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Lack of α -1 integrin alters lesion morphology during pulmonary *Mycobacterium tuberculosis* infection

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Abstract

The hallmark of *Mycobacterium tuberculosis* infection is the granuloma, a highly dynamic immune structure that contains the bacilli during chronic infection. Here, we examined if α 1 β 1 integrin is required in the development and maintenance of the granulomatous structure during pulmonary infection using the α 1-integrin knockout (α 1-null) mouse. The α 1 β 1 integrin is expressed on activated macrophages and T cells, and interacts with collagen molecules in the extracellular matrix (ECM), and thus may play a role in the granulomatous process. Following pulmonary infection with virulent *M. tuberculosis*, lungs of α 1-null infected mice had striking differences in granuloma structure, as well as distinct and markedly thickened alveolar septae. By day 180, there were regions of cell death within granulomatous lesions, characterized by cellular debris in these mice. To determine if this molecule was necessary for T cell trafficking within the lungs, the expression of CD4, CD44 and CD62L was monitored. The numbers of activated and IFN- γ -producing CD4⁺ T cells increased in the lungs of α 1-null mice during the chronic phase of infection, although they had decreased concentrations of TNF- α and MMP-9. These results suggest that while α 1 β 1 integrin is not required for trafficking or maintenance of T cells in *M. tuberculosis* infected lungs, it does play a role in granuloma structure and integrity during the chronic phase of infection.

Keywords

α -1 integrin; VLA-1; adhesion; granuloma; tuberculosis

INTRODUCTION

Declared a global health emergency in the early 1990s, tuberculosis (TB) remains a major health problem today and is one of the leading causes of morbidity and mortality worldwide, particularly in settings of poverty¹. The World Health Organization (WHO) estimates that approximately 1.7 million people die annually from TB, and in 2004, approximately 8.9 million people developed the disease¹. The emergence of multidrug-resistant TB (MDR TB) and, more recently, extensively drug-resistant TB (XDR TB), highlights the urgency of understanding and preventing this ancient disease².

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The hallmark of *Mycobacterium tuberculosis* infection is the granuloma, a highly dynamic immune structure that is thought to contain the bacilli during the latent period of infection. Granulomas are composed of multiple immune cells, mainly monocytes and lymphocytes, which are recruited to the site of infection through a complex network of cytokines and chemokines^{3,4,5}. Macrophages infected with *M. tuberculosis* release inflammatory cytokines interleukin-1 (IL-1), IL-6, IL-12 and tumor necrosis factor alpha (TNF- α)^{6,7,5}. Acquired immunity to *M. tuberculosis* is mediated by IFN- γ -producing T cells, which activate infected macrophages to eliminate the bacilli, or at least contain mycobacterial growth^{8,9,10}. In humans, upon reactivation of the bacterium, necrosis can be followed by caseation, which in turn leads to a break down in the granuloma structure and bacteria are released into the airways for transmission to other hosts¹¹.

Integrins belong to a large family of transmembrane, heterodimeric adhesion receptors consisting of α and β chains and are involved in cell trafficking¹². They can also serve as primary sensors of the extracellular matrix (ECM) environment, which initiate signaling pathways that regulate cell migration, growth and survival^{13,14}. The $\alpha 1 \beta 1$ integrin, also known as “very late antigen” (VLA)-1, is a major receptor for collagen molecules on the ECM¹⁵. The $\alpha 1$ integrin is expressed on, amongst other cell types, T cells, natural killer (NK) cells, NK T cells, and macrophages, and thus may be expected to play a significant role in mediating cell adhesion during inflammatory responses. In particular, $\alpha 1 \beta 1$ integrin has been shown to be expressed on activated T cells¹⁶ and on activated macrophages¹⁷ and associated with inflammatory diseases such as arthritis, sarcoidosis and asthma. While thus far few studies have been reported to show that $\alpha 1 \beta 1$ integrin plays a role in tuberculosis, it is up-regulated on T cells in humans during tuberculosis infection¹⁸, and several studies have implicated the involvement of other integrins such as VLA-4 in the recruitment of leukocytes into infected lungs in a mouse model of tuberculosis¹⁹.

