Journal of Virology, Nov. 1995, p. 6658–6664 Vol. 69, No. 11
0022-538X/95/$04.00+0
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Role of T-Lymphocyte Subsets in Recovery from Respiratory Syncytial Virus Infection in Calves

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Received 12 June 1995/Accepted 3 August 1995

The role of T-cell subsets in respiratory syncytial virus (RSV) infection was investigated by using monoclonal antibodies (MAbs) to selectively deplete gnotobiotic calves of $CD4^+$, $CD8^+$, or $WC1^+$ $\gamma\delta$ T-cell receptor$^+$ lymphocytes. Injection of these MAbs produced specific reductions of the target cell populations in the circulation and tissues. Ten days after RSV infection, immunoglobulin M (IgM), IgG1, and IgA antibodies were detected in sera and lung washings from control calves. Depletion of $CD8^+$ T cells had no effect on either the serum or local antibody responses to RSV, whereas depletion of $CD4^+$ T cells suppressed the antibody responses in two of three calves. The IgM and IgA responses were significantly increased in the lung washings of calves from which $WC1^+$ T cells were depleted. Depletion of $CD4^+$ or $WC1^+$ T cells caused no significant delay in virus clearance, although an increase in the extent of pneumonic consolidation was observed in anti-CD4-treated calves. Nasopharyngeal excretion of RSV was prolonged in calves depleted of $CD8^+$ T cells, and virus was isolated in high titers from lung washings of these animals 10 days after infection, whereas virus had been cleared from lung washings of all other animals. The delayed virus clearance was associated with an increase in the severity of pneumonic consolidation in three of four of the calves from which $CD8^+$ T cells were depleted. This study shows that $CD8^+$ T cells play a dominant role in the recovery of calves from RSV infection.

The occurrence of unusually severe lower respiratory tract disease in more than three-quarters of the children given a formalin-inactivated respiratory syncytial virus (RSV) vaccine that failed to protect against infection (30, 32) has highlighted the need to understand the role of the immune response in RSV infections. Such studies have been carried out predominantly with small laboratory animals. Thus, passively transferred monoclonal antibodies (MAbs) and polyclonal antibodies can protect the lungs of mice and cotton rats against RSV infection, and there is no evidence that antibody can exacerbate disease (20, 39). An increase in the severity of lung lesions has not been observed even after passive transfer of nonprotective MAbs or antibody obtained from animals vaccinated with formalin-inactivated RSV that developed enhanced lung lesions after RSV challenge (8). Further studies of anti-μ-treated mice showed that although antibody was not required to terminate RSV replication in a primary infection, only partial immunity against challenge was induced in the absence of antibody (16).

In contrast to these studies with antibodies, T cells appear to contribute to the pathogenesis of disease in mice. Thus, passive transfer of RSV-specific, $CD8^+$ cytotoxic T-lymphocyte (CTL) lines or clones caused rapid clearance of virus from the lungs of RSV-infected mice but resulted in a lethal pulmonary disease (6). Passive transfer of RSV-specific $CD4^+$ T cell lines also clears RSV from the lungs of infected mice but can cause more severe pathological changes in the lungs than $CD8^+$ T cells (4). The type of lung pathology appears to vary depending on the T cell transferred to RSV-infected mice. For example, passive transfer of $CD4^+$ T-helper 2 (TH2) cells resulted in a severe, potentially fatal illness which was characterized by lung hemorrhage, pulmonary neutrophils, and intense pulmonary eosinophilia in RSV-infected mice, whereas a mixed $CD8^+$ CTL and $CD4^+$ TH1 cell line resulted in only a minimal enhancement of lung pathology with no eosinophilia (3). The role of $CD4^+$ and $CD8^+$ T cells in RSV infection has also been studied in mice from which these T-cell subsets were selectively depleted by using specific MAbs (17). Depletion of either $CD4^+$ or $CD8^+$ T cells alone had little or no effect on RSV infection; however, when both T-cell subsets were depleted, virus replication was prolonged. Furthermore, the lungs of mice from which both T-cell subsets were depleted had virtually no lymphocyte infiltration, and these mice did not develop signs of illness, indicating that host immune response, rather than virus cytocidal effect, was the main cause of disease.

A key question raised by these studies in small laboratory animals is to what extent they are applicable to natural RSV infections. Immunocompromised infants and adults are at risk for prolonged RSV excretion and severe lower respiratory tract disease, which can be fatal (13, 18, 19, 21). This contrasts with T-cell-depleted mice, in which prolonged virus shedding is associated with a reduction in the severity of lung lesions and illness (17) and highlights the need to study immune responses in a natural host of RSV. Bovine RSV is a major cause of respiratory disease in young calves (37), and the epidemiology and pathogenesis of RSV infection of calves closely mirror those of children. The availability of MAbs to bovine T-cell subsets provides the opportunity to examine the role of T cells in RSV infection in a natural host, i.e., calves. Three major subsets of bovine T lymphocytes have been identified in the circulation and secondary lymphoid organs. In addition to $CD4^+$ and $CD8^+$ T cells, there is a subpopulation of cells which are $CD2^-$, $CD5^+$, $CD4^-$, $CD8^-$, and $\gamma\delta$ T-cell receptor$^+$ (TcR) and express the 215,000- to 300,000-molecular-weight molecule WC1 (7, 25). Howard et al. (27) have depleted blood of specific T-cell subsets by intravenous injection of calves with the appropriate murine MAbs. In the present study, we caused depletion of $CD4^+$, $CD8^+$, or $WC1^+$ cells by treat-

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ment in vivo with MAbs to investigate the role of these cells in the recovery of calves from primary infection with bovine RSV.

