

Pharmacological Modulation of the Antigen-Induced Expression of the Low-Affinity IgE Receptor (FcεRII/CD23) on Rat Alveolar Macrophages

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Abstract. Brown-Norway (BN) rats were sensitized by 3 aerosol exposures to ovalbumin (OA; 10 mg/ml) at days 1, 3 and 14. At day 21, the rats were challenged with the antigen or vehicle by aerosol. Alveolar macrophages (AM) were obtained by bronchoalveolar lavage and the expression of FcεRII/CD23 was assessed by flow cytometry after staining with the BB10 monoclonal antibody. A maximum of 74% of the AM from sensitized and challenged BN rats expressed FcεRII/CD23 24 h after OA exposure, compared to 12% of the cells from rats exposed to vehicle. Sprague-Dawley rats were passively sensitized by intravenous injection of 0.1 or 0.05 ml/kg mouse ascitic fluid containing dinitrophenyl (DNP)-specific monoclonal IgE (2682-1) and after 24 h exposed to an aerosol of 5 mg/ml of DNP-bovine serum albumin for 30 min. In this case also, antigen exposure induced the expression of FcεRII/CD23 on 75% AM, compared to 17% AM from saline-challenged rats. Such an induction of FcεRII/CD23 on AM was, however, not observed when the animals were challenged with either histamine, serotonin or acetylcholine by aerosol. The antigen-induced expression of FcεRII/CD23 on AM was inhibited upon treatment of the rats with ketotifen or beclomethasone. In addition, oral or aerosol administration of respectively BN 50730 or BN 52021 (two structurally unrelated platelet-activating factor antagonists), inhibited the antigen-induced FcεRII/CD23 expression on AM, indicating the participation of this lipid mediator in this process.

Allergic reactions are part of the vast field of immunology, in which many inflammatory mediators have been implicated [1]. It is now well recognized that allergic diseases, and particularly asthma, are associated with marked inflammatory changes in the tissues. The resident inflammatory cell types most characteristic of asthma are mast cells and alveolar macrophages, whereas the role of T lymphocytes still awaits a precise definition. It is now well established that murine mast cells and alveolar macrophages from various species generate various cytokines and inflammatory mediators that may play a role in the development of allergic reactions. Since they govern the immunological processes associated with inflammatory disorders, the production of these mediators and cytokines may play a critical role in the pathology of asthma.

Atopic diseases are usually associated with the production of IgE specific for one or several allergens. Up to now, the role of these immunoglobulins has been linked to their interaction with the high-affinity

receptor (FcεRI) present on basophils and mast cells. Upon interaction with the antigen, these cell types degranulate and release in the microenvironment a plethora of mediators, leading to the development of the immediate symptoms of the allergic reactions.

Since the discovery of the low-affinity receptor for IgE (FcεRII/CD23), another role for this immunoglobulin class can be envisioned. However, since FcεRII/CD23 is not usually spontaneously present on the cell surface, the interaction of IgE, or IgE immune complexes, with this receptor requires the priming of the cells with cytokines, especially interleukin-4 (IL-4). Therefore, IL-4-stimulated cells may become responsive to IgE-dependent mechanisms and this can explain why the *in vivo* role of this receptor has been mostly investigated up to now in pathological situations such as allergic and parasitic diseases and transplantation [2–5].

We have previously proposed that mast cell degranulation induced by the antigen may lead to alterations in lung cell functions [1]. Indeed, the produc-

tion of cytokines by degranulating mast cells may evoke hematopoietic precursor cells to differentiate into eosinophils (via e.g. IL-5, granulocyte/macrophage-colony-stimulating factor), regulation of IgE synthesis (via e.g. IL-4, IL-6) and alteration in macrophage functions (via e.g. IL-4, IL-6, tumor necrosis factor- α). Besides the production of mediators, including platelet-activating factor (PAF), leukotriene B₄ (LTB₄), histamine, proteases and acid hydrolases, whose consequence is the development of the immediate episode of pulmonary obstruction, the release of these cytokines from mast cells may also induce, because they often require protein synthesis for action, late changes in pulmonary and cell functions. Thus, we speculated that the possible release of IL-4 from antigen-stimulated murine mast cells may affect alveolar macrophage functions and evoke the expression of Fc ϵ RII/CD23 on this cell type. The aim of the present review is to summarize the data obtained in our laboratory on the pharmacological modulation of the antigen-induced expression of Fc ϵ RII/CD23 on rat alveolar macrophages.

