A COMPARISON OF THE RECRUITMENT OF ANTIBODY FORMING CELLS IN THE NOSE AND LUNG: PRELIMINARY FINDINGS

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Abstract -- Instillation of a particulate antigen into a selected lung lobe leads to an accumulation of antibody forming cells in the exposed lung lobe. Our goal in this preliminary study was to determine if an immune response could be elicited in the nasal mucosa of Beagle dogs exposed to a particulate antigen, and if so, to compare this immune response with that of the lungs when the nasal mucosa and the lungs are each immunized with a different particulate antigen. An immune response was observed when the nasal mucosa was exposed to particulate antigen, but numbers of antibody-forming cells and levels of antibody in the nose were much lower than observed in an immunized lung lobe.

The structure of the nasal cavity acts as a physical barrier in its protection of the respiratory tract.[1] When an inflammagen is instilled in the nasal cavity, there is a large influx of neutrophils to the mucosa.[2] Local production of secretory IgA results when the nasal cavity is exposed to antigen.[3] However, it is not known if the exposure of the nasal cavity to a particulate antigen results in a response similar to that observed following the immunization of a selected lung lobe with a particulate antigen. After the immunization of a lung lobe with antigen, antibody forming cells (AFC) are first found in the blood and then are recruited to the immunized lobe in significant amounts.[4] It is also known that this recruitment of AFC is not antigen specific.[5]

In this study, the nasal cavity of Beagle dogs was immunized with sheep red blood cells (SRBC). The left cardiac lung lobe (LC) was used as a positive control and was immunized with rabbit red blood cells (RRBC). The positive control lobe was necessary to allow recruitment of SRBC AFC. There is no significant cross-reactivity between RRBC and SRBC. The right cardiac lung lobe (RC) was the negative control lobe, exposed to sterile saline.

METHODS

Two male Beagle dogs, 2 yr of age were used in this study. Prior to exposure, a physical examination, chest radiographs, complete hematology and serum chemistries (urea nitrogen, alkaline phosphatase and alanine amino transferase) were completed to ensure that the animals were healthy. Both dogs remained healthy throughout the study and were returned to the ITRI colony at the end of the study.

The dogs were anesthetized by halothane inhalation and intubated, prior to instillation. Food was withheld 18 h before anesthesia. The LC was instilled with 5×10^{10} RRBC in 1 mL of sterile saline; the RC was the saline control lobe. Instillation was via a catheter through a fiberoptic bronchoscope (Olympus Corp., Model BF, Type 4B2, Lake Success, NY; 5.5 mm O.D.). For the nasal cavity exposure, with the dogs in dorsal recumbency, a pediatric fiberoptic bronchoscope was introduced into the dog's mouth, inserted just pass the soft palate, and then directed retrogradely into the nasal cavity. The nasal cavity was then exposed to 10^{10} SRBC in 1 mL of saline. Lavage of the LC, RC, and nasal cavity was performed at days 1, 3, 5, 7, 10, 12, and 14.

Five aliquots of 10 mL of saline were used to lavage each lung lobe, and 10 aliquots of 3 mL of saline were used to lavage the nasal cavity.

Lavage cells from the lungs and nasal cavity were washed three times by centrifugation, resuspended in RPMI supplemented with 10% fetal calf serum, and counted. Cytocentrifuge smears were stained with Wright's stain and evaluated for differential cell counts. The Cunningham modification of the Jerne plaque assay was used to determine the number of antibody forming cells producing anti-RRBC and anti-SRBC IgM antibody in the blood, nasal lavage fluid, and the lavage fluid from the exposed and control lung lobes. The Lowry method of protein analysis was used to measure the total protein of the nasal and lung lavage fluids. The anti-SRBC IgA, IgG, and IgM antibody levels in the serum, and in the nasal and lung lavages were determined with an enzyme-linked immunosorbent assay (ELISA).

RESULTS

The peak total number of bronchoalveolar cells was observed at day 1 after instillation of RRBC into the exposed lung lobe (data not shown). These cells were predominately polymorphonuclear leukocytes (PMNs). There was also an increase in the total number of cells in the nasal cavity at 1 day after immunization. However, the number of total cells seen in the nasal cavity was significantly less than the number of total cells in the immunized or control lung lobes.

