# The Antigen-presenting Activities of Ia$^+$ Dendritic Cells Shift Dynamically from Lung to Lymph Node After an Airway Challenge with Soluble Antigen

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## Summary

Dendritic cells (DC) are widely distributed in the lung where they are distinguished by their morphology and class II major histocompatibility complex (Ia) antigen expression. Although a role for DC as pulmonary antigen-presenting cell (APC) has been suggested, little is currently known concerning how these cells respond to inhaled antigens in vivo. Hen-egg lysozyme (HEL) was injected intratracheally into Lewis rats; DC were subsequently purified from the lung and regional lymph nodes (LN) at intervals of up to 14 d and examined for their ability to stimulate the proliferation of HEL-immune T cells in vitro in the absence of added HEL. Pulmonary DC displayed APC activities at 3 h and for up to 7 d after the injection of antigen. Dendritic cells in the draining hilar LN showed APC activities that appeared at 24 h, peaked at day 3, and then diminished progressively. After the primary sensitization, HEL-immune T cells were detected in hilar LN but not in the lung. A second airway challenge with HEL at day 14 yielded an antigen-specific pulmonary immune response, characterized histologically by the accumulation of mononuclear cells around lung venules. We conclude that APC activities shift from lung to lymph node during the response to inhaled antigen.

Pulmonary immune responses can be generated by the introduction of antigen into the airways (1–3). The development of specific cell-mediated pulmonary immunity requires an afferent phase, during which inhaled antigens are captured, processed, and presented to T cells by class II MHC (Ia)$^+$ accessory cells. Until recently, it was widely believed that macrophages were the dominant APC in the lung. However, pulmonary macrophages generally do not provide accessory support for T cell mitogen responses (4–8).

Investigators have demonstrated that Ia$^+$ dendritic mononuclear cells are the effective APC of the lung (9–14). These cells are strategically located in the pulmonary airways, pulmonary vessels, alveolar septa, and pleura, where they may function as sentinels for inhaled or circulating antigens. Holt et al. (11) have shown that pulmonary dendritic cells (DC)[1] can present inhaled antigens to immune T cells and that they traffic from the lung to the draining LN, after exposure to an airway bacterial stimulus (15). In the present study, we examined changes in the APC activities of DC in the lung and draining LN after an intratracheal challenge with hen-egg lysozymes (HEL).

[1] *Abbreviations used in this paper:* DC, dendritic cells; HEL, hen-egg lysozyme; i.t., intratracheally.

## Materials and Methods

### Animals

Inbred, pathogen-free, 6–8-wk-old female Lewis rats (150–250 g) were obtained from Charles River Laboratories (Kingston, MA). Rats were housed in a restricted access animal care facility, and permitted access to food and water ad libitum.

### Reagents and Antibodies

*HEL.* HEL (Sigma Chemical Co., St. Louis, MO) was prepared at a concentration of 1 mg/ml in normal saline, filtered through a 0.45-$\mu$m low protein-binding filter (Acrodisc no. 4184; Fisher Scientific, Boston, MA) and stored at 4°C.

*Heat-killed Listeria monocytogenes (HKL).* HKL was obtained from the M.G.H. Bacteriology Laboratory (Boston, MA). The concentration of organisms was determined by McFarland Nephelometer standards and the bacteria were heat-inactivated in a 63°C water bath for 90 min. Viability was assessed by the failure of the bacteria to grow on blood agar plates. Aliquots ($10^9$ bacteria/ml in saline) were stored at −20°C.

*Indocyanine Green.* Indocyanine green dye (2.5 mg/ml; Becton Dickinson Microbiology Systems, Mountain View, CA) was used as a marker for the distribution of HEL in the lung after intratracheal administration.

*Chloral Hydrate.* Chloral hydrate (Fisher Scientific) was administered as an anesthetic at a dose of 400 mg/kg.

*Collagenase.* Collagenase (150 U/ml; Worthington Biochem-

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ical Corp., Freehold, NJ) and DNase (50 U/ml; Sigma Chemical Co.) were freshly prepared for digestion of rat lung.

*BSA.* BSA was prepared by a modification of the procedure of Steinman and Cohn (16). 10.5 g BSA powder, fraction V (Intergen, Purchase, NY), was solubilized in 2.9 ml 1 N NaOH, 5.8 ml double distilled water, and 18.6 ml PBS to yield a final pH of 7.35 ± 0.05 and a density of 1.080, as judged by density refractometry (ABBE-3L, 33-46-10; VWR Scientific, Boston, MA). The solution was filtered using a 0.45-$\mu$m filter with prefilter (Nalgene; Fisher Scientific) and maintained at 4°C before use.

*Complete Medium (CM) and Culture Conditions.* Cells were cultured in RPMI-1640 (JRH Biosciences, Lenexa, KS), 10% heat-inactivated fetal bovine serum (FBS; Sigma Chemical Co.), 50 $\mu$g/ml Gentamycin (GIBCO BRL, Gaithersburg, MD), 0.5% 1 M Hepes buffer (GIBCO BRL), and 2-mercaptoethanol ($5 \times 10^{-5}$ M) (Sigma Chemical Co.), and incubated at 37°C in a humidified chamber of 95% air and 5% $CO_2$.

