

Human bronchial epithelial cells modulate CD3 and mitogen-induced DNA synthesis in T cells but function poorly as antigen-presenting cells compared to pulmonary macrophages

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This study was undertaken to investigate the ability of bronchial epithelial cells (ECs) to function as accessory cells. Pulmonary monocytes were our reference cells. ECs and pulmonary monocytes were isolated from the bronchial mucosa and pulmonary parenchyma of subjects undergoing lobectomy for standard clinical reasons. Circulating autologous T cells were rigorously depleted of accessory cells to the extent that they lost the capacity to respond to mitogenic lectins alone. ECs restored mitogen-induced DNA synthesis and DNA synthesis triggered by CD3 cross-linking in T cells, as did pulmonary macrophages, and this was unrelated to HLA-DR expression. The ability of promoting T cell proliferation after CD3 cross-linking was, in part, due to the secretory products of ECs, since their supernatants were also effective. Interferon- γ -treated ECs were capable of presenting antigens to autologous T cells. This was an HLA-DR-restricted phenomenon, but EC efficiency in this system was <40% of efficiency demonstrated by pulmonary monocytes. However, ECs greatly upregulated antigen-specific responses supported by pulmonary monocytes. (J ALLERGY CLIN IMMUNOL 1991;87:930-8.)

T cell recognition of Ag is the result of complex interactions between Ag-presenting cells and the responding T cells. Although the complete repertoire of signals provided by the Ag-presenting cells has not been elucidated, it is clear that there are certain requirements that those cells must fulfill to be effective

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Supported by National Research Council grants (United States-Italy bilateral project and finalized projects).

Presented in abstract form at World Health Conference on Lung Health, Boston, Mass., May 1990.

Received for publication June 6, 1990.

Revised Dec. 7, 1990.

Accepted for publication Dec. 14, 1990.

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*Dr. M. Soloperto is a postgraduate student supported by Fisons plc, U.K.

**Dr. S. Mattoli is recipient of a scholarship from the Minister of University and of Scientific and Technologic Research, Italy.

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Abbreviations used

EC:	Epithelial cell
PGE ₂ :	Prostaglandin E ₂
Ag:	Antigen
Ia:	Immune response gene-associated antigen
MHC:	Major histocompatibility complex
Ab:	Antibody
FCS:	Fetal calf serum
PHA:	Phytohemagglutinin
INF- γ :	Interferon- γ
IL-1, IL-2, IL-6:	Interleukin 1, 2, and 6

accessory cells and support the cellular events that lead to T cell expression of IL-2 receptor, as well as IL-2 production and proliferation in response to Ag.

To be effective, Ag-presenting cells must express class II (Ia) molecules of the MHC on their surface and be able to provide further activation signals, such as IL-1 and IL-6¹⁻³ or adhesion molecule expression.⁴

Of central importance is the requirement for Ag-presenting cells to generate the immunogenic moiety recognized by T cells. Thus, these cells take up Ag, degrade it to reveal the relevant determinant, and present it in the context of MHC gene products (Ia) to Ag-committed T cells.^{3, 5}

In the normal mammalian host, constitutive expression of Ia molecules is mostly limited to lymphoid cells, such as macrophages, dendritic cells, and B cells.⁶ Other nonlymphoid cells, such as human fibroblasts, endothelial cells, and thyroid ECs, can be induced to express Ia in vitro with INF- γ .⁷⁻⁹ Studies of these cells have revealed Ag-presenting cell activity in endothelial cells¹⁰ and thyroid ECs¹¹ incomplete Ag-presenting activity in keratinocytes,⁹ and a lack of activity in fibroblasts.¹² Thus, there is evidence that only some nonlymphoid cells, which are induced to express Ias can present Ags as lymphoid cells present.

Bronchial ECs form the interface between the environment and bronchial mucosa and thus are located in a unique position to play a role in initiating or regulating the interactions of resident T cells and monocytes with inhaled Ags. Some recent observations from our laboratory and by other authors suggest the possibility that bronchial ECs may have the potential to support lymphocyte activation, since they express MHC class II Ags in vivo¹³ and release cytokines, which promote activation and proliferation of T cells induced by Ag-receptor triggering in vitro.^{14, 15}

The present study was undertaken to examine the Ia status of a homogenous population of cultured human bronchial ECs and to determine whether ECs are capable of functioning as Ag-presenting cells and/or completely sufficient accessory cells for mitogen-induced T cell proliferation. Pulmonary monocytes were evaluated as positive controls.

