HISTOLOGICAL FEATURES OF DENDRITIC CELLS IN ALLERGIC ASTHMA - EXPERIMENTAL MODEL

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Abstract

It has been already shown that allergic reactions are driven by the continuous flow of antigen take-up and presentation processes, which are maintained mainly by the dendritic cells (DCs). The ability of allergens to cause allergic inflammation is conditioned by the presence of an immunological mean and of a micro-milieu that either favors Th2 responses, or hampers these reactions by inducing anti-inflammatory contra-regulatory activities of the immune system. The contact with allergens initiates a series of events that offers DCs the necessary "equipment" to migrate to regional lymph nodes and to activate allergen-specific Th2 cells (N. Novak). As immune system guardians, APC circulate from blood to peripheral tissue in order to capture self or non-self cells. After that, they migrate to the lymph drainage organs in order to transform naïve T cells into Th1 or Th2 effector cells. In the human immune system two functionally different subtypes of DCs were discovered: myeloid DCs that preferentially direct naïve T cells differentiation into Th1 cells, thus called DC1, and plasmacytoid DCs, which represent the type 2 DCs, namely plasmacytoid dendritic cells (pDC) and have a Th2 polarization profile.

Key words: DCs, pulmonary, der p1, morfology

Introduction

Asthma is a very frequent disease that is present in all age groups, but mainly in children. It is estimated that up to 5% of United States population is affected. In Romania, bronchic asthma prevalence is of 7-8% of population, which accounts for over one million patients. Estimative, 1 out of 20 school-age children has bronchic asthma (diagnosed or not). Almost half of asthma cases occur before the age of 10 years and another third before the age of 40 years. In children, the male/female ratio is 2:l, but this ratio equalizes until the age of 30 years. Allergy is one of the factors that favor asthma installation. The tendency to be allergic is usually inherited. If one of the parents is allergic, the child presents 30% chances to develop an allergy; if both parents are allergic, the probability increases to 50-60%, and the child may have allergies that are not present in any of the parents.

Although today we have excellent medicines and treatment schemes, the number of children that suffer from asthma at 7 or 10 years after the initial diagnosis varies between 26 and 78 %, with an average of 46%, while the proportion of those that continue to present severe forms is of 6 up to 19%.

The main feature of asthma diathesis is represented by the unspecific hyper-excitability of tracheobronchic tree. In both asthmatic and normal children, bronchic reactivity increases after viral infections of respiratory tract and after exposure to oxidant atmospheric pollutants. The viruses have a more important consequence, and after an apparently benign infection of upper respiratory airways, the reactivity may remain high for more weeks. The allergens may cause the increase of airways reactivity in minutes and can maintain it for several weeks. If antigen dose is sufficiently high, acute obstruction episodes may occur daily for a longer period of time, after a single exposure.

Aims

Our main aim was to identify and mark the pulmonary DCs. The use of experimental animal models played and essential role in the understanding of mechanisms involved in the pathogenesis of bronchic asthma. Some animal species can be sensitized to various proteins and, during the time, they can develop atopic asthma that displays bronchic hyper-reactivity. Setting up of bronchic hyper-reactivity animal models was an important way to investigate the possible mechanisms involved in the pathogenesis of this phenomenon.

Material and methods

The experiment was realized on two groups of eight Spraque-Dawley rats each, weighting between 250 – 300 grams. One of the groups was the control group. The sensitization consisted in two phases, after the following protocol (see figure 1). In the first phase, the rats were injected intra-peritoneal with der p1 solution (10.000AU/ml, 99%, ph=7.4) in aluminum hydroxide suspension (allergen extracts are usually applied as aqueous solutions or they are adsorbed to the adjuvants with deposit role, as aluminum hydroxide; while aqueous solutions present a high risk of anaphylactic reactions, the aluminum hydroxide is known as a potent agent that induces Th2 and stimulates the IgE synthesis) in the day 0 at the beginning of the experiment, followed by another intra-peritoneal injection in the day 12. The second phase consisted in the direct nebulization (with aerosols - Happyneb I) for an hour of 1 ml allergen in 10 ml of physiologic serum.

