Specifically, the smokers' alveolar macrophages from smokers and nonsmokers. Our initial studies compared a population of older patients undergoing diagnostic fiberoptic bronchoscopy. The \( O_2 \) uptake by smokers was approximately 50 percent greater than the \( O_2 \) uptake by nonsmokers. We then evaluated alveolar macrophages from young smoking volunteers and age-matched nonsmokers. The \( O_2 \) uptake was approximately the same in these two groups, but the pathways of oxygen utilization appeared different. Specifically, the smokers' alveolar macrophages when stimulated had a greater superoxide release than the nonsmokers. So I think that \( O_2 \) uptake above doesn't tell you how the oxygen is utilized by the cell.

Dr. Hoidal: I think that a good part of the anesthetic which produced an effect on the cells came from the smaller airways. In the study all tubing was changed after anesthetization of lower airways and prior to recovering any fluid during the lavage.

Dr. Lynn: Did you happen to do any of your studies in the absence of protein or serum?

Dr. Hoidal: Yes, we sedimented the cells and washed with balanced salt solution.

Dr. Reiss: Do you wish to speculate as to the mechanism of action of lidocaine in the light of the fact that it's highly soluble in lipid.

Dr. Hoidal: We've tried to determine the mechanism and action of the lidocaine on these cells. Lidocaine has been reported to have numerous actions. It removes the adsorbed calcium from the cell membrane. Secondly, it probably blocks the sodium and potassium channels in the cell membrane. And thirdly, it fluidizes the phospholipid bylayers of the cell membrane. To date, the study has been directed at determining whether removal of adsorbed calcium from the cell membrane was the mechanism of lidocaine action. We have not been able to establish this. We've made two types of preliminary studies. First we've replaced the calcium and tried to override the block, without success. Secondly we've tried to utilize ionophores to transport calcium across the membrane. Again this did not prevent the effect of lidocaine.

Dr. Musson: Dr. Hoidal, were you looking at production of superoxide and the consumption of oxygen with adherent cell cultures or were those cultures in suspension?

Dr. Hoidal: The biochemical tests were done on recently harvested cells in suspension.

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**Local and Systemic Immunity following Localized Deposition of Antigen in the Lung**

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The results of previous studies using dogs indicated that intrapulmonary immunization resulted in an increase in antigen-specific antibody-forming cells in the population of cells removed by lung lavage. However, it remains unclear whether these cells were produced by antigen deposition and stimulation of cells in airway-associated lymphoid tissues, or in regional and distant lymphoid tissues followed by a migration and accumulation of antibody-forming cells in the lung. To obtain additional data concerning the role of local and systemic immune responses following instillation of antigen into the lung, dogs were immunized in specific airways and the resulting immune response was measured in immunized as well as control airways, in regional lymphoid tissues and in blood and spleen.

**Materials and Methods**

Beagle dogs two and three years of age were immunized by instillation of 10^10 sheep red blood cells (SRBC) in 1.0 ml saline solution into airways of individual lung lobes. A fiberoptic bronchoscope was used to locate specific airways and the SRBC suspension was delivered through 1.6 mm diameter polyethylene tubing into airways approximately 2 mm in diameter. The SRBC were instilled into the airways of the right or left apical and right or left diaphragmatic lung lobes of 16 dogs. These dogs were sacrificed five days after immunization and cell suspensions were prepared from individual lung-associated lymph nodes and the spleen. The Jerne plaque assay as modified by Cunningham was used to determine the number of anti-SRBC plaque-forming cells (PFC). To evaluate the local response in immunized and control airways, nine additional dogs were immunized in airways of either the left or right apical lung lobes. The immunized airway and a control airway in the opposite apical lobe were lavaged with five saline solution washes of 10 ml each using the fiberoptic bronchoscope on days 3, 5, 7, 10, 12, 14 and 21 after SRBC exposure. An average volume of 42.2 ml lavage fluid was recovered. The cells recovered were counted and the percentage of macrophages, lymphocytes and granulocytes was determined. Blood samples were also taken and the number of direct (IgM) and indirect (IgG) PFC in the blood, and in the cells from immunized and control airways was determined.

**Results**

The number of plaque-forming cells in lung-associated

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lymph nodes five days after immunization indicates that lymphatic drainage from localized antigen deposition was basically ipsilateral. For example, immunization in the right apical or right diaphragmatic lobes resulted in a higher number of PFC in the right tracheobronchial lymph node than in the left tracheobronchial lymph node and the PFC number in the right mediastinal lymph nodes was higher than in the left mediastinal lymph nodes. This observed ipsilateral drainage was statistically significant \((P < 0.05)\), and ipsilateral drainage was observed in all but two of 16 dogs. In each of these two instances, a single lymph node exhibited contralateral drainage. The highest level of PFC was obtained by immunization into the right apical lobe with a mean response of 278,000 PFC/organ in the right tracheobronchial lymph nodes. The response in the spleen following immunization was minimal regardless of the lung lobe immunized.

