescing lesions.

**Histology.** Tissue biopsy specimens were fixed in 10% neutral buffered forma-
tolin for at least 7 days before being processed. The biopsy specimens were
embedded in wax, and 4-μm-thick sections were cut and stained with hematox-
ylin and eosin using standard procedures.

**RESULTS**

*MAb CC63 transiently depletes CD8⁺ T cells in cattle.* Injec-
tion of MAb CC63 has been shown to deplete CD8⁺ T cells in
peripheral blood and in the lungs and to influence the
progress of an experimental respiratory syncytial virus infec-
tion in cattle (52). We confirmed that the regime followed in
our experiments indicated that this strain at this dose and by this route induced TB
in calves 6 to 8 weeks of age. The inoculum titer was confirmed by colony counts
(55) and incubated at 37°C. After 4 weeks, colonies
were counted; the results are expressed as log₁₀ CFU per gram of tissue. This
period of incubation with this strain has proven to be enough to determine
whether the cultures are negative.

Injection of MAb CC63 into calves infected with *M. bovis* induced, as expected, a
marked transient depletion of CD8⁺ T cells in peripheral blood (Fig.
1), which was statistically significant at week 2 (P = 0.01 by the
* t* test) and occurred in all five inoculated calves. Shortly after antibody treatment was stopped, the CD8⁺ T cells reappeared.

**Proliferative responses of 1:10-diluted blood to mycobacte-
ria antigens.**[^3]H-TdR incorporation in response to PPD-B was
detectable in all animals from both groups by week 4 postin-
fec tion (Fig. 2). Some variability was noted throughout the
experiment; however, the SI never dropped below 10 for any of
the animals in either group.[^3]H-TdR incorporation in response to
PPD-A (not shown) was detectable in some animals of both
groups at different times, but it was much lower than that seen
with PPD-B. No differences were detected in the proliferative
responses of the depleted and nondepleted groups.

**Production of IFN-γ in blood in response to mycobacterial
antigens in vitro.** Production of IFN-γ against PPD-A (not shown)
or PPD-B (Fig. 3) was evaluated in the plasma of 24-h whole-blood cultures. The production of IFN-γ in response to
medium alone remained undetectable throughout the experi-
ment, and therefore it is not shown. IFN-γ against PPD-B was
detected in some animals in the two groups by 3 weeks after
inoculation and in all animals in both groups by 4 weeks after
inoculation and remained relatively high throughout the
experiment. A comparison of the production of IFN-γ against
PPD-B in the two groups from week 4 onward shows that
control calves produced more IFN-γ than depleted calves (P = 0.0367 by a Mann-Whitney test). At the peak of the response at
week 5, control calves produced IFN-γ with a range from
40.7 to 469.8 pg/ml, while depleted calves in the same week
produced IFN-γ with a range of 55.3 to 117.9 pg/ml. Thus,
depletion of CD8⁺ T cells early during infection appeared to
compromise the ability of the animals to mount high IFN-γ
responses to mycobacterial antigens later in infection. IFN-γ
production against PPD-A (not shown) was detectable by week
4 postinfection and reached a peak at 4 to 7 weeks postinfc-
tion, with a median production of 6.25 pg/ml for the control

group and 4.18 pg/ml for the CD8-depleted group. However, the production of IFN-γ against PPD-A compared to that in response to PPD-B was minor. No correlation was found between proliferative responses and production of IFN-γ in response to PPD-B.

**Evaluation of TB lesions at postmortem.** Table 1 shows the outcome of the evaluation of the presence of TB lesions at postmortem, 8 weeks after challenge. No differences were detected between the two groups in the levels of lesions in the lungs and associated lymph nodes. This is reflected in the lesion scores for these organs, with a cumulative score of 66 for calves depleted of CD8+ T cells and 69 for control calves. However, the score for TB lesions in the head lymph nodes showed a difference between the two groups: CD8+ T-cell-depleted animals had a total score of 21 in these tissues. On the other hand, the control calves had a score of 53 in these tissues. Although not statistically significant (P = 0.115), these differences show a trend indicating that CD8 cells contribute to pathology.

