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Respiratory Syncytial Virus Infection in Anti-μ-Treated Mice

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BALB/c mice were depleted of B cells by anti-μ treatment to investigate the pathogenesis of respiratory syncytial virus (RSV) infection in the absence of antibody. Termination of RSV replication after primary infection occurred with the same kinetics in anti-μ-treated mice as in phosphate-buffered saline (PBS)-treated controls. Yet, when rechallenged, anti-μ-treated mice were more permissive to RSV replication than PBS-treated controls. Anti-μ-treated mice also experienced greater illness than PBS-treated controls during both primary infection and rechallenge. Passive transfer of RSV-specific immune serum to anti-μ-treated mice before rechallenge reconstituted complete protection from RSV replication and diminished illness. Thus, RSV-specific antibody is not required to terminate RSV replication in primary infection, but without antibody, only partial immunity against rechallenge is induced. While it is unknown whether the mechanism is a direct effect on RSV titer or modulation of the illness-causing cellular immune response, the presence of RSV-specific antibody reduces illness in both primary RSV infection and rechallenge of mice.

The determinants of illness following respiratory syncytial virus (RSV) infection are incompletely defined. Our fragmentary understanding of the pathogenesis of primary RSV infection, susceptibility to reinfection, and the immunopathological features of the enhanced illness following parenteral immunization for RSV have stymied vaccine development. We have developed a BALB/c mouse model of RSV infection for the purpose of investigating the immunological determinants of disease pathogenesis in primary infection, reinfection, and the enhanced illness. Although mice are not fully permissive to human strains of RSV, a large nasal inoculum in mice results in high-titer RSV replication in the nose and lung, histopathological features of bronchiolitis and pneumonia, and illness (9). Passively administered antibody has been shown to reduce virus titer in RSV-infected mice (19) or cotton rats (16) without inducing pathology or illness. However, in primary RSV infection of mice, antibody cannot be detected in the lung until after virus has been cleared (1). Anderson et al. (1) have shown that a successive series of immune responses occur in the lungs of RSV-infected mice. Clearance of RSV after primary infection of mice correlated with the appearance of RSV-specific, major histocompatibility complex class I-restricted cytotoxic T cells (CTL) between days 6 and 10 (1). This supported the findings of Cannon et al. (6) in which passive transfer of RSV-specific CTL terminated RSV replication in the lungs of immunodeficient mice and suggested that CTL are the dominant immune mechanism for viral clearance after primary RSV infection. It was also shown that while CTL are important for viral clearance, the same effector cells enhance pathology and illness when given in larger numbers (5). It was therefore considered important to further define the influence of RSV-specific antibody on the immune response to primary RSV infection and the importance of preformed RSV-specific antibody in protection from reinfection.

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In this report, we describe the effects of experimental depletion of B cells on the pathogenesis of primary RSV infection and rechallenge in mice. Anti-μ treatment of neonatal mice results in depletion of B cells and their products, such that treated mice are unable to mount an antibody response (14). This approach has been used in other model systems to explore the relative contribution of humoral versus cellular immune responses to the pathogenesis of infectious diseases (7, 13, 17). Further delineating the role of isolated components of the immune response to RSV should accelerate the progress toward developing an effective RSV vaccine that can be safely tested in seronegative infants.

MATERIALS AND METHODS

Mice. Pathogen-free female BALB/c mice were purchased from Charles River Breeding Laboratories (Raleigh, N.C.). They were shipped in filtered crates and housed in a HEPA-filtered Duo-flo isolator (Lab Products, Inc.) remote from the central animal facility. Cages, bedding, food, and water were sterilized before use. Room temperature was maintained at 25°C, and a 12-h-on, 12-h-off light cycle was provided. Breeding was accomplished by housing two females with each male and removing females on the appearance of vaginal plugs. Pregnant females were housed individually during the third week of gestation. In caring for animals, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals" prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (15).

