Induction of Indoleamine 2,3-Dioxygenase in Alveolar Interstitial Cells of Mouse Lung by Bacterial Lipopolysaccharide*

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The cellular localization of indoleamine 2,3-dioxygenase was studied in the mouse lung after induction by lipopolysaccharide treatment. No significant indoleamine 2,3-dioxygenase activity was detected in alveolar macrophages and type II epithelial cells, which were recovered by alveolar lavages and trypsin-treatment, respectively. To determine this enzyme activity in other types of lung cells, we prepared monodispersed lung cells (6.5 x 10⁷ cells/lung) by incubation with 0.1% collagenase and 0.1% trypsin. In a Percoll isopycnic gradient, the dispersed cells were distributed with two peaks at the densities of 1.040 and 1.080 g/ml. The enzyme activity was recovered exclusively in the lighter fractions. As examined by electron microscopy or more quantitatively by using various marker enzyme activities, endothelial cells (angiotensin-converting enzyme as a marker enzyme of these cells), alveolar interstitial cells (prostaglandin dehydrogenase), type I epithelial cells, type II epithelial cells, alveolar macrophages (β-gluconuridase), Clara cells (counarin hydroxylase), and polymorphonuclear leucocytes (arylsulfatase) were distributed with peaks at the densities of 1.033, 1.040, 1.042, 1.045, 1.070, 1.082, and 1.093 g/ml, respectively. The distribution pattern of the indoleamine 2,3-dioxygenase activity exactly coincided with that of alveolar interstitial cells. The localization of this enzyme in alveolar interstitial cells was immunohistochemically confirmed with the anti-indoleamine 2,3-dioxygenase antibody.

Indoleamine 2,3-dioxygenase (indoleamine:oxygen 2,3-oxidoreductase (decyclizing)) catalyzes the oxygenative cleavage of the pyrrole ring of various indoleamine precursors and indoleamines such as tryptophan, 5-hydroxytryptophan, tryptamine, and serotonin (1-5). After an intraperitoneal administration of bacterial LPS† (6, 7), or during influenza virus infection (8), the enzyme was induced about 100-fold in the mouse lung. The induction was also observed in the lung slices incubated with LPS in vitro (9), indicating that the induction occurred in the cells which originally existed in the lung. We therefore attempted to isolate and identify the cells responsible for the indoleamine 2,3-dioxygenase induction. However, many dissociation procedures previously reported on the rabbit or rat lung (10-15) failed to disperse the mouse lung cells enriched in the indoleamine 2,3-dioxygenase activity after the LPS treatment. We developed a new dissociation method for the mouse lung in which the intact lung without mincing was dispersed by the incubation with 0.1% collagenase and 0.1% trypsin. By this method, about 40% of lung cells was recovered in the dispersed cell preparation, as judged by the recoveries of protein and various enzyme activities. The dispersed cells were further fractionated in a Percoll isopycnic gradient. For the assessment of the distribution patterns of various lung cells in the gradient, we used two independent criteria. One is the morphological identification by electron microscopy. In the course of this study, we have noted some morphological characteristics of dispersed alveolar interstitial cells. To the best of our knowledge, this is the first report for the characterization of these dispersed cells. The other is the biochemical examination by using various marker enzymes specific for each type of lung cells. This biochemical approach allowed us more quantitative assessment of the distribution profile of various lung cells than the morphological approach. The cellular localization of indoleamine 2,3-dioxygenase was further examined by the immunofluorescence staining with the monospecific antibody for this enzyme. The results described in this report indicate that the indoleamine 2,3-dioxygenase induction occurred exclusively in the alveolar interstitial cells.

MATERIALS AND METHODS

Chemicals—Trypsin, LPS derived from Escherichia coli 055:B5 prepared by the Westphal method (Difco), soybean trypsin inhibitor, deoxyribonuclease I (DNase) type 1 (Sigma), collagenase type 3 (Worthington), and Percoll (Pharmacia) were purchased from the manufacturers. Fluorescein isothiocyanate-labeled goat anti-rabbit Immunoglobulin (IgG) (the molar ratio of fluorescein to protein = 1.3) was a product of Medical Biological Laboratory, Nagoya, Japan, and was further purified by column chromatography on Sephadex G-200. L-[ring-2,4 C]Tryptophan (36 Ci/mol) was obtained from Commissariat a l'Energie Atomique, France, and purified by column chromatography on Dowex 50W-X2 (H⁺ form) (0.5 x 20 cm), as previously described (5). [1,2 C]Prostaglandin E₂ (66 Ci/mol) was purchased from Amersham Radiochemicals. All other chemicals were of reagent grade.