*[$^3$H]Thymidine.* Radiolabel was used at 1 $\mu$Ci/well (specific activity 81.0 Ci/mmol; Dupont/New England Nuclear, Boston, MA).

*rh-IL-2.* rhIL-2 (Cetus Corp., Emeryville, CA) was added to culture wells at a final concentration of 100 U/ml.

*Monoclonal Mouse Anti-rat Antibodies.* Anti-rat mAbs were used to purify and characterize cells in these studies. These included: W3/13 (CD43), W3/25 (CD4), OX-8 (CD8), OX-39 (CD25), OX-1 (CD45R), OX-22 (CD45RC), OX-43, OX-6 (Ia), (all from Accurate Chemical & Scientific Co., Westbury, NY); ED1 and ED2 (a generous gift from C. Dijkstra, Free University, Amsterdam, the Netherlands), anti-IgM, OX-12 (anti-$\kappa$ light chain), and OX-33 (CD45RA); (all from Pharmingen, San Diego, CA). The mAbs were prepared either as ascites or supernatants at predetermined optimal concentrations. For in situ localization, DC or lung tissues were stained by an avidin-biotin immunoperoxidase technique (13). Surface immune phenotype was examined in a cytofluorimeter (FACS® 440; Becton Dickinson) after direct staining with fluorescein-conjugated anti-rat mAbs or indirect staining with goat F(ab')$_2$ anti-mouse IgG-FITC (Tago Immunologicals, Camarillo, CA) (17).

*Generation of HEL and HKL Immune T Cells.* Rats were immunized with an emulsion of 100 $\mu$g HEL or $10^7$ HKL in CFA (Difco Labs., Detroit, MI). The emulsion (0.1 ml) was injected bilaterally at the base of the tail; inguinal LN were harvested at 10–14 d, mechanically dispersed, and separated on Isolymph (Gallard Schlesinger Industries Inc., Carle Place, NY). LN mononuclear cells ($2 \times 10^6$) were incubated in 24-well culture plates with HEL (100 $\mu$g/ml) or HKL ($10^7$/ml) antigen in a humidified chamber of 95% air and 5% $CO_2$. IL-2 (100 U/ml) was added to the medium at day 5 and then 2–3 times weekly. Every 3–4 wk, the cultures were restimulated with HEL or HKL, respectively, in the presence of normal irradiated (3,000 cGy) syngeneic spleen cells at a ratio of 10:1 spleen cells to T cell blasts. Immune phenotyping by cytofluorimetry showed the antigen-specific lymphoblasts were >95% W3/25$^+$(CD4) OX22$^-$(CD45RC). The specificity of the response was judged by a greater than threefold difference in the magnitude of [$^3$H]thymidine incorporation by T cell blasts to HEL compared to an irrelevant antigen (HKL).

*Intratracheal Instillation.* Rats were lightly anesthetized with chloral hydrate (400 $\mu$g/kg), and the trachea was surgically exposed. Equal volumes (0.1 ml) of HEL (10 or 100 $\mu$g/rat), normal saline, and indocyanine green (2.5 mg/ml) were introduced into the trachea slowly via a 25-gauge needle. The rats were subsequently killed at 3 and 24 h, and at days 3, 7, and 14. In some experiments, rats were rechallenged with HEL (100 $\mu$g/rat) intratracheally on day 14 and killed at 72 h, to determine whether HEL-specific T cell responses were generated in the lung.

*Dendritic Cell Purification.* Dendritic cells were purified from lung, spleen, hilar LN, and inguinal LN, as previously described (13). Briefly, lungs were perfused with saline via the pulmonary artery to diminish blood contaminants. Excised lung tissue was digested with collagenase and DNase, fractionated by BSA density gradient sedimentation, and the desired mononuclear cell fraction was attached to tissue culture dishes (no. 3003/Falcon; Fisher Scientific) with CM for 2 h at 37°C. Nonadherent cells were discarded and the adherent fraction was cultured overnight at 37°C. Loosely adherent cells enriched for DC were further subjected to immunopanning with OX-6. The selected cells were retrieved by gentle scraping with a rubber policeman and applied to plastic tissue culture dishes for 1 h at 37°C to remove adherent macrophages. The nonadherent cells were judged to be highly enriched (>90%) for DC based on their morphology, failure to stain for nonspecific esterase, immunostaining characteristics, and ability to stimulate a primary allogeneic MLR (13).

