THE EFFECT OF IN-VIVO INTERLEUKIN-1 ON RECRUITMENT OF IMMUNE CELLS INTO THE LUNG

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Abstract — The mechanisms responsible for recruitment of lymphocytes into the lung are not known. Data suggest that interleukin-1 (IL-1) is chemotactic for neutrophils (PMN), macrophages, and lymphocytes. Therefore, the release of IL-1 in the lung in response to antigen exposure might be important in lymphocyte recruitment. The data from this study indicate that the instillation of a low dose of human IL-1b (50 ng) into lung lobes of dogs recruited only PMN with no increase in lymphocytes. A dose of 2000 ng recruited large numbers of PMN, with a slightly increased number of lymphocytes. The instillation of 2000 ng of IL-1 into the lungs of dogs that were immunized with sheep red blood cells did not result in the recruitment of antibody-forming cells (AFC) into the treated lung lobe. These data do not support the hypothesis that IL-1 by itself is chemotactic for lymphocytes in vivo, or that inflammation induced by IL-1 alone allows the entry of AFC and antibody at the same level as in lung lobes instilled with antigen.

The recruitment of lymphocytes into the lungs is essential in providing pulmonary defense against infectious organisms. However, large accumulations of lymphocytes in the lung can also lead to disease. After the inhalation of antigenic materials, large numbers of lymphocytes accumulate in the lungs, resulting in hypersensitive lung diseases in a small percentage of humans. An understanding of the mechanisms involved in the recruitment of lymphocytes to the lungs could be important not only in treatment of individuals with recurrent pulmonary infections, but also in the therapy of diseases induced by the development of abnormal immune responses in the lungs.

One mediator that is chemotactic for lymphocytes in vitro, and that might recruit lymphocytes into localized areas of the lung, is interleukin-1 (IL-1) released by activated alveolar macrophages.[1] The goal of this study was to determine if IL-1 instilled into the lung is chemotactic in vivo for lymphocytes, and capable of recruiting lymphocytes and AFC from the blood into the lung. Our results indicate that IL-1 instilled into the lung is not directly chemotactic for lymphocytes or antibody-forming cells (AFC) in vivo.

METHODS

Four 2-yr old Beagle dogs were used in this study. Physical examinations, thoracic radiographs, venous blood cell counts, and serum chemistry determinations of blood urea nitrogen, alkaline phosphatase, and alanine aminotransferase were used to determine the health of each dog before exposure to IL-1 or immunization. All dogs were healthy throughout this study.

Based on the results of studies of inflammation induced in the lung by instillation of IL-1, we selected two doses to determine if IL-1 would recruit AFC from the blood into the lung. A high dose of 2000 ng IL-1 was selected because the degree of inflammation induced (total PMN and total protein) was similar to the inflammation produced by instillation of particulate antigen.[2] Low doses of IL-1 may selectively attract T-cells, whereas higher concentrations attract a variety of

cells. Therefore, we also included a second dose of 50 ng to allow comparisons of accumulation of AFC in lung lobes exposed to inflammatory and noninflammatory doses of IL-1.

On day 0, dogs were immunized by instillation of 10^{10} sheep red blood cells (SRBC) into the left cardiac lung lobe, while saline (1 mL) was instilled into the right intermediate lobe as a control. These lung lobes were lavaged on 1, 3, and 5 days after instillation. On day 6, around the time of peak numbers of AFC in the blood,[2] 50 ng IL-1 was instilled into the left diaphragmatic lung lobe, and 2000 ng IL-1 was instilled into the right cardiac lung lobe. As a positive control, 10^{10} rabbit red blood cells (RRBC) were instilled into the right diaphragmatic lung lobe. AFC do not enter the lung on an antigen-specific basis, and the instillation of RRBC into a separate lung lobe would result in the accumulation of AFC from the blood into the lung. Each of the immunized, IL-1 exposed, and control lung lobes were lavaged on days 7, 9, 12, and 14 immunization with SRBC (1, 3, 6, and 8 days after instillation of IL-1 and RRBC).

The number of AFC producing anti-SRBC IgM in blood and in lavage fluid from the lung lobes exposed to SRBC, IL-1, RRBC, or saline was determined by the Cunningham modification of the Jerne plaque assay.[3] The level of anti-SRBC IgM, IgG, and IgA antibody in the lavage fluids and in serum was evaluated with an enzyme-linked immunoassay (ELISA).[2] Total protein and total cells in lavage fluids were determined, and the numbers of PMN, lymphocytes, and alveolar macrophages present were evaluated by microscopic evaluation of cytocentrifuge slides.

