Journal of Leukocyte Biology 44:87–92 (1988)

Inhibition of Phagocytosis and Interleukin-1 Production in Pulmonary Macrophages From Rats With Sialodacryoadenitis Virus Infection

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To test whether or not sialodacryoadenitis virus (SDAV) infection in rats affects pulmonary macrophage function, we intranasally inoculated pathogen-free F344 rats with SDAV and collected alveolar and interstitial macrophages 5 d later. We assessed Fc receptor-mediated attachment and phagocytosis by phase-contrast microscopic examination of monolayers of alveolar and interstitial macrophages incubated with zymosan, nonopsonized sheep erythrocytes, or erythrocytes opsonized with rabbit antisheep-erythrocyte IgG. Alveolar macrophages from virus-infected rats had significantly ($P \leq .05$) lower indices of attachment and phagocytosis of opsonized erythrocytes than control macrophages, but there was no difference in attachment of zymosan particles. Interstitial macrophages were not affected. Alveolar macrophages from SDAV-infected rats produced significantly less interleukin-1 than those from control rats, as assessed by testing supernatants from lipopolysaccharide-stimulated macrophage cultures for induction of mouse thymocytes to take up tritiated thymidine. Effects of SDAV infection on lung macrophages could increase host susceptibility to other pathogens or complicate studies of respiratory tract immunity.

Key words: alveolar macrophages, respiratory tract infections, coronavirus infections

INTRODUCTION

Alveolar macrophages, by virtue of their phagocytic and microbicidal capabilities, constitute the primary nonspecific defense of the lungs against inhaled microorganisms and particulate matter [7,11]. Inasmuch as phagocytosis of most microorganisms by alveolar macrophages is enhanced by opsonization, especially with subclasses of IgG [7,11], Fc receptor-mediated phagocytosis by alveolar macrophages probably contributes to pulmonary resistance to infection.

Alveolar macrophages also can serve as accessory cells in specific immune responses by presenting antigen and regulating lymphoid cell responses, and they secrete a wide variety of substances, including enzymes, enzyme inhibitors, arachidonic acid derivatives, reactive oxygen metabolites, binding proteins, and growth-regulating factors such as interleukin-1 (IL-1) [7,21]. IL-1 is a principal mediator of immune and inflammatory responses and is produced in response to a wide variety of stimuli [5,6,25]. It stimulates, or enhances the effects of other lymphokines on, T and B lymphocytes, NK cells, and macrophages; it induces fever, neutrophilia, and synthesis by hepatocytes of acute-phase proteins; and it promotes fibroblast growth [5,6,25]. Thus, IL-1 probably is important in pulmonary responses to infection.

Many viral infections of the respiratory tract result in changes in phagocytic [8,10,13,14,19], bactericidal [9,13,14], cytotoxic [1,8,19], secretory [1], metabolic [16], or other functions of alveolar macrophages [1,8–10,13,14,16]. The significance of some of these effects is uncertain, but those contributing to decreased intrapulmonary bactericidal activity probably have an important role in the predisposition to bacterial pneumonia that commonly occurs in respiratory viral infections [13,14].

SDAV is a common coronavirus of rats that causes mild, acute disease characterized by necrotizing inflammation of the respiratory mucosa and the salivary and lacrimal glands [12,15]. Although rats commonly are used in investigations of respiratory system physiology, immunology, and pathology, studies of effects of SDAV infection on respiratory tract defense mechanisms have not been reported. Such effects could increase susceptibility of the host to other respiratory diseases and could complicate studies in rats of pulmonary macrophages and

Received February 8, 1988; accepted April 12, 1988.

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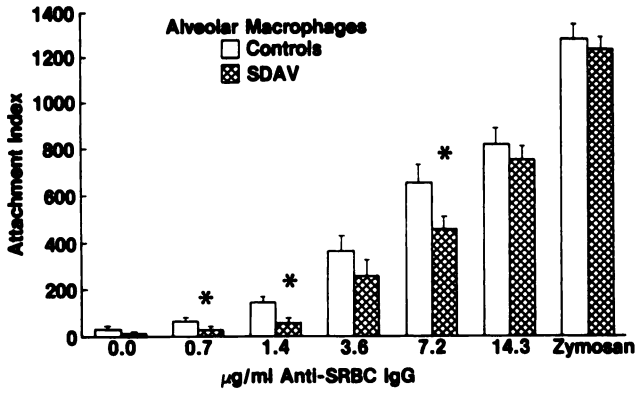

Fig. 1. Indices of attachment of zymosan particles or of SRBCs opsonized with various concentrations of anti-SRBC IgG to alveolar macrophages from rats 1 wk after SDAV inoculation and from control rats. Means ± standard deviations, each n = 9. $P \leq .05$ for differences between means marked *.

