Treatment with Anti-LFA-1 Delays the CD8\(^+\) Cytotoxic-T-Lymphocyte Response and Viral Clearance in Mice with Primary Respiratory Syncytial Virus Infection

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Cytotoxic T lymphocytes (CTLs) play an important role in the immune response against respiratory syncytial virus (RSV) infection. The cell surface molecule lymphocyte function-associated antigen 1 (LFA-1) is an important contributor to CTL activation, CTL-mediated direct cell lysis, and lymphocyte migration. In an attempt to determine the role of LFA-1 during RSV infection, we treated BALB/c mice with monoclonal antibodies to LFA-1 at days −1, +1, and +4 relative to primary RSV infection. Anti-LFA-1 treatment during primary RSV infection led to reduced illness and delayed clearance of virus-infected cells. CTLs from RSV-infected mice that were treated with anti-LFA-1 exhibited diminished cytolitic activity and reduced gamma interferon production. In addition, studies with BrdU (5-bromo-2′-deoxyuridine) and CFSE [5-(and 6)-carboxyfluorescein diacetate succinimidyl ester]-labeled lymphocytes showed that anti-LFA-1 treatment led to delayed proliferation during RSV infection. These results indicate that LFA-1 plays a critical role in the initiation of the immune response to RSV infection by facilitating CTL activation. These results may prove useful in the development of new therapies to combat RSV infection or other inflammatory diseases.

Human respiratory syncytial virus (RSV) is a pneumovirus of the Paramyxoviridae family of viruses (14). The majority of infants and toddlers with RSV develop only a mild upper respiratory infection. However, 20 to 30% of infected children fall victim to more dangerous lower respiratory tract infections and bronchiolitis, resulting in an excess of 130,000 hospitalizations annually in the United States alone (48). RSV infection among the institutionalized elderly is also associated with high rates of mortality (20). In immunocompromised patients, particularly bone marrow transplant recipients, RSV leads to acute respiratory failure with exceptionally high mortality rates (30). These data clearly make RSV infection a high priority for vaccine development. However, a formalin-inactivated, alum-precipitated virus (FI-RSV) produced in the 1960s caused more severe illness, increased rates of hospitalization, and some mortality (35). This history of vaccine-enhanced illness has stymied efforts to produce a safe and efficacious vaccine for RSV infection.

RSV-specific cytotoxic T lymphocytes (CTLs) have been isolated from humans and mice. In the murine model, primary RSV infection normally results in mild to moderate disease initiation of the immune response to RSV infection by facilitating CTL activation. These results may prove useful in the development of new therapies to combat RSV infection or other inflammatory diseases.

accompanied by a lack of visible illness. When mice are depleted of CD8\(^+\) T cells, virus clearance is delayed but the moderate illness observed during primary infection is abolished (23). Conversely, illness is more severe when CD8\(^+\) T cells are present in excess (12). These data indicate that T lymphocytes not only shoulder the burden of RSV clearance during primary infection but are also major contributors to the observed illness. Recent data from RSV-infected infants suggest that in primary infection, disease severity correlates with gamma interferon (IFN-γ) levels, and this finding is consistent with immunopathology mediated by an overly exuberant CD8\(^+\)-CTL response (9).

Lymphocyte function-associated antigen 1 (LFA-1) is an integrin composed of noncovalently associated CD11a and CD18 chains (50). It has been well documented that LFA-1 is of paramount importance in multiple cellular processes, including activation, migration, and CTL effector functions (6, 10, 11, 15, 19, 29, 32, 49, 55). Through its role as an adhesion molecule, LFA-1 helps define the immunological synapse (16). Briefly, LFA-1, along with CD2, constitutes a peripheral supramolecular activation complex, which surrounds a central supramolecular activation complex comprising the T-cell receptor and CD28. The immunological synapse is the site of T-cell activation, which is governed by a complex series of signaling events and cytoskeletal rearrangements (17–19, 38). The primary ligand for LFA-1 is intercellular adhesion molecule 1 (ICAM-1) (39, 46). Past studies have identified ICAM-1 as the receptor for the major groups of human rhinoviruses (27, 53, 54).

