Immunology 1991 **74** 215–222

Long-term maintenance of localized antibody responses in the lung

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Accepted for publication 25 June 1991

SUMMARY

Immune memory cells for antibody production in the lung respond to antigen challenges, providing an important pulmonary defence. However, studies reported here suggested that long-term antibody production in the lung might also be important in pulmonary immune memory. After localized lung immunization and challenges, antibody production of specific IgG and IgA continued in the immunized lung lobes of dogs for months after the last antigen exposure. In addition, the evaluation of lung immunity in dogs immunized and challenged 3 years or 5 years previously (without additional antigen challenge) showed significantly higher levels of specific antibody in lavage fluid from the lung lobe exposed to antigen than in lavage fluids from control lung lobes. Cells from blood or from control lung lobes did not produce significant levels of specific antibody *in vitro*, whereas cells lavaged from the immunized lung lobes were producing specific antibody. Therefore, long-term antibody production by cells in lung lobes exposed to antigen probably contributed to antibody levels in serum and unexposed lung lobes. Traditionally, lymphoid tissues are believed to be responsible for long-term antibody production. However, antibody production in the lung for years without repeated antigen exposure suggested that other tissues might also be important in long-term antibody production. Maintenance of localized antibody production in the lung would be an important pulmonary defence against infectious agents, but might also play a key role in hypersensitivity lung diseases.

INTRODUCTION

Pulmonary defences are efficient, and except in the aged or in individuals with immunodeficiencies, severe pulmonary infections in humans are not frequent. Although pulmonary defences that prevent infections from inhaled or aspirated pathogens are complex, the development of immunity is essential in pulmonary defence. Dogs and non-human primates have provided excellent animal models to describe pulmonary immunity.[1] In contrast to some other species, intense localized immune responses are produced in lung lobes of dogs and non-human primates after instillation of particulate antigen.[1-4] Immune cells that enter the lung after primary immunization are produced in the lung-associated lymph nodes.[3,5,6] Antigen-specific immune cells are released into the blood, and large numbers are recruited into the lung lobes exposed to antigen.[7,8] Immune cells recruited into the lung actively produce antigen-specific antibody, and mature plasma cells are found in the interstitial tissues and alveoli.[9] This local production of antibody provides high levels of antibody in lung lining fluid, which plays an important role in phagocytic pulmonary defences.[10]

In addition to the recruitment of large numbers of antibody-forming cells into the lung after a primary exposure to antigen, immune memory cells are recruited and/or produced only in the immunized lung lobe.[4,11,12] These memory cells respond to challenges with low doses of antigen,[11] and cells dividing in response to antigen challenge are present in interstitial lung tissues.[4] Because immune memory for antibody production is probably essential to prevent recurrent pulmonary infections, this study was initiated to determine how long memory cells remain in the lung after antigen exposure. Cells in lymphoid tissues are thought to be responsible for long-term immune memory,[13] but there are no data concerning the retention of immune memory cells in the lung. Therefore, dogs in this study were immunized, challenged twice with a high dose of antigen, and then challenged with low doses of antigen at increasing intervals to determine how long cells would be available in the lung to respond to an antigen challenge. Although the results of this study support the concept that antibody immune memory cells are present in the lung, our results also show that antibody production continues in the lung for several months after the last antigen challenge. Therefore, we evaluated the level of immunity in dogs exposed to antigen 3 or 5 years earlier. Without any additional exposure to antigen, we observed that localized antibody production in the lung continued for several years after instillation of particulate antigen into the dog lung.

Local production of antibody in the lung for years after exposure to antigens would provide a high level of antibody in lung lining fluid that could play a central role in protecting the

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lung from recurrent infections. However, localized immune responses in the lung may also be involved in the induction of some immunologically mediated lung diseases. For example, local production of IgE would provide an important mechanism for the maintenance of allergic respiratory disease.