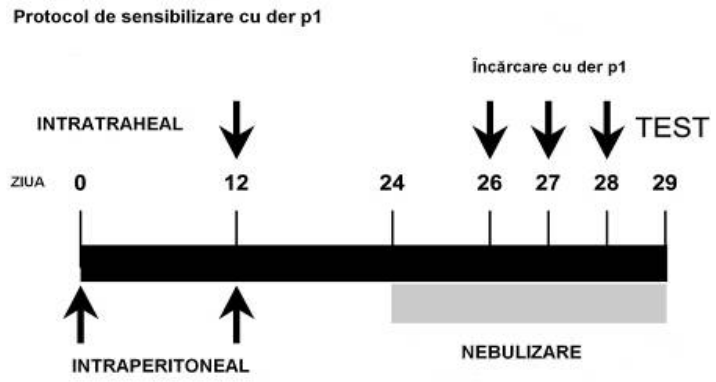

Fig.1 Sensitization protocol (der p1).

After the sensitization period, the animals were sacrificed by decapitation, after intra-peritoneal administration of sodium pentobarbital, dose of 50 mg / body kg.

Trachea fragments sampled were washed of blood and maintained in a recipient with Krebs – Henseleit solution, at 4^0C, at most 15 minutes after harvesting. Macroscopic integer parts were chosen and spiral-type preparations were made, with a length of 15 mm and a width of 2-3 mm. The preparation was put in the 10 ml volume organ bath, containing Krebs – Henseleit solution, at 37^0C, continuously aerated with a mixture of O_2 95% and CO_2 5%. The Krebs – Henseleit solution used had the following composition: NaCl 118 mM, KCl 4.7 mM, $CaCl_2$ 2.5 mM, $MgSO_4$ and KH_2PO_4 1.2 mM, $NaHCO_3$ 25 mM, glucose 5.55 mM. The solution pH was verified both in the beginning and the end of the experiment, as well as every 30 minutes during the experiment (pH = 7.4). Trachea spirals were pretensioned at 1.5 g and they were left to equilibrate 60 – 90 minutes, while the liquid in the bath was replaced every 15 minutes. Before the experiments were performed, the contractile response of trachea preparation was verified at 10-5 M acetylcholine. Preparation in which two similar contraction were not obtained were excluded from study. Between experiments, the preparation was washed with Krebs-Henseleit solution for three times at 1-2 minutes so that to regain the initial tonus.

Preparation was put in the organ bath with the inferior end fixed to a metallic ring by an inextensible silk lock. The superior end of preparation was connected through a similar lock to an isometric force transductor type FORT 10 (World Precision Instruments, WPI Inc.). The results were represented graphically by connecting the transductor to a unit of data acquisition in computerized system BIOPAC MP100, while the data processing and graphic representation were performed using „AQKNOWLEDGE" version 3,72 soft. The curves dose-effect for acetylcholine and methacoline at concentrations between 10^{-7} M and 10^{-4} M were determined. The preparation was incubated for 60 min with Der p1. After the incubation solution was removed, the preparation was repeatedly washed with Krebs – Henseleit solution and preparation reactivity to Mch was tested again.

After the animals were sacrificed, integer parts of trachea, lung and ganglions were harvested from both the sensitized lot and the control one. The fragments were studied histologically, through simple (HE), trichromatic colorations or immuno-histochemical marking (S_{100}).

Results

Under physiological conditions, the tracheo-bronchic smooth muscles are in a state of permanent tonus, induced by the complex interaction of various regulatory factors. The main effector of bronchic hyper-reactivity is the tracheo-bronchic smooth musculature that presents an increased reactivity under conditions of disturbance of one or more musculature tonus regulatory factors.

