Pulmonary Responses to Antigen*

David E. Bice, Ph.D.

A fter exposing the lung to an immunizing dose of antigen, a coordinated series of cellular and mediator responses result in the induction of pulmonary immunity that defends the lung. Although these cellular and molecular events are not fully understood, studies have helped clarify the mechanisms responsible for the induction of immunity to primary antigen exposures and the development of localized immune responses after antigen challenges. This presentation will discuss the cellular responses important in the induction of immunity after a primary exposure to antigen, the recruitment of lymphocytes into the lung, the response of pulmonary immune memory cells to antigen challenges, and the induction of long-term antibody production in the lung.

Inflammatory Responses to Antigens in the Lungs

Exposure of the lung to low doses of antigen does not usually induce immune responses. It is likely that phagocytic and mucociliary clearance mechanisms in the lung effectively remove low doses of antigens without transport of immunizing levels to the lung-associated lymph nodes (LALN). Even repeated exposures to low doses of antigen do not result in localized production of antibody in the lung. The induction of immunity to antigens deposited in the lung appears to require an antigen dose that overwhelms normal phagocytic and clearance mechanisms, resulting in the induction of inflammatory responses in the lung. Inflammation at a site of antigen deposition seems to be necessary in the translocation of antigen from the lung to the LALN. An elevated immune response is produced in the LALN if antigen is deposited in the lungs of animals that have inhaled inflammmagens, further supporting the importance of pulmonary inflammation in the translocation of antigen from the lung to the LALN. Pulmonary inflammation also increases the clearance of insoluble particles from the lung to the LALN. The mechanisms responsible for the clearance of antigen from the lung to the LALN are not completely understood. Because pulmonary inflammation appears to be important, phagocytic cells may phagocytize the antigen in the alveoli and transport it to the LALN. However, it is also possible that cell-free antigen passes via the lymphatics to these lymphoid tissues.

A large number of neutrophils enter the lung from the vasculature with a peak response about 1 day after instillation of antigen, and these cells may be important in the transport of antigen to the LALN (Fig 1). Although dogma suggests that macrophages are responsible for the transport of antigen to lymph nodes draining the site of antigen deposition, neutrophils can phagocytize particles in the alveoli and migrate to the LALN with the phagocytized particles, suggesting that neutrophils may play an important role in the transport of antigen to the LALN. Alveolar macrophages can also phagocytize particles in the lung and transport them to the LALN. However, neutrophils with phagocytized particles reach the LALN earlier than alveolar macrophages and therefore may be more important in the transport of antigen from the lung to these lymph nodes. The relative contribution of neutrophils and alveolar macrophages in the translocation of antigen from the lung to the LALN is not known.

Even though neutrophils or alveolar macrophages may be important in the transport of antigen from the lung to the LALN, these cells may have little or no function as antigen-presenting cells in these tissues. It is reasonable to assume that antigen transported to the LALN in phagocytic cells is released and that dendritic cells and/or lymph node macrophages are responsible for antigen presentation in these tissues.

If lung inflammation and the accumulation of neutrophils in the lung enhance the transport of antigens from the lung to the LALN, it seems possible that inhalation of materials that induce pulmonary inflammation might lead to increased recognition of airborne antigens. This possibility increases the concern that pulmonary inflammation caused by inhaled pollutants and passive cigarette smoke might increase the immune recognition of allergens and be responsible for increasing rates of asthma. In addition, inflammation induced by pulmonary viral infections may also be important in the induction of immunity to low levels of environmental antigens (eg, allergens responsible for asthma).

Recruitment of Lymphocytes into the Lung

Antigen-specific IgG, IgA, and IgM antibody-forming

FIGURE 1. Time course for total neutrophils in the saline solution control lung lobe (right intermediate) and the lung lobe instilled with SRBC (left cardiac). Data shown are from a single animal. Although differences are observed between individual animals, all animals show peak numbers of neutrophils at 1 to 3 days and 7 to 10 days after instillation of antigen.

