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Definition of the Pulmonary Antibody Response to Ovalbumin following Local Challenge in Systemically Immunized Rats (42707)

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Abstract. The *in vivo* pulmonary immune response of rats to local stimulation with antigen was assessed by measuring antigen-specific antibody and antibody-secreting cells utilizing enzyme-immunoassay technology. Sprague–Dawley rats were immunized subcutaneously with ovalbumin (OA) emulsified in Freund's incomplete adjuvant, challenged with OA intratracheally on Day 19 and sacrificed 1, 2, 3, or 4 days later. Specific antibody-secreting cells in the lung-associated lymph nodes were enumerated with the ELISA–SPOT assay and antibody concentration in the pulmonary lavage fluids and sera was assessed with the ELISA. The greatest response for each parameter was on Day 2. Cellular infiltration of the lung was minimal. Cellular infiltrates consisted mainly of polymorphonuclear leukocytes and were most numerous in the lavage fluid on Days 1 and 2 and in the lung parenchyma on Day 2 after challenge. Local production versus serum transudation of antibody was evaluated by comparing the levels of OA-specific antibody to albumin in the lavage fluid and serum. The data showed that antibody in the lungs was primarily produced locally.

A better understanding of the *in vivo* humoral immune response would entail not only the measurement of antibody but also the simultaneous assessment of antibody production at the cellular level. In the respiratory tract of immunized animals, specific antibody has been extensively measured with such sensitive assays as radioimmunoassay (1) and ELISA (2, 3) while in separate studies, pulmonary antibody-forming cells have been measured utilizing the hemolytic plaque assay (4–6). A few recent studies using these and other methods have reported quantitation of antibody and of cells containing or forming antibody in the respiratory tract in the same *in vivo* model (7–9) but, to our knowledge, in no reports have both parameters been measured utilizing two assays based on the same principle. It was the purpose of the present study to accurately compare antibody presence in the lungs with cellular production of antibody in the lung-associated lymph nodes (10) of animals sensitized and challenged with soluble antigen. The same (enzyme-immunoassay) technology was utilized to measure both parameters.

The change in the antibody-mediated response over time was ascertained in rats sensitized systemically with ovalbumin (OA) emulsified in Freund's incomplete adjuvant and challenged with OA intratracheally (i.t.). The number of anti-OA IgG secreting cells in lung-associated lymph nodes and the quantity of anti-OA IgG in the bronchial alveolar lavage fluids and sera were assessed. The question of whether specific antibody was produced locally in the lungs or was transudated from the peripheral circulation was addressed by a comparison of the quantities of antibody and albumin in the lavage fluid and serum. Cellular infiltration of the lungs was monitored by evaluating lung histological sections and cellular content of the lavage fluid.

Materials and Methods. *Animals.* Male, Sprague–Dawley rats (Harlan–Sprague–Dawley, Indianapolis, IN), approximately 10 months of age, were used for all studies. Animals were housed two per cage, received Purina Rodent Chow No. 5001 and water *ad libitum,* and were maintained in animal rooms having ambient temperatures of 20–23°C with 12 hr light–dark cycles.

Immunization and challenge. Sixteen rats were immunized subcutaneously (sc) with 1 mg OA (5× crystallized, U.S. Biochemical

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Corp., Cleveland, OH) emulsified in Freund's incomplete adjuvant (FIA, Difco, Detroit, MI) and challenged 19 days later by the i.t. route with 1 mg OA in saline. Eight rats (controls) were injected with saline in FIA and 19 days later saline was instilled i.t.

Cell and fluid collection. Four OA-inoculated and two saline-inoculated rats on each of Days 1, 2, 3, or 4 after challenge were anesthetized with 2 ml of pentobarbital (Nembutal, Abbott, North Chicago, IL) intramuscularly and exsanguinated and the serum was collected. The lung-associated lymph nodes were excised and the cells were suspended to a concentration of 1×10^6 viable cells/ml (trypan blue dye exclusion) in culture medium: RPMI 1640 (M.A. Bioproducts, Walkersville, MD) supplemented with 2% fetal calf serum (heat inactivated at 56°C for 30 min), 2 m*M* L-glutamine, 50 units/ml penicillin, 50 μg/ml streptomycin, 50 μg/ml gentamicin, and 25 m*M* Hepes buffer. These suspensions were used for assessment of antibody-forming cells using the ELISA–SPOT assay (defined below). The bronchial alveolar lavage fluid was withdrawn from the lungs *in situ* 5 min after instilling 5 ml of saline (37°C). The fluid then was centrifuged at 300*g* for 20 min to sediment cells. The sera and fluid were frozen at −20°C for later assay of albumin and antibody to OA.

