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Review article

The role of alveolar macrophages and dendritic cells in allergic airway sensitization

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"Respiratory immunology" concerns the unique situation that the lungs can perform their vital functions only during continuous, direct contact with the environment. A large quantity of foreign antigens and allergens impinge on the epithelial surface of the respiratory tract and are taken up. The airway compartment has to remain completely sterile and immunologically tolerant of the environmental agents. Therefore, the immune system in the airways very much specializes in keeping up the first line of immune defence against inhaled allergens or colonizing antigens. They are eliminated via various routes. The immune system in the respiratory tract comprises immunocompetent cells in the respiratory epithelium, the lung parenchyma, and the alveolar lumen; lymphoid structures such as bronchus-associated lymphoid tissue (BALT); and regional lymph nodes (RLN) draining the local compartment.

An immune induction event in the respiratory tract is determined by two sets of interacting factors: host immune status, and the physicochemical properties of the inhaled antigens. In respiratory allergy, the immune status shows hyperreactivity of the T-cell-dependent immune system, whereas inhaled allergens are defined as low-molecular-mass, soluble antigens penetrating easily into the respiratory mucosal surface via intracellular (pinocytic) or intercellular pathways (1). There they are taken up rapidly by immunocompetent cells, such as antigen-presenting cells (APC), *in situ*. APC can "store" allergens in an immunogenic form while *in situ* in the airway tissue and have the capacity for efficient priming to naive T cells. Entry of allergens to the airways then gives rise to normal development of immunity and memory immune function. Relevant APC require the additional capacity to migrate selectively to the paracortical T-cell zones of the RLN, in order to induce a primary immune response in a naive host (2). Studies on the distribution of total and antigen-specific IgE plasma cells and IgE mRNA during antibody production have identified the RLN draining the lower respiratory tract as the primary site for initiation of the IgE response to inhaled allergens (3). Under normal conditions, repeated passive exposure to nonpathogenic, protein antigens induces a state of T-cell-mediated immunologic tolerance. Experimental exposure of animal airways to a T-cell-dependent allergen (ovalbumin) aerosol once weekly induced transient allergen-specific IgE responses (4), which spontaneously stopped after 3–4 weeks, despite continuation of the exposure. This tolerance develops most rapidly in the IgE antibody isotype, and is usually followed by suppression of the IgG response and delayed-type hypersensitivity (DTH) (5). In respiratory allergy, there is special evidence that this tolerance of T-cell-dependent allergens does not develop in an appropriate way. Infancy is the period of greatest risk of T-cell sensitization to ubiquitous environmental antigens. Individuals at "high risk" of atopy show, in the same way as children below the age of 4 years, maturational "deficiency" in CD4+ T-cell cytokine profiles (6), suggesting that this may be a cause of the development of a different immunologic responsiveness to environmental allergens.

Atopic disease is further associated with the occurrence of allergen-specific CD4+ T cells of the

Th2-cell subset (7, 8). Th2-cell products induce the increased production of IgE (7, 8). In allergic asthmatics, house-dust-mite-specific Th2-cell clones showed restricted cytokine production profiles with the production of high levels of interleukin (IL)-4 and IL-5 and relatively little interferon-gamma (IFN-γ) (8). IL-4 is an essential cytokine in the B-cell switch to IgE production. It is produced *in vitro* by expanding, oligoclonal-proliferating, allergen-epitope-specific Th2 populations, sensitized by various epitopes of different major allergens (9, 10). Therefore, Th2-cell cytokines are thought to play an important role in the regulation of pulmonary immunologic and inflammatory reactions in allergic asthmatic patients (11). On the one hand, APC factors are of specific importance in the modulation of Th cells. In atopic patients, they may have the ability to induce type 2 cytokine secretion profiles in Th cells. On the other hand, supernatants of activated Th2 cells strongly enhance expression of the low-affinity IgE-receptor ($Fc_{\varepsilon}RII/CD23$) on the cell surface of APC *in vitro* (12); this is explained predominantly as another effect of the Th2-derived cytokine IL-4 (13, 14). However, the Th2-like production profile does not appear to be an intrinsic property of the peptide-specific T-cell populations. It is also dependent upon their mode of activation.

This review will focus on the role of alveolar macrophages and airway dendritic cells in processing clinically relevant inhaled allergens, such as house-dust mite (HDM) (*Dermatophagoides pteronyssinus*) or cat dander (*Felis domesticus*) (Fd), with special reference to the induction of locally aberrant, Th2-dependent, atopic immune reactions in allergic individuals.

Accessory cells and allergic sensitization

As everywhere else in the body, a T-cell-dependent immune reaction in the airways is induced by uptake of the antigen by APC. The discovery that this process is restricted by the presence of class II MHC (major histocompatibility complex) molecules on the surface of the APC has focused attention on the main role of APC in the activation of T helper (Th)cells. Various cell populations present in the respiratory tract tissues can express these appropriate, MHC II, function-associated molecules on their surface, which are required for antigen presentation to T-cell receptors (TcR). The major APC subsets in the airways consist of the pulmonary alveolar macrophages (PAM), the intraepithelial and subepithelial dendritic cells (DC), the intraluminal (specific) B cells, type II alveolar epithelial cells, and, presumably to a lesser extent, bronchial epithelial cells. PAM and DC are cells that stem from CD34-expressing precursor cells in the bone marrow. Functionally, they are considered extreme maturation products. Although many macrophage types can present antigen to memory T cells, only DC can act as APC for naive T cells. However, the potential role of individual cell populations in regulation of local T-cell-dependent immune reactions in the airways depends on various additional factors, including their precise localization, migration characteristics, expression of T-cell "costimulatory" signals, responsiveness to cytokine stimuli, host immune status, and the nature of the antigen challenge (2).