MATERIALS AND METHODS

Experimental animals

Two groups of Beagle dogs (five dogs per group), born and raised in the Institute's colony, were used in this study. Physical examinations, thoracic radiographs, venous blood cell counts, and serum chemistry determinations for blood urea nitrogen, alkaline phosphatase, and alanine aminotransferase were used to determine the health of each dog before immunization. All dogs were healthy, by these criteria, at the beginning of the study and were observed daily. The dogs lived in kennel buildings with indoor and outdoor runs. Each dog was fed 350 gm of dry dog food once a day (Allied Mills, Chicago, Illinois), and water was available at all times. All instillations and lavages were performed under halothane anaesthesia. Food was withheld for 18 hr before giving anaesthesia. No visible signs of emotional effects or discomfort from the procedures used in our experiments were observed. At the end of this study, the dogs were returned to our dog colony.

Longevity of immune memory cells in the lung

The first group of five dogs was immunized by instillation of 10^{10} sheep red blood cells (SRBC) in 1 ml saline into the left cardiac lung lobe using a fiberoptic bronchoscope, as described previously[14] (Table 1). As controls, the right cardiac lung lobe was instilled with 1 ml saline, and the right intermediate lung lobe was instilled with 10^6 SRBC, a non-immunogenic dose of antigen.[11] These same doses of antigen and saline were instilled into these lung lobes at 21 and 42 days (challenges 1 and 2) after the primary exposure. Serum, lavage fluid and lavage cells obtained at 3, 5, 7, 10, 12 and 14 days after primary immunization, at Day 7 after the first challenge, and at 3, 5, 7, 10, 12 and 14 days after challenge at 42 days; were evaluated to determine the level of immune response produced after primary immunization and challenge. Total cells, and the percentage lymphocytes, alveolar macrophages and neutrophils (PMN) in lavage fluids, were quantified by microscopic evaluation of cytocentrifuge slides. A microdetermination assay (Sigma Diagnostics, St Louis, MO) was used to determine total protein in lavage fluids. The levels of anti-SRBC IgM, IgG, and IgA antibody in lavage fluid and in serum samples taken at the time of lavage were determined with an enzyme-linked immunosorbent assay (ELISA).[11] A control well containing known anti-SRBC antibody was used in each evaluation. To reduce assay variability, all plates were counted when this well reached an optical density (OD) of 1·0.

Table 1.

Antigen exposure	SRBC antigen LC	RI	RC	Days after immunization	Interval between antigen exposures (weeks)
Primary	10^{10}	10^6	saline	0	
					3
Challenge 1	10^{10}	10^6	saline	21	
					3
Challenge 2	10^{10}	10^6	saline	42	
					3
Challenge 3	10^6	10^6	saline	63	
					5
Challenge 4	10^6	10^6	saline	98	
					7
Challenge 5	10^6	10^6	saline	147	
					9
Challenge 6	10^6	10^6	saline	210	
					26
Challenge 7	10^6	10^6	saline	398	

LC, left cardiac lung lobe; RI, right intermediate lung lobe; RC, right cardiac lung lobe.

To determine how long immune memory cells remained in the lung, left cardiac and right intermediate lung lobes were challenged with 10^6 SRBC at increasing intervals. The right cardiac was instilled at the time of each challenge with 1 ml saline as a control. Serum, lavage fluid and lavage cells obtained at 3, 5, 7, 10, 12 and 14 days after each challenge were evaluated to determine the level of immune response produced. After reviewing data from challenges at increasing intervals of 3, 5, 7 and 9 weeks (challenges 3, 4, 5 and 6) (Table 1), we observed that high levels of anti-SRBC antibody were present at the time of each challenge, and the level of anti-SRBC antibody in lavage fluid from the immunized left cardiac lung lobe never fell to the level of antibody in lavage fluid from the control lung lobes. Therefore, challenges were stopped; immunized and control lung lobes were lavaged and serum was collected approximately monthly to determine when the local production of antibody in the immunized/challenged left cardiac lung lobe would fall to levels observed in control lung lobes. After 6 months, the local production of antibody had still not returned to background levels. Therefore, the dogs were challenged a final time (challenge 7) (Table 1) by instilling 10^6 SRBC into the previously immunized and challenged lung lobes. The control lung lobes were instilled with saline or 10^6 SRBC as described above.