The data obtained from the evaluation of AFC, specific antibody, and cell cytologies in lavage fluid from lung lobes exposed to antigen, IL-1, or saline were compared by ANOVA and multiple group comparisons (BMDP Statistical Software, Inc., Los Angeles, CA). Only differences at $p < 0.05$ were considered significantly different.

RESULTS

Instillation of IL-1 or antigen did not induce fever or other clinical signs in any of the dogs used in this study. However, the instillation of SRBC, RRBC, and 2000 ng IL-1 all resulted in significantly increased numbers of PMN in lung lavage fluid (Fig. 1). The peak numbers of PMN

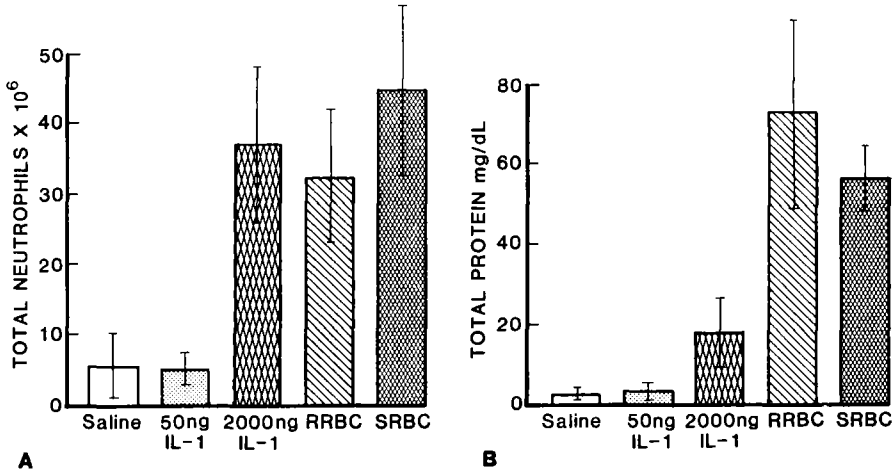

Figure 1. Total neutrophils (A) and total protein (B) after instillation of saline (right intermediate lobe), 50 ng interleukin-1 (IL-1) (left diaphragmatic lobe), 2000 ng IL-1 (right cardiac lobe), 10^{10} rabbit red blood cells (RRBC) (right diaphragmatic lobe), or 10^{10} sheep red blood cells (SRBC) (left cardiac lobe). All instillations were in 1 mL saline. Saline and SRBC were instilled on day 0, while RRBC and IL-1 were instilled on day 6. The values represent means ± S.E. of peak responses (n = 4) in lavage fluids from selected lung lobes instilled with saline, SRBC, IL-1, or RRBC.

occurred at 1 or 3 days after instillation of antigen or IL-1. Although the number of PMN in lung lavage fluid from lung lobes exposed to 2000 ng IL-1 was the same as observed after exposure to SRBC and RRBC, instillation of 2000 ng IL-1 did not significantly increase the amount of total protein in lavage fluid in comparison to total protein in control lung lobes instilled with saline (Fig. 1). The 50 ng dose of IL-1 did not increase the total number of PMN nor the level of total protein present in lung lavage fluids (Fig. 1). As observed for the number of PMN, the peak levels of total protein were present at 1 or 3 days after instillation of antigen or IL-1.

Both the 50 and 2000 ng doses significantly increased the percentage of PMN in lavage fluids. The peak percentage of PMN for all lung lobes were as follows: saline - 7.7%, 50 ng IL-1 - 37.2%, 2000 ng - 67.0%, RRBC - 58.2%, and SRBC 63.0%.

Exposure to 2000 ng IL-1, SRBC, and RRBC all significantly increased the total number of cells present in lung lavage fluid (Table 1). Although 2000 ng IL-1 appeared to elevate the peak percentage of lymphocytes present (at day 1 only), the total number of lymphocytes was only slightly elevated by instillation of IL-1 (Table 1). However, the instillation of SRBC and RRBC significantly elevated both the percentage and total number of lymphocytes. The time course of the number of lymphocytes in the SRBC- and RRBC-exposed lung lobes was also different in comparison to numbers of lymphocytes in the lung lobes instilled with 2000 ng IL-1 (Table 1, Fig. 2). The mean peak number of lymphocytes in the lung lobes instilled with SRBC or RRBC occurred at 14 days after antigen exposure. Lymphocytes entered lung lobes exposed to SRBC or RRBC starting around 5 to 7 days after immunization with SRBC or 1 day after instillation of RRBC. Few lymphocytes entered the lung at first day after exposure to SRBC or RRBC. In contrast, instillation of 2000 ng IL-1 resulted in a peak response at 1 day after instillation, and the number of lymphocytes returned to control levels by 3 days after instillation (Fig. 2). Instillation of 50 ng IL-1 had no effect on percentage or number of lymphocytes.