Identification of cells in bronchial alveolar lavage. White blood cells collected from the lavage fluid were brought to 1×10^6 viable cells/ml in culture medium. Cell preparations were centrifuged at 500 rpm for 30 sec in a cytocentrifuge (Shandon-Southern Instruments, Ltd., Camberely, Surrey, UK). The slides were fixed in absolute methanol for 30 min at room temperature and were stained with Wright's stain (Camco Quik stain, Cambridge Chemical Products, Inc., Ft. Lauderdale, FL) for 2 min and washed with distilled water for 2.5 min. After air drying, the slides were examined under oil immersion and 200 cells were identified per slide. Data were expressed as relative and absolute numbers of large and small mononuclear and polymorphonuclear cells per milliliter of fluid.

Lung pathology. After the lungs of each animal were lavaged, they were excised and inflated with 10% buffered formalin. Five-micrometer paraffin sections were cut and stained with hematoxylin and eosin and the pathology was assessed by light microscopy. Additionally, selected specimens were post-fixed in 1% OsO_4 in Millonig phosphate buffer, embedded in Epon 812, sectioned, stained with uranyl acetate and lead citrate, and examined with a Philips 201 electron microscope.

Absorption of antisera conjugate. The method of Porath *et al.* (11) was used to make columns consisting of 125 mg OA conjugated to 200 mg cyanogen bromide activated Sepharose 4B (Sigma, St. Louis, MO). One milliliter of rabbit anti-rat IgG (H- and L-chain specific) alkaline phosphatase conjugate (Zymed, South San Francisco, CA) was eluted through each column at 4°C and 0.1-ml fractions of the OA-absorbed eluate were collected, diluted 1:10 with 0.85% saline, and measured for protein content using a DU spectrophotometer (Beckman, Fullerton, CA). Those fractions displaying an OD_{280} of 0.100 or greater were pooled, sodium azide was added (0.02%), and antisera were stored at 4°C until use in the ELISA–SPOT or ELISA. Lack of cross-reaction with OA was verified with double immunodiffusion utilizing prepared plates (No. 642761, Miles Scientific, Naperville, IL).

ELISA–SPOT enumeration of antibody-forming cells. A method similar to that developed by Sedgwick and Holt (12) was used to enumerate anti-OA IgG secreting cells. The OA, at a concentration of 1 mg/ml in 0.1 *M* carbonate buffer (pH 9.6) was filter-sterilized (0.45 μm), added at 1 ml/well to 24-well tissue culture plates (Costar, Cambridge, MA), and incubated at 4°C overnight. The plates were washed twice with phosphate-buffered saline (PBS, pH 7.4) containing 0.05% Tween 20 and twice with PBS at 2 ml/well for 3 min. A mixture of 1% bovine serum albumin (BSA, Miles Scientific) and 1% normal rabbit serum (NRS, Pel-Freez, Rogers, AR) in 0.1 *M* phosphate buffer (pH 7.3) was added at 0.2 ml/well. The lymph node cell suspension was immediately added, 1 ml/well. After 2 hr incubation at 37°C, the wells were washed twice with PBS–Tween 20 and

a 1:300 dilution of rabbit anti-rat IgG–alkaline phosphatase conjugate was added at 0.5 ml/well and incubated overnight at 4°C.

The substrate, 2.3 m*M* 5-bromo-4-chloro-3-indolyl phosphate, was diluted in 1 *M* 2-amino-2-methyl-1-propanol buffer (pH 10.25), brought to 45°C, and then added to 3% agarose (Seakem, FMC Corp., Rockford, ME) which had been autoclaved and brought to 56°C. The mixture was maintained at 45°C with a 0.6% final concentration of agarose. The wells were washed twice with PBS–Tween 20 and 1 ml of substrate–agarose was added/well and allowed to harden at room temperature. The substrate reacted with enzyme–antisera conjugate to produce a blue-colored product. Antibody-forming cells were enumerated with the aid of indirect light under low magnification approximately 2 hr after the addition of the substrate. The counts were expressed as number of antibody-forming cells/10^6 viable cells.