An animal model adequate for this disease study has to ensure a high similarity with the disease pathology in humans, to allow the objective measurement of physiological parameters and to present sensitivity and reproducibility. Therefore, the ideal model of asthma should reproduce paroxysmal broncho-constriction, development of early and delayed response to allergen challenge, respiratory airways inflammation, including eosinophilia, bronchic obstruction variability, as well as pulmonary remodeling with the impairment of pulmonary function. Histological, der p1 sensitization induced the activation of lymphatic follicles from the lymph organs, as a sign of hyper-sensitivity reaction.

Histo-pathological changes consisted in inflammatory infiltrate rich in lymphocytes and rare eosinophils under basal membrane, glandular epithelium metaplasia and absence of significant changes of muscular layer. In the pulmonary tissue an aspect of alveolitis developed, with rich alveolar infiltrate. On the transversal section of trachea wall, in the unsensitised lot it can be seen a discrete edema in the submucosa (fig.2). In the sensitized lot it can be seen the immune marking of leucocytes, numerous in lamina propria, disposed subepithelial and perivascular (fig.3).

In the pulmonary areas in the sensitized lot we evidenced an abundant interstitial and perivascular lympho-plasmocitary infiltrate and immune marking with S_{100} anti-protein antibodies of some antigen-presenting cells, with stellar aspect in the periphery of lymphoid infiltrate (fig. 4). Also, in the bronchiolar wall of the lung allergenized with abundant lymphoid infiltrate, with nodular organization here and there, with numerous dendritic cells at the periphery of infiltrate, intra- and sub-epithelial in lamina propria of bronchic mucosa (fig. 5).

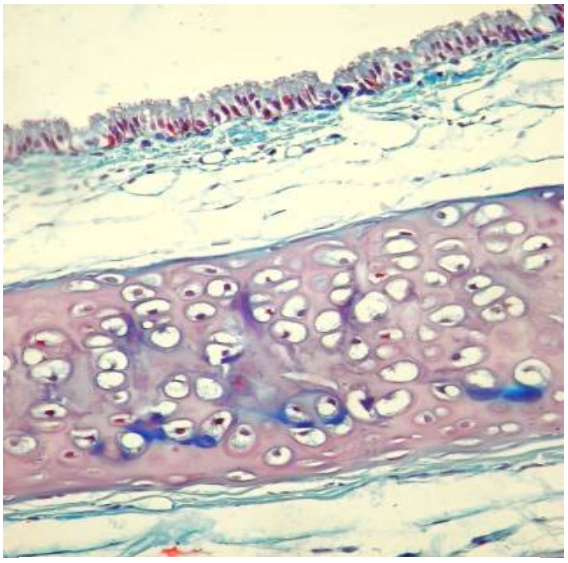

Fig.2 Transversal section trough trachea wall in unsensitized rats (trichromatic coloration; objective x200).

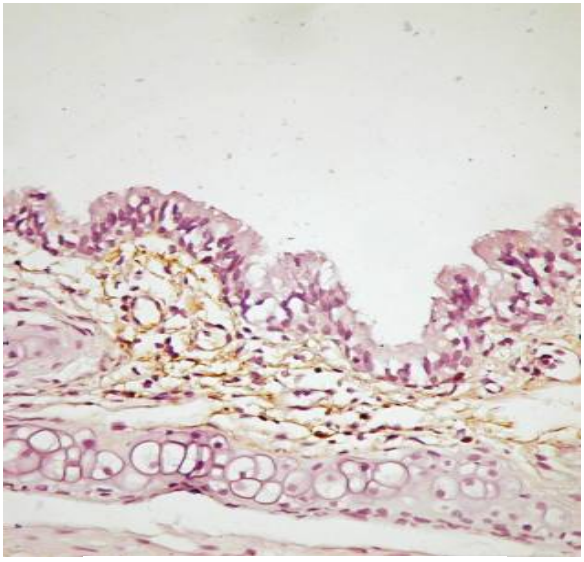

Fig. 3 Trachea in allergized lot (anti LCA antibodies, chromogen DAB; objective x200).

