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Lung localization of antibody-forming cells stimulated in distant peripheral lymph nodes*

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Summary. Previous studies have shown that antibody-forming cells (AFC) produced in the lung-associated lymph nodes after lung immunization enter the blood and are subsequently extravasated into immunized lung lobes. This study evaluated AFC in blood and lung lavage fluids following simultaneous stimulation of the thoracic and popliteal lymph nodes with two antigenically distinct immunogens. Five dogs were immunized in the hind feet with rabbit red blood cells (RRBC) and in the left cardiac lung lobe with sheep red blood cells (SRBC). The number of anti-SRBC and anti-RRBC AFC in the blood and lavage fluids was periodically evaluated. The results indicated that both immunizations significantly increased the number of AFC in the blood. The number of AFC to RRBC and SRBC antigens was significantly higher in the immunized lung lobes than in the control lung lobes. A comparison of the number of RRBC and SRBC AFC in the immunized or control lung lobes, relative to the number of RRBC and SRBC AFC in the blood, indicated that AFC to both antigens entered the lung at the same rate. We conclude that AFC produced in distant lymphoid tissues enter the lung from the blood as readily as AFC produced after lung immunization.

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INTRODUCTION

Lymphocytes are mobile cells capable of migrating through lymphoid and non-lymphoid tissue via circulating blood and lymph. Interest in their recirculation began when Griscelli, Vasalli & McCluskey (1969) reported that lymphocyte migration and subsequent localization was not a random process, but that a true dichotomy existed in the *in vivo* behaviour of peripheral and mesenteric lymphoblasts. Other investigations confirmed that, whereas blast cells from mesenteric lymph nodes preferentially home to the gut wall, blast cells from peripheral lymph nodes return to these nodes (Hall, Hopkins & Orlans, 1977; McDermott & Bienenstock, 1979).

Many factors appear to influence specific lymphocyte recruitment but none has been shown to be the sole mechanism governing the localization and retention of mucosal lymphoblasts. It has been shown that localized antigen will promote the appearance of specific antibody-forming cells (AFC) in mucosal organs (Husband & Gowans, 1978), but it is not the primary stimulus of recruitment. This was determined following observations that the selective migration of

thoracic duct and mesenteric lymph node lymphocytes occurred both in the implanted antigen-free grafts of fetal gut (Parrott & Ferguson, 1974) and in the sterile environment of fetuses *in utero* (Hall *et al.*, 1977).

The presence of organ-specific determinants on the cells of post-capillary, high endothelial cell venules (HEV) may also influence lymphocyte localization. Stevens, Weissman & Butcher (1982) postulated that the HEV determinants are recognized by lymphocyte receptors at the site of diapedesis. Similarly, the secretory component of IgA, regional blood flow, hormones, regulatory T cells and nutrition have all been implicated in the localization of lymphoblasts in mucosal tissue (McDermott, Befus & Bienenstock, 1982). The exact mechanism of lymphocyte recruitment most likely involves a number of these factors acting in concert.

The theory that lung and gut subepithelial lymphoid aggregates are part of a common mucosal immunological system has recently become popular, and is supported by work by Rudzik *et al.* (1975). They observed that, in contrast to cells from the bronchus-associated lymphoid tissue (BALT) and Peyer's patches, cells from popliteal lymph nodes would not repopulate BALT. Later repopulation studies revealed that cells from bronchial lymph nodes demonstrated a marked propensity for lung localization. McDermott & Bienenstock (1979) concluded from these studies that lymphoblasts from bronchial lymph nodes were strikingly different from those prepared either from mesenteric or peripheral lymph nodes. This contradicts results of Butler *et al.* (1982), which indicate that a common mucosal immune system for the lung and the intestine does not exist.