An immune response was elicited by instillation of particulate antigen into the nasal cavity and the lung, as demonstrated by the presence of SRBC and RRBC AFC in blood. The blood contained peak numbers of RRBC AFC at Day 5 and peak numbers of SRBC AFC at Day 7 (data not shown).

An antigen-specific antibody titer was also observed in the blood, with anti-SRBC IgG and IgM antibodies in greater amounts than anti-RRBC IgG and IgM antibodies. Both anti-RRBC IgA and anti-SRBC IgA antibody levels were low.

The SRBC and RRBC AFC were then recruited back to both the immunized and control lung lobes and to the nasal cavity. AFC to SRBC and anti-SRBC antibody levels were found to be higher in the LC lobe (immunized with RRBC), then in the RC lobe (saline control lobe) or in the nasal cavity (immunized with SRBC) (Figs. 1-3).

Both dogs showed bilateral enlargement of the tonsils, one dog at days 5 and 7 after exposure, and the other at day 5 only. Impression smears of the tonsils contained many PMNs, and tonsillitis was diagnosed.

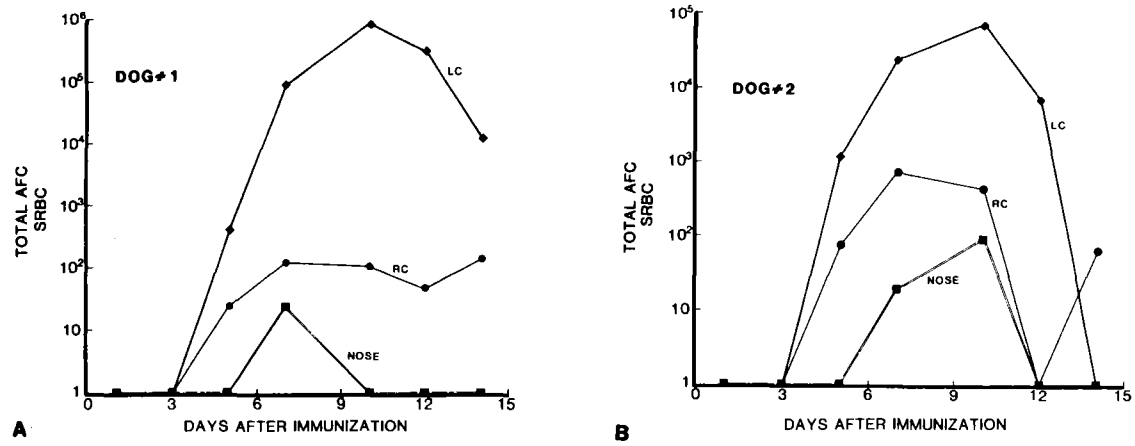

Figure 1. Total number of anti-SRBC AFC present in (A) dog #1 and (B) dog #2. The left cardiac (LC) lobe was exposed to 5 x 10^{10} RRBC and the nose was exposed to 10^{10} SRBC. The right cardiac (RC) was used as the saline control.