Animals—All animals used in this study were male SlcICR mice, 8 to 12 weeks-old, weighing 35 ± 2 g. All mice were purchased from the Shizuoka Agricultural Cooperative Association for Laboratory Animals, Shizuoka, Japan, and raised under specific pathogen-free conditions in an air-conditioned room at 25 ± 2 °C and about 50% humidity at the Institute of Laboratory Animals, Kyoto University. Mice were used for the experiments 24 h after an intraperitoneal administration of LPS (20 μg/mouse) (6). Control mice were treated with sterile pyrogen-free 6.9% NaCl solution.

Preparation of Alveolar Free Cells—Under pentobarbital anesthesia and heparinization, lungs were perfused thoroughly with saline via a
pulmonary artery and were lavaged five times with 1.5 ml of saline. Alveolar free cells were recovered from the pooled lavage fluid by centrifugation at 500 \( \times \) g for 10 min, and washed twice in ice-cold saline by the same method.

**Dispersion of Type II Epithelial Cells—** Alveolar type II epithelial cells dispersion was obtained by the method of previous investigators (16-18). The procedure consisted of three modifications. A solution containing 100 mm NaCl and 20 mm Tris-HCl (pH 7.4) was used as a buffer unless otherwise stated. A 1.0% trypsin solution in the buffer (1.5 ml/lung) was introduced through a tracheal catheter into the lung after perfusion and alveolar lavages. The trachea was tied and the lung instilled with the trypsin solution and was emulsified intact. Three lungs were immersed in 10 ml of the buffer and then incubated at 37 \( ^\circ \)C for various intervals. After the incubation, a 2.0% soybean trypsin inhibitor solution (5 ml) was added. The lung lobes were minced into about 2-\( \mu \)m fragments, agitated with a DNase solution (50 \( \mu \)g/ml) for 5 min at 37 \( ^\circ \)C to prevent the aggregation of cells, and placed into an ice-bath. The cold suspension was filtered through a nylon cloth (40-\( \mu \)m mesh), and the filtrate was centrifuged at 500 \( \times \) g for 30 min to recover the dispersed cells. The recovered cells and the undisguised tissue trapped on the nylon cloth were washed twice with the cold buffer in the same way.

**Isopycnic Gradient Centrifugation of Dispersed Lung Cells—** Lungs were perfused with saline following with 0.1% collagenase and 0.1% trypsin solution in the buffer containing 5 mm CaCl\(_2\). After alveolar lavages with saline, the protease solution (1.5 ml) was introduced into the lung. The lung instilled with the protease solution was excised intact with a small portion of lung tissue from the base. The pieces were minced into 10 ml of the buffer solution and incubated at 37 \( ^\circ \)C for 20 min with gentle mixing. At this stage, the lung tissue was almost completely disintegrated. After major airways and blood vessels were removed, the suspension was transferred into a spinner flask (Bellco Glass). A 0.1% collagenase solution (10 ml) containing 0.2% soybean trypsin inhibitor was added to minimize inactivation of collagenase and cell damage by excess trypsin-treatment, and then the cell suspension was further incubated at 37 \( ^\circ \)C for 30 min with mixing. After the incubation, the suspension was centrifuged at 500 \( \times \) g for 5 min and washed twice with the cold buffer to remove the excess trypsin inhibitor. The pellet was resus- pended in the 0.1% collagenase and 0.1% trypsin solution (5 ml) containing 50 \( \mu \)g/ml of DNase, and incubated in the spinner disk in the same way to obtain the monodispersed cells. After 10 min, a 0.2% soybean trypsin inhibitor solution (5 ml) was added and then the suspension was placed into an ice-bath. The cell suspension was filtered through two kinds of nylon cloths in sequence (40- and 40-\( \mu \)m mesh). The filtrate was centrifuged and the pellet was washed twice with the cold buffer by the same method.