*Accessory Cell Activities.* Dendritic cells from the lung and LN were irradiated (3,000 cGy), suspended in CM, and plated ($10^4$ cells/well) into 96-well flat-bottom culture plates (Falcon; Fisher Scientific). Nylon wool-treated and OX-6$^+$ cell-depleted splenic lymphocytes, HEL-immune, or HKL-immune T cells ($5 \times 10^4$) were added to DC with or without HEL (100 $\mu$g/ml) or HKL ($10^7$ bacteria/ml). The culture plates were incubated for 72 h at 37°C, and the wells were pulsed with [$^3$H]thymidine (1 $\mu$Ci/well) for 6 h before harvesting. The pulsed wells were harvested in a semi-automatic cell harvester (Skatron Inc., Sterling, VA) and counted in a Tri-Carb liquid $\beta$-scintillation spectrometer (Packard Instrument Co., Sterling, VA). The percent specific response was calculated by the formula:

$$\text{cpm}\ \frac{\text{(HEL immune T cells + HEL pulsed DC)}}{\text{(HEL immune T cells + HEL pulsed DC + HEL)}} -$$

$$\text{cpm}\ \frac{\text{(HEL immune T cells + control DC)}}{\text{(HEL immune T cells + control DC + HEL)}} \times 100$$

*Limiting Dilution Analysis (LDA).* The frequency of DC functioning as APC in vivo was estimated by LDA. HEL-immune T cells ($5 \times 10^4$/well) were plated in >20 replicate wells of 96-well flat-bottom culture plates with irradiated DC (9.75–1250/well) isolated from HEL-challenged lungs. The plates were incubated at 37°C for 72 h and the wells were pulsed with [$^3$H]thymidine (1 $\mu$Ci/well) for 6 h before harvesting. A positive response was scored when the mean cpm per well exceeded the mean cpm $\pm 3 \times$ SD of [$^3$H]thymidine incorporation of wells that received antigen-specific T cells alone. When the number of DC providing APC activity is limiting, a positive response (Fo) is not detected. The mean number of limiting cells, designated $\mu$, was estimated by the Poisson distribution. Wells showing no response were scored, and the fraction of nonresponding wells was plotted against cell number for each condition. As estimates of $\mu$ are most accurate in the range, $0.1 < \text{Fo} < 0.37$, the limiting number of DC was estimated for $F = 0.37$.

## Results

*Dendritic Cell Yield and Purity.* Visual inspection of the freshly excised lungs showed that the intratracheal instillates were well distributed, as judged by the bilateral discoloration of pulmonary parenchyma by the indocyanine green dye (Fig. 1). The immune phenotype of the putative pulmonary DC fraction was assessed by staining the cells in suspension with a panel of mAbs followed by cytofluorimetry or by anal-

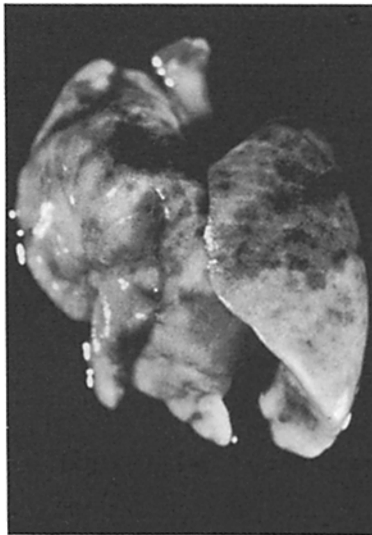


**Figure 1.** Distribution of HEL after intratracheal instillation. The lung parenchyma shows diffuse bilateral green discoloration due to the distribution of indocyanine green in the instillate.

ysis of immunostained cytocentrifuge preparations. Table 1 shows that virtually all of the cells were $CD45R^+$, consistent with their origin in the bone marrow. Approximately 95% were $OX\text{-}6^+$ but <5% stained for T cell (CD4, CD8) or B cell (CD45RA, sIgM, κ light chain) markers. Less than 10% of the putative DC population stained for either ED1 or ED2 antigens, that are expressed by pulmonary macrophages (18).

The primary instillation of HEL i.t. did not yield a substantial difference in the number of pulmonary DC at 48 h compared to saline controls ($1.4 \pm 0.5$ vs. $1.6 \pm 0.5 \times 10^5$ cells/g wet lung). However, the secondary challenge with HEL at day 14, resulted in a sixfold increase ($p = 0.01$) in the number of pulmonary DC at 72 h compared to the saline control (Fig. 2).

Purified DC and unfractionated pulmonary mononuclear cells from the lung digests were compared for their ability to serve as APC. Pulmonary DC were substantially more effective than the unfractionated lung cells in supporting [$^3$H]thymidine incorporation by HEL-immune T cells (Fig. 3).

*APC Activities of Antigen-pulsed Pulmonary DC.* Next, pulmonary DC purified from the HEL-challenged rats were examined for their ability to specifically promote [$^3$H]thymidine incorporation in vitro by (*a*) HEL-immune T cells, (*b*) HKL-immune T cells, and (*c*) nonimmune spleen cells in the absence of added HEL. At 3 h after the intratracheal administration of HEL (100 μg/ml), pulmonary DC yielded a 92-fold increase in the response of HEL-immune T cells, but did not stimulate HKL-immune T cells or nonimmune spleen cells. The level of the specific response seen at 3 h was comparable to that achieved when control pulmonary DC were incubated with both HEL-immune T cells and HEL (100 μg/ml) (Table 2).

Having established the specificity of the APC response, we examined its kinetics in vivo. After a single intratracheal challenge with HEL (100 μg/rat), pulmonary DC were able to present antigen for up 7 d (Fig. 4). The magnitude of the response varied directly with the dose of HEL (10–100 μg/rat) administered i.t. (Fig. 5). When the lungs were rechallenged with HEL i.t. at day 14, a second peak in the specific APC activities of pulmonary DC was observed at 72 h (Fig. 4).