The characteristics of typical RSV infection and the importance of LFA-1 in the immune response led us to hypothesize that LFA-1 may play a major role in RSV-induced illness.
Other work has demonstrated that treatment with anti-LFA-1 monoclonal antibody can assist in neutralizing human immunodeficiency virus infection in vitro (31) and blocks the induction of experimental autoimmune encephalomyelitis in a murine model (21). We therefore examined the effect of anti-LFA-1 treatment during primary RSV infection. Our results demonstrate that treatment with anti-LFA-1 during primary RSV infection delayed viral clearance and diminished illness. This was associated with diminished CTL activation and migration to the lungs. However, antibody responses were unaltered, resulting in sufficient memory immune responses to protect mice from subsequent RSV challenge. We conclude that anti-LFA-1 treatment during primary RSV infection in mice leads to delayed T-cell trafficking and activation, resulting in a different balance of responses used to clear virus, with the consequence of reduced immunopathology.

MATERIALS AND METHODS

Mice. Pathogen-free, BALB/c female mice between the ages of 8 and 10 weeks were purchased from Harlan Industries (Indianapolis, Ind.) or Charles River Laboratories (Raleigh, N.C.). The mice were cared for in accordance with the Guide for the Care and Use of Laboratory Animals (42), as described previously (25). Experiments were performed with age-matched groups.

Cell lines and antibodies. HEp-2 cells, used to determine titers of RSV in lungs, were maintained in Eagle’s minimal essential medium containing 10% fetal bovine serum (10% EMEM). CTL cytolytic activity was measured using persistently RSV-infected BH4 CH cells, with BC cells as uninfected controls. Both cell lines were also maintained in 10% EMEM. Hybridoma cell lines producing a monoclonal antibody directed against the CD11a subunit of murine LFA-1 (clone number M175.2) (47) and HLA-DR5 (BB151), an isotype control monoclonal antibody, were purchased from the American Type Culture Collection (Rockville, Md.). Monoclonal antibodies were prepared as clarified ascites fluid from hybridoma-inoculated, pristane-primed BALB/c mice. Total protein and albumin concentrations were quantitated using a Multistat III microcentrifugal translator (Instrumentation Laboratory, Lexington, Mass.). Protein electrophoresis was performed with a Titan Gel high-resolution REP SP-30 kit, and the gamma globulin fraction was determined by densitometry with an electrophoresis data center (Helena Laboratories, Beaumont, Tex.). Ascs fluid was diluted in phosphate-buffered saline to 1 μg of immunoglobulin/ml before injection. All cell lines were supplemented with 2 mM glutamine, 10 U of penicillin G per ml and 10 μg of streptomycin sulfate per ml and were determined to be free of mycoplasma contamination by analysis with the PCR (American Type Culture Collection).

Virus infection. The RSV challenge stock was derived from the A2 strain of RSV by sonication of HEp-2 monolayers as previously described (25). Mice were anesthetized intramuscularly with ketamine (40 μg/g body weight) and xylazine (6 μg/g body weight) prior to intranasal inoculation with 10^5 PFU of live RSV in 100 μl of 10% EMEM. In most experiments, mice received intraperitoneal injections of 200 μg of either isotype control or anti-LFA-1 on days −1, +1, +4 relative to the time of infection. In lungs were isolated from RSV-infected mice at days 8 and 10 postinfection. Lungs were washed twice in phosphate-buffered saline containing 5% fetal bovine serum to sequester any free CFSE that had failed to diffuse into the cells. The lymphocytes were then resuspended in 10% RPMI medium in six-well plates and supplemented with 1 mg of anti-CD28 and anti-CD49d antibodies/ml, 10 ng of interleukin-2 (IL-2), which was replenished late during day 2, and 4 mg of the RSV M2 peptide (aa 82 to 90). One group of cells was treated with 500 μg of isotype control antibody, whereas the second group was treated with 500 μg of anti-LFA-1. Cells were incubated at 37°C for 5 days and were then analyzed by flow cytometry. Cells were stimulated with the aforementioned flu virus peptide (nucleoprotein aa 147 to 155) as a negative control.