ELISA anti-OA antibody determination. The ELISA method used was a modification of that developed by Voller *et al.* (13). To each well of 96-well tissue culture plates (3075, Becton–Dickenson, Oxnard, CA) was added 0.2 ml filter-sterilized (0.45 μm) OA diluted in carbonate buffer at a concentration of 1 mg/ml. After overnight incubation at 4°C, plates were emptied and a mixture of 1% BSA and 1% NRS in phosphate buffer was added at 0.2 ml/well and incubated overnight at 4°C. Following three washes with PBS–Tween 20 at 0.2 ml/well for 5 min/wash, serum and lavage fluid samples were diluted in PBS–Tween 20 with 1% BSA, dispensed at 0.1 ml/well, and incubated for 1 hr at 37°C. Wells were washed three times and rabbit anti-rat IgG–alkaline phosphatase was added at a dilution of 1:300 in PBS–Tween 20 at 0.1 ml/well and incubated for 1 hr at 37°C. After washing as before, the substrate, *p*-nitrophenol phosphate (Sigma), was added at a concentration of 1 mg/ml in 10% diethanolamine buffer (pH 9.8). When a positive serum control on each plate reached an optical density (OD) value close to 1.0, 0.1 ml of 0.3 *N* NaOH was added/well to stop the enzyme–substrate reaction. The yellow product was quantitated by absorption at 405 nm using a spectrophotometer (Biotek, EL310, Burlington, VT). Each ELISA plate was standardized by dividing each value by a positive reference serum taken from rats inoculated sc with OA emulsified in Freund's complete adjuvant and boosted sc with OA emulsified in FIA. In addition, the averaged conjugate assay control value from each plate was subtracted from each ELISA result.

Rat albumin quantitation. The method of Mancini *et al.* (14) was employed for single radial immunodiffusion quantitation of rat albumin in lavage fluid and serum. Goat anti-rat albumin (Cooper Biomedical, Malvern, PA) was diluted in PBS (pH 7.3), warmed to 45°C, and added to purified agar (0560-01, Difco) at 56°C to achieve final concentrations of 5 and 1.5% for the antisera and agar, respectively. Five milliliters of mixture was added to each immunodiffusion plate (Miles Scientific) and was allowed to harden on a level surface. Three-millimeter-diameter holes were cut in the agar and 70 μl of test sample was added in triplicate. Each plate also received four different known concentrations (1000, 500, 100, and 50 g/ml) of a rat albumin standard (Pel-Freez). After incubation at 37°C in a humid atmosphere for approximately 90 hr, the diameters of the precipitin rings were measured, the areas were calculated, and the quantity of rat albumin (in mg/ml) present in the unknown samples was calculated from a standard curve.

Statistics. Statistical significance between experimental and control animals was determined using Student's *t* test. A *P* value of ≤ 0.05 was considered significant. In addition, statistical significance of the day-to-day variation in absolute and relative numbers for the differential cell evaluations in lavage fluid of the OA-inoculated rats was determined with one-way analysis of variance followed by pairwise comparisons using Student's *t* test.

Results. The number of OA-specific IgG antibody-forming cells (AFCs) in the lymph nodes of OA-inoculated rats was significantly greater than saline controls ($P < 0.001$) on Days 2, 3, and 4 following OA challenge (Fig. 1). The numbers of antibody-forming cells were greatest on Day 2 (109 antibody-forming cells/10^6 viable cells) with decreasing

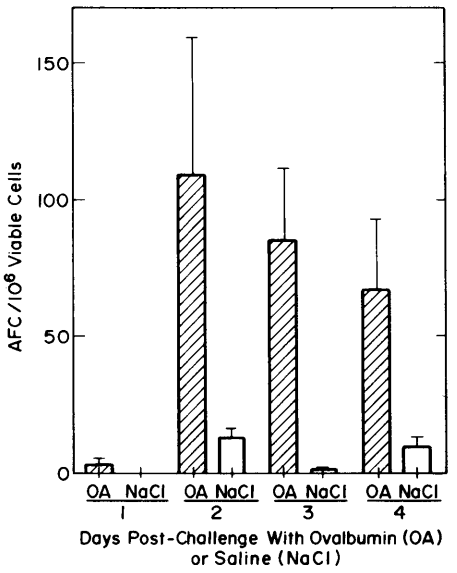

FIG. 1. ELISA–SPOT determination of OA-specific IgG secreting cells in lung-associated lymph nodes from rats inoculated with OA. Rats were immunized with 1 mg OA emulsified in FIA sc, challenged 19 days later with 1 mg OA i.t., and exsanguinated 1, 2, 3, or 4 days after challenge. Saline was used in place of OA for control animals. Mean ± SE: OA, $n = 4$; control, $n = 2$.

numbers on Days 3 and 4 (85 and 65, respectively). Numbers of AFCs from animals injected with saline were at background level. Conjugate and substrate backgrounds were uniformly negative.