Anti-μ treatments. Rabbit anti-μ antibody was purchased from Southern Biotechnology (Birmingham, Ala.). Pregnant females were examined every 6 to 12 h as they neared their gestational term, and Thorazine (50 mg/250 ml) was added to their water. Treatment of neonatal mice was initiated upon the first appearance of milk in the stomach (between 2 and 6 h after birth). Neonatal mice handled by this method had a survival rate of 91%. Treatment began with 50 μg of anti-μ in 0.05 ml of phosphate-buffered saline (PBS) intraperitoneally delivered through a 30-gauge needle and was maintained with 50 μg intraperitoneally every other day until a schedule

of 50 μg Monday, 50 μg Wednesday, and 100 μg Friday was achieved (11). Treatment was continued through the period of rechallenge or until the end of each experiment. There was no difference between the appearance or growth of mice treated with anti-μ and the appearance and growth of those injected with PBS three times weekly before their entry into an experiment.

Cells and virus. HEp-2 cells were maintained in Eagle's minimal essential medium supplemented with glutamine, amphotericin, gentamicin, penicillin G, and 10% fetal bovine serum (10% EMEM). The A2 strain of RSV was used in all experiments, and the stocks were prepared as previously described (9). Working stocks maintained a titer between 1×10^8 and 2×10^8 PFU/ml for over 6 months.

Mouse infection and harvest. Four-week-old mice were used in all primary infection experiments, and rechallenge was done at 8 weeks of age. Nasal inoculation was performed as previously described with 100 μl of RSV stock in mice anesthetized with xylazine and ketamine (9). A clinical illness score was assigned to each mouse in a blinded fashion on each day of the study: 0, healthy; 1, barely ruffled; 2, ruffled, but active; 3, ruffled and inactive; 4, ruffled, inactive, hunched, and gaunt; 5, dead. Mice were exsanguinated via retro-orbital puncture before cervical dislocation and sterile dissection. The right lung was removed, and then the nose was removed en bloc. The specimens were quick frozen in 10% EMEM and stored at −70°C. The left lung was inflated via the left main bronchus and fixed with 10% formalin phosphate before paraffin embedding. Lung sections were stained with hematoxylin and eosin. In each nasal challenge experiment either for primary infection or rechallenge, an age-matched untreated control group was used to standardize potency of stock virus.

Plaque assays and neutralization tests. Two-day-old HEp-2 monolayers, 80% confluent in Costar 12-well plates, were used for plaque assay and neutralization (NT) tests as previously described (9).

BCH4 ELISA. Immulon II 96-well plates (Nunc, Roskilde, Denmark) were used in all assays. BCH4, a persistently RSV-infected BALB/c fibroblast cell line, was a gift from Bruce Fernie, Georgetown University. BCH4 and BC, its parent cell line, were bound to the solid phase in the antigen-positive and antigen-negative wells, respectively. The preparation of enzyme-linked immunosorbent assay (ELISA) plates and performance of the assay have been previously described in detail (8). The final titer was expressed as the $\log_2$ reciprocal of the highest positive dilution.

Anti-μ ELISA. Rabbit anti-μ antibody in mouse sera was detected by an ELISA constructed and analyzed by the same method described above except that immunoglobulin (IgM) (Accurate Biochemicals, Westbury, N.Y.) (20 ng) was bound to the solid phase with carbonate buffer (pH 9.6). All assays were done in a blinded fashion on coded samples. Serum was analyzed at a dilution of 1:20 and considered positive if the mean absorbance in the two sample wells was twice that of the antigen-negative control well and the mean A_{405} was greater than 0.1.

FACS analysis. Spleens were ground between the frosted ends of two sterile glass microscope slides in RPMI medium containing 10% fetal bovine serum. Cells were separated from spleen capsules, and then the suspension was layered on a 3-ml cushion of Ficoll-Hypaque (1.090 specific gravity) and centrifuged at room temperature for 20 min at $1,500 \times g$. The lymphocyte band was aspirated and washed twice in PBS containing 5% fetal bovine serum and adjusted to a concentration of 5×10^6 cells per ml, and then the cells were labeled with fluorescein isothiocyanate-conjugated antibody against Thy1.2 (Becton Dickinson, Mountain View, Calif.), IgM, or IgG (Southern Biotechnology) by incubation for 60 min at room temperature. Cells were washed twice in PBS containing 5% fetal bovine serum and analyzed on a Coulter Epic 753 fluorescence-activated cell sorter (FACS).

