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Effect of Respiratory Syncytial Virus Infection on the Uptake of and Immune Response to Other Inhaled Antigens[1] (42727)

JOACHIM FREIHORST,[2,3] PEDRO A. PIEDRA,[4] YOSHITAKA OKAMOTO, AND PEARAY L. OGRA[5]

Departments of Pediatrics and Microbiology, State University of New York at Buffalo, and Division of Infectious Diseases and Microbiology Laboratories, The Children's Hospital of Buffalo, Buffalo, New York 14222

Abstract. Groups of BALB/c mice were sham infected or inoculated intranasally (IN) with live RSV. From Day 4 to 8 after infection, the animals were exposed IN to ovalbumin (OVA) with or without alum adjuvant. At different intervals, levels of OVA concentration in serum, IgG-anti-OVA antibody activity in serum, and IgA-anti-OVA antibody activity in bronchial washings were determined, employing the ELISA technique. IgE-anti-OVA antibody titers in serum and bronchial washings were assessed by PCA. OVA concentrations in serum were significantly higher in RSV-infected animals compared to uninfected controls. The use of alum adjuvant also increased OVA uptake in uninfected animals but to a lesser extent than RSV infection. RSV-infected animals developed significantly higher OVA-specific antibody titers of IgG isotype in serum and IgA isotype in bronchial washings than the uninfected controls, while alum enhanced the immune response less markedly but still significantly in uninfected mice. An IgE antibody response to OVA in serum was demonstrable in 50% of RSV-infected mice immunized IN with OVA and alum, while all uninfected animals and RSV-infected animals immunized with OVA alone (without adjuvant) failed to develop a detectable IgE response. These findings suggest that infections with viral agents such as RSV may function as adjuvants for other antigens inhaled during acute respiratory infection. These observations may explain the alterations in the immune response to other antigens in patients with acute viral-induced bronchopulmonary diseases.

Observations in infants and children with viral respiratory tract infections have suggested that such infections can trigger manifestation of allergic airway disease especially in allergy-prone individuals (1). In addition, patients with infection-induced bronchospasm often exhibit other broad-based clinical and immunologic abnormalities affecting other dietary and inhaled antigens (2). Possible mechanisms responsible for these alterations include increased permeability of the respiratory epithelial barrier during viral infections, thus facilitating access of antigens to the antibody-forming cells in the airway, development of virus-specific IgE activity, and virus-induced suppression of regulatory T-cell subpopulations (3, 4).

In a recent study, serum IgE antibodies to aerosolized ovalbumin were observed to develop in mice only after exposure to influenza A virus (5). Since RSV is the most common respiratory pathogen in infancy, we undertook this study to investigate the effects of RSV infection on the kinetics of antigen uptake and nature of serum and secretory antibody response to ovalbumin administered concurrently via the respiratory tract during acute infection with RSV in BALB/c mice.

Materials and Methods. *Animals.* Male BALB/c-Ros mice, 8–10 weeks of age (West Seneca Laboratories, West Seneca, NY),

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[3] Present address: Kinderklinik der Medizinischen, Hochschule Hannover, Konstanty-Gutschow-Str. 8, 3000 Hannover 91, FRG.

[4] Influenza Research Center, Texas Medical Center, Department of Microbiology and Immunology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030.

[5] To whom all correspondence and reprint requests should be addressed at Division of Infectious Diseases, Children's Hospital of Buffalo, 219 Bryant Street, Buffalo, NY 14222.

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raised on ovalbumin-free food, were used. The animals were maintained in isolation in the animal facility of the Children's Hospital of Buffalo. Sprague–Dawley rats (Hilltop Lab Animals, Scottsdale, PA) were used for passive cutaneous anaphylaxis.

Virus. Long strain of RSV was prepared as previously described (6) and stored in aliquots at −70°C. The stock virus had a titer of 2×10^7 plaque-forming units/ml (PFU/ml).