T cells do not recognize free antigens. They recognize them only when presented on the surface of APC. This function of APC is called "antigen processing". During the antigen processing, large antigens are degraded to fragments (linear peptides) recognizable by T cells. Presumably, the most important APC in the airways are the intraepithelial DC. DC always express class II MHC molecules. After antigen processing, the "antigen presentation" to the CD4+ T cells is MHC-haplotype; e.g., genetically restricted. The single T-cell receptor (TcR) recognizes the MHC-antigen combination. For a better understanding of the role of APC in the pathogenesis of allergic reactions in the airways, it is important to trace those factors, derived from PAM or DC, that lead to the predominant occurrence or activation and clonal proliferation of antigen-specific Th2 cells. Basically, a predominance of Th2 cells may result from selective outgrowth of this subset by the effect on T-cell proliferation and/or from selective modulation of cytokine secretion. Studies in mice have provided evidence of the occurrence of Th0 cells with the same antigen specificity, suggesting that the cytokine profile of responding T cells changes during the course of the immune response (15).

Other APC, such as alveolar macrophages, type II alveolar cells, and (airway) epithelial cells, require class II molecule-inducing signals such as IFN-γ (2, 16). B cells have moderate amounts of class II antigens on their surface. This is increased by cytokines such as IL-4, and inhibited by IFN-γ. Antigen presentation by cells other than DC might differ from the normal immune induction in atopic patients. Epidermal accessory APC (17), such as keratinocytes in the skin, showed lack of HLA-DR expression after allergen (Der p) challenge in the late-phase response compared with the T-cell-dependent, delayed-type response induced by hemocyanin (18). This may be compatible with a Th2-cytokine profile produced by the local lymphocytic infiltrate. Although data derived from T-cell-cloning studies in man have revealed that allergen-reactive T-cell subsets are present in both normal and atopic individuals in the RLN, a

preferential homing of allergen-specific Th2 cells is also present in the inflamed mucosal airway tissue of allergic asthmatics (19). Recently, we showed that Th2 cells are the predominant differentiated cells giving T-cell help in the IgE-antibody response in HDM-sensitive allergic asthmatics (7). This Th2-cell-mediated response appears to depend on an HDM- or Fel d 1-specific, but HLA-DR-heterogeneous, response (9). Under these circumstances, B-cell activation depends on T- and B-cell interactions. Moreover, the specific B-cell response is facilitated through allergen uptake by B cells through pinocytosis. Antibody-producing B-memory cells are present in the airway lumen of sensitized individuals and make this facilitation possible (20). There is some evidence that bronchial epithelial cells, in upregulation of their cytokine production and/or of their expression of adhesion molecules, can activate local T cells via an antigen-nonspecific system (21). However, this may entail entrance of APC or sensitization of T cells.

Antigen-specific suppression

In the steady state, respiratory tract T cells are tightly regulated by a series of inherent and acquired immunosuppressive control mechanisms, which normally limit local T-cell activation to situations where antigenic exposure is accompanied by an inflammatory stimulus, with invading pathogenic microorganisms. As stated above, allergic airway diseases may result from inappropriate responses to inhalant allergens by the respiratory tract T-cell system. "Natural" exposure of the healthy respiratory mucosal surface to inhalants produces a characteristic pattern of immune responsiveness, which follows initial transient specific low-level IgE antibody production, and finally results in selective suppression of DTH and IgE responses, with the concomitant preservation of variable degrees of allergen-specific IgA, IgG, and T-helper-cell reactivity (22, 23). This tolerance is antigen specific and transferrable to naive recipient animals by T cells of the CD8+ suppressor/cytotoxic phenotype (24–26). T-suppressor cells appear to be initially generated in the draining lymph nodes of the upper respiratory tract (25, 27). They expand and spread systemically, providing an antigen-specific tolerance throughout the whole body (23). However, the ability to build up a tolerogenic threshold may vary widely among individuals. A major factor appears to be the overall maturational status of the immune system; e.g., age (28). Immune induction in immature individuals may lead to induction of sensitization (29). In addition, susceptibility to tolerance induction can be markedly influenced by other confounding factors, such as Th1-dependent reactions to bacterial or viral infection, which can aggravate a specific, local immune response not related to the microorganism involved, and sometimes may even stimulate the development of hypersensitivity (23) (see below). It has been suggested that tolerance transfer might also occur through T cells which may be part of the γ/δ T-cell lineage (26). This also has been described as a form of genetically determined tolerance, at least under experimental conditions (30, 31).