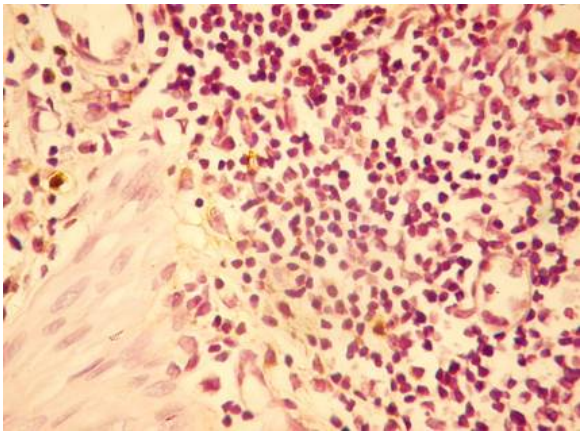

Fig. 4 Lung section in allergized lot; (chromogen DAB, objective x400).

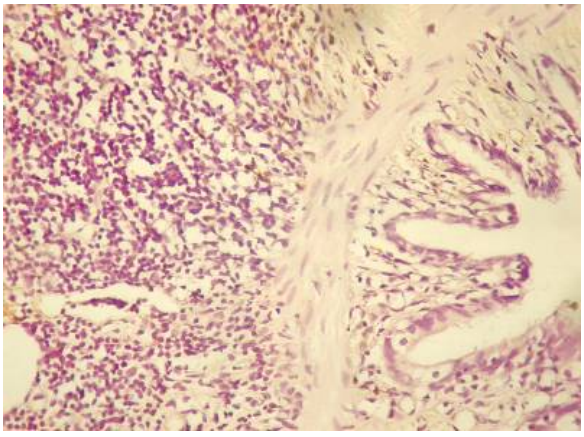

Fig. 5 Bronchiolar wall in allergized lung, (immune marking with S100anti-protein antibodies, chromogen DAB; objective x200).

Conclusions

Chronic inflammatory processes in the respiratory tract of asthmatic patients turned the attention to the mechanisms involved in the induction and maintaining of allergic immune response in these tissues. Distinct DCs subpopulations were identified at all the levels of respiratory tree, including epithelium and airways submucosa, lung interstitium, parenchyma and tissues surrounding blood vessels in the pleura and alveolar surface. Unlike DCs in the skin that display a turnover rate of about three weeks in animal models, respiratory tree DCs turnover rate is more rapid, ranging between three and ten days.

As response to an allergen challenge, airways DCs are immediately recruited from blood myeloid by releasing chemotactic factors like MIP-3α and epithelial β-defensines or MDC, TARC, IL-8 and RANTES. This process involves an essential role of DCs in the allergen-induced immune responses, as DCs are able to induce a pulmonary inflammatory reaction mediated through the activation of T cells and eosinophils that infiltrate the airways and are responsible for the increased production of Th2 IL-4 and IL-5 cytokines found in the bronchial lavage fluid. They are also responsible for an increased local production of IgE in mucosa by the plasmatic cells. During their migration, DCs undergo maturation and increase their stimulatory capacity towards T cells. Later, the antigens processed and presented to T cells activate naïve T cells into effector cells, which is a unique feature of DCs in the immune system. Polarized effector cells leave the lymph nodes and migrate to peripheral inflammatory tissue like airways, where they take part in the allergic inflammatory process.

It can be hypothesized that allergen exposure normally induces the development of cells for T cell tolerance mediated by regulatory T cells. As an important

therapeutic action mean, increased expression of TARC in the airways epithelium of asthmatic patients can be down-regulated by the glycocorticoids treatment. This indicates that effective therapeutic strategies intervene in the initial stages of ongoing allergic cascade, like inflammatory DCs recruitment to airways. Taken together, these discoveries show that respiratory tract DCs represent the engine of allergic-inflammatory immune responses acceleration, as well as of inhibition of these processes.

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