Antigen preparation. Aluminum hydroxide (Amphojel tablets, Wyeth Laboratories, Philadelphia, PA) was suspended in PBS at 5 mg/ml and homogenized by sonication. Ovalbumin, grade V (Sigma Chemical Co., St. Louis, MO), was dissolved at a concentration of 400 μg/ml, either in PBS or in the alum suspension.

Experimental protocol. Four groups of mice were either infected with RSV or sham infected on Day 0 and subsequently 4 to 8 days later received five doses of 5.0 ml (0.4 mg/ml) of OVA administered IN, with or without alum (Fig. 1). Respiratory infection was established by intranasal administration of 50 μl (=10^6 PFU) of stock virus to animals lightly anesthetized by inhalation of methoxyflurane (Metofane, Pitman–Moore, Washington Crossing, NJ). Virus replication in the lungs was confirmed 6 days after inoculation by a plaque-forming assay from lung homogenates. Control animals were sham infected with virus-free HEp-2 cell suspension processed in a manner similar to stock RSV. Intranasal antigen administration (20 μg OVA, dissolved in 50 μl of PBS or alum suspension) also was performed after inhalation anesthesia with methoxyflurane. Determination of OVA concentrations in bronchial washings obtained 1 hr after antigen administration indicated that with this procedure approximately 15–25% of the antigen dose reached the lower airways. Samples were taken from 10 mice from each group according to Fig. 1. Additional mice were used to assess OVA uptake and for histopathology.

Samples. Blood samples were obtained by retroorbital bleeding or cardiac puncture. Serum was stored in aliquots at −20°C (−70°C for IgE determination). For bronchial washings mice were sacrificed by CO_2 overdose and exsanguinated. A 24-gauge Teflon catheter (Quick-Cath, Travenol Laboratories, Deerfield, IL) was inserted into the exposed trachea and secured with a suture. Then 0.5 ml of PBS was slowly injected and withdrawn three times. The recovered fluid gave a volume of 0.35–0.45 ml and an average protein concentration of 0.5 mg/ml (Bio-Rad Protein Assay, Bio-Rad Laboratories, Richmond, VA). After centrifugation at 2000 rpm for 5 min, the supernatant was stored at −20 and −70°C, respectively.

Antigen and antibody assays. OVA concentration in serial samples of serum and bronchial secretions was determined by a sandwich-type ELISA (7). Rabbit anti-OVA antiserum (IgG fraction) was coated to polyvinyl chloride microtiter plates, and peroxidase-conjugated rabbit anti-OVA antiserum (IgG fraction) was used for detection (both Cooper Biomedical, Malvern, PA). Results were expressed as optical density (OD) obtained for a 1:200 dilution of samples. OVA-specific antibodies of IgG (in serum) and IgA

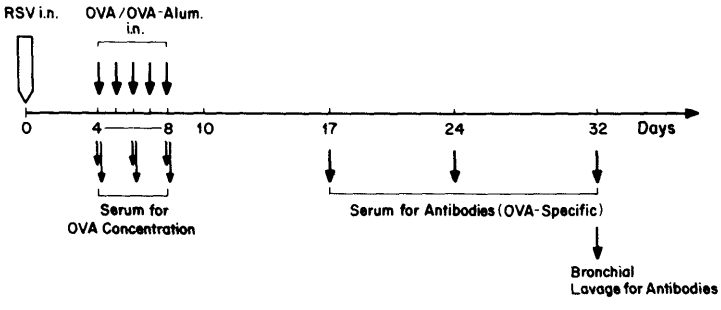

FIG. 1. Experimental protocol performed with four groups of mice: group A was RSV infected and given OVA alone, group B was sham infected and treated with OVA alone, group C was RSV infected and treated with OVA/alum, and group D was sham infected and given OVA with alum.