Evaluation of long-term antibody production in the lung

Because localized antibody production continued in the immunized and challenged lung lobe for at least 6 months after the last challenge with a low dose of antigen, it was necessary to determine if antibody production could continue for even longer times after localized immunization and challenge. To do this, we evaluated the immune responses in the lungs of three dogs that had been immunized 3 years prior to the present study and two that had been immunized 5 years earlier. These dogs were originally immunized as controls in studies to evaluate the effects of age on pulmonary immune responses[15] or to determine the effects of localized lung immunity on phagocytosis.[10] In these studies, dogs were immunized and challenged by instillation of 10^{10} SRBC in 1 ml saline into specific airways in individual lung lobes by using a fiberoptic bronchoscope.[2,14] At the end of these studies, the dogs were returned to our dog colony and were not used in other studies that would interfere with their use in the evaluations presented here.

To determine if antibody was still being produced at 3 or 5 years after the last antigen exposure, the exposed (left cardiac lung lobe) and a control lung lobe were lavaged (5 × 10 ml saline

washes). The left apical had not been exposed previously and was selected as the control lung lobe. Total cells, and the percentage lymphocytes, alveolar macrophages, PMN, and total protein in lavage fluids were quantified as described above. The levels of anti-SRBC IgM, IgG, and IgA antibody in lavage fluid (no dilution) and in serum samples (diluted 1:50) taken at the time of lavage were determined as described above.

To determine whether immune cells producing anti-SRBC antibody were present in the blood or in lavage samples, blood or lavage cells were cultured for 7 days in RPMI-1640 (Grand Island Biologicals, Grand Island, NY) with 10% foetal calf serum (Hyclone, Logan, UT). The cells were cultured at 10^5 cells/well in round-bottomed, 96-well plates (Corning, Corning, NY), with or without the addition of pokeweed mitogen (PWM; Grand Island Biologicals) (10 μl/ml) to stimulate antibody production. Because of the possibility that antibody measured in the culture medium was released from the surfaces of cells, rather than being produced *de novo*, cycloheximide (Calbiochem, San Diego, CA) (100 μg/ml) was added to some cultures to inhibit protein synthesis. Culture supernatants were collected at 7 days. Supernatants from five culture wells, with or without cycloheximide or PWM, were pooled and frozen at -90°. The levels of anti-SRBC IgM, IgG and IgA antibody in undiluted culture supernatants were evaluated by ELISA,[11] and data are presented as the ELISA OD minus the OD observed in cultures with cycloheximide. These data, therefore, represent only the levels of antibody being produced by cells in culture and not antibody passively released from the cells.

The data obtained from the evaluation of specific antibody, total protein, and cell cytologies in lavage fluid from lung lobes exposed to antigen or saline were compared with ANOVA and multiple group comparisons (BMDP Statistical Software, Inc., Los Angeles, CA). Only differences with $P<0.05$ were considered significant.

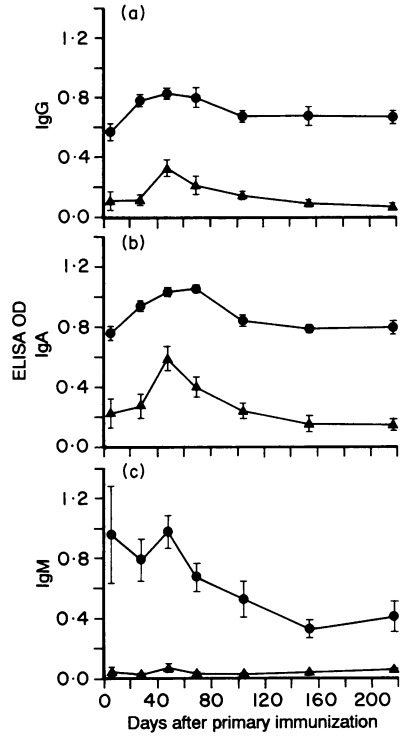

Figure 1. Levels of anti-SRBC IgG (a), IgA (b) and IgM (c) in lavage fluid from the immunized and challenged lung lobe (left cardiac; ●) and the control lung lobe instilled with saline (right cardiac; ▲). Except for challenge 1 (Day 21), the values presented are the means ± SE of samples from five dogs; each point was calculated by using the peak response in each dog measured during time-course evaluations (Days 3, 5, 7, 10, 12 and 14) after primary immunization and challenges 2–6 (Days 42, 63, 98, 147 and 210, respectively).