Lung histopathology. Mice were sacrificed 8 days postinfection, and the left lungs were inflated with 0.2 to 0.3 μl of 10% formalin. The formalin-fixed lungs were OCT-embedded, and thin sections were cut and stained with hematoxylin and eosin. Slides were viewed with a Zeiss Axiosplan light microscope at a magnification of ×40 under oil immersion.

RPA for detection and quantitation of mRNA species. Mice were sacrificed 4 days after infection, and lungs were quick-frozen in liquid nitrogen. Total RNA was isolated, and RNase protection assays (RPAs) were performed as previously described (33) using the Pharmingen RibonQuant mCK-1 and mCK-2b template sets. These templates include IL-1α, IL-1β, IL-1 receptor antagonist, IL-6, IL-10, IL-12p35, IL-12p40, IFN-γ, and IFN-γ-inducing factor. ELISAs. IFN-γ production was measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (R & D Systems, Minneapolis, Minn.). Briefly, 50 μl of supernatant from ground lungs of RSV-infected mice was thawed and added to precoated 96-well microtiter plates. Peroxidase-labeled anti-cytokine antibody was added to bind bound cytokine, and the plates were developed by addition of tetramethylbenzidine substrate. Separate ELISAs were performed to quantitate the isotypes and titers of RSV F protein-specific antibodies. Wells were coated with purified RSV F protein (a gift from Wyeth-Lederle-Praxis, Pearl River, N.Y.), and the assay was performed as previously described for RSV G protein-specific antibodies (34).

Statistical analysis. Data from individual mouse experiments were maintained in a Paradex database. Statistical analysis was performed by transferring data from the database into the Student’s t-test (Statistical Analysis System, Cary, N.C.) and performing analysis of variance by using Kruskal-Wallis and Wilcoxon rank sum tests. Values of P < 0.05 were considered statistically significant.
RESULTS

Illness is reduced in mice treated with anti-LFA-1. BALB/c mice received 200 μg of an isotype control antibody or anti-LFA-1 antibody intraperitoneally at days −1, +1, and +4 relative to the time of RSV infection. On day 0, mice were infected intranasally with 10^7 PFU of RSV in 100 μl of 10% EMEM. Mice treated with the isotype control exhibited a typical pattern of RSV-induced illness. Peak weight loss was about 18% of original weight on day 7 postinfection (Fig. 1A). In contrast, peak weight loss in mice that received anti-LFA-1 treatment was only 4% of original weight on days 8 to 9 postinfection. Clinical illness score patterns paralleled the weight loss data in both groups (Fig. 1B). The peak illness occurred earlier in isotype control-treated mice and was more severe. These illness profiles suggest that anti-LFA-1 treatment offers some protection against RSV-induced illness.

Viral clearance from the lungs is delayed during primary RSV infection in anti-LFA-1-treated mice. RSV titers were measured in the lungs on days 4, 6, 8, 10, and 12 postinfection (Fig. 2). In the lungs, viral titers on day 4 showed no significant difference between the control group and the anti-LFA-1-treated group (P > 0.05). On days 6 and 8 postinfection, mice treated with anti-LFA-1 retained significantly more virus than the isotype control-treated mice (P < 0.05). By day 8, RSV could not be detected in the lungs of isotype control-treated mice but was still present in the lungs of anti-LFA-1-treated mice. By day 10 postinfection, virus had been cleared in both groups. These data indicate that treatment with anti-LFA-1 results in a delay in viral clearance.

