III. PEDIATRICS

THE ROLE OF DENDRITIC CELLS IN ATOPIC DISEASE

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Abstract

Large populations of dendritic cells (DCs) are found throughout the respiratory tract, the most prominent comprising a contiguous network dispersed throughout the epithelium and underlying mucosa of the conducting airways. These populations of DCs in the lung and airway wall are now known to play a central role in the maintenance of immunological homeostasis in the respiratory tract. Dendritic cells play a critical role in the initiation of allergic pulmonary inflammation. Pulmonary DCs total number is increased in the asthmatic pulmonary tissue, even though it is not known if these pulmonary DCs are phenotypically or functionally different from those present in the bronchia and bronchioles of non-asthmatic individuals.

Key words: dendritic cell, allergy, Th_2, asthma

Atopic diathesis is characterized by three main diseases: allergic rhino-conjunctivitis, allergic asthma and atopic dermatitis, usually associated with increased levels of IgE. Approximately 3 millions Romanians are suffering of allergic diseases. The spring is accompanied for them with an exacerbation of symptoms, determined by an increase in aeroallergens. In many cases, the symptoms are only annoying (rhinorea, sneezing etc.); however, more severe consequences, as exacerbation of bronchic asthma, are possible.

Research regarding asthma revealed the essential role of airway dendritic cells in inducing allergen sensitivity. This paper describes the physiology of DCs, as well as the mechanisms of allergic sensitization through dendritic cells and provides a summary of a recent proved theory that DCs function regardless of sensitization degree.

Many factors may influence the establishment of allergic disease, including genetic susceptibility, environmental factors as microbial exposure and allergen dose, the time of allergen exposure, and the subtype/function of dendritic cells that initiate Th_2 polarization. Although genetic component is important, it seems that environmental interactions during the first years of life, a concept called hygiene hypothesis, are crucial in rhinitis development. Childhood exposure to microbial endotoxines with Th_1 immunologic programming may have a protective effect. For example, animal contacts during the childhood (children that grow in farms or that have pets), provide protection against atopic sensitization.

Allergy thus may originate in a fail to change allergen-specific Th2 response in protective Th1 response. It has been also suggested that impaired regulatory T cells activity in atopic individuals would be a cause of disease development.

Asthma is a common, hardly treatable disease, with an incidence that doubled in the last two decades, its global costs exceeding those for tuberculosis and HIV/AIDS together. Asthma is a Th_2-type inflammatory disease of the airways characterized by airway eosinophilia, increase of mucus production and structural remodeling or airway walls. All these features lead to airways obstruction and bronchial hyper-reactivity (BHR) to non specific stimuli.

The presence of high levels of allergen-specific IgE in allergic asthma is the reflection of a Th_2 immune reaction to common environment allergen as HDM or aeroallergens. This Th_2 sensitization process to inhaled allergens occurs in the childhood and is influenced by genetic factors such as infections and exposure to microbial compounds.

Naive T cells require antigen-presenting cells (APC), such as dendritic cells, to clonally proliferate and to acquire Th_2 effector function so that to react at the time of antigen contact. The studies conducted in the late 90's clearly showed that DCs play a vital role in deciding the result of antigen integration in the immune system and in the integration of antigen-derived signals, inflammation context and host environment into a signal that can be read by naïve T cells in lymphoid tissues.

DCs play a unique role in the initiation of specific immune responses, beside their role in the process of differentiation and polarization of antigen-specific T cell responses. Even though it has been suggested that other cells, such as B cells, macrophages, epithelial cells and even eosinophils, take part in antigen presentation, it became more and more clearly that DCs are the most important APC. For instance, ovalbumin (OVA)-sensitized B cell-deficient mice further develop airway inflammation. Similarly, the eosinophils seem to amplify Th_2 responses, but they lack the capacity to present the antigen to naïve T cells. In a murine model of human severe combined immunodefficiency, reconstituted with peripheral blood mononuclear cells from *Dermatophagoides pteronyssinus*

(Der p1)-sensitive patients, it has been shown that human DCs are mainly localized in the alveolar spaces of the lungs of mice, which developed a pulmonary inflammatory infiltrate. After exposure to Der p1, the number of DCs in the airways decreased and subsequently it was detected an increased production of IgE as compared to mice in the control group. It has been recently shown that adoptive transfer of DCs from mice with cow's milk food allergy also induces allergen-specific IgE in naïve syngenic mice in absence of antigen challenge. Interestingly, allergen-specific IgE response was induced without altering Th_1/Th_2 balance, indicating that Th_2-skewed responses were not involved in early phases of allergic responses. Moreover, OVA-sensitized transgenic mice, with selectively depleted airway DCs, but not macrophages and B cells, displayed the suppression of eosinophilic airway inflammation after OVA exposure, compared to control mice, thus proving DCs contribution to the pathogenesis of this disease.