RESULTS

Primary immunization

Increasing levels of anti-SRBC IgM, IgG and IgA antibody were observed in lavage fluid from the immunized lung lobe after primary immunization. Peak responses in individual dogs were observed 10 or 12 days after instillation of antigen, as reported previously[2,11,15] and, for simplicity, only the mean peak response is presented (Fig. 1). Significantly lower responses were observed in the control lung lobes exposed to saline or 10^6 SRBC (non-immunogenic dose). There were no differences in the levels of antibody in these control lobes (saline and 10^6 SRBC).

Challenges 1–6

In comparison to the peak primary immune response, the level of anti-SRBC IgG, IgA and IgM antibody was higher in lavage fluid from the challenged lung lobe at 7 days after the first challenge (10^{10} SRBC), the only day evaluated after this challenge (Fig. 1). After the second challenge with 10^{10} SRBC, and after the third to sixth challenges with 10^6 SRBC, anti-SRBC IgM, IgG or IgA in lung lavage fluid from the challenged lung lobe did not show a time-course increase in antibody levels. Unlike the primary response, the level of anti-SRBC antibody was as high on Day 3 as in lavage fluid at 5, 7, 10, 12 or 14 days after challenge. Therefore, to simplify data presentation, only the mean peak IgG, IgA, and IgM response observed after immunization and each challenge is presented (Fig. 1). There was never a difference in the level of anti-SRBC antibody in lavage fluid from the control lung lobes exposed to saline or 10^6 SRBC. Therefore, data from the lung lobe exposed to 10^6 SRBC are not included for clarity.

The levels of anti-SRBC IgG and IgA remained relatively high in lavage fluid from the immunized and challenged left cardiac lung lobe through to the sixth challenge (Fig. 1a, b). In comparison, the level of anti-SRBC IgM tended to fall in lavage fluid from the immunized lung lobe (Fig. 1c). Relatively low levels of anti-SRBC IgG and IgA antibody were observed in lavage fluid from the control lung lobes (exposed to saline or 10^6 SRBC), with the peak response after the second antigen challenge (Fig. 1a, b). No significant increase of anti-SRBC IgM was observed in the control lung lobes (Fig. 1c).

Unlike lavage fluid, where anti-SRBC IgG and IgA remained high, the levels of these antibodies in serum were highest after the second challenge and then continually fell, even though lungs were repeatedly challenged with 10^6 SRBC (Fig. 2a, b). The level of anti-SRBC IgM was highest after primary immunization, tended to fall with repeated challenges, but remained relatively high in comparison to anti-SRBC IgG and IgA (Fig. 2c).

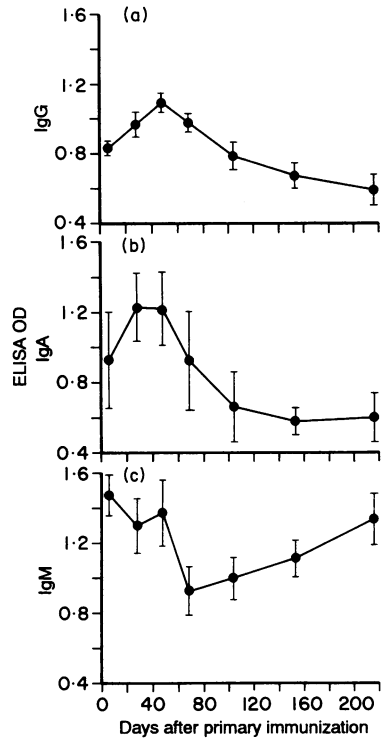

Figure 2. Levels of anti-SRBC IgG (a), IgA (b) and IgM (c) in serum. Except for challenge 1 (Day 21), the values presented are the means ± SE of samples from five dogs; each point was calculated by using the peak response in each dog measured during time-course evaluations (Days 3, 5, 7, 10, 12 and 14) after primary immunization and challenges 2–6 (Days 42, 63, 98, 147 and 210, respectively).