Pulmonary alveolar macrophages

The pulmonary alveolar macrophages (PAM) are the representatives of the mononuclear phagocyte system in the lungs. They are strategically situated at the air–tissue interface in the peripheral ducti alveoli and alveoli. PAM are the first cells to encounter inhaled antigens in the lower respiratory tract. By their phagocytic capacity, these cells exert potent antimicrobial effects (32). In addition to phagocytosis of antigens, PAM can produce and secrete various immunoregulatory products upon stimulation (33). Moreover, PAM are primary sources of several proinflammatory cytokines, such as tumour necrosis factor-alpha (TNF-α), IFN-γ, IL-1, chemokines such as IL-8, and an array of chemotactic factors, including leukotriene B_4 and platelet-activating factor (PAF) (34–36).

PAM arise from monocytic migration from the vasculature (37), from interstitial macrophage proliferation and differentiation which is followed by migration into the alveolar compartment (38), and from cellular proliferation *in situ* (39). The PAM-derived monocyte chemoattractant protein-1 (MCP-1) is considered to play an important role in monocyte recruitment during acute inflammatory reactions in the lung (40). MCP-1 modulates the adhesion of peripheral blood monocytes to endothelial cells (41), after which these cells migrate to the tissue site and are modulated to alveolar macrophages. In addition, colony-stimulating factors (CSF) promote the survival (42) and the proliferation (43) of murine and human PAM *in vitro*, a fact which suggests that CFS seem to be a group of regulatory factors that support the local functions of these cells.

During allergic inflammatory responses in the airways, changes occur in the patterns of adhesion molecules expressed on endothelial cells of the vasculature, depending on the type of inflammatory mediators that are produced at that specific site (44). As mentioned above, IL-4 is one of the cytokines produced by activated Th2 cells and mast cells in the local tissue where such responses occur. IL-4 has been shown to increase the adhesiveness of endothelial cells to lymphocytes (45). Recently,

it has been shown that IL-4 induces the selective upregulation of the adhesion molecule, vascular cell adhesion molecule-1 (VCAM-1), on endothelial cells, thereby increasing their adhesiveness to the VCAM-1-ligand-bearing, very late antigen-4 (VLA-4)-positive peripheral blood monocytes (46). Additional endothelial accessory factors, such as MCP-1 and TNF-α, are later necessary for transendothelial migration of these cells into the tissues (47, 48).

It is noteworthy that small numbers of PAM can migrate from the alveoli to the RLN (49), where they home to T-cell regions. Mature PAM, as they are present at the local site in the alveolar compartment, can be divided into subpopulations that exhibit morphologic, phenotypic, and functional heterogeneity, which is, in addition, species-dependent. Further functional differentiation of subsets is consistent with the current view that PAM play an important immunoregulatory role within their microenvironment (see below). Lung macrophages from specific-pathogen-free (SPF), lipopolysaccharide (LPS) hyporesponsive C3H/HeJJ mice appeared to be relatively homogeneous no matter what lung-tissue compartment they were obtained from (50), indicating that further functional specialization takes place at the local site. Data from human studies support this view that the various functions observed within the macrophage family found in the peripheral blood or in lymphoid tissue are performed by the same cells (51). For identification of functional subpopulations of alveolar macrophages, well-recognized correlations between the surface antigen expression and function of these cells have been exploited (52). By using monoclonal antibody (mAb) probes that can distinguish mature classic macrophages (RFD7+) and APC (RFD1+) in normal tissue (53) and within the human PAM population, three different subpopulations of PAM have been identified (RFD1+D7− is the phenotype of DC, RFD1-D7+, and RFD1+D7+) (54, 55). The possible significance of these subsets was revealed by the observation that T-cell responsiveness might be influenced by relative changes within these heterogeneous PAM populations (56). Increased numbers of RFD1+ cells in the bronchial mucosa of allergic asthmatics have been reported, numbers which decreased during treatment with inhaled corticosteroids (57).

PAM and allergic sensitization

Studies on the role of PAM in the development of hypersensitivity reactions in the airways showed somewhat controversial results, as variations have been reported in PAM activity in *in vitro* T-cell activation or suppression when different T-cell activation stimuli were used.

Suppressive effects of PAM

As mentioned above, resident PAM play an important immunoregulatory role at the local site in the airways. PAM have a role in T-cell proliferation, as it has been demonstrated that the production of IL-1β by PAM is essential for primary immune responses to T-cell antigens. Absence of IL-1 in the initial stages leads to abrogation of the immune responses to antigens (58, 59). In contrast to these findings, it has been recognized for many years that an immunologic homeostasis directed toward suppression of the T-cell-dependent immunity is present in the respiratory tract, even in relation to secondary immunity in primed animals. Soluble, suppressive signals (putative cytokines) derived from PAM and lung macrophages from the lung tissue, but also cell-to-cell contact between macrophages and lymphocytes, play a central role in this process (60). Besides, a considerable body of evidence points to the relative inefficiency of PAM of various species in functioning as accessory cells. Most studies show that animal and human PAM function poorly as APC compared to blood monocytes or macrophages from other tissues (60). This poor antigen-presenting capacity of PAM seems to be a general phenomenon (61–63) and may, in addition to the downmodulation of local and *in vitro* T-cell responses in the steady state, be an important factor in the maintenance of immunologic homeostasis in the lung.