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Reprint requests: David E. Bice, Lovelace Biomedical and Environmental Research Institute, Bldg. 9217, Area Y, Kirtland AFB East, Albuquerque 87115

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cells are found in the blood of dogs, cynomolgus monkeys, chimpanzees, and humans after localized pulmonary immunization. 19-21 These B lymphocytes are recruited into the lung lobes exposed to antigen, 19-22 and most antigen-specific antibody in the lung after a primary exposure to antigen is produced locally by these cells. 22 Few antibody-forming cells enter the control lung lobes exposed to saline solution. 19-22

Some correlations can be made between pulmonary inflammation and the recruitment of immune cells in the blood into lung lobes exposed to antigen. Two peaks of inflammation are observed after lung immunization (Fig 1). The first peak of neutrophils and total protein occurs on day 1 with a secondary inflammatory response between 7 and 10 days after immunization (Fig 1).

Few lymphocytes enter the lung at 24 h after exposure to antigen when inflammation is high as indicated by the large numbers of neutrophils present. However, lymphocytes do enter lung lobes exposed to antigen between 7 and 10 days, the time of the second influx of neutrophils (Fig 2). 23 These observations suggest that either these 2 inflammatory responses differ, or that lymphocytes capable of entering a site of inflammation are available only during the second inflammatory response.

The presence of antibody-forming cells in the blood makes it possible to determine if inflammation produced at 24 h after antigen instillation can recruit lymphocytes into the lung. To accomplish this, 1 lung lobe in each of 5 Beagle dogs was immunized with sheep red blood cells (SRBC) resulting in large numbers of anti-SRBC antibody-forming cells in the blood. 24 To determine if antibody-forming cells could be recruited by inflammation produced at day 1 after antigen instillation, a second antigen, rabbit red blood cells, (RRBC) was instilled into a different lung lobe at 6 days after immunization with SRBC. Lymphocytes producing antibody to SRBC were recruited into the lung lobe exposed to RRBC at a rate equal to the lung lobe immunized 6 days earlier with SRBC. 25 These data indicate that lymphocytes can be recruited into the lung during inflammatory responses on day 1 as well as at 7 to 10 days after immunization.

The most likely reason for a lack of lymphocyte recruitment during the initial inflammatory response on day 1 after antigen exposure is that an appropriate lymphocyte population that can enter a site of inflammation in the lung is not present in the vasculature at this time. 26 The release of lymphocytes from the LALN into blood probably provides cells capable of entering a site of inflammation. However, it is not known if the inflammatory response observed at 7 to 10 days after immunization is responsible for the recruitment of lymphocytes into the lung, or if lymphocytes that enter the lung and produce antibody cause this inflammatory response.

Information is limited on the adhesion molecules on the surface of B lymphocytes that would allow them to enter lung lobes recently exposed to antigen. However, recent data suggest that most T lymphocytes which enter the lung are recently activated immune memory cells (CD45RO). 27 In addition to the generation of lymphocytes with adhesion molecules, inflammation causes phenotypic changes of endothelial cells that are important in the recruitment of lymphocytes. The expression of endothelial ligands for lymphocyte binding is either increased (ICAM-1) or induced de novo (VCAM-1 and E-selectin) by inflammation. 28 The interaction between adhesion molecules on the surface of lymphocytes recently activated with endothelial cells at sites of inflammation in the lung would result in recruitment. Recruitment by lymphocyte adhesion molecules and endothelial ligands would occur regardless of antigen-specificity 29 or the lymph node responsible for lymphocyte production. 30

In addition to lymphocyte recruitment, the comparison of immunity produced after immunization with two antigens helps identify the mechanisms responsible for primary immune responses in the lung. Results show that blood is the source of the antibody-forming cells that enter the lung after a primary lung immunization rather than from an antigen stimulation of lymphoid tissues in the lung, and
also, that lymphocytes entering the lung after a primary immunization are not amplified locally by interaction with antigen retained in the lung.19 If antigen stimulated a local production of antibody-forming cells in the lung, or if antigen retained in the lung stimulated lymphocytes entering the lung to proliferate, a higher level of anti-SRBC antibody would be found in the lung lobe exposed to SRBC, and a higher level of antibody to RRBC would be found in the lung lobe exposed to RRBC.