Anti-LFA-1 therapy impairs CTL-mediated killing. Since anti-LFA-1 treatment caused a delay in viral clearance, we reasoned that the cytolytic activity of CTLs was impaired in these mice. To this end, lungs were harvested on day 8 postinfection from RSV-infected mice that received 200 μg of either isotype control or anti-LFA-1 antibody at days −1, +1, and +4 relative to the time of RSV infection. We chose to examine the cytolytic effector function of virus-specific CTLs on day 8 because that is the day of peak CD8 T cell-mediated cytolytic activity in response to primary infection. Cytolytic activity of these lymphocytes was measured by incubation with a persistently RSV-infected mouse fibroblast cell line, BCH4, in a direct 51Cr release assay (Fig. 3). A high percentage of RSV-specific CTL activity was observed in isotype control-treated mice. In contrast, lymphocytes from mice treated with anti-LFA-1 demonstrated a sixfold lower effector activity at the same dilution. No specific lysis was observed when CTLs from either group were incubated with an uninfected fibroblast cell line. From this result, we conclude that anti-LFA-1 treatment significantly impairs the lytic activity of RSV-specific CTLs.

Anti-LFA-1 treatment delays the kinetics of the CTL response during primary RSV infection. The binding of LFA-1 to ICAM-1 facilitates lymphocyte migration, as well as adhe-
tion and costimulation during antigen presentation. Since our previous data indicated a delay in the immune response during anti-LFA-1 treatment, we hypothesized that extending the treatment with anti-LFA-1 would further delay the RSV-specific immune response. To test this, we produced a primary RSV infection in three additional groups of mice. Two independent groups of mice were treated with either the isotype control or anti-LFA-1 at days –1, +1, and +4, exactly as in our previous experiments. In addition, we administered anti-LFA-1 antibody to a third group of mice on days –1, +1, +4, and +7. Mice were sacrificed at days 6, 8, 10, and 12, and the right lungs were used in ICS for IFN-γ to define the effect of LFA-1 on CTL function during primary RSV infection.

Prolonged treatment with anti-LFA-1 further delayed detection of functional CTLs at the site of infection, as evidenced by a reduction in the percentage of IFN-γ-producing CD8+ T cells (Fig. 4A). In isotype control-treated mice, peak CTL activation was observed on day 6 postinfection, when approximately 5% of CD8+ T cells produced IFN-γ. IFN-γ production by CD8+ T cells then steadily declined for the duration of the experiment, indicative of a normal pattern of CTL activation during RSV infection. In mice that received anti-LFA-1 through day 4, CTL production of IFN-γ was delayed, steadily increasing until it peaked on day 10. A similar pattern of delayed IFN-γ production was seen in mice that received anti-LFA-1 through day 7. When we examined IFN-γ production by cytokine ELISA of lung supernatants from these mice, we observed the same trends (Fig. 4B). The peaks of IFN-γ production were therefore not only delayed but also reduced in both anti-LFA-1-treated groups, with production slightly more depressed in the mice that received prolonged anti-LFA-1 treatment. These results suggest that anti-LFA-1 treatment delays the appearance of functionally active CD8+ T cells responding to the viral infection.

Anti-LFA-1 treatment impairs lymphocyte activation. LFA-1 is known to contribute to lymphocyte migration as well as play a potential role in activation. For this reason, we chose to examine the effect of anti-LFA-1 treatment on the pulmonary lymphocytic infiltrate during RSV infection. The absolute number of CD8+ T cells was lower in the lungs of anti-LFA-1-treated mice at the earliest time points (Fig. 5), but by the conclusion of the experiment, CD8+ T-cell numbers were equivalent to those in isotype control-treated mice. These data suggested that anti-LFA-1 treatment might also be inhibiting CD8+ T-cell migration to the lungs. However, delayed activation of CD8+ T cells in the lymph nodes would also preclude their migration into the periphery in search of infected target cells. Consequently, a significant impairment in activation would similarly result in reduced lymphocyte numbers in the lungs. In addition, a late schedule of anti-LFA-1 treatment on days 5, 7, and 10 postinfection had no effect on illness, viral...
clearance (Fig. 6), or cytolysis. In addition, no significant difference in lung pathology or degree of inflammatory infiltrate was noted in mice starting treatment on day 5 (data not shown). Treatment on day 5 begins after activation has been initiated but before activated CTLs begin to infiltrate the lungs. The results of this experiment strongly argue against anti-LFA-1 treatment causing a significant effect on lymphocyte migration. We therefore focused on the role of impaired activation as the potential mechanism for the effect.