The ELISA values in the lavage fluid and serum from the OA-inoculated animals also were significantly greater than controls ($P < 0.001$) (Figs. 2 and 3, respectively). Ovalbumin-specific IgG antibody in the lavage fluid (assayed at a 1:2 dilution) was increased on Day 2 ($OD_{405} = 0.487$) and had decreased slightly by Day 4 ($OD_{405} = 0.417$). The amount of OA-specific IgG in the serum (assayed at 1:100 dilution) also was greatest on Day 2 with an OD_{405} of 0.694 and decreased on Days 3 and 4 with values of 0.389 and 0.326, respectively. The saline control results were negligible on all days, ranging from 0.015 to 0.027 (OD_{405}). Conjugate and substrate control OD_{405} values were consistently near or below 0.100.

Determination of local production or transudation of anti-OA IgG in rats immunized and challenged with OA is summarized in Table I. Ratios of specific IgG values (OD_{405}) to rat albumin concentrations (mg/ml) of lavage fluid and serum were calculated. The lavage fluid ratio was then divided by the serum ratio with a value greater than 1

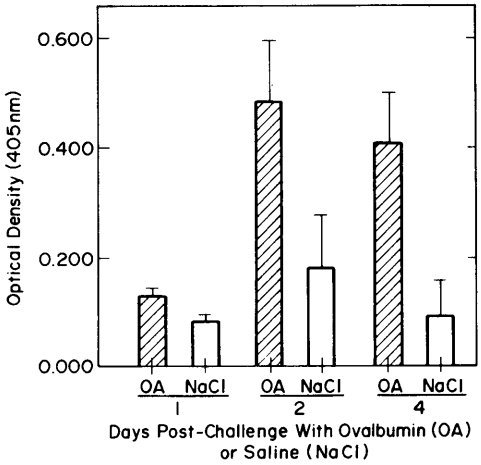

FIG. 2. ELISA determination of OA-specific IgG in lung lavage fluid. Lavage fluid samples (diluted 1:2) were from the rats described in Fig. 1. Samples from Day 3 were not available.

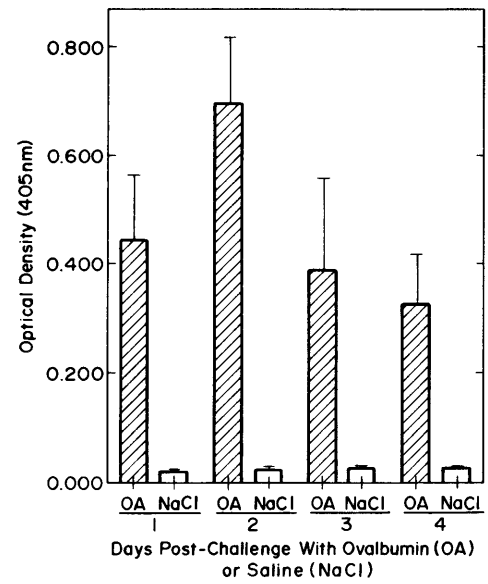

FIG. 3. ELISA determination of OA-specific IgG in serum. Serum samples (diluted 1:100) were from the rats described in Fig. 1.

TABLE I. DETERMINATION OF LOCAL PRODUCTION/TRANSUDATION OF ANTI-OA IgG IN RATS IMMUNIZED AND CHALLENGED WITH OA[a]

Days post OA challenge[b]	Lavage fluid[c]	Serum[c]	Lavage fluid ratio[d] / Serum ratio
1	0.14/0.08 = 1.7	0.45/0.64 = 0.7	2.4
2	0.49/0.10 = 4.9	0.69/0.47 = 1.5	3.3
4	0.42/0.11 = 3.8	0.33/0.70 = 0.5	7.6

[a] See legend to Fig. 1.
[b] Samples from Day 3 were not available.
[c] Anti-OA IgG values (OD: 405 nm) were divided by rat albumin concentration (mg/ml) for lavage fluid and serum.
[d] A value greater than 1.0 indicates local production of anti-OA IgG.

indicating primarily local production of OA-specific antibody (15). The data show local production of OA-specific IgG and suggest additional persistence of antibody in the lungs.