Several recent studies suggest that PAM have a major role in regulating the magnitude of secondary IgE responses to inhaled allergen. Both soluble immunosuppressive factors and cell-to-cell contact among mature PAM, lymphocytes, and DC have been shown to take part in PAM-mediated suppression of *in vitro* lymphocyte proliferation induced by antigens or mitogens (60) and suppression of the APC functions of pulmonary DC *in vivo* (64), respectively. Another consistent finding in these studies has been the presence of an endogenous macrophage population in the lung-tissue digests which inhibits DC-induced T-cell activation *in vitro* (65, 66). Under experimental conditions, the pathway of T-cell suppression appeared to be reversible, at least transiently, via GM-CSF derived from LPS-induced, cytokine-rich, lung-conditioned medium (67), suggesting that secretion of GM-CSF by mesenchymal cells or other local inflammatory cells, during acute inflammatory reactions such as late-phase allergic reactions, provides a potential mechanism for upregulation of local T-cell responsiveness.

In the same study, experiments with a range of other cytokines indicated that IL-4, transforming growth factor β1 (TGF-β1), and TNF-α exhibited weaker (but significant) modulatory effects on PAM, and (in the case of TNF-α and TGF-β) amplified the effects of GM-CSF. Such findings suggest that production of high levels of these cytokines, as in local inflammatory processes such as those of asthma, may abrogate PAM-mediated T-cell suppression and potentiate antigen presentation, thus contributing to the immunomodulation of the local allergic reaction. The same study demonstrated that T cells activated in the presence of PAM demonstrate normal Ca^{++}-flux, TcR downmodulation, cytokine secretion (including IL-2), and IL-2R expression, but are prevented from further oligoclonal expansion, thus limiting their contribution to the immune response (Holt PG, personal communication, 68). Counter experiments show that the downregulation of the local T-cell responsiveness is indeed PAM mediated. Selective *in situ* elimination, by the intratracheal administration of liposome-encapsulated dichloromethylene diphosphonate (DMDP), of PAM from ovalbumin-presensitized rats and mice showed that this elimination greatly amplified immune responses to the inhaled antigen (69–71). In particular, T-memory-cell-dependent secondary antiovalbumin IgE antibody responses increased dramatically, locally and systemically. In addition, the antigen challenge was accompanied by massive mononuclear cell infiltration (including activated T cells) of the connective tissues surrounding the small airways and the vasculature, suggesting that the PAM-induced suppressive control mechanism is preferentially directed against homing of activated $CD4^{+}$Th2-memory cells (70). Furthermore, soluble signals from PAM inhibit the APC function of DC (65) and their capacity to upregulate their APC functions in response to GM-CSF. *In vivo* elimination of alveolar macrophages increases the antigen-presenting capacity of the pulmonary DC *in vitro* (64) and the clonal expansion of pulmonary T cells (72).

In summary, these studies show that eradication of the PAM-induced suppressive effects gives rise to an increased antigen-presenting activity of resident DC, an increased cloning efficiency of lung-derived T-cells – especially that of Th2 cells presumably, with, as a result, an increased Th2-dependent IgE response – and, finally, a significant increase in the secondary immune response to inhaled allergens. Therefore, together with the already mentioned stimulatory influence of the release of proinflammatory cytokines such as GM-CSF and IL-4 (67), this release of PAM-induced suppression may aggravate local allergic reactions.

Facilitating effects on PAM functions

The predominant Th2 type response in allergic reactions may explain the elevated local allergen-specific IgE levels. As mentioned before, supernatants of activated Th2 cells strongly enhance expression of the low-affinity IgE receptor ($Fc_{\varepsilon}RII$/CD23) on the cell surface of APC-like B cells. Recently, antigen-specific-mediated facilitated antigen presentation (FAP) was described, suggesting more efficient trapping of IgE-complexed antigens by CD23-expressing, antigen-presenting B cells (73, 74). The studies indicate that FAP is based on facilitated binding of preformed IgE-allergen complexes mediated by CD23 expressed by APC, and subsequent endocytosis followed by processing and functional re-exposure of allergen-peptide fragments in association with class II MHC molecules. Probably other APC bearing low-affinity CD23 receptors such as PAM can also mediate FAP. APC such as epidermal Langerhans' cells and dermal DC (see below) express high-affinity $Fc_{\varepsilon}R$ ($FC_{\varepsilon}R$-I) (75, 76), and therefore can bind uncomplexed IgE (77, 78) and mediate FAP (79). This may also be true of DC in the epithelium of peripheral airways in allergic asthmatic patients. The studies do not support a role for IgG in FAP (74). FAP seems to be allergen-selective; that is, predominantly HDM-derived major allergens, such as Der p 2, are subject to FAP (74). Therefore, inhalation of only minute doses of Dp protein may induce FAP in HDM-allergic asthmatics, in addition, counteracting the immunosuppressive local milieu which is, as we have seen before, present in the peripheral airways of healthy individuals.