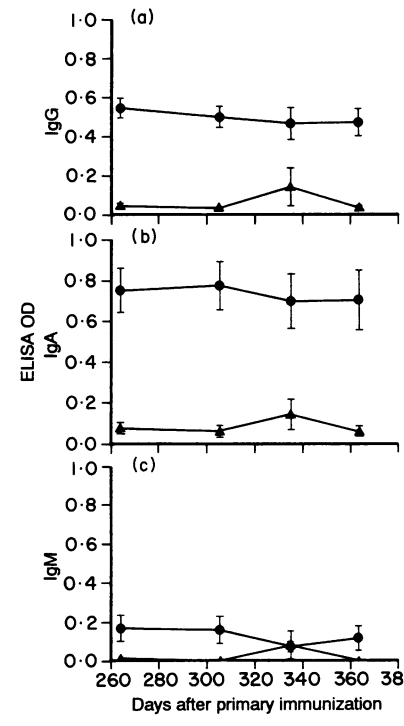

Figure 3. Levels of anti-SRBC IgG (a), IgA (b) and IgM (c) in lavage fluid from the immunized and challenged lung lobe (left cardiac ●) and the control lung lobe instilled with saline (right cardiac; ▲). The four lavages were taken over 6 months with no additional challenges. The values presented are the means ± SE of samples from five dogs.

Maintenance of pulmonary antibody without challenge

The maintenance of anti-SRBC antibody in lavage fluid from challenged lung lobes, with no increase in antibody response after each challenge, weakened our ability to determine how long memory cells remain in the lung. Therefore, after the sixth challenge, all challenges were stopped, and serum and lavage samples were collected four times over the next six months to attempt to determine when antibody levels in lavage fluid from the immunized-challenged lung lobe would return to the background level observed in the control lung lobes. Data showed that both anti-SRBC IgG and IgA remained high in lavage fluid from previously challenged lung lobes for 6 months, while anti-SRBC IgM fell to background levels (Fig. 3). Anti-SRBC IgG, IgA, and IgM were present in serum for 6 months, with IgM being somewhat higher (data not shown).

Seventh challenge

Because it seemed that high levels of anti-SRBC IgG and IgA would be maintained indefinitely, the seventh challenge with 10^6 SRBC was given at approximately 6 months (188 days) after the sixth challenge. Anti-SRBC IgG, IgA and IgM all tended to increase after the seventh challenge, but only the increase of IgM in lavage fluid was significant (Fig. 4). Although significant, the IgM response was not high and was significantly elevated only because the background level was low at the time of the seventh challenge. The increased levels of IgG and IgA were not significantly higher because of relatively high background levels

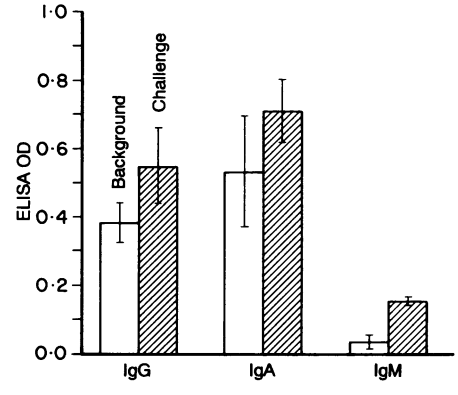

Figure 4. Peak levels of anti-SRBC IgG, IgA and IgM in lung lavage fluid before challenge (background) and after challenge 7 (Day 398 after primary immunization). The background values represent the mean values of samples from five dogs, while the challenge data are the peak mean response ± SE of samples from five dogs measured during a time-course evaluation (Days 3, 5, 7, 10, 12 and 14) after challenge. Only the IgM response was significantly elevated over the background level of anti-SRBC antibody.

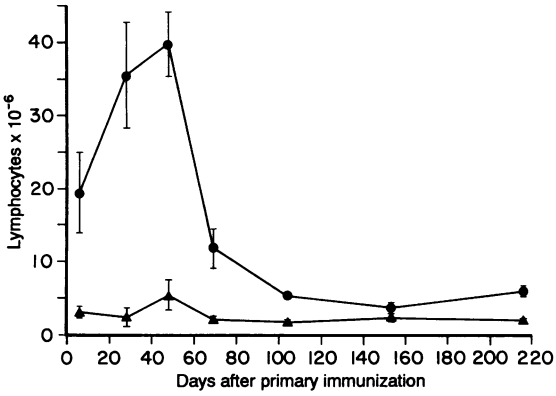

Figure 5. Total lymphocytes in lavage fluid from the immunized and challenged lung lobe (left cardiac; ●) and the control lung lobe (right cardiac; ▲) instilled with saline. Except for challenge 1 (Day 21), the values presented are the peak mean response ± SE of samples from five dogs measured during time-course evaluations (Days 3, 5, 7, 10, 12 and 14) after primary immunization and challenges 2–6 (Days 42, 63, 98, 147 and 210, respectively).