The impact of anti-LFA-1 treatment on lymphocyte proliferation was evaluated both in vivo and in vitro. First, we treated mice with BrdU to examine lymphocyte proliferation during anti-LFA-1 treatment in the context of primary RSV infection. We used two groups each of isotype control-treated mice and anti-LFA-1-treated mice. The first pair of groups received a single daily intraperitoneal injection of 100 μg of BrdU on days 0 to 5 postinfection, and the second pair of groups received BrdU on days 5 to 9. The staggering of the BrdU treatments was performed to determine the timing of CTL activation and expansion. All mice received 200 μg of either isotype control or anti-LFA-1 antibody at days -1, +1, and +4 relative to the time of RSV infection. We observed that proliferation of CD8+ T cells occurred earlier in the isotype control-treated mice (Fig. 7A). Isotype control-treated mice were characterized by a dramatic reduction in proliferation between days 8 and 12. In contrast, proliferation of CD8+ T cells increased in anti-LFA-1-treated mice between days 8 and 12. Moreover, the level of proliferation in anti-LFA-1-treated mice was not as robust as that observed in isotype control-treated mice. These data indicate that the administration of anti-LFA-1 to RSV-infected mice resulted in a delayed and inefficient activation and expansion of CD8+ T lymphocytes.

To better understand the timing of the anti-LFA-1 effect, we performed additional in vitro studies. We isolated lymphocytes from the spleens of RSV-immune BALB/c mice, labeled them with CFSE, and incubated them in the presence of 500 μg of either the isotype control antibody or anti-LFA-1. The lymphocytes were also supplemented with costimulatory antibodies, IL-2, and the immunodominant RSV M2 epitope for 5 days at 37°C. When we examined the cells by flow cytometry, it was very clear that anti-LFA-1 compromised lymphocyte proliferation (Fig. 7B). Isotype control-treated lymphocytes that were stimulated with the RSV peptide exhibited a maximum proliferation of 4.13% ± 2.26%, as determined by CFSE dilution. In contrast, proliferation of anti-LFA-1-treated lymphocytes was only 0.07% ± 0.09%. The cumulative results of these experiments confirm that anti-LFA-1 treatment impairs lymphocyte activation, suggesting that an early step in the antigen presentation process.

Lung pathology in mice treated with anti-LFA-1 is similar to that in control mice. Since a significant portion of the illness...
observed during primary RSV infection is immune mediated, anti-LFA-1 has the effect of reducing illness, as shown in Fig. 1. However, it also leads to delayed clearance of the virus. We therefore examined pathology in the lungs of isotype control (Fig. 8A)- and anti-LFA-1 (Fig. 8B)-treated mice on day 8 postinfection, the day of peak cytolytic activity. The overall level of perivascular and peribronchiolar infiltration was similar in anti-LFA-1-treated mice and isotype-treated controls. However, the anti-LFA-1-treated mice had a more diverse cell population in the infiltrate that included a higher frequency of macrophages and polymorphonuclear leukocytes. The composition of the infiltrate in the isotype-treated controls was more uniform, with a higher frequency of lymphocytes. This difference in the composition of the infiltrate may reflect a compensatory response to the lack of effective RSV-specific CTLs in the lungs of anti-LFA-1-treated mice.