TABLE 1. Evidence that anti-μ treatment was effective in depleting B cells and antibody

Treatment	BCH4 ELISA[a]	% B cells in spleen[b]		Rabbit anti-μ present in serum[c]
		IgG	IgM	
Anti-μ	<7.3	<5	<5	149/150
PBS	11.2 ± 0.7	55 ± 7	54 ± 10	0/119

[a] $\log_2$ reciprocal serum dilution ± standard deviation in mice 4 weeks after primary RSV infection with 10^7 PFU.
[b] Determined for four pools of splenocytes from two separate experiments.
[c] All serum samples drawn before primary infection or rechallenge were tested in this assay.

RESULTS

Anti-μ treatment of mice. We first determined whether the anti-μ treatment was adequate to effectively deplete B cells and abolish serum antibody responses. Three approaches were taken to document efficacy (Table 1). First, it was determined that RSV-specific antibody was absent in serum samples from all anti-μ-treated mice 4 weeks after primary infection, whereas PBS-treated mice uniformly had detectable antibody to RSV. Next, we analyzed by FACS the phenotypic distribution of lymphocytes in three pools of splenocytes from anti-μ-treated mice and four pools of splenocytes from PBS-treated mice collected in two separate experiments. In spleens from anti-μ-treated mice, less than 5% of the lymphocytes expressed surface IgG or IgM compared with 55 ± 7% and 54 ± 10%, respectively, in the PBS-treated mice. These data indicated that no measurable RSV-specific antibody was induced in anti-μ-treated mice and that B lymphocytes were absent or depleted to very low levels. Finally, all serum specimens were tested for circulating rabbit anti-μ by ELISA. In 65 anti-μ-treated mice, 149 of 150 serum samples had detectable rabbit anti-μ by ELISA, while in 51 PBS-treated mice, 0 of 119 serum samples had detectable rabbit anti-μ. The documented excess of rabbit anti-μ verified the absence of μ-expressing B lymphocytes. We also attempted to measure mucosal antibody in nine anti-μ-treated and seven PSB-treated rechallenged mice at the time of harvest by lavage with 1.0 ml of PBS through the nasopharynx out the nostrils. Specimens collected in this way were assayed at a single dilution of 1:2 in the BCH4 ELISA. The mean A_{405} ratios of antigen-positive/antigen-negative wells in samples from μ-suppressed mice were 0.75 ± 0.26 for IgG and 0.67 ± 0.15 for IgA, and those from PBS-treated mice were 7.32 ± 2.75 for IgG and 1.70 ± 0.35 for IgA. These data suggest that mucosal IgG ($P < 0.0001$ by two-tailed t test) and IgA ($P < 0.0001$ by two-tailed t test) responses were also suppressed by the anti-μ treatment.

Primary RSV infection in anti-μ-treated mice. Primary RSV infection was examined in 4-week-old anti-μ-treated mice to determine the role of antibody in terminating RSV replication. In normal mice, a peak titer of RSV can be detected in the lungs and nose on day 5 after primary infection, and by day 8 RSV can no longer be isolated (9). Day 7 is a transition point when RSV recovery from the lungs is diminished but detectable and is the most sensitive

TABLE 2. Primary RSV infection in anti-μ- and PBS-treated mice[a]

Treatment	Harvest day	No./group	GMT ± SD[b]		Wt change (g)	Mean illness score
			Log_{10} PFU/g of lung	Log_{10} PFU/nose		
Anti-μ	5	9	5.1 ± 0.1	2.6 ± 0.2	−2.6 ± 2.0[c]	3.0
	7	3	2.7 ± 0.2	ND[d]	ND	ND
	8	8	<1.8	<1.1	−1.8 ± 1.2	2.0
PBS	5	12	5.5 ± 0.2	2.4 ± 0.1	+0.8 ± 1.2	0
	7	4	2.8 ± 0.3	ND	ND	ND
	8	8	<1.8	<1.1	−1.8 ± 0.7	0

[a] All mice were challenged with 10^7 PFU of RSV.
[b] GMT, geometric mean titer.
[c] $P = 0.005$ by two-tailed t test for difference between day 5 weight loss in anti-μ- versus PBS-treated controls.
[d] ND, not done.

time to determine the effects of an intervention on the kinetics of RSV replication. In anti-μ-treated mice, the pattern of RSV recovery in the lungs and nose was not significantly different from that of PBS-treated controls (Table 2). This suggests that termination of RSV replication in the lungs or nose after primary infection of mice can occur in the absence of RSV-specific antibody.