MATERIALS AND METHODS

MAbs. The MAbs used for flow cytometry have been described at the International Workshop on Bovine, Sheep, and Goat Leukocyte Differentiation Antigens (25) and were CC8 (anti-CD4), CC63 (anti-CD8), and CC15 (anti-WC1). These MAbs were also used to deplete lymphocytes from calves. MAb F79EB6 to *Mycoplasma dispar* was administered to control animals and served to control for any effects due to inoculation of mouse proteins. All MAbs were murine immunoglobulin G2a (IgG2a), except F79EB6 (which is IgG2b), and were produced at the Institute for Animal Health, Compton, United Kingdom.

Virus and cells. The Snook strain of bovine RSV (43) was isolated from a calf with pneumonia and had been passaged twice in calf testis cells and three times in calf kidney cells. A stock of virus with a titer of 6×10^4 PFU/ml was prepared in primary calf kidney cells and stored in liquid N_2.

Animals and experimental design. Gnotobiotic, RSV-seronegative calves were derived, were reared on a milk-based diet as described previously (10, 23), and were maintained individually in plastic isolators. Calves, aged 7 to 16 days (mean, 9 ± 2 days), were inoculated with approximately 10^5 PFU of a standard stock of bovine RSV in a volume of 20 ml, 10 ml of which was administered intranasally and 10 ml of which was administered intratracheally on day 0. All calves were inoculated intravenously with 100 mg of the nonsteroidal antiinflammatory drug flunixin meglumine (Finadyne; Schering-Plough Animal Health) on day −1 only and with 4 mg of MAb daily for 10 days, starting on the day before infection. Flunixin meglumine, which is active only for 24 to 36 h, was used to reduce the occasional adverse reaction observed in some calves following the first inoculation of the anti-CD8 MAb. Seven calves were inoculated with MAb to *M. dispar* (control group), three were inoculated with anti-CD4 MAb, four were inoculated with anti-CD8 MAb, and three were inoculated with anti-WC1 MAb. The calves were killed 10 days after infection. Data were accumulated from a series of experiments which involved two or three calves, from one or two of which lymphocytes were depleted (one calf was always a control animal from which lymphocytes were not depleted).

During the course of the experiments, heparinized blood was obtained at 2 to 3 day intervals for analysis of circulating lymphocytes by flow cytometry. Nasopharyngeal swabs were obtained daily to monitor virus shedding. At postmortem examinations, macroscopic lung lesions were recorded on a standard lung diagram, and the extent of pneumonic consolidation was expressed as percent pneumonia. Lung washings (LW) were collected by irrigating the lungs with 400 to 800 ml of phosphate-buffered saline (PBS) (44). The LW were centrifuged at $1,200 \times g$ for 15 min at 4°C; the LW cells were resuspended in 5 ml of Hanks balanced salt solution containing 25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; pH 7.8), 200 U of penicillin per ml, 200 μg of streptomycin per ml, 50 U of mycostatin per ml, 0.218 M sucrose, 4.8 mM glutamate, and 30 mM magnesium chloride (lung buffer) for virus assays; and a sample of the supernatant was stored at −20°C for antibody detection. Three pieces of pneumonic lung taken from different lobes were homogenized in lung buffer to give a 20% (wt/vol) suspension. Homogenates were centrifuged at $9,000 \times g$ for 1 min, and the supernatant was assayed for virus. Samples from nasopharyngeal swabs, LW cells and lung homogenates were inoculated into calf testis and secondary calf kidney cells to detect virus (44). Tissues were also removed for analysis of mononuclear cells by flow cytometry and for immunocytochemistry.

Immunocytochemistry. RSV antigen was detected in snap-frozen lung samples by using MAb 8, which is specific for the M2 protein (39), and MAb 16, which is specific for the F protein of RSV (40), and then horseradish peroxidase-labelled goat anti-mouse IgG and 3,3′-diaminobenzidine as substrate.