Table 1
Peak Total Lavage Cells and Lymphocytes
After Instillation of Antigen or IL-1

Exposure	Total Cells[a] x 10^6	Percent Lymphocytes[b]	Total Lymphocytes x 10^6
Saline	26.3 ± 8.4	13.0 ± 2.2	1.3 ± 0.4
50 ng IL-1	13.1 ± 2.0	10.7 ± 1.3	0.8 ± 0.2
2000 ng IL-1	55.5 ± 16.3	18.0 ± 3.0	3.5 ± 0.7
SRBC[c]	66.0 ± 14.3	33.5 ± 7.2	13.6 ± 7.7
RRBC[d]	61.9 ± 12.4	22.2 ± 2.9	12.5 ± 7.8

[a]Total cells were observed at 1 or 3 days after exposure. Values are the means of peak responses observed for each dog.

[b]Total lymphocytes were observed at 9, 12, or 14 days after exposure to SRBC or RRBC, or 1 or 3 days after exposure to IL-1. Values are the means of peak responses observed for each dog.

[c]Sheep red blood cells.

[d]Rabbit red blood cells.

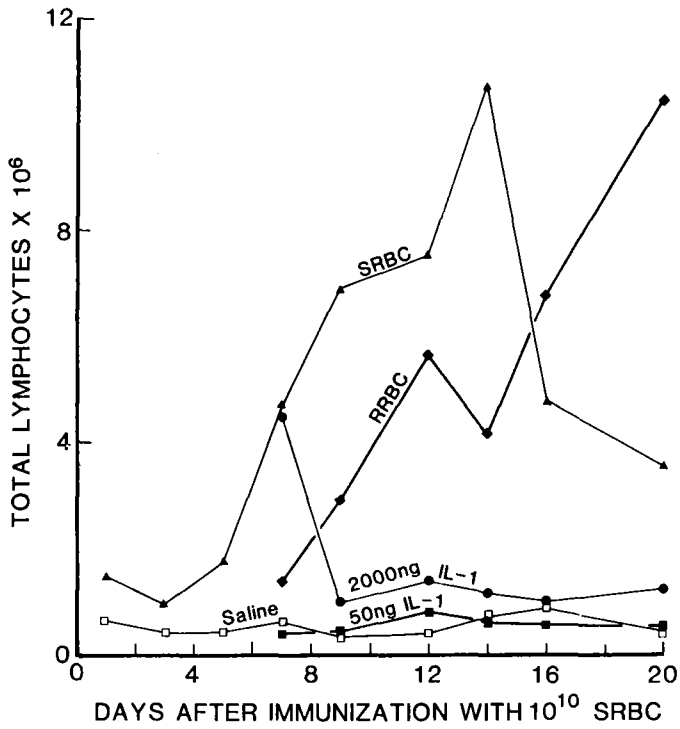

Figure 2. Total numbers of lymphocytes in lavage fluid after instillation of saline (right intermediate lobe), 50 ng interleukin-1 (IL-1) (left diaphragmatic lobe), 2000 ng IL-1 (right cardiac lobe), 10^{10} rabbit red blood cells (RRBC) (right diaphragmatic lobe), or 10^{10} sheep red blood cells (SRBC) (left cardiac lobe). All instillations were in 1 mL saline. Saline and SRBC were instilled on day 0, while RRBC and IL-1 were instilled on day 6. The values represent means observed in lavage fluids from 4 dogs. Error bars are not presented for clarity. In comparison to saline controls, the numbers of lymphocytes were significantly elevated only in the lung lobes instilled with SRBC or RRBC.

The peak number of anti-SRBC AFC in blood occurred around 7 to 9 days after instillation of SRBC (Fig. 3). These results indicate that large numbers of anti-SRBC AFC were in the blood when IL-1 and the second antigen (RRBC) were instilled into the lung. Peak levels of specific serum IgG were observed at 12 days after instillation of SRBC. Therefore, the level of specific anti-SRBC IgG antibody in serum when IL-1 and RRBC were instilled into the lung was low.