After neglecting DCs research for some years, investigators are now showing a great interest on the subject, because of the central role these cells in the complex processes of adaptive immune reaction. Furthermore, understanding the role of DCs in physiopathologic conditions might be an important step in developing a therapy for many diseases. As several types of DCs, including follicular and thymic DCs, were identified in the last years, this paper will focus on classic DC.

Over the last decade, airways DCs were shown to be vital in Th_2 allergic sensitization process, especially in asthmatic gerbils. The gerbils were induced with transgenic disease to study DCs role in pulmonary allergic reactions. These experiments lead to the conclusion that airways DCs are vital not only in the regulation of inhaled allergen sensitization process, but also in controlling established allergic inflammation. Change of DCs function is a therapeutic concept that will be able either to prevent establishment of sensitization, or to treat the already established disease.

DCs were found in all epithelial types (subcutaneous tissue, mucosa, lungs), as well as in heart, kidneys and other organs. In addition, various DCs subtypes were found in the blood and lymphatic system. These represent different maturation stages (depending on antigenic load) and are connected through circulatory pathways. Beside the typical dendritic structure in tissue and suspension, DCs are initially characterized by the expression of major histocompatibility complex (MHC), class II HLA-DR and by high stimulatory activity towards allogeneic T cells. Immature DCs are distributed all over the lungs area, playing a pivotal role in the control of immune reactions of inhaled auto-antigens. A network of airway DCs is situated immediately above or beneath the basement membrane of respiratory epithelium in all studied species.

Even though they act as specialized antigen-presenting cells (APC), DCs must undergo 4 main differentiation and maturation stages before effectuating their main function in lymphoid organs.

Once situated in the peripheral blood, DCs are considered functionally immature. This means that tissue DCs are specialized in capturing and processing both self and non-self antigens. Another DCs feature is the stability of MHC class I or class II molecules on the cell surface, enabling them to remain loaded with defined antigens for a long time. In this maturation state, DCs are able to stimulate memory T cells by migrating throughout the tissue, and initiating a secondary immune response in this site of captured antigen contact. However, as the macrophages ant other cells are equally effective in this type of stimulatory activity, it is conceivable that activation of secondary immune response is the main role of DCs under normal conditions.

Over the last years DCs migration has been shown to be up-regulated by chemokines. Expression of chemokines in various anatomic sites and under different pathological conditions, combined with expression of chemokine receptors on the cells during different maturation stages represent the basic events for the initiation of a complex signaling network that directs their migration and interaction in the process of immune response. It has been shown (characteristic for DCs) that chemokine receptor profile expressed on immature DCs(CCR1, CCR2, CCR5 and CCR6) recognizes mainly the chemokines released during the inflammatory process. This allows for DCs to accumulate for the antigen uptake at sites of inflammation. When chemokines like IL-1 and TNF-α occur, this process continues by induction of immature DCs that will release more chemokines. Mature DCs regulate their inflammatory chemokines receptors and also express different chemokine receptors (CCR4, CCR7, CXCR4, SLC and ELC).

After antigen uptake, DCs migrate from the tissue to the regional lymph nodes. For instance, LC appear to migrate quite fast, several millimeters in 30 minutes. On their way to the lymph nodes, DCs start a profound metamorphosis leading to significant changes in structure and phenotype. DCs in afferent lymphatic vessels were described as veil (circulating) cells, while DCs in the T cell-rich paracortical areas of secondary lymph tissues as interdigital cells. Mature DCs loose their antigen uptake capacity in the process and acquire their antigen-presentation function. One of the major steps of this development is the regulation of costimulatory and peptide-loaded MHC class II molecules (CD80, CD86) on these cells surface. Meanwhile, DCs rapidly regulate Fc receptors expression. DCs migration and maturation seem to be inter-connected *in vitro*, as factors like lipopolysaccharide (LPS), TNF-α and IL-1 induce the both processes. *In vitro* TNF-α induces the maturation of monocytes-derived DCs, leading to regulation of CD80, CD86, CD83 and MHC class II molecules, which are all crucial in effective antigen presentation.