MATERIAL AND METHODS

Isolation of bronchial ECs

Bronchial ECs were isolated from the grossly normal bronchial mucosa of 18 subjects undergoing lobectomy for standard clinical reasons (N = 13, lung cancer not involving the bronchi used for experiments; N = 5, cystic lesions) by enzymatic digestion, according to a standard protocol.¹⁴⁻¹⁶

Resulting cell suspension was depleted of dendritic cells, T cells, B cells, and monocytes by repeated panning¹⁷ with the monoclonal Abs against the specific markers of these cells, CD1a, CD2, CD22, and CD14 (OKT6, OKT11, OKB22, and OKM14, Ortho Diagnostic System, Milan, Italy).¹⁴⁻¹⁶ Cell count and viability (trypan blue exclusion method) were assessed by use of a Burker chamber (Cecchinato, Venice, Italy). Cells were finally resuspended in medium 199 supplemented with epidermal growth factor

(25 ng/ml), transferrin, (5 μ g/ml), insulin (5 μ g/ml), penicillin (100 U/ml), streptomycin (100 μ g/ml), and 10% heat-inactivated FCS (all from GIBCO Laboratories, Paisley, Scotland), and plated in collagen-treated 24-well culture plates at a density of 5×10^5 cells per well per milliliter. Cells were then incubated at 37° C, and 5% CO₂, and 100% humidity for 3 days. Under these conditions the cells grew to confluence.

For preparation of EC supernatants, confluent cells were cultured without growth supplements for 48 hours. At the end of the incubation period, the supernatants were harvested and frozen at -70° C until use.

EC characterization and evaluation of HLA-DR expression

EC monolayers were characterized by phase-contrast microscopy and immunospecific labeling with the monoclonal Ab against human cytokeratin (Dako CK1, DAKOPATTS, Glostrup, Denmark) after detachment of the cells by brief incubation with dispase and transfer onto glass slides.¹⁶ Only the monolayers that demonstrated the characteristic cobblestone appearance under phase-contrast microscopy were composed by >99% keratin-containing cells and did not contain monocytes, T cells, or dendritic cells, as assessed by immunospecific labeling, were used for the study.

The expression of HLA-DR Ags was evaluated in freshly isolated cells and in the cells cultured for 48 hours in serum-free medium with and without the addition in culture of human recombinant INF- γ 100 U/ml (Genzyme Corp., Boston, Mass.). For immunospecific labeling, cultured cells were detached from the culture surfaces by brief exposure to dispase. Washed cells were then transferred onto glass slides. Labeling was accomplished by use of the anti-HLA-DR monoclonal antibody, OKIa1, and the binding of the Ab was revealed by use of an immunoperoxidase staining.¹⁸ Control slides were labeled with an irrelevant isotype-matched Ab. At least 400 nucleated cells were counted in each slide, and the number of labeled cells was expressed as percent of the total.

Isolation of pulmonary monocytes

Autologous pulmonary monocytes were isolated from the pulmonary parenchyma of the same donors of ECs. The parenchyma was cut in small pieces that were placed in 60 mm dishes containing 5 ml of Hanks' buffer, 1% collagenase, 0.05% elastase, 100 U/ml of penicillin, 100 μ g/ml of streptomycin, and 25 mmol/L of *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (all from Sigma Chemical Co., St. Louis, Mo.) for 2 hours. The resulting cell suspension was washed repeatedly by centrifugation, and the pellet was resuspended in Hanks' buffer. Macrophage/monocytes were purified by affinity chromatography with the anti-CD14 monoclonal antibody coupled to CNBr-activated Sepharose 4 MB beads (Pharmacia Diagnostics, Uppsala, Sweden) and resuspended in RPMI 1640 supplemented with 5% heat-inactivated FCS, nonessential amino acids, 0.3 mg/ml of L-glutamine, and antibiotics (GIBCO Laboratories). These cells were cultured for 48 hours in 24-well culture plates at

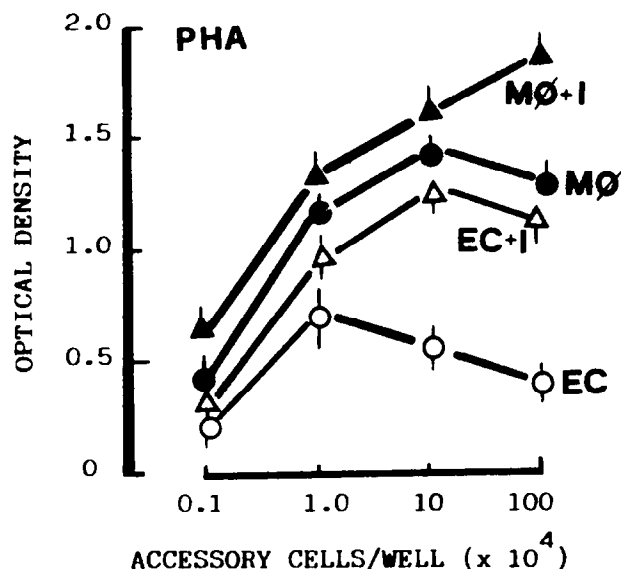


FIG. 1. Accessory cell function of human bronchial ECs and pulmonary macrophages (Mo) in PHA-induced T-lymphocyte proliferation. T cells (1×10^5 per well) were cultured either alone or with varying numbers of epithelial cells or pulmonary macrophages for 72 hours. Indomethacin, $5 \mu\text{g/ml}$ (I) was added to the cultures at the initiation of incubation where it was indicated. Data represent mean values $\pm$ SD of triplicate experiments performed by use of the cells from five donors.

