# Development of the Airway Intraepithelial Dendritic Cell Network in the Rat from Class II Major Histocompatibility (Ia)-negative Precursors: Differential Regulation of Ia Expression at Different Levels of the Respiratory Tract

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## Summary

The relative inefficiency of respiratory mucosal immune function during infancy is generally attributed to the immaturity of the neonatal T cell system. However, immune competence in the adult lung has recently been shown to be closely linked to the functional capacity of local networks of intraepithelial dendritic cells (DC). This study examines the density and distribution of these DC throughout the neonatal respiratory tract in rats, focusing particularly on microenvironmental regulation of their class II major histocompatibility complex (MHC) (Ia) expression. In animals housed under dust-controlled conditions, airway epithelial and alveolar $Ia^+$ DC detectable by immunostaining with the monoclonal antibody (mAb) Ox6 are usually not seen until day 2–3 after birth, and adult-equivalent staining patterns are not observed until after weaning. In contrast, the mAb Ox62 detects large numbers of DC in fetal, infant, and adult rat airway epithelium. Costaining of these $Ox62^+$ DC with Ox6 is rare in the neonate and increases progressively throughout infancy, and by weaning $Ia^+$ DC comprised, on average, 65% of the overall intraepithelial DC population. In infant rats, $Ia^+$ DC are observed first at the base of the nasal turbinates, sites of maximum exposure to inhaled particulates, suggesting that their maturation is driven in part by inflammatory stimuli. Consistent with this suggestion, densitometric analysis of Ia staining intensity of individual DC demonstrates that by 2–3 d after birth, Ia expression by nasal epithelial DC was comparable with that of $Ia^{high}$ epidermal Langerhans cells in adjacent facial skin, at a time when expression by tracheal epithelial DC was 7–10-fold lower. Additionally, the rate of postnatal appearance of $Ia^{high}$ DC in the airway epithelium was increased by administration of interferon $\gamma$, and decreased by exposure of infant rats to aerosolized steroid. These findings collectively suggest that Ia expression by neonatal respiratory tract DC is locally controlled and can be upregulated by mediators that are produced within the lung and airway epithelium in response to inhalation of proinflammatory stimuli. It was also noted that $Ia^{low}$ neonatal airway DC expressed adult equivalent levels of class I MHC, which suggests differences in capacity to prime for $CD8^+$-dependent versus $CD4^+$-dependent immunity to inhaled pathogens, during the early postnatal period.

Retrospective seroepidemiological studies in humans have identified early infancy as a period of high risk for primary T cell sensitization to nonpathogenic environmental antigens, in particular those associated with allergic respiratory disease (1). The mechanism(s) underlying the heightened susceptibility of the infant immune system to induction of potentially harmful T memory to airborne antigens has not yet been defined.

Delayed postnatal maturation of T cell competence has been invoked as one potentially important etiologic factor in this process (for a review see reference 2). Infants are recognized to manifest increased susceptibility to infectious diseases (3), especially to those of the respiratory tract, and this is usually attributed to a generalized state of developmental immaturity in systemic immune function(s) during the birth-to-weaning period (3, 4), which is likely to contribute to compromised immune defense in all tissues.

However, recent evidence indicates that the kinetics of post-

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natal development of immune competence can vary markedly between different peripheral tissue sites. For example, local antigenic challenge of the gastrointestinal mucosa in newborn experimental animals effectively "primes" the T cell system for subsequent hypersensitivity responses (5, 6), whereas comparable challenge of the respiratory mucosa in infant animals fails to elicit a T cell response (7). Similarly, the capacity to develop protective oral tolerance to dietary antigens appears within a few days of birth (5), whereas the parallel process in the respiratory tract does not operate effectively until after weaning (7, 8), despite the attainment of adult-equivalent levels of systemic T cell competence well before this time.

These latter findings suggest that site-specific developmental factors can play an important role in the induction and expression of T cell immunity within individual tissue microenvironments, during early postnatal life. The present study focuses upon the postnatal development and regulation of networks of class II MHC (Ia)–bearing dendritic cell (DC)[1] populations within the epithelium lining the conducting airways and the alveoli of rats. Evidence presented below indicates that these cells commence seeding into respiratory tract tissues during fetal life as $Ia^{negative}$ or $Ia^{low}$ precursors, and subsequently develop in situ into $Ia^{high}$ DC during the birth-to-weaning period. MHC class I expression by the neonatal DC was comparable with adults. Both the numerical density and intensity of surface Ia expression on individual DC increase markedly within alveolar and airway epithelial tissues during the early postnatal period, and moreover, these changes proceed at markedly different rates at different levels of the respiratory tree, and are independent of changes occurring within the developing epidermal Langerhans cell (LC) populations of the same animals. Previous data indicate that as the principal APC population in the conducting airways (9, 10), these DC play a key role in the regulation of host responses to inhaled environmental antigens, in particular potential allergens. Given the recent evidence that the outcome of these responses depends on the interplay between antigen-specific MHC class II–restricted $CD4^+$ and MHC class I–restricted $CD8^+$ T cells during initial encounters with the antigens (1, 11), the differential expression of these MHC molecules by airway DC in the early postnatal period may be an important etiologic factor in allergic respiratory disease.

## Materials and Methods

*Animals.* Specific pathogen-free (SPF) rats were obtained from the Animal Resources Centre (Murdoch University, Perth, Western Australia), and were barrier housed and maintained on dust-free shredded paper bedding. Neonatal animals were born onto dust-free bedding. The majority of this study employed animals of the WAG strain, but various aspects of the work were validated in the BN, Wistar Furth, and PVG strains.

*Antibodies.* The mAb Ox19 (CD5), Ox6 (MHC class II), Ox52 (pan rat T cells), and Ox21 (anti–human C3bi used as isotype control) were kindly supported by Dr. Don Mason (Dunn School of Pathology, Oxford, UK); Ox27 (MHC class I) was a gift from Dr. Jon Sedgwick (Centenary Institute, Sydney University, Sydney, Australia); they are described (12). ED2 (pan tissue macrophage [13] was provided gratis by Drs. C. Dijkstra and G. Kraal (Vrije University, Amsterdam, The Netherlands). Ox62 (anti–rat DC) is detailed (14). Anti–rat CD3 mAb was purchased from Pharmingen (San Diego, CA). Biotinylated and FITC conjugates of the antibodies were prepared in-house (Western Australian Research Institute for Child Health). Streptavidin–horseradish peroxidase (HRP), biotinylated sheep anti–mouse IgG (SHAM), and streptavidin-alkaline phosphatase (AP) conjugates were purchased from Amersham (Sydney, Australia). SHAM conjugated to $F(ab')_2$-PE was purchased from Silenus (Melbourne, Australia).