*LDA of DC Activity.* LDA, established 24 h after the HEL intratracheal challenge, showed that $\sim 10^2$ in vivo antigen-pulsed pulmonary DC were able to generate a detectable mitogen response. A similar level of response was observed

**Table 1.** *Surface Immune Phenotype of Pulmonary DC*

| Surface antigen | Percent positive staining |
|---|---|
| CD45R (OX-1) | 99 ± 5 |
| Class II MHC (OX-6) | 95 ± 4 |
| CD45RC (OX-22) | 4 ± 1 |
| CD4 (W3/25) | 2 ± 2 |
| CD8 (OX-8) | 1 ± 0.2 |
| sIgM | 2 ± 1 |
| CD45RA (OX-33) | 4 ± 1 |
| κ light chain (OX-12) | 3 ± 1 |
| ED1 | 5 ± 2 |
| ED2 | 9 ± 3 |

Pulmonary DC were stained with anti-rat mAbs and analyzed by cytofluorimetry or by immunoperoxidase staining. The vast majority of cells were $Ia^+$ and lacked markers of T cell, B cell, or macrophage lineage. Data are the mean ± SD of three experiments.

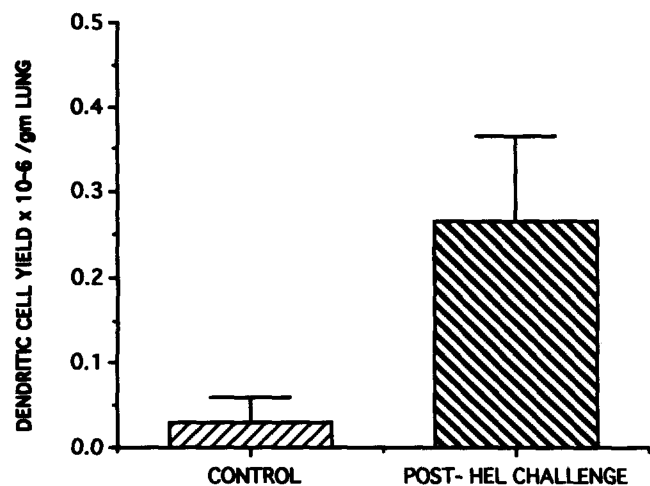



**Figure 2.** Pulmonary DC increase after a repeat intratracheal antigen challenge in previously sensitized rats. Rats sensitized with HEL (100 μg/rat) i.t. were rechallenged on day 14. There was a significant increase ($p = 0.1$) in the number of pulmonary DC purified from the lung digests at 48 h. Data are the mean ± SD of three experiments.

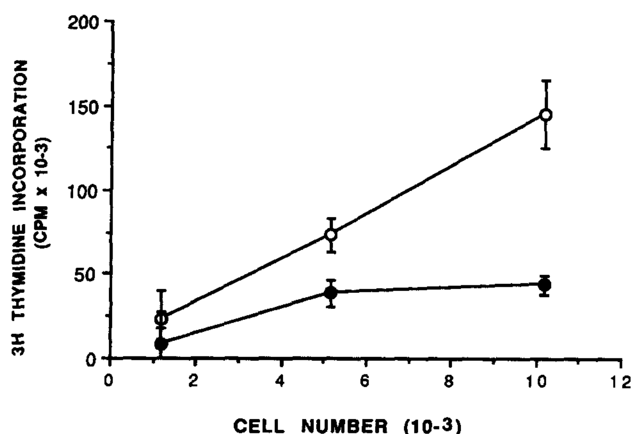



**Figure 3.** Differences in antigen presentation by purified lung DC and bulk lung digest cells. HEL-immune T cells were cultured with HEL (100 μg/ml) and either purified lung DC or unfractionated mononuclear cells from the pulmonary digests ($10^3$–$10^4$) for 72 h. The cultures were pulsed with [$^3$H]thymidine (1 μCi/well) for 6 h, harvested, and counted in a β-spectrometer. ○, Lung DC; ●, lung mononuclear cells.

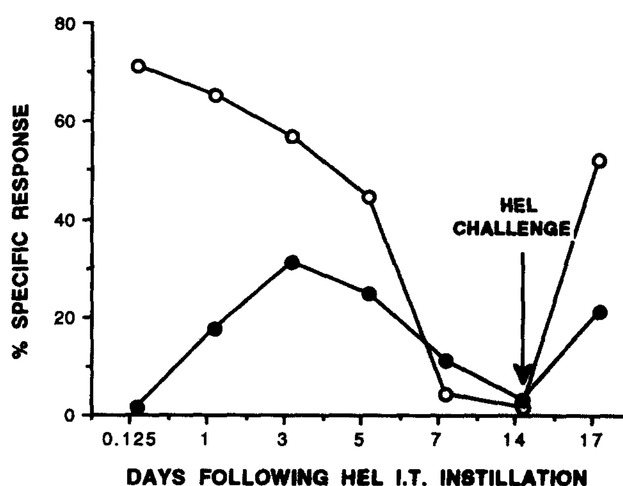



**Figure 4.** The kinetics of DC-associated APC activities after an airway challenge with HEL. Dendritic cells were purified from the lung and draining hilar LN at intervals after an airway challenge with HEL. DC from the lung showed peak APC activity at 3 h, which subsequently decreased gradually to day 7. APC activity in the LN peaked at day 3 and was detectable until day 14. When rats received a second challenge with HEL i.t., specific DC-associated APC responses were again stimulated in lung and LN. ○, Lung DC; ●, LN DC.

when LDA was repeated for DC harvested at day 3 (data not shown).