Anti-LFA-1 treatment leads to reduced cytokine mRNA levels in the lungs of RSV-infected mice. The kinetics of viral clearance and CTL induction may be influenced by cytokine expression. For this reason, we performed RPAs to determine cytokine mRNA levels in the lungs of isotype control- and anti-LFA-1-treated mice following primary RSV infection. We examined mRNA production of the cytokines included in the RiboQuant mCK-1 and mCK-2b template sets from BD Pharmingen. No significant differences ($P > 0.05$) between the groups were detected in levels of IL-1α, IL-1β, IL-12p35, and IFN-γ-inducing factor (Fig. 9A). However, administration of anti-LFA-1 to RSV-infected mice did result in significant reductions ($P < 0.05$) in levels of IL-1 receptor antagonist, IL-6, IL-10, IL-12p40, and IFN-γ mRNA (Fig. 9B). Interestingly, the significant reductions were seen in cytokines that are largely produced by antigen-presenting cells (APC) and lym-
phocytes after their reciprocal activation following interaction through the immunological synapse. This observation supports the concept that blocking LFA-1 function negatively impacts T lymphocytes by inhibiting the initial APC-T lymphocyte interaction required for activating the expansion and cytolytic function of CD8$^+$ CTLs.

The RSV-specific antibody response is unaltered in anti-LFA-1-treated mice. We next examined the RSV-specific antibody response to primary RSV infection in mice treated with anti-LFA-1. To accomplish this, sera of mice obtained 4 weeks after primary infection were evaluated by measuring the total immunoglobulin to the fusion protein of RSV (F). The titers of...
F-specific immunoglobulin G antibody after primary infection were similar in both groups (Fig. 10). In control mice, the titer was measured as a $14.7 \pm 0.29\log_2$ reciprocal serum dilution, whereas the titer of mice treated with anti-LFA-1 was a $13.9 \pm 0.74\log_2$ reciprocal serum dilution. The similarities in antibody titers after infection indicate that anti-LFA-1 treatment does not affect the humoral immune response to primary RSV infection. Furthermore, when these findings are combined with our other data, it appears that LFA-1 is more important for major histocompatibility complex class I costimulation than for major histocompatibility complex class II stimulation during primary RSV infection and has a more profound effect on CD8$^+$ T cell-mediated events than on CD4$^+$-T-cell function.

**DISCUSSION**

With this report, we provide evidence that the role of LFA-1 is important in RSV pathogenesis and that interference with LFA-1 interactions during the initial phases of virus infection can delay the CD8$^+$-T-cell response. It has been shown in the murine model that RSV disease is mediated by CD8$^+$ T cells (22, 23, 25). For this reason, we concentrated our efforts on the CD8$^+$-T-cell response, although many different cells express LFA-1. Our present study shows that RSV disease was significantly reduced when infected mice were treated with anti-LFA-1 (Fig. 1). Anti-LFA-1 treatment also caused a delay in viral clearance (Fig. 2) during RSV infection. An impaired CD8$^+$-CTL response was demonstrated by weakened killing activity as measured in $^{51}$Cr release assays (Fig. 3), diminished IFN-γ production (Fig. 4), and reduced proliferation both in vivo and in vitro (Fig. 7). Furthermore, administration of anti-LFA-1 on days 5, 7, and 10 postinfection, a regimen which began prior to the time of CTL infiltration into the lungs but well after the period of initial T-cell activation, had no statistically significant impact on illness or viral clearance (Fig. 6). Nevertheless, anti-LFA-1-treated mice were still able to establish protective immunity, as they were immune to RSV infection upon challenge (data not shown). These data lead us to conclude that anti-LFA-1 treatment interferes primarily with the process of CTL activation.

The interaction of LFA-1 with ICAM-1 is a critical event in the process of T-cell activation and effector function. Over the last decade, a significant amount of research has appeared in the literature supporting the idea that lymphocyte activation and effector function are triggered at the immunological synapse. One recent report has suggested that the formation of the immunological synapse does not occur in vivo (28). This study showed that naïve T cells and dendritic cells fail to form immunological synapses in vitro in collagen gels, which were intended to recreate the environment of the peripheral organs. However, Dustin and de Fougerolles argue that since activation of naïve T cells by APC occurs in the secondary lymphoid tissues, where collagen fibers are sequestered in reticular fibers, this interference with immunological synapse formation is not a significant problem (19). Furthermore, a recent study