**Isopycnic Gradient Centrifugation of Lung Cells—** The dispersed cells were applied to two kinds of linear gradient. One which ranged from 5% (v/v) Percoll in the buffer (density 1.037 g/ml) to the upper interface with the cover layer to minimize inactivation of collagenase and cell damage by excess trypsin-treatment, and then the cell suspension was further incubated at 37 \( ^\circ \)C for 30 min with mixing. After the incubation, the suspension was centrifuged at 500 \( \times \) g for 5 min and washed twice with the cold buffer to remove the excess trypsin inhibitor. The pellet was resus- pended in the 0.1% trypsin solution containing 0.1% trypsin solution (5 ml) containing 50 \( \mu \)g/ml of DNase, and incubated in the spinner disk in the same way to obtain the monodispersed cells. After 10 min, a 0.2% soybean trypsin inhibitor solution (5 ml) was added and then the suspension was placed into an ice-bath. The cell suspension was filtered through two kinds of nylon cloths in sequence (40- and 40-\( \mu \)m mesh). The filtrate was centrifuged and the pellet was washed twice with the cold buffer by the same method.

**Biochemical Determinations—** Lung tissues and cells were homogenized with 5 volumes of ice-cold 0.14 M KCl, 20 mm Tris-HCl buffer (pH 7.4) by using a Kinematica Polytron homogenizer (Lacern, Switzerland) and a Bronson Sonifier disruptor (Heat Systems Ultrasonic, Plainview, NY), respectively. The homogenate was centrifuged at 30,000 \( \times \) g for 30 min, and the resulting supernatants were used as the enzyme source, except for the assays of coumarin hydroxylase and ACE, for which the homogenates were used as the enzyme source. The indoleamine 2,3-dioxygenase activity was determined as previously described (5). \( \beta \)-Glucuronidase (EC 3.2.1.31) (20), arylsulfatase (EC 3.1.6.1) (21), coumarin hydroxylase (22, 23), and angiotensin-converting enzyme (EC 3.4.15.1) (24) activities were estimated as described by previous investigators. The 15-Hydroxyprostaglandin dehydrogenase (EC 1.1.1.41) activity was assayed by the formation of 15-ketoprostaglandin E\(_2\) and 13,14-dihydro-15-ke- toprostaglandin E\(_2\) as described by Moore and Hout (25), using 1-\( ^{14} \)C prostaglandin E\(_2\) as a substrate. Protein concentration was deter- mined by the method of Lowry et al. (26) using bovine serum albumin as a standard.

**Morphological Examination—** For light microscopy, fresh cell smears were stained with a May-Grünwald-Giemsa solution. To identify type II epithelial cells, we used the fluorescence technique with phosphine SR (17). For electron microscopy, the lung tissue and the cells were fixed for 0 h in 2% glutaraldehyde in 0.1 M potassium phosphate buffer (pH 7.4), postfixed in 2% osmium tetroxide, dehydrated in ethanol, and embedded in Epon by the routine technique. Thin sections were cut with a Porter-Blum ultramicrotome, stained with uranyl acetate and lead citrate, and examined in a JEM 100CX electron microscope.

**Immunofluorescence Staining—** Preparation of the anti-indolea- mine 2,3-dioxygenase rabbit IgG was described previously (7). The monospecific antibody for indoleamine 2,3-dioxygenase was isolated by the method described by Dean and Coon (27). After perfusion and alveolar lavages, the lung was cut into 2-mm slices, fixed in a peroxide-free solution (28) at 4 \( ^\circ \)C for 1 h, and washed overnight in phosphate-buffered saline containing 10% sucrose at 4 \( ^\circ \)C. The fixed tissue was cut into 4-\( \mu \)m thick sections on a cryostat. The sections were mounted on a glass slide, air-dried, and washed in phosphate-buffered saline at 4 \( ^\circ \)C for 15 min. The washed sections were incubated with the monospecific IgG for indoleamine 2,3-dioxygenase (100 \( \mu \)g/ml) in a constant humidity chamber at 25 \( ^\circ \)C for 1 h. Control was incubated with preimmunized rabbit IgG or the residual IgG after absorption of the monospecific IgG. The slides were washed with phosphate-buffered saline by immersing and agitating three times, and then incubated with fluorescein isothiocyanate-labeled goat anti-rabbit IgG (1 mg/ml) at 25 \( ^\circ \)C for 30 min. After washing as above, the slides were viewed with a Nikon microscope with an ep-illumination system.