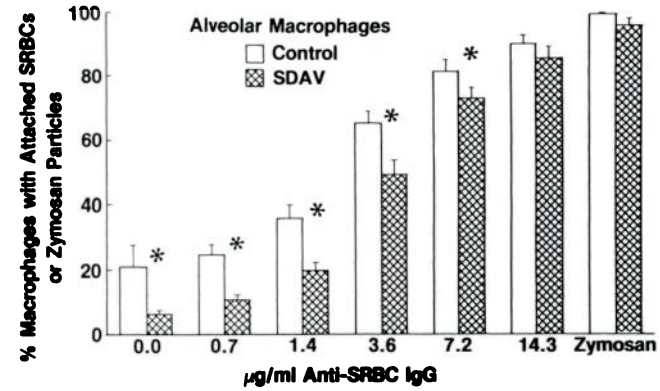

Fig. 2. Percentages of SDAV-infected rat and control rat alveolar macrophages with attached zymosan particles, or SRBCs opsonized with various concentrations of anti-SRBC IgG. Means ± standard deviations, each n = 9. $P \leq .05$ for differences between means marked *.

other respiratory tract defenses. To test whether or not SDAV infection could affect pulmonary macrophage functions, we assessed Fc receptor-mediated attachment and phagocytosis of sheep red blood cells (SRBCs) and release of IL-1 by pulmonary macrophages from pathogen-free rats inoculated with SDAV. Alveolar macrophages collected 5 d after inoculation from virus-infected rats bound and ingested fewer SRBCs and produced less IL-1 than macrophages from control rats.

MATERIALS and METHODS

Animals

Eight- to 10-wk-old pathogen-free male F344 rats and 6–8-wk-old pathogen-free female C3H/HeJ mice (Frederick Cancer Research Facility, Frederick, MD) were transported behind biological filters, housed in microisolators (Lab Products, Inc., Maywood, NJ), and provided with autoclaved food (Agway, Inc., Syracuse, NY), water, and hardwood bedding (Sani-Chip; P.J. Murphy Forest Products Corp., Rochelle Park, NJ).

Virus

SDAV (American Type Culture Collection, Rockville, MD) was grown in primary rat kidney cell culture [12] and stored at −70°C. For inoculations, rats were anesthetized by intramuscular injection of 0.05 ml of a 10:1 mixture of 100 mg/ml ketamine (Ketalar; Parke-Davis, Morris Plains, NJ) and 100 mg/ml xylazine (Rompun; Haver, Shawnee, KS). In each experiment, six rats were inoculated intranasally with 0.05 ml of virus stock (10^5 50% rat infectious doses) [22], and six control rats were given sterile medium.

Macrophage Collection

In three experiments, macrophages were collected 5 d after virus inoculation. In a fourth experiment, the cells were harvested 2 wk after inoculation. We obtained alveolar macrophages by repeated lung lavage [2–4]. Rats were anesthetized with pentobarbital (85 mg/kg given intraperitoneally) and then exsanguinated by severing the abdominal aorta. The trachea was cannulated with sterile polypropylene tubing fixed to an 18-gauge needle, and with gentle massage of the thoracic cavity, the lungs were lavaged six times in situ with a total of 50 ml cold (4°C) Dulbecco's phosphate-buffered saline without Ca^{+2} and Mg^{+2} (Gibco, Grand Island, NY). The lavage fluid was filtered through a single layer of sterile gauze to remove mucus and then centrifuged at 400*g* for 10 min at 4°C. Cell pellets from two rats were combined, washed twice with Dulbecco's solution, and resuspended in RPMI-1640 medium (Gibco) supplemented with 10% heat-inactivated fetal calf serum (Hyclone Laboratories, Logan, UT), 100 U/ml penicillin, and 100 μg/ml streptomycin (RPMI-FCS).

Interstitial or lung parenchymal macrophages were harvested according to described methods [4]. The left atrium was cut and the lung vasculature was perfused with sterile saline via the right ventricle until the fluid was clear. The lung parenchyma was dissected free of airways and vessels, placed in RPMI-FCS, teased, and crushed. The resulting cell suspensions were filtered through a single layer of sterile gauze to remove tissue fragments and then centrifuged at 400*g* for 10 min at 4°C. Residual red blood cells were lysed with hypotonic saline and the suspensions were again centrifuged. Cell pellets from two rats were combined, washed twice with Dulbecco's saline, and resuspended in RPMI-FCS. Cell viability and number were determined by trypan blue exclusion and counting in a hemocytometer.

