

EDITORIAL

The role of CD23 in allergic disease

CD23, also known as the low affinity receptor for Immunoglobulin (Ig)E (FcεRII), has been widely implicated in the synthesis of IgE as well as in IgE-mediated immune and inflammatory functions. As a result, CD23 may have a pivotal role in the development and maintenance of allergic inflammation, however, it has been a subject of debate ever since its discovery in 1975. Indeed, the relationship of CD23 expression to disease and the specific functions of CD23-positive cells are still unclear, in particular into the light shed by murine experiments.

In humans, CD23 is a Ca^{2+} -dependent C-type lectin of 45 kDa. It has a wide distribution among haematopoietic and structural cells, and exists under two forms, CD23a and CD23b, resulting from an alternative splicing at the N terminus and differing by five amino acids in the cytoplasmic domain. The two isoforms of CD23 are found on B cells, one is constitutively expressed (CD23a), whereas the other form (CD23b) is induced in particular by IL-4 [1]. CD23b is also found on non-B cells such as T cells, Langerhans cells, monocytes, macrophages, platelets and eosinophils [2,3] and mediates different biological functions. The b form has been shown to be associated with phagocytosis of soluble IgE complexes, while the a form is associated with endocytosis of IgE-coated particles [4]. Structurally, CD23 presents a single membrane-spanning domain followed by an extracellular domain that consists of three regions: the α -helical coiled coil stalk region, which mediates the formation of trimers, followed by the lectin head, which binds IgE, and at the C terminus, a short tail containing an inverse RGD sequence, a common recognition site of integrins [5]. CD23 at the cell surface is turned over to yield a series of soluble fragments. sCD23 of molecular weight 37, 33, 29, 25, and 17 kDa, arise by an autocatalytic process involving a matrix metalloprotease cleavage of membrane-bound CD23. CD23 has been implicated in different functions, ranging from cellular adhesion, antigen presentation, growth and differentiation of B and T cells, rescue from apoptosis, release of cytotoxic mediators, and regulation of IgE synthesis [6].

In vitro experiments have shown that CD23 is upregulated by its ligand IgE, or following stimulation with IL-4 and several other cytokines. IFN γ counteracts the inducing effect of IL-4. IL-4-induced CD23 expression has an enhancing effect on IgE synthesis through CD21 binding [7]. Binding of IgE or IgE-immune complexes to the lectin region transduces a feedback inhibitory signal that prevents further IgE synthesis [8]. In contrast, soluble forms above 25 kDa upregulate IgE production, and their release has

been found to be inhibited by IgE binding [9]. It has been recently shown that the protective effect of IgE on the initial cleavage of CD23, through binding to the lectin domain, results from a stabilization of the integrity of the coiled coil structure which contains the site of cleavage. The unravelling of the coiled coil domain through interaction with the leucine zipper domain of CD23 induces its cleavage [5]. It could be speculated that any autologous or exogenous agent able to interact with the leucine zipper domain of CD23 would disrupt the IgE inhibitory mechanism thereby leading to enhanced IgE synthesis. It is therefore of interest that Der p 1, the major house dust mite allergen has been shown to selectively cleave CD23, and to promote IgE synthesis [10]. CD23b can also, via IgE binding, promote the generation of different mediators such as cytotoxic mediators, chemokines, pro- or anti-inflammatory mediators in alveolar macrophages, platelets, or eosinophils [11–13] leading to an enhancing effect on the inflammatory reaction. Finally sCD23 is able through ligation to CD11b/CD11c to promote release of inflammatory mediators in particular from monocytes such as IL-1 β , IL-6 and TNF- α [14].