![FIG. 9. Cytokine mRNA levels after RSV infection of anti-LFA-1-treated mice. RSV-infected mice were treated with isotype control or anti-LFA-1 antibody as previously described. Four days postinfection, induction of cytokine mRNA was examined by RPA by using radiolabeled riboprobes. The data are represented as results of densitometric analysis of RPA radiographs, with cytokine mRNA levels normalized to GAPDH (glyceraldehyde-3-phosphate dehydrogenase) levels (mean ± standard error of the mean). Five mice per group were used. (A) $P > 0.05$ for all mRNA levels shown. IGIF, IFN-γ-inducing factor. (B) $P < 0.05$ for all mRNA levels shown.](http://jvi.asm.org/)

![FIG. 10. RSV-specific antibody titers. RSV-infected mice were treated with isotype control or anti-LFA-1 antibody as previously described. Serum samples were collected from RSV-infected mice on day 28 postinfection. The total amount of antibody specific for the fusion protein of RSV was determined by ELISA. Data are represented as means ± standard errors of the means of the log$_2$ reciprocal serum dilution producing an optical density at 450 nm of 0.1 for each group. Five mice per group were used, and $P$ was 0.768.](http://jvi.asm.org/)
has provided high-resolution microscopic evidence that synap-
se formation does occur in vivo (41). While our data do not
address the issue of the existence of immunological synapse
formation in vivo, they demonstrate that disruption of LFA-1
function delayed T-cell activation in a well-established murine
model of viral infection and support the validity of an immu-
nological synapse in vivo.

A large body of literature addresses adhesion molecule func-
tion during RSV infection. This work has clearly demonstrated
that a consequence of RSV infection is an increase in ICAM-1
mRNA levels and surface expression on epithelial cells and
neutrophils (2, 3, 40, 44, 52, 56, 57). Another report has further
shown that ICAM-1 mRNA levels are increased in RSV-infec-
ted epithelial cells via NF-κB and C/EBP activation (13). In
addition, one study has provided evidence that the increased
ICAM-1 expression is the result of an autocrine mechanism of
IL-1α secretion by RSV-infected epithelial cells (45). Produc-
tion of IL-1α/β and tumor necrosis factor alpha leads to a
subsequent release of IL-8 during the inflammatory immune
response, and increases in IL-8 production by epithelial cells
and mononuclear phagocytes have been demonstrated during
RSV infection (1, 2, 8, 43). These consequences of the inflam-
matory response to RSV are likely to facilitate T-cell chemo-
taxis and activation via the upregulation of adhesion molecules
such as ICAM-1, which is the primary ligand for LFA-1 (7, 37,
51). These facts indicate the LFA-1 is an important component
of T-cell activity at multiple points over the course of an im-
une response. We recognize that anti-LFA-1 treatment may,
therefore, influence T-cell activity at multiple stages of the
immune response and that these effects may not occur inde-
pendently of one another. However, when we administered a
late treatment regimen of anti-LFA-1 at days 5, 7, and 10
post-RSV infection, no statistically significant impact on virus
clearance, illness (Fig. 6), or pathology was observed. It is
important to note that in this scenario, anti-LFA-1 treatment
begins after CTL activation has been initiated but prior to CTL
infiltration into the lungs. Our data suggest that the primary
effect of anti-LFA-1 treatment is interference with lymphocyte
activation, but a minor impact on lymphocyte migration cannot
be excluded.

The findings presented in this report demonstrate that the
treatment of mice with neutralizing antibodies to LFA-1 dur-
ing primary RSV infection resulted in diminished illness and
delayed viral clearance. We hypothesize that our observations
stem from a disturbance of the early immune response to RSV.
Specifically, we propose that neutralization of LFA-1 function
inhibits the activation of CTLs and the induction of other
cytolytic functions at the level of antigen presentation and
CD8+ T-cell activation. This work has clear implications for
the development of immunotherapeutic strategies that could
be combined with new antivirals for the treatment of RSV-
duced disease.

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