We also asked what impact the absence of antibody had on illness and pathology in RSV-infected mice. The illness in anti-μ-treated mice was most severe on days 5 and 6 after primary infection, and there was evidence of partial recovery by day 8 (Table 2). In contrast, PBS-treated mice exhibited no clinical illness. Anti-μ-treated mice consistently developed a diffuse peribronchovascular infiltrate composed of macrophages, lymphocytes, and polymorphonuclear leukocytes (PMNs) (Fig. 1). There was also an alveolar component to the immune infiltrate of the same cellular composition (Fig. 2). The lungs of the PBS-treated controls had minimal cellular infiltrate. Thus, both illness and pathology were enhanced in the anti-μ-treated mice after primary RSV infection.

RSV rechallenge of anti-μ-treated mice. One of the features of RSV is that repeated infections are common throughout life (2). Using anti-μ-treated mice, we asked whether antibody was important in preventing RSV replication or illness after rechallenge of mice. Data on rechallenge of anti-μ-treated mice with RSV were compiled from four separate experiments. All mice underwent primary infection at 4 weeks of age. Rechallenge was performed 14 or 28 days after primary infection with two different concentrations of RSV stock virus, and mice were harvested on day 4 after rechallenge. In the first rechallenge experiment, no serum pretreatment was given. In subsequent experiments, all mice received 200 μl of RSV-immune mouse serum or normal nonimmune mouse serum intraperitoneally 16 h before rechallenge. The pool of RSV-immune sera was derived from mice convalescent from primary RSV infection and had an NT titer of 1:240 (log_2 reciprocal dilution = 7.9) and a BCH4 ELISA titer of 1:10,240 (log_2 reciprocal dilution = 13.3). Because there was no discernible difference in RSV recovery, pathology, or illness in mice that received no serum versus nonimmune sera, data from those groups were combined.

First, we asked whether the absence of antibody would permit the isolation RSV from the lungs after rechallenging mice. In normal mice of this age rechallenged 4 weeks after primary infection with 7.0 log_{10} PFU, RSV is barely detectable in the lungs and nose 24 h after rechallenge and is undetectable by 48 h (8). In μ-suppressed mice that did not receive immune sera, RSV was isolated from the lungs on day 4 after rechallenge in 15 of 18 mice. RSV was not isolated from the nose in any of the rechallenged mice (Table 3). The titer of RSV isolated from the lungs of the μ-suppressed groups was a function of the rechallenge dose (Table 3). Anti-μ-treated mice that received immune sera before rechallenge had no detectable RSV in the lungs at day 4 after rechallenge. Just before rechallenge, the geometric mean NT titer in anti-μ-treated mice given immune sera was 1:17 (log_2 reciprocal dilution = 4.1 ± 0.5), and the geometric mean log_2 reciprocal dilution for the BCH4 ELISA was 9.4 ± 0.6. Thus, without antibody, protection from RSV replication is incomplete. Yet, only a modest titer of NT antibody is sufficient to restore the rapid elimination of RSV after rechallenge. RSV was not isolated from the lungs or noses of anti-μ-treated mice that were rechallenged on day 14 after primary infection (Table 3). The explanation for this is not known, although in the shortened interval between primary infection and rechallenge, there may have been residual natural killer cell activity or CTL activity or other mediators of inflammation that may have attenuated RSV replication at the time of inoculation. Local natural killer cell activity and CTL activity have been documented in the lower respiratory tract of mice after primary infection (1), although the relative contributions of specific elements of the immune system to protection against RSV replication have not been defined.

Despite partial protection from RSV replication, rechallenged μ-suppressed mice became ill. Anti-μ-treated mice exhibited significant illness on day 4 following rechallenge (Fig. 3) that was diminished by pretreatment with immune sera or by using a lower-titer rechallenge inoculum (Table 3). In contrast, PBS-treated mice exhibited little or no clinical illness during rechallenge, although a small amount of weight loss occurred. These findings were consistent with historical controls. Hence, as in primary infection, RSV rechallenge of mice induces an enhanced illness in the absence of RSV-specific antibody. Since the anti-μ-treated mice have no antibody and RSV replication did not attain a high level after rechallenge, we asked whether there was histologic evidence of altered cellular immune responses in the lungs of anti-μ-treated rechallenged mice. The characteristic histopathological findings seen in normal mice undergoing rechallenge were seen in the PBS-treated mice. Aggregates of small, uniform, basophilic lymphocytes were present around the bronchovascular bundles (Fig. 4A). In anti-μ-treated mice, the lymphocytic accumulations around bronchovascular bundles were less prominent and included the presence of 10 to 15% PMNs. The immune infiltrate in anti-μ-treated mice also contained an increase in lymphocytes, PMNs, and macrophages in the alveolar spaces (Fig. 4B). Thus, the distribution and composition of the cellular immune response in anti-μ-treated mice was different from that seen in PBS-treated controls and was associated with enhanced illness.