The studies described indicated a possible relationship between production of IFN-γ and pathology. The amounts of IFN-γ produced by PBL in response to PPD-B in vitro from each calf throughout the experiment were added, and the total was plotted against the pathology score. The plot (Fig. 4) shows a direct correlation between production of IFN-γ and gross pathology. Thus, it would appear that a general effect of depleting CD8 cells is diminished production, but not total absence, of IFN-γ, which is associated with diminished pathology.

**Mycobacterial viable counts in lymph nodes and histological examination.** Table 2 shows the results of the culture of selected lymph node biopsy specimens on 7H11 agar. The limit of detection of the technique employed is five bacteria. Mycobacteria were isolated from mediastinal lymph nodes (Table 2), but not from head lymph nodes (retropharyngeal or parotid). No differences were detected between depleted and control calves. Although no bacteria were detected in parotid or retropharyngeal nodes, upon histological examination, it was confirmed that the lesions were typical of *M. bovis*, showing giant Langhan’s cells within developing areas of necrosis, and in more advanced lesions, calcification was evident. Thus, the depletion of CD8 cells early after infection did not have consequences for the bacterial load at postmortem, 8 weeks after infection.

**DISCUSSION**

In this work, we evaluated the role of CD8+ T cells in immunity to *M. bovis* in cattle in vivo by depleting target cells with MAb. PBL from animals depleted of CD8 cells produced reduced amounts of IFN-γ in vitro in response to *M. bovis* antigens, indicating that in vivo these cells play a role in the immune response to mycobacteria by contributing to the amount of IFN-γ produced postchallenge. However, CD8 cells
also appeared to contribute to the immunopathology of bovine TB, possibly through the regulation, or the production, of IFN-γ. The importance of CD8 cells in protection against TB in vivo was first shown in adoptive-transfer experiments with mice (35, 36). Later experiments, using depletion of CD8 cells by MAb in mice infected with *M. bovis*, further showed that these cells play a role in immunity to mycobacteria (30). More recently, mice genetically deficient in β2m or TAP have been used to demonstrate a role for major histocompatibility complex class I-restricted cells in immunity to tuberculosis (13). Further evidence comes from immunization studies with dendritic cells pulsed with CD8 cell-restricted epitopes that were found to be protective (28). In humans, a role for CD8 T cells in immunity is suggested by studies showing the presence of specific immune cells after infection or vaccination with *M. bovis* BCG (45–47, 49). In cattle, like humans, a role for CD8 cells can be inferred from the presence of antigen-specific immune CD8 T cells following infection with *M. bovis* or vaccination with BCG (15, 26, 27).

To study the role of CD8 cells in immunity to mycobacteria in cattle, we used MAb to deplete the target population. However, depletion using MAb can only be carried out for a limited time, as an anaphylactic reaction to murine IgG develops in calves ~10 days after initial inoculation (16). Therefore, any effects seen in this experiment would be the result of the temporary depletion of the target population while MAb was being inoculated. We have previously used similar depletion protocols to target T-cell subpopulations for depletion in cattle to investigate their roles in different infections. The clearest findings have been with viruses causing acute transient disease, and they have clearly shown the involvement of CD4 and CD8 cells in immunity to infection (16, 34, 52). Using a similar protocol, depletion of WC1+ cells in cattle infected with *M. bovis* provided evidence that these cells contributed to the IFN-γ response in vivo and to the Th1 bias seen in bovine TB (21).

We chose to start CD8 cell depletion 10 days after inoculation with *M. bovis*, as in our experience and that of others, specific immune responses to mycobacteria are detected 2 to 4 weeks after infection (3, 39, 40, 55). Furthermore, it has been reported that CD8 cells appear early, after 1 to 2 weeks, in response to infection with mycobacteria in mice (44). Thus, the timing of the inoculation of MAb should have coincided with the early stage of the immune response at the site of infection, ensuring that the CD8 response would be delayed and that any effect would be magnified over the ensuing period. Measure-

![FIG. 2. Proliferative responses of PBL from individual control (A) and CD8-T-cell-depleted (B) calves to PPD-B expressed as SI. The continuous line represents the median SI of the group, and the number next to each bar is its numeric value of the average. Each symbol represents the results for one animal.](image1)