*APC Activity of DC from Hilar LN.* In parallel, we examined the APC activities of DC purified from the draining hilar LN after the HEL intratracheal challenge. Dendritic cells from the LN did not stimulate a specific T cell response by HEL-immune cells at 3 h. However, APC activity was detected at 24 h, peaked at day 3, and then gradually diminished to control levels by day 14 (Fig. 4). When sensitized rats were rechallenged i.t. with HEL and examined at 72 h, a second rise in APC activities was observed (Fig. 4). After intratracheal injection of HEL, DC harvested from inguinal LN (Table 3) or spleen (not shown) did not elicit a mitogen response unless HEL was added directly to the culture wells.

*Immune Phenotype of Pulmonary T Cells In Vivo.* Lymphocytes isolated from the lungs were examined for their surface immune phenotype. Table 4 shows that pulmonary lymphocytes from saline controls displayed a slight dominance of $CD4^+$ cells and few expressed the OX-39 antigen (IL-2R). The primary HEL challenge yielded a small decrease in the percentage of $CD4^+$ pulmonary lymphocytes and little change in $IL\text{-}2R^+$ lymphocytes. The secondary challenge at day 14 increased the percentage of pulmonary $CD4^+$ lymphocytes but also did not increase the percentage of $IL\text{-}2R^+$ lymphocytes.

Less than 5% of $CD4^+$ cells in the lung expressed the

**Table 2.** *Antigen Presentation by HEL-pulsed Pulmonary DC*

| Responding cells | Additions to culture wells | HEL-pulsed DC | Saline-control DC | Proliferation index |
|---|---|---|---|---|
| | | *cpm × $10^{-3}$ ± SD* | | |
| HEL-immune T cells | None | 275 ± 50 | 3 ± 1 | 92 |
| | HEL | 309 ± 53 | 194 ± 24 | |
| | HKL | 226 ± 18 | 3 ± 1 | |
| HKL-immune T cells | None | 2 ± 1 | 3 ± 1 | 0.7 |
| | HEL | 1 ± 1 | 2 ± 1 | |
| | HKL | 295 ± 25 | 235 ± 20 | |
| Nonimmune splenic T cells | None | 8 ± 3 | 14 ± 2 | 0.6 |
| | HEL | 6 ± 1 | 16 ± 1 | |

HEL-pulsed DC isolated from the lung 3 h after the i.t. injection of HEL (100 μg/rat) i.t. were able to support the specific proliferation of HEL-immune T cells but not HKL-immune or non-immune splenic T cells in a 72 h proliferation assay. HEL-immune T cells did not respond to saline injected control DC unless HEL antigen (100 μg/ml) was added to the culture wells. Data is mean ± SD of one of three representative experiments.

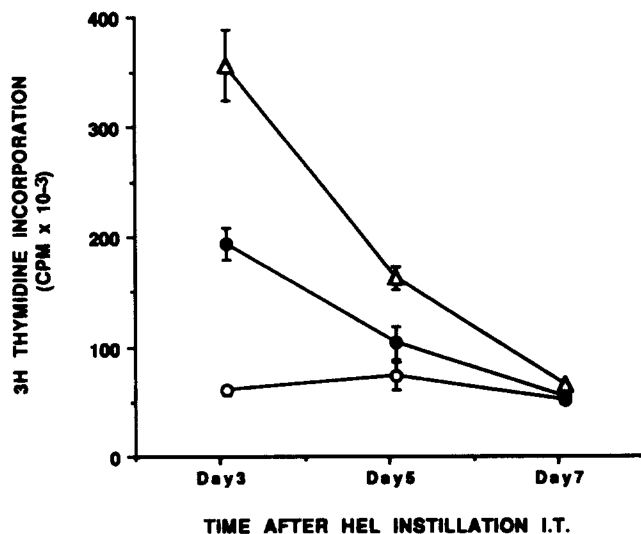



**Figure 5.** The APC activities of pulmonary DC after intratracheal challenge with HEL are dose dependent. Pulmonary DC from rats receiving HEL (100 μg/rat) showed greater specific APC activities at day 3 and 5 (both $p < 0.01$) compared to DC from rats that received a lower dose of HEL (10 μg/rat) or saline. Data are the mean ± SD of three experiments. ○, Saline; ●, HEL (10 μg/rat); △, HEL (100 μg/rat).

OX-22 (CD45RC) isoform of the common leukocyte antigen, whereas 97% of pulmonary $CD8^+$ cells were OX-$22^+$. As naive and sensitized T cells may display distinct pathways of lymphocyte circulation in vivo (19), we examined whether the expression of OX-22 was modulated on naive spleen cells by the polyclonal T cell activator Con A (5 μg/ml). At 72 h, 93% of W3/25 lymphocytes were OX-$22^-$, whereas virtually all OX-$8^+$ cells were OX-$22^+$, suggesting that there is a major difference in how class I ($CD8^+$) and II ($CD4^+$) MHC-restricted T cell subsets regulate their OX-22 expression.