DISCUSSION

The serial injection of antibody to μ chain beginning in newborn mice was originally developed as a method of investigating isotype diversity. Anti-μ-treated mice are known to have reduced immunoglobulin-bearing lymphocytes in the spleen, have depressed synthesis of all immunoglobulin isotypes, and lack humoral response to immuni-

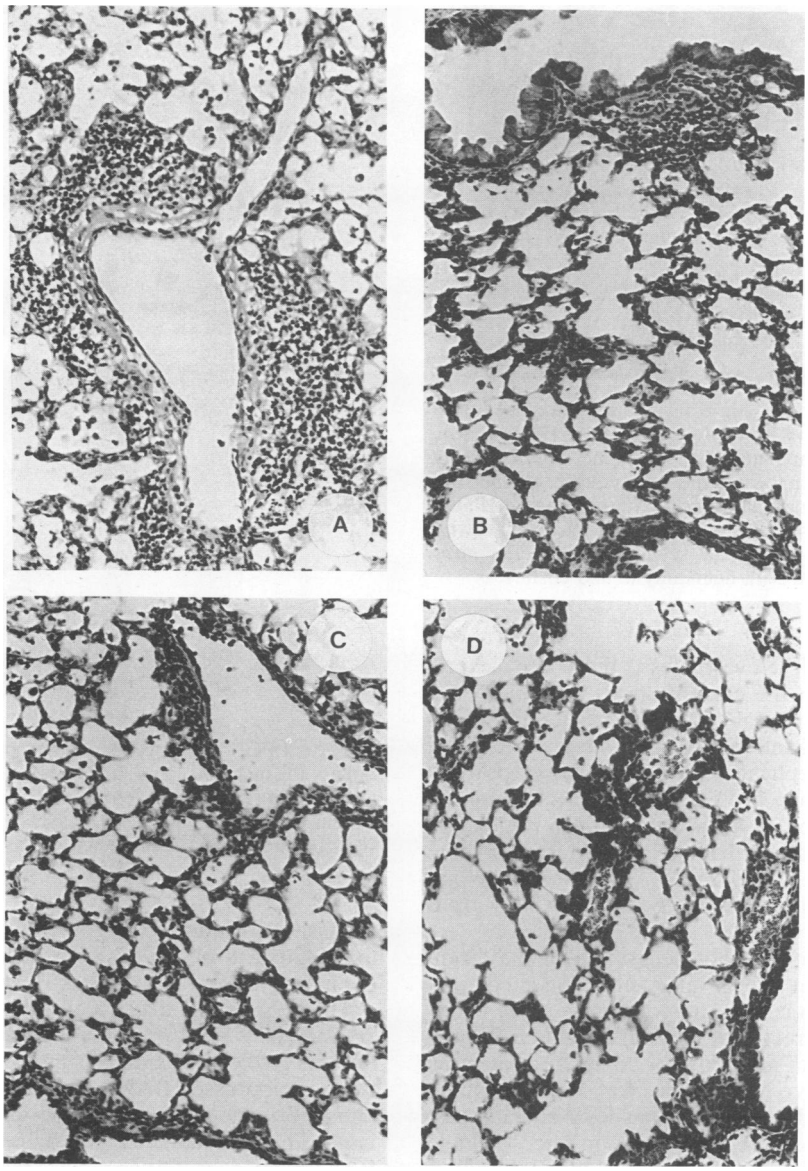

FIG. 1. Lung histology in anti-μ-treated mice 5 days (A) and 8 days (B) after nasal challenge with live RSV (10^7 PFU) or in PBS-treated controls 5 days (C) and 8 days (D) after challenge. Anti-μ-treated mice developed a diffuse peribronchovascular infiltrate with alveolar involvement composed of macrophages, lymphocytes, and PMNs (PMNs identified by multilobed nuclei) by day 5 (A) which became better localized in the perivascular and peribronchiolar spaces by day 8 (B). In PBS-treated control mice, the immune infiltrate was composed predominantly of lymphocytes, and involvement of the alveoli was less prominent than in anti-μ-treated mice. There were no PMNs seen in the lungs of PBS-treated control mice. (Magnification ×250; stained with hematoxylin and eosin.)