In humans, the expression of CD23 is strikingly increased in allergic disorders, in terms of membrane expression on B cells and monocytes, but also in terms of sCD23 production [15]. Moreover reduction of allergen-induced CD23 expression on B cells has been observed after successful hyposensitization [16]. Altogether, the large body of evidence previously mentioned suggests that in humans CD23 plays a central role in IgE regulation with antagonistic effects. On one hand, crosslinking FcεRII at the cell surface by IgE delivers a negative feedback for IgE production and inhibits the release of sCD23. On the other hand, sCD23 fragments larger than 25 kDa that retain a part of the stalk region promote continuing IgE production by at least two mechanisms: (1) sCD23 directly stimulates IgE production possibly through CD21 triggering; (2) sCD23 by its ability to trap IgE in the medium prevents negative feedback through membrane-bound CD23. Dysregulation of the CD23 pathway in atopic patients might be part of their increase propensity to develop IgE antibodies and of an enhancement of the inflammatory reaction through the action of sCD23 as well as through IgE-dependent triggering of CD23⁺ non-B cells.

In rodent models, somehow different *in vivo* results have been obtained questioning the *in vivo* relevance of many of these observations, and in particular the relevance of murine models to man. Most of the studies performed

in mice have implicated CD23 as an inhibitor of this IgE response. It has been shown that CD23-deficient mice exhibit no phenotypic difference, normal T and B cell development, and in some studies an increased production of IgE upon sensitization to antigen, but not at baseline [17,18]. Furthermore, using heterozygous CD23^{+/-} populations of gene-targeted mice, an inverse relationship was found between the expression of CD23 and levels of IgE produced after parasite infection. However some other studies did not find modifications of IgE production in CD23^{-/-} mice [19,20]. These data suggest that the presence of CD23 in the mouse model may exert an inhibitory effect on IgE production and that the membrane bound form of CD23 may be the dominating IgE regulatory factor. Contradictory results between humans and mice have also been obtained at the level of the inflammatory reaction. Indeed it has been shown that in knockout CD23 mice, there is an increased allergic airways hyperresponsiveness following sensitization with ovalbumin [17,20] and that treatment with a transducing anti-CD23 mAb produces attenuation of the allergic pulmonary inflammation [22], again providing clues as CD23 being an inhibitor of the inflammatory reaction in the mouse model.

Although availability of the transgenic and knockout mouse models have opened new insights in the understanding of immunological functions, there are a number of limitations to these models. In particular there is a redundancy in the genome which allows multiple genes to perform overlapping functions, as well as to compensate for the absence of function of the targeted gene. Therefore the enhanced IgE production observed in knockout mice might be explained by other membrane structures able to bind and to mediate functions of IgE including the high affinity FcεRI, or molecules such as Mac2 [23] which could potentially compensate for the lack of CD23. Recent data obtained in our laboratory support this hypothesis. Indeed cross-regulatory effects between CD23 and FcεRI expression have been observed in terms of IgE-dependent platelet activation. Human platelets bear the low as well as the high affinity receptor for IgE, and it has been shown that antibodies to CD23 are able to inhibit the IgE-dependent activation of platelets induced through FcεRI [24]. The mechanism of action could be through the promotion of the autoproteolytic cleavage of the receptor. Indeed, sCD23 mimics the inhibiting effects of anti-CD23 antibodies.

On the other hand, the impaired ability to synthesize IgE upon stimulation in CD23-transgenic mice as well as with monoclonal activating antibodies, suggests that ligation of CD23 plays an inhibitory role on IgE production again in the mouse model [22,25,26].

The clinical relevance of the mice observations is difficult to extrapolate to humans. Indeed there are marked differences in CD23 immunobiology between the two

species. First at the molecular level, there is only 57% amino acid sequence homology between human and mouse CD23. Furthermore the murine CD23 is naturally truncated at its carboxy terminus, deleting the RGD motif [2]. Secondly, in humans, most of the regulatory effect of sCD23 is ascribed to its IgE-binding capacity, whereas in mice sCD23 does not retain IgE-binding [9] or binds with a much lower affinity [27]. Most of the effects ascribed to sCD23 in humans could not be confirmed in the CD23-deficient mice. *In vivo* experiments using sCD23-transgenic mice showed no particular dysregulation of IgE-induced production [25]. The relative importance of membrane CD23 vs the cytokine-like activities of sCD23 remains unclear. Thirdly, the discrepancy of CD23 function in mouse and human in the context of the inflammatory reaction, might be related to differences in the cellular distribution between species. In the mouse the existence of isoforms of CD23 is controversial [28,29] and CD23 has been found predominantly expressed on mature B cells, follicular dendritic cells, and some T cells, whereas in humans IgE-dependent activation of CD23⁺ non-B cells seems to increase the inflammatory part of the allergic reaction. In this context it is of note that the rat model shows similarities with human findings. For example, *in vivo* anti-CD23 antibody treatment of rats was shown to inhibit specific IgE responses [30], supporting the role for CD23 as an enhancer of the IgE response. Rat macrophages, eosinophils and platelets have been shown to bear receptors for IgE [31], while Langerhans cells and eosinophils in mice do not express CD23 either at baseline or after IL-4 stimulation [32,33]. Therefore rat models might be more relevant to humans than mouse models.