*Tissue Sectioning and Immunostaining.* With the exception of experiments employing anti-CD3 staining, fixation and sectioning of tracheal and lung tissues, and single-color immunoperoxidase staining for cell surface antigens was performed as described previously (15). Fixation in 2% paraformaldehyde proved optimal for preservation of rat CD3 and was used accordingly where required. Dual-color staining employed biotinylated Ox6 linked to streptavidin-AP (employing Fast blue/naphthol substrate, producing a blue color) in conjunction with mAb Ox62. Binding of the latter was detected by the use of SHAM-HRP (color development employing 3-amino-9-ethyl carbazole, yielding a red color. Double-stained cells developed a distinct purple color. With the exception of experiments involving densitometric analysis (see below), both single and double staining employed reagent concentrations/incubation times that produced maximal color development. Quantitation of DC density per square millimeter tracheal epithelium in stained sections was as detailed (15).

In experiments involving quantitative analysis of intensity of Ia expression on individual DC, sections were stained via Ox6 linked to biotinylated SHAM and streptavidin-HRP, and the timing of the substrate incubation step was set at the minimum required to reveal 100% of DC in adult tissue sections (prolonged incubation produced more intense staining, but obscured differences in staining intensity between individual DC). Computer-assisted densitometric scanning of individual immunoperoxidase-stained DC in such sections was performed employing the Image Analysis System (model MD20; Leica Instruments, Sydney, Australia), which provides a readout of mean optical density per unit surface area of each selected cell. Staining conditions were rigorously standardized between individual runs, which included common internal (adult) controls.

*Cell Preparation from Lung and Airway Tissue.* Enriched DC and T cell preparations were prepared from collagenase digests of sliced lung by the method detailed (16) and cytospins were immunostained as with the Ox6 and Ox19 mAbs. Tracheal epithelial cell suspensions were prepared via incubation of tracheal segments with shaking in Dispase (Boehringer, Sydney, Australia), followed by rapid filtration through gauze and collection at the interface of a Ficoll gradient. Histological examination of the segments after incubation confirmed detachment of the bulk of the epithelium, the submucosa below the basal lamina remaining intact.

*Modulation of Airway DC Maturation by Exogenous Agents.* Recombinant rat IFN-$\gamma$ (a kind gift from Dr. Peter van der Meide, TNO, Rijswijk, The Netherlands) was administered intraperitoneally at $2 \times 10^4$ U per infant rat per day for the period specified. In other experiments, infant animals exposed for 30 min daily to a respirable aerosol of the steroid Fluticasone propionate (Glaxo, Greenford, Herts, UK), generated from a stock solution of 0.3 mg/ml saline, over the birth-to-weaning period.

[1] *Abbreviations used in this paper:* DC, dendritic cell; HRP, horseradish peroxidase; MFI, mean fluorescence intensity, SHAM, sheep anti–mouse IgG.

*Flow Cytometry.* Single cell suspensions were immunostained via biotinylated or FITC-conjugated mAbs, in conjunction with avidin-conjugated FITC and SHAM-PE; mAbs Ox6, Ox27, and Ox62 were employed in various combinations, with Ox21 or FITC-Ox21 (anti-human C3bi) as the background control. Fluorescence intensity analyses were performed on stained preparations using a cytometer (Epics Elite; Coulter Instruments, Hialeah, FL).

## Results

*Kinetics of Postnatal Accumulation of Ia$^+$ DC in the Tracheal Epithelium.* Fig. 1 *A* illustrates a tangential frozen section of adult rat tracheal epithelium, cut parallel to the underlying basement membrane. Immunostaining with the mAb Ox6 demonstrates an intraepithelial network of highly pleomorphic Ia$^+$ cells, with the typical morphology of DC. Previous studies (15, 17) have established that these cells do not stain with the pan-macrophage-specific mAb ED2, reinforcing the view that they are DC.

Fig. 1 *B* shows an identical immunostained tracheal section from a newborn rat, indicating the absence of Ia-staining cells within the epithelium. As depicted in Fig. 2, the development of adult-equivalent densities of Ia$^+$ DC within the airway epithelium is not complete until around the time of weaning (mean of 21 d after birth). It should be noted that cells were scored here as DC on the basis of pleomorphic morphology and positive staining for Ia, but regardless of staining intensity (see below). Parallel staining of sections with the pan-macrophage-specific mAb ED2 did not reveal positive cells within the epithelium, and T cells staining with mAb against CD3, CD5, or the pan-T marker Ox52 were rarely seen, and the few detected were of small lymphocyte size and regular shape (data not shown).