**Table 3.** *Antigen Presentation after Intratracheal HEL Injection*

| Days after HEL i.t. | Addition to culture wells | Hilar LN-DC | Inguinal LN-DC |
|---|---|---|---|
| | | *CPM $10^{-3}$ ± SD* | |
| 3 | None | 33 ± 3 | 1 ± 0.05 |
| 7 | None | 18 ± 5 | 0.8 ± 0.01 |
| 14 | None | 4 ± 0.3 | 0.4 ± 0.02 |
| 3 | HEL | 70 ± 10 | 62 ± 3 |
| 7 | HEL | 70 ± 7 | 69 ± 4 |
| 14 | HEL | 66 ± 5 | 55 ± 3 |

Dendritic cells from hilar and inguinal LN were isolated on days 3, 7, and 14 after the introduction of HEL (100 μg/rat) i.t., cultured with HEL-immune T cells ± HEL (100 μg/ml) for 72 h, and examined for [$^3$H]thymidine incorporation after a 6-h pulse. Inguinal LN-DC show no capacity to support the mitogen response of HEL-immune blasts in the absence of added HEL.

**Table 4.** *Immune Phenotype of Pulmonary Lymphocytes*

| Condition | Surface antigen percent positive + SD CD4 | CD8 | IL-2R | CD4/CD8 ratio |
|---|---|---|---|---|
| Control | 40 ± 13 | 31 ± 6 | 5 ± 1 | 1.2 |
| Post-HEL 1° challenge | 34 ± 8 | 32 ± 9 | 6 ± 2 | 1.1 |
| Post-HEL 2° challenge (day 17) | 39 ± 4 | 28 ± 8 | 9 ± 4 | 1.7 |

Lymphocytes harvested from enzymatic digests of lung were treated with appropriate mAbs, stained with goat anti-mouse F(ab)'$_2$ IgG-FITC, and analyzed in a FACS® 440 cytofluorimeter. Data are the mean ± SD of at least three experiments.

*Immune Specificity.* To assess whether the responses in the lung and LN were antigen specific, T cells were purified from the lungs or hilar LN of HEL-challenged animals, incubated with irradiated syngeneic spleen APC ± HEL, and examined for [$^3$H]thymidine uptake at 72 h. After the primary challenge with HEL, positive responses, as judged by a stimulation index of ≥5 compared to the saline control, peaked in the draining LN at day 3 and subsequently rapidly declined. Antigen-specific responses were not detected in the lung before day 14. However, when the sensitized rats were rechallenged with HEL intratracheally, lymphocytes harvested from both lung and LN at 72 h showed positive responses (Fig. 6).

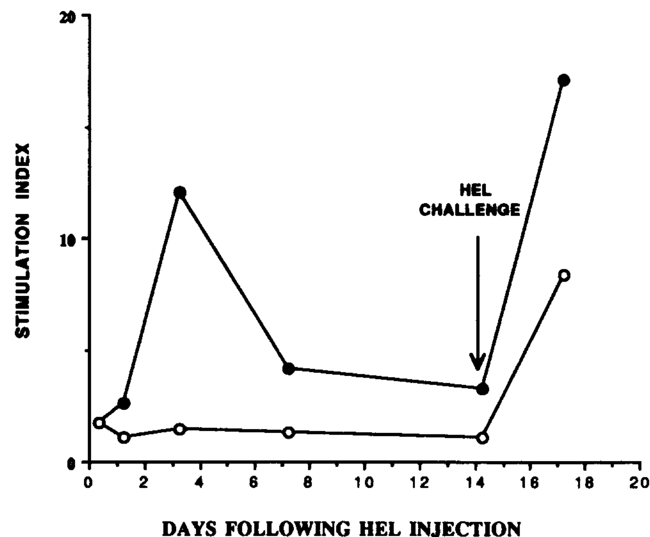
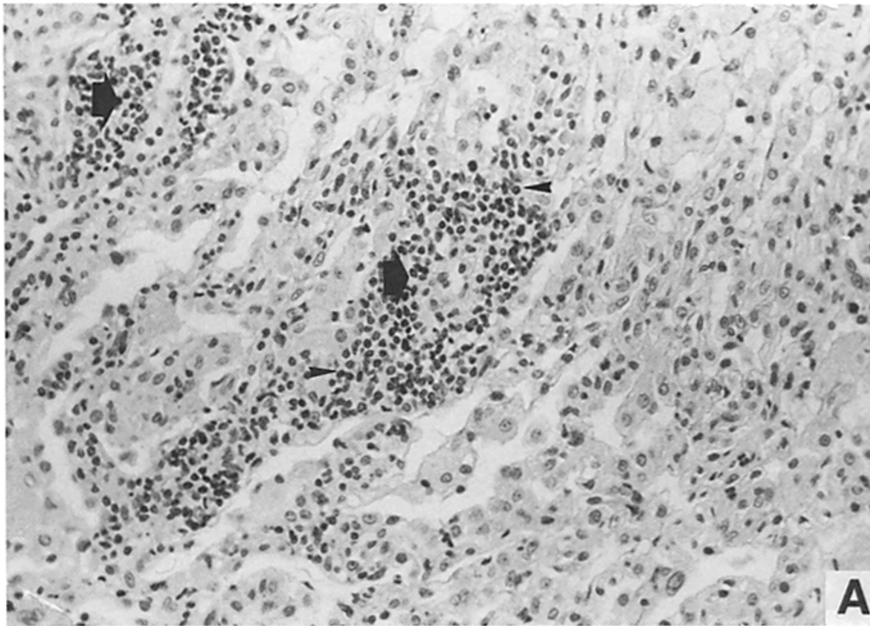