Naïve T cells activation is a crucial role of DCs. For this, DCs and naïve T cells must meet in the paracortical area of lymph nodes. An interesting finding is that naïve T cells express chemokine receptors (e.g. CCR7) which enable them to receive signals from mature DCs releasing ELC and specific chemokine receptors. After reaching the T cell zone, a single DC can activate hundreds of naïve T cells. In this process, peptides bound to MHC class II or MHC class I by DCs are presented to T cells *versus* T cell receptor complex (TCR). Recently, in addition to the signals received *versus*

TCR, co-stimulatory were shown to be of key importance in initiating and directing the T cell response. The interaction between co-stimulatory molecules CD80/CD86 and CD28 or CTLA-4 T cells determines whether the stimulation process will result in a T cell antigen- or tolerance-specific proliferation. Indeed, additional factors, as IL-10, present in the site of T cell – DCs interaction can change CD80/CD28 signaling by blocking the events of signal delivery, thus leading to an antigen-specific tolerance.

An important observation is that DCs releases IL-12. This cytokine is involved in the induction of a Th_1 response of T cell. In the same way, other cytokines, like IFN-γ, may induce a Th_1 response, even though IL-4 directs T cell response towards Th_2. This ability to influence the type of T cell response may explain the reason why some antigens induce an allergic reaction, while the others don't. It is interesting that cytokines and factors released during T cell activation induce a different chemokine repertoire on stimulated T cells. Although Th_1 cells express CCR1, CCR2, CCR5, CXCR3 and CXCR5, Th_2 cells are characterized by the expression of CCR2, CCR3, CCR4 and CXCR5.

Differential expression pattern could recruit these cells to specific types of inflammation. As the allergic reactions concern, Th_2, eosinophil and basophil cells are known to share the expression of chemokine receptors CCR3, even if Th_1 cells and monocytes, that are able to differentiate into DCs, share CCR1 and CCR5.

Once antigen presentation accomplished, DCs cannot recirculate in the peripheral blood or lymph vessels; it is assumed DCs will be killed by T cells and will die by apoptosis.

DCs are thought to be the best candidates for T cell activation against environmental allergens. In the context of Th_1/Th_2 dichotomy that dominated immunologic research over the last years, it has been intensively discussed the way T cells are directed towards Th_1 or Th_2 during antigen presentation. Although it became clear that IL-12 secreted by DCs is responsible for the skewing towards Th_1, which cells are the source of IL-4 – that skews the T cell response towards Th_2 remains to be further discussed. Kalinski *et al.* showed that prostaglandin E_2 (PGE_2) may be the signal that directs Th_0 cells towards Th_2. Recently Rissoan *et al.* showed that myeloid DCs are responsible for the skewing of T cells towards Th_1 (DC_1), while lymphoid DCs are skewed towards Th_2 independently of IL-4 (DC_2). Moreover, there are feedback mechanisms acting between DCs and T cells.

LC, monocytes and myeloid DCs were reported to express the IgI and $Fc_\varepsilon RI$ high affinity receptor. $Fc_\varepsilon RI$ on LC and DC_1 shows important differences from this receptor on effector cells of anaphylaxis.

It is indeed not expressed on these cells, but seems to be regulated by the signals of inflammatory micromilieu that surrounds the cells. Therefore, the highest $Fc_\varepsilon RI$ expression is displayed on LC and a recently described inflammatory dendritic epidermal cell (IDEC, presumably DC_1) from lesional skin of atopic dermatitis.

The lack of receptor complex is due to the low expression of signal transmission chain that is important for the expression of heterotrimeric structure surface, whereas the chain IgE-bound is present inside the cells. Furthermore, $Fc_\varepsilon RI$ on LC and DC_1, as well as on monocytes, lacks 4 transmembrane chains. As a consequence, in contrast with LC and DC_1 from atopic individuals, normal LCs (with low expression receptors) are not activated. There are proofs of $Fc_\varepsilon RI$ role in antigen presentation that emphasizes blood monocytes, LC and DC. Multimeric ligations taken over by the endocytosis-mediated FcεRi receptors are effectively directed in MHC class II compartments, like the organs in which processing-dependent catepsin S and MHC class II-loading peptides occur. This results in the optimal antigen presentation by $CD4^+$, similar to a mechanism in the first line of antigen recognition. In this context, a carrier role for DCs expressing $Fc_\varepsilon RI$ in the regulation of IgE synthesis is conceivable. It is generally accepted that IgE molecules and effector cells are the result of the efficient anti-parasitic defending mechanism. This system was proposed to be redirected towards a harmless allergen environment because of the lack of physiologic/pathologic partners.