zation (14). The effects of μ suppression have been evaluated in a number of other mouse models of viral infection. Anti-μ-treated mice infected with influenza A virus can recover from primary infection in the absence of detectable antibody, although termination of viral replication may be slightly delayed. Anti-μ-treated mice were not susceptible to lung reinfection with influenza A virus but ocassionally had virus isolated from the nose, whereas normal controls were solidly protected (13). Lymphocytic choriomeningitis virus (LCMV) has a longer period of viremia in anti-μ-treated mice after primary infection, although virus can eventually be cleared. On rechallenge, μ-suppressed mice allow LCMV replication up to 6 days, whereas no virus can be recovered from normal rechallenged mice (7). Primary cutaneous herpes simplex virus infection in normal and μ-suppressed mice resolves with the same kinetics, but the μ-suppressed mice are more likely to have ganglion involvement. Normal mice are protected from reinfection with herpes simplex virus, but μ-suppressed mice can be reinfected with a large inoculum (17).

The determinants of immunity may be different for viruses that are surface restricted in their tissue tropism (e.g. RSV) compared with viruses with an obligatory viremic phase in their pathogenesis (e.g., LCMV) or viruses with a predilection to become latent (e.g., herpes simplex virus), so translation of pathogenetic mechanisms from one system to

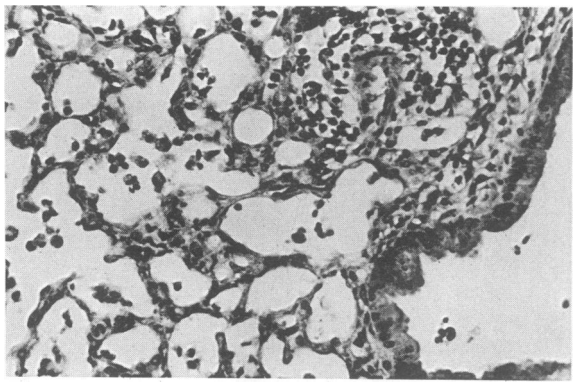

FIG. 2. Photomicrograph of lung from an anti-μ-treated mouse sacrificed 5 days after primary RSV infection with 10^7 PFU (same as Fig. 1A). The alveolar component of the immune infiltrate in the RSV-infected μ-suppressed mice had the same cellular composition as that in the peribronchovascular space. The presence of PMNs and the degree of increase in alveolar macrophages are what distinguished the response from that seen in PBS-treated mice. (Magnification ×400; stained with hematoxylin and eosin.)

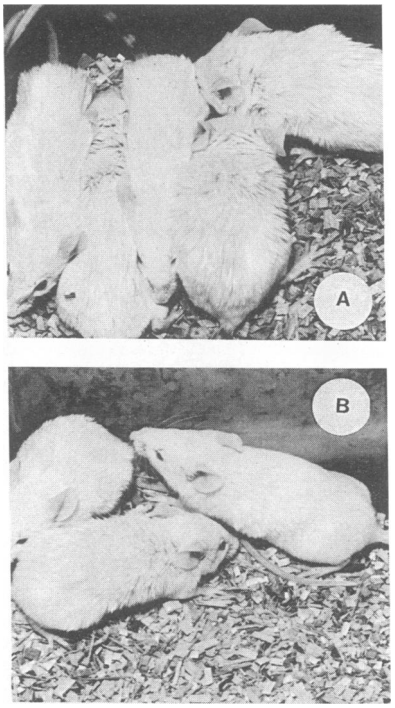

FIG. 3. (A) Anti-μ-treated mice 4 days after nasal rechallenge with 10^7 PFU of RSV 28 days after primary infection. The fur is ruffled, the mice are gaunt, and some of the mice maintain a hunched posture. These mice are representative of the group in Table 3 with a mean illness score of 3.8. (B) PBS-treated control mice show no signs of illness after rechallenge.

another may be limited. Nevertheless, there are several themes that evolve from the current study which are supported by work in other viral systems with μ-suppressed mice. One is that lack of antibody has a modest or no effect on the course of RSV replication and clearance in primary infection. Another is that protection from rechallenge is incomplete in the absence of antibody, although reinfection of the lung can occur after challenge with large inocula. Finally, illness and pathology in RSV-infected mice are increased when induced in the absence of antibody or B cells.