**RESULTS**

**Effects of Perfusion and Alveolar Lavages on Indoleamine 2,3-Dioxygenase Activity in Lung—** To examine whether blood components and alveolar free cells could account for the increase in the pulmonary indoleamine 2,3-dioxygenase activity after the LPS treatment, we determined the indoleamine 2,3-dioxygenase activity after perfusion and alveolar lavages (Table I). The perfusion resulted in about 45% decrease in the total protein and about 40% increase in the total indoleamine 2,3-dioxygenase activity in the lung from LPS-treated or control mice. The indoleamine 2,3-dioxygenase activity in the perfused lung was completely recovered in the lung tissue after alveolar lavages. However, little or no indoleamine 2,3-dioxygenase activity was detected in alveolar free cells or alveolar fluid. These results showed that indoleamine 2,3-dioxygenase induced by the LPS treatment was localized in lung cells tightly attached to the tissue.

**Indoleamine 2,3-Dioxygenase Activity in Type II Epithelial Cells—** Since type II epithelial cells are known as one of the main types of lung cells and their isolation method had already been established (16-18), the indoleamine 2,3-dioxygenase activity was determined by the digestion with 1.0% trypsin and by the digestion with 1.0% trypsin. After a 40-min incubation, when type II epithelial cells were maximally recovered (1.0 \( \times \) 10\(^7\) cells/lung), the isolated cells contained 12.8% of protein (30,000 \( \times \) g supernatant fraction) and less than 0.2% of the indoleamine 2,3-dioxygenase activity originally observed in the perfused lung. More than 80% of the indoleamine 2,3-dioxygenase activity in the perfused lung was recovered in the undisguised tissue trapped on the nylon cloth with a higher specific activity
**Effects of perfusion and alveolar lavages on pulmonary indoleamine 2,3-dioxygenase activity on control and LPS-treated mice.**

Animals were used for the experiments after the intraperitoneal administration of either sterile saline or LPS (E. coli, 20 μg/mouse). Tissue fractions were separated following specified procedures as described under "Materials and Methods." The protein contents and the indoleamine 2,3-dioxygenase activities were determined with the supernatant fractions after the centrifugation at 30,000 × g for 30 min. Each value represents the mean ± S.E. of 10 determinations.

<table>
<thead>
<tr>
<th>Control mice</th>
<th>LPS-treated mice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Protein mg/lung</td>
</tr>
<tr>
<td>Whole lung</td>
<td>14.96 ± 0.22</td>
</tr>
<tr>
<td>After perfusion</td>
<td>8.21 ± 0.32</td>
</tr>
<tr>
<td>After perfusion and alveolar lavages</td>
<td>8.30 ± 0.50</td>
</tr>
<tr>
<td>Alveolar free cells*</td>
<td>0.07 ± 0.01</td>
</tr>
<tr>
<td>Alveolar fluid</td>
<td>0.24 ± 0.06</td>
</tr>
</tbody>
</table>

*The number of alveolar free cells obtained from either control or LPS-treated mice was almost unchanged (3.2 ± 0.7 × 10⁶ cells/mouse). The cell population was also similar (about 90% was alveolar macrophages and the rest was lymphocytes). 

**ND,* not detectable.**

### TABLE II

**Indoleamine 2,3-dioxygenase activity in type II epithelial cell-rich fraction from LPS-treated mouse lung.**

Three lungs from LPS-treated mice were used for the experiment. After perfusion and alveolar lavages, the right lobe (the wet weight was about ½ of the whole lung) was incubated with saline containing 20 mM Tris-HCl (pH 7.4) as the control perfused lung. The other lobes were incubated with 1.0% trypsin at 37 °C for 40 min. After the incubation, the epithelial cell-rich fraction and the undigested tissue were recovered as described under "Materials and Methods." The protein and the indoleamine 2,3-dioxygenase activity were determined with the 30,000 × g supernatant fraction. Each value of total protein and indoleamine 2,3-dioxygenase activity is expressed as percentage of those in the control perfused lung, where the protein content was 7.4 mg/lung and the indoleamine 2,3-dioxygenase activity was 63.5 nmol/h/lung.

<table>
<thead>
<tr>
<th>Total protein</th>
<th>Indoleamine 2,3-dioxygenase activity</th>
<th>Total activity</th>
<th>Specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>%</td>
<td>mg/lung</td>
<td>nmol/h/mg protein</td>
</tr>
<tr>
<td>Control perfused lung</td>
<td>100</td>
<td>100</td>
<td>2.57</td>
</tr>
<tr>
<td>Type II epithelial* cell-rich fraction</td>
<td>12.8</td>
<td>&lt;0.2</td>
<td>&lt;0.13</td>
</tr>
<tr>
<td>Undigested tissue</td>
<td>64.8</td>
<td>80.5</td>
<td>10.66</td>
</tr>
</tbody>
</table>

*The cell number was 2.5 × 10⁶ cells/lung. About 86% of these cells was viable. The population of type II epithelial cells was about 40% as estimated with the phosphine 3R staining.

(10.66 nmol/h/mg of protein) than that of the perfused lung (8.57 nmol/h/mg of protein). Increasing the incubation time from 30 min did not modify the results. These results showed that the indoleamine 2,3-dioxygenase activity did not exist in type II epithelial cells but in other types of cells hardly detached from the lung tissue by the trypsin-treatment.

### Dispersion of Lung Cells Responsible for the Indoleamine 2,3-Dioxygenase Induction—When the dispersed lung cells were subjected to isopycnic gradient (1.021 to 1.100 g/ml) centrifugation by the floating method, the cells obtained from LPS-treated mice were distributed in two peaks at the densities of 1.040 and 1.050 g/ml. When the sedimentation method was used, cells of heavy density were trapped in the layer of cells of light density and a good separation of lung cells was not achieved. The indoleamine 2,3-dioxygenase activity was found exclusively in the lighter fractions and showed about 2-fold increase in the specific activity in the peak fraction than that of the unfractinated cells. The indoleamine 2,3-dioxygenase activity showed a distribution pattern similar to those of prostaglandin dehydrogenase (a marker enzyme of alveolar interstitial cells) and ACE (endothelial cells) activities with a peak at the density of 1.040 g/ml. However, arylsulfatase, coumarin hydroxylase, and β-glucuronidase activities were mainly localized in the heavier fractions with peaks at the densities of 1.093, 1.082, and 1.070 g/ml, respectively.

When the dispersed lung cells were fractionated in a narrow range density gradient (1.021 to 1.053 g/ml) (Fig. 2), the indoleamine 2,3-dioxygenase activity was again associated with a peak at the density of 1.040 g/ml and the distribution profile coincided with that of the prostaglandin dehydrogenase activity. On the other hand, the ACE activity was distributed in less dense fractions with a peak at the density of 1.033 g/ml. Arylsulfatase, coumarin hydroxylase, and β-glucuronidase activities again showed the distribution patterns clearly different from that of the indoleamine 2,3-dioxygenase activity.

When the cells from control mice were subjected to these gradients, these cells showed an almost identical distribution...
Indoleamine 2,3-Dioxygenase in Alveolar Interstitial Cells

FIG. 1. Distribution of cells, indoleamine 2,3-dioxygenase and various marker enzyme activities after Percoll isopycnic gradient (1.021 to 1.100 g/ml) centrifugation of dispersed lung cells obtained from LPS-treated mice. Experimental conditions are described under “Materials and Methods.” The yield of dispersed cells was 5.9 X 10^7 cells/lung. The specific activities of indoleamine 2,3-dioxygenase and other marker enzymes in the unfractionated cells were as follows: indoleamine 2,3-dioxygenase, 6.0 nmol/h/mg of protein; prostaglandin dehydrogenase 2.5 nmol/h/mg of protein; ACE, 0.2 pmol/min/mg of protein; β-glucuronidase, 1.6 nmol/min/mg of protein; coumarin hydroxylase, 1.2 pmol/min/mg of protein; and arylsulfatase, 32.2 nmol/min/mg of protein.

profile with that of the cells from LPS-treated mice (data not shown). Although the indoleamine 2,3-dioxygenase activity in the cells from control mice was about 1% of that in the cells from LPS-treated mice, other marker enzyme activities and their distribution patterns in these gradients were almost unchanged. These results indicated that the indoleamine 2,3-dioxygenase activity was exclusively induced in alveolar interstitial cells.

Morphological Identification of Lung Cells Responsible for the Indoleamine 2,3-Dioxygenase Induction—With an electron microscope, dispersed type I epithelial cells (36, 37), type II epithelial cells (16–18), Clara cells (31, 38), and endothelial cells (39, 40) were identified by their morphological characteristics reported previously. However, no information was available concerning the morphology of dispersed alveolar interstitial cells. We examined by electron microscopy the structural alteration of these cells during the dissociation procedure and found some morphological characteristics of dispersed alveolar interstitial cells. The typical electron micrograph of the dispersed alveolar interstitial cell is shown in Fig. 3. The dispersed cells showed an irregular shape with well branched and pseudopodal cell processes. These cells contained several lipid droplets and a large amount of fibrils in the cytoplasm, as observed in the tissue (41–44).

With the morphological criteria as described above, the distribution profiles of all of the main types of lung cells were determined in the narrow range density gradient by electron microscopy (Fig. 4). Clara cells, ciliated airway epithelial cells, lymphocytes, and polymorphonuclear leucocytes were almost completely recovered in the fraction 1. Alveolar macrophages were concentrated in the fraction 2. Type II epithelial cells and type I epithelial cells were mainly distributed in the fractions 2 and 3, and 3 and 4, respectively. The middle fractions (fractions 4 and 5) contained alveolar interstitial cells with the highest enrichment. Endothelial cells were more enriched in less dense fractions (fractions 6 and 7). The distribution profile of the indoleamine 2,3-dioxygenase activity was closely associated with that of alveolar interstitial cells but not with those of other types of lung cells. These results suggested again that indoleamine 2,3-dioxygenase induced by the LPS treatment was exclusively localized in alveolar interstitial cells.

Immunohistochemical Identification of Lung Cells Responsible for the Indoleamine 2,3-Dioxygenase Induction—The lung section obtained from LPS-treated mouse was stained with anti-indoleamine 2,3-dioxygenase IgG (Fig. 5). The immunofluorescence was not observed in endothelial cells faced to the capillary lumen or in type I epithelial cells covering the surface of alveolar lumen. The distribution profile of the immunofluorescence was distinct from that of type II epithelial cells, since these cells usually develop a part of their cytoplasm into the alveolar space. On the other hand, Clara
Indoleamine 2,3-Dioxygenase in Alveolar Interstitial Cells

Indoleamine 2,3-dioxygenase (IDO) is an enzyme that catalyzes the breakdown of tryptophan to kynurenine. It plays a crucial role in the immune system, particularly in regulating the immune response to pathogens.

**Fig. 3.** Electron micrograph of a dispersed alveolar interstitial cell. Magnification × 13,500. Well branched and pseudopodal cell projections are seen at the upper part of the figure. An interstitial cell contains some lipid droplets (*) and large amount of fibrils (arrowheads) in the cytoplasm.

Cells and ciliated airway epithelial cells scarcely exist in the alveoli but are mainly located in the bronchus or bronchioles, where no significant immunofluorescence was observed. The fluorescence appeared to be localized in the cytoplasm of alveolar interstitial cells, as judged by the observed positions and the morphological characteristics. 1) The fluorescence was located within the alveolar septum and not faced to the alveolar or capillary lumen, and 2) pseudopodal projections of the fluorescent cells were sometimes observed (arrowhead in Fig. 5). In a control staining with preimmunized IgG or IgG preabsorbed with purified indoleamine 2,3-dioxygenase, and in sections obtained from control mice, the specific fluorescence was not observed. These results, together with the data of fractionation of lung cells, showed that indoleamine 2,3-dioxygenase induced by the LPS treatment was exclusively localized in alveolar interstitial cells.

**DISCUSSION**

The lung contains more than 40 cell types (45), and no one cell type constitutes the major mass of the lung. Therefore, studies on lung homogenates do not yield information about individual cell types. The isolation of various types of lung cells is essential to evaluate the functions of this complex organ on a cellular level. In this paper, we describe the method for dispersion and fractionation of various types of mouse lung cells, and provide several lines of evidence that indoleamine 2,3-dioxygenase induced by the LPS treatment is exclusively localized in alveolar interstitial cells.

**Fig. 4.** Distribution of the indoleamine 2,3-dioxygenase activity and various types of lung cells after Percoll isopycnic gradient (1.021 to 1.053 g/ml) centrifugation of dispersed lung cells obtained from LPS-treated mice. Experimental conditions are the same as described in the legend for Fig. 2. The cell population was determined on a group of 200 cells in each fraction by electron microscopy, as described under “Materials and Methods.” The symbols used in per cent cells (middle and bottom) represent alveolar interstitial cells (○), type I epithelial cells (□), type II epithelial cells (△), endothelial cells (●), alveolar macrophages (△), and Clara cells (□), respectively. The yield of dispersed cells was 5.7 X 10⁶ cells/lung. The specific activity of indoleamine 2,3-dioxygenase in the unfractionated cells was 10.9 nmol/h/mg of protein.