Table II summarizes the total and differential cell counts over a 4-day period of control rats or rats immunized and challenged with OA. As expected, large mononuclear leukocytes were predominant in the lavage fluid of control animals. There were significantly fewer of these cell types present in the OA-inoculated animals on Days 1 and 3 because polymorphonuclear leukocytes (PMN) predominated. High PMN counts in one control animal on Day 2 prevented significant differences, compared with OA-challenged animals, from being seen.

The variation in differential and total cell counts for the OA-inoculated animals over time was analyzed for statistical significance by analysis of variance (ANOVA). When the ANOVA was significant, pairwise comparisons were made using Student's t test. Absolute and relative numbers of large mononuclear and PMN cells on Day 1 were significantly different than those on Days 3 and 4

TABLE II. IDENTIFICATION OF CELLS FROM BRONCHIAL ALVEOLAR LAVAGE OF OA-INOCULATED OR CONTROL RATS[a]

Days post OA challenge and test groups	Large mononuclear cells: Relative (% ± SE)	Large mononuclear cells: Absolute (number/ml × 10^3 ± SE)	Small mononuclear cells: Relative (% ± SE)	Small mononuclear cells: Absolute (number/ml × 10^3 ± SE)	Polymorphonuclear leukocytes: Relative (% ± SE)	Polymorphonuclear leukocytes: Absolute (number/ml × 10^3 ± SE)	Total cells (number/ml × 10^3 ± SE)
Day 1							
OA inoculated	34.3 ± 9.3[b]*	712 ± 96[c]	3.6 ± 1.3	72 ± 32	62.1 ± 10.3*	1866 ± 662*	2650 ± 715
Control	97.5 ± 0.0	999 ± 463	2.0 ± 0.5	18 ± 4	0.5 ± 0.5	8 ± 8	1025 ± 475
Day 2							
OA inoculated	39.8 ± 9.8	196 ± 151	6.0 ± 5.0	12 ± 5	54.3 ± 4.8	218 ± 129	425 ± 275
Control	75.0 ± 20.0	573 ± 188	5.7 ± 0.8	43 ± 3	19.3 ± 19.3[d]	135 ± 135	750 ± 50
Day 3							
OA inoculated	74.3 ± 5.5*	333 ± 71	18.4 ± 6.6	70 ± 16	7.4 ± 2.2*	35 ± 14*	438 ± 75
Control	95.5 ± 0.5	526 ± 194	4.5 ± 0.5	24 ± 6	0.0 ± 0.0	0 ± 0	550 ± 200
Day 4							
OA inoculated	89.0 ± 1.7	422 ± 100	10.4 ± 1.3	50 ± 13	0.6 ± 0.3	3 ± 1	475 ± 113
Control	90.5 ± 1.0	228 ± 97	8.8 ± 1.1	19 ± 5	0.5 ± 0.4	3 ± 2	250 ± 104
Day 1 vs Day 2	—	—	—	—	—	—	—
Day 1 vs Day 3	**	**	—	—	**	**	**
Day 1 vs Day 4	**	—	—	—	**	**	**
Day 2 vs Day 3	**	—	—	—	**	—	—
Day 2 vs Day 4	**	—	—	—	**	—	—
Day 3 vs Day 4	**	—	—	—	**	—	—

[a] See legend to Fig. 1.
[b] Mean % ± SE derived from 200 cells counted/sample.
[c] Number of cells/ml sample recovered.
[d] High value due to one animal.
* $P \leq 0.05$ OA inoculated versus respective control (Student's t test).
** $P \leq 0.05$ OA inoculated analyzed over time (analysis of variance, Student's t test).

with the exception of absolute number of large mononuclear cells on Day 4. On Days 2 and 3, the relative numbers of both cell types were still significantly different from those on Days 3 and 4.

The total cell numbers in the lavage fluid from the lungs of OA-inoculated rats were greatest on Day 1 at 2650×10^3 cells/ml, a significant increase over Days 3 and 4. The relative and absolute numbers of small mononuclear cells in lung lavage fluid did not change significantly over the 4-day period in the OA-inoculated rats.