Whether some of the effects ascribed to CD23 are specific for the human situation remains to be resolved. For obvious ethical problems, *in vivo* experiments are difficult to perform in humans, and models more relevant to humans are needed. In this context, the paper published in this issue of the journal by Mayer and colleagues is of interest [34]. They show that compounds selectively able to inhibit the proteolytic cleavage of CD23 are able to inhibit sCD23 release and IgE synthesis not only in an *in vitro* model but also in an *in vivo* model. They used a SCID mouse model, which exhibits a mutation in the VDJ recombinase system, and thereby does not have functional T and B lymphocytes. These mice were reconstituted with IL-4-stimulated PBL from atopic donors and treated with inhibitors of CD23 processing parallel to anti-CD23 antibodies. They were able to show that these reagents inhibited IgE production without inhibiting IgG production providing evidence that targeted inhibition of sCD23 production is sufficient for inhibition of IgE synthesis. Indeed more than 90% inhibition was observed at the peak of IgE production in the Hu-PBL-SCID model with both inhibitors of processing

and anti-CD23 antibodies. Although Hu-SCID mice constitute a chimeric model with its own limits, they represent a strong advance in the *in vivo* evaluation of human pathophysiological mechanisms, and might allow a pre-evaluation step of therapeutics before clinical trials in man. Therefore the availability of inhibitors of CD23 processing might be of potential therapeutical interest to modulate IgE synthesis in allergic patients. However it is still not clear if the targeting of IgE will have any influence on the allergen-mediated T-cell effects of the allergic inflammatory reaction. In the paper by Mayer *et al*, the effects of CD23 processing inhibitors were only evaluated on the IL-4-induced IgE response. It would be of interest to evaluate such compounds in the context of an *in vivo* allergen-driven Th-2 reaction. Again the use of SCID mice should help to answer these questions, since a model of human allergen-induced pulmonary inflammation has been described in these mice [35]. In the case of other clinical trials focused on IgE, for example using anti-IgE antibodies, it has been shown to have good efficacy although not total on asthmatic symptoms [36] showing that IgE-independent mechanisms and probably direct T-cell activation play an important role in the pathophysiology of the allergic reaction.

It is therefore difficult, at this point to conclude on the potential usefulness of inhibitors of CD23 processing on a fully developed allergic reaction. It appears however, that IgE-binding structures are essential in the definition of guidelines for regulating allergic disorders. As far as CD23 is concerned, and as underlined above, clinical improvements in allergic patients were associated with the decrease of sCD23 in biological fluids. Investigations on the precise functions of CD23 are therefore of outstanding importance for the development of effective means of controlling allergic diseases.