Preparation of mononuclear cells from blood and tissues. Peripheral blood mononuclear cells were isolated from heparinized blood by centrifugation at $1,200 \times g$ for 40 min at 20°C over Histopaque 1083 (Sigma). Mononuclear cells were prepared from bronchial lymph nodes (BLN), prescapular lymph nodes (PSLN), and spleens by being teased in PBS containing 5% fetal calf serum. Lung mononuclear cells were prepared from 3 g of finely chopped pneumonic lung incubated in 100 ml of RPMI 1640–25 mM HEPES buffer–100 U of penicillin per ml–100 μg of streptomycin per ml (RPMI-HEPES) containing 200 U of collagenase type 1 (Worthington Biochemical Corporation) per ml, 50 U of DNase (Sigma) per ml, and 5% heated fetal calf serum. After 2 h of incubation at 37°C in an orbital shaker at 200 rpm, the lung fragments were disrupted with a syringe. Cell suspensions from all tissues were filtered through sterile gauze and centrifuged over Histopaque 1083, and the cells at the interface were washed three times with PBS and resuspended in RPMI-HEPES containing 10% heated fetal calf serum and 2 mM glutamine.

Flow cytometry. Cell suspensions from blood, lymph nodes, spleens, and lungs were stained with MAbs as described previously (27), except that fluorescein isothiocyanate-conjugated anti-mouse Ig (Southern Biotechnology Associates Inc., Birmingham, Ala.) was used, and the samples were analyzed on a FACScan (Becton Dickinson, Sunnyvale, Calif.).

ELISA. The presence of antibodies to RSV in sera and LW was determined by enzyme-linked immunosorbent assays (ELISA) with lysates prepared from calf kidney cells infected with the Snook strain of bovine RSV and mock-infected cells as control antigens as described previously (14). Antibody isotypes were detected by using MAbs to bovine IgM (B67), IgG1 (B37), IgG2 (B192), and IgA (B84/11) obtained from the Department of Veterinary Medicine, Bristol University, and then by using horseradish peroxidase-conjugated goat anti-mouse IgG (Sigma). ELISA titers were calculated after subtraction of the optical density (OD) with control antigen from OD with RSV antigen and plotting of this specific OD against sample dilution. By regression analysis of the linear part of this curve, the end point was deduced at an OD that was 1.5 times the background (38).

Statistical analysis. The differences between the mean values of individual groups were compared by a two-tailed independent Students *t* test.

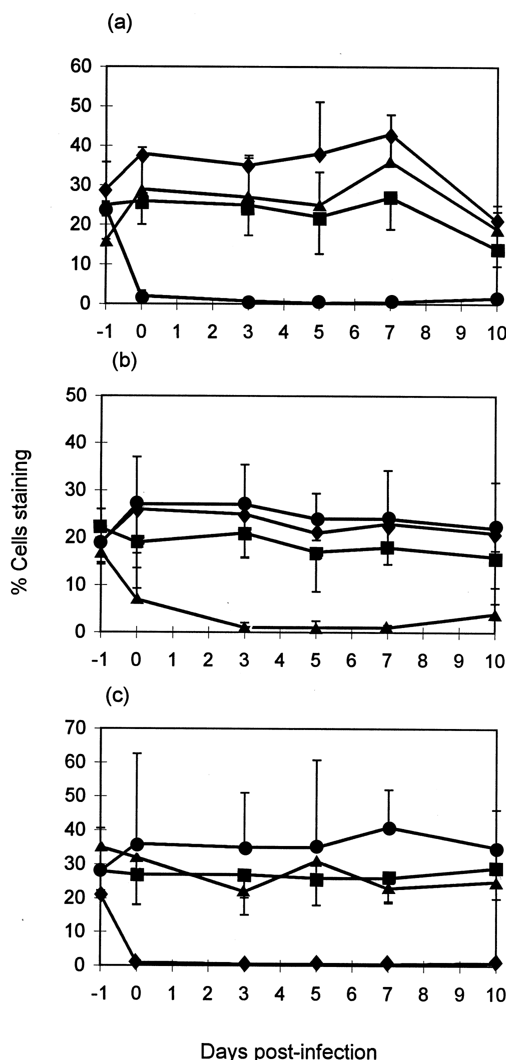

FIG. 1. Effect of injection of MAbs on the percentage of T-lymphocyte subpopulations in peripheral blood. The results show the mean percentages (± standard deviations) of lymphocytes staining with MAb CC8 (anti-CD4) (a), MAb CC63 (anti-CD8) (b), and MAb CC15 (anti-WC1) (c) for groups of calves injected with a control MAb (anti-*M. dispar*) (■), MAb CC8 (anti-CD4) (●), MAb CC63 (anti-CD8) (▲), or MAb CC15 (anti-WC1) (◆). Calves were injected intravenously with MAbs daily for 10 days, starting on day −1.

RESULTS

Effect of MAb injections on circulating lymphocytes. The total of $CD4^+$, $CD8^+$, and $WC1^+$ T cells in calves treated with the control MAb decreased from a mean of 75% ± 8% on day −1 to 57% ± 7% on day 10 of RSV infection ($P < 0.002$) (Fig. 1). This decrease was associated with a slight increase in the proportion of B cells from 12% ± 4% on day −1 to 16% ± 5%

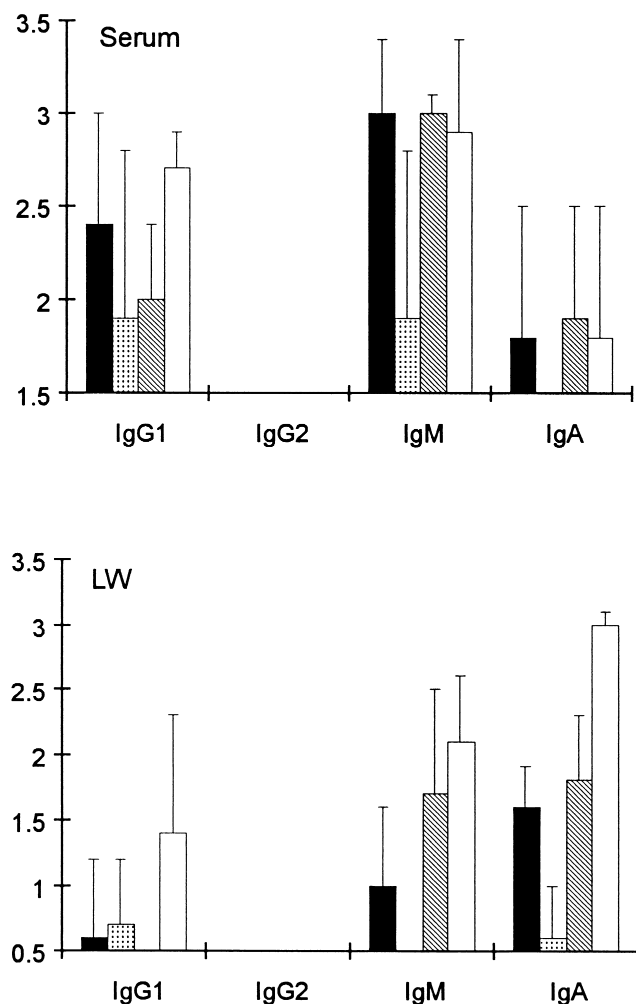

FIG. 2. Effect of T-cell depletion on the antibody response of calves to RSV in sera and LW obtained 10 days p.i. Values are mean titers (± standard deviations) of calves injected with control MAb (■), MAb to CD4 (▤), MAb to CD8 (▧), or MAb to WC1 (□) as described in the legend to Fig. 1. Since antibody was undetectable ($\log_{10}$ of serum antibody is <1.5 and $\log_{10}$ of LW antibody is <0.5) in two of three calves from which CD4 cells were depleted, means ± standard deviations were calculated by using values of $\log_{10}$ of serum antibody titer of 1.4 and $\log_{10}$ of LW antibody titer of 0.4 for the two calves which failed to develop an antibody response.

on day 10 (results not shown). Injection of MAbs to CD4, CD8, or WC1 antigens specifically reduced the percentage of cells in blood expressing the respective markers (Fig. 1). Injection of anti-CD4 reduced the percentage of circulating $CD4^+$ cells from a mean of 24% ± 4.5% to a mean of 2% ± 1.3% within 24 h of the initial MAb injection. The percentage of $CD4^+$ cells remained below this level for the duration of the experiment. Similarly, treatment with anti-WC1 MAb reduced the level of $WC1^+$ cells from 21% ± 7.1% to 0.9% ± 0.7% within 24 h, and the percentage of these cells remained below this level for the duration of the experiment. Depletion of $CD8^+$ cells in anti-CD8 MAb-treated calves was maximal on day 3 postinfection (p.i.). The proportion of $CD8^+$ T cells was reduced from a mean of 17% ± 9% on day −1 to a mean of 1% ± 1% on day 3; however, $CD8^+$ cells were detected returning to the circulation at 10 days p.i. (4% ± 2%) (Fig. 1). The level of nonspecific binding was less than 2% in all animals.

Effect of MAb injection on antibody responses to RSV. Sera and LW were analyzed for isotype-specific antibodies to RSV at 0 and 10 days p.i. (Fig. 2). At day 0, titers of antibody in sera and LW were $<\log_{10}$ 1.5 and $<\log_{10}$ 0.5, respectively. Similarly, antibody was undetectable in the sera or LW of two uninfected control calves 10 days after inoculation with tissue culture fluid. Ten days after RSV infection, the predominant antibody responses in sera from control calves were IgM and IgG1, with little or no detectable IgA or IgG2 antibody, and the predominant antibody responses in LW were IgM and IgA, with little or no detectable IgG1 (detected in one of seven calves) or IgG2 antibody (Fig. 2). Antibody to RSV was detected in sera and LW from only 1 of 3 calves given anti-CD4 MAb compared with 14 of 14 of the calves in the other experimental groups. There were no statistically significant differences in the mean titers of antibody in the sera of calves treated with anti-CD8 or anti-WC1 MAbs compared with those of controls or in the levels of antibodies in LW from calves treated with anti-CD8 MAbs compared with those of control animals. However, the levels of IgM and IgA antibody in LW from calves given MAb to WC1 were significantly greater than those in control animals ($P < 0.05$ and $P < 0.002$, respectively).

Effect of lymphocyte depletions on RSV infection. Nasopharyngeal swabs were obtained daily to examine the level and duration of virus excretion, and calves were killed at 10 days p.i. to examine the lungs for virus infection. Virus was first detected in the nasopharynges from about days 2 to 3 p.i., and peak titers of virus were isolated on days 4 to 6. Animals that were given anti-CD8 MAb excreted RSV for a significantly greater number of days (>8.3 ± 0.5 days) than controls (5.0 ± 0.6 days) ($P < 0.001$) and than animals given anti-CD4 (5.2 ± 0.6 days) or anti-WC1 MAb (4.3 ± 0.6 days) and were still excreting virus, at almost peak titers, when they were killed 10 days p.i. (Fig. 3). All other calves had cleared virus from their nasopharynges by day 8 p.i. RSV was detected in higher titers on day 7 p.i. from the nasopharynges of calves depleted of $CD4^+$ cells (3.2 ± 0.4 $\log_{10}$ PFU/ml) compared with those for controls (2.3 ± 1.2 $\log_{10}$ PFU/ml), and the difference in mean titers was statistically significant ($P < 0.02$) (Fig. 3). Although RSV was isolated on day 7 from the nasopharynges of calves depleted of $WC1^+$ T cells (0.9 ± 1.6 $\log_{10}$ PFU/ml) in titers lower than those for control calves, the difference was not statistically significant. RSV was isolated from LW from three of four calves treated with MAb to CD8 on day 10 p.i., whereas virus was not isolated from LW of any of the other animals (Table 1). Although RSV was not isolated from the LW of one calf treated with anti-CD8, there was widespread distribution of RSV antigen in all lung samples from this animal. In fact, the distribution of RSV antigen in the lungs of three of four calves treated with anti-CD8 was more extensive than that in any of the other groups of animals (Table 1). However, RSV was isolated only from two samples of lung homogenate (one from a control calf and one from a calf treated with anti-CD8 MAb [Table 1]).

Clinical signs of respiratory disease were not observed in any of the calves, with the exception of one calf treated with anti-CD4 MAb. The extent of pneumonic consolidation in all infected calves ranged from 0 to 35% (Table 1) and was significantly greater in calves treated with anti-CD4 MAb than in controls ($P < 0.05$). Extensive lesions were also observed in three of the four calves treated with anti-CD8 MAb; however, one animal failed to develop gross pneumonic lesions even though virus was isolated from LW obtained 10 days p.i. However, little or no viral antigen was detected in the lungs of this calf (Table 1). Two calves injected with MAb to CD8 and two untreated calves that were inoculated intranasally and intra-

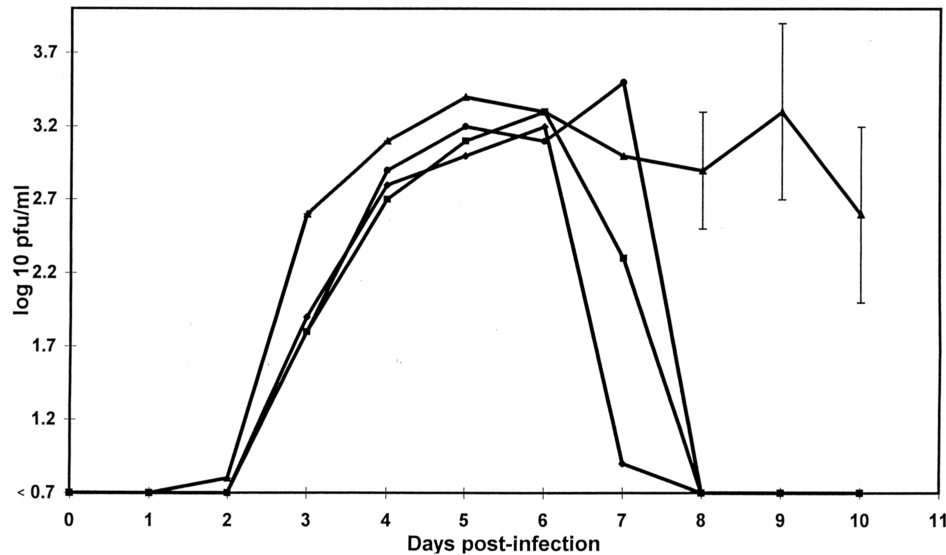

FIG. 3. Effect of T-cell depletion on excretion of RSV from the nasopharynges of calves. Values are mean virus titers of groups of calves as described in the legend to Fig. 1.

tracheally with control tissue culture fluid failed to develop pneumonic lesions.

Effect of MAb injections on lymphocytes in lungs, BLN, PSLN, and spleens. The effects of MAbs on the lymphocyte subpopulations present in the lungs and in secondary lymphoid tissues of calves treated with MAbs for 10 days are shown in Table 2. At 10 days p.i., calves treated with MAb to CD4 or WC1 showed a significant reduction in the percentages of $CD4^+$ or $WC1^+$ cells, respectively, in the lungs, BLN, PSLN, and spleens compared with those in controls. However, although calves treated with anti-CD8 MAb had a significant reduction in the percentages of $CD8^+$ cells in the lungs, BLN, and PSLN, no such reduction was observed in the spleen (Table 2). Treatment with anti-CD4 or anti-WC1 reduced the levels of $CD4^+$ and $WC1^+$ cells to 1 and 2%, respectively, in the lungs 10 days after infection, whereas there were 9% $CD8^+$ cells in the lungs of calves treated with anti-CD8, compared with 27% $CD8^+$ cells in the lungs of control animals.

A comparison of the lymphocyte subpopulations obtained from two uninfected calves that were not treated with any MAbs and RSV-infected calves that were treated with MAb to *M. dispar* showed a significant increase in the proportion of $CD8^+$ cells in the lungs of the RSV-infected calves compared with that in uninfected controls 10 days p.i. ($P < 0.003$).

DISCUSSION

The depletion of lymphocyte subsets by administration of MAbs clearly demonstrates the importance of $CD8^+$ T cells in the clearance of RSV from the lungs and nasopharynges of calves. This delayed virus clearance was reflected in an increase in the severity of pneumonic lesions in three of the four calves from which $CD8^+$ T cells were depleted. Even though the pneumonic lesions were more extensive in these animals, they still did not develop signs of clinical disease. It is possible that the duration of these experiments (10 days) was not sufficient for the development of clinical disease. These findings of more severe lung lesions associated with prolonged RSV infection are similar to those seen for immunocompromised infants and adults who develop severe and often fatal pneumonia (13, 18, 19, 21) but contrast with those for mice, whereby persistent RSV infection in mice from which both

TABLE 1. Effect of T-cell depletion on RSV infection of the lower respiratory tracts of calves 10 days after challenge

Treatment group[a]	No. of calves	Virus titer in LW[b]	RSV in lung tissue		% Pneumonia[c]
			Proportion infected[d]	RSV antigen[e]	
None[f]	2	<0.7	0/6	0, 0	0
Control MAb	7	<0.7	1/21	1, 2, 1, 1, 2, 1, 1	7.4 ± 5 (4–18)
Anti-CD4	3	<0.7	0/9	2, 1, 2	16.7 ± 5 (13–22)
Anti-CD8	4	3.1 ± 1.8	1/12	3, 3, 0, 3	16.3 ± 14 (0–35)
Anti-WC1	3	<0.7	0/9	1, 1, 2	10 ± 7 (4–18)[g]

[a] Groups of calves were treated as described in the legend to Fig. 1 with MAbs to *M. dispar*, bovine $CD4^+$, $CD8^+$, and $WC1^+$ T cells.
[b] Mean $\log_{10}$ of PFU/ml ± standard deviation.
[c] Percent areas of lungs showing pneumonic consolidation, with ranges in parentheses.
[d] Numbers of lung samples containing infectious virus/total numbers of samples examined.
[e] Extent of virus antigen in lungs stained with MAbs to RSV and horseradish peroxidase-labelled goat anti-mouse IgG. 1, one focus of cells showing intracytoplasmic RSV antigen; 2, at least two foci of cells showing intracytoplasmic RSV antigen; 3, several extensive foci of cells showing intracytoplasmic RSV antigen.
[f] Calves were inoculated intranasally and intratracheally with control tissue culture fluid.
[g] The extent of pneumonic consolidation in RSV-infected calves from which CD4 cells were depleted was significantly different from that in infected control animals ($P < 0.05$ [Student's *t* test]).

TABLE 2. Analysis of T-cell subsets in lungs, BLN, PSLN, and spleens of RSV-infected calves treated with MAbs to bovine T-cell subpopulations

Treatment group[a]	RSV	No. of calves	T-cell subset	% Cells staining with MAbs (mean ± SD) in[b]:			
				Lungs	BLN	PSLN	Spleens
None	—	2	CD4	12 ± 2	ND	25 ± 4	15 ± 4
			CD8	13 ± 1	ND	17 ± 1	28 ± 12
			WC1	18 ± 8	ND	9 ± 3	8 ± 1
Control MAb	+	7	CD4	13 ± 2	26 ± 7	27 ± 5	12 ± 3
			CD8	27 ± 7	17 ± 4	21 ± 5	35 ± 8
			WC1	20 ± 5	2 ± 0.6	9 ± 2	9 ± 3
Anti-CD4	+	3	CD4	1 ± 0.5[c]	8 ± 5[c]	7 ± 4[d]	3 ± 2[d]
			CD8	29 ± 5	20 ± 3	20 ± 5	33 ± 8
			WC1	17 ± 4	3 ± 0.8	11 ± 4	11 ± 3
Anti-CD8	+	4	CD4	16 ± 2	27 ± 6	24 ± 5	17 ± 5
			CD8	9 ± 4[c]	4 ± 1[c]	6 ± 3[c]	19 ± 13
			WC1	22 ± 10	2 ± 2	4 ± 1	9 ± 2
Anti-WC1	+	3	CD4	18 ± 2	24 ± 6	23 ± 9	11 ± 0.9
			CD8	28 ± 5	15 ± 2	19 ± 9	41 ± 10
			WC1	2 ± 0.5[c]	0.3 ± 0.5[d]	0.3 ± 0.5[d]	2 ± 0.8[d]

[a] Groups of calves were treated as described in the legend to Fig. 1.
[b] Calves were killed 10 days after RSV infection. ND, no data.
[c] T-cell-depleted and control RSV-infected calves were compared statistically. The level of significance was $P < 0.001$.
[d] T-cell-depleted and control RSV-infected calves were compared statistically. The level of significance was $P < 0.01$.

CD8$^+$ and CD4$^+$ T cells were depleted resulted in a reduction in pneumonic lesions (17). The finding that RSV infection of mice can be terminated by either CD4$^+$ or CD8$^+$ T cells is similar to that seen with influenza virus-infected mice, whereby such mice lacking functional major histocompatibility complex (MHC) class I and/or CD8$^+$ class I MHC-restricted effector T cells or from which CD4$^+$ T cells are depleted can still clear influenza virus from their lungs, although simultaneous removal of both T-cell subsets leads to death (2, 11, 12). Other studies have shown a more central requirement for MHC class I-restricted CD8$^+$ T cells in recovery of mice from Sendai virus infection (24, 31). However, an alternative mechanism of recovery from Sendai virus infection involving CD4$^+$ T cells exists and can compensate, in time, for loss of CD8$^+$ T-cell function. Simultaneous depletion of both CD4$^+$ and CD8$^+$ T cells in calves may result in more prolonged RSV shedding than that which was observed in calves from which CD8$^+$ T cells alone were depleted. However, the return of CD8$^+$ T cells to the circulation and their presence in substantial, albeit reduced, numbers in the lungs of anti-CD8-treated calves 10 days p.i. would make it difficult to determine if CD4$^+$ T cells could compensate for the loss of CD8$^+$ T-cell function. The induction of an antibody response in calves to the mouse MAbs within 7 days of injection (27) probably accounts for the reappearance of lymphocytes at day 10. Since depletion of CD4$^+$ T cells can delay or reduce the antibody response, combined treatment of calves with anti-CD4 and anti-CD8 MAbs might be expected to prolong the T-cell depletion. However, other studies showed that CD8$^+$ T cells returned to the circulation in calves given a combined treatment of MAbs to CD4 and CD8 at the same rate as that in animals treated with anti-CD8 MAb alone (34). To prolong T-cell depletion, it may be necessary to produce either chimeric bovine-murine MAbs or bovine MAbs to bovine T-cell subsets by grafting of complementarity-determining regions (1).

Other studies have shown that RSV can be isolated from homogenates of lung tissues of immunologically intact calves 7 days p.i. (41). In the present studies, RSV could not be isolated from the lung homogenates of control animals 10 days p.i. The presence of CD8$^+$ T cells in the lungs of anti-CD8-treated calves 10 days p.i. may have partially cleared infectious RSV from the lungs at this time, since virus was isolated only from LW and not from lung homogenates. However, failure to isolate virus may not provide definitive evidence that virus has been cleared, since antibody may have neutralized the virus when the lung tissue was homogenized. The extensive RSV antigen in the bronchial epithelia of three of four calves from which CD8$^+$ T cells had been depleted 10 days p.i. correlated with more extensive pneumonic consolidation compared with those for animals treated with anti-WC1 MAb or controls (42). However, the more severe lung lesions seen in calves from which CD4$^+$ T cells had been depleted compared with those in control animals could not be explained by more extensive viral antigen in the lungs, since the amount of antigen in the lungs 10 days p.i. was similar to that seen in controls. A substantial increase in alveolar lymphocytes was observed in the lungs of CD4-depleted mice 7 days after RSV infection, although such animals had few lymphocytes in the bronchovascular spaces (17). In mice from which CD8$^+$ T cells had been depleted, there was a peribronchovascular infiltrate of lymphocytes and a moderate increase in alveolar lymphocytes compared with RSV-infected controls (17). The increased lymphocyte infiltrations in the lungs of mice from which either CD4$^+$ or CD8$^+$ T cells alone had been depleted may have resulted from higher titers of virus in the lungs of these animals than in controls 7 days p.i. (17). It is possible that RSV replicated to higher titers in the lungs of CD4$^+$ T-cell-depleted calves and caused more extensive lung lesions compared with those in controls and yet was cleared at the same rate as that for control animals. Although the lungs were not examined for virus earlier than 10 days p.i., higher titers of virus were detected in the nasopharynges of calves from which CD4$^+$ T cells were depleted at day 7 than in those from controls. A detailed description of the histology of the lung lesions and an analysis of the distribution of T-cell subsets in the lungs of the T-cell-depleted calves are given elsewhere (42).