Figures 4 and 5 are light and electron microscopic views, respectively, of representative lung tissue from OA-inoculated rats 2 days postchallenge. The greatest degree of cellular infiltrate occurred 2 days after challenge with decreasing amounts on Days 3 and 4 (Figs. 4 and 5). The least amount of cellular infiltrate in these animals was observed on Day 1. In contrast, in saline-inoculated controls, no appreciable pulmonary cellular infiltrate was seen at any time. Lung lesions consisted of perivasculitis around small but not large blood vessels with a cellular infiltrate comprised mainly of polymorphonuclear cells with occasional plasma cells, lymphocytes, and monocytes. The infiltrate was moderate and unevenly distributed, in terms of both the number of lung lobes involved and the amount or area of each lobe affected. The bronchus-associated lymphoid tissue (BALT) appeared to be normal and no bronchiolitis was present.

Discussion. The kinetics of the *in vivo* antibody-mediated response was investigated utilizing a novel approach based on enzyme-

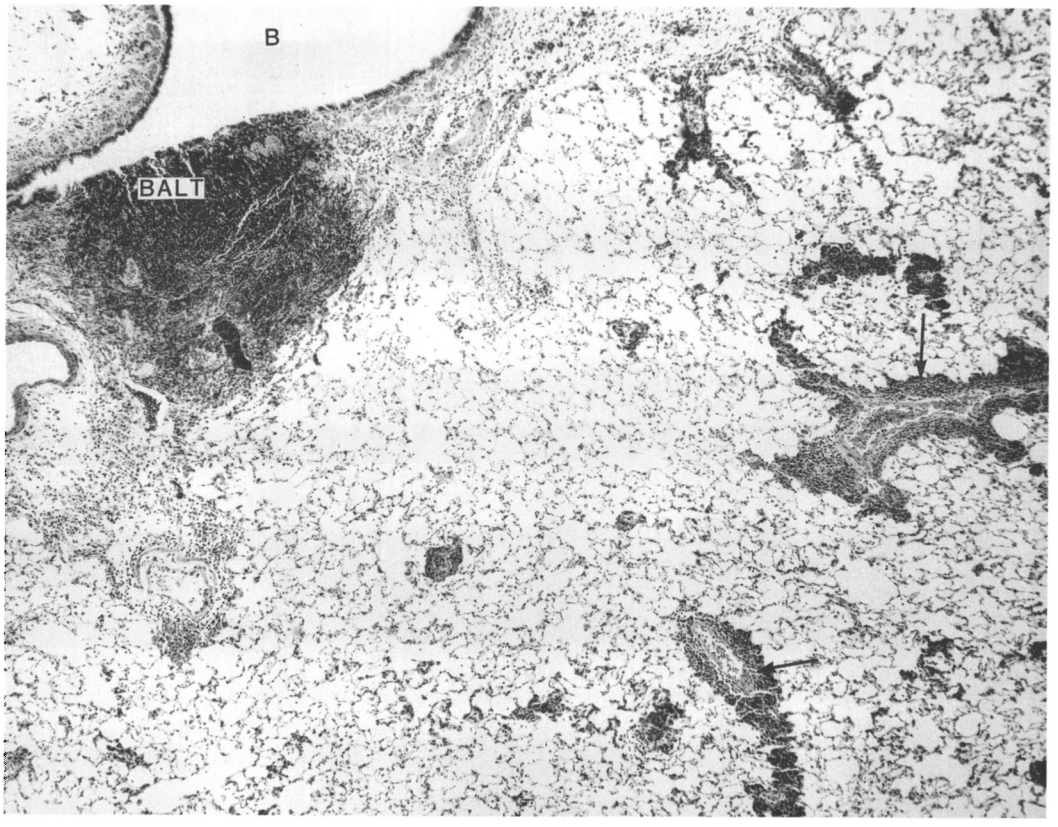

FIG. 4. Perivasculitis around blood capillaries (arrows) in a 5-μm lung section from an OA-inoculated rat exsanguinated 2 days after challenge. Bronchiole (B), bronchus-associated lymphoid tissues (BALT). Hematoxylin and eosin stain. Magnification 46.7×.