The number of PFC in the cell population removed from control and immunized airways by regional lavage is presented in Figures 1 and 2. The number of IgM PFC was consistently higher \((P < 0.005)\) in the immunized airway than in the control airway with a peak response at 7-12 days after antigen exposure (Fig 1). The number of IgM PFC in the control airways and blood were similar following SRBC instillation in either the left or right apical lobe. However, immunization in left apical airways induced significantly higher \((P < 0.05)\) numbers of PFC in immunized airways than immunization in right apical. This heightened response in left apical immunizations was also apparent when IgG responses were evaluated (Fig 2) with a mean peak of 13,900 IgG PFC/10^6 lymphocytes at 12 days after exposure in the left apical lobe. A significantly lower mean response of 1,086 PFC/10^6 was observed in immunized airways ten days after antigen deposition in the right apical lobe. The immunized airways in the left apical lobe had higher numbers of IgG antibody forming cells \((P < 0.0005)\) than control airways while no differences were seen in IgG PFC numbers in control and immunized airways after immunization in the right apical lobe. As seen in the IgM response, there was no difference in the level of IgG response in blood whether immunization was in the left or right apical lobe.

An additional difference in the response of the right and left apical lobes was indicated by the total cells recovered in lavage fluid after SRBC exposure. Immunization in the left apical lobe resulted in a maximum number of cells ten days later \((44 \times 10^6)\) while a lower peak response of \(22 \times 10^6\) cells was found three days after immunization in the right apical lobe. The increase in cells in the left apical was due predominately to
pulmonary alveolar macrophages (59 percent) and lymphocytes (29 percent), with a slight increase in granulocytes (11 percent). Maximum granulocytes were found in the immunized left apical lobe three days after immunization (30 percent) followed by a decline in granulocyte numbers through day 21.

DISCUSSION

An evaluation of immune responses in lung-associated lymph nodes indicated that maximum numbers of antibody-forming cells were found in lymph nodes on the same side of the lung that received antigen. This indication of ipsilateral lymphatic drainage of antigen to lung-associated lymph nodes supports the conclusions of Correll and Langston who reported that dye injected in specific lung lobes stained only ipsilateral lymph nodes. The minimal response observed in the spleen of our animals probably results from migration of antibody-forming cells from lung-associated lymph nodes rather than translocation of antigen to the spleen.

Previous studies have shown that both IgM and IgG antigen-specific antibody forming cells are found in the bronchoalveolar spaces. However, the source of these antibody forming cells is not understood. In the present study there were significantly more IgM and IgG antibody-forming cells in immunized than control airways. This increase of PFC in immunized airways probably represents a local production of antibody forming cells in airway-associated lymphoid tissues. It is possible that antibody forming cells observed in blood, which were apparently produced in lung-associated lymph nodes, may be an additional source for PFC found in airways. However, if PFC in blood were a direct source of airway PFC, a uniform distribution of antibody forming cells in both control and immunized airways would be expected. A possible mechanism by which blood-borne antibody-forming cells might be attracted to a localized airway would be by retention of antigen in immunized airway tissues.

The unexpected observation that the number of antibody forming cells, especially IgG, was lower in the immunized airway of the right apical lobe in comparison to the response in the left apical lobe cannot be explained at this time. It is possible that this difference could be due to technique or anatomic differences in lung airways. However, even though fewer cells were obtained from the right apical lobe by lavage, the lower number of antibody-forming cells observed in animals immunized in the right apical lobe was not due to the lower number of cells removed by lavage because these data were expressed as a concentration (PFC/10⁶ lymphocytes). In addition, it was clear that an adequate level of immunization was induced after immunization in the right apical lobe because the number of PFC in the blood was equal to the level observed following immunization in the left apical lobe. Also, the response in lung-associated lymph nodes was actually higher following right apical immunization than after SRBC exposure of the left apical lobe. The observation that local immunity was not as high in the right apical lobe suggests that regional defense mechanisms in the lung may vary and might explain the regional distribution of certain lung diseases.

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REFERENCES


DISCUSSION

Dr. Dannenberg: Dr. McGregor and his group showed that when a lymphocyte divides in response to an antigen, ie., forms a lymphoblast, it will "home" nonspecifically to any site of inflammation. Could some of your results be explained by irritation of the lobe where you injected the antigen, resulting in a nonspecific inflammation into which blast cells produced elsewhere would home?

Dr. Bice: The only thing that leads me away from that conclusion is that a maximum inflammation probably occurred in the early hours after injection of the sheep red blood cells. The lavage fluid at 3 days contained dog red blood cells which indicated that there was an inflammatory response produced by injection of the antigen. However, after 3 days we never saw any dog red blood cells indicating that the inflammatory response appeared to resolve rather soon after immunization.

Dr. Reynolds: When you immunize one side, do you sham immunize the other side with the carrier solution?

Dr. Bice: Yes, the opposite lung was sham injected with 1 ml saline solution which was the carrier fluid.

Dr. Turner-Warwick: I may be quite wrong here, but when you suppose that your main response is local, are you implying that this is mainly an IgM response? If so, it is rather unusual to find a lot of IgM producing plasma cells in the peripheral parts of the lung.

Dr. Bice: Even though the dog on a microscopic basis has very limited lymphoid tissues in the airway, I think that they are present. When we do some histologic studies, i believe we will find that they're greatly increased only in the airway that we have immunized. And so there is the possibility that IgM antibody-forming cells are produced locally, dumped into the airways and that they could have a real function. However, the maximum number of cells present were producing IgG antibody.