Treatment of calves with MAb to CD4$^+$ T cells caused little delay in virus clearance, even though both the serum and local antibody responses were inhibited in two of three of the calves. Furthermore, the presence of levels of IgM and IgA antibodies in the lungs of calves treated with MAb to WC1 that were higher than those in control animals did not significantly enhance clearance of RSV from the respiratory tract. Taken together, these findings suggest that the early antibody response to RSV does not play a major role in virus clearance. An increase in the IgA response to Sendai virus has been observed in mice lacking CD8$^+$ T cells (28), and an enhancement of IgG production has also been observed in mice treated with anti-CD8 antibody (9). However, depletion of CD8$^+$ T cells in calves did not appear to affect the antibody response to RSV at least up to 10 days p.i. In contrast, an increase in RSV-specific IgM and IgA antibody was detected in the lungs of calves from which WC1$^+$ T cells were depleted. Previous studies have shown that depletion of WC1$^+$ T cells resulted in an enhancement of serum IgG antibody to inert antigens (27) but did not affect either the serum or the local antibody response of calves to rotavirus (34). The failure to detect an effect on the antibody response to rotavirus in calves treated with anti-WC1 MAb may be related to the fact that WC1$^+$ T

cells are only a minor T-cell population in the bovine small intestine (34), whereas they represent a major population in the lungs of calves (Table 2). The role of γδ T cells in protection against infections is unclear, and it is not known if bovine WC1$^+$ T cells possess a function similar to that of nonruminant γδ T cells. TcR-γδ antigen recognition is largely MHC unrestricted, and γδ T cells have been shown to recognize MHC class Ib antigens, heat shock proteins, and bacterial antigens (5, 29, 45). A herpesvirus-specific TcR-γδ clone which appeared to recognize unprocessed g1 protein of herpes simplex virus type 1 in an MHC-independent fashion and in a manner similar to that of immunoglobulin recognition has been identified (35). Since many TcR-γδ cells reside in epithelial tissues, they may serve as a first line of defense against infections by combining immunoglobulin-like recognition of whole, cell surface proteins on bacteria and viruses with cellular effector functions. γδ T cells have been shown to play a protective role at an early stage in *Listeria monocytogenes* infection (22). If WC1$^+$ T cells have a protective role early in RSV infection, depletion of these cells could have resulted in higher levels of virus antigen during the early stages of infection, which may have induced a more rapid IgM and IgA response in the lungs. However, the titers of virus isolated from the nasopharynges of calves from which WC1 T cells were depleted were the same as those from control animals.

These studies confirmed earlier ones (34) which showed that MAb treatment was much less effective in depleting CD8$^+$ T cells in calves compared with depletion of either CD4$^+$ or WC1$^+$ T cells. Furthermore, different tissues varied in the levels of depletion or rates of repopulation of T-cell subsets. It may be that the MAb used to deplete CD8$^+$ T cells is much less effective than those used to deplete CD4$^+$ or WC1$^+$ T cells. Alternatively, it may be that CD8$^+$ T cells are inherently less susceptible to lysis, since a MAb to bovine CD6, which binds to both CD4$^+$ and CD8$^+$ T cells, caused depletion of CD4$^+$ cells in calves more effectively than CD8$^+$ cells (26).

The present study highlights the importance of CD8$^+$ T cells in the clearance of RSV from both the nasopharynges and the lungs of calves, which are a natural host of RSV infection, and contrasts with studies of RSV-infected mice which suggested that both CD4$^+$ and CD8$^+$ T cells were involved in terminating RSV replication. The observation that persistent RSV infection in CD8-depleted calves and in immunocompromised infants and adults is associated with enhanced pneumonic lesions, whereas persistent RSV infection in mice from which CD4$^+$ and CD8$^+$ T cells were depleted was associated with a reduction in the severity of lung lesions (17), suggests that virus cytocidal effects may play a greater role in the pathogenesis of disease in the natural host than they do in the mouse. It is likely that termination of RSV replication in calves is mediated by RSV-specific, MHC class I-restricted, CD8$^+$ CTLs. Such CTLs have been demonstrated in the peripheral blood of calves 6 to 10 days after RSV infection, and high levels of RSV-specific CTL activity are present in the lungs of calves infected 10 days previously (15). In addition, there was a significant increase in the proportion of CD8$^+$ T cells in the lungs and a decrease in the proportion of circulating T cells in calves 10 days after RSV infection. A reduction in the number of T cells has also been observed in the peripheral blood of lambs infected with bovine RSV (36). These observations have been confirmed and extended in a subsequent study which also showed an increase in the proportion of CD8$^+$ T cells in both the trachea and the nasopharynges (33). Further analysis of the role of T cells in protection of calves against RSV infection and in the pathogenesis of disease should aid the development of a safe and effective vaccine for use not only in cattle but also in humans.

ACKNOWLEDGMENTS

This work was supported by the Ministry of Agriculture, Fisheries, and Food.

We thank Brenda Jones for growing the hybridoma cells and the staff of the Gnotobiotic Unit for their care of the experimental animals.

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