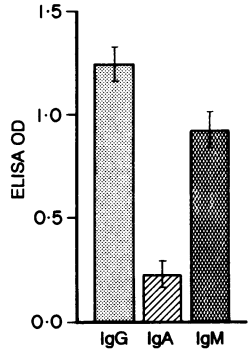

Figure 6. Levels of anti-SRBC IgG, IgA and IgM in serum. The values presented are the means ± SE of samples from five dogs at 3 years (three dogs) or 5 years (two dogs) after the last exposure to antigen.

of anti-SRBC antibody at the time of the seventh challenge. There were no changes in the control lung lobe instilled with saline at the seventh challenge (data not shown). It is possible that challenge with a higher dose of antigen would have resulted in increased levels of antibody in the exposed lung lobe. However, increased responses with higher antigen doses would probably have been by recruitment of antibody from the blood, rather than stimulation of local memory cells.[11] It is also possible that, by waiting longer than 6 months, the levels of IgG and IgA anti-SRBC antibody would have dropped, and significant increases would have been observed after challenge.

Lavage cytology after primary immunization and challenges

Large numbers of PMN and high levels of total protein were observed in lavage fluids after primary immunization and the first and second challenges with 10^{10} SRBC. However, no increase in total protein or number of PMN was observed after third to seventh challenges with 10^6 SRBC. The instillation of 10^6 SRBC into a control lung lobe never stimulated an increase in total protein or PMN.

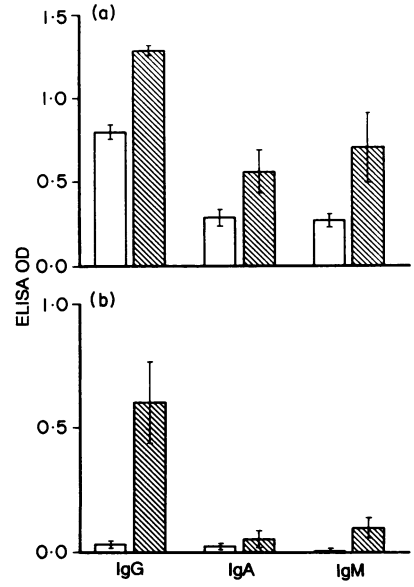

Figure 7. (a) Levels of anti-SRBC IgG, IgA and IgM in lung lavage fluid in previously immunized (left cardiac; ▨) and a control lung lobe (left apical; □) and (b) the production of anti-SRBC IgG, IgM and IgA by lavage cells from the left cardiac (▨) and left apical (□) lung lobes. The values presented are the means ± SE of samples from five dogs at 3 years (three dogs) or 5 years (two dogs) after the last exposure to antigen. Data for antibody production by lavage cells are presented as ELISA OD minus OD values obtained by evaluation of cultures treated with cycloheximide.

The maximum numbers of lymphocytes in lavage fluid from the immunized and challenged lung lobes were observed after primary immunization and the first two challenges with 10^{10} SRBC (Fig. 5). Although the number of lymphocytes fell after challenges with 10^6 SRBC, the numbers were higher in the challenged lung lobes compared to control lung lobes. Even though the number of lymphocytes was higher in the challenged lung lobe, the seventh challenge did not significantly increase the number of lymphocytes in lavage fluid. Immunization and challenge also significantly increased the number of alveolar macrophages in the lavage fluid, with a time-course similar to that observed for lymphocytes (data not shown).