Large numbers of anti-SRBC AFC were recruited from the blood into the lung lobe exposed to SRBC, and a high level of specific IgG was observed in lung lavage fluid from this lobe (Fig. 4). The peak number of AFC occurred on day 9, with large numbers also found at 12 and 14 days. Recruitment of AFC from the blood into the lung is not antigen specific,[4] and large numbers of AFC producing antibody to SRBC entered the lung lobe exposed to RRBC (Fig. 4). The time course for the accumulation of anti-SRBC AFC in the lung lobes exposed to RRBC was the same as for the lung lobe exposed to SRBC, even though the RRBC were instilled at 6 days after immunization with SRBC. Some AFC were also recruited into the lung lobe exposed to 2000 ng IL-1, although the numbers of anti-SRBC AFC were not as high as observed in lung lobes exposed to SRBC or RRBC. The peak AFC response in the 2000 ng lobe occurred at 9 days, but unlike lung lobes exposed to either SRBC or RRBC, there was a precipitous drop in AFC number by the next lavage day, day 12. Few AFC were present in this lobe at 14 days after instillation of 2000 ng IL-1. The numbers of AFC in the lung lobes exposed to 50 ng IL-1 or saline were basically the same.

The levels of anti-SRBC IgG in the lung lobes exposed to SRBC or RRBC reached peak values at 12 days after immunization with SRBC (Fig. 4). There was a slight increase in anti-SRBC IgG at 9 days in the lung lobe exposed to IL-1. However, by 14 days this response was the same as observed

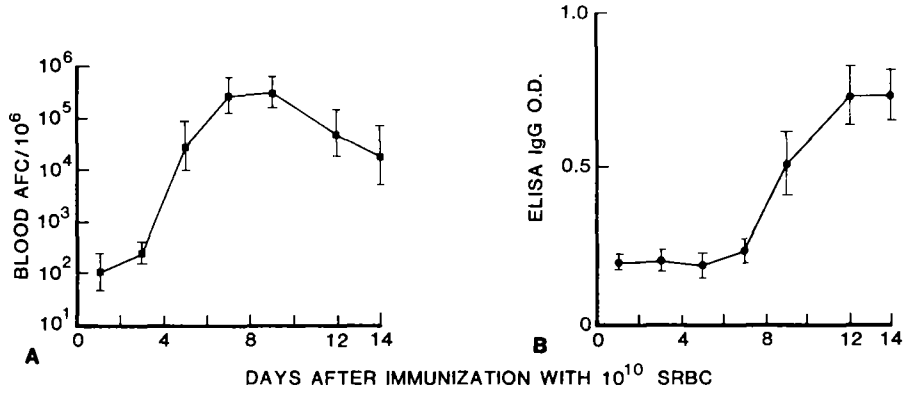

Figure 3. Number of anti-sheep red blood cell (SRBC) antibody-forming cells (AFC) (A) and level of anti-SRBC IgG (B) in blood after instillation of SRBC on day 0. Rabbit red blood cells, 50 ng IL-1, and 2000 ng IL-1 were instilled on day 6.

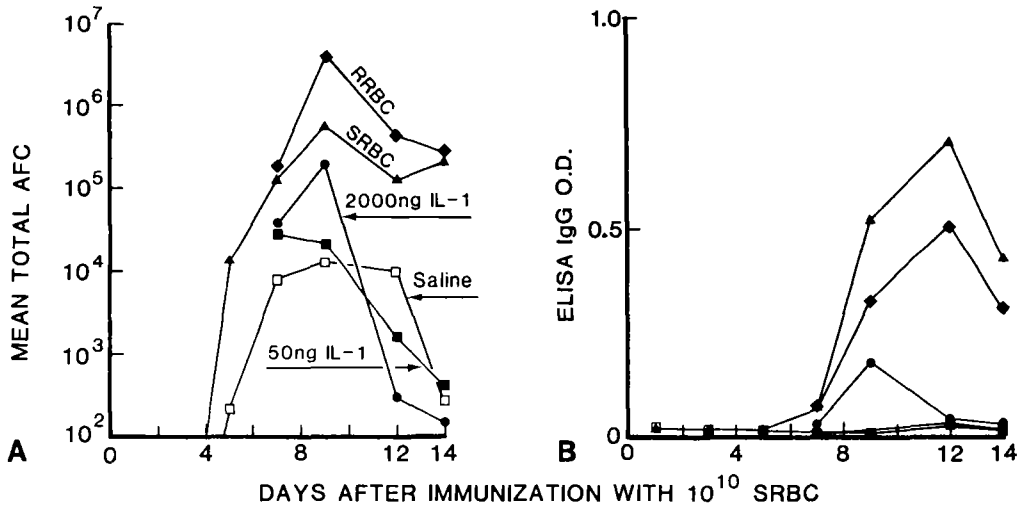

Figure 4. Number of anti-sheep red blood cell (SRBC) antibody-forming cells (AFC) (A) and peak level of anti-SRBC IgG antibody (B) in lung lavage fluid from lung lobes after instillation of saline (right intermediate), 50 ng interleukin-1 (IL-1) (left diaphragmatic), 2000 ng IL-1 (right cardiac), 10^{10} rabbit red blood cells (RRBC) (right diaphragmatic), or 10^{10} sheep red blood cells (SRBC) (left cardiac). All instillations were in 1 mL saline. Saline and SRBC were instilled on day 0, while RRBC and IL-1 were instilled on day 6. The values represent means observed in lavage fluids from 4 dogs. Error bars are not presented for clarity. In comparison to the saline control, the numbers of AFC and the level of anti-SRBC antibody were significantly elevated only in the lung lobes instilled with SRBC or RRBC.