Dendritic cells (DC) in the airways

DC were first recognized by Steinman & Cohn (80) in the peripheral lymphoid organs of mice. It is now accepted that the immune system contains a DC lineage whose members are widely distributed throughout the body. In nonlymphoid organs, they can be found either close to body surfaces, as in the skin, where the Langerhans' cells (LC) are thought to be members of the DC family and precursors of the interdigitating cells in the lymph nodes (81). They are also prominently found in the gut, and the human airways (82–86), where they are situated primarily in the epithelium and subepithelial tissue of the bronchus and the bronchilolus (86, 87). Most lung DC initially arrive via the blood as monocyte-like DC precursors (88), and home selectively to intraepithelial microenvironments, where they become mature. In the epithelium of the airways, a subpopulation of the DC carry the LC marker CD1a (OKT6) (approximately 30%) (89,

90). DC can also be found in the body fluids (91, 92), whereas DC are present as interdigitating cells (IDC) in the T-cell-dependent areas of lymphoid tissues (93). Only a few CD1a-negative DC are found free in the alveolar compartment in nonatopic individuals (87) as well as in patients with allergic asthma (Jansen HM, Hoefsmit B, unpublished observations). However, the bronchoalveolar lavage fluid of healthy, nonsmoking individuals contains a number of precursors of DC which show strong morphologic and immunocytologic similarities to DC after *in vitro* maturation (90).

DC appear to be the most potent accessory cells to induce primary T-cell responses (94, 95) after generation of MHC-peptide complexes and presentation of them to the TcR, and formation of T-cell-dependent antibodies (96). DC can prime MHC-restricted T cells and initiate a primary humoral response *in vivo* without using adjuvants (95, 96). Typical immunohistologic features of DC are their bean-shaped, eccentric nucleus, the prominent cytoplasmatic protrusions, and the strong MHC class II expression (97). Recent studies have identified the class II MHC-bearing DC as a contiguous network in the airway epithelium of several species, including man (86). Immune competence in the adult lung has been shown to be closely linked to the functional capacity of this locally situated cell system (reviewed by Holt [2]). The intraepithelial DC network seems comparable to the epidermal LC population (98), and it accounts for virtually all local immunostaining for class II MHC in bronchial mucosal biopsies. In general terms, the density of class II MHC (e.g., Ia antigens) on the surface of the cells correlates with the potency of the APC in T-cell activation (99). Experimental cell kinetic studies indicate a half-life of ≤ 2 days for airway epithelial DC (100). In contrast, the epidermal LC half-life is ≥ 15 days. This indicates a very rapid turnover of mucosal DC, as consistent with an important role in immune surveillance of inhalant antigens. Inhalation of bacterial antigens, e.g., *Moraxella catarrhalis*, gives rise to a rapid recruitment of putative class II MHC-positive DC precursors into the airway epithelium, up to 3000 cells/mm^2 within 24 h, developing the dendriform structure typical of resident DC (101). These cells then migrate to the RLN after the ensuing 48-h period. This rapid, DC-mediated response might be typical of the immune surveillance in the airways (101), and disturbance of this possibly plays a role in local allergic reactions. Recent findings suggest that Ia expression by neonatal respiratory tract DC is locally controlled and can be upregulated by mediators produced within the airways in response to inhalation of proinflammatory stimuli such as bacterial antigens or allergens (102).

Immunoregulatory functions of respiratory DC

The role of DC in the respiratory system can be clarified in part by dermatology. A series of studies by Holt et al. has recently identified airway intraepithelial and alveolar septal DC populations as the primary APC in the lung, both in man and in experimental animals (103) (reviewed above). In the airway mucosa, putative allergens and the immune system directly interact, and any situation facilitating the contact with immunocompetent cells residing within the airway mucosa (e.g., MHC class II-positive DC) is likely to enhance specific IgE production in genetically susceptible individuals (2, 103).

Facilitating effects on DC function

Although the airway epithelium represents a highly regulated and tight barrier, allergen macromolecules gain access to the immunocompetent cells under various pathologic conditions. In asthma, transepithelial solute permeability is increased (104). This is governed by a number of factors, of which the most important are solute concentrations, molecular radius, and the presence of active transport processes (105, 106). In addition, allergen deposition itself causes the bronchial epithelium to become increasingly permeable to macromolecules (107), whereby allergens are more prominently presented to the intraepithelial dendritic network. Under normal circumstances, DC present antigens in the RLN, and not locally in the epithelial layer. However, under the influence of proinflammatory cytokines, especially GM-CSF, which is present in high local levels in allergic inflammation, direct antigen presentation to local T cells takes place, intraepithelially, aggravating the secondary immune response. DC-dependent T-cell activation *in vitro* is significantly enhanced by DC precultured with GM-CSF (Holt PG, personal communication). PAM-induced suppression of DC function is always restricted to the RLN and will presumably not control this intraepithelial increased antigen presentation.

A predominantly Th2-like response induced by allergens may become established at or around the time of the first exposure; that is, in man, during early childhood. As reported above, the animal-model literature suggests that the outcome of these initial responses can be influenced by various factors, including the suppressive nature of PAM, the functional maturity and competence of APC and CD4+ T cells, and the presence of inflammatory or infectious stimuli at the local site of the airways. Recent studies indicate that respiratory syncytial

virus (RSV) infections, during the first year of life, enhance allergic sensitization in individuals with a family history of allergy (reviewed by Martinez [108]). Especially in the initial phase, as well as during a sustaining-memory T-cell response, such factors can create an IFN-γ-rich milieu which selects against Th2 cells (109, 110). The airway intraepithelial DC network becomes functional after maturation, a development which can occur only after birth. It has been suggested that this is driven by *in vivo* exogenous inflammatory stimuli (102).

