Brief Definitive Report

Rapid Dendritic Cell Recruitment Is a Hallmark of the Acute Inflammatory Response at Mucosal Surfaces

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Summary

Immunohistochemical analysis of challenge sites such as skin and the peritoneal cavity has identified neutrophils as virtually the sole cellular participants in acute bacterial inflammation, peak influx occurring 24–48 h in advance of mononuclear cell populations associated with adaptive immunity. This study challenges the general applicability of this paradigm. We demonstrate here that the earliest detectable cellular response after inhalation of *Moraxella catarrhalis* organisms is the recruitment of putative class II major histocompatibility complex–bearing dendritic cell (DC) precursors into the airway epithelium, the initial wave arriving in advance of the neutrophil influx. Unlike the neutrophils which rapidly transit into the airway lumen, the DC precursors remain within the epithelium during the acute inflammatory response where they differentiate, and develop the dendriform morphology typical of resident DC found in the normal epithelium. During the ensuing 48-h period, these cells then migrate to the regional lymph nodes. No comparable DC response was observed after epidermal or intraperitoneal challenge, and it may be that mucosal surfaces are unique in their requirement for rapid DC responses during acute inflammation. We hypothesize that the role of the DC influx during acute inflammation may be surveillance for opportunistic viruses, and that this covert protective mechanism is operative at a restricted number of mucosal tissue sites.

Previous reports from this laboratory have established the importance of dendritic cells (DC) as APC within normal lung tissue (1, 2), and have additionally shown that a functionally and morphologically identical DC population exists within the epithelial lining of the conducting airways of both humans (3) and rodents (4, 5), where they form a contiguous network analogous to the Langerhans cell (LC) population in the epidermis.

We have also recently presented evidence that the density, distribution, and surface phenotype of airway DC populations reflects the level of stimulation provided by inhalation of airborne irritant stimuli (5). Moreover, brief exposure to aerosolized bacterial LPS was demonstrated to induce a transient increase (~50%) in the density of airway intraepithelial DC during the 24–48-h period after exposure, suggesting active participation of DC in the acute inflammatory response (5). The present study sought to further elucidate the role of DC in acute inflammation in the airways, employing a much more potent inhaled stimulus in the form of whole bacteria.

Materials and Methods

Animals. Specific pathogen-free (SPF) adult PVG rats were used in these experiments. They were barrier housed under dust-free conditions, as detailed previously (5).

Aerosol Exposure. The animals were exposed for 60 min to an aerosol of heat-killed *Moraxella catarrhalis* organisms (clinical hospital isolate) suspended in normal saline at $\sim 10^9$ CFU/ml.

Antibodies and Immunostaining. The mAbs Ox6 (Ia), Ox19 (CD5; pan T cell), Ox12 (κ light chains; pan B cell) (6), and Ox 42 (β chain of CD11a/18) (7) were a gift from Dr. D. Mason (Medical Research Centre Cellular Immunology Unit, Oxford, UK), and ED2 (pan tissue macrophage) (8) was a gift from Dr. C. Dijkstra (Vrije University, Amsterdam, The Netherlands). Fixation and single color immunostaining procedures were as described (5); dual color immunostaining was as detailed (9).

Quantitation of DC. For enumeration of DC in lymph nodes, single cell suspensions were prepared and rigorously depleted of macrophages by adherence to nylon wool (2), and nonadherent cells collected by slow elution were immunostained with combinations of mAbs against Ia, κ light chains (to exclude B cells), and ED2 (to exclude macrophages), and analyzed by flow cytometry. The cells that were scored as DC were nonadherent, Ia^+, $Ox12^-$, and $ED2^-$. Enumeration of DC in frozen sections employed the technique previously described (5), and employed the same immunostaining criteria.

Antigen Presentation in Primary MLR. Semi-purified DC were prepared from lymph nodes by cell sorting, gating for the population which was Ia^+, $Ox12^-/ED2^-$; this procedure yielded DC preparations that were 58–70% pure. The MLR procedure was a micromodulation of that in our earlier study (9), and employed 2×10^4 responder cells per microplate well.