The finding that antibody is not necessary for the termination of RSV replication after primary infection is compatible with the work of Anderson and colleagues (1) in which CTL responses were correlated with viral clearance. Nevertheless, the importance of antibody for complete protection from reinfection may have implications for immunoprophylaxis of RSV infection. The findings that RSV-specific passive antibody can reduce RSV replication in mice (19) and cotton rats (16) without inducing illness and that antibody is necessary for complete protection from reinfection suggest that induction of RSV-specific neutralizing antibody is an important and safe goal for RSV immunoprophylaxis.

The recognition that T-cell repertoires are altered in the absence of B cells or immunoglobulin limits the interpretation of studies utilizing μ suppression. The helper T cells from μ-suppressed BALB/c mice fail to induce a response to phosphorylcholine with the T15 idiotype, which is the dominant idiotype induced by helper T cells from normal BALB/c mice (3). Other reports have shown that μ suppres-

TABLE 3. Rechallenge of anti-μ- and PBS-treated mice with RSV[a]

Treatment	Serum pretreatment	Interval (days)	Log_{10} PFU/ inoculation	No./ group	GMT ± SD[b]		Wt change (g)	Mean illness score
					Log_{10} PFU/g of lung	Log_{10} PFU/nose		
Anti-μ	Nonimmune	14	7.0	8	<1.8	<1.1	−4.1 ± 2.4	ND[c]
		28	7.0	10	3.3 ± 0.9 (8/10)[d]	<1.1	−5.0 ± 0.8[e]	3.8
		28	6.5	8	2.0 ± 0.3 (7/8)	ND	−1.4 ± 1.4	2.1
	Immune	28	7.0	5	<1.8	ND	−5.7 ± 1.4	2.8
		28	6.5	4	<1.8	ND	−1.7 ± 0.7	2.0
PBS	Nonimmune	14	7.0	6	<1.8	<1.1	−0.3 ± 0.3	ND
		28	7.0	3	<1.8	<1.1	−3.4 ± 1.4	1.0
		28	6.5	3	<1.8	ND	−0.4 ± 0.6	0
	Immune	6.5	28	3	<1.8	ND	−0.3 ± 1.0	0

[a] All mice were inoculated with 10^7 PFU at the time of primary infection.
[b] GMT, geometric mean titer. Mice were sacrificed on day 4 after rechallenge.
[c] ND, not done.
[d] Numbers in parentheses represent number of mice in group from which RSV was isolated.
[e] $P < 0.0001$ versus anti-μ-treated mice challenged with 6.5 log_{10} PFU and $P = 0.01$ versus PBS-treated mice challenged with 7.0 log_{10} PFU by two-tailed t test.