As mentioned before, allergen uptake and presentation are the primary functions of DCs. Among the methods of allergen capture, classically including nonspecific absorption, fluide phase pinocytosis and cell surface receptor endocytosis, the last is the most effective.

Expression of high $Fc_\varepsilon RI$ density in atopic DCs patients involves several important features. Firstly, DCs extend their ability to react to allergens by binding large amounts of IgE molecules with specific variants. This significantly increases the probability of FcεRI cross-linking to a different allergen on the cell surface.

Secondly, IgE/FcεRI complexes allow the allergen capture; under normal circumstances, they don't undergo fagocytosis via normal route (e.g: by pinocytosis). Thirdly, FcεRI aggregation on DCs is followed by their internalization through receptor-mediated endocytosis. However, similar to B cell receptor, where Igα and Igβ specialize on various endosomal behaviors, this route used for the antigen uptake by DCs, especially via IgE and FcεRI, can determine if the foreign structures will be effectively processed and orientated to MHC class II-rich compartments, finally leading to a higher density of specific peptides on MHC class II molecules surface; lastly, DCs that express high receptor densities show the total activation of the cell after FcεRI ligation, most probably including synthesis and release of mediators to be finalized. Such mediators are able to influence antigen presentation.

The conclusion is that DCs expressing FcεRI coupled with IgE can activate the second immune response and IgE synthesis by recruiting and activating antigen-specific Th_2 cells. In the case of FcεRI mediated antigen, the uptake and the presentation seem not to occur in the primary response, as specific IgE should be present from the beginning. However, the hypothesis that allergen structures captured via FcεRI on DCs are processed by these cells in a manner that leads to peptide presentation to T cell should not be excluded. This initiates a response to the antigens, resulting in the increase of specific IgE diversity. This concept of DCs expressing FcεRI study remains to be further explored, considering particularly the recent research

suggesting an important role of DC-derived IL-12 from PGE_2 in skewing T cells towards Th_2 or Th_1, respectively.

Although most asthmatic patients are atopic, only some of them develop the disease. Asthma is a complex clinic entity, characterized by chronic and acute stages. Characteristic for the acute stage is histamine release from respiratory airways cells, while the chronic stage is induced by an inflammatory intruder in the airways mucosa. In the end, chronic inflammation leads to a permanent lesion of respiratory airways. Asthma is a proto-typical allergic disease associated with Th_2 response and with an increased level of IgE serum.

Recently it has been speculated that increasing incidence of asthma or any other allergic disease could be caused by the high hygiene standards. However, the newborns encounter less pathogens that activate the Th_1 immune response. In addition, postnatal stimulation of a recently allergen-activated immune system predisposes to the positive Th_2 selection, thus favoring the atopic diseases-associated type of immune response. Maturation of airways DCs function after birth represents an important factor for the result of Th_1/Th_2 memory cell selection. Variations in the effectiveness of maturation process may play a key role in determining the genetic risk of asthma.

The first requirement for the induction of an immune response to allergens is for these molecules to gain access to the immunocompetent cells. Even if the airway epithelium is a regulated barrier, transepithelial permeability is increased in asthma. Even the bronchic epithelium becomes permeable to macromolecules after allergen deposition. Moreover, allergen exposure induces the expression of GM-CSF by asthmatic epithelium, which attracts DCs to the antigen contact site.

As for the antigen uptake by DCs, the most rapid cell reaction detectable in the tracheal tissue is the recruitment of complex MHC class II-carrying DCs. The cells remain in the epithelium, reaching their maximum within an hour since the antigen exposure. After that, DCs morphologically convert from the round form to the veil cells form. Surveillance of active DCs in the epithelium is amplified and an increase of their traffic form epithelium to the lymph nodes results. Another mechanism that may contribute to an increased reaction of asthmatic patient to inhaled allergens can be the same as in the inflammatory process, i.e. recruitment of "new" DCs from monocytes. Monocyte-derived DCs in patients with allergic asthma are known to show phenotypic variations in HLA-DR, CD11b and IgE high affinity receptor expression and even a B7-2 (CD86) regulation, and develop in stronger accessory cells than in normal patients.

Data from mice and humans show that DCs play a crucial role in the pathogeny of pulmonary allergic reaction, both during the sensitization and the disease. Therefore, the dendritic cells are leading a complex multi-cellular process, in which T cell aberrant responses, genetic influence in the process of allergen reconstitution, structural changes of respiratory airways walls and inherent epithelial defects play an important role.

Airways DCs are critical for the activation of the immune system to inhaled allergens, their interaction with APC, as well as with other effector cells remaining an active research field.

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