**Figure 6.** T cell responses to HEL from rats injected with HEL intratracheally. Rats received HEL (100 μg/rat) i.t. on day 0 and again on day 14. Nylon wool separated lymphocytes from lung and LN were incubated with HEL (100 μg/ml) and irradiated splenic accessory cells at each time point for 72 h. Data represent the stimulation index of one of two experiments conducted at each time point. ○, Lung T cells; ●, LN T cells.



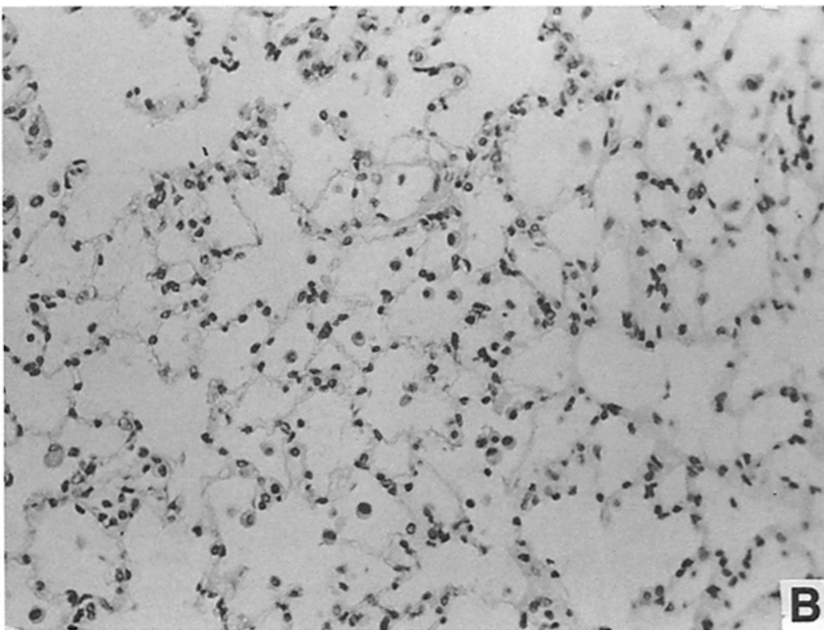




**Figure 7.** An intratracheal HEL challenge on day 14 after airway sensitization produces a perivenular lymphoid infiltrate in the lung. 72 h after the repeat challenge with HEL (100 μg/rat) i.t. on day 14; (*A*) a perivenular (*arrows*) and modest interstitial lymphocytic infiltrate (*arrowhead*) was seen in the lung; (*B*) the lung of an HEL-sensitized rat challenged with saline i.t. shows no accumulation of immune cells. Immunohistology showed that the majority of the lymphocytes in the perivascular cuffs were $CD4^+$ (not shown). × 150. Hematoxylin and Eosin.

Microscopic examination of the lungs after the primary challenge with HEL revealed a small increase in pulmonary macrophages compared to saline controls. 72 h after the secondary HEL intratracheal challenge, a cuff of mononuclear cells was seen surrounding small pulmonary veins (Fig. 7). Immunohistology showed that the majority of lymphocytes in the perivenular infiltrates were $W3/25^+$(CD4) (not shown). Minimal inflammation of large or small airways was observed.

## Discussion

Holt et al. (11) have previously reported that pulmonary DC exposed to aerosolized OVA in vivo present antigen specifically to OVA-immune T cells in vitro. We examined the sequential changes in the APC activities of pulmonary and hilar LN DC after an intratracheal antigen challenge.

Pulmonary DC were able to present HEL shortly after it was introduced into the airways. These APC activities were

judged to be specific, as the pulmonary DC exposed to antigen in vivo selectively stimulated a response by HEL-immune T cells but were unable to promote the proliferation of either naive syngeneic spleen T cells or HKL-immune T cells. The APC activities of pulmonary DC were detected for up to 7 d, suggesting that antigen had been retained in vivo over that interval. Although, we did not directly demonstrate that HEL was stably associated with a population of pulmonary DC, the short half-life of inhaled soluble antigens in vivo (20–22) makes it unlikely that free HEL was taken up by a renewable population of DC. Nevertheless, the possibility that pulmonary DC can effectively present antigen in the lung for prolonged intervals must be interpreted with caution, as Holt et al. have demonstrated that the half-life of DC in airways is relatively brief (23) and that the APC activities of DC are downregulated by pulmonary macrophages in vivo (25).