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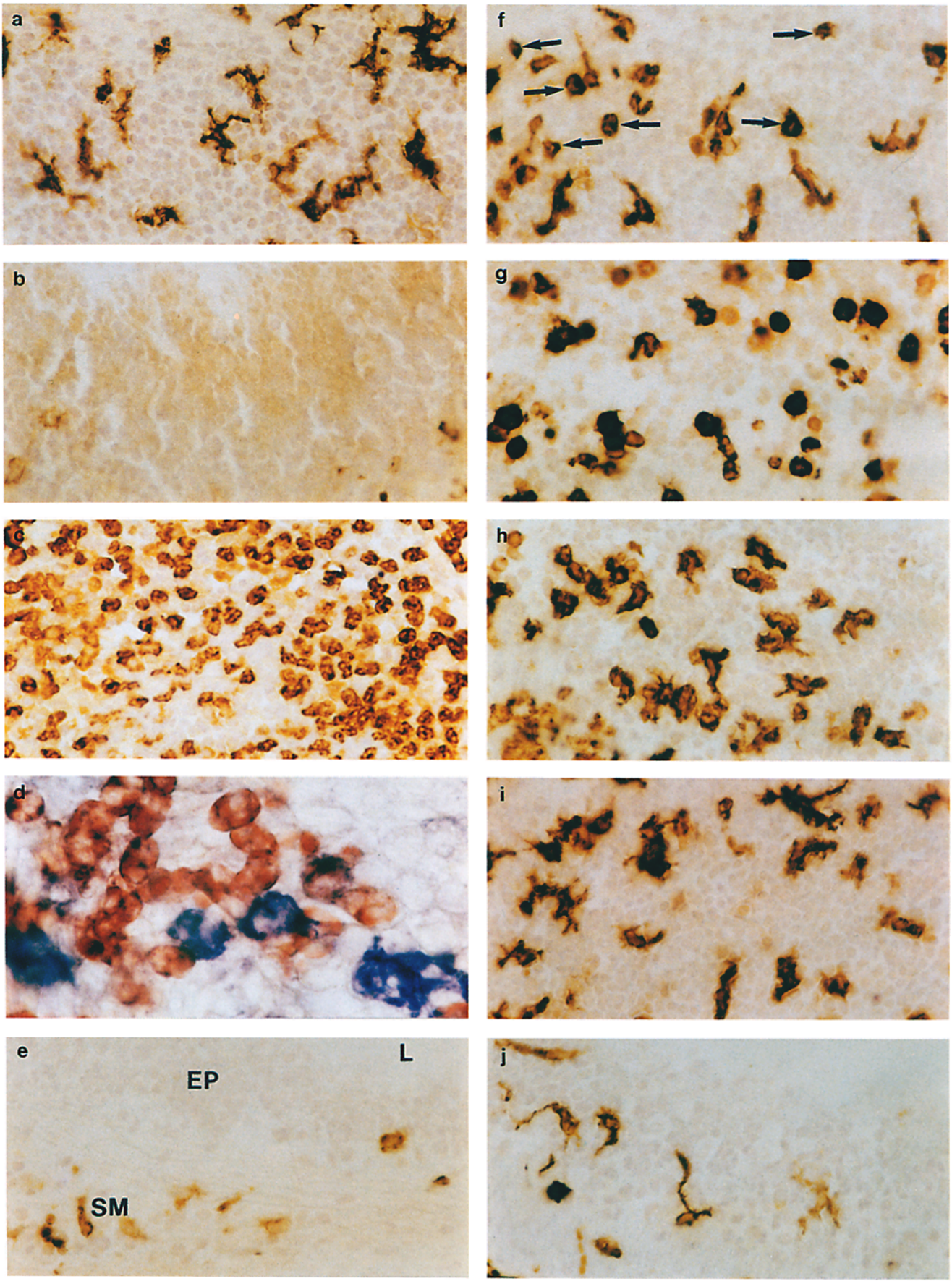
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Results and Discussion

In the rat, histological identification of DC in tissue sections is based upon demonstration of a characteristic pleomorphic, dendriform morphology, coupled with intense surface staining for class II MHC (Ia) and a lack of reaction with mAb that are pan specific for tissue macrophages or B cells. Immunoperoxidase staining of airway tissues from normal control rats with the anti-Ia mAb Ox6, reveals a pattern of staining typified by Fig. 1 *a*, in which virtually 100% of Ia^+ cells display typical DC morphology; <1% of these cells stain with the macrophage-specific mAb ED2 (2, 5; see also below).

To examine the response of the airway intraepithelial DC network to microbial-induced inflammation, normal PVG rats were exposed for 60 min to an aerosol containing heat-killed *M. catarrhalis* organisms. We chose to use this organism because of its known ability to induce an acute inflammatory tracheitis in humans. As can be seen in Fig. 2 *A*, this provoked a rapid inflammatory response characterized by the migration of large numbers of neutrophils onto the lumenal surface of the airways. These cells, which were recovered by bronchoalveolar lavage, reached maximum numbers within 4–8 h after challenge and waned thereafter. The number of recoverable alveolar macrophages did not change until 48 h when the typical secondary wave of influxing macrophages had begun (Fig. 2 *A*). This response is highly reproducible and the changes reported here for cell populations in the airway lumen and adjacent tissues are typical of a large series. Resident alveolar macrophages in SPF rats are Ia^-; Ia^+ cells were absent in the lavage fluids of these animals throughout the 48-h sampling period.