Localized antibody production 3–5 years after antigen exposure

Because the dogs in our study continued to produce antibody in the lung 6 months after the last antigen exposure, we next evaluated the levels of anti-SRBC antibody in serum and in lavage fluids from control lung lobes and lung lobes that were last exposed to antigen 3 or 5 years earlier. Anti-SRBC IgG, IgM and IgA were present in serum from these dogs (Fig. 6). Significantly higher levels of anti-SRBC IgG, IgM and IgA were present in lavage fluid from lung lobes exposed to antigen 3 or 5 years earlier than in lavage fluid from the control lung lobes (Fig. 7). Nevertheless, lavage fluid from the control lung lobes did contain anti-SRBC antibody.

In vitro production of anti-SRBC antibody

Lavage cells from the immunized lung lobes produced significantly more anti-SRBC IgG antibody after being cultured for 7

days than did lavage cells from the control lung lobes (Fig. 7b). Although more IgM and IgA anti-SRBC antibody was produced by cells in lavage fluids from immunized lung lobes than from control lung lobes, these differences were not significant (Fig. 7b). The levels of anti-SRBC IgM and IgA antibody produced in culture were small in comparison to the production of anti-SRBC IgG antibody. An insignificant level of specific antibody was produced by cells lavaged from the control lung lobes. Likewise, cultured blood lymphocytes did not produce measurable anti-SRBC antibody (data not shown).

The addition of PWM to cultures of cells lavaged from immunized or control lung lobes did not stimulate production of anti-SRBC IgG antibody by resting B lymphocytes (data not shown). Furthermore, addition of PWM to blood lymphocyte cultures did not stimulate production of measurable levels of anti-SRBC antibody (data not shown). The addition of cycloheximide to cultures of cells obtained by lavage of immunized lung lobes eliminated the production of IgG antibody (data not shown).

Lavage cytology 2 or 5 years after antigen exposure

Altough there were more alveolar macrophages in lavage fluid from the immunized lung lobes, the difference was not significant. There were no differences between immunized and control lung lobes in total protein or in total numbers of lymphocytes or PMN in lavage fluids. The percentages of lymphocytes present in lavage fluid from the immunized and control lung lobes were $5{\cdot}0 \pm 1{\cdot}4$ SE and $6{\cdot}2 \pm 1{\cdot}5$, respectively. There were no differences in the levels of total protein in lavage fluids from the immunized and control lung lobes (data not shown).

DISCUSSION

Relatively long-term antibody production continues after systemic immunization with a variety of non-replicating antigens (e.g. tetanus). However, long-term, localized antibody production has not been described in the lung, and the initial observation that antibody production continued only in immunized and challenged lung lobes for 6 months after the last antigen exposure was unexpected. The significance of long-term antibody production in the lung was further strengthened by identification of antibody in lavage fluid from lung lobes last exposed to antigen 3–5 years earlier. Our observations that the levels of specific IgG antibody are higher in lavage fluids from immunized lung lobes than from control lung lobes, and that cells producing anti-SRBC antibody are present in lung lavage fluids from these same lobes, support the conclusion that long-term antibody production continued only in lung lobes exposed to antigen.

Although cells obtained from lung lavage fluids produced antibody *in vitro*, it is possible that antibody production by these cells represents only a fraction of the total antibody produced in the lung. Data from other studies suggest that cells in the interstitium are also involved in immune memory and antibody production.[4,9,16,17] Therefore, antibody produced by interstitial cells could contribute significantly to antibody levels in lavage fluid. Additional data are needed to determine the relative contributions of alveolar and interstitial cells in providing long-term antibody production in the lung.

The retention of antigen–antibody complexes on follicular dendritic cells in lymph nodes that drain the site of antigen injection has been proposed as the mechanism responsible for long-term antibody production after systemic immunization.[13] Theoretically, as the level of circulating antibody falls, antigen is revealed on dendritic cells, stimulating cell division and antibody production by antigen-specific B lymphocytes.

Because antigen retention in peripheral lymph nodes appears to be important in long-term antibody production, it is possible that antigen is also retained in the lung-associated lymph nodes after lung immunization, and that this antigen continues to stimulate antibody production. However, the presence of cells producing anti-SRBC antibody in lavage fluid from the immunized lung lobes for months to years after the last antigen exposure indicates that long-term antibody production occurs in lung tissues exposed to antigen.