in the control lung lobe exposed to saline. Exposure of the lung to 50 ng IL-1 did not result in an increase in specific IgG. Anti-SRBC IgM and IgA responses in the lung lobes exposed to SRBC or RRBC were similar to IgG responses in these lung lobes, but levels were lower, as previously reported.[2] As observed with IgG, the instillation of 50 or 2000 ng IL-1 did not result in elevated levels of anti-SRBC IgM or IgA.

DISCUSSION

The importance of inflammation in the entry of lymphocytes several days after antigen exposure, and the mechanisms that allow lymphocytes to enter the lung are not understood. Although large numbers of PMN enter the lung within 24 h after instillation of particulate antigen, the peak number of lymphocytes are not observed until several days after antigen exposure.

Previous studies show that IL-1 is chemotactic for PMN *in vitro*, and our results show that IL-1 was effective in the recruitment of PMN into the lung.[5] Even the low dose of IL-1 increased the percentage of PMN in lung lavage fluids. However, *in vivo* exposures to IL-1 appeared to be poorly chemotactic for lymphocytes. In the present study, some lymphocytes entered the lung after *in vivo* instillation of IL-1, but this occurred only at the high dose that also increased vascular permeability as indicated by increased total cells and total protein. Therefore, it is likely that lymphocytes entered the lung after instillation of the high dose of IL-1 primarily due to an overall increase in vascular permeability, resulting in increased lymphocytes due to nonspecific mechanisms rather than chemotaxis.

The possibility that lymphocytes entered the lung lobe exposed to 2000 ng IL-1 only because of changes in vascular permeability is also supported by comparisons of responses in lung lobes exposed to the high and low dose of IL-1. The low dose of IL-1 clearly did not recruit any AFC into the lung, even though 37.0% of the cells in this lung lobe were PMN at 1 day after instillation. The high dose of IL-1 did result in the entry of some AFC from the blood. However, not only was the response not as high as observed in the lung lobe exposed to SRBC or RRBC, but the time course was also different. Exposure to 2000 ng IL-1 resulted in a peak PMN and lymphocyte response at 1 day, suggesting that the small number of lymphocytes and anti-SRBC AFC observed entered not by specific chemotaxis by IL-1, but by changes in increased vascular permeability. In contrast to exposures to IL-1, lymphocytes did not start to enter the lung lobe exposed to SRBC until around 7 days after instillation of antigen.

These data suggest that IL-1 is likely important in the recruitment of PMN into the lungs. The release of IL-1 from alveolar macrophages could be pivotal in the production of inflammation in immunized lung lobes. However, IL-1 instilled into the lung was not strongly chemotactic for AFC or lymphocytes, and those lymphocytes that did enter the lung, appeared to enter primarily due to nonspecific mechanisms resulting from the increased vascular permeability.

REFERENCES

1. Hunninghake, G. W., A. J. Glazier, M. M. Monick, and C. A. Dinarello. Interleukin-1 is a Chemotactic Factor for Human T-lymphocytes, Am. Rev. Respir. Dis. 135: 66-71, 1987.

2. Bice, D. E., and B. A. Muggenburg. Effect of Age on Antibody Responses After Lung Immunization, Am. Rev. Respir. Dis. 132: 661-665, 1985.

3. Cunningham, A. J., and A. Szenberg. Further Improvements in the Plaque Technique for Detecting Single Antibody-Forming Cells, Immunology 14: 599-600, 1968.

4. Bice, D. E., M. A. Degen, D. L. Harris, and B. A. Muggenburg. Recruitment of Antibody-Forming Cells in the Lung After Local Immunization is Nonspecific, Am. Rev. Respir. Dis. 126: 635-639, 1982.

5. Goto, K., S. Nakamura, F. Goto, and M. Yoshinaga. Generation of an Interleukin-1-Like Lymphocyte-Stimulating Factor at Inflammatory Sites: Correlation with the Infiltration of Polymorphonuclear Leukocytes, Br. J. Exp. Pathol. 65: 521-632, 1984.