In vivo Regulation of the Expression of Fc ϵ RII/CD23 in the Rat

We initially investigated the possible modulation of Fc ϵ RII/CD23 expression on alveolar macrophages from sensitized rats, challenged or not with the antigen. Brown-Norway (BN) rats of both sexes were placed twice at a 48-hour interval in a plexiglass chamber (30 $\times$ 50 $\times$ 30 cm) and exposed to aerosols of a 0.9% NaCl solution in water (saline solution) containing 10 mg/ml ovalbumin (OA) for 30 min. A booster administration (OA aerosol, 10 mg/ml for 30 min) was performed under the same condition at day 14. Twenty-one or 28 days after the initial sensitization procedure, the rats were placed again in the plexiglass chamber and exposed to aerosol administration of 4 successive solutions of OA of, respectively, 1, 5, 10 and 50 mg/ml for 15 min each. In a separate set of experiments, it was established that antigen challenge of sensitized animals evokes a bronchoconstriction, although of limited extent (i.e. 30–40%). Bronchoalveolar lavages from rats receiving various treatments were performed at defined time intervals after OA or saline exposure. The expression of Fc ϵ RII/CD23 on alveolar macrophages was determined by indirect immunofluorescence staining using the BB10 mono-

clonal antibody (IgM isotype) [6], and fluorescence was measured with a fluorescence-activated cell sorter.

No expression above the background level of Fc ϵ RII/CD23 on alveolar macrophages from antigen-challenged, nonsensitized rats was observed. In contrast, 62% of the alveolar macrophages expressed Fc ϵ RII/CD23 after antigen stimulation by aerosol, compared to 5.5% following challenge with the saline solution. Kinetics studies demonstrated that the expression the Fc ϵ RII/CD23 started after 6 h, was maximal by 24–48 h and then declined progressively. In contrast, no expression of Fc ϵ RII/CD23 on rat alveolar macrophages was observed upon exposure of sensitized rats to an aerosol of acetylcholine (500 μ g/ml), 5-hydroxytryptamine (60 μ g/ml) or histamine (30 μ g/ml) for 30 min, demonstrating that this phenomenon was not in relation with the protracted bronchoconstriction.

These results indicated that antigen challenge of sensitized animals induced mast cell degranulation and the release of IL-4, which in turn, modulates the expression of Fc ϵ RII/CD23 on the alveolar macrophages. To confirm that mast cells were indeed involved in this process, the antidegranulating agent disodium cromoglycate (DSCG) was used. Sensitized BN rats were exposed or not to a 30-min aerosol of a 10-mg/ml DSCG solution and after 30 min challenged with the antigen by aerosol. After 24 h, the Fc ϵ RII/CD23 expression on rat alveolar macrophages was analyzed by flow cytometry. The marked increase in Fc ϵ RII/CD23 expression on rat alveolar macrophages induced by the antigen was not observed in those animals treated with the drug, indicating that mast cells may indeed play a role in this phenomenon.

Another evidence for the involvement of mast cells in the antigen-induced expression of Fc ϵ RII/CD23 was obtained in experiments implicating passive sensitization of the rats with mouse monoclonal IgE. Antigen challenge of these passively sensitized animals evoked a dose-dependent expression of Fc ϵ RII/CD23 on alveolar macrophages, a process that was also blocked upon treatment of the animals with DSCG.

The pharmacological modulation of the antigen-induced expression of Fc ϵ RII/CD23 on rat alveolar macrophages from sensitized rats by various antiasthma drugs was also investigated. The antigen-induced Fc ϵ RII/CD23 expression on alveolar macro-

phages was significantly reduced by 49 and 28% upon pretreatment of the rats with beclomethasone dipropionate or ketotifen, respectively. Glucocorticosteroids also inhibited in a dose-dependent fashion the IL-4-induced expression of FcεRII/CD23 on U937 cells, suggesting a direct effect of this drug on alveolar macrophages. By contrast, the inhibitory effect of ketotifen, which does not operate *in vitro* on the IL-4-induced FcεRII/CD23 expression on U937 cells, is at this stage unexplained. Since ketotifen has been shown to exhibit PAF-inhibitory activity, the effect of specific antagonists of this phospholipid mediator was analyzed. Treatment of BN rats with two structurally unrelated PAF antagonists, BN 50730 (25 mg/kg *p.o.*) or BN 52021 (10 mg/ml aerosol), markedly inhibited (above 70%) the expression of FcεRII/CD23 on alveolar macrophages (table 1), suggesting the involvement of this phospholipid in this process. This hypothesis was confirmed since aerosol administration of PAF to rats led to an increased expression of FcεRII/CD23 on alveolar macrophages, whereas lyso-PAF was ineffective. As well, *in vitro* incubation of rat alveolar macrophages in the presence of PAF induced the expression of FcεRII/CD23 in a dose-dependent manner and with a maximum after 24 h. Here again, lyso-PAF was ineffective in inducing the expression of FcεRII/CD23 on rat alveolar macrophages *in vitro*. However, this effect on FcεRII/CD23 expression is not restricted to PAF since aerosol administration of LTB₄ also increased it.

Thus, lipid mediators appear to regulate the expression of FcεRII/CD23 on rat alveolar macrophages. In addition, PAF and LTB₄ induce such an expression of FcεRII/CD23 *in vitro*, suggesting a direct effect on rat alveolar macrophages. Whether or not PAF produced during antigen challenge of sensitized rats is only of mast cell origin is also to be determined. As well, the possible contribution of IL-4 on the FcεRII/CD23 expression observed in the *in vivo* experiments is still to be clarified.