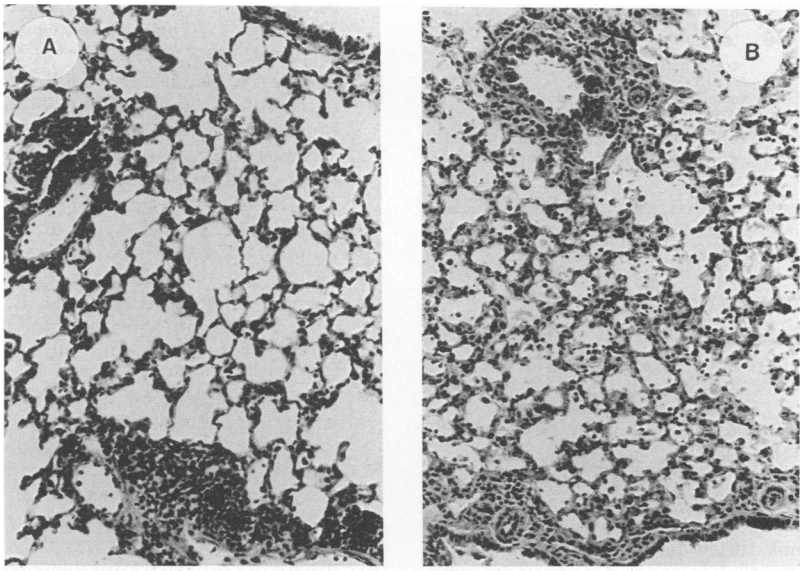

FIG. 4. (A) Lung histology in PBS-treated mice 4 days after day 28 nasal rechallenge with 10^7 PFU of RSV showed aggregates of small, uniform basophilic lymphocytes around the bronchovascular bundles but no alveolar infiltrate. (B) Lung histology in anti-μ-treated mice 4 days after day 28 nasal rechallenge with 10^7 PFU of RSV showed lymphocytic accumulations around the bronchovascular bundles composed of lymphocytes and PMNs and a diffuse alveolar infiltrate consisting of lymphocytes, PMNs, and an increased number of macrophages. (Magnification, ×250; stained with hematoxylin and eosin.)

sion alters the IgH restriction of T-cell idiotopes. Anti-μ-treated mice had delayed-type hypersensitivity and major histocompatibility complex-restricted CTL responses to azobenzenearsonate equivalent to those of their normal counterparts. However, suppressor T-cell factors derived from μ-suppressed mice immunized with azobenzenearsonate had altered Igh restriction in suppression of the delayed-type hypersensitivity response (18). Such studies suggest that the development of T-suppressor-cell idiotopes is strongly influenced by the presence of immunoglobulin-bearing B cells and demonstrates the importance of immunoglobulin in the genesis of the T-cell-receptor repertoire. Another mechanism by which μ-suppressed mice might have altered T-cell function is through modified antigen presentation (10, 11). In the absence of B cells, antigen presentation occurs by alternative routes and may induce different populations of T cells to different extents. Other lymphocyte populations, such as natural killer cells, can also be influenced by the absence of antibody. Natural killer cells have been shown to have increased activity in μ-suppressed mice (4).

In this context, one can only propose partial explanations for the enhanced illness after RSV rechallenge of μ-suppressed mice. Because there is evidence that RSV replication is diminished and antibody is absent, the illness is presumably mediated by T lymphocytes, although macrophages, natural killer cells, or PMNs may play a role. Passive transfer experiments have shown that CTL have the potential to terminate RSV replication in the lung when given in small doses (6) but cause enhanced pathology and illness if given in larger doses (5), which makes them plausible candidates for the mediator of illness in this system. It has been reported that μ-suppressed LCMV-infected mice inoculated either intracranially or intraperitoneally have more inflammatory infiltrate and higher mortality than nonsuppressed controls (12). LCMV given by the intraperitoneal route has also caused more extensive hepatocellular injury in μ-suppressed mice than in normal mice (21). In LCMV, the illness has been highly correlated with induction of virus-specific *H-2* class I-restricted cytotoxic T cells (21). From our studies, we suggest that either RSV-specific antibody or B cells modulate the intensity of the T-cell response which determines the degree of illness. The μ-suppressed and PBS-treated mice had equivalent titers of RSV after primary infection in the lung, yet illness was enhanced and the cellular infiltrate was more prominent in μ-suppressed mice. The phenotypic constitution of the lymphocytic component in the lungs of RSV-infected μ-suppressed mice was not defined in situ, but the documented absence of B cells in the spleen suggests that the lymphoid elements were predominantly T lymphocytes. The explanation for the presence of PMNs in the lung infiltrate or their role in pathogenesis is unknown. A similar modulation of T-lymphocyte-mediated immunopathology has been described in the LCMV mouse model, in which it is known that virus cannot be cleared without CTL. The presence of neutralizing or nonneutralizing antibody allowed the clearance of LCMV to occur without immunopathology (20). It is proposed that the mechanism by which antibody diminishes the T-lymphocyte-mediated immunopathology could be reduction of viral antigen load on target cells combined with reduced viral antigen available to induce clonal expansion and activation of CTL.

In summary, studies in anti-μ-treated mice indicated that antibody is not necessary for termination of RSV replication after primary infection. However, mice without antibody demonstrated enhanced histopathology in the lung and experienced more severe illness than mice with a complete immune response. In rechallenged mice, only partial reduction of RSV replication in the lungs is achieved without antibody, and illness is more severe than in mice with both humoral and cellular responses intact. These findings, together with the previous work of others, support the postu-

late that antibody is an illness-sparing mechanism for immune-mediated protection from RSV.

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