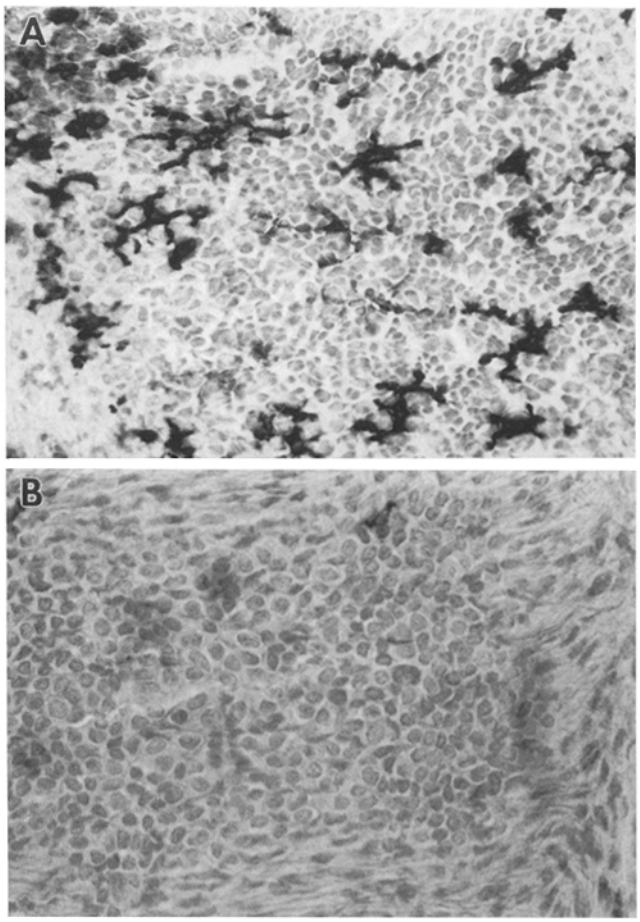


**Figure 1.** Ia immunostaining of adult and neonatal rat tracheal epithelium. Tangential frozen sections of adult (*A*) and newborn (*B*) tracheal epithelium, immunoperoxidase stained with mAb Ox6.

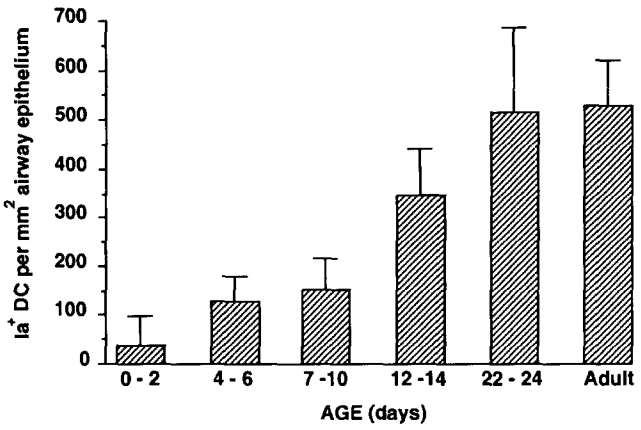


**Figure 2.** Postnatal development of the airway intraepithelial DC network. Data shown are $X \pm$ SD derived from five to seven animals in each age group; at least 200 cells were counted for each animal.

*Independent Regulation of DC Accumulation and Ia Expression within the Airway Epithelium.* Fig. 3 illustrates the results of experiments involving densitometric scanning of Ia staining intensity on individual cells. It can clearly be seen that mean intensity of Ia expression per cell follows a postnatal development pattern similar to that observed in Fig. 1 for overall DC accumulation, i.e., extremely low at birth, rising rap-

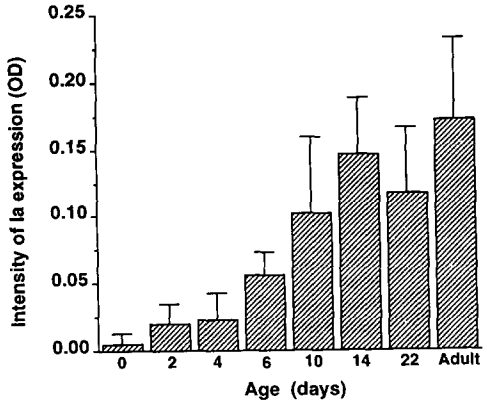


**Figure 3.** Ia expression on individual airway epithelial DC in rats. Frozen sections of tracheal epithelium were immunoperoxidase stained with mAb Ox6, and the intensity of staining determined on randomly selected DC; data shown are $X \pm$ SD derived from three litters in each age group, and seven adult animals.

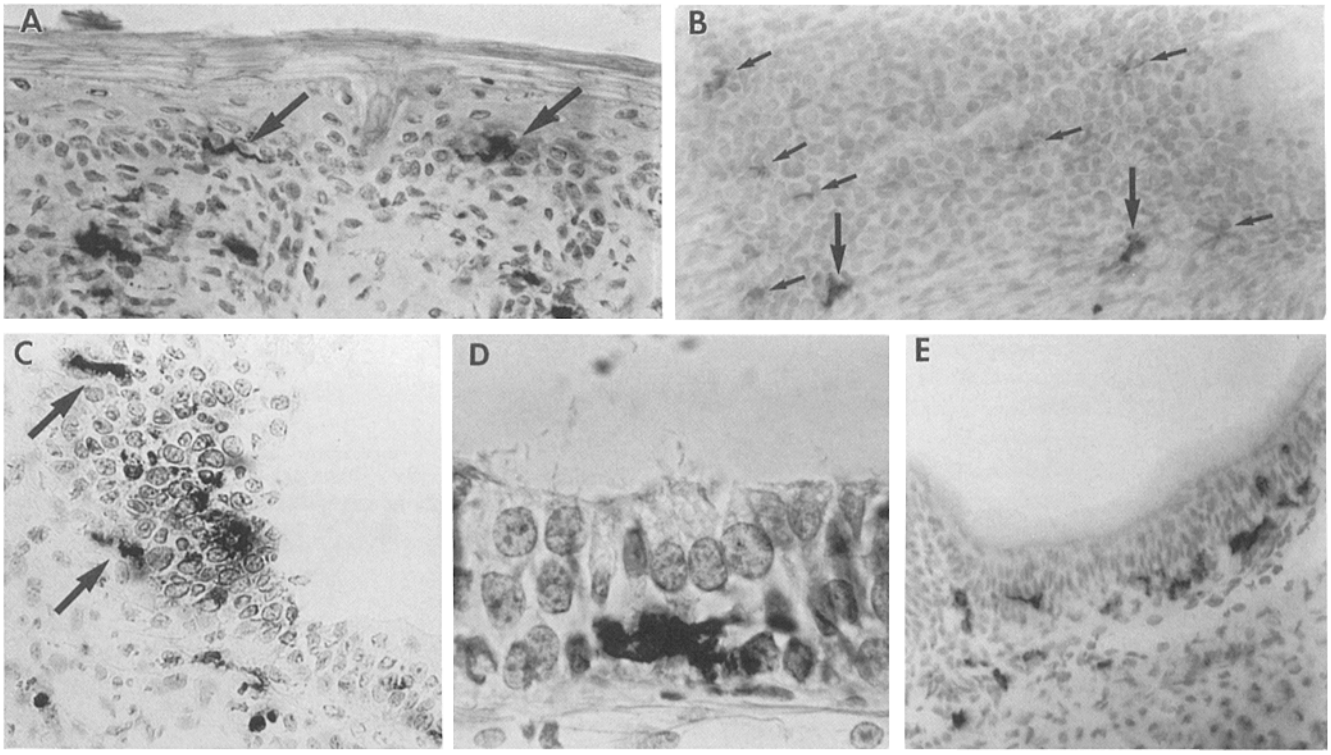


**Figure 4.** Variations in intensity of Ia immunostaining of respiratory tract DC in individual animals. Frozen sections of facial skin (*A*), tracheal epithelium (*B*), and epithelium at the base of a nasal turbinate (*C* and *D*) from a single 3-d-old rat, and nasal turbinate epithelium from a young adult rat (*E*), immunostained in parallel with mAb Ox6.

idly after an initial lag in the first week, to adult-equivalent levels around the time of weaning.

*Microenvironmental Influences of Ia Expression on DC in the Infant Rat.* Fig. 4, *A–D* illustrates a series of tissue sections immunoperoxidase stained for Ia, from the same 3-d-old rat, and the pattern shown is representative of a large series. Fig. 4 *A* demonstrates typical $Ia^{high}$ epidermal LC, which stain intensely with the Ox6 mAb, equivalent to those in sections from adults treated under identical conditions (data not shown). In contrast, intraepithelial DC in tracheal sections from the same animal contained predominantly $Ia^{low}$ with a few $Ia^{moderate}$ DC (*small* and *large arrows*, respectively, Fig. 4 *B*); staining intensity of the latter are generally so low that visualization of the overall morphology of individual cells is only possible via computer-assisted manipulation of backgrounds during the image analysis process.

The micrographs in Fig. 4, *C* and *D* represent sections taken from the nasal mucosa in the same animal, and reveal heavily stained $Ia^{high}$ DC (which were again $ED2^-$). These cells are typically associated with the epithelial basement membrane (Fig. 4 *D*), as noted previously for their tracheal intraepithelial counterparts (10), and at this early age are usually restricted to the base of the nasal turbinates (Fig. 4 *C*). Ia staining was also frequently observed on the epithelial cells at the base of the turbinates (Fig. 4 *C*). For purposes of comparison, an immunostained adult nasal turbinate is depicted in Fig. 4 *E*.

Image analysis of a series of individual Ia-stained DC in

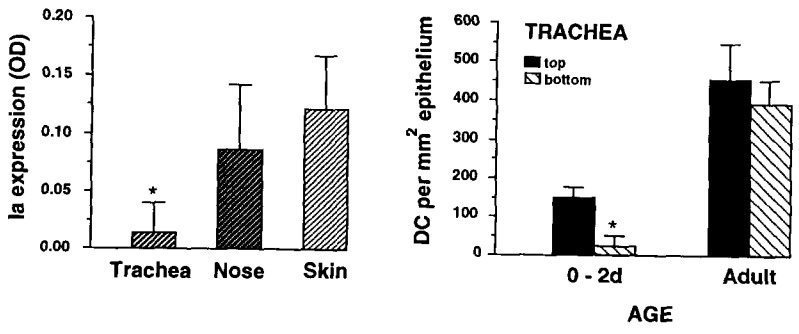


**Figure 5.** Interanimal variation in DC density and Ia expression in different tissue sites. Ia staining intensity of DC at different tissue sites within a litter of 3-d-old rats (*left*; $X \pm$ SD from five littermates), and intraepithelial DC density in the trachea of 2-d-old littermates versus adults ($X \pm$ SD, six animals per group).

tissue sections taken from 3-d-old littermate animals indicate that the magnitude of the variations in Ia expression at different levels of the neonatal respiratory tract are on the order of 5–10-fold, and intensity of expression appears related to proximity to the outside environment (Fig. 5, *left*). These differences become progressively less marked with age, and are not seen after weaning (data not shown). The accumulation of $Ia^+$ DC within the epithelium of the trachea appears to follow a similar postnatal pattern as illustrated in Fig. 5 (*right*), where quantification of the total number of $Ia^+$ DC per square millimeter epithelium in a pooled sample of 0–2-d-old animals revealed differences on the order of sixfold between sections prepared from the top (viz. pharyngeal end) versus bottom of the trachea; these differences again disappeared with age.

*DC Populations in the Lower Respiratory Tract.* Immunoperoxidase staining of frozen sections of parenchymal lung tissue from adult rats reveals a large population of $Ia^+$ cells (10, 11), including a major subset of highly pleomorphic DC with prominent processes, and which stain heavily for Ia but do not express the tissue macrophage marker ED2 (17).

The postnatal development of these and related cells is illustrated in Figs. 6 and 7. As shown in Fig. 6, immunoperoxidase staining of newborn lung sections reveals only rare $Ia^{low}$ cells. In the ensuing weeks, $Ia^{high}$ cells appear which can be classified readily into three groups by morphology (17). (*a*) Dendritic as per Fig. 6 *B*: irregular (pleomorphic) shape including prominent cellular processes (in the adult these are $ED2^-$ as revealed by dual-color immunostaining [17], as are their counterparts in the neonates [data not shown]); (*b*) pleo-

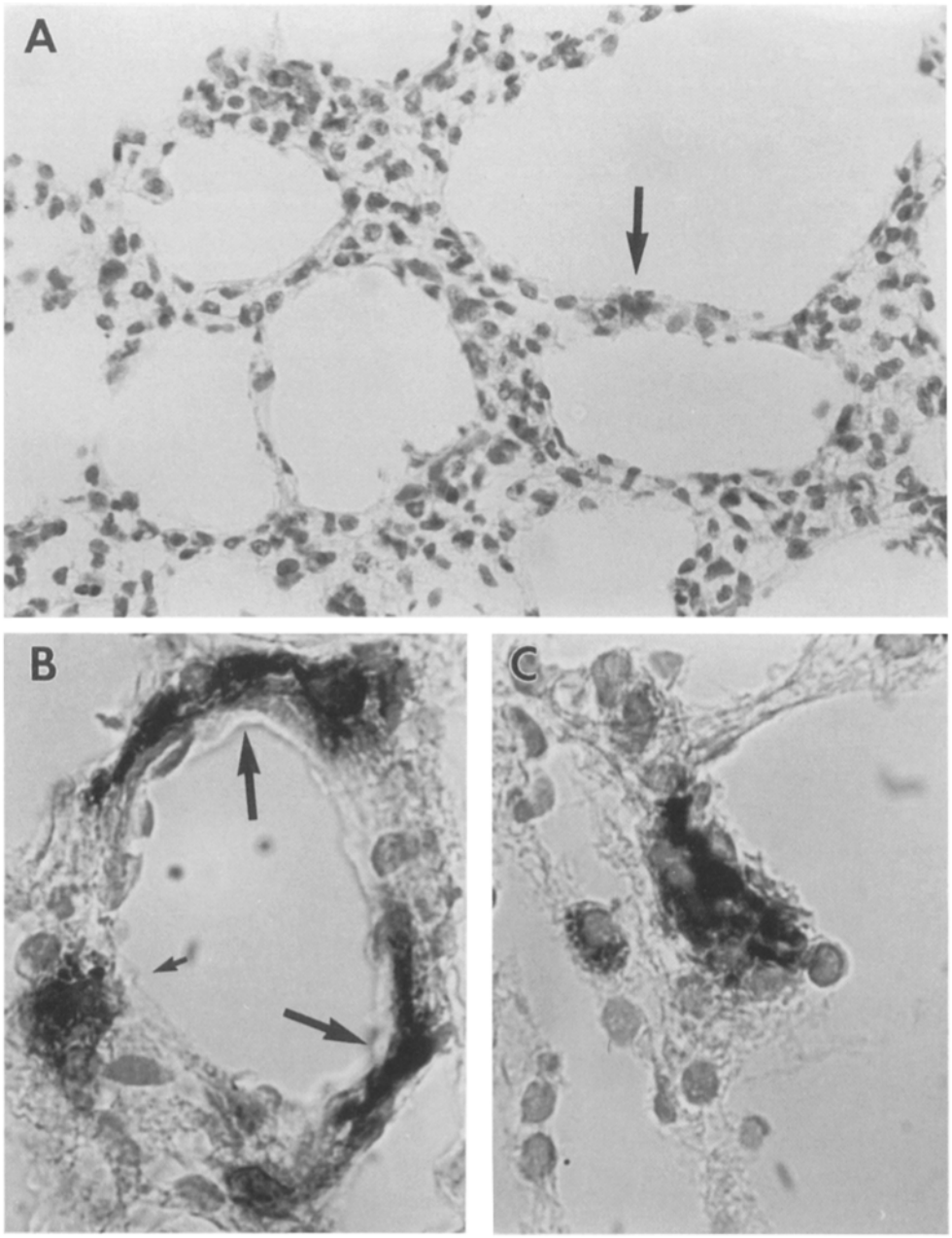


**Figure 6.** Ia immunostaining of frozen lung sections from neonatal rat lung. Immunoperoxidase staining with mAb Ox6 of lung from newborn (*A*; note the single pale staining cell) and 3-wk-old lung (*B* and *C*). Darkly staining cells are frequent in sections from animals >10 d old and can be classified as overtly dendritic (*large arrow*, *B*), regular (*small arrows*, *B*), or irregular pleomorphic (*C*).

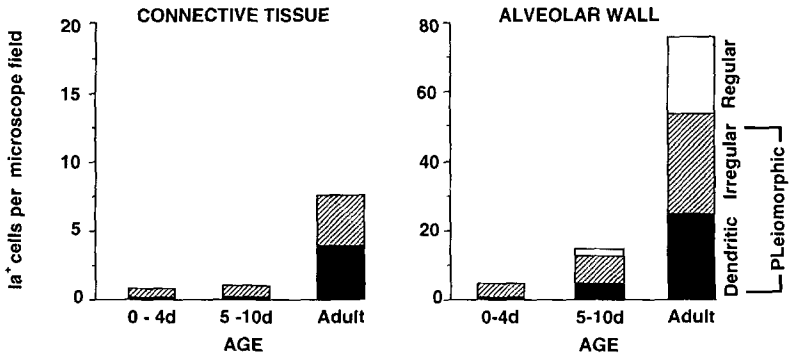


**Figure 7.** Ia+ cells in parenchymal lung tissue. Data shown are mean number of Ia+ cells per microscope field, derived from six animals per age group. ×25.

morphic as per Fig. 6 *C*: irregular but without processes (a variable proportion express ED2); and (*c*) regular as per Fig. 6 *B*, usually round (ED2⁻).

As shown in Fig. 7, Ia+ cells with dendritic or pleomorphic morphology are virtually absent at birth, and during infancy, accumulate in both the alveolar septa and the connective tissues surrounding the vessels and small airways in the deep lung. Whereas detailed time course studies were not performed with respect to postnatal development of these lung parenchymal DC populations, it appears likely from the data in Fig. 7 that their overall kinetics resemble those of their counterparts within the epithelium of the conducting airways. Consistent with this view, quantitation of total numbers of Ia+ cells recoverable via collagenase digestion of lung samples as demonstrated in Fig. 8 (*left*), revealed a pattern closely resembling that of Fig. 2. The accumulation of Ia+ cells in the lung parenchyma correlated strongly with total T cell numbers in the tissue (Fig. 8, *right*). It should be emphasized that this relationship is not evident within the airway epithelium, where total T cell densities remain extremely low in normal animals throughout life (data not shown).

*Pharmacomodulation of Neonatal Airway Intraepithelial DC.* A series of studies were performed in which littermates were

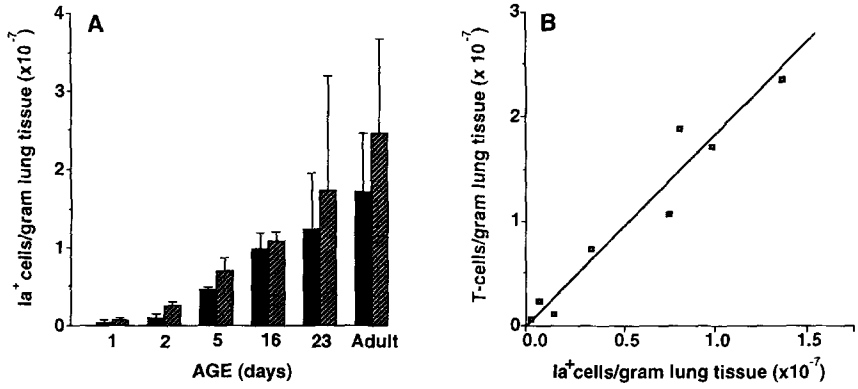


**Figure 8.** Enumeration of Ia+ cells and CD5+ T cells in collagenase digests of lung tissue. Data shown are $X \pm SD$ derived from ⩾10 animals per age group.

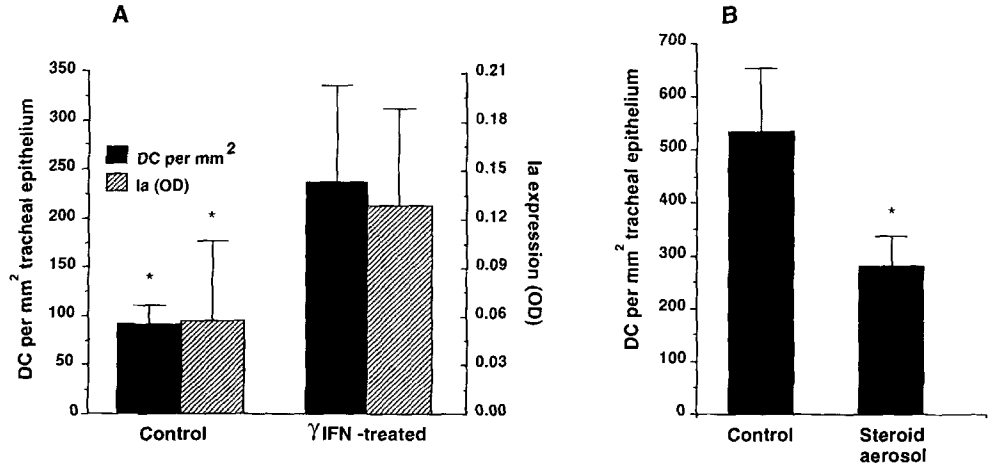


**Figure 9.** Pharmacomodulation of neonatal airway intraepithelial DC. Intraepithelial DC density and Ia staining intensity was compared between normal neonates and littermates treated with IFN-γ (*A*) or aerosolized Fluticasone (*B*) as detailed in Materials and Methods. Preliminary experiments established that placebo treatments were without effect on controls. Data shown are $X \pm SD$ from five animals per group. *Test >controls, $p < 0.05$ for *A*; test <controls, $p < 0.05$ for *B*.

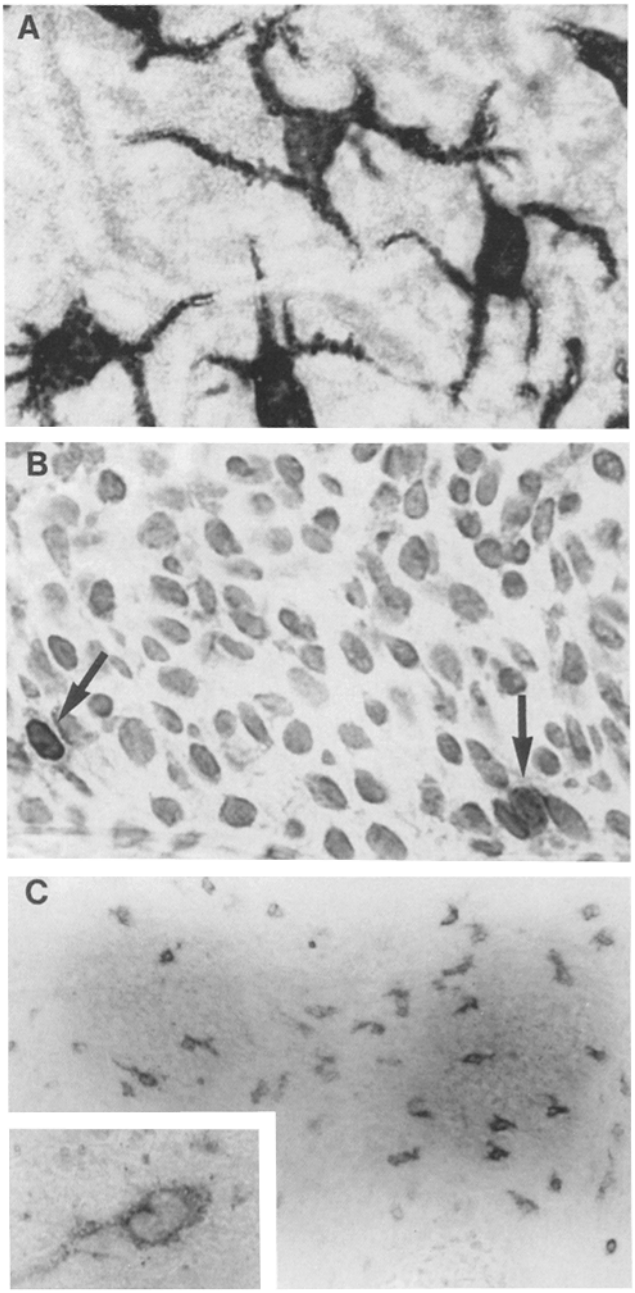


**Figure 10.** Detection of Ia$^-$ DC in neonatal airway epithelium. Adult epidermal sheet (*A*) and tracheal epithelium (*B*) immunostained with anti-CD3, and fetal tracheal epithelium (*C*) immunostained with mAb Ox62 (*inset*, individual Ox62$^+$ DC at a high magnification). Dual-color immunostaining with mAb Ox6 (data not shown) indicated that the DC in *C* were uniformly Ia$^-$. (*Arrows*) CD3$^+$ T cells in airway epithelium.

inoculated intraperitoneally with either saline or rIFN-$\gamma$ for 3–5 consecutive days before killing, for analysis of tracheal intraepithelial DC density. The time of initial inoculation varied between days 1 and 7 after birth. The body weight dose of IFN-$\gamma$ chosen was based on a recent report (18) on upregulation of adult rat lung and airway DC by this cytokine, and preliminary experiments confirmed the effectiveness of this dosage on adult animals from our colony (data not shown).

Fig. 9 *A* illustrates representative data from an individual litter, in which IFN-$\gamma$ administration was performed over days 2–4 after birth, before analysis on day 5. IFN-$\gamma$–treated animals demonstrated a mean threefold increase in the total number of airway intraepithelial Ia$^+$ DC relative to untreated controls, with a concomitant increase in the intensity surface Ia expression per individual DC.

In parallel experiments, neonates were exposed daily to aerosolized topical steroid Fluticasone, throughout the weaning period, and their tracheal intraepithelial DC density compared with littermate controls. Fig. 9 *B* is typical of a series of three experiments, and demonstrates downmodulation of postnatal accumulation of Ia$^+$ DC in steroid-treated animals.

*Airway Intraepithelial Ia$^-$ DC.* A recent report (14) describes a mAb (Ox62) which stains a cell surface marker on rat DC in lymphoid and nonlymphoid tissues, and CD3$^+$ dendritic epithelial T cells (DET; putative TCR-$\gamma/\delta$) in epidermis. The prominent DET population found in adult rat epidermis (Fig. 10 *A*) does not have a counterpart in tracheal epithelium, where the few T cells present that stained with either anti-CD3 (Fig. 10 *B*) or anti-TCR-$\alpha/\beta$ (data not shown) exhibit conventional lymphocyte morphology. Similar results were obtained with neonatal trachea (data not shown). Immunostaining of a tangential section of fetal rat trachea with Ox62 (Fig. 10 *C*) reveals a highly developed network of pleomorphic cells with prominent processes (*insert*) which, accordingly, are likely to be DC. Parallel single staining with the anti-Ia mAb Ox6, and its concomitant use in dual-color immunostaining (data not shown), indicated that these cells are uniformly Ia$^-$. In addition, they failed to stain with pan-T cell mAbs such as Ox19 or the pan-tissue–macrophage mAb ED2, reinforcing the view that they are Ia$^-$ DC.

Dual-color immunostaining of tracheal samples from postnatal rats with Ox6/Ox62 revealed a time-dependent increase in the frequency of double-staining cells from birth-to-weaning (Fig. 11). Parallel staining with Ox62 alone provides figures for the total number of DC present in these tissues, and it can be seen from Fig. 11 that the Ox6$^+$ (Ia$^+$) subset of this population increases markedly in frequency during the preweaning period. It should be noted, however, that Ia$^-$ DC remain a major subset ($\sim$35%) of the overall airway intraepithelial DC population in adult rats, and their distribution throughout the respiratory tract appears heterogeneous (data not shown). Similar proportions of Ia$^-$ and Ia$^+$ DC were observed in adult rats housed under conventional or dust-controlled conditions.

*Flow Cytometric Analysis of Dissociated Epithelium.* Single-cell suspensions of tracheal epithelium were dual-color immunostained with various combinations of mAb against CD3, MHC class I and II, and the mAb Ox62, before analysis on an Epics Elite cytometer. Consistent with the observations above on in situ staining in frozen sections, minimal overlap was observed within adult cell suspensions between populations staining for CD3 and Ox62, whereas gating for high

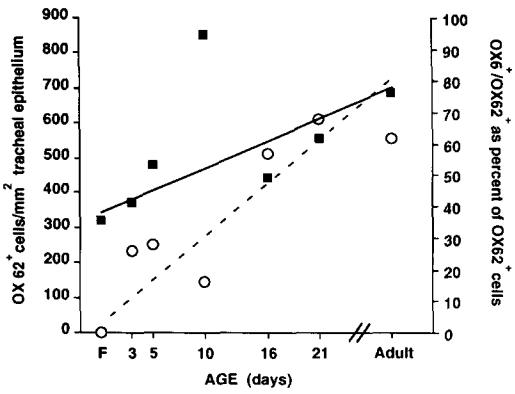


**Figure 11.** Quantitation of $Ox6^+/Ox62^+$ DC in tracheal epithelium of neonatal rats. Data shown are means derived from individual litters of fetal (*F*; day 20 of gestation) and infant rats of various ages, together with three adults. Frozen sections of tracheal epithelium were dual color immunostained with mAbs Ox6 and Ox62; (■) total number of $Ox62^+$ per $mm^2$ epithelium, and (O) double-stained cells as percent total $Ox62^+$ population. Comparable data were obtained in follow-up studies with a range of adults varying in age from 6 wk–1 yr (data not shown). The line of best fit is shown for each set of figures.

intensity Ox62 expression also selected for the high intensity $Ox6^+$ cells (data not shown).

Accordingly, for experiments involving assessment of MHC class I expression on adult airway epithelial DC, cell suspensions were stained with mAb Ox27 in combination with either Ox6 or Ox62. DC were then selected by gating $Ox6^{high}$ or $Ox62^{high}$ cells, and mean fluorescence intensity (MFI) of Ox27 expression determined on the gated population. Comparable data were obtained by gating adult DC employing either of the antibodies, and the range of Ox27 staining intensity observed for normal adults was 8.4–10.4 MFI units. Selection for airway DC in neonatal tracheal suspensions was restricted to gating for $Ox62^{high}$ cells (as $Ox6^{high}$ cells were not detected), and subsequent analysis of intensity of Ox27 staining on these cells yielded figures between 8.6 and 9.4 MFI units 1–7 d before birth.

*Stimulation of Postnatal Maturation of Lung and Airway DC Populations in Infant Rats by Housing on Dusty Bedding.* At the outset of these experiments, it was observed that housing in cages containing conventional bedding (e.g., wood shavings, chaff, vermiculite) led to highly variable stimulation of lung and airway DC influx and Ia expression in infant rats. Reproducible age-related changes in these variables were detected only subsequent to the introduction of dust-control measures in our animal house, in particular the use of shredded paper bedding. These findings are in line with our recent experience with adult animals which indicate marked stimulation of respiratory tract DC by dust generated from conventional bedding materials (15), and emphasize the potentially important role in inflammation in regulation of the lung and airway DC populations.

## Discussion

The kinetics of postnatal development of immunocompetence varies between species (19), but the increased susceptibility of the newborn to infectious diseases is essentially universal among mammals and is generally attributed to variations in the rate of postnatal maturation of key functions in the central immune system, in particular within T and B cell populations. By extension, strategies to protect "at risk" pediatric populations against common infections in infancy presuppose that the vaccine-driven induction of immunological memory against pathogenic antigens should per se compensate for this developmental defect in immune function and accordingly translate into effective immunity.

Although such maneuvers are demonstrably successful in stimulating systemic IgG response in neonates, it is not clear that they are universally effective in inducing local immunity in peripheral tissues, particularly in secretory antibody responses and local effector T cell activation. The expression of host immunity within peripheral tissues is limited by the availability of a central pool of lymphocytes with appropriate antigen receptors, but in addition requires efficient local APC for initial T cell signaling, especially during the critical early stages of the immune response. In relation to the respiratory tract, highly developed networks of resident DC have recently been identified within the epithelium of the conducting airways and alveoli, and in vitro functional studies (20, 21) indicate they are the most potent APC present in these tissues.

The postnatal maturation of these respiratory tract DC networks has not been subjected to previous detailed investigation, and was the subject of the study reported herein. The salient findings are as follows.

First, in both the conducting airways and the alveolar septa, $Ia^+$ DC are virtually absent (or undetectable) at birth, and their distribution and density does not resemble that of adults until around the time of weaning.

Second, the first DC that are detected within the neonatal airway epithelium contrast markedly with their $Ia^{high}$ adult counterparts in expressing Ia at extremely low levels that barely exceed the threshold for detection via immunoenzymatic techniques. This suggests that they initially migrate into the tissues as $Ia^-$ or $Ia^{low}$ precursors. Consistent with this possibility, the mAb Ox62 detects a large population of $Ia^-$ DC in fetal and newborn airways, and this population progressively acquires surface Ia throughout the birth-to-weaning period. This finding echoes an earlier report (22) on the development of murine $Ia^+$ epidermal LC from $Ia^-$ precursors.

Third, it is clear that exogenous stimuli play a central role in the postnatal development of the airway intraepithelial DC network. This may be inferred from the relationship between proximity to the outside environment (and hence intensity of exposure to inhaled irritants) and the rate of local accumulation of $Ia^{high}$ DC within individual tissue microenvironments in the respiratory tract. This is particularly evident with respect to the epithelium at the base of the nasal turbinates which represents the area of maximum impaction of inhaled particulates, and is the first site within the respiratory tract at which $Ia^{high}$ DC appear postnatally. The inhibitory effect of the antiinflammatory steroid Fluticasone on DC accumulation in the neonatal airways provides further indirect support for the role of inflammation. In this context, the overall distribution pattern of airway DC in SPF adult rats

reported earlier by our laboratory (15) and subsequently confirmed independently elsewhere (23), viz., an inverse relationship between intraepithelial $Ia^{high}$ DC density and airway diameter, is also consistent with a role for inhaled stimuli in maintenance of the tonus of the respiratory tract DC network in the steady state.

The mechanism(s) by which local inflammation upregulates Ia expression on neonatal airway DC remains to be defined. The data in Fig. 9 suggest a potential role for cytokines such as IFN-$\gamma$; the strong correlation between the numbers of $Ia^+$ and T cells in the lung parenchyma during the neonatal period appears consistent with this possibility, but infiltrating lymphoid cells are normally extremely sparse in airway tissue from both neonates and adults, suggesting that other mediators (possibly derived from mesenchymal cells) may regulate this process in the airway epithelium under steady state conditions. In this context, Ia expression on neonatal monocytes in several species is maintained at low levels, a process which appears to be controlled by IFN-$\beta$ (24, 25). This cytokine has been shown to suppress MHC class II but not I gene expression (26), and it is feasible that its local production within or adjacent to the actively remodeling airway epithelium may be a contributing factor to the differential surface expression of class I versus II MHC gene products on neonatal airway DC. This possibility is currently being investigated.

Previous immunohistochemical studies (27, 28) on the ontogeny of Ia-bearing cells in the rat lung have focused on parenchymal tissue, and have revealed small numbers of putative DC in the neonatal alveolar wall, the frequency of which appeared to increase postnatally. Quantitation of these changes was attempted in a more recent study (29), which demonstrated a 500% increase in lung parenchymal DC density between birth and adulthood, the majority of this increase occurring in the first 4 d after birth. This observation contrasts with our present findings which demonstrate a slower rate of increase in the peripheral lung DC population, postnatally (Fig. 7). The more rapid postnatal appearance of lung parenchymal $Ia^{high}$ DC in (29) may reflect covert stimulation of DC influx and/or Ia expression by airborne particulates associated with conventional bedding material (see Results; 15). Genetic factors may also have contributed to these differences, on the basis of reports on strain-related variations in Ia ontogeny in other rat tissues (30).

The functional capacity of airway and lung parenchymal DC populations in local immune defense in the neonatal lung, remains to be established. The virtual absence of Ia expression on these cells in the newborn and their low expression levels during the first week of life, considered in the context of the literature demonstrating a direct correlation between Ia expression and the competence of APC in the induction of MHC class II–dependent immunity (for a review see reference 31), suggests that their functional capacity is likely to be low in the absence of exogenous stimulation. The sole reported study relevant to this issue employed neonatal lung parenchymal DC, which were used successfully to induce primary MLR in vitro (29). However, the method of DC preparation employed in the latter study included an overnight incubation step, a procedure which we have recently demonstrated to markedly upregulate the APC activity (and Ia expression) of adult lung DC (11, 17), analogous to the stimulation observed with epidermal LC in culture (32, 33). More detailed studies are clearly required to define the extent of the APC activity of neonatal DC when freshly isolated from lung and airway tissue, and to elucidate the factor(s) required to drive their functional maturation in vivo.

It is also interesting to note that the surface expression of class I MHC on the neonatal airway DC appears equivalent to their adult counterparts, which suggests that they may be competent in initiation of class I MHC–restricted immune responses (in particular to viral antigens) during infancy, and this is currently being tested. The related possibility that the APC function(s) of the $Ia^-$ DC subset in adult airway epithelium may also differ from their $Ia^+$ counterparts is also under investigation.

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