Immunostaining of tangential sections of airway epithelial tissue at various time points from 2 h after challenge revealed large numbers of intraepithelial neutrophils. These cells were readily identifiable by characteristic multilobed nuclear morphology (evident at high magnification), regular shape and small size, and intense surface staining for CD11a/18 (7, c.f. Fig. 1, *b* versus *c*); they were additionally $ED2^-$ (Fig. 1 *e*) and endogenous peroxidase negative, and did not costain for Ia with the mAb Ox6 (Fig. 1 *d*).

Fig. 1, *f–j* shows airway epithelial sections taken sequentially after bacterial challenge, and immunostained for Ia antigen. Fig. 1 *f* typifies the cellular changes occurring after 30 min and illustrates the appearance within the epithelium of regular shaped intensely Ia^+ cells (*arrows*) which were not seen in control sections (Fig. 1 *a*). It is clear that the degree of "arborization" of the resident DC at this time point was significantly reduced relative to that seen in control sections.

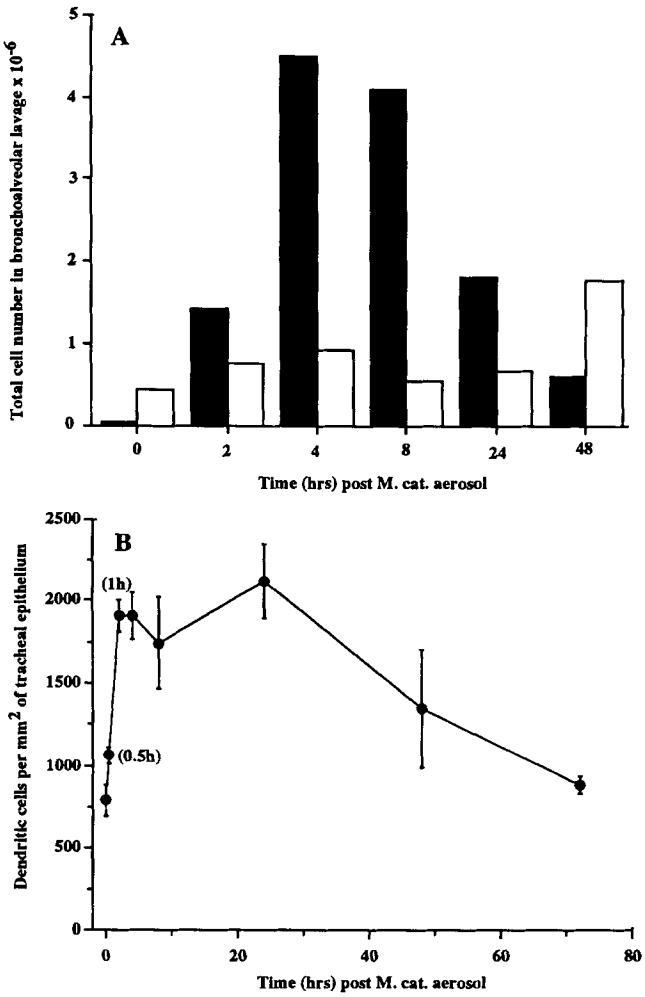

Figure 2. Cellular inflammatory response in the lung and airways after inhalation of whole bacteria. Adult PVG rats were exposed to aerosolized *M. catarrhalis* as per Fig. 1. At various times after the aerosol exposure, bronchoalveolar lavage was performed using medium containing 0.35% (wt/vol) lignocaine. Cytospin preparations of the cells obtained were stained with Leishmans stain and differential counts performed. Tracheas were removed at each time point and frozen sections prepared and immunostained as detailed in Fig. 1. (*A*) Recovery of neutrophils (*filled bars*) and macrophages (*open bars*) from bronchoalveolar lavage fluids. Cytospin preparations stained with Ox6 contained little or no Ia^+ cells. (*B*) Fluctuations in the density of Ia^+ DC in the tracheal epithelium during the inflammatory response. The data shown are derived from three to five animals per time point.