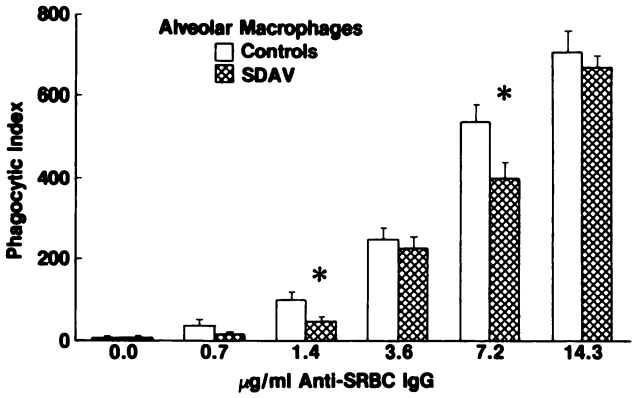

Fig. 3. Indices of phagocytosis of SRBCs opsonized with various concentrations of anti-SRBC IgG by alveolar macrophages from rats 1 wk after SDAV inoculation and from control rats. Means ± standard deviations, each n = 9. $P \leq .05$ for differences between means marked *.

Preparation of Antibody-Coated Erythrocytes

SRBC in Alsever's solution (Colorado Serum Co., Denver, CO) were washed three times in Dulbecco's saline without Ca^{+2} and Mg^{+2} and suspended in veranol-buffered glucose containing Ca^{+2} and Mg^{+2} and 0.1% gelatin (VBG) to a concentration of 5% (vol/vol). One-milliliter aliquots of 5% SRBC suspension were incubated with 14.3, 7.2, 3.6, 1.4, or 0.7 μg rabbit anti-SRBC IgG (Cordis Laboratories, Miami, FL) for 15 min at 37°C and then centrifuged at 400*g* for 10 min at 4°C. The pelleted SRBCs were resuspended in VBG at a concentration of 0.1%.

Phagocytosis and Attachment Assays

Macrophages were cultured as previously described [3, 4]. Triplicate samples of up to 50,000 pooled alveolar or interstitial macrophages were allowed to adhere to eight-chamber tissue culture slides (Lab-Tek; Miles Laboratories, Naperville, IL) for 1 hour at 37°C and 5% CO_2. Nonadherent cells were then removed. Adherent cells were washed with Dulbecco's PBS with Ca^{+2} and Mg^{+2}, cultured for 24 hr in RPMI-FCS, washed twice with Dulbecco's PBS with Ca^{+2} and Mg^{+2}, and supplied with 0.25 ml of RPMI in each well. Attachment and phagocytic activities of the macrophages were determined by incubating monolayers with opsonized SRBCs, nonopsonized SRBCs, or 0.1% zymosan (Sigma, St. Louis, MO) for 30 min at 37°C. (Zymosan was included as an additional control because, unlike nonopsonized SRBCs, zymosan particles attach well. Also, zymosan attachment is mediated by mannose receptors [23], and is thus independent of Fc receptor-mediated attachment.) Cultures were then washed with PBS and fixed with 2.5% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.4. Erythrocyte attachment and internalization were scored by microscopic examination with a 100× phase-contrast oil-immersion objective. In attachment assays, all SRBCs associated with a macrophage were counted. In assessments of phagocytosis, external SRBCs were lysed with water, and only internalized SRBCs were counted. Raw results were corrected for nonopsonized erythrocyte attachment and internalization. Percentages of alveolar and interstitial macrophages with attached or internalized SRBCs and numbers of attached or internalized SRBCs per macrophage were obtained by examining at least 100 cells in each well. Results were expressed both as percentages of macrophages with internalized or attached SRBCs and as phagocytic and attachment indices, which for each sample of pooled cells was the percentage of macrophages with internalized or attached SRBCs multiplied by the average number of SRBCs internalized or attached per macrophage. Results with zymosan were assessed in the same fashion, but phagocytic indices were not determined because of the lack of a reliable method for distinguishing internalized particles from attached ones overlying the cells.