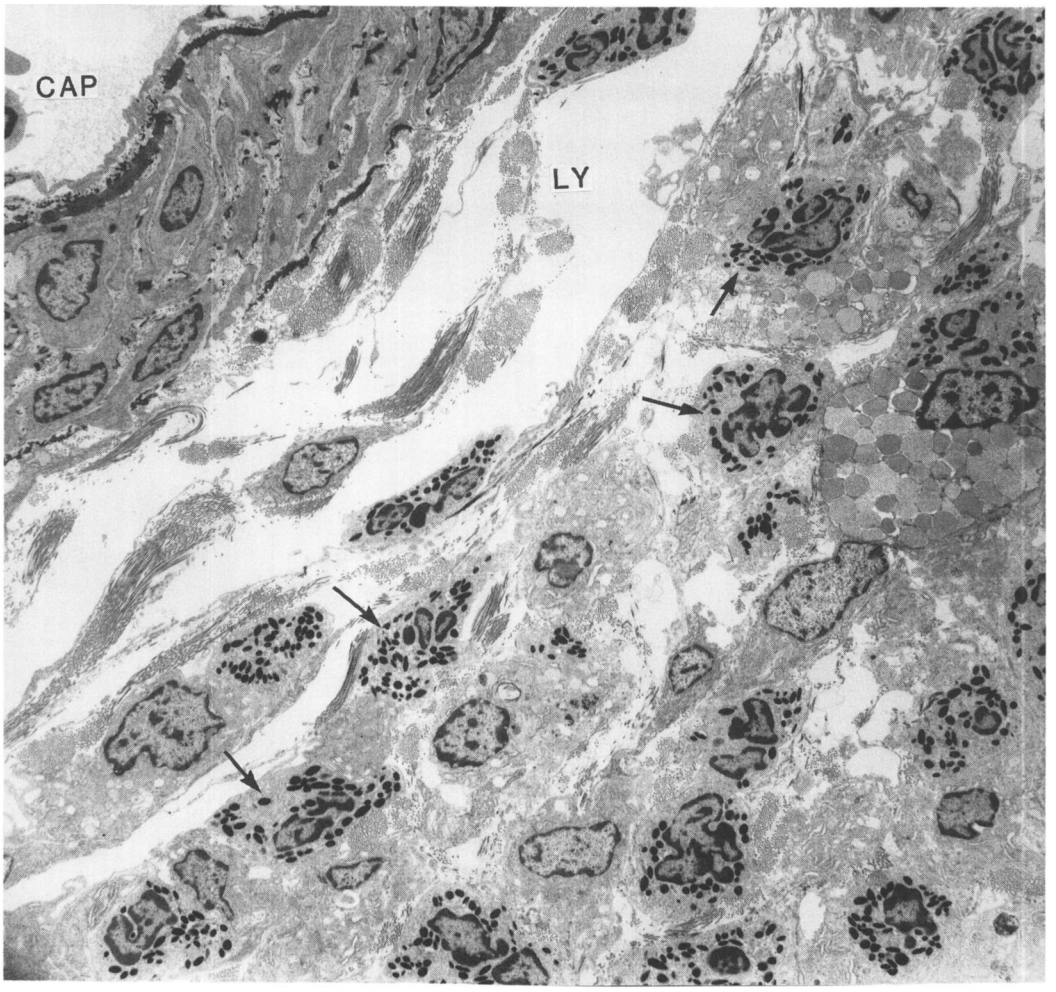

FIG. 5. Infiltrate near blood capillary consisting of polymorphonuclear leukocytes (arrows). Electron micrograph of the same lung lobe shown in Fig. 2b. Blood capillary (CAP), lymphatic vessel (LY). Magnification 2600×.

immunoassay technology. Antibody concentration in the lungs of rats sensitized and challenged with soluble antigen was assessed with the ELISA and antibody-forming cells in the lung-associated lymph nodes were enumerated with the ELISA–SPOT. A similar response over time both for antibody in the lungs and serum and for antibody-forming cells in the lung-associated lymph nodes of OA-immunized rats was seen. In both cases, the peak response was 2 days after challenge while the total number of cells present in alveolar wash was greatest 1 day after challenge.

In other studies, the kinetics of the maximum IgG amnestic response after a local injection of antigen in the lungs of previously immunized animals varied with type of antigen and species. Kaltreider *et al.* (6) demonstrated elevated numbers of antigen-specific IgG-forming cells in the hilar lymph nodes 5 days and in the lungs 7 days after i.t. challenge of mice previously sensitized by the i.t. route with SRBC. Nash (16) and Thomas *et al.* (17), using the i.t. immunization route, demonstrated peak responses at 6 days in lung-draining lymph nodes using solubilized SRBC stroma or whole SRBC as antigen, re-

spectively. Mason *et al.* (9) demonstrated peak anti-SRBC IgG antibody responses in lavage fluid approximately 7 days after four i.t. injections of cynomolgus monkeys. In none of the above studies, however, were both antigen-specific IgG and IgG-producing cells assessed after local challenge of previously sensitized animals.

In the monkey, rabbit, and sheep, antibody and antibody-producing cells in the respiratory tract have been measured in the same animals, but with dissimilar assays (7–9). Monkeys were immunized and challenged by deposition of SRBC in distal airways and alveoli of selected lung lobes. Antibody was assessed by ELISA with solubilized SRBC as antigen, and antibody-forming cells were identified by the Jerne plaque assay. In rabbits, the antigen utilized was OA. Animals were sensitized intradermally in the toepads with OA emulsified in complete Freund's adjuvant and subsequently challenged by OA aerosol. Antibody was assessed with the ELISA and antibody-forming cells were identified in tissue sections utilizing tetramethylrhodamine isothiocyanate–OA followed by fluorescein-conjugated guinea pig anti-rabbit immunoglobulin. The sheep model was of the normal respiratory tract immunoglobulins using radioimmunoassay to assess antibody and immunofluorescence to identify the antibody-forming cells.

As previously stated, the present study utilized similar assays to measure both antibody and antibody-forming cells. The immunization regimen was designed to favor antibody formation with minimal to moderate degrees of cellular infiltrate in the lung. This design, similar to that developed by Richerson in rabbits (18), demonstrated high levels of antibody and antibody-forming cells with minimal cellular infiltrate. The pathology of the lung was characterized by moderate, scattered vasculitis with infiltrates consisting primarily of polymorphonuclear leukocytes and no bronchiolitis. These results are characteristic of a classic antibody-mediated inflammatory response (type III) (19).

The source of the specific IgG found in the lung lavage fluid was determined by comparing amounts of anti-OA IgG to those of albumin in lavage fluid and in serum. These determinations indicated that the majority of anti-OA IgG present in the lungs was locally produced. Local production in this study was also suggested by the presence of plasma cells in the lung parenchyma. That no cellular infiltrate was present in the saline-challenged controls on Day 2 also suggests that the plasma cells were recruited from a different site.

Several studies employing animal models of a humoral immune response have shown that the antibody-forming cells specific to the antigen deposited into the lungs are recruited there from extrapulmonary lymphoid tissue (5, 6, 15, 20, 21). Antigen exposure alters the lung to allow the recruitment of cells. For example, Bice *et al.* (5) demonstrated this phenomenon in the dog in an experiment in which separate lung lobes were immunized with antigenically different particulate antigens. Similar numbers of antibody-forming cells to both antigens were found in each immunized lobe. Antibody-forming cells also were found in control lung lobes, but at significantly lower numbers at the peak immune responses. Kaltreider *et al.* (6) supported this finding in mice utilizing immunization, ablation, and adoptive transfer to define that intrapulmonary boosting with antigen was responsible for the appearance of antibody-forming cells in the lungs.

In contrast, in hypersensitivity pneumonitis, a model of lung disease in rabbits which is primarily cell-mediated (22), most of the specific IgG present in the lavage fluid was found to be transudated directly from the systemic circulation (7). In this model, the animals were immunized systemically with OA in Freund's complete adjuvant and challenged with OA aerosol 3 weeks later. A chronic challenge of antigen in the same study (three aerosol challenges weekly for 4 weeks) demonstrated large numbers of OA-containing cells in the lungs, suggesting that cellular recruitment is a function of antigen concentration or duration.

In conclusion, the enzyme-immunoassay technology utilized in this study has proven sensitive and reliable in assessing the kinetics of the *in vivo* pulmonary immune response of rats immunized to favor antibody formation. The data demonstrated that increased numbers of antibody-forming cells in lymph nodes draining the lungs are accompanied by

increased levels of locally produced antibody in lung lavage fluid. In addition, the kinetics of these reponses was similar with greatest values occurring 2 days after challenge.

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