If antigen retained in lung tissues is responsible for long-term antibody production in the lung, only minimal data are available concerning which lung cells might be responsible for the retention and presentation of antigen to B lymphocytes. Some data suggest that alveolar macrophages could be responsible for antigen and presentation in the lung.[18,19] However, other studies show that these cells are poor antigen presenters,[20,21] and macrophages apparently maintain antigen on their surfaces only for short times.[13,22] In addition, antigen challenge of lung lobes showed that the cells responding by division were present in the interstitial tissues rather than in the alveoli,[4] further suggesting that antigen presentation in the lung is not by cells in the alveoli. These observations make it seem unlikely that alveolar macrophages are responsible for long-term maintenance and presentation of antigen in the lung.

It is possible that dendritic cells could be responsible for maintenance and presentation of antigen in the lung. Dendritic cells are present in the interstitial tissues of the lung, and some data indicate that these cells can present antigen to T lymphocytes.[23,27] However, only follicular dendritic cells, found in lymph nodes and spleen, retain antigen for long times.[13,28] Lymphoid dendritic cells, found in tissues other than lymph nodes, have not been shown to have this capability. Additional data are required to determine whether antigen retained in the lung is responsible for long-term antibody production, and if so, what cell types are responsible for maintaining the antigen.

In addition to antigen retention, it is also possible that other mechanisms may be responsible for long-term antibody production in the lung. Mice immunized intraperitoneally continued to produce antibody for 8 months[29] or 1 year after immunization.[30] The exposure of immunized mice and rats to high radiation doses did not eliminate antibody production in these animals, suggesting that cell division is not necessary for antibody production, which continued for more than 30 days after radiation exposure.[30,31] The results of other studies suggested that the life span of rat plasma cells could be considerably longer than 6 months and that the bulk of antibody produced at extended times after immunization is being made in cells developed shortly after the original antigenic stimulus.[32] Plasma cells have been observed in the alveoli and interstitial lung tissues in lung lobes exposed to antigen,[9] and it is possible that these cells continue to produce antibody for long times after localized immunization. Although T-helper cells may be important in the localization and regulation of plasma cells in mucosal tissues,[33] the exact roles played by these cells in long-term antibody production in the lung are not known.

The dose of antigen instilled into the lung appeared to be

important in the establishment of long-term antibody production. Repeated instillations of a low dose of antigen, 10^6 SRBC in the right intermediate lung lobe, did not produce a measurable immune response even after the induction of immunity by instillation of a high dose of antigen into the left cardiac lung lobe. The low dose of antigen instilled into the right intermediate lung lobe did not induce measurable inflammation, and lymphocytes were not recruited from the blood into this lung lobe. Therefore, it is possible that a dose of antigen that results in the recruitment of lymphocytes into the lung might be essential in the establishment of long-term antibody production. Once long-term antibody production has been established, our data, and the results of other studies, suggest that additional antigen exposures are not necessary to maintain antibody production.[34]

Long-term antibody production at localized sites in the lung has important implications for both pulmonary defence and pulmonary disease induced by immune responses. The production of antibody for years after a pulmonary infection could provide high titres of specific antibody in fluids lining the lung and could also contribute to the pool of systemic antibody. Localized high titres of antibody would effectively remove pathogens in future exposures. Although minimal data are available, long-term antibody production may be important in protecting humans after influenza virus infection.[35,36] Mice also produce antiviral antibody for at least 6 months in the lung and 18 months in the spleen after viral infection.[16,17]

Although long-term antibody production is likely essential in pulmonary defence, long-term local production of antibody may also play a key role in hypersensitivity lung diseases. Not only could locally produced antibody be more important than systemic antibody in lung hypersensitivity, but long-term antibody production could maintain an individual's pulmonary sensitivity for years, even if not re-exposed.

ACKNOWLEDGMENTS

The authors thank K. J. Everhart, C. I. Kenyon and A. J. Williams for their technical assistance and the personnel at the Inhalation Toxicology Research Institute for the care of the animals used in this study. We also thank our colleagues for review of this manuscript and their helpful suggestions concerning these studies.

This research was performed with support from the U.S. Department of Energy, Office of Health and Environmental Research, Contract Number DE-AC04-76EV01013 and in facilities fully accredited by the American Association for the Accreditation of Laboratory Animal Care.

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