(in bronchial washings) isotypes were assessed by ELISA with OVA coated to polyvinyl chloride microtiter plates, followed by incubation with serial dilutions of samples. Peroxidase-conjugated, affinity-purified γ-chain-specific goat anti-mouse IgG (Cooper Biomedical) or γ-chain-specific rabbit anti-mouse (ICN Immunobiologicals, Lisle, IL; N.B., some lots contain anti-OVA activity) were used as detecting antibodies. For serum samples, antibody activity was expressed as ELISA titer (reciprocal dilution) giving an OD of 0.5, calculated from log–log transformations of the dose–response curves for each sample. Antibody activity in bronchial washings was expressed in ELISA units, calculated as the reciprocal of the highest dilution giving positive readings $\times$ OD of the dilution. No correction for protein concentration was done since little variation was found in the experimental groups. Negative controls and positive reference samples were included in each assay; each sample was run in the same assay with the corresponding control sample.

OVA-specific IgE antibody was detected by passive cutaneous anaphylaxis (PCA) (8). Briefly, 100 μl of samples and twofold dilutions were injected intradermally into the dorsal skin of shaved rats, which were challenged intravenously with OVA in PBS (1 mg/ml) with 1% Evans blue 48 hr later. A blue lesion of a diameter greater than 5 mm, judged 30 min later, was considered positive. PCA titers were expressed as the reciprocal of the highest dilution giving a positive reaction.

Histopathology. Mice were sacrificed by CO_2 overdose and exsanguinated. The lungs were inflated *in situ* with buffered 10% formalin solution (Lyne Laboratories, Stoughton, MA) and fixed in the same reagent. Lung sections were embedded in paraffin and stained with hematoxylin–eosin. Slides were examined blindly and judged for the following criteria: (1) perivascular inflammation; (2) peribronchial infiltrates; (3) septal thickening; and (4) magnitude of cellular inflammatory response.

Statistical analysis. OVA concentrations in serum were calculated as arithmetic mean $\pm$ standard error of the mean (SEM) for each experimental group and assessed for significance of differences by Student's t test. Antibody activities in serum and bronchial washings were expressed as geometric mean titers for each group and compared by the Wilcoxon rank sum test. Differences in OVA-specific IgE response were evaluated by the Fisher exact test.

Results. *OVA uptake.* OVA concentrations were determined in serum samples collected at 1, 2, 4, 6, and 24 hr after IN administration of OVA. Peak concentrations were observed 2 hr after administration of the antigen. Representative data obtained from samples collected after 2 hr of OVA inhalation on Day 6 are shown in Table I. Both groups of RSV-infected mice (group A, immunized with OVA alone, and group C, immunized with OVA/alum) attained significantly higher peak concentrations compared to uninfected mice (group B, immunized with OVA alone, and group D, immunized with OVA/alum). On the other hand, serum OVA concentrations observed in group D mice were significantly higher than group B mice. In RSV-infected mice no additional

TABLE I. SERUM OVA CONCENTRATIONS[a] MEASURED BY ELISA IN RSV-INFECTED AND NONINFECTED CONTROLS

Experimental group/route	OD $\pm$ SEM at 1/200 dilution	Significance of difference vs group B	Significance of difference vs group D
(A) RSV infected, OVA/IN[b]	0.644 $\pm$ 0.103	$P < 0.001$	$P < 0.001$
(B) Sham infected, OVA/IN	0.179 $\pm$ 0.035	—	$P < 0.01$
(C) RSV infected, OVA–alum/IN	0.571 $\pm$ 0.119	$P < 0.001$	$P < 0.001$
(D) Sham infected, OVA–alum/IN	0.243 $\pm$ 0.048	$P < 0.01$	—

[a] Representative data observed on samples taken 2 hr after IN administration of 20 μg OVA with or without alum adjuvant on Day 6 after RSV inoculation or sham inoculation. Eight to ten mice were used from each group.
[b] IN = intranasal.

enhancement of OVA concentration in serum could be achieved by the use of adjuvant (group C vs group A). Higher OVA concentrations observed at 2 hr in RSV-infected animals were not due to differences in absorption kinetics between these and the noninfected controls. Increased serum concentrations of OVA were observed in infected animals at each sampling interval after administration of OVA (data not shown). These findings could be reproduced over the entire period of OVA administration. Intragastric administration of 20 μg of OVA did not produce measurable OVA levels in serum.