The study presented here was designed to evaluate the effects of the tissue of origin on the localization of immune cells in immunized and control lung lobes. Previous histological evaluation of immunized lung tissue determined that the antigen-specific AFC found in lung lavage fluid (Brownstein *et al.*, 1980; Bice *et al.*, 1980) were produced in the lung-associated lymph nodes and subsequently recruited from the blood to the immunized lung (Brownstein *et al.*, 1980). Additional data indicate that lung immunization results in alterations which induce a non-specific recruitment of immune cells, regardless of their antigen specificity, from the blood into immunized lung lobes (Bice *et al.*, 1982). In the present study, dogs were simultaneously immunized in the lung and toe-pads with antigenically distinct immunogens. Since the peripheral lymphoid system includes both the popliteal and thoracic lymph nodes, one probable result was the localization of AFC from both sources in the immunized lung lobes. If, however, cell localization in the lung is either antigenically determined, or if lymphocytes possess unique organ-specific receptors, the AFC produced in the lung-associated lymph nodes would predominate in the lung. Our results demonstrate that AFC produced in distant peripheral lymph nodes are recruited to the lung at the same rate as the antigenically distinct AFC produced in the lung-associated lymph nodes.

MATERIALS AND METHODS

Using a BF3 fibreoptic bronchoscope (Olympus Corp., New Hyde Park, NY), five male Beagle dogs, 2·5 years of age, were anaesthetized with halothane and then immunized in the left cardiac lobe with 10^{10} sheep red blood cells (SRBC) in 1 ml sterile saline as previously described (Bice *et al.*, 1980). One ml of sterile saline was instilled in the control right cardiac lobe. While the dogs were still anaesthetized, 1 ml sterile saline containing 10^{10} rabbit red blood cells (RRBC) was divided into equal parts and injected into each toe-pad of both hind feet. Published data indicate that virtually all particulate antigen is removed by draining lymph nodes (Hall & Morris, 1963). Although somewhat dose-dependent, SRBC antigen instilled into the lung should stimulate the production of AFC primarily in the lung-associated lymph nodes, while AFC to RRBC injected into the feet should be primarily produced in the popliteal lymph nodes. On Days 5, 7, 10, 12 and 14 after immunization, both lung lobes were relocated with the bronchoscope and lavaged with five saline washes of 10 ml each.

A separate investigation was conducted with two additonal Beagle dogs to insure that antibody produced against either SRBC or RRBC had minimal or no cross-reactivity. One dog received 10^{10} SRBC in the left cardiac lung lobe while the other similarly received 10^{10} RRBC. The immune response against both antigens was subsequently monitored on Days 7, 9, 12 and 14 after instillation.

Lavage fluid cell counts were obtained using a ZBI Coulter Counter. Differential cell determinations were routinely performed on Diff-Quick (Dade Diagnostics Inc., Puerto Rico) stained cytocentrifuge preparations. Differential morphological features of at least 400 cells were determined for each specimen. The bronchoalveolar cells in the lavage fluids were recovered by centrifugation (250 ***g***) for 10 min. The cells

were washed with RPMI-1640 (Gibco, Grand Island, NY), and resuspended in RPMI-1640 supplemented with 10% heat-inactivated, SRBC and RRBC absorbed, fetal calf serum. The number of lymphoid cells producing IgM antibody to SRBC and RRBC was determined by the Cunningham & Szenberg (1968) modification of the Jerne plaque assay. The supernatant lavage fluids recovered after centrifugation were evaluated for total albumin by single radial immunodiffusion, and for SRBC and RRBC haemagglutinating antibody using standard techniques.

Venous blood samples were taken immediately prior to each lavage. Serum samples were analysed for SRBC and RRBC haemagglutinating antibody. Blood lymphoid cells were isolated from heparinized blood samples using Isolymph (Gallard-Schlesinger Chemical Mfg. Corp., Carle Place, NY) density centrifugation and analysed for AFC.

Statistical comparisons of the number of AFC and haemagglutinating antibody titres against each of the antigens in the lung and blood were made. A logarithmic transformation of the data was utilized to stabilize the variance (Gottlieb, 1974). Statistical analysis of the transformed data was performed using the one-sided, paired Student's *t*-test. Only those differences with $P < 0.05$ were considered to be statistically significant.

RESULTS

The data obtained to evaluate the possible cross-reactivity of RRBC and SRBC are presented in Table 1.