IL-1 Assay

Pooled alveolar macrophages collected 5 d after virus inoculation were washed once in RPMI medium containing 2 mM L-glutamine (Flow Labs, McLean, VA), 10% fetal calf serum, and 90 μg/ml gentamicin (Schering Corp., Kenilworth, NJ). After adjustment of the cell suspension to 1×10^6 cells/ml, 0.20 ml of suspension was added to wells of 96-well flat-bottom plates (Costar, Cambridge, MA). After culture for 24 hr with or without 10 μg/ml *E. coli* lipopolysaccharide (LPS) (Sigma, St. Louis, MO), medium was collected from the cultures and assayed for IL-1 activity [17]. C3H/HeJ thymocytes were suspended in RPMI 1640, adjusted to 1.5×10^7 cells/ml, and transferred in 0.10-ml aliquots to wells of 96-well flat bottom plates. The thymocytes were incubated for 72 hr with 0.2 μg phytohemagglutinin (PHA) (Sigma, St. Louis, MO) in 0.10 ml RPMI 1640 and with either triplicate 0.05-ml samples of macrophage culture supernatants combined with 0.05 ml of RPMI 1640, or with 0.10 ml of control RPMI 1640 medium. Cultures were pulsed with 1 μCi ^{3}H-thymidine (Amersham International, Amersham, UK) for the final 6 hr of incubation. Thymocytes were harvested with a semiautomated cell harvester (Skatron, Sterling, VA), and incorporated radioactivity was measured by liquid scintillation counting. We recognize that although this method is widely used and accepted, as a biological assay it does not directly identify IL-1. Therefore, we cannot exclude the possibilities, however unlikely they might be, that stimulatory macrophage supernatants could contain growth-promoting factors other than IL-1, or that less active superna-

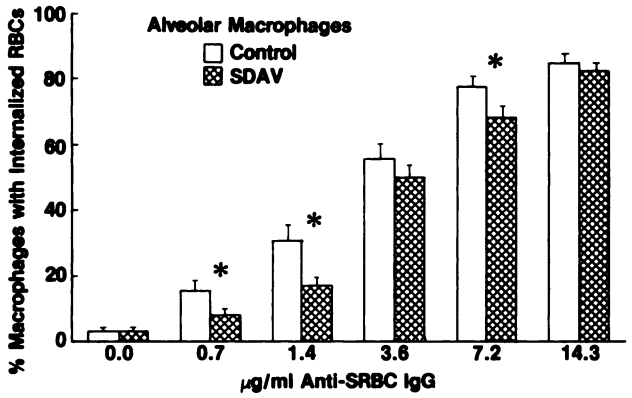

Fig. 4. Percentages of SDAV-infected rat and control rat alveolar macrophages with internalized SRBC opsonized with various concentrations of anti-SRBC IgG. Means ± standard deviations, each n = 9. $P \leq .05$ for differences between means marked *.

tants could contain inhibitory substances. We refer to IL-1 activity or IL-1 production with this understanding.

Statistics

We used Student's t-test [24] to test differences between macrophages from SDAV-infected and control rats and considered differences significant when $P \leq .05$.

RESULTS

None of the rats inoculated with SDAV had clinical signs other than cervical swelling or had gross lesions other than edema of the connective tissue surrounding the salivary glands. We did not histologically examine tissues from the rats, but in previous studies [22] the virus caused acute sialoadenitis with cervical edema and mild necrotizing inflammation of the respiratory mucosa. These lesions are typical of SDAV infection [12].

Each rat yielded three million to five million macrophages. Cytospin analysis (Shandon Southern Corp., Sewickley, PA) indicated that ≥ 95% of lavaged cells were macrophages, and their viability as determined by trypan blue exclusion was about 90% in all experiments. SDAV infection had no statistically significant effect on the numbers or viability of recovered macrophages.

Attachment indices for alveolar macrophages collected from virus-infected rats 5 d after virus inoculation were significantly lower than those for macrophages from control rats for SRBCs opsonized at 7.2, 1.4, and 0.7 μg/ml IgG (Fig. 1). Virus-infected rats also had significantly smaller percentages of alveolar macrophages with attached SRBCs opsonized at antibody concentrations of 7.2, 3.6, 1.4, and 0.7 μg/ml and significantly fewer macrophages with attached unopsonized SRBCs (Fig. 2). Phagocytic indices for alveolar macrophages from virus-infected rats were significantly lower for SRBCs opsonized at 7.2 and 1.4 μg/ml IgG (Fig. 3), and smaller percentages of alveolar macrophages from infected rats ingested SRBCs opsonized with 7.2, 1.4, and 0.7 μg/ml antibody (Fig. 4). Indices of zymosan attachment and percentages of macrophages with attached zymosan particles were not statistically different between macrophages from virus-infected and control rats. At 2 wk after virus inoculation, alveolar macrophages from virus-infected rats did not differ from those from uninoculated control rats in SRBC attachment or ingestion (data not shown). Interstitial macrophages from SDAV-infected rats were not significantly different from those from control rats in attachment or phagocytic indices or in the percentages of cells with attached or phagocytized SRBC at either 5 d or 2 wk after inoculation (data not shown).