![FIG. 3. IFN-γ production in picograms per milliliter by PBL from individual control (A) and CD8-depleted (B) calves against PPD-B. The continuous line represents the median of the group, and the number next to each bar is its numeric value. Each symbol represents the results for one animal.](image2)
TABLE 1. Lesions in lymph nodes of control or CD8-depleted calves at postmortem, 8 weeks after inoculation

<table>
<thead>
<tr>
<th>Site</th>
<th>Score*</th>
<th>Summary of control calves</th>
<th>Summary of depleted calves</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>47</td>
<td>64</td>
</tr>
<tr>
<td>Head nodes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parotid L</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Parotid R</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Submand L</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Submand R</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Retrophar L</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Retrophar R</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Tonsils</td>
<td>L</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>R</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Total head nodes</td>
<td>24</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>Lower respiratory tract</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Lung nodes</td>
<td>Mediastinal</td>
<td>3</td>
<td>3</td>
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<td>3</td>
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<td>Bronch 2</td>
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<td>3</td>
<td>3</td>
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<tr>
<td>Lungs</td>
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<td>5</td>
<td>5</td>
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<tr>
<td>Apical R</td>
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<td>3</td>
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<tr>
<td>Cardiac L</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cardiac R</td>
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<td>3</td>
<td>3</td>
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<td>4</td>
<td>5</td>
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<tr>
<td>Inter R</td>
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<td>5</td>
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</tr>
<tr>
<td>Total general</td>
<td>49</td>
<td>27</td>
<td>12</td>
</tr>
</tbody>
</table>

* Scores were given as described in Materials and Methods.
* a L, left; R, right; Submand, submandibular; Retrophar, retropharyngeal; Diaphragmatic, Bronch, bronchial; Inter, intermediate.

FIG. 4. Correlation between cumulative production of IFN-γ by whole blood cells against PPD-B and gross pathology score (P ≤ 0.001; R² = 0.7898). Squares, control calves; crosses, depleted calves.

TABLE 2. Mycobacterial viable counts in lymph nodes of control or CD8-depleted calves at postmortem (8 weeks after inoculation) expressed as \( \log_{10} \) CFU

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control calves</th>
<th>CD8-depleted calves</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>47</td>
<td>64</td>
</tr>
</tbody>
</table>

* The limit of detection using the isolation technique described in Materials and Methods is five colonies; thus, <1 indicates that no bacteria were cultured from that sample. NA, not available.
* a Animal number.

counterparts (18). In mice, it has been demonstrated that one mechanism by which IFN-γ contributes to protection is by activating macrophages to kill intracellular mycobacteria (1, 10). However, in humans and cattle, the role of IFN-γ in the activation of macrophages for the killing of mycobacteria has been more difficult to pinpoint (2, 53), as preincubation of human or bovine macrophages with IFN-γ alone does not induce the activation of macrophages to kill intracellular mycobacteria in vitro. In the human and bovine systems, the presence of lymphocytes is required for the control of mycobacteria, although in the bovine system, this phenomenon is not antigen specific (5). In this work, we found no differences between CD8-depleted and control groups in the incorporation of 3H-TdR by PBL from CD8-depleted calves produced less IFN-γ in response to mycobacteria than PBL from control calves. Thus, depletion of CD8 cells in vivo resulted in a diminished ability of PBL to produce IFN-γ in response to mycobacterial antigens in vitro. It is unlikely that lower production of IFN-γ was due to a lower percentage of CD8+ cells in peripheral blood at the time the in vitro test was carried out. From week 4 onward, when the differences between CD8-depleted and control calves became apparent, the CD8 population in the peripheral blood of depleted calves had recovered from the trough of depletion at week 2. Further, PPD-B would have been loaded into the exogenous antigen presentation pathway and therefore would have been unlikely to stim-

