

# Immunoglobulin Secreting Cells in Normal Human Bronchial Lavage Fluids

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**ABSTRACT** Immunoglobulin secreting cells were quantitated in the bronchial lavage fluids of 12 normal volunteers and compared with immunoglobulin secreting cells in peripheral blood, by a reverse hemolytic plaque assay. The mean number of cells secreting immunoglobulin (Ig)G in bronchial lavage fluids was 489 per million lymphocytes vs. a mean of 175 IgG secreting cells per million lymphocytes in peripheral blood ( $P < 0.02$ ). The mean number of IgA secreting cells in bronchial lavage fluids was 633 per million lymphocytes as compared to 100 per million lymphocytes in peripheral blood ( $P < 0.005$ ). Thus, compared to peripheral blood, cells from the lavage fluids were relatively enriched for both IgG and IgA secreting cells. However, IgA secreting cells were the major class of immunoglobulin secreting cells in bronchial lavage fluids, whereas IgG secreting cells predominated in peripheral blood. The prominence of IgA secreting cells in bronchial lavage fluids was further demonstrated by a mean ratio of IgA/IgG secreting cells in bronchial lavage fluids of 1.26 compared to a ratio in peripheral blood of 0.57 ( $P < 0.02$ ). Cells secreting IgM were identified in only four of seven bronchial lavage fluid samples studied but in all peripheral blood samples. IgE secreting cells were not present in normal peripheral blood but could be demonstrated in 5 of 11 lavage fluid specimens. Thus, cells actively secreting immunoglobulins can be identified in the lower bronchial-alveolar tree of normal human subjects. Cells secreting IgG, IgA, or IgM may function in local lung defenses against infection; cells secreting IgE may contribute to hypersensitivity reactions in the lung.

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## INTRODUCTION

The lung is an immunocompetent organ capable of an antibody response to particulate antigen (1-3). Cells with immunologic capabilities can be demonstrated in the lung parenchyma (3, 4), the bronchial associated lymphoid tissue (5), and the distal bronchial-alveolar airways (6, 7) in animal models as well as in man. The relative composition of cells from the human lower bronchial-alveolar tree, obtained by fiberoptic bronchoscopy and bronchial lavage, has been well defined (8-10), and the functional capabilities of these cells are now being investigated by several laboratories (11, 12).

In the present study, we have assessed the immunoglobulin secretory capacity of normal human bronchial-alveolar cells, by a modified reverse hemolytic plaque assay (13). In this assay, indicator sheep erythrocytes (SRC)<sup>1</sup> that have been precoated with Protein A (*Staphylococcus aureus*) are mixed in agar with mononuclear cells in the presence of rabbit IgG developing antisera specific for either human IgG, IgA, IgM, or IgE. Because Protein A has an affinity for the Fc portion of IgG, the developing antisera, bound to the secreted immunoglobulin of the appropriate class, bind to the Protein A-coated SRC as an immune complex. With the addition of complement, the immune complex-laden SRC surrounding an immunoglobulin secreting cell are lysed, producing a plaque. Such plaques have been shown to be inhibitable by cycloheximide (14, 15), a reversible inhibitor of protein synthesis, but not by maneuvers to remove cytophilic immunoglobulins (16). Thus, with monospecific developing antisera, it is possible to identify cells actively secreting either IgG, IgA, IgM, or IgE in bronchial lavage fluids or peripheral blood.

<sup>1</sup> Abbreviation used in this paper: SRC, sheep erythrocytes.

## METHODS

**Study subjects.** 12 normal volunteers at Baylor College of Medicine, Houston, Tex. served as the subjects for this study after giving informed written consent. Seven were male and five were female, ranging in age from 23 to 41 (mean age, 28.5) yr. Four of the subjects were cigarette smokers, but because of the small size of the study population, no differentiation was made between cigarette smokers and non-smokers in data analysis.

**Preparation of mononuclear cells.** Bronchial lavage was performed on each volunteer as previously described (11). The recovered cells (mean  $\pm$  SEM,  $31.8 \pm 5.7 \times 10^6$ ) were washed twice in saline and twice more in McCoy's 5a medium (modified) Grand Island Biological Co., Grand Island, N. Y.). Mononuclear cells were obtained from blood by Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, N. J. and Winthrop Laboratories, Sterling Drug, Inc., New York) centrifugation as previously described (11), and were washed once with sterile saline and three more times with McCoy's medium.