LPS-induced production of IL-1 activity by alveolar macrophages from rats inoculated with SDAV 5 d earlier was significantly less than that from macrophages from control rats. Thymocytes incubated in culture supernatants from macrophages from virus-infected rats had 7,180 ± 1,438 counts per minute (CPM) (mean ± standard deviation, n = 6), whereas thymocytes stimulated by supernatants from control rat macrophages had 15,450 ± 2,186 CPM (n = 6). Spontaneous (unstimulated) release of IL-1 activity was not statistically different between macrophages from virus-infected rats (102 ± 45 CPM, n = 6) and those from control rats (250 ± 154 CPM, n = 6). We did not assess IL-1 production in the experiment in which macrophages were collected 2 wk after virus inoculation.

DISCUSSION

Results of this study show that alveolar macrophages from rats with acute SDAV infection had decreased capacities for Fc receptor-mediated attachment and phagocytosis of SRBCs and for production of IL-1, suggesting that, like other respiratory viral infections [13,14], SDAV infection could increase susceptibility of the host to bacterial infection and disease. This possibility is supported by the finding that concurrent SDAV infection increases severity of murine respiratory mycoplasmosis [22], a common condition in rats maintained conventionally in research facilities [15]. However, it has not been determined that this results from inhibition of alveolar macrophage functions.

Our results are consistent with those of most previous studies of effects of viral infections on alveolar macrophage functions. In most cases, phagocytosis mediated nonspecifically or by Fc or complement receptors was inhibited [8,10,13,14,19], although in some studies transient stimulation preceded inhibition [8,19], and one report describes only enhancement of phagocytosis [1]. Effects of viral infection on IL-1 production by alveolar

macrophages have not been extensively studied. In many cases, IL-1 production is stimulated by in vitro exposure of macrophages to viruses [6,20]; however, IL-1 production by alveolar macrophages from calves inoculated with bovine herpesvirus was inhibited [1]. Various other alveolar macrophage functions also can be altered in viral infections [1,8,10,13,14,16,19].

The mechanisms responsible for these effects are not known in most instances. However, results of studies in mice of Sendai virus and influenza virus infections, which are known to predispose the host to secondary bacterial pneumonia, indicate that most of the pulmonary macrophage functional defects in these infections result from effects of antiviral immune responses on macrophages containing viral antigen [13,14]. Viral infections that damage type II pneumonocytes also could impair alveolar macrophage antibacterial activity by reducing availability of pulmonary surfactant, inasmuch as surfactant can aid phagocytosis and killing of bacteria by alveolar macrophages [18].

Further studies will be necessary to determine the basis for the effects of SDAV infection on alveolar macrophages. It is not known, for example, whether or not the virus infects alveolar macrophages and replicates in them. In immunofluorescence studies [12,26], SDAV antigens have been found only in epithelial cells of the airways, salivary glands, and lacrimal glands, although infectious virus was present in cervical lymph nodes as well as respiratory organs [12]. In any case, it seems likely that SDAV affects phagocytosis of particles mediated by different receptors to different degrees, because although attachment and phagocytosis of SRBC were decreased, zymosan attachment was not affected. SDAV infection did not affect interstitial macrophages, possibly because SDAV infection affects only the epithelium of the respiratory tract, causes mild or no pneumonitis in adult rats, and does not result in viremia, insofar as is known [12, 26]; therefore, interstitial macrophages may not have been exposed to the virus. The transience of the effects of the viral infection on alveolar macrophages in our experiments could have been because the virus does not persist in tissues more than 8–10 d after inoculation [12].

Our results also indicate that naturally occurring SDAV infection could complicate pulmonary macrophage research in which rats are used. Therefore, such rats should be purchased from a source known to be free of the virus, and should be maintained in effective barrier housing.

ACKNOWLEDGMENTS

We thank Frank M. Griffin, Jr., for use of the phase-contrast microscope.

This work was supported by Public Health Service grants RR00463, RR07003, and HL07553 from the National Institutes of Health and by the Veterans Administration Research Service.

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