Figure 1. Cellular influx into the tracheal epithelium after aerosol exposure to *M. catarrhalis*. Adult PVG rats were exposed to an aerosol of heat-killed (60°C for 30 min) *M. catarrhalis* (clinical hospital isolate) suspended in normal saline. (*a–j*) Immunostaining of tracheal tissue sectioned through the epithelium parallel to underlying basal lamina (9), except (*e*) (see below). (*a*) immunoperoxidase staining of normal tracheal epithelium with Ox6, revealing a network of constitutively Ia^+ highly dendriform DC; (*b*) 30-min postinhalation of bacteria, immunostaining for Ox42 showing the absence of neutrophils; (*c*) 2 h after exposure stained for Ox42, revealing a large infiltrate of $Ox42^+$ neutrophils into the epithelium; (*d*) dual colour immunostaining for Ox42 (*red*) and Ox6 (*blue*) at the 2-h time point, demonstrating the lack of overlap between these two cell populations (note the indented nucleus characteristic of DC in central $Ox6^+$ cell); (*e*) tissue taken at 4 h after exposure, cut in an oblique plane of section and stained with ED2 illustrating the position of the airway lumen (*L*), epithelium (*EP*) and submucosa (*SM*). The low levels of intraepithelial staining with ED2 was consistent throughout the time course; (*f–j*) immunoperoxidase staining of tracheal epithelium for Ia at 30 min and, 4, 8, 24, and 72 h after exposure to *M. catarrhalis*.

At 2 h, the total number of Ia^+ cells within the epithelium had attained peak levels, equivalent to a 3.5-fold increase over controls, and numbers remained at this level over at least the ensuing 24 h (see Fig. 2 *b* and below).

At 4 h after challenge, densely staining cells with a regular morphology comprised the dominant Ia^+ population within the epithelium, and few dendriform cells were visible (Fig. 1 *g*). It should be emphasized that neither at this time point nor any stage during the acute inflammatory response did the cells within the epithelium express the pan-specific macrophage marker defined by the ED2 mAb, although macrophages were prominent within the submucosa beneath the epithelial basal lamina (example shown in Fig. 1 *e*). Also, no influx of T cells was seen.

At the 8-h time point (Fig. 1 *h*), the morphology of the intraepithelial Ia^+ cell population had changed markedly towards a more pleomorphic form reminiscent of DC in their "veiled cell" form (10) and, by 24 h, many of these cells appeared to have developed into typical mature, highly branched DC (Fig. 1 *i*). We interpret the gradual temporal change in the morphology of the Ia^+ cells in the airway epithelium over the 8–24-h period of this response in the absence of concomitant change in their number per unit area, to indicate the morphological maturation of incoming DC precursors, and this rationale forms the basis for the DC counts expressed in Fig. 2 *B*.

By 72 h after challenge, the density of Ia^+ DC within the airway epithelium again approximated control levels (Fig. 2 *B*), with close to 100% of these cells expressing typical highly dendriform morphology. (Fig. 1 *j*).

Cytospin preparations of cells obtained by bronchoalveolar lavage during the course of these experiments were also stained for Ia antigen with the Ox6 mAb. As no increase in Ia^+ cells was detected, it appears that the influxing DC were restricted to the epithelial layer. Similar experiments, in which the bacterial preparation was injected intraperitoneally, resulted in large increases in lavageable neutrophils with no apparent increase in constitutively Ia^+ cells. These results suggest the existence of specific DC surface receptors responsible for maintaining the influxing DC within the epithelial layer.

Previous studies on DC traffic from our laboratory (10) and elsewhere (11), suggest that the majority of emigrant DC-exiting epithelial tissue are filtered out in respective draining lymph nodes, and the data of Table 1, which indicate that a large increase in DC numbers occurs in the lymph nodes draining the upper and lower respiratory tract during the acute inflammatory response to inhaled bacteria, are consistent with this notion. These "inflammatory" DC manifest MLR-stimulatory activity which superficially appears comparable to controls (Fig. 3), but more detailed experiments are required to accurately determine their capacity to process and present different types of antigen.

The DC build-up in the regional lymph nodes during acute inflammation presumably comprises both DC resident within the airway epithelium before challenge and which are subsequentially "purged" from the tissue during the early phase of the response (the early disappearance of dendriform Ia^+ cells noted in Fig. 1 *g* being consistent with this suggestion), as well as freshly recruited cells cycling rapidly through the inflamed epithelium. In this context, it is noteworthy that gavage of rats with bacterial LPS has been shown to stimulate the passage of large numbers of DC from the gut wall into draining lymph (12).