**Reagents.** Antisera against either human IgG, IgA, IgM, or IgE were raised in rabbits and purified by solid phase absorption until each antiserum could be shown to be monospecific by ochterlony analysis and immunoelectrophoresis. Protein A (*S. aureus*) (Pharmacia Fine Chemicals) was coupled to SRC with  $\text{CrCl}_3$  as previously described (13). The Protein A-SRC were washed three times and diluted to a 30% suspension in balanced salt solution.

**Enumeration of immunoglobulin secreting cells.** To  $10 \times 75$ -mm glass culture tubes, preheated to  $43^\circ\text{C}$ , were added 0.25 ml of 1.12% agarose (SeaPlaque, Marine Colloids, Inc., Rockland, Me.).  $2.5 \times 10^5$  or  $1 \times 10^6$  mononuclear cells from either peripheral blood or bronchial lavage fluids were then added in a 0.1-ml vol to duplicate tubes, followed by 0.025 ml of 30% Protein A-SRC.

The contents of the tubes were then mixed with a vortex mixer and transferred with a pipet to  $60 \times 15$ -mm Petri

dishes that had been precoated with 4 ml of 2.5% agarose. The petri dishes were quickly swirled to uniformly distribute the cell-agar mixture into a monolayer. The agarose was allowed to solidify and 1 ml of appropriately diluted developing antisera, or media as a control, was added to each dish. All petri dishes were placed in a humidified 5%  $\text{CO}_2$  incubator at  $37^\circ\text{C}$  for 1 h. Then 1 ml of a 1:8 dilution of SRC-absorbed guinea pig complement was added to each dish. The dishes were incubated for an additional 2 h and the fluid was aspirated.

After overnight incubation at  $4^\circ\text{C}$ , the dishes were examined under  $\times 10$  magnification and the number of plaques in the duplicate dishes were determined for each class of developing antisera, as well as for the control dishes which had not been exposed to developing antisera. The number of plaques in the control dishes (usually 0–5) represents cells secreting IgM anti-SRC antibodies, and this number was subtracted from the number counted in dishes containing developing antisera for IgG, IgA, and IgE. The data were then adjusted for the percentages of lymphocytes in Ficoll-Hypaque preparations of peripheral blood and the bronchial lavage (from Wright-Giemsa stains) and expressed as immunoglobulin secreting cells per million lymphocytes.

## RESULTS

**IgG secreting cells.** The mean number of IgG secreting cells in bronchial lavage fluids was 489 per million lymphocytes vs. a mean of 175 in peripheral blood (Table I). This difference was statistically significant ( $P < 0.02$ , by Student's paired  $t$  test, two-tailed). Thus, cells from bronchial lavage fluids were relatively enriched in IgG secreting cells compared to peripheral blood.

**IgA secreting cells.** The mean number of IgA

TABLE I  
Comparison of Immunoglobulin Secreting Cells in Normal Bronchial Lavage Fluids and Peripheral Blood

Subject no.	IgG secreting cells		IgA secreting cells		IgM secreting cells		IgE secreting cells	
	Blood	Lavage	Blood	Lavage	Blood	Lavage	Blood	Lavage
1	ND	*ND	ND	837	ND	233	ND	188
2	192	833	199	2,083	32	0	0	0
3	459	408	343	284	35	46	0	0
4	67	560	13	160	8	ND	ND	570
5	225	143	71	57	40	0	ND	0
6	44	343	13	1,043	23	443	ND	143
7	431	720	392	2,610	ND	ND	ND	290
8	316	1,256	1,416	2,950	ND	ND	0	575
9	53	57	36	91	ND	ND	0	0
†10	253	454	68	546	200	111	ND	0
†11	355	1,033	89	2,408	20	ND	ND	ND
†12	ND	1,688	ND	950	ND	0	ND	0
Geometric mean	175	489	100	633	§32	§18	§0	§13
(95% confidence)	(102–301)	(268–891)	(38–261)	(290–1,380)	(15–67)	(2–144)		(2–83)
‡P value	<0.02		<0.005		>0.40		ND	

\* ND = not determined.

† Samples were stored at  $4^\circ\text{C}$  overnight, then washed and assayed.

‡ For determination of geometric mean values, data of 0 were entered into the calculations as ln 1 (or 0).