Table 1. Evaluation of cross-reactivity between sheep red blood cells (SRBC) and rabbit red blood cells (RRBC) instilled in the left cardiac lung lobe

Antigen used for immunization	Days after immunization	Antibody-forming cells	
		Anti-SRBC	Anti-RRBC
Dog 1—SRBC	7	14,000*	50
	9	15,000	0
	12	14,000	0
	14	9000	0
Dog 2—RRBC	7	200	13,000
	9	0	26,000
	12	0	9000
	14	0	1000

* All values are expressed as the number of IgM antibody-forming cells/10^6 lymphoid cells recovered in the lung lavage fluid.

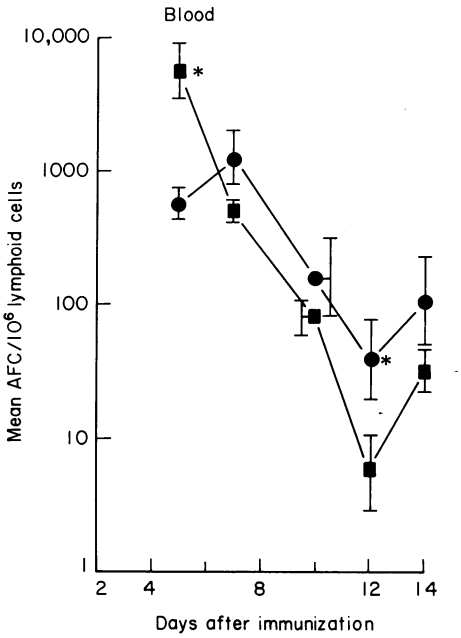

Figure 1. Direct (IgM) antibody-forming cells (AFC) to sheep red blood cells (SRBC) and rabbit red blood cells (RRBC) in the blood, 5–14 days after immunization. Each point represents the geometric mean and 95% confidence intervals of data from five dogs: (●), anti-SRBC; (■), anti-RRBC.
* Significant differences ($P < 0.05$).

These results indicate that only in the initial stage of the immune response was there any cross-reactivity. The heterologous reactivity was responsible for only 0·4% of the SRBC and 1·5% of the RRBC AFC determinations.

Both the direct lung instillation of SRBC and the toe-pad injection of RRBC resulted in a significant immune response against both antigens in the blood and lung as measured by haemagglutinating antibody and AFC. The numbers of AFC in the blood producing antibody to SRBC and RRBC are presented in Fig. 1. With the exceptions of Days 5 and 12, there were no significant differences in the numbers of AFC against these two antigens in the blood. These two exceptions, analogous to those observed examining antibody titres (data not shown), most likely reflect differences in the effect of the immunization route on the time of peak response.

There was a significant ($P < 0.05$) to highly significant ($P < 0.01$) comparative difference in the haemagglutinating antibody titre of lavage fluids from the immunized lung lobe and from the control lobe (data

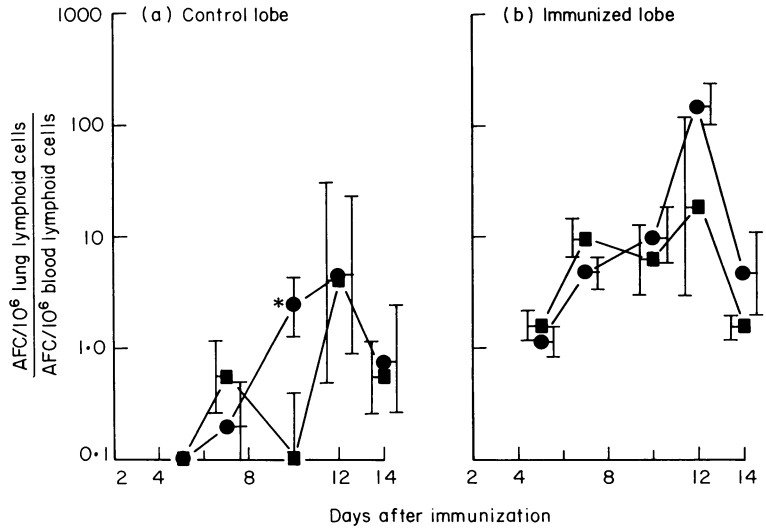

Figure 2. The ratio of blood IgM antibody-forming cells (AFC) and lung AFC producing antibody to either sheep red blood cells (SRBC) or rabbit red blood cells (RRBC). Each point represents the geometric mean and 95% confidence intervals of data from five dogs: (●), anti-SRBC; (■), anti-RRBC.
* Significant differences ($P < 0{\cdot}05$).