Matrix metalloproteinases (MMPs) play a major role in degrading the ECM and tissue remodeling during inflammation²⁰, thus it may be anticipated that MMPs might be involved in regulating the interaction of activated T cells and macrophages during the inflammatory process. The interaction of the $\alpha 1 \beta 1$ integrin with the ECM down-regulates the expression of MMPs²¹. Furthermore, $\alpha 1$ subunit knockout mice have increased MMP-9 activity, suggesting a down-regulatory role for this molecule²².

In order to gain further understanding of granuloma development and progression and the role that $\alpha 1 \beta 1$ integrin may play in the process we employed a mouse model of pulmonary tuberculosis, using an $\alpha 1$ -integrin ($\alpha 1$ -null) mouse strain. Pulmonary infection with *M. tuberculosis* is known to cause recruitment of immune leukocytes leading to the formation of a granulomatous lesion. Given the fact $\alpha 1$ integrin is utilized by leukocytes to bind to the ECM, we hypothesized that it is required by T cells and macrophages for maintenance of granuloma integrity.

MATERIALS AND METHODS

Mice

Specific pathogen-free female, 6–8 week-old BALB/c were purchased from Jackson Laboratory (Bar Harbor, ME) and integrin $\alpha 1$ null breeder mice were kindly provided by the laboratory of Dr. Ambra Pozzi at the Vanderbilt University School of Medicine (Nashville, TN). The integrin $\alpha 1$ null breeder mice were maintained as a colony at Colorado State University. Both mouse strains were maintained under barrier conditions with sterile mouse chow and water *ad libitum*. The specific pathogen-free nature of the mouse colonies was demonstrated by testing sentinel animals, which were shown to be negative for 12 known

mouse pathogens. All experimental procedures were approved by the Colorado State University Animal Care and Use Committee.

Infection with *M. tuberculosis*

Mycobacterium tuberculosis strain H37Rv (TMC #102), obtained from the Trudeau Mycobacterial Culture Collection, was passed three times through pellicle and then stored frozen as a seed stock. Working stocks were made by passage through liquid culture of Proskauer-Beck medium containing 0.01% Tween 80, and aliquots of the organism in mid-log phase growth were stored at -80°C until needed. Mice were infected using procedures described previously⁸. Briefly, bacterial stocks were diluted in 5 ml of sterile distilled water to 2×10^6 CFU/ml and placed in a nebulizer attached to an airborne infection system (Glass-Col, Terre Haute, IN). Mice were exposed to 40 minutes of aerosol, during which approximately 50–100 bacteria were deposited in the lungs of each animal. Bacterial load was determined by plating whole organ homogenates onto nutrient 7H11 agar supplemented with OADC. Colonies were enumerated after 21-day incubation at 37°C .

Lung Cell Digestion

Mice were euthanized by CO_2 asphyxiation and the pulmonary cavity opened. The lungs were cleared of blood by perfusion through the pulmonary artery with 10 ml of cold PBS containing 50 U/ml heparin (Sigma-Aldrich, St. Louis, MO). The lungs were removed from the thoracic cavity, teased apart, and treated with a solution of DNase IV (Sigma-Aldrich; 30 $\mu\text{g/ml}$) and collagenase D (Roche, Nutley, NJ; 0.7 mg/ml) for 30 minutes at 37°C . To obtain a single-cell suspension, the lungs were passed through cell strainers (BD Biosciences, Mountain View, CA). Remaining red blood cells were then lysed with Gey's solution (0.15 M NH_4Cl and 10mM KHC_3O_3), and the cells washed with RPMI-1640 (Invitrogen, Carlsbad, CA) containing 5% heat-inactivated fetal bovine serum (Atlas Biologicals, Fort Collins, CO). Total viable cell numbers were determined using a Neubauer chamber (IMV International, Minneapolis, MN) and 2% Trypan Blue solution.