**Fig. 5.** Immunostaining of indoleamine 2,3-dioxygenase in the LPS-treated mouse lung. Magnification × 1,000. The section from the LPS-treated mouse lung was stained with monospecific IgG for indoleamine 2,3-dioxygenase as described under “Materials and Methods.” Bottom, diagrammatic representation of the top of figure. The arrowheads point to the pseudopodal projections of immunofluorescent cells. The immunofluorescent cells are located within the alveolar septum and not faced to alveolar (AI) or capillary space (small arrows, bottom).
Although the alveolar interstitial cells are one of the main types of lung cells (about 40%) and occupy about 15% of the cellular space in the lung (46), their biological function has not yet been elucidated. The main reason is the difficulty in identification of these cells in the tissue section and the lack of a method for isolation of these cells. However, numerous studies have suggested that alveolar interstitial cells are not only the structural component of the lung but also have various other important functions. 1) Kapanci et al. (41) reported that these cells are the contractile cells and might regulate the ventilation/perfusion ratio in the respiration; 2) Yamada and Hirosawa (42) reported that these cells have the abilities of uptake and storage of vitamin A; 3) Etherton and Conning (33) reported that prostaglandin dehydrogenase, a key enzyme of inactivation of prostaglandins, was localized specifically in these cells; and 4) we demonstrated in this report that the indoleamine 2,3-dioxygenase induction by the LPS treatment occurred exclusively in these cells. Since alveolar interstitial cells isolated and enriched by our method are viable and grow under the cell culture condition, the studies using cultured cells of this type would make it possible to clarify the biological functions of these cells and also of indoleamine 2,3-dioxygenase induction.

The total dissociation procedure of the mouse lung described in this report allowed us to obtain dispersed cells with about 5-fold higher yield (2.5 × 10^6 cells/g of tissue, wet weight) than that previously reported with rat lung (4.4 cells/g of tissue, wet weight) (14, 15). Almost all of these cells were viable, morphologically intact, and composed on a comparable population of various types of lung cells with that in the tissue, as judged by the data regarding the recoveries of various marker enzyme activities. On a Percoll isopycnic gradient, each type of lung cells showed a distinct distribution profile (Figs. 1, 2, and 4). These techniques of dispersion and fractionation of mouse lung cells may be useful methods for the studies of various enzymes or biological activities whose cellular localization in the lung has not yet been elucidated. By this method, we found that two forms of cytochrome P-450s are heterogeneously distributed in the cells of the mouse lung (31), and that monoamine oxidase, an enzyme involved in the metabolism of biogenic amines, is almost exclusively localized in endothelial cells in the mouse lung.2

Marker enzymes used in this study except for arylsulfatase were closely associated with the specific cells in the isopycnic gradient as examined by electron microscopy (Figs. 2 and 4). Paterson et al. (29) used arylsulfatase as a specific marker of mast cells in their study of the isolation of mast cells from the human lung. However, this enzyme activity was widely distributed in various types of lung cells of mouse (Figs. 1 and 2). The peak fraction of this enzyme activity (fraction 3 in Fig. 1) was mainly composed of polymorphonuclear leucocytes but not mast cells. It is well known that the content of mast cells in the mouse lung is very low as compared with that in the human lung, and that arylsulfatase is also enriched in polymorphonuclear leucocytes of rats (47). Therefore, the arylsulfatase activity in our study may be derived mainly from polymorphonuclear leucocytes.

We previously reported that indoleamine 2,3-dioxygenase was induced specifically in the mouse lung after the LPS treatment (6, 7) and showed in this paper that the enzyme induction occurred exclusively in alveolar interstitial cells. Preliminary experiments in our laboratory indicated that the indoleamine 2,3-dioxygenase induction resulted in an about 10-fold increase in the levels of pyrrole ring-cleaved metabolites of indoleamine derivatives in the mouse plasma. It may be reasonable to conjecture that indoleamine 2,3-dioxygenase in alveolar interstitial cells may regulate the levels of indoleamine derivatives in the circulating system similar to the role of prostaglandin dehydrogenase in these cells in the prostaglandin metabolism (39).

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