However, during infections, bacterial products and possibly also viral epitopes stimulate macrophages and DC to produce IFN-α and natural killer cell (NK) stimulatory factor (NKSF or IL-12). Both cytokines induce such an IFN-γ-rich environment by stimulation of NK cell- and T-cell-derived IFN-γ production (111, 112). When Th cells are simultaneously presented by processed antigen and these endogenously produced cytokines, the differentiation is pushed to a Th1 response (113). However, virus-induced differentiation of allergen-specific T cells can be driven toward the Th2 profile by simultaneous addition of IL-4 (113). *In vitro*, naive T cells with identical TcRs can develop preferentially into Th2 phenotypes under the influence of IL-4, whereas IL-10, a cytokine produced by Th2 cells, macrophages, and Ly-1$^+$ B cells (114), inhibits the capacity of some, but not all, APC to drive Th1 development (115, 116). These effects of IL-4 occur regardless of the APC used to activate the naive T cells, and are not seen in stimulation of T cells previously activated in the absence of IL-4. The non-T-cell cellular source of this IL-4 at the local site in the airways may be local mast cells or basophils. Up to 90% of the IL-4 secretion cells in normal or atopic human lung are mast cells (117). The effects of IL-10 may operate by regulating the expression of costimulatory molecules such as B7 on some, but not all, APC (see below) and/or by suppressing the already mentioned production of IL-12 (115, 116).

Later, in atopic reactions, an additional Th2-skewing factor appears to be the APC-derived prostaglandin E_2 (PGE_2) (118). PGE_2 suppresses selectively the production of the Th1 cytokines IL-2 and IFN-γ, whereas in some cases the secretion of the Th2 cytokine IL-5 is induced. However, no differences were found between PGE_2-induced effects on T cells of atopic and nonatopic individuals. Moreover, the differential effects induced by PGE_2 were associated with endogenous IL-2 production. Suppression of endogenous IL-2 production abolished production of Th1 as well as Th2 cytokine (119). Like IL-12, PGE_2 is secreted by APC subsets upon activation, and there is evidence that the balance of PGE_2/IL-12 production by APC determines the balance of IL-4/IFN-γ production by Th cells activated by these APC (120). IL-12 and PGE_2 production by accessory cells is very much influenced by production of other soluble factors derived from locally activated Th cells or APC. For instance, IL-12 production appears to be strongly stimulated by IFN-γ and inhibited by IL-10 and IL-4 (see above).

These findings imply a tightly interactive network of factors which regulate the production of Th1- and Th2-type cytokines, and finally determine the course of the immune response. Th1-type factors (such as IL-12 and IFN-γ) promote the course of a determined delayed-type cellular reaction, whereas Th2-type factors (PGE_2, IL-10, IL-4, and IgE) facilitate a mainly humoral response and early- and late-phase allergic reactions. Yet there is slight evidence that APC from atopic individuals show preferential susceptibility in production of PGE_2. In addition, soluble signals from activated T-cell-suppressive PAM (see above) suppress the APC function of DC in the lung, and inhibit the capacity of DC to upregulate their APC function in response to GM-CSF (67). As already mentioned, the enhanced responsiveness of PAM-deficient animals to inhaled antigen is an IgE response suggesting that PAM-induced T-cell suppression is mainly directed against Th2 cells. Certain exogenous factors, such as infections occurring at an early stage in the development of the immune response, may eliminate this PAM-regulated suppression.

Suppressive effects of DC function

As already discussed, DC present antigens to MHC class II-restricted CD4+ T cells. However, DC are also potent inducers of class I MHC-restricted CD8+ cytotoxic T cells, as has been shown for viruses and viral antigens (121). Recent studies have demonstrated that inhaled soluble protein antigens may be processed by a subpopulation of spleen APC in an MHC class I-restricted pathway, priming antigen-specific CD8+ T cells (122). The same studies have shown that a similar APC population operates at the level of the RLN in the airways. The antigen-specific CD8+ T cells need IL-2, derived from primed CD4+ T cells, for proliferation, and they then may suppress the Th2 response by producing large amounts of IFN-γ, thus creating a milieu which supports a shift to the Th1 response. The study by Nobel et al. supports the latter possibility, because the depletion of CD8+ T cells results in the upregulation of Th2 cytokine expression (123). Not much is known of the occurrence of allergen-specific CD8+ T cells in man and their relation to APC. Recently, we described one Der p 2-specific CD8+ T-cell clone generated from the peripheral blood of a Der p 2-allergic asthmatic patient (9). This T-cell clone was HLA-DR

restricted, producing IFN-γ, but not IL-4. However, a panel of 10 Der p 2-specific CD4+ T cells from the same patient were clearly Th2 cells that produced high levels of IL-4, but not IFN-γ, suggesting that the two cell types have different functional characteristics. This finding suggests that allergen-specific CD8+ T cells do exist in allergic patients and, together with those discussed above, may explain certain aspects of inducing tolerance of inhaled antigens. In allergy, this induced tolerance loop may be disturbed by local factors. Recently, it has been shown that the presence of IL-4 during the generation of antigen-specific CD8+ effector populations promoted synthesis of IL-4 and significant levels of IL-5, and dramatically downregulated secretion of IFN-γ by these cells, whereas priming with IL-12 generated CD8+ effector cells producing elevated quantities of IFN-γ (124). Future studies will be needed to determine the frequency of these allergen-specific CD8+ T cells in allergic patients and control subjects, the HLA class II or HLA class I restriction, and the cytokine profile of these cells, in order to gain insight into the relevance of the cells in the inhibition of the allergic response induced through the APC-dependent pathway.