Flow Cytometric Analysis of Cell Surface Markers

Single-cell lung suspensions were stained with fluorescent-labeled, monoclonal antibodies against CD3 (145-2C11), CD4 (RM4-5), CD8 (53-6.7), CD44 (IM7) and CD62L (MEL-14) surface markers on T lymphocytes. Antibodies were purchased from BD PharMingen (San Diego, CA) unless otherwise stated, and were used at 0.2 $\mu\text{g}/10^6$ cells. Cells were gated on lymphocytes by forward and side scatter according to their characteristic scatter profile, and further gated upon based on CD3 and CD4 or CD8 expression. Individual cell populations were identified according to the presence of specific fluorescent-labeled antibody, and all analyses were performed with an acquisition of at least 100,000 events on a Becton Dickinson FACSCalibur flow cytometer (BD Biosciences, San Jose, CA), and the data analyzed using Cell Quest software (BD Biosciences).

Intracellular Cytokine Staining

Intracellular IFN- γ was measured by preincubation of single-cell lung suspensions with monensin (3 μM), anti-CD3 and anti-CD28 (clone 37.51; both at 0.2 $\mu\text{g}/10^6$ cells) for 4 hours at 37°C in 5% CO_2 . The cells were then stained with PerCP anti-CD4 and APC anti-CD8 for 30 minutes, washed with PBS containing 0.1% sodium azide, fixed and permeabilized with Per Fix/Perm Wash (BD Pharmingen), and stained for intracellular IFN- γ using FITC anti-IFN- γ antibody (XMG1.2) or FITC rat immunoglobulin G1 (IgG1) isotype control (BD Pharmingen) for 30 additional minutes.

Cytometric Bead Array

A Cytometric Bead Array Mouse Inflammation Kit (BD Biosciences) was used to measure TNF- α and MCP-1 in the lung homogenate supernatants. The assay procedure was performed according to kit instructions, and the beads were analyzed on a FACSCalibur flow cytometer (BD Biosciences).

Measurement of MMPs

The concentration of active MMP-9 was measured in lung homogenate supernatants using a MMP-9 Biotrak Activity Assay System (Biotrak® Amersham Biosciences, Piscataway, NJ) according to the manufacturer's instructions. Briefly, standards and samples were incubated in microtitre wells coated with anti-MMP-9 antibody. Any MMP-9 present remained bound to the pre-coated wells during subsequent washing and aspiration. MMP activity was detected through activation of the modified pro-detection enzyme and the subsequent cleavage of its chromogenic peptide substrate. The resulting color was read at 405 nm on a spectrophotometer.

Immunohistochemistry

Whole lungs from euthanized BALB/c wild type and α -1 null mice were inflated with 10% formalin. The tissues were then paraffin-embedded and sectioned. Following deparaffinization, endogenous peroxidase was inhibited by incubation in 3% H₂O₂. Nonspecific staining was blocked with DAKO Protein Block Serum-Free (Dako, Carpinteria, CA). The cells expressing TNF- α were immunohistochemically stained with a polyclonal rabbit anti-human TNF- α antibody (Sigma, St. Louis, MO for 30 minutes at room temperature, the tissue sections were sequentially incubated with Envision+ Rabbit System Labeled Polymer, HRP (Dako, Carpinteria, CA). Staining was developed with Liquid DAB+ (Dako, Carpinteria, CA) and counterstained with Hematoxylin.

Histological Analysis

Whole lungs from euthanized BALB/c wild type and α -1 null mice (n=3) were inflated with 10% formalin, embedded in paraffin and five, 4 μ m serial sections were cut. Sections 1 and 5 were then stained with Hematoxylin and Eosin or Masson's trichrome and examined by a veterinary pathologist with no prior knowledge of the experimental design.

Pathological correlation and lesion analysis

Lung volumes and total nodular burden were quantitated from the cross-sectional images by using three-dimensional analytical volume software (Voxar, Ltd., Edinburgh, United Kingdom) as previously described²³.

Statistical Analysis

Statistical significance was determined with the Student's *t*-test assuming unequal variances.