The remarkable antigen presenting capacities of pulmonary DC in vivo were underscored by the results of the LDA, which showed that as few as 100 pulmonary DC were sufficient to yield a specific mitogen response after HEL challenge. This may explain how DC function as the dominant APC in the lung, despite the fact that they constitute <1% of pulmonary mononuclear cells (11–13). The comparable levels of APC activity yielded at 24 h and day 3 in the LDA further suggests that the HEL antigen was not substantially catabolized over that interval in vivo.

The present study supports the position that primary sensitization of naive T cells after an airway antigen challenge occurs predominantly, if not exclusively, within local LN, and not in the lung or bronchial-associated lymphoid tissues (25, 26). The APC activities of DC in the hilar LN were peaking at the same time that they were decreasing in the lung. This suggests that migration of antigen-pulsed pulmonary DC to local LN may contribute to the delivery of intrapulmonary antigens. In recent reports, Havenith et al. demonstrated that antigen-pulsed DC introduced directly into the airways migrate to the local LN (27) and sensitize naive T cells for a subsequent specific immune response (28). The possibility that pulmonary DC traffic to LN after an inflammatory airway challenge has recently been suggested by McWilliam et al. (15). The shifts in APC activities from lung to LN, parallels the sequence of changes reported for dendritic cells in the skin after contact sensitization (29).

Gong et al. (30) have recently reported that approximately half of the DC obtained by microdissection of the airways were FcR$^+$ and that airway DC were more efficient APC for sensitized T cells than DC in the lung parenchyma. They further demonstrated that ~95% of DC in the lung were FcR$^-$ and phenotypically resembled connective tissue DC (30). In a separate study from the same laboratory, it was concluded that there was little difference in the APC activities of purified FcR ± DC subsets in the lung with respect their abilities to stimulate sensitized T cells (31). The DC examined in the current studies were a heterogeneous population of FcR ± DC (31) that presumably reflect contributions from DC in the intraparenchymal airways and lung parenchyma.

The procedures used to isolate pulmonary DC are lengthy and may have affected the apparent APC activities observed in vitro. For example, the ability of lung DC to stimulate an MLR, a function primarily mediated by FcR$^-$ DC (31), increases substantially with overnight culture (32). It is recognized that FcR$^+$ Langerhans cells differentiate into FcR$^-$ cells in the presence of GM-CSF (33) and that lung DC function can mature in the presence of TNF-$\alpha$ that is produced by lung cells during the purification process (32).

The current study does not distinguish whether all, or a subset, of pulmonary DC, actually mediate the afferent immune response to inhaled antigen in vivo. Based on their substantial phenotypic similarities with Langerhans cells in the epidermis, we speculate that the FcR$^+$ DC in the pulmonary airway epithelium may be primarily concerned with the transport and presentation of antigen to the draining LN during the afferent response to inhaled antigen, whereas a functionally mature subset of FcR$^-$ DC, localized in the connective tissues around pulmonary vessels and other sites in the lung, may be primarily responsible for amplifying the efferent immune response.

Compartmental shifts of T lymphocytes after an airway challenge with antigen are likely to contribute to how the pulmonary cellular immune response is integrated. The T cells isolated from the lung before the secondary antigen challenge did not respond to HEL in vitro, which suggests that the numerical frequency of antigen-specific T cells in the lung was low. However, after the secondary airway antigen challenge at day 14, relatively weak but specific T cell responses were detected in the lung, associated with the accumulation of perivenular T lymphocytes. The localization of predominantly CD4$^+$ T cells around postcapillary venules is reminiscent of the histologic response seen in cutaneous delayed type hypersensitivity (34).

The vast majority of lung T cells do not express the high molecular weight isoforms of CD45R but do show neoexpression of the low molecular weight CD45R0 isoform (35). As loss of high molecular weight isoforms of CD45R is associated with the acquisition of T cell "memory," we have previously speculated that immunologically naive T cells may be selectively excluded from the lung (35). Compared to LN, which contained substantial numbers of CD4$^+$ OX-22$^+$ lymphocytes, virtually all CD4$^+$ lymphocytes in both the control and HEL-sensitized rat lung were OX-22$^-$, suggesting that these pulmonary lymphocytes had undergone sensitization and compartmentalization in vivo.

Based on these findings, we suggest the following pathways in the generation of a pulmonary cell–mediated response to airway antigen. After the inhalation of antigen, we speculate that a subset of FcR$^+$DC in the airways entraps the antigen, transports it to the draining LN, and presents it to naive T cells, in a scenario that is comparable to how Langerhans cells function in the skin (36). Subsequently, sensitized T cells leave the draining LN and enter the circulation. The virtual exclusion of OX-22$^+$ "naive" CD4$^+$ T cells from the lung coupled with rapid clearance of inhaled antigen effectively assures that a pulmonary immune response

will not occur after a primary airway antigen challenge. However, after a secondary challenge lymphocytes are rapidly recruited to the lung, where they may be expanded by perivascular FcR⁻ DC.

This model applies only to the pulmonary handling of soluble antigen. In other experiments, we have demonstrated that particulate antigens are rapidly ingested by macrophages and sequestered away from pulmonary DC (manuscript in preparation). Thus, the lung may use distinct cellular mechanisms in its handling of inhaled antigens.

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