Based upon the biphasic nature of the DC response in the airway epithelium, it also appears likely that at least two sets of stimuli are generated locally, viz. a chemoattractant stimulus that rapidly attracts immature DC, and a concomitant signal that remobilizes the recruited cells soon after arrival. Recent studies in mouse skin have demonstrated that exogenous TNF-α is capable of rapidly mobilizing epidermal DC which then migrate to regional lymph nodes. Inhalation of bacterial LPS by rats has recently been shown to trigger local production of high levels of this cytokine (13), suggesting it is likely to play a role in regulating DC traffic in the airway epithelium during inflammation. Little is known of the nature of the signals involved in attraction of DC to specific tissue

Table 1. *DC "Traffic" from the Airway and Lung Wall to Respective Draining Lymph Nodes during Acute Inflammation*

	Mean number of DC per lymph node $\times 10^{-5}$			
Lymph node	Control	*M. catarrhalis* aerosol	Difference	Percent increase
Superficial cervical	20.7	24.3	3.6	17
Internal jugular	0.88	2.67	1.79	203
Parathymic	0.40	1.53	1.13	282
Posterior mediastinal	1.39	2.54	1.15	83

Data shown are mean number of DC per lymph node from groups of three animals. PVG rats were exposed to an aerosolized bacterial preparation as per Figs. 1 and 2. After 48 h, the superficial cervical, internal jugular, and parathymic lymph nodes draining the upper respiratory tract and conducting airways, and the posterior mediastinal nodes draining the peripheral lung, were removed, a single cell suspension was prepared, and macrophages were depleted by adherence to nylon wool. DC were enumerated by flow cytometric analysis, as detailed in Materials and Methods. Parallel analysis of lymph nodes taken at the 24 h time point revealed a similar order of increase in DC numbers, however the changes were more consistent at 48 h.

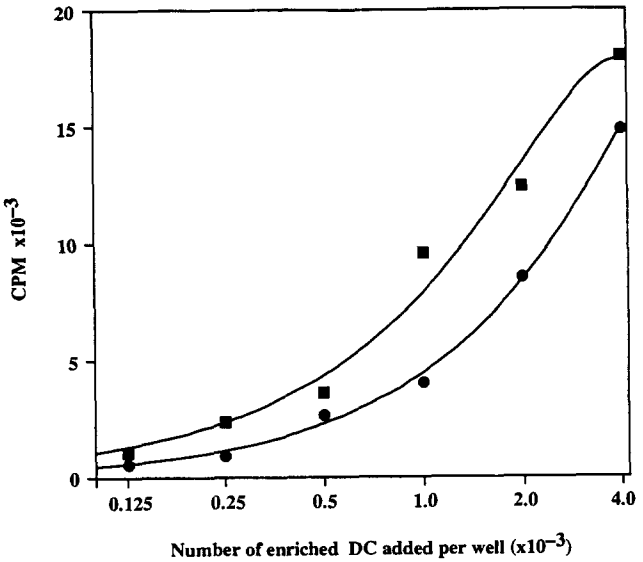

Figure 3. MLR stimulating activity of DC from normal and *M. catarrhalis*–exposed rats. Preparations of isolated DC from control or exposed PVG rats were titrated into lymph node cells isolated from Lewis rats; the cell preparations used contained 58.3 and 61.8% DC, the latter accounting for all Ia$^+$ cells present (the principal contaminants being Ia$^-$ epithelial cells). ^{3}H-DNA synthesis (shown as median CPM from replicate cultures) was determined at 5 d. No MLR activity was obtained employing purified mononuclear cells from the lavage fluids from these animals (data not shown). Control animals (●), *M. catarrhalis*–exposed animals (■).

microenvironments. However, as we were unable to demonstrate any changes in Langerhans cell numbers after subcutaneous injection of *Moraxella* (data not shown), it appears that the DC population in the airway epithelium is, perhaps, a more dynamic population and is highly reactive to locally produced cytokines. We are currently investigating the nature of these cytokines, and are seeking to identify the cells from which they derive.

In summary, we have demonstrated that during the course of an acute inflammatory response to bacterial stimuli in the conducting airways, active DC "surveillance" of the adjacent lining epithelium is amplified, resulting in an increase in the traffic of these cells between the airway epithelium and the regional lymph nodes. Recent studies on DC from a variety of peripheral tissues (11) including the lung and airways (9, 14) emphasize the important role of these cells in the induction of primary immune responses, in particular through the presentation of viral antigens to T cells (15–17), and we speculate that the function of this hitherto covert DC response during acute inflammation may accordingly be to protect the host against concomitant infection with opportunistic viruses. Given the known association between airway inflammation and primary T cell sensitization to inert airborne environmental antigens (18), it is also likely that this DC response may play a pivotal role in the inductive phase of respiratory allergic disease.

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