37°C and $5\% \text{CO}_2$ in air and 100% humidity, at a density of 5×10^5 cells per milliliter.

Isolation of T-lymphocytes

Autologous T-lymphocytes were isolated from non-adherent, Ficoll-Hypaque gradient-separated peripheral blood mononuclear cells. To eliminate non-T cells, cell suspension was subjected to repeated panning¹⁷ with the monoclonal Abs against the surface marker of monocytes (CD14), B cells (CD22), dendritic cells (CD1a), and natural killer cells (CD16). Depletion of activated T cells was accomplished by incubation of the resulting cell suspension with the monoclonal Ab against the HLA-DR Ag (OK1a, Ortho Diagnostic System), followed by lysis of the cells bearing the Ag with rabbit complement.

The final population consisted of $>99\%$ T cells, as judged by specific labeling with the anti-CD2 monoclonal Ab and an immunoperoxidase staining. Those cells did not proliferate after incubation with optimal concentrations of PHA ($1 \mu\text{g/ml}$) alone (15).

Effect of ECs or EC-conditioned medium on T cell proliferation induced by PHA or CD3 cross-linking

To evaluate the ability of ECs to promote the proliferation of autologous T cells activated with mitogenic lectin, T-lymphocytes (1×10^5 per well) were cocultured with bronchial ECs (5×10^4 per well) in RPMI 1640 medium supplemented with 10% FCS, 0.3mg/ml of L-glutamine, in-

domethacin, $5 \mu\text{g/ml}$, and Abs in 96-well microtiter plates with flat-bottomed wells, with or without PHA, $1 \mu\text{g/ml}$, for 72 hours.

To study the effect of ECs on T cell activation induced by CD3 cross-linking, T cells were incubated for 4 hours with suboptimal concentrations of a monoclonal Ab against CD3 (OKT3, Ortho Diagnostic System) coupled to CNBr-activated Sepharose 4 MB beads, as described,^{18,19} and were then cocultured at the concentration of 1×10^5 cells per well with 5×10^4 per well of ECs in complete RPMI medium for 72 hours.

Analogous cocultures of 1×10^5 of T-lymphocytes and 5×10^4 of autologous pulmonary macrophages were used as controls.

Before coculturing, bronchial ECs and pulmonary macrophages were exposed to $40 \mu\text{g/ml}$ of mitomycin C (Sigma Chemical Co.) to prevent replication.

To evaluate whether any effect of bronchial ECs on T cell DNA synthesis was due to some factor(s) released from ECs, in parallel experiments, T cells (1×10^5 per well) were cultured alone in complete RPMI medium with the addition of 10% (vol:vol) supernatants of bronchial ECs for 72 hours, with or without PHA or previous activation by CD3 cross-linking.

DNA synthesis in T-lymphocytes was evaluated as indicated below.

ECs as Ag-presenting cells

To evaluate the ability of bronchial ECs to present Ag to the responding autologous T cell population, mitomycin-treated ECs (5×10^4 per well) were cocultured with autologous T cells (1×10^5 per well) in RPMI 1640 complete medium supplemented with different concentrations of the Ag (mumps antigen, Eli Lilly & Co., Indianapolis, Ind., 1:25 to 1:50; tetanus toxoid, Wyeth Laboratories, Philadelphia, Pa., 1 to $10 \mu\text{g/ml}$; *Candida*, and Dermatophyton O, Hollister-Stier Laboratories, Spokane, Wash., 100 to 500U/ml) for 5 days.

Analogous cocultures of T cells (1×10^5 per well) and autologous mitomycin-treated pulmonary macrophages (5×10^4 per well) were used as controls.

To evaluate the importance of HLA-DR expression in any Ag-presenting cell activity of bronchial ECs, replicate cocultures of T cells and INF- γ -treated ECs were prepared after lysing Ia-bearing ECs by sequential incubation with OKIa1 and rabbit complement.

T cell DNA synthesis was evaluated as indicated below.

Effect of ECs or EC-conditioned medium on Ag-presenting activity of pulmonary macrophages

To test the ability of ECs of regulating the Ag-presenting activity of pulmonary macrophages, T cells (1×10^5 per well) were cocultured with mitomycin-treated macrophages (5×10^4 per well) alone or with pulmonary macrophages (2.5×10^4 per well) and mitomycin-treated ECs (2.5×10^4 per well), exposed or not to INF- γ , in complete RPMI medium supplemented with suboptimal doses of Ag for 5 days.