RESPONSE OF PULMONARY IMMUNE MEMORY CELLS TO ANTIGEN CHALLENGE

In addition to antigen-specific, antibody-forming cells, immune memory cells are also recruited to and/or produced in lung lobes after a primary exposure to antigen.2,3,30-31 The response of these memory cells to an antigen challenge is clearly demonstrated by comparing the responses in the 2 lung lobes exposed to 2 distinct antigens. For this comparison, 5 Beagles were anesthetized, 109 SRBC was instilled into the left cardiac lung lobe, and 109 RRBC was instilled into the right cardiac lung lobe. As discussed above, lymphocytes producing antibody to SRBC or to RRBC are recruited equally into lung lobes exposed to either SRBC or RRBC20 (Fig 3).

In contrast to the primary immune response, the challenge of these 2 lung lobes with the same antigens (SRBC—left cardiac; RRBC—right cardiac) results in an intense localized response that is antigen-specific (Fig 4). The most logical explanation for this antigen-specific response after an antigen challenge is antigen recognition by immune memory cells recruited into the lung after a primary immunization.3,5

An important question remaining to be answered is whether retained antigen plays any role in the development of immune memory cells that enter the lung. Immune memory cells may enter lung lobes exposed to antigen randomly as do antibody-forming cells, with no need for further antigen contact for their development. If this is true, the challenge of a lung lobe exposed to RRBC with SRBC antigen would result in a secondary response to SRBC.

Many other questions also remain in order to understand the mechanisms of pulmonary immune memory. These include the identification of the cells responsible for antigen presentation and the possible roles of T lymphocytes in the stimulation of localized antibody production. Although antigen in the alveoli could be presented by alveolar macrophages, the results of recent studies suggest that dendritic cells in the interstitial tissues of the alveoli could present antigen in the lung.4 In addition, cells responding to antigen challenge are located in the interstitial tissues, rather than the alveoli, supporting the possibility that dendritic cells and/or tissue macrophages are the antigen-presenting cells.8

LONG-TERM PRODUCTION OF ANTIBODY IN THE LUNG

The long-term maintenance of immune memory cells in the lung is important in pulmonary defense. Experiments to evaluate the maintenance of immune memory cells in the lung provided results not only about the function of memory lymphocytes, but also showed that long-term antibody production is induced after immunization and challenge of the lung. Local production of antigen-specific antibody continues in exposed lung lobes for several years after immunization and challenge.4 More anti-SRBC IgG, IgA, and IgM are found in lavage fluid from immunized and challenged lung lobes than in lavage fluid from control lung lobes at 3 to 5 years after the last antigen challenge.4 An evaluation of the cells responsible for the production of antibody in immunized and control lung lobes showed that only cells lavaged from lung lobes exposed to antigen were producing antibody to SRBC. Therefore, in addition to pulmonary immune memory, the continued production of antibody in the lung for extended times after antigen challenge could also be important in the prevention of recurrent pulmonary infections.

The mechanisms responsible for long-term antibody production in the lung are not understood. For example, it is not known if small amounts of antigen maintained in tissues of a lung lobe exposed to antigen are responsible for stimulating antibody production by antigen-specific B lymphocytes, or if long-lived lymphocytes residing in the lung continue to produce antibody.

Although cells producing antibody can be lavaged from exposed lung lobes several years after antigen challenge, it is also possible that cells in lung tissues, in draining LALN, and in distant lymphoid tissues (eg, from other mucosal sites) may also play important roles in long-term antibody production. To compare the level of long-term antibody production in each of these tissues, dogs immunized and challenged 2 years earlier were killed and cell suspensions prepared. A preliminary evaluation of antigen-specific antibody produced in vitro suggests that most long-term antibody production occurs in the interstitial tissues of the lung. Significant antibody production was not observed in the LALN or other lymphoid tissues evaluated. These data suggest that lung tissues exposed to antigens are important sources of antibody production to protect the specific lung tissues exposed. However, antibody produced in lung tissue exposed to antigen enters blood, also protecting distant lung and other body tissues.
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