RESULTS

α 1 integrin is necessary for pulmonary granuloma formation

To determine if the α 1 β 1 integrin is involved in the pulmonary granuloma that forms during infection with *M. tuberculosis*, the lungs from infected α 1-null mice and wild type mice were examined at days 14, 28, 40, 60, 120 and 180 post-infection. Granulomatous lesions were apparent in both the wild type and α 1-null mouse strains by day 28 of infection and the lesions were more focally extensive in the wild type compared to the α 1-null group. This difference, although present at all time points became more obvious during the later chronic phase of infection. Figure 1 shows that by days 120 and 180, the granulomatous lesions in the α 1-null

strain were smaller in size and more discrete, with a lesser tendency to coalesce with surrounding lesions as in the wild type mice. However, at these later time points, there was distinctly more cell death, characterized by cellular debris within granulomatous lesions in the $\alpha 1$ -null mice compared to the wild type mice. There were obvious foamy macrophages present in the wild-type mice at these later time points (Figure 1, Panel F). Morphometric analysis of pulmonary lesions indicated that the total lesion volume was similar in both groups for all the time points examined (data not shown). Sections stained with Masson's trichrome showed the presence of collagen in the granulomatous lesions of both the $\alpha 1$ -null and wildtype mouse strains, with more collagen deposition in the knockout strain as shown in Figure 2. Sections were also examined for collagen type I and type IV, and for both the staining pattern reinforced the trichrome stain in that collagen deposition was greater in the knockout strain (data not shown). In addition, alveolar septae were visibly distinct and markedly thickened due to fibrosis in the $\alpha 1$ -null strain compared to the wild type mice, in which septae were effaced by lymphocyte infiltration. These data suggest that the absence of the $\alpha 1\beta 1$ integrin affected the progression of the pulmonary lesion and consequently the lesion appeared more focal. Moreover, during the chronic phase, these lesions were characterized by more extensive cell death.

Lungs of $\alpha 1$ -null mice contain more activated lymphocytes during the chronic phase of infection

To determine if the absence of the $\alpha 1$ -integrin subunit also affected the cellular composition and activation state of cells within the pulmonary lesions, single cell suspensions were analyzed by flow cytometry for activation markers at various time points after infection. There was a significant increase in the number of CD4 T cells expressing CD44^{high} (Figure 3A) and CD62L^{low} (Figure 3B) in the lungs of the $\alpha 1$ -null mice beginning at day 60 of infection until day 180 when the experiment ended. Next, we analyzed the ability of pulmonary CD4 T cells to produce IFN- γ . Single-cell lung suspensions were stained for intracellular IFN- γ (Figure 3C). While IFN- γ -producing CD4 T cells were increased in the wild type group at day 28 of infection, by day 60 and 120, there were more IFN- γ -producing CD4 T cells in the $\alpha 1$ -null mouse group. An increase in IFN- γ -producing CD8 T cells was also observed, but only at day 60 of infection (data not shown). These results indicate that in the absence of the $\alpha 1$ -integrin subunit, mice were not only capable of maintaining effector T cells in the lungs, but in fact possessed greater numbers of activated T lymphocytes during the chronic stage of infection. This may have contributed to the increased cell death in $\alpha 1$ -null mice in the chronic phase of infection.

$\alpha 1$ -null mice produce decreased levels of TNF- α during mycobacterial infection

Given that $\alpha 1$ -null mice had a different pulmonary pathology from that of wild type mice, we then wanted to determine if this difference resulted from a reduced capacity to induce TNF- α , an important cytokine required for the integrity of granulomatous lesions⁶. Lung supernatants from days 14, 28, 40, 60, 120 and 180 were analyzed for the presence of TNF- α . Figure 4A shows that the concentration of TNF- α was significantly decreased at days 60 and 120 in the $\alpha 1$ -null mice compared to the wild type strain, suggesting that the $\alpha 1\beta 1$ integrin may be involved in up-regulating TNF- α during the latent phase of infection and thus involved in attracting, and potentially maintaining macrophages in the granulomatous lesion during infection. Immunohistochemical staining of lung sections (Figures 4B and 4C) showed that TNF- α production was decreased in the $\alpha 1$ -null mice, from day 14 and continued throughout the course of infection. These results suggest that $\alpha 1\beta 1$ integrin signaling may play a role after cells have migrated into the lungs and that it is required for optimal granuloma formation.