References

- Yokota A, Kikutani H, Tanaka T, Sato R, Barsumian EL, Suemura M, Kishimoto T. Two species of human Fc epsilon receptor II (FcεRII): tissue-specific and IL-4-specific regulation of gene expression. *Cell*, 1988; 55:611–8.
- Delespesse G, Suter U, Mossalayi D, Bettler B, Sarfati M, Hofstetter H, Kilcherr E, Debre P, Dalloul A. Expression, structure, and function of the CD23 antigen. *Adv Immunol*, 1991; 49:149–91.
- Soussi Gounni ABL, Morita M, Aldebert D, Sarfati M, Capron A, Capron M. Molecular characterization of the low-affinity IgE receptor Fc epsilonRII/CD23 expressed by human eosinophils. *Int Immunol*, 1998; 10:395–404.
- Yokota A, Yukawa K, Yamamoto A, Sugiyama K, Suemura M, Tashiro Y, Kishimoto T, Kikutani H. Two forms of the low affinity Fc receptor for IgE differentially mediate endocytosis and phagocytosis: identification of the critical cytoplasmic domains. *Proc Natl Acad Sci*, 1992; 89:5030–4.
- Munoz O, Brignone C, Grenier-Brossette N, Bonnefoy JY, Cousin JL. Binding of anti-CD23 monoclonal antibody to the leucine zipper motif of Fc epsilonRII/CD23 on B cell membrane promotes its proteolytic cleavage. Evidence for an effect on the oligomer/monomer equilibrium. *J Biol Chem* 1795–80; 1998 (273):3., 0.
- Bonnefoy JY, Lecoanet-Henchez S, Gauchat JF, Graber P, Aubry JP, Jeannin P, Plater-Zyberk C. Structure and functions of CD23. *Int Rev Immunol*, 1997; 16:113–28.
- Aubry JP, Pochon S, Graber P, Jansen KU, Bonnefoy JY. CD21 is a ligand for CD23 and regulates IgE production. *Nature*, 1992; 358:505–7.
- Sherr E, Macy E, Kimata H, Gilly M, Saxon A. Binding the low affinity Fc epsilon R on B cells suppresses ongoing human IgE synthesis. *J Immunol*, 1989; 142:481–9.
- Lee WT, Rao M, Conrad DH. The murine lymphocyte receptor for IgE. IV. The mechanism of ligand-specific receptor upregulation on B cells. *J Immunol*, 1987; 139:1191–8.
- Shakib F, Schulz O, Sewell H. A mite subversive: cleavage of CD23 and CD25 by Der, p. 1 enhances allergenicity. *Immunol Today*, 1998; 19:313–6.
- Gosset P, Tillie-Leblond I, Oudin S, Parmentier O, Wallaert B, Joseph M, Tonnel AB. Production of chemokines and pro-inflammatory and anti-inflammatory cytokines by human alveolar macrophages activated by IgE receptors. *J Allergy Clin Immunol*, 1999; 103:289–97.
- Klouche M, Klinger MH, Kuhnel W, Wilhelm D. Endocytosis, storage, and release of IgE by human platelets: differences in patients with type I allergy and nonatopic subjects. *J Allergy Clin Immunol*, 1997; 100:235–41.
- Capron M, Truong MJ, Aldebert D, Gruart V, Suemura M, Delespesse G, Tourvieille B, Capron A. Heterogeneous expression of CD23 epitopes by eosinophils from patients. Relationships with IgE-mediated functions. *Eur J Immunol*, 1991; 21:2423–9.
- Lecoanet-Henchoz S, Gauchat JF, Aubry JP, Graber P, Life P, Paul-Eugene N, Ferrua B, Corbi AL, Dugas B, Plater-Zyberk C et al. CD23 regulates monocyte activation through a novel interaction with the adhesion molecules CD11b-CD18 and CD11c-CD18. *Immunity*, 1995; 3:119–25.
- Gagro A, Rabatic S. Allergen-induced CD23 on CD4+ T lymphocytes and CD21 on B lymphocytes in patients with allergic asthma: evidence and regulation. *Eur J Immunol*, 1994; 24:1109–14.
- Jung CM, Prinz JC, Rieber EP, Ring J. A reduction in allergen-induced Fc epsilon R2/CD23 expression on peripheral B cells correlates with successful hyposensitization in grass pollinosis. *J Allergy Clin Immunol*, 1995; 95:77–87.
- Haczku A, Takeda K, Hamelmann E, Oshiba A, Loader J, Joetham A, Irvin C, Kikutani H, Gelfand EW. CD23 deficient mice develop allergic airway hyperresponsiveness following sensitization with ovalbumin. *Am J Respir Crit Care Med*, 1997; 156:1945–55.
- Yu P, Kosco-Vilbois M, Richards M, Köhler G, Lamers MC. Negative feedback of IgE synthesis by murine CD23. *Nature*, 1994; 369:753–6.
- Stief A, Texido G, