MHC CLASS II ANTIGEN-BEARING DENDRITIC CELLS IN PULMONARY TISSUES OF THE RAT

Regulation of Antigen Presentation Activity by Endogenous Macrophage Populations

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The low prevalence of immunologically mediated respiratory hypersensitivity disease, despite almost continuous exposure to plant- and animal-derived antigens present in ambient air, indicates that the induction of T cell–dependent immune responses in bronchopulmonary tissues is tightly controlled, and is normally restricted to encounters with potentially pathogenic antigens. Recent evidence (1) suggests that active immune suppression plays an important role in the process by which the respiratory immune system discriminates between trivial and potentially pathogenic antigens, as passive inhalation of physiological levels of antigen is a potent means of inducing long-lasting immunological tolerance. This phenomenon closely parallels the better known process of oral tolerance in response to antigen feeding (2), which is believed to play a key role in protection against immunologically mediated enteropathies that may be triggered by dietary antigens (3).

The nature of the mechanism(s) that govern tolerance induction at mucosal surfaces remains to be defined, but it is apparent from the results of drug intervention studies that endogenous APC play a key role in immune regulation at these sites (3–5).

In relation to the respiratory tract, the nature of the local cells engaged in processing inhaled antigen remains controversial. The most readily accessible phage population, the alveolar macrophages (AM),[1] express no or limited APC activity, depending upon the species examined, and in some circumstances appear to actively suppress T cell activation (reviewed in reference 6). The limited information available on macrophages from solid lung tissues also suggests low APC activity (7, 8). However, two recent studies have identified populations of highly active APC in solid lung tissues, which exhibit the physical (9) and phenotypic (10) properties of dendritic cells (DC). The present experiments examine the distribution of MHC class II antigen–bearing cells with DC-like

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[1] *Abbreviations used in this paper:* AEC, airway epithelial cells; AM, alveolar macrophages; BRBC, bovine red blood cells; DC, dendritic cells; HRP, horseradish peroxidase; LNC, lymph node cells; LWC, lung wall cells.

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morphology within the rat respiratory tract, and demonstrate that endogenous lung tissue macrophages actively suppress their in vitro APC activity.

Materials and Methods

Rat Tissues. Young adult female WAG rats were obtained from the Animal Resource Centre, Murdoch University, Perth, Western Australia. After they were killed by an overdose of Nembutal, lung wall cells (LWC) were prepared as detailed previously (10). Briefly, the rib cage was removed, and the lung vascular bed was flushed with 20 ml chilled Dulbecco's saline (DAB) containing 10 U/ml heparin introduced via cannulation of the heart, first severing the aorta above the liver. Perfusion was continued until the lungs appeared completely blood-free, and then the vessels entering and leaving the lung were immediately tied off. Repeated endobronchial lavage was then performed, using 6 × 10 ml RPMI medium supplemented with 10% FCS and 12 mM lignocaine hydrochloride, and this yielded a cell population that consisted predominantly of AM. The lungs were then sliced mechanically to 500 μM, and the separated slices were digested for 90 min at 37°C in a mixture of collagenase and DNAse, as described (10). After teasing the digested tissue and after rapid filtration through cotton wool, single cell suspensions were collected. The viability of these lung wall cells (designated LWC) was assessed by trypan blue exclusion and ranged from 80 to 90%. Airway epithelial cells (AEC), were prepared by a modification of the method of Sertl et al. (9). Individual tracheas in batches of 5–10 were opened with scissors, and submerged for 20 min in the collagenase/DNAse mixture, after which the loosened epithelium was removed with fine forceps; some contamination with underlying mucosal cells undoubtedly occurred at this point. The detached tissue was teased apart and incubated for a further 60 min at 37°C in collagenase/DNAse, resuspended by vigorous pipetting, and sieved rapidly through cotton wool, resulting in a single cell suspension that was >90% viable.

Cell Fractionation Procedures. Populations of LWC were fractionated by buoyant density on Percoll (Pharmacia Fine Chemicals, Uppsala, Sweden) gradients. Five-step discontinuous gradients were prepared by sequentially layering 5-ml volumes of 55–35% Percoll (in steps of 5%) into centrifuge tubes; the LWC were finally overlayed in 2 ml of 20% Percoll. After centrifugation at 400 *g* for 30 min at 20°C, the top 2 ml was discarded (containing mainly dead cells and debris), and cells were harvested from the ensuing 5,5 and 10 ml portions of the gradient at densities equivalent to 1.033, 1.048, and 1.062 g/ml, respectively (fractions 1–3 in the text below).

In other experiments, LWC and AEC were depleted of either adherent cells via nylon wool columns (11), or FcR-bearing cells via rosetting with IgG-coated bovine erythrocytes (12).

Depletion of surface Ig^+ or MHC class II (Ia) antigen–bearing cells from nonadherent LWC preparations was performed by a modification of the method described by Parish and Hayward (12). LWC were initially incubated with a mouse mAb against either rat κ chains (0x12; see below) or rat Ia (0x6; see below) at 4°C for 60 min, washed, and then incubated for 20 min at 4°C with bovine erythrocytes (BRBC) conjugated to sheep $F(ab')_2$ anti–mouse IgG. Rosette-positive cells were subsequently removed by differential centrifugation; these procedures reduced Ia^+ and $0x12^+$ cells to below 2% and 0.5%, respectively, frequencies equivalent to those of endogenous peroxidase-positive cells.

Antibodies. The mAbs 0x19 (13) and 0x52 (14) against surface markers on rat T cells; 0x41 and 0x42 (15), against surface markers on rat macrophages; 0x6 (16) against framework determinants on rat Ia molecules; and 0x12 against rat κ chains (14), were kindly provided by Drs. Alan Williams, Don Mason, Anne Robinson, and Jon Sedgwick, MRC Cellular Immunology Unit, Oxford, United Kingdom. ED1 (17), which stains most rat macrophages and some dendritic-like cells, was a gift from Dr. Christine Dijkstra, Dept. of Histology, Free University, Amsterdam, The Netherlands. Sheep anti–mouse IgG was immunopurified from hyperimmune sheep serum, and $F(ab')_2$ fragments were prepared as described previously (18). Biotinylated rabbit anti–mouse IgG, streptavidin–horseradish peroxidase (HRP) conjugates, and streptavidin-FITC conjugates, were purchased from Amersham, Sydney, Australia.

Staining Procedures. For fresh frozen sections, lengths of trachea were initially filled with OCT freezing medium (Tissue-Tek II; Miles Laboratories Inc., Naperville, IL), and embedded by freezing in OCT using liquid nitrogen–cooled isopentane. Lungs were initially filled with a dilution of OCT in PBS via the trachea, and 5-mm cubes were excised and embedded as above. Longitudinal or transverse sections were cut respectively from trachea and lung using a Bright cryostat, air dried at room temperature for 1 h, fixed in absolute ethanol at 4°C for 10 min, and rehydrated through 70% ethanol to Dulbecco's PBS, before incubation with mAbs.

Immunoperoxidase detection of antigens was by an indirect technique detailed previously (18). A mouse mAb against a human lymphocyte surface marker was used as a negative control. Incubation of tissue sections with mAbs was performed at 4°C for 1 h, followed sequentially by biotinylated sheep anti–mouse IgG and the streptavidin-HRP conjugate to detect bound mouse antibodies. After washing, enzyme-linked antibody was revealed by reacting with 3,3′-diaminobenzidine and hydrogen peroxide for 10 min at room temperature. The sections were then lightly counterstained with hematoxylin. In experiments involving double staining, counterstaining was preceded by a second sequence of incubations, commencing with PBS/1% BSA for 30 min, followed by another mAb, biotinylated sheep anti–mouse IgG, preformed streptavidin-biotin-alkaline phosphatase (Amersham), and finally bromo-chloro-idolylphosphate/nitro-blue tetrazolium.

For detection of antigens on alveolar lavage cells, cytocentrifuge preparations were initially air dried, ethanol fixed, and then rehydrated as above, before immunoperoxidase staining.

Antigen Presentation. Female WAG rats were immunized subcutaneously at the base of the tail, with 100 μg OVA (Grade *V*, Sigma Chemical Co., St. Louis, MO) emulsified in CFA. Single cell suspensions were prepared from inguinal and para-aortic lymph nodes 14–30 d after immunization, for use in in vitro T cell–activation assays. For the latter, 4×10^5 OVA-immune lymph node cells (LNC) in 200 μl RPMI 1640 supplemented with 5% syngeneic rat serum and 5×10^{-5} M 2-ME were dispensed in triplicate into wells of microtest plates, together with optimal stimulatory levels of OVA, or the control antigen BSA, as described (8). After 72 h, all cultures were inoculated with [^{3}H]thymidine, and cells were harvested for determination of [^{3}H]DNA synthesis (as Δ disintegrations per minute, Δ dpm per culture) at 96 h (8).

LNC from control (nonimmune) rats did not respond to OVA in vitro. Coculture of the LNC from immunized rats with OVA but not BSA stimulated significant levels of [^{3}H]DNA synthesis, and selective removal of T cells from these preparations by panning with the anti–rat T cell mAbs 0x19 and 0x52 (detailed in reference 19) abrogated OVA responsiveness (data not shown), indicating that the assay detected antigen-specific T cell activation.

To assay APC activity, the LNC were first depleted of endogenous APC via passage through nylon wool and fractionation on Percoll, which yields a population of >95% T cells. This process, which is described in detail in an earlier publication (20), renders the OVA-immune LNC preparation refractory to stimulation with soluble OVA, unless exogenous APC are first added.

Antigen Aerosol Exposure. WAG rats were placed unrestrained in a plexiglass chamber, and exposed for 30 min to an aerosol generated from a solution of 20 mg/ml OVA in PBS, as described (21). Control animals were exposed to an aerosol of PBS alone.

Results

Cell Yields from Rat Lungs. In these experiments, rat lungs were lavaged repeatedly in situ, until no further cells appeared in the washout (6 volumes of fluid). As shown in Table I, this procedure yielded on average 2.4×10^6 cells, of which 87% were phagocytic. 95–98% of these cells stained positively for the macrophage surface markers ED1 and 0x41, while 22% expressed the 0x42 marker, which is the rat homologue of the human and murine iC3b receptor (15). <5% of the lavage cells expressed the 0x19 or 0x52 pan–T cell markers,

TABLE I
Cell Yields from Rat Lung

Cell preparation method	Cell yields per 1.0 g lung						
	Total	Percent phagocytic*	Percent staining with:				
			ED1	OX6	OX19/52	OX41	OX42
Lavage	2.5×10^6	87	≥95	≤1	2	≥95	22
Lung digestion	1.1×10^8	19	26	15	35	22	21
Tracheal digestion	0.2×10^6	ND	3	26	11	20	18

Mean recovery figures are shown, derived from 12 animals.
* Colloidal gold uptake.

and Ia staining was not observed. Digestion of rat lung slices in a mixture of collagenase and DNAse yielded ~10^8 viable mononuclear cells per lung. The enzyme mixture used has previously been shown by us to not affect FcR activity in rat spleen cell preparations (7), or FACS profiles of human peripheral blood mononuclear cells stained with FITC-conjugated mAbs against an array of lymphocyte and macrophage surface markers (22). Accordingly, the cell surface markers detected on rat LWC illustrated in Table I are likely to reflect the surface phenotypes of these cells in vivo. This LWC population differed markedly from that obtained by lavage. In particular, tissue digestion yielded large numbers of T cells (~3.5×10^7 per lung) and Ia^+ cells (1.5×10^7 per lung), neither of which were observed in lavage fluids. Additionally, the expression of macrophage surface markers differed both qualitatively and quantitatively from that of lavage cells. In particular, a significant population of cells was observed that stained with the ED2 mAb, which has been shown to mark a subset of BALT macrophages, as well as cells in the perivascular and peribronchial regions of the lungs (17). It can also be seen that while the 0x42 mAb stains <25% of macrophages from the airways (viz., 30% $0x42^+$ versus 95% $0x41^+$), these frequencies are identical in the lung digest, and thus the 0x42 marker appears to be present on all macrophages in the LWC preparations. This finding of a relatively lower frequency of the monocyte marker 0x42 (equivalent to Mac-1 and OKM1) on the alveolar lavage population, is consistent with the more advanced stage of maturation/activation of AM relative to LWC macrophages, a conclusion born out by recent studies on the functional activity of these cell types (Bilyk, N., and P. G. Holt, manuscript in preparation).

Enzymatic digestion of tracheal tissue yielded on average 2×10^5 mononuclear cells per animal, of which 16% expressed Ia. Small numbers of $0x19/52^+$ T cells were also present, and equivalent staining (~20%) was observed with the macrophage markers 0x41/42.

APC Activity in Lung Tissue Digests. In the experiments illustrated in Fig. 1, OVA-immune LNC exhibited high levels of [^{3}H]DNA synthesis ($\bar{x}$ = 78, 850 dpm per culture) after coculture with an optimal stimulating concentration of OVA, and depletion of endogenous APC (by the methods detailed above) abrogated this response. These latter LNC were used as indicators in APC assays, using various fractions of the cell population released by digestion of solid lung tissue. Thus, in column A, APC-depleted lymph node cells incubated with OVA

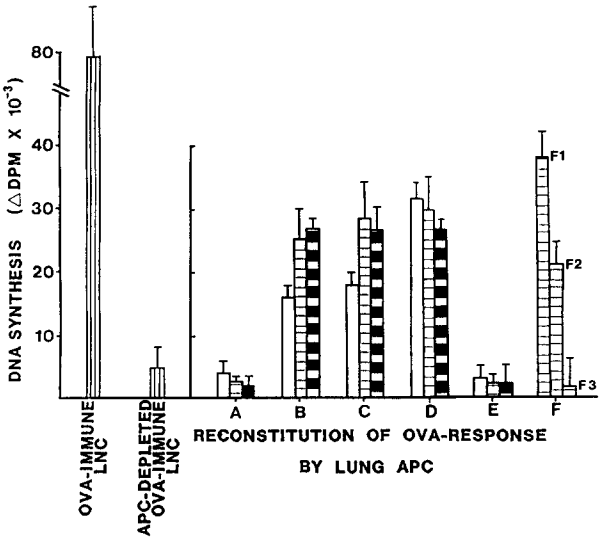

FIGURE 1. APC activity in lung tissue digests. Data shown are $\bar{x} \pm$ SE derived from triplicate cultures after subtraction of background (unstimulated) proliferation (≤2,000 dpm/culture). Cultures for the Exps. *A–F* comprised 4.0×10^5 APC-depleted OVA-immune LNC, cocultured with 200 μg/ml OVA, plus 1.25 (□), 2.5 (▤) or 5.0×10^3 (▦) mononuclear cells from lung tissue digests, as follows: (*A*) unfractionated lung digest cells; (*B*) FcR-depleted digest cells; (*C*) adherent cell–depleted digest cells; (*D*) *C*, after further depletion of $0x12^+$ cells; (*E*) *C*, after further depletion of Ia^+ cells; (*F*) *C* cells, three fractions from discontinuous Percoll gradient at densities of 1.033 (*F1*), 1.048 (*F2*), and 1.062 (*F3*) g/ml.

failed to proliferate, despite the addition of $1–5 \times 10^3$ LWC. However, if the LWC were first depleted of FcR-bearing (column B) or adherent cells (column C), their subsequent addition to the cultures reconstituted the capacity of the lymph node T cells to respond to OVA antigen, indicating the presence in the lung wall of APC.

Examination of the adherent lung cell population by immunoperoxidase showed a predominance of $0x42^+$ (47–56%) and $ED1^+$ (45–49%) macrophages, plus 67–75% $0x12^+$ cells. In contrast, the nonadherent population comprised mainly T cells (75–85%) and Ia^+ cells (up to 17%), plus small numbers (4–6%) of $0x12^+$ B cells; contamination of the latter with cells staining for the macrophage markers was low, in particular ED1, which was generally <1%. Depletion of $0x12^+$ cells by rosetting with conjugated BRBC (column D) did not affect the APC activity of the nonadherent LWC, indicating that B cells are not significant presenters of antigen in this system. 0x12 depletion also removed residual $ED1^+$ and $0x42^+$ cells from the nylon wool effluent population (to <0.1%). In contrast, rosette depletion of Ia^+ cells (column E) from the lung cells completely ablated their APC activity. Finally, fractionation of nonadherent LWC on Percoll before addition to the T cell cultures (column F) indicated that the most active APC appeared in the lowest density (≤1.033 g/ml) fraction, which comprised ~10% of the original nylon wool effluent population loaded onto the gradient.

Immunoperoxidase staining of this APC-enriched low-density lung fraction revealed 12–15% Ia^+ cells. Approximately one-third of these displayed prominent veils that stained strongly for Ia (Fig. 2), and they appeared indistinguishable from the veiled (dendritic) cells we have previously observed in rat lymph (20). The remainder of the Ia^+ cells in this fraction were smaller and highly pleiomorphic. This fraction also contained low numbers (up to 5%) of $0x42^+$ cells, the majority of which were small and regular in shape (not shown). The low-density lung cell preparation additionally contained ~20% $ED1^+$ cells. While a proportion of these were of large size, they were again round and regular in

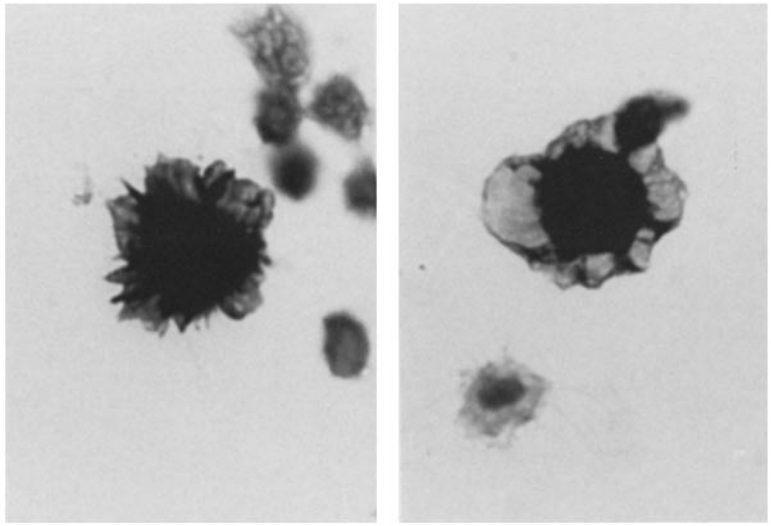

FIGURE 2. Low-density Ia^+ cells in lung tissue digests. Large DC-like cells settled onto alcian blue–treated slides, stained by immunoperoxidase for Ia (× 340). Note heavy staining above cytoplasm, and on prominent veils. The cells were collected from the low-density (≤1.033 g/ml) band from a discontinuous Percoll gradient.

shape, and dendritic or veiled $ED1^+$ cells were not observed. However, some DC have been reported to stain positively with the ED1 mAb (17). Consequently, despite the failure of ED1-depletion to diminish the overall APC activity of nonadherent lung cells (see above), the possibility remains that a small $ED1^+$ subset of DC may contribute to the APC activity of the low-density fraction. This possibility will be tested in future experiments involving positive selection of low-density $ED1^+$ cells.

Collectively, these data indicate that the major APC population in rat lung wall are nonadherent, FcR^-, surface Ig^-, Ia^+ cells of ultra low density, properties consistent with DC (23, 24). Additionally, the demonstration of APC activity in LWC preparations requires initial rosette depletion of FcR^+ cells, or passage through nylon wool, which depletes the majority of adherent cells expressing the 0x42 and ED1 surface markers (putative tissue macrophages).

Light Microscopy. Immunohistochemical staining of 5–7-μm frozen sections of respiratory tract tissues was performed to locate Ia^+ cells, particularly at sites of potential interaction with inhaled antigens. Information was also sought on the presence in the same tissue microenvironments, of cells staining with mAbs against known macrophage surface markers. We initially screened the 5–7 μm sections with the mAbs via peroxidase, and subsequently by a double-staining technique. We also examined sequential peroxidase-stained thin (3 μm) sections.

Ia^+ cells were dispersed throughout the lung parenchyma (Fig. 3, *A* and *B*). The stained cells were pleiomorphic, and included large DC-like (see particularly 3*A*) as well as small cells (immature macrophages or B cells?) within the alveolar septa, and large rounded, vacuolated cells apparently adherent to the air side of the lung wall (Fig. 3*B*). Free AM in these rats (not shown) were uniformly Ia^-, unlike the mouse (9). However, adherent cells of the type shown in Fig. 3*B* also

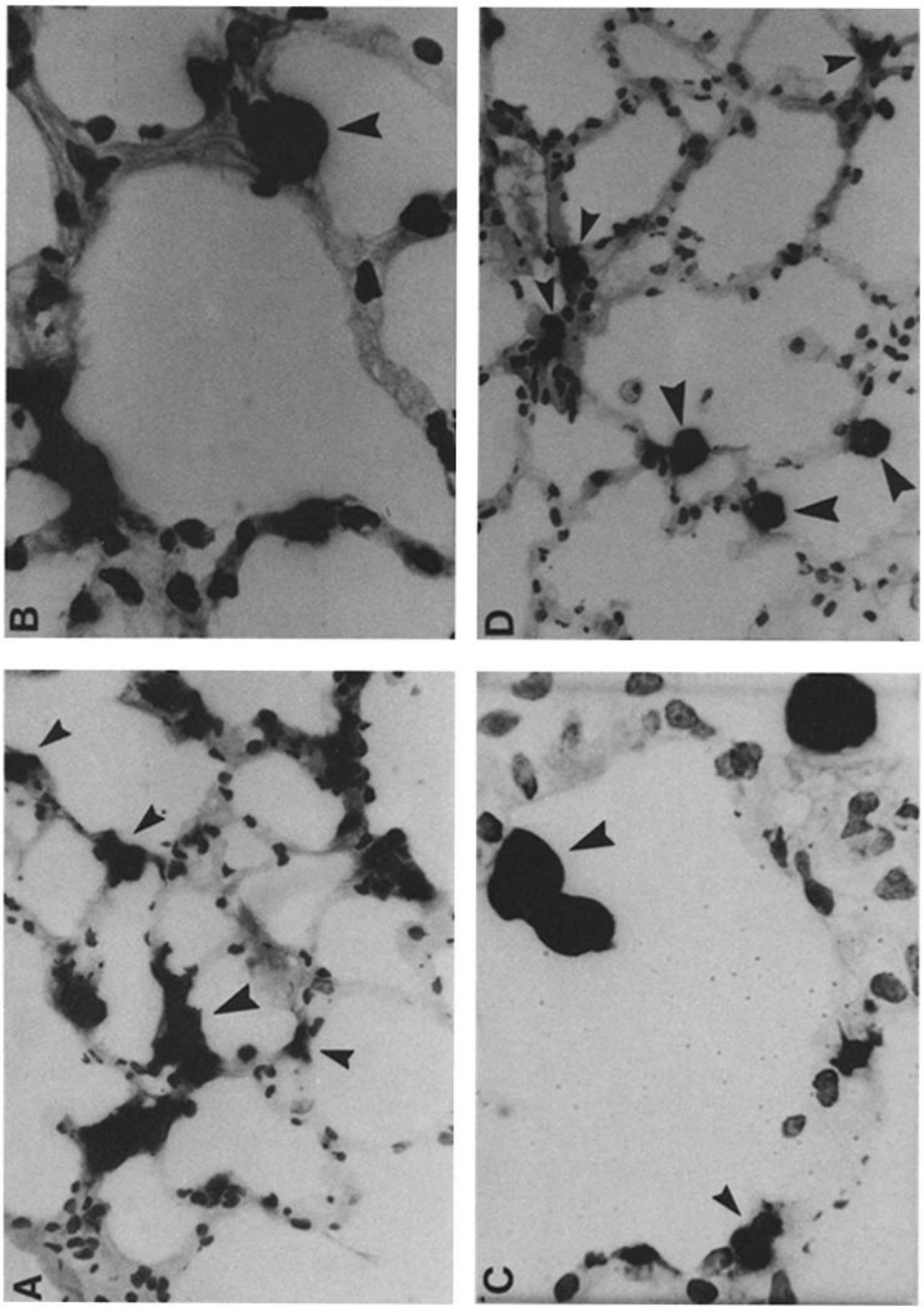

FIGURE 3. Immunoperoxidase staining of cells in alveolar region of rat lung. (*A*) DC-like (*large arrow*) and small Ia^+ cells (*medium-sized arrows*) in alveolar septal walls (magnification × 135); (*B*) large vacuolated Ia^+ cells attached to septal wall; vacuolation obscured in reproduction (× 340); (*C*) large $ED1^+$ cells in alveolar space (*large arrow*), and small stained cell within wall (× 340); (*D*) $0x42^+$ cells in alveolar spaces, attached to septal walls (*large arrows*), and within alveolar septa (*small arrows*; × 135).

stained with the ED1 (Fig. 3*C*) and 0x42 (Fig. 3*D*) mAbs, which together with their obvious vacuolation under the light microscope, suggests they are AM that had become attached to the lung wall; expression of Ia antigen here may indicate AM activation, associated with adherence to the alveolar epithelium. $0x42^+$ macrophages were also observed randomly distributed within the alveolar septa (Fig. 3*D*).

Fig. 4 illustrates longitudinal 5-μm sections of rat trachea. DC-like Ia^+ cells can be seen both on and below the epithelial basement membrane (Fig. 4, *A* and *B*). These cells were distributed at regular intervals throughout the airway epithelium in the trachea and bronchi, and ED1-staining cells with similar morphology showed a comparable distribution (Fig. 4*C*). In contrast, $0x42^+$ cells were comparatively rare within the airway epithelium, but were present in significant numbers in the underlying mucosa (Fig. 4*D*).

In experiments using sequential thin (3 μm) sections, cells staining positively for Ia and negatively for ED1 and 0x42, together with Ia^- cells that were positive for either ED1 or 0x42, could be readily observed in close proximity within the same sites in sequential sections (not shown). Using a double-staining procedure, we also observed peroxidase-stained Ia^+ DC-like cells juxtaposed to peroxidase-negative alkaline phosphatase–stained $0x42^+$ and peroxidase-negative alkaline phosphatase–stained $ED1^+$ cells. We could not readily identify individual double-staining cells with this procedure, although these undoubtedly occur (15, 17); this question will be addressed in more detail in future experiments using alternative enzyme substrates for tissue staining.

APC Activity of Alveolar Cells and AEC. Fig. 5 compares the APC activity of intact (columns A and C) vs. adherent cell–depleted (columns B and D) populations prepared from rats by endobronchial lavage, or by enzymatic digestion of tracheal epithelium. The APC assay system was as per Fig. 1. As noted previously (7), free cells from the airways of this species were incapable of transmitting antigen-specific activating signals to immune T cells in vitro (columns A and B). In contrast, significant APC activity was demonstrable in cell preparations derived from the airway epithelium (column C), which was enhanced by removal of endogenous adherent cells (column D).

The experiments of Fig. 6 demonstrate the capacity of APC in the tracheal wall to bind antigen that is inhaled under conditions of natural exposure in vivo. Groups of rats were initially exposed to an aerosol of DAB (column A) or DAB plus OVA (column B) for 30 min before they were killed, and their tracheal epithelial cells were extracted. The capacity of these cells to transmit an OVA-specific signal to T cells was assessed with the assay system used in Fig. 1, using OVA-immune LNC depleted of endogenous APC. It can be seen that AEC from OVA-exposed (but not control) rats triggered significant levels of [^{3}H]DNA synthesis in the T cell cultures.

Discussion

This report demonstrates the presence of Ia^+ cells with characteristic dendritic morphology within and below the airway epithelium, and within the alveolar septal walls of rats; this population is not represented, however, among the free airway cells accessible by endobronchial lavage. These putative DC appear in

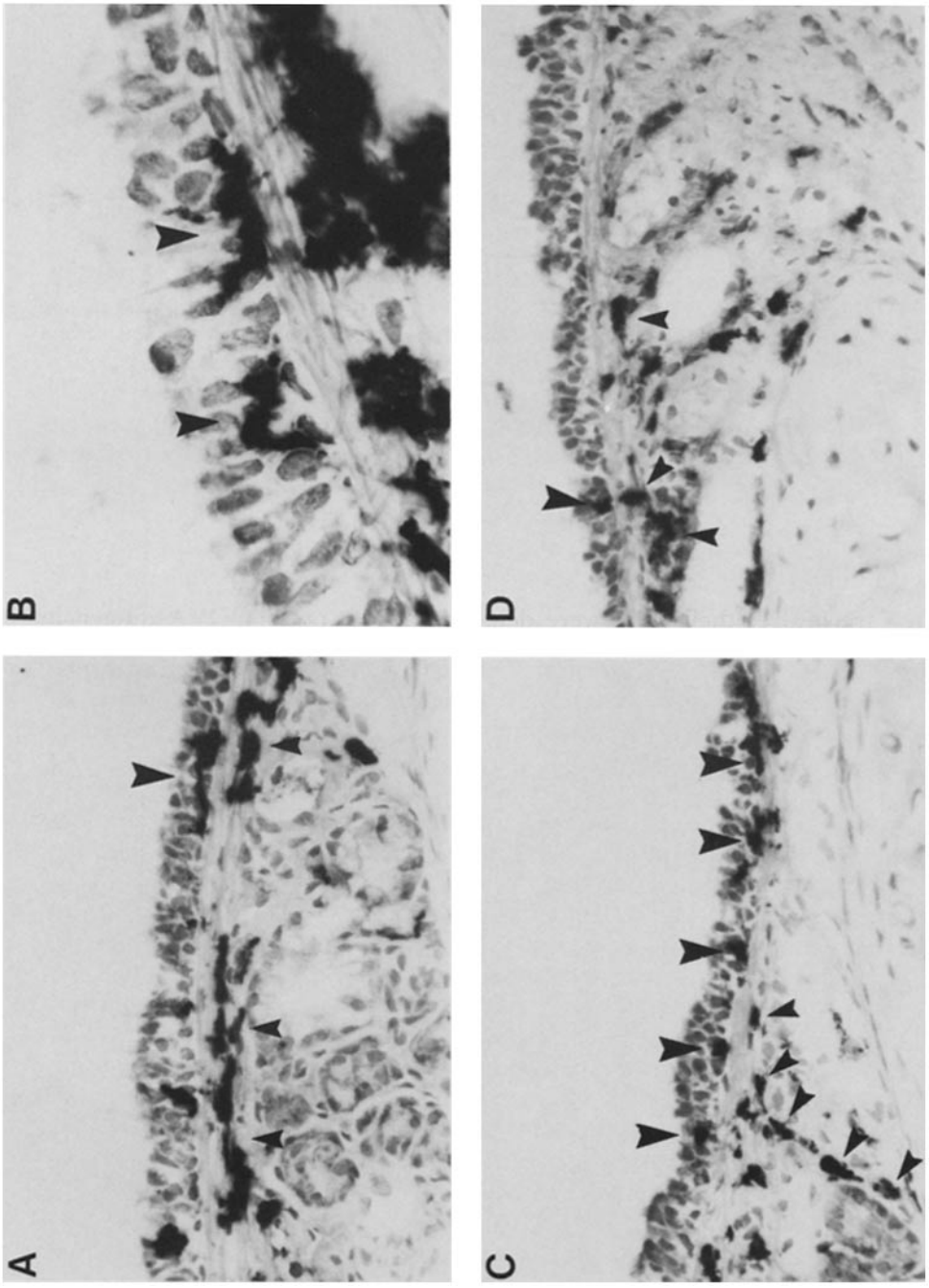

FIGURE 4. Immunoperoxidase staining of cells in and below tracheal epithelium. (*A* and *B*) Ia$^+$ DC-like cells stained within (*large arrows*) and below (examples designated by *small arrows*) the epithelium (× 135 and × 340); (*C*) ED1$^+$ cells within (*large arrows*) and below (e.g., *small arrows*) the epithelium (× 135); (*D*) occasional 0x42$^+$ cell within epithelium (*large arrow*), and more frequent staining (e.g., *small arrows*) in underlying mucosa (× 135).

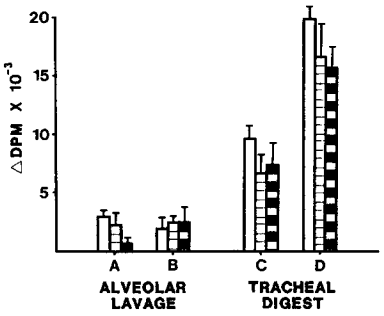

FIGURE 5. APC activity of cells in lavage fluids or in tracheal tissue digests. OVA-induced proliferation of APC-depleted OVA-immune LNC, reconstituted with 1.25 (□), 2.5 (▤), or 5.0×10^5 (▤) cells prepared by alveolar lavage, or digestion of tracheal tissue. Putative APC were added as unfractionated cell preparations (*A* and *C*) or after removal of adherent cells (*B* and *D*).

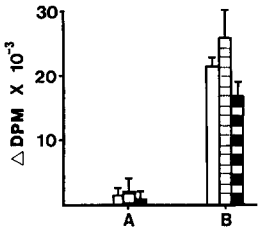

FIGURE 6. Binding of OVA-antigen in immunogenic form in vivo by tracheal epithelial cells. 1.25 (□), 2.5 (▤), or 5.0×10^3 (▤) nonadherent tracheal cells prepared from rats exposed to a control saline aerosol (*A*), or an aerosol containing OVA (*B*), were cocultured with 4.0×10^5 APC-depleted OVA-immune LNC, and resulting proliferation was determined 96 h later.

close proximity to cells expressing a variety of the macrophage surface markers, most of which are also found on AM.

The distribution of pulmonary DC in rat is thus comparable with that recently reported for DC in mouse and human respiratory tissues (9). In the latter study, mouse tracheal epithelial DC were demonstrated to present OVA-antigen in vitro to T cells, and our present results with cell preparations derived from rat trachea and lung indicate that cells of this type are important APC throughout the respiratory tract.

An important difference between our results with rat tissue and those from the mouse (9) concerns the expression of Ia antigens by, and the APC activity of, endogenous bronchopulmonary macrophages. While the available data from the mouse demonstrate that AM are capable of transmitting antigen-specific activation signals to T cells in vitro (9, see also review in reference 6), they are considerably less active than APC from other sources (6), particularly DC, which on a cell-for-cell basis appear to be the most active APC in that species (23). In the rat, the functional differences between DC and macrophages in relation to T cell activation appear even more extreme. In this situation, in vitro presentation of protein antigens to T cells appears to be the exclusive province of DC, whereas macrophages actively suppress T cell activation under identical assay conditions in vitro (6, 20, 24). The failure of free rat AM to function as APC in vitro is paralleled by their lack of expression of Ia, in contrast to the mouse, where 10–30% of AM are Ia^+ (9). However, as shown in earlier studies, rat AM also elaborate lymphocytostatic molecules that directly suppress T cell activation (6), and the dampening effect of FcR^+ adherent cells upon APC activity in lung and tracheal digests (Figs. 1 and 4) indicate that macrophages present in solid pulmonary tissue function in a similar fashion.

While this interplay between DC and macrophage populations provides a theoretical mechanism for downregulation of local helper T cell activation in the rat lung in the steady state, it begs the question of how immune induction occurs

when it is patently necessary for host survival, i.e., in the face of challenge with pathogenic antigen(s). Indirect evidence from a number of sources (reviewed in reference 6, see also reference 4) suggests that local inflammation, resulting in an influx of mononuclear cells from the peripheral blood, may temporarily disturb the balance between these regulatory cell populations, resulting in net T cell activation.

However, recent observations suggest that factors relevant to cell efflux from the tissue are also likely to be important. In this context we have reported that under steady-state conditions in SPF rats, up to 2×10^6 DC migrate from the gut wall per day, 90% of which are trapped in the draining (mesenteric) lymph nodes (20). These DC functioned as highly active APC in vitro (20). In addition, they were shown to transport antigens previously fed to the rats, to the mesenteric lymph nodes (20), which suggests an important role for these cells in regulation of immune responses to dietary antigen.

The present study, as illustrated in Fig. 6, demonstrates that putative DC in the airway walls also exhibit a high degree of APC activity in vitro, and are also capable of trapping antigen that impinges upon the epithelium that they occupy. It is therefore reasonable to postulate an important regulatory role for their airway cells and their equivalents in the alveolar septa, which includes antigen sampling at the respiratory epithelial surface, a transport function linking the epithelium to its draining lymph node, and the subsequent engagement of the antigen-bearing DC with relevant T cells in the lymph node. An important function of endogenous macrophages in this process may be to limit T cell activation (and attendant tissue damage) within the fragile epithelial and mucosal microenvironments during the transit of antigen-laden DC from the epithelium to the regional lymph nodes.

There is clearly a need to gain further information on the population dynamics of these cell populations in lung tissue, in both the steady state and during inflammation, to elucidate their roles in local immune regulation.

Summary

Collagenase digestion of tissue slices from perfused, lavaged SPF rat lung released ~10^8 viable mononuclear cells per gram tissue, which comprised 35% T lymphocytes and up to 26% macrophages. A subset of these cells that were Ia^+, surface Ig^-, nonadherent, FcR^- and of ultra low density (putative dendritic cells [DC]), presented protein antigen to immune T cells in vitro, and this function was inhibited by the presence of low numbers of endogenous adherent, FcR^+ cells (putative macrophages). APCs were also identified in digests from tracheal epithelium, and were shown to bind antigen in immunogenic form as a result of natural (inhalation) exposure in vivo. Immunoperoxidase staining of frozen sections revealed populations of strongly Ia^+ cells with prominent DC-like morphology within the alveolar septal walls and the tracheal epithelium; in both areas, they were closely associated with pleiomorphic cells that expressed macrophage surface markers. We accordingly postulate that interactions between Ia^+ antigen-presenting DCs and endogenous tissue macrophages play an important role in regulating T cell activity in the respiratory tract.

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