not shown). This difference existed both for SRBC and RRBC antibody. Transudation of antibody from the serum to the lung may be a source of some of this specific antibody. However, high titre/albumin ratios were observed in lavage fluid from immunized and control lobes, indicating that a significant portion of the antibody to SRBC or RRBC was produced by cells in either the lung interstitum or alveoli.

Antibody to SRBC and RRBC, as well as antigen-specific AFC, were found in control lung lobes and lung lobes immunized with SRBC. Some kinetic differences were observed in the number of AFC producing antibody to SRBC and RRBC in the blood (Fig. 1). Therefore, the number of AFC in the control and immunized lung lobes are presented as a ratio of the number of antigen-specific AFC found in the lung relative to those found in the blood (Fig. 2). As observed in past studies, a larger total number of AFC were observed in the immunized lung lobes than in the control lung lobes (Bice *et al.*, 1980). With only one exception (day 10 in the control lobe), there were equal numbers of AFC producing antibody to SRBC and RRBC within each lung lobe, regardless of treatment.

DISCUSSION

Earlier studies have shown that immunoblast localization depends upon tissue origin. The data presented in this study, however, indicate that immune cells are recruited to either immunized or control lung lobes, regardless of their tissue of origin. One source of the lymphocytes obtained during bronchoalveolar lavage is the blood stream (Daniele, Beacham & Gorenberg, 1977). Despite the fact that during this study the RRBC injected into the feet would have principally stimulated the popliteal lymph nodes (Hall & Morris, 1963), and that the SRBC instilled into the lung would primarily drain into the lung-associated lymph nodes (Bice *et al.*, 1980), both cell types ultimately appeared in the blood. As indicated in Fig. 2, the number of SRBC- or RRBC-specific AFC found in the lung directly reflected their respective number in the blood. Their presence in the lung therefore appears to be due to their extravasation from the blood to the lung.

The results of other studies also support this conclusion. In a repopulation study, Hall, Hopkins & Orlans (1977) found that the immunoblasts from the efferent lymph of peripheral lymph nodes primarily relocated in the lungs, spleen, liver and peripheral lymph nodes. This was in sharp contrast to the immunoblasts from intestinal lymph which went to the small gut and mesenteric lymph nodes. Spencer & Hall (1984) also showed that immunoblasts released into lymph after antigen stimulation of the caudal mediastinal lymph node went principally to the lungs and

spleen. In a study similar to that reported here, dogs were immunized by instillation of SRBC and RRBC in separate lung lobes, and the immune response was evaluated in both the blood and the lung. Results indicated that AFC to both antigens were found in equal numbers in both lung lobes, regardless of immunizing agent (Bice *et al.*, 1982). Earlier, Daniele, Altose & Rowlands (1975) also observed that the subpopulations of lymphocytes from human lungs were present in the bronchoalveolar air spaces in the same ratio as they existed in peripheral blood. We conclude that, regardless of their tissue of origin, immune cells are released into the blood and may subsequently localize in the lung in the same ratio as in the blood.

Bice *et al.* (1982) have previously demonstrated the lung localization of IgA AFC after pulmonary immunization; however, the specific migration of IgA AFC to the lung after peripheral lymph node stimulation has yet to be investigated. The methods used in this study concentrated on the production and presence of IgM. It is, therefore, unknown if the IgG and IgA AFC induced by lung and toe-pad immunization possess similar localization patterns.

The importance of lymphocyte recruitment, and the subsequent appearance of immune cells in the alveoli, in pulmonary defence has not been adequately studied. There appear to be mechanisms which allow immune cells to leave the blood and enter into immunized lung tissues, suggesting that these cells play a significant role in pulmonary defence.

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