Serum antibody response. Serum IgG OVA-specific antibody activity was measured prior to and 9, 16, and 24 days after the last administration of OVA (Days 17, 24, and 32 after virus inoculation). Results are shown in Fig. 2. RSV-infected animals (groups A and C) exhibited significantly higher anti-OVA titers when compared to the titers in uninfected controls (groups B and D). However, uninfected animals exposed to OVA with alum (group D) had significantly higher anti-OVA IgG titers in the serum compared to uninfected animals exposed to OVA without adjuvant (group B).

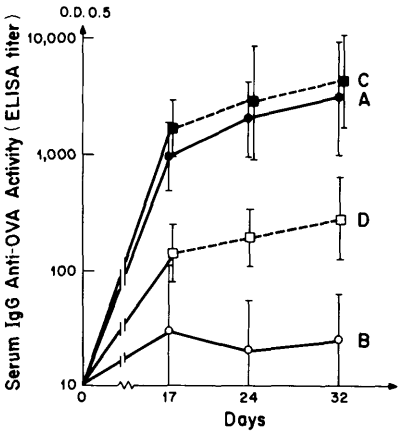

FIG. 2. Serum IgG anti-OVA antibody response in groups of mice infected with RSV (A and C) or sham infected (B and D) on Day 0 and given five IN doses of OVA without (A and B) or with (C and D) alum adjuvant on Days 4–8. Results are shown as geometric mean titers ±SEM. Differences were statistically significant ($P < 0.01$) for groups C, A, and D vs group B, and groups C and A vs group D.

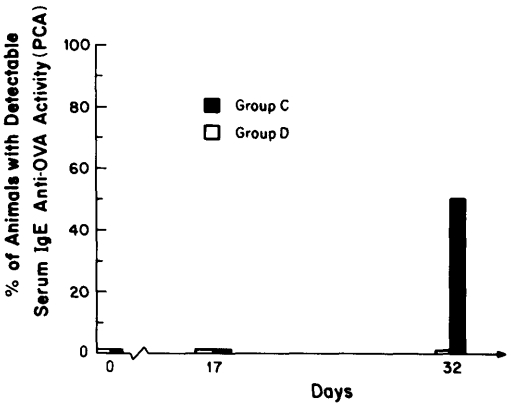

FIG. 3. Serum IgE anti-OVA antibody response in mice infected with RSV (C) or sham infected (D) on Day 0. Samples were taken before and after five IN doses of OVA with alum adjuvant (Days 4–8). The difference observed at Day 32 was statistically significant ($P < 0.02$).

IgE anti-OVA activity was determined in serum samples collected before RSV infection or sham infection and 17 and 32 days postinfection (9 and 24 days after OVA administration). As shown in Fig. 3, no anti-OVA-specific IgE activity was observed in RSV-infected or uninfected animals before and up to 9 days after administration of OVA with adjuvant. However, 50% of the adjuvant-treated, RSV-infected animals exhibited a detectable OVA-specific IgE response 2 weeks later (Day 32), PCA titers ranging from 1:1 to 1:16. All remaining animals failed to elicit any IgE response.

Antibody response in bronchial washings. Bronchial washings were obtained from all mice at Day 32 and assessed for protein concentration and for OVA-specific antibodies, results shown in Fig. 4. No OVA-specific IgA was found in uninfected mice, treated with OVA alone (group B). Low levels of anti-OVA IgA were detected in RSV-infected mice, treated with OVA alone (group A) and in uninfected animals, treated with OVA and alum (group D). The highest antibody activity was found in the bronchial washings of mice infected with RSV and exposed to OVA with alum (group C). This difference was significant, compared to all other groups.

Histopathology. Representative samples of lungs were taken from three animals in each

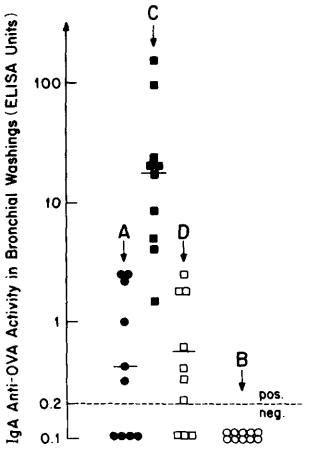

FIG. 4. IgA anti-OVA antibody activity in bronchial washings obtained 32 days after virus inoculation/sham inoculation (24 days after last dose of OVA or OVA/alum). Groups are named according to legend of Fig. 1. Differences were statistically significant between group B vs groups C and D ($P < 0.01$), group B vs group A ($P < 0.05$), and group C vs groups A and D ($P < 0.01$).

group on Day 6 after virus inoculation (after three daily OVA or OVA/alum administrations) and at Day 32. Uninfected mice treated with OVA alone had normal histological features of the lungs and airways. RSV-infected mice, whether treated with OVA alone or with OVA/alum, showed slight changes, characterized by peribronchial and perivascular infiltration by mononuclear cells and few polymorphonuclear leukocytes. In addition, minimal flattening of the bronchial epithelium was observed during acute infection in samples collected at Day 6 (data not shown). By Day 32, these findings had completely resolved in all infected animals. In animals treated with OVA/alum, noticeable tiny particles of solid material, most likely alum particles, were found near bronchi and in peripheral lung tissue, with beginning lymphoid infiltration seen in samples taken at Day 32.

Discussion. The data reported here suggest that acute respiratory infection with RSV results in significantly increased uptake of OVA administered into the respiratory tract during the acute phase of viral infection. Such infection was also associated with development of significantly increased serum IgG and IgE and secretory IgA anti-OVA antibody responses. It is, however, of interest to note that use of an adjuvant with OVA, without any viral infection, also resulted in enhancement of OVA antigen uptake and development of serum IgG and secretory IgA anti-OVA response, although the magnitude of such enhancement was significantly lower than observed with viral infection alone.

The mechanisms underlying the increased antigen uptake observed here cannot be defined based on the present data. However, several possible explanations may exist. These include suppression of phagocytic or mucociliary clearance of OVA, development of mucosal damage during acute viral infection resulting in increased permeability of the respiratory epithelium to concurrently available antigens for systemic uptake, or possible alterations of mucosal transportation mechanisms secondary to the release of potent pharmacologic mediators from mucosal mast cells and polymorphonuclear leukocytes, or other lymphokines released during acute RSV infection (2, 9, 10).

The role of mucosal damage in the uptake of antigens has been investigated in a number of experimental models. Studies with chronic inhalation of irritants (11, 12), mucosal inflammation due to hypersensitivity reactions (13), and BCG-induced granulomatous inflammation (14) have suggested that macromolecular absorption across the alveolar capillary barrier may be controlled by inflammatory as well as immunologic mechanisms. Pulmonary infection with cytomegalovirus in immunosuppressed mice has been shown to cause increased leakage of plasma proteins into the alveolar space (15), but no quantitative data were available until now to demonstrate the effect of viral respiratory infections on the transport of protein molecules across the epithelium of respiratory mucosal surface or the distal airspaces. Although our data clearly show enhancement of systemic antigen uptake during acute RSV infection, the histopathological findings of the lungs of RSV-infected mice do not exhibit marked damage of the bronchioalveolar epithelium in light microscopy. In view of the observations that the virus can be demonstrated in alveolar and bronchiolar epithelial cells in the mouse model of RSV infection (16), one can anticipate that the in-

fected cells may undergo subtle structural changes which may be apparent only in electron microscopy studies. Observations with im or ip injection of alum and other adjuvants suggest a similar effect of adjuvant on antigen uptake at mucosal sites, including a depot effect with prolonged local persistence of the adsorbed antigens, alterations in antigen presentation, and local inflammation due to complement activation and attraction of leukocytes, especially eosinophils, to the site of action (17–19). Earlier studies have demonstrated absorption of intact protein molecules across the pulmonary air–tissue barrier following their instillation directly into the lungs (20). This is thought mainly to be due to overloading of the pulmonary clearance mechanisms (21). Thus, by instillation into the lung, the antigen additionally may reach and stimulate distant lymphoid tissues not associated with the airways. The increased serum IgG and IgE antibody responses to OVA in RSV-infected and adjuvant-treated animals observed here may be explained in part by the larger amount of antigen entering the circulation. Interestingly, however, it has been shown with a model of BCG-induced granulomatous lung inflammation in rabbits that immunization of the respiratory tract elicits a substantially greater systemic IgG immune response in these animals than the same dose of antigen given iv (22). Thus, stimulation of the lymphoid tissue of the respiratory tract seems to play an important potentiating role for the systemic response. Increased access of antigen to the bronchus-associated lymphoid tissue, paralleling increased antigen uptake into the blood, may in part be responsible for the enhanced IgA antibody response in bronchial secretions of RSV-infected and adjuvant-treated animals observed in this study.

A variety of other known effects of viral agents and adjuvants may play a role in enhancement of systemic and local immune responses. Release of lymphokines and other mediators during acute viral infections may alter antigen processing and number and function of B lymphocytes and regulatory T cells (23). The latter might be of particular significance for the IgE antibody response, which is to a greater extent regulated by isotype-specific T suppressor cells (24). Earlier data from our laboratories have indicated that infants recovering from RSV bronchiolitis have reduced numbers of OKT8 antigen-positive lymphocytes in their venous blood, a lymphocyte subpopulation that includes largely suppressor cytotoxic phenotype cells (4). It is possible that during RSV infection, depression of IgE-specific suppressor cell function might contribute to the observed increase in the anti-OVA IgE antibody responses.

On the other hand, alum adjuvant has been shown to stimulate the formation of carrier-specific helper cell populations for the IgG and IgE response *in vitro* (25). Earlier studies have also suggested that the nucleic acids or naked viral RNA function as excellent immunologic adjuvants (26). Thus, in the experiments described in the present report, RSV infection and alum may both act synergistically, in part via different pathways, to enhance the immune response to OVA. This possibility is supported by the observation that both viral infection and use of adjuvant were necessary for the induction of reaginic antibodies to OVA, in accordance with the findings of others with a model of influenza virus infection (5).

Based on the observations reported here and in view of the known effects of other adjuvants in modulation of the immune response (17), it is suggested that *in vivo* infections with viral agents such as RSV may function as adjuvants for other inhaled allergens, dietary proteins, and environmental antigens available in the mucosal lumen during acute viral infection. It would appear that mucosal viral infection may alter the immunologic and structural homeostasis of the respiratory mucosa sufficiently enough to mount enhanced immunologic reactivity to otherwise less immunogenic antigens present in the mucosal lumen. These observations may thus explain the broad-based atopic manifestations to other inhaled and dietary antigens in patients with viral-induced and IgE-associated bronchospasms and other bronchopulmonary allergic disorders.

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1. Frick OL, German DF, Mills J. Development of allergy in children. I. Association with virus infections. J Allergy Clin Immunol **63**:228, 1979.
2. Ogra PL, Welliver, RC, Riepenhoff-Talty M, Faden HS. Interaction of mucosal immune system and infections in infancy: Implications in allergy. Ann Allergy **53**:523–534, 1984.
3. Dolin R, Richman DD, Murphy BR, Fauci AS. Cell-mediated immune responses in humans after induced infection with influenza A virus. J Infect Dis **135**:714–719, 1977.
4. Welliver RC, Kaul TN, Sun M, Ogra PL. Defective regulation of immune responses in respiratory syncytial virus infection. J Immunol **133**:1925–1930, 1984.
5. Sakamoto M, Ida S, Takishima T. Effect of influenza virus infection on allergic sensitization to aerosolized ovalbumin in mice. J Immunol **132**:2614–2617, 1984.
6. Wong DT, Rosenband M, Hovey K, Ogra PL. Respiratory syncytial virus infection in immunosuppressed animals: Implications in human infection. J Med Virol **17**:359–370, 1985.
7. Edevåg G, Eriksson M, Granström M. The development and standardization of an ELISA for ovalbumin determination in influenza vaccines. J Biol Stand **14**:223–230, 1986.
8. Mota I, Wong D. Homologous and heterologous passive cutaneous anaphylactic activity of mouse antisera during the course of immunization. Life Sci **8**:813, 1969.
9. Jakab GJ, Green GM. The effect of sendai virus infection on bactericidal and transport mechanisms of the murine lung. J Clin Invest **51**:1989, 1972.
10. Carson JL, Collier AM, Hu S-CS. Acquired ciliary defects in nasal epithelium of children with acute viral upper respiratory infections. N Engl J Med **312**:463–468, 1985.
11. Gordon RE, Case BW, Kleinerman J. Acute NO_2 effects on penetration and transport of horseradish peroxidase in hamster respiratory epithelium. Amer Rev Respir Dis **128**:528, 1983.
12. Watson AY, Brain JD. The effect of SO_2 on the uptake of particles by mouse bronchial epithelium. Exp Lung Res **1**:67, 1980.
13. Braley JF, Peterson LB, Dawson CA, Moore VL. Effect of hypersensitivity on protein uptake across the air–blood barrier of isolated rabbit lungs. J Clin Invest **63**:1103–1109, 1979.
14. Tenner-Rácz K, Racz P, Myrvik QN, Ockers JR, Geister R. Uptake and transport of horseradish peroxidase by lymphoepithelium of the bronchus-associated lymphoid tissue in normal and *Bacillus* Calmette-Guérin-immunized and challenged rabbits. Lab Invest **41**:106, 1979.
15. Brody AR, Kelleher PC, Craighead JE. A mechanism of exudation through intact alveolar epithelial cells in the lungs of cytomegalovirus-infected mice. Lab Invest **39**:281, 1978.
16. Taylor G, Stott EJ, Hughes M, Collins AP. Respiratory syncytial virus infection in mice. Infect Immun. **43**:649–655, 1984.
17. Warren HS, Vogel FR, Chedid LA. Current status of immunological adjuvants. Annu Rev Immunol **4**:369, 1986.
18. Ramanathan VD, Badenoch-Jones P, Turk JL. Complement activation by aluminium and zirconium compounds. Immunology **37**:881, 1979.
19. Walls RS. Eosinophil response to alum adjuvants: Involvement of T-cells in non-antigen-dependent mechanisms. Proc Soc Exp Biol Med **156**:431–435, 1977.
20. Bensch KG, Dominguez E, Liebow AA. Absorption of intact protein molecules across the pulmonary air–tissue barrier. Science **157**:1204, 1967.
21. Willoughby JB, Willoughby WF. In vivo responses to inhaled proteins. I. Quantitative analysis of antigen uptake, fate and immunogenicity in a rabbit model system. J Immunol **119**:2137–2146, 1977.
22. Yoshizawa Y, Hostetter MW, Nakazawa T, Ripani LM, Dawson CA, Moore VL. Enhancement of systemic immune response by immunization into chronically inflammed lungs. J Lab Clin Med **100**:61, 1982.
23. Quinnan GV. Immunology of viral infections. In: Belshe RB, Ed. Textbook of Human Virology. Littleton, MA, PSG Pub Co, pp103–138, 1984.
24. Katz DH. Recent studies on the regulation of IgE antibody synthesis in experimental animals and man. Immunology **41**:1, 1980.
25. Kishimoto T, Ishizaka K. Regulation of antibody response in vitro. VI. Carrier-specific helper cells for IgG and IgE antibody response. J Immunol **111**:720–732, 1973.
26. Mozes E, Shearer GM, Sela M, Braun W. Conversion with polynucleotides of genetically controlled low immune response to high response in mice immunized with a synthetic polypeptide antigen. In: Beers P, Braun W, Eds. Biological Effects of Polynucleotides. New York, Springer-Verlag, pp197–213, 1971.

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