§ P values were calculated by Student's paired  $t$  test, with two tails.

secreting cells in bronchial lavage fluids was 633 per million lymphocytes as compared to a mean of 100 in peripheral blood. This difference was highly statistically significant ( $P < 0.005$ ), indicating that bronchial lavage fluids were particularly enriched for IgA secreting cells when compared to peripheral blood. Moreover, as again shown in Table I, the major class of immunoglobulin secreting cells in bronchial lavage fluids was IgA, whereas that of peripheral blood was IgG. This relative prominence of IgA secreting cells in bronchial lavage fluids is further emphasized in Fig. 1, which compares the ratio of IgA/IgG secreting cells in bronchial lavage fluids and peripheral blood. The mean ratio of IgA/IgG secreting cells in bronchial lavage fluids was 1.26 vs. a mean ratio of 0.57 in peripheral blood ( $P < 0.02$ ). This relative enrichment of the bronchial fluids for IgA secreting cells may reflect the importance of locally produced IgA to local pulmonary immunity.

**IgM secreting cells.** IgM secreting cells were identified in only four of seven bronchial lavage

samples studied, with a geometric mean value for all samples studied of 17 IgM secreting cells per million lymphocytes. Low numbers of IgM secreting cells were found in all peripheral blood samples studied, with a mean of 32 per million lymphocytes (difference not significant). Because free IgM has not usually been detected by conventional means in normal bronchial lavage fluids (17), the relative paucity of IgM secreting cells in bronchial lavage fluids is not surprising.

**IgE secreting cells.** IgE secreting cells were present in 5 of 11 lavage fluid specimens, with a geometric mean for all samples studied of 13 IgE secreting cells per million lymphocytes. Statistical comparison with peripheral blood IgE secreting cells was not possible because only four peripheral blood samples from these normals were studied. However, we have assayed numerous other normal peripheral blood samples not included in the present study and have never found IgE secreting cells. Thus, the finding of IgE secreting cells in bronchial lavage fluids is of particular significance, perhaps reflecting the role of the lung in hypersensitivity reactions.

## DISCUSSION

Cells obtained by fiberoptic bronchoscopy and bronchial lavage in humans have been shown to mediate cellular immunity, as measured by proliferative responses to mitogens (12). In the present study, we have shown that bronchial lavage cells may also mediate humoral immunity, as measured by the capacity to secrete immunoglobulins of the four major human classes. The prominence of IgA secreting cells in bronchial lavage fluids may indicate the importance of locally produced IgA to local pulmonary immunity. The presence of IgE secreting cells in bronchial lavage fluids suggests a role for these cells in hypersensitivity lung diseases such as asthma.

The ability to enumerate class-specific immunoglobulin secreting cells in bronchial lavage fluids, as described here, brings a new dimension to the functional analysis of immunocompetent pulmonary cells. With this or similar techniques, it may become feasible to better define the local pulmonary immunoregulatory mechanisms that are operative in normal and diseased lungs.

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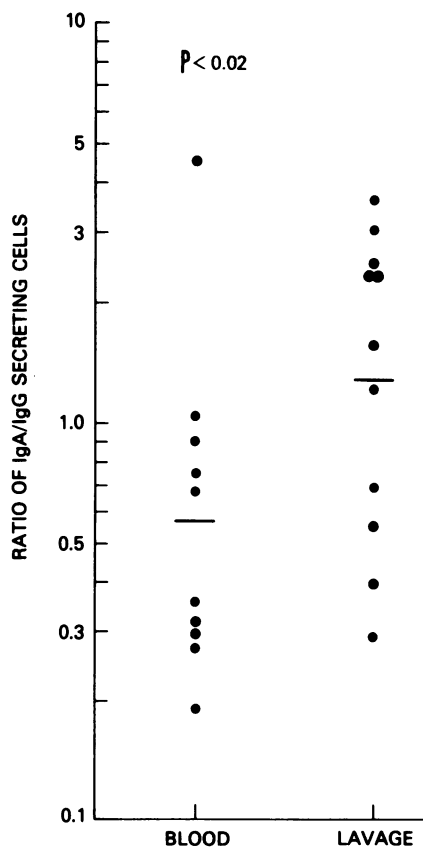


FIGURE 1. Ratio of IgA/IgG secreting cells in peripheral blood and bronchial lavage. The geometric mean ratio for peripheral blood was 0.57 vs. geometric mean for bronchial lavage of 1.26 ( $P < 0.020$ , Student's paired  $t$  test, two tailed).

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