MMP-9 activity is decreased in $\alpha 1$ -null mice

Given the change in pulmonary histology that was most prominent during the chronic phase of infection, we next determined if this difference transcended to an alteration in the level of pulmonary MMP-9. In addition, this gave us the opportunity to investigate a potential relationship between MMP-9 and $\alpha 1\beta 1$ integrin during pulmonary infection with *M. tuberculosis*. Supernatants from lung homogenates, as described above, were analyzed for their concentration of MMP-9. As shown in Figure 5, the concentration of active MMP-9 was decreased in the lungs of $\alpha 1$ -null mice compared to wild type mice from day 60 of infection onwards. These results suggest that the $\alpha 1\beta 1$ integrin is required for up-regulation of MMP-9 activity in the lungs during *M. tuberculosis* infection, and that this relationship is important for granuloma formation during the chronic phase of infection.

Bacterial growth in $\alpha 1$ -null and wild type mice

To assess if the $\alpha 1\beta 1$ integrin is involved in protection during pulmonary infection with *M. tuberculosis*, $\alpha 1$ -null mice and wild type mice were examined for the 180 day period after infection with a low dose of virulent *M. tuberculosis*, H37Rv. At each time point, mice were sacrificed and their lungs, spleens and lung associated lymph nodes (LALN) were harvested to determine the number of viable organisms in each organ. The data in Figure 6 show that there was no difference between $\alpha 1$ -null and wild type mice in terms of the number of colony forming units in any organ tested, although there was a transient trend at day 120 for the $\alpha 1$ -null mice to have fewer organisms in all the organs tested. These results indicated that mice deficient in the $\alpha 1\beta 1$ integrin do not have an impaired ability to combat pulmonary *M. tuberculosis* infection, when compared to the wild type mouse.

DISCUSSION

The current set of experiments was performed to determine if the adhesion molecule, $\alpha 1$ integrin, played a significant role in the pulmonary pathology during *M. tuberculosis* infection. Our data have shown that $\alpha 1\beta 1$ integrin does indeed contribute to the structure of the granuloma and to the cytokine milieu, particularly during the chronic phase of infection. The absence of the $\alpha 1$ -integrin subunit resulted in a relative increase in the number of pulmonary IFN- γ -producing CD4 T cells, but there was a reduction in the concentration of TNF- α and MMP-9 when compared to wild type mice. However there was no significant effect on the bacterial burden in any of the organs examined.

Previous studies by others have shown that the $\alpha 4$ -integrin played a significant role in the migration of lymphocytes into the lungs of *M. tuberculosis* infected mice, which compromised their ability to reduce the number of CFU¹⁹. In contrast, our data show that the $\alpha 1$ -integrin is not as absolutely required as the $\alpha 4$ -integrin. This may reflect the different roles that each integrin plays in the process with the latter required more for leukocytes migration into tissue, and the former for adhesion to the ECM after leukocytes have extravasated into tissue. Others that have also examined adhesion molecules during *M. tuberculosis* infection have shown that LFA-1 was required for protective immunity and efficient T cell trafficking to the lung, and LFA-1 knockout mice had prominent neutrophil accumulation associated with early central necrosis²⁴. Interestingly, lymphocyte trafficking into the lungs was not impaired in the $\alpha 1$ -null mouse strain as shown in Figure 3; and while the granulomas were smaller overall, there was little difference in the amount of lung affected between the two groups according to morphometric analysis. Thus, although the $\alpha 1$ -null mice had smaller lesions, there may have been a greater number of these lesions, which totaled the same area as the lesions in the WT mice. Taken together the data would suggest a sequential involvement of each integrin whereby leukocytes use the $\alpha 4$ -integrin and LFA-1 to migrate into *M. tuberculosis* infected lungs and then $\alpha 1$ -integrin for maintaining the integrity of the granulomatous lesion possibly through the