Costimulation effects of DC function

It is now recognized that for effective antigen presentation APC must deliver simultaneous signals to the TcR, as there are, firstly, processed antigen peptides complexed with surface MHC class II (Ia), and, secondly, a "costimulating" signal supplied via a second group of cell-surface molecules on the T cell with their ligands expressed on APC (125) such as the B7-1/B7-2-CD28 pathway (126). These costimulation receptor pathways have been shown to be prominent in functional DC (127) (Fig. 1). Ligation of CD28 on the T cell with B7-1 (CD80) or B7-2 (CD86) on the APC is the best characterized mode of costimulation (128, 129). Signalling through CD28 stimulates IL-2 mRNA production and stabilizes IL-2 mRNA, enabling T cells to proliferate in response to antigens (130). Chelen et al. (63) recently showed that antigen presentation by PAM could be restored by the addition of anti-CD28 mAb to cultures of T cells and macrophages, indicating that costimulation by PAM via the CD28 pathway was defective. In addition, these authors found that PAM activated by IFN-γ failed to express B7-1 or B7-2 antigens. These observations suggest that reduced expression of B7-1 and B7-2 by PAM may explain the already mentioned ineffectiveness of PAM in presenting antigens to T cells, and, moreover, that this mechanism may be involved in the induction of immune tolerance at the local site.

In general, it has been shown that the nature of the APC present during the first encounter with the antigen may influence the magnitude and the quality of the humoral immune response, whether it results in the production of Th1-dependent isotypes or the induction of a Th2-associated antibody response (131). This feature could be related to the differential expression of the required costimulatory molecules. The correlation of B7 expression and IgG2 production may suggest that B7 is a costimulatory molecule for IFN-γ production (131). Recent studies have shown that CTLA-4, expressed on activated T cells, is a second ligand for CD80 and CD86 that can cooperate with CD28 as a costimulatory molecule (132). CTLA4-Ig, a fusion protein of the extracellular domain of CTLA-4 and the constant domain of IgG, which has the ability to bind to CD80 and CD86 with high affinity, can block this costimulatory pathway. Transplantation rejection can be inhibited by CTLA4-Ig (133). However, several studies have suggested that blocking CD80/CD86-CD28/CTLA4 interaction may not result in nonresponsiveness of Th2 cells (134), although CD28 signalling is required for IL-4 production of T cells and increases IL-4 responsiveness in Th2 cells (135). We recently demonstrated that T-cell responses of allergic asthma patients and T-cell responses of control donors to HDM allergens are inhibited equally well by blocking with CTLA4-Ig (van Neerven RJJ, et al., unpublished observations). Because, in these studies, CTLA4-Ig was shown to inhibit the proliferation of Th2 cells and the production of Th2 cytokines, it may be a useful tool to inhibit Th2 responses to allergens in a relatively specific manner. CTLA4-Ig will probably also inhibit most T-cell-mediated immune responses *in vivo*, resulting in compromised immune responses after administration. However, if B7 expression on APC that present allergens to T cells can be modulated, this indirect approach of blocking costimulation may prove to be a good alternative to the use of CTLA4-Ig in combination with allergens to suppress specific allergic responses.

Barcy et al. (136) have demonstrated that B7-1 and B7-2 expression can be downregulated *in vitro* by $Fc_{\gamma}RII$ signalling in monocytes. This has been shown to block the function of these cells as APC. The formation of allergen-immune complexes that lead to $Fc_{\gamma}RII$ signalling and antigen presentation may result in the downregulation of B7-1 and B7-2 on only these APC and may therefore prevent allergen-specific T-cell activation *in vivo* without affecting the immune response.

Conclusions and clinical considerations

Understanding the cellular and molecular mechanisms of recruitment and the local function of APC