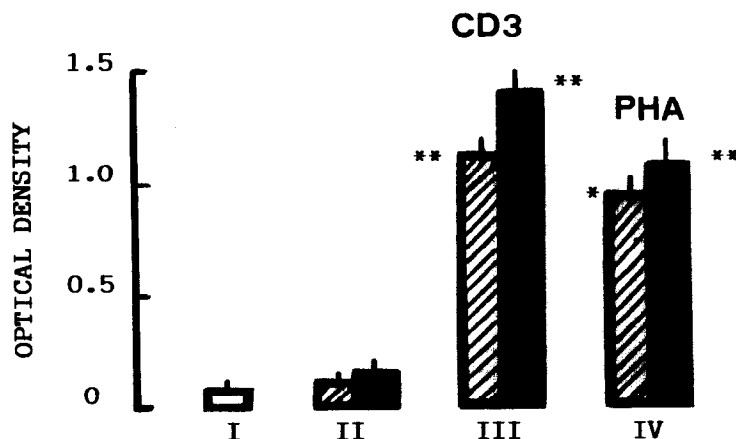


FIG. 2. Accessory cell function of bronchial ECs (▨) and pulmonary macrophages (■) in T-lymphocyte proliferation triggered by CD3 cross-linking (///) and by PHA (IV); 1×10^5 T cells were cocultured with 5×10^4 ECs or 5×10^4 pulmonary macrophages for 72 hours with PHA or after CD3 triggering. Parallel cocultures of T cells and accessory cells were incubated without any stimulus (I). Control T cells were cultured alone (II). Indomethacin, 5 μ g/ml, was added to each culture. Mean values $\pm$ SD from triplicate experiments performed by use of the cells of eight donors; * $p < 0.05$; ** $p < 0.025$ versus I and II.

The ability of EC-conditioned medium of replacing ECs in the regulation of Ag-specific responses supported by monocytes, was evaluated by coculturing T cells and pulmonary monocytes with the addition of 10% supernatants of EC monolayers.

T cell DNA synthesis was evaluated as indicated below.

Evaluation of DNA synthesis in T cells

Sixteen hours before the end of the incubation of T cells with the accessory cells, singly or in combination, an aqueous solution of 5-bromo-2'-deoxyuridine and 5-fluoro-2'-deoxyuridine (10:1 ratio) was added (1:1000 final dilution) (cell proliferation kit, Amersham plc, Buckinghamshire, England). At the end of the culture period, the cells into each well were air-dried and fixed with 2% hydrogen peroxide in methanol. Additional protein-binding sites were blocked with bovine serum albumin. Cells were then sequentially incubated with the monoclonal Ab against bromodeoxyuridine and nuclease for DNA denaturation for 1 hour,²⁰ affinity-purified peroxidase-conjugated sheep anti-mouse Ab for 30 minutes, and o-phenylenediamine substrate. Optical density was measured at a wavelength of 492 nm with an ELISA reader (laboratory system Titertek Uniskan, Flow Laboratories, Milan, Italy), according to the cell ELISA technique described by Baumgarten.²¹ Specific labeling of the T cell nuclei was confirmed by visual inspection of the cells into the wells by use of an inverted microscope.

Controls were replicate cultures of the cells treated in the same manner except that incubation with the monoclonal antibody was omitted.

All data were expressed as the difference between the means of triplicate experimental and control cultures (Δ optical density). Differences in T cell DNA synthesis under different experimental conditions were analyzed with Wil-

coxon's signed-rank test or Friedman's free rank-order analysis.²²

RESULTS

Expression of HLA-DR Ags on bronchial ECs

Freshly isolated human bronchial ECs expressed surface HLA-DR Ags (mean percent number of positive cells $\pm$ SD, 38.6% $\pm$ 8.6%; N = 8). Culturing of the cells in serum- and hormone-free medium for 48 hours markedly decreased the expression of those Ags to the extent that, at the end of the incubation period, <1% of the cells were still labeled with the specific Ab against HLA-DR.

The addition of INF- γ , 100 U/ml, to the serum- and hormone-free medium prevented the lack of surface HLA-DR Ags, and at the end of incubation, the percentage of ECs demonstrating DR Ags was even greater than in the fresh samples (mean percent positive cells $\pm$ SD, 86.8% $\pm$ 8.5%; N = 8; $p < 0.05$). Pulmonary monocytes maintained HLA-DR expression at high levels during similar periods of incubation without the addition of INF- γ (mean percent positive cells $\pm$ SD; freshly isolated pulmonary monocytes, 98.6% $\pm$ 0.9%; pulmonary monocytes cultured for 48 hours, 97.6% $\pm$ 0.7%; N = 6; $p > 0.1$).

Accessory function of bronchial ECs

We initially examined the ability of ECs to support mitogen-induced T cell DNA synthesis and to promote the DNA synthesis in T cells triggered by Ag-receptor cross-linking.

As illustrated in Fig. 1, a small number of bronchial ECs restored and supported the responsiveness of

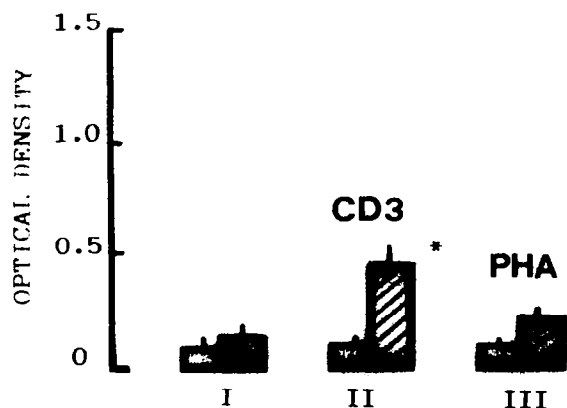


FIG. 3. Comparison of the effect of bronchial ECs (▨) or their conditioned media (10%) (□) on the proliferation of T cells induced by CD3 cross-linking (II) or PHA (III). Control T cells were cultured with ECs (I) or their supernatants (□) in absence of any stimulus (I). Indomethacin, 5 μ g/ml, was added to each culture. Mean values + SD from duplicate experiments performed with the cells of five donors; * p < 0.05 versus the effect of conditioned media alone (□).

T cells to PHA as effectively as pulmonary macrophages. However, the addition of larger numbers of ECs resulted in decreased responses (Fig. 1). Since prostaglandins, like PGE₂, inhibit many lymphocyte functions^{23, 24} and bronchial ECs release PGE₂ in culture,¹⁶ we investigated the possibility that the depressed responsiveness observed in T cell cultures containing large numbers of ECs might have resulted from the production of arachidonic acid metabolites. Indomethacin (5 μ g/ml in 1 μ l of ethanol) was used to inhibit cyclooxygenase activity; ethanol (1 μ l) was the control. Indomethacin markedly enhanced the proliferative response of mitogen-triggered T-lymphocytes supported by ECs, whereas it had only a minimal effect on responsiveness in cultures containing pulmonary monocytes (Fig. 1). These data demonstrated that the suppressive activity of ECs was mediated, in part, by one or more products of the cyclooxygenase pathway, and therefore, all the subsequent experiments were performed by adding indomethacin to the media used for evaluating T cell proliferation.

In this way, 5×10^4 bronchial ECs restored and supported the responsiveness of T cells to PHA and to Ag-receptor cross-linking as effectively as a comparable number of pulmonary macrophages (Fig. 2). The effect was not an HLA-DR-restricted phenomenon because the bronchial ECs used in the experiments were not previously exposed to INF- γ and had lost the expression of HLA-DR Ags, as assessed by immunospecific labeling.

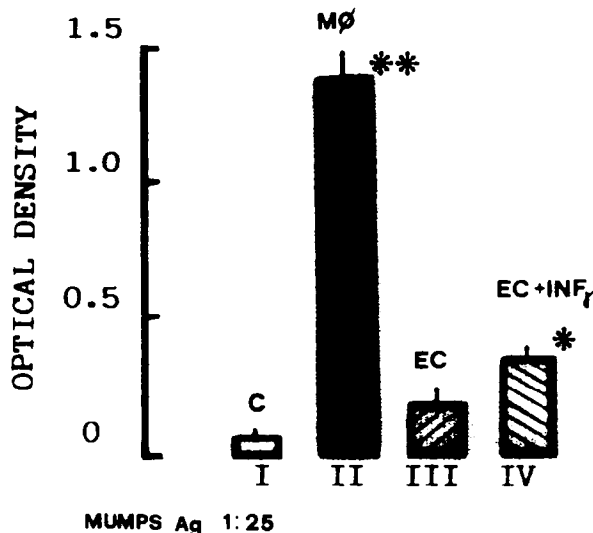


FIG. 4. Comparison of the Ag-presenting activity of bronchial ECs (▨) exposed (IV) or not exposed (III) to INF- γ (100 U/ml) and of pulmonary macrophages (■) (II); 1×10^5 responding T cells were cocultured with 5×10^4 ECs or pulmonary macrophages in presence of mumps Ag (1:25) for 5 days. Control cultures of T cells (C) were incubated with the Ag in absence of accessory cells (I). Indomethacin, 5 μ g/ml, was added to each culture. Mean values + SD from five experiments carried out in duplicate with the cells from five donors; * p < 0.05; ** p < 0.025 versus I.

Experiments were next performed to determine whether soluble factors produced by bronchial ECs could be responsible for their ability to support T cell proliferation induced by mitogen or CD3 cross-linking. As illustrated in Fig. 3, culture supernatants of bronchial ECs were not sufficient to support mitogen responsiveness of purified T cells in the absence of accessory cells but slightly promoted DNA synthesis in T cells sensitized by CD3 cross-linking, in which cross-linkage is known to replace, in part, the effect of accessory cells.¹⁹ In this respect, however, the supernatant of ECs alone was at least 50% less effective than bronchial ECs (Figs. 2 and 3).

Bronchial ECs as Ag-presenting cells

The possibility that bronchial ECs might function as Ag-presenting cells was next examined. To examine this possibility, ECs, grown to confluency and cultured in serum- and hormone-free medium, with or without the addition of INF- γ , for 48 hours, were cocultured with autologous T cells and Ag for 5 days. Autologous pulmonary macrophages were tested as positive controls.

Bronchial ECs not exposed to INF- γ did not present Ags to T cells that had demonstrated marked DNA synthesis when pulmonary macrophages had been

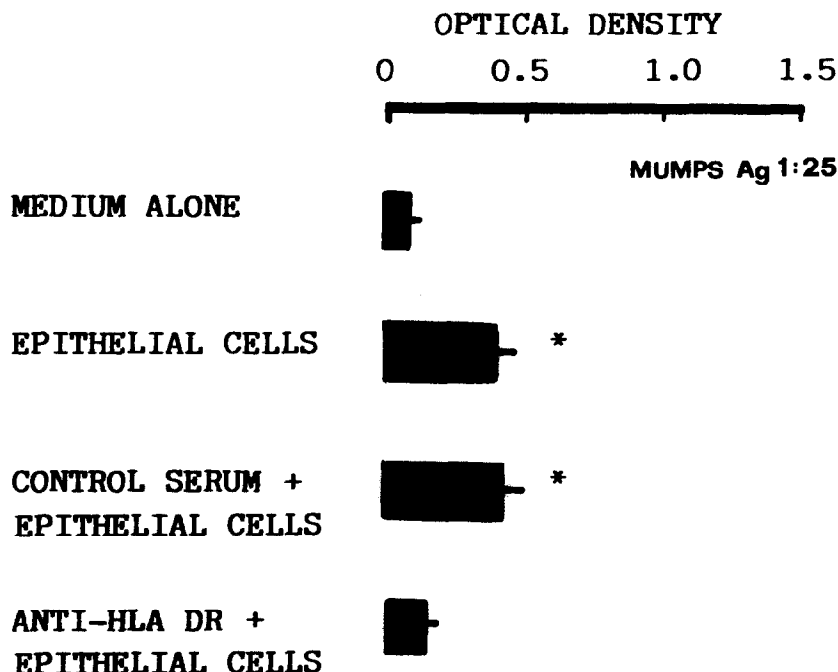


FIG. 5. Importance of HLA-DR expression by INF- γ -treated ECs for their Ag-presenting activity; 1×10^5 T cells were cocultured with 5×10^4 INF- γ -exposed epithelial cells before or after the incubation of ECs with anti-HLA-DR antiserum or control antiserum, as indicated. The cultures were incubated for 5 days in presence of mumps Ag (1:25). Control T cells were cultured with the Ag in absence of accessory cells. Indomethacin, 5 μ g/ml, was added to each culture. Mean values $\pm$ SD from duplicate experiments performed with the cells of five donors; * $p < 0.05$ versus control cultures.

used as Ag-presenting cells (Fig. 4). Bronchial ECs cultured in the presence of INF- γ were able to induce significant DNA synthesis in T cells exposed to Ags, but their effect was $<40\%$ of the effect demonstrated in parallel experiments by pulmonary macrophages.

The ability of INF- γ -treated ECs in presenting Ags to autologous T cells was an HLA-DR-restricted phenomenon because treatment of those cells with anti-Ia Ab and complement, to kill the Ia-bearing cell population, completely abolished EC-induced DNA synthesis in T cells (Fig. 5).

Modulatory effect of bronchial ECs on Ag-induced T cell proliferation supported by pulmonary monocytes

When both ECs and pulmonary macrophages were added to cultures of autologous T cells in the presence of suboptimal doses of Ags, ECs greatly increased the ability of pulmonary macrophages to elicit T cell DNA synthesis ($p < 0.01$). This phenomenon was only, in part, associated with the expression of Ias by bronchial ECs because the ECs that were not exposed to INF- γ and had lost surface HLA-DR were also ineffective in upregulating Ag-presenting cell function of

pulmonary macrophages (Fig. 6). It was probably due both to a direct cell-to-cell interaction and to the release of soluble factors, because in similar experimental conditions, the supernatant of ECs alone replaced, in part, ECs in promoting the T cell DNA synthesis supported by pulmonary macrophages (Fig. 7).

DISCUSSION

The present study demonstrates that human bronchial ECs can function as accessory cells for mitogen-induced T-lymphocyte DNA synthesis and for T cell proliferation triggered by CD3 cross-linking. The study also indicates that those cells exert a number of other immunomodulatory effects. By contrast, although bronchial ECs can be induced to express Ias in culture by exposure to INF- γ , they are much less effective Ag-presenting cells than autologous pulmonary macrophages.

To examine the ability of human bronchial ECs in supporting mitogen or Ag-induced T cell proliferation, it was necessary to prepare populations of ECs and responding autologous lymphocytes that were not contaminated with other types of accessory cells. Al-

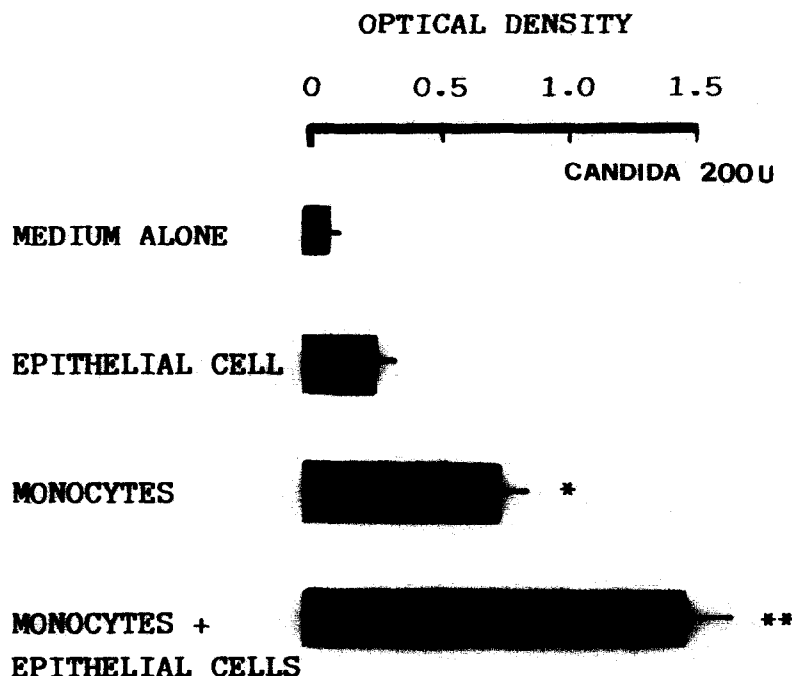


FIG. 6. Effect of bronchial ECs on the Ag-presenting activity of pulmonary macrophages. Responding T cells (1×10^5 per well) were cultured with either ECs (5×10^4 per well) or pulmonary macrophages (5×10^4 per well) or with a 50:50 mixture of each (total number of accessory cells 5×10^4 per well) in the presence of suboptimal doses of *Candida* (200 U/ml) for 5 days. Control T cells were cultured with the Ag in absence of any accessory cell. Indomethacin, 5 μ g/ml, was added to each culture. Mean values $\pm$ SD from duplicate experiments performed by use of the cells of five donors; * $p < 0.05$; ** $p < 0.025$ versus control cultures.

though macrophages and/or other potential accessory cells were initially coisolated with ECs, they were lost as a result of the subsequent purification steps involving depletion of cells bearing the surface Ags of monocytes, B cells, natural killer cells, and dendritic cells. In addition, cultured ECs uniformly demonstrated positive labeling with the monoclonal Ab against cytokeratin. Thus, the monolayers of ECs we used in our study appeared to be composed of a uniform cell population without apparent contamination with other cell types. The responding autologous lymphocyte populations were also rigorously depleted of accessory cells; thus, they lacked mitogen responsiveness unless they were supplemented with intact accessory cells. Therefore, the use of these techniques made it possible to examine the ability of ECs to support lymphocyte mitogenesis in a proper well-controlled manner.

Bronchial ECs were found to be fully capable of supporting T-lymphocyte DNA synthesis in response to mitogenic stimulation and after CD3 cross-linking when the ratio between T cells and ECs was 2.0. High numbers of ECs also inhibited T-lymphocyte responses, and this phenomenon was partly reversed by

indomethacin, suggesting that a cyclooxygenase product of ECs was responsible for the inhibitory effect. Appreciable amounts of PGE₂ are known to be constitutively produced in culture by human bronchial ECs,¹⁶ and PGE₂ can suppress T-lymphocyte functions in vitro, including T cell mitogenesis.^{23, 24} Our in vitro observations suggest that, although bronchial ECs can promote T cell responsiveness, their cyclooxygenase products may also restrict T cell function. Therefore, in vivo regulation of the biosynthesis of arachidonic acid metabolites in bronchial ECs may be one physiologically important determinant of the magnitude of local immune responses developing in the bronchial mucosa.

Although ECs could support the initiation of mitogen responsiveness, they were not as effective Ag-presenting cells as pulmonary macrophages were. It has been well documented that Ag-specific T cell activation requires T cell recognition of a component of the Ag as well as class II products of the MHC on the Ag-presenting cells.²⁵ When bronchial ECs were induced to reexpress HLA-DR in culture by the addition of INF- γ , their efficiency as Ag-presenting cells was still <40% of efficiency demonstrated by pul-

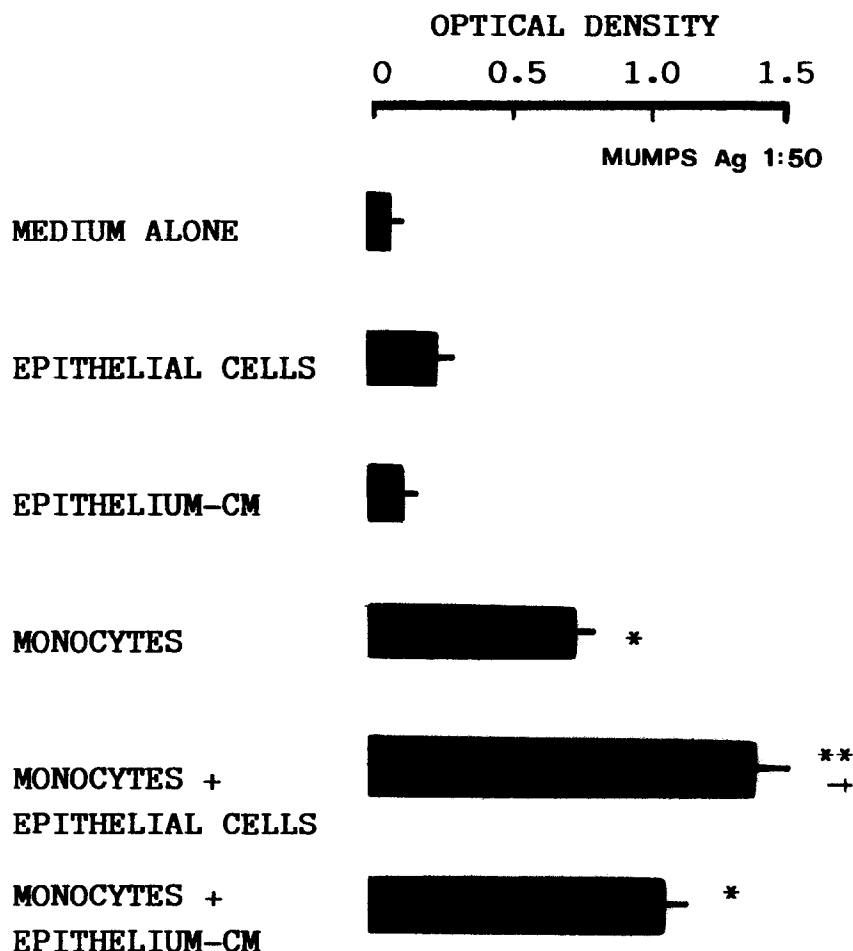


FIG. 7. Comparison of the effect of ECs or their conditioned medium (CM) on the Ag-presenting activity of pulmonary macrophages. Responding T cells (1×10^5 per well) were cultured with 5×10^4 per well of ECs, pulmonary macrophages or with a combination of these cells (each added at 2.5×10^4 per well), or with pulmonary macrophages and 10% EC-conditioned medium (CM) for 5 days in the presence of suboptimal doses of mumps Ag (1:50). Control T cells were cultured with the Ag in absence of accessory cells. Indomethacin, $5 \mu\text{g/ml}$, was added to each culture. Mean values $\pm$ SD from duplicate experiments carried out by use of the cells from five donors; * $p < 0.05$, ** $p < 0.025$ vs control cultures; † $p < 0.05$ versus the effect of pulmonary monocytes alone.

monary monocytes, which demonstrated comparable pattern of Ia expression. The ability of bronchial ECs to support mitogen-induced proliferation or proliferation triggered by CD3 cross-linking suggested that they had the capacity to support the entry of T cells into the cell cycle when generation of the appropriate immunogenic moiety and its presentation in the context of class II products of MHC were not required. Thus, the lack of effective Ag presentation may have related to EC inability to process Ag efficiently, as demonstrated with murine ECs.⁹

Although bronchial ECs were not highly effective as Ag-presenting cells, they dramatically enhanced Ag responsiveness in cultures containing pulmonary mac-

rophages. At least part of this amplifying effect was mediated through the action of products found in EC-culture supernatants. These supernatants, however, could not themselves support T-lymphocyte activation induced by Ag in the absence of intact accessory cells. We have already demonstrated that bronchial ECs may release IL-1 and IL-6 in culture and that by the synergistic effect of these two cytokines, ECs can promote T cell proliferation triggered by CD3 cross-linking, although resting T cells depleted of accessory cells are not affected.^{14, 15} In this study, we confirm the ability of EC-conditioned medium to promote T cell proliferation triggered by CD3 cross-linking in which the cross-linkage mimics the effect of accessory

cells, and we also demonstrate that EC-conditioned medium can, in part, substitute for ECs in augmenting the Ag-induced T cell responses in presence of pulmonary macrophages. Although we did not measure IL-1 and IL-6 in EC-conditioned media, these cytokines were probably secreted by ECs, as observed in our previous work,¹⁵ and contributed to the biologic activity of the supernatants.

In conclusion, this study has delineated the potential of bronchial ECs to function as accessory cells and to upregulate the magnitude of Ag-induced T cell responses supported by macrophages. These in vitro observations suggest that bronchial ECs, which are located at the interface between the environment and the internal milieu in the airways, may exert in vivo a number of regulatory influences that affect the ultimate result of the local immunologic reactions.

Dr. S. Mattoli is grateful to Prof. L. Allegra, Department of Respiratory Disease, University of Milan, for helpful advice and support.

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