Recent studies have suggested that bronchodilators, such as β₂-adrenoceptor agonists (salbutamol), may influence the immunological reactions linked to allergic asthma. In addition, increasing evidences indicate that repeated β₂-adrenergic agonist administration produces various side effects including an increase in the mortality of sensitized guinea pigs after antigen challenge [7]. As postulated above, mast cell mediators could contribute not only to the triggering

Table 1. Effects of BN 52021 or BN 50730 on FcεRII/CD23 expression on alveolar macrophages collected from OA-challenged BN rats

Treatment	Dose	Route	FcεRII/CD23 expression, %
Control			5
OA			76.5
BN 52021	10 mg/ml	aerosol	15
BN 52021 + OA	10 mg/ml	aerosol	13
BN 50730	25 mg/kg	<i>per os</i>	5.5
BN 50730 + OA	25 mg/kg	<i>per os</i>	5

Sensitized BN rats were pretreated by administration of BN 52021 (10 mg/ml, aerosol) or BN 50730 (25 mg/kg, *p.o.*) 1 h before the challenge with OA. FcεRII/CD23 expression on alveolar macrophages was evaluated by flow cytometry.

of the immediate reaction but also to the initiation of the late-phase reaction. However, the late-phase reaction may be influenced by mast-cell-independent mechanisms since aerosolized salbutamol induced spontaneously CD23 on rat alveolar macrophages and potentiates this expression when induced by antigen challenge [8].

Concluding Remarks

The entry of allergen in lung tissue is followed by complex reactions whose consequence is the triggering and/or the perpetuation of a pulmonary inflammatory state. Indeed, the antigen can stimulate several immunocompetent cell types present in the lung and responsible of the allergic reaction such as IgE-producing cells and antigen-primed or activated T cells. In addition, antigen evokes the degranulation of mast cells and the release of various mediators whose consequence is the initiation of the early phase of the allergic reaction. In addition, these mast-cell-derived mediators may induce alterations of the tissue response to further stimulation, this latter process being one of the characteristic feature of the allergic asthma. It is now suspected that IgE-sensitized murine mast cells degranulate on challenge with the specific antigen and release PAF and various mediators, including IL-4 and IL-5, in the microenvironment.

IL-4, as well as PAF, induces within a few hours the expression of the low-affinity receptor for IgE which can then interact with IgE-antigen immune complexes, a phenomenon possibly leading to a second wave of mediator release. These data indicate that mast cells play a central role in the development of the allergic reaction, although by a mechanism very different from that initially suspected.

References

- 1 Mencia-Huerta JM, Dugas B, Braquet P: Immunologic reactions in asthma. *Immunol Allergy Clin North Am* 1990; 10:337-353.
- 2 Spiegelberg HL, O'Connor RD, Simon RA, Mathison DA: Lymphocytes with immunoglobulin E Fc receptors in patients with atopic disorders. *J Clin Invest* 1979;64:714-720.
- 3 Bruynzeel-Koomen C, Van der Donk EMM, Bruynzeel PLB, Capron M, De Gast GC, Mudde GC: Associated expression of CD1 antigen and Fc receptors for IgE on epidermal Langerhans cells from patients with atopic dermatitis. *Clin Exp Immunol* 1988;74:137-142.
- 4 Joseph M, Auriault C, Capron A, Vorng H, Viens P: A new function for platelets: IgE dependent killing of schistosomes. *Nature* 1983;303:810-812.
- 5 Bengtsson M, Gordon J, Flores-Romo L, Cairns JA, Smedmyr B, Öberg G, Simonsson B, Tötterman TH: B-cell reconstitution after autologous bone marrow transplantation: Increase in serum CD23 ('IgE-binding factor') precedes IgE and B-cell regeneration. *Blood* 1989;73:2139-2144.
- 6 Capron M, Jouault T, Prin L, Joseph M, Ameisen JC, Butterworth AE, Papin JC, Kusnier JP, Capron A: Functional study of a monoclonal antibody to IgE Fc receptor (FcεRII) of eosinophils, platelets, and macrophages. *J Exp Med* 1986;164:72-89.
- 7 Sanjar S, Aoki S, Kristersson A, Smith D, Morley J: Antigen challenge induces pulmonary airway eosinophil accumulation and airway hyperreactivity in sensitized guinea pigs: The effect of anti-asthma drugs. *Br J Pharmacol* 1990;99:679-686.
- 8 Braquet P, Dugas B, Paul-Eugène N, Lagente V, Cairns JA, Katria A, Hosford D, Mencia-Huerta JM, Gordon J: Enhancement of the expression and release of the human monocyte low affinity IgE receptor FcεRII/CD23 by β₂-adrenoreceptor agonists. *Clin Exp Allergy* 1991;21(suppl 1):268-271.

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