counterparts (18). In mice, it has been demonstrated that one mechanism by which IFN-γ contributes to protection is by activating macrophages to kill intracellular mycobacteria (1, 10). However, in humans and cattle, the role of IFN-γ in the activation of macrophages for the killing of mycobacteria has been more difficult to pinpoint (2, 53), as preincubation of human or bovine macrophages with IFN-γ alone does not induce the activation of macrophages to kill intracellular mycobacteria in vitro. In the human and bovine systems, the presence of lymphocytes is required for the control of mycobacteria, although in the bovine system, this phenomenon is not antigen specific (5). In this work, we found no differences between CD8-depleted and control groups in the incorporation of 3H-TdR by PBL from CD8-depleted calves produced less IFN-γ in response to mycobacteria than PBL from control calves. Thus, depletion of CD8 cells in vivo resulted in a diminished ability of PBL to produce IFN-γ in response to mycobacterial antigens in vitro. It is unlikely that lower production of IFN-γ was due to a lower percentage of CD8+ cells in peripheral blood at the time the in vitro test was carried out. From week 4 onward, when the differences between CD8-depleted and control calves became apparent, the CD8 population in the peripheral blood of depleted calves had recovered from the trough of depletion at week 2. Further, PPD-B would have been loaded into the exogenous antigen presentation pathway and therefore would have been unlikely to stim-
ulate CD8+ cells during a 24-h incubation. Rather, it is likely that through the production of IFN-γ in vivo, CD8+ T cells contribute to the Th1 polarization of the immune response, the results of which are reflected in the in vitro production of IFN-γ against mycobacterial antigens, mainly by CD4+ T cells (reference 56 and our unpublished observations).

Due to their reduced ability to produce IFN-γ in response to PPD-B in vitro, it would have been expected that CD8-depleted calves would be more susceptible to infection than controls. Postmortem examination revealed that depleted and control calves had similar levels of pathology in the lower respiratory tract lymph nodes, but CD8-depleted calves had a lower lesion score in the head lymph nodes than control calves. Further analysis of these results also revealed a positive correlation between production of IFN-γ and the gross pathology score, indicating that IFN-γ could be involved in the pathogenesis induced by M. bovis.

It might have been expected that a reduced ability to produce IFN-γ in response to mycobacterial antigens in CD8-depleted calves would have consequences for the bacterial load in these calves. On the other hand, the number of lesions might be expected to be related to the bacterial load and therefore to be greater in control calves. No indication of differences in the numbers of mycobacteria detected in depleted or control lymph nodes was found, although histological examination revealed lesions typical of TB. It is necessary to interpret bacterial counts with caution; for instance, it has been shown that the level of pathology in cattle is disproportionate to the bacterial load compared to the pathology levels and bacterial loads seen in other animal species (14). Also, to determine the bacterial load, we used 1 g of tissue, which may not be fully representative.

Thus, although it has been shown that CD8+ cells are required for protection against mycobacteria, it is possible that through regulation of the production of IFN-γ, CD8 cells may also contribute to pathogenesis in the bovine model of TB. This would also imply that, like CD8+ cells, IFN-γ, although necessary for protection, also contributes to the pathology of M. bovis in cattle. It is possible that following CD8 depletion in the early stages of infection, an altered balance of the immune response occurred from increased CD4 cell contact with infected antigen-presenting cells, which permitted the establishment of a more protective immune response. By the time CD8 cells returned to normal levels, their response would have been directed by the already established immune response toward protection rather than pathology. Although production of IFN-γ is regarded as necessary for protection, it has been shown in mice to be involved in the immunopathology induced by M. avium (11) and respiratory syncytial virus (38). In humans, the levels of IFN-γ in the serum or pleural fluid have been shown to be positively correlated with the level of disease (41, 54, 58). Recently, it has been proposed that the induction of a strong unregulated Th1 response may be a strategy of mycobacteria for survival in the host. The formation of the granuloma, followed by its liquefaction, may provide a rich environment to which the immune system has no access and in which the mycobacteria can replicate extracellularly (20). Immunity against mycobacteria is multifactorial and dependent on the balance between an inflammatory response that allows the host to develop a granuloma, which contains the microorganism, and an anti-inflammatory response that restricts the extent of the granuloma and allows contact of effector T cells with the infected cells, which results in the killing of the infecting pathogen. While a Th1 response is necessary for protection, it may also have immunopathological consequences.

ACKNOWLEDGMENTS

We thank M. Vordermeier and G. Hewinson for providing the virulent M. bovis AF212227 strain. We also acknowledge the contribution of staff in the HSU.

This work was supported by grants from the BBSRC and DEFRA.

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Editor: S. H. E. Kaufmann

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