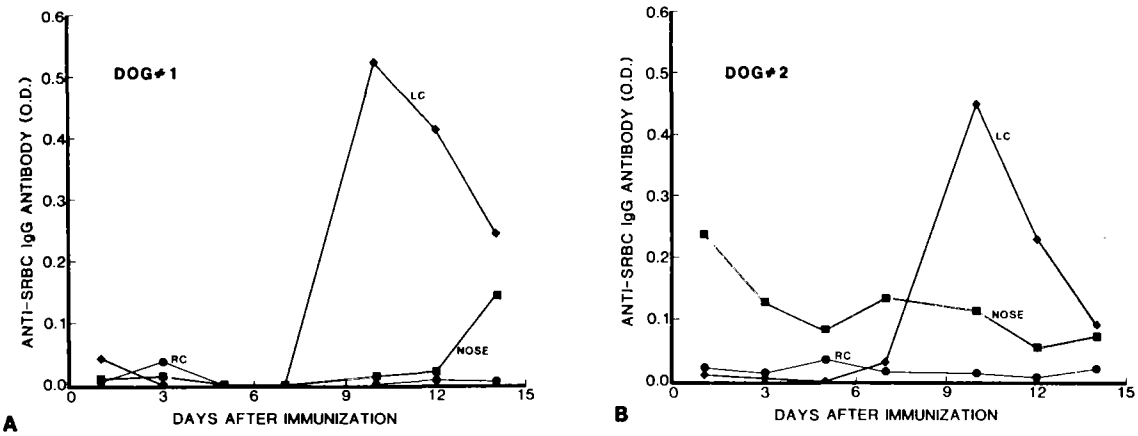

Figure 2. Anti-SRBC IgG antibody levels in (A) dog 1 and (B) dog 2. See legend for Figure 1 for explanation of exposures.

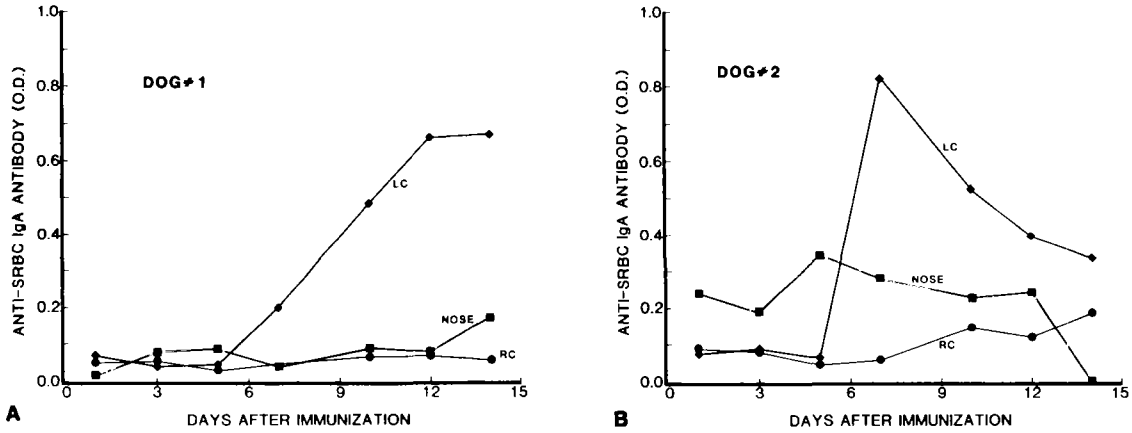

Figure 3. Anti-SRBC IgA antibody levels in (A) dog 1 and (B) dog 2. See legend for Figure 1 for explanation of exposures.

DISCUSSION

The immunization of a selected lung lobe with a particulate antigen, such as SRBC, results in a systemic immune response. AFC to that specific antigen are first observed in the blood and then are recruited to the immunized lung lobe, with much lower numbers appearing in nonimmunized lobes.[4] However, the recruitment of AFC into immunized lung lobes is not antigen specific.[5] When selected lung lobes are exposed to distinctly different antigens, the number of AFC from each antigen entering each exposed lobe is similar. This recruitment of nonspecific AFC into an immunized lung lobe is also seen when other distant lymph nodes are stimulated.[6]

Our preliminary data show that when the nasal cavity is exposed to particulate antigen, AFC and antibody are produced. It is hypothesized that the antigen deposited in the nasal cavity is phagocytized and carried to nasal lymphoid tissue. The enlargement of the tonsils was probably due to the incidental drainage of the antigen from the nasal cavity. AFC and antibody that were released into blood were recruited to the lung lobe exposed to a different antigen (RRBC). The

numbers of AFC and levels of antigen specific antibody in the nasal cavity were low, as compared to those in the immunized lung lobe. These low values may relate to clearance mechanisms of the nasal cavity, which may allow the rapid removal of AFC, and hence of antibody, as compared to clearance mechanisms of the lungs. Also, the interstitium and alveolar space of the lung can provide an area that allows accumulation of cells (AFC) and antibody, whereas the structure of the nasal cavity restricts such accumulation.

Additional studies are necessary to provide supplementary data. A biopsy of the nasal mucosa and examination of the tonsils would also be beneficial in such studies.

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