regulation of MMP-9 activity. Reduced MMP-9 levels in α 1 null mice may have also caused the thickening of alveolar septae that was observed in these mice (Figure 1), a finding that was similar to MMP-9 KO mice during infection²⁵. It is possible that decreased MMP-9 levels in the α 1-null mice may have also been associated with increased collagen deposition in Figure 2, although further studies would need to be performed to determine potential involvement of other MMPs. It has been shown that α 1-null mice accumulate more collagen in the dermis than wildtype mice due to a loss of feedback regulation of collagen synthesis²⁶, and likewise, α 1-null mice in the present study also produced more collagen than the wild type strain. In light of this finding and the more recent finding by Richter et al.²⁷ that T cell subsets localize differently according to collagen distribution and integrin markers in influenza-infected lung tissue, future experiments in this laboratory will be performed to investigate possible differential expression of α 1- and α 2-integrins on CD4 and CD8 T cells, respectively, as well as localization of these cell populations according to collagen distribution within the granulomatous environment during infection with *M. tuberculosis*. More recently, semaphorin (SEMA) 7A has been shown to interact directly with the α 1 β 1 integrin on T cells and SEMA7A knockout mice are deficient in their T cell mediated immune responses²⁸. Given that the α 1 integrin interacts with multiple ligands, it will be very interesting to determine if the interaction of SEMA7A with α 1 integrin plays a role in the immune response to tuberculosis.

Our data suggest that during infection, α 1 β 1 integrin is required for effective activation of MMP-9 (Figure 5), which contrasts with previous findings in a tumor vascularization model that showed α 1-null mice had increased MMP-9 activity, suggesting a down-regulatory role for this molecule²² and thus may reflect the diverse functions of this integrin in specific pathologies depending on factors such as the cytokine milieu. Certainly, in the absence of the α 1-integrin subunit during *M. tuberculosis* infection, there were increased numbers of activated T cells from day 60 of infection producing IFN- γ (Figure 3), although the concentration of TNF- α was consistently lower during this same period (Figure 4). Indeed, immunohistochemical analysis showed that the greatest accumulation of TNF- α occurred in the pulmonary lesions of wild type mice (Figure 4C). The greater number of effector T cells in the lungs of α 1-null mice may have contributed to increased cellular death due to the presence of activated macrophages, suggesting a TNF- α -independent mechanism. Thus, we presume that the increased numbers of effector T cells in the α 1-null mice to be located within the granulomatous regions. Indeed others have shown that CD4 T cells tend to be localized within granulomatous regions²⁹. Other studies using influenza virus infection in α 1-null mice showed that virus-specific memory CD8 T cells were decreased suggesting that α 1 β 1 integrin is responsible for retaining protective memory CD8 T cells in the lung via attachment to the ECM³⁰. The current study would suggest that during infection with an intracellular pathogen that persists in the lung for long periods, α 1 β 1 integrin expression on T cells is not required (Figure 6). The ability of *M. tuberculosis* to persist in the lung, while influenza virus is cleared may be the determining factor as to whether T cells can be retained in the lung via the ECM binding integrin. The fact that there were increased numbers of effector T cells may be due to multiple factors that could be attributed to the continuous presence of antigens made by the organism or that the absence of signaling through the α 1 β 1 integrin may maintain the T cells in an effector state. Increased effector T cells may also be due to the decreased levels of TNF- α in these mice, as other have shown that TNF- α can induce T cell apoptosis and thus less TNF- α would result in fewer T cells undergoing apoptosis³¹. Alternatively others have shown an increase in regulatory T cells associated with lower levels of TNF- α ³², and this may also be related to the ability of α 1-null mice to limit granulomas to smaller well focused lesions. Further studies would be needed to elucidate which of these was true. Interestingly, the presence of increased numbers of effector CD4 T cells did not affect the mycobacterial burden, and may suggest that the number of CD4 T cells in the wild type was sufficient to cause a plateau in CFU and any increase above this level had no effect.

In general the current data suggest that $\alpha 1\beta 1$ integrin may be utilized by T cells during the chronic phase of infection to down-regulate their activity and thus may be involved in conversion to a memory phenotype as suggested by others ^{30, 33}, since in its absence, T cells were significantly more activated than in wild type mice. In addition, our data suggest that it may play a part in the sequential use of integrins by T cells during their trafficking into and through infected tissue. Indeed, within the environment of an infected lung, it would seem likely that leukocytes use multiple integrins, depending on the stimuli received and incorporated. Taken together, it would seem probable that integrins are utilized by multiple cell phenotypes as they traffic from the vasculature into infectious foci. For tuberculosis it would be of great interest to determine what functions, other than adhesion and trafficking, that these molecules play throughout the infectious process.

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Figure 1.

Representative hematoxylin & eosin stained lung sections from $\alpha 1$ -null mice and WT mice at days 120 and 180 of infection with *M. tuberculosis*. From 28 days post-infection, when lesions became apparent in both WT and $\alpha 1$ -null mice, and at all subsequent time points except at days 120 and 180 of infection, the lesions were more numerous and tended to be more focally extensive in the WT compared to $\alpha 1$ -null animals. By days 120 and 180, this difference was less apparent due to coalescence of lesions. The granulomatous lesions in the $\alpha 1$ -null strain (panels A and B, respectively) were considerably smaller in size and discrete, with less tendency to coalesce compared to the lesions in the WT mouse lung (panels D and E). By day 180, there were distinct regions of cell death (panel C, box) within the granulomas in the $\alpha 1$ -

null mice, characterized by cellular and nuclear debris (C, arrow head). Alveolar septae were clearly distinct and markedly thickened in the $\alpha 1$ -null strain (C, arrows) compared to the WT mice, in which septae were effaced by lymphocyte infiltration or dissolution (panel F). The total magnification is 20X for panels A, B, D and E, and 100X for panels C and F.

Figure 2.

Representative Masson's trichrome-stained lung sections from $\alpha 1$ -null mice (A and C) and WT mice (B and D) infected with pulmonary *M. tuberculosis*. By day 180 of infection, collagen deposition (arrows) was greater in the lungs of the $\alpha 1$ -null mice than in the WT strain. The total magnification is 10X for panels A and B, and 20X for panels C and D.

Figure 3.

Phenotypic effector T lymphocytes reside in greater numbers in the lungs of $\alpha 1$ -null mice. Single-cell suspensions from the lungs were stained with antibodies specific for CD4 and surface activation markers CD44 (A) and CD62L (B), or intracellular IFN- γ (C). Cells were gated on lymphocytes by forward and side scatter according to their characteristic scatter profile. The number of effector CD4 T cells was higher in the $\alpha 1$ -null mice from day 60 to day 120, and the number of IFN- γ -producing CD4 T cells was significantly higher in the $\alpha 1$ -null mice at days 60 and 120 of infection. *Open circles*, $\alpha 1$ -null; *closed circles*, WT. Data are expressed as the mean ($n = 5$ mice) $\pm$ SEM. p values were calculated using a Student's t -Test comparing WT versus $\alpha 1$ -null mice at each time point; * $p < 0.05$.

Figure 4.

Decreased production of TNF- α in the lungs of $\alpha 1$ -null mice. Lungs were harvested over a period of 180 days of pulmonary infection with *M. tuberculosis* and were analyzed by cytometric bead array for the production of TNF- α . The level of TNF- α was significantly decreased in the lung homogenates of the $\alpha 1$ -null mice (A). Day 180 lung sections stained with anti-TNF- α antibody also showed a decrease in TNF- α staining in the $\alpha 1$ -null mouse strain (B) compared to the WT mice (C). Open circles, $\alpha 1$ -null; closed circles, WT. Data are expressed as the mean ($n = 5$ mice) $\pm$ SEM. p values were calculated using a Student's *t*-Test comparing WT versus $\alpha 1$ -null mice at each time point; * $p < 0.05$.

Figure 5.

Production of active MMP-9 in the lungs of WT versus $\alpha 1$ -null mice. Lungs were harvested over a period of 180 days of pulmonary infection with *M. tuberculosis* and were analyzed for the production of active MMP-9. The level of active MMP-9 protein was significantly higher in the WT mouse strain beginning at day 60 of infection through day 180. *Open circles*, $\alpha 1$ -null; *closed circles*, WT. Data are expressed as the mean ($n = 5$ mice) $\pm$ SEM. p values were calculated using a Student's *t*-Test comparing WT versus $\alpha 1$ -null mice at each time point; * $p < 0.05$.

Figure 6.

Bacterial load in the lungs of WT and α1-null mice. Lungs (A), spleen (B) and LALN (C) were harvested over 180 days of pulmonary infection with *M. tuberculosis*. No differences in CFU numbers were observed between the two strains of mice over the course of the infection. Data are expressed as the mean ($n = 5$ mice) $\pm$ STDEV. Open circles, α1-null; closed circles, WT.