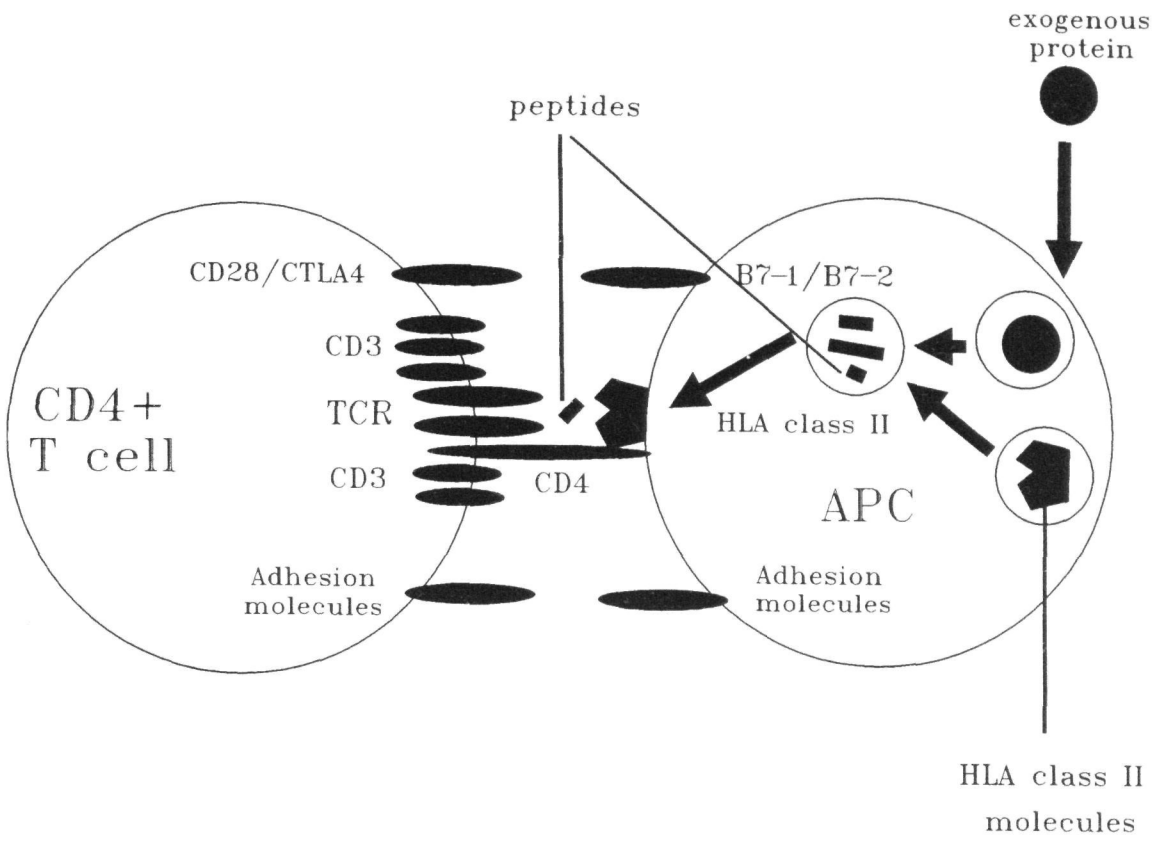


Fig. 1. Schematic representation of antigen-recognition, antigen-presentation, and antigen-processing pathways of CD4+ T-cell and antigen-presenting cell (APC). Courtesy of RJJ van Neerven.

in nonlymphoid organs such as the lung parenchyma, the alveolar space, and the airway mucosa is an important step toward improvement of our insight into the role of these cells in the lymphocyte-mediated immune reactivity in allergic reactions.

As we may conclude from the studies reviewed here, this specific immune response of T cells to allergen challenge, which defines at least the nature of the established atopic response, is highly dependent on the presence of various types of accessory cells, such as pulmonary alveolar macrophages, DC, B cells, and epithelial cells, and the changes in their activation profile. The potential role of PAM and DC populations in the regulation of local T-cell-dependent immune reactions in the airways and regional lymph nodes depends on various additional factors, including their precise localization, migration characteristics, maturation, expression of T-cell "costimulatory" signals, responsiveness to cytokine stimuli, and, last but not least, host immune status and the nature of the allergen challenge. The effective phase of the immune response represents the final outcome of the interactions within this cell-to-cell and cell-to-cytokine network.

The question remains whether this knowledge from the already mentioned basic research areas will lead to the development of useful suppressive modulations of established allergic respiratory diseases. As HLA restriction patterns of allergen-specific T cells have been shown to be as diverse as T-cell epitope recognition, there is only a weak correlation between HLA haplotype and specific IgE to major allergens. However, because allergen-specific Th cells seem to be causally related to the pathophysiology of allergic disease, and available evidence suggests that this Th2-cell selection process is antigen-driven, information on the antigen presentation of T-cell epitopes they recognize is needed to evaluate the potential use of synthetic peptides in the suppression of this response. Besides the induction of allergen-specific CD4+ T-cell anergy, and/or allergen-specific suppressor cell activity, an increased Th2-Th1 cytokine switch may be effective.

Because the T-cell selection process, resulting in either Th1 or Th2 dominance, presumably takes place in early childhood, manipulation and prevention of this reaction should be performed at that age. However, the data reviewed here suggest that even under established Th2-cell memory, the response remains dependent on allergen-booster challenge, and therefore under the influence of antigen presentation by APC. Therefore, this process should also be susceptible to manipulation.

In contrast to previous data, there is evidence, as already mentioned, that blocking of APC to Th2-cell costimulation pathways may inhibit the Th2 proliferation and, as a result, may prevent or suppress allergen-specific T-cell activation (137).

Suppression or activation of less specific pathways in the induced secondary allergic reactions may be effective as well. In the human system, considerably less attention has been paid to the concept of active restoration of the PAM-mediated T-cell suppression or the induction of a high, PAM- and DC-derived IL-12/PGE_2 production balance, to restore Th1 proliferation. As already mentioned,

such a strategy would theoretically be feasible. For instance, suppression of production of proinflammatory cytokines with corticosteroids or, more specifically, with cytokine blockers, may be effective in susceptible individuals (138).

The ultimate goal for clinicians confronted with established "adult" atopy will continue to be suppression of allergen-specific, T-cell-mediated secondary responses, to prevent the occurrence of IgE-mediated adverse reactions. As we still face huge difficulties in peptide-specific immunomodulation in established allergic reactions, we may try to intervene at the level of accessory cells to suppress the reactions, but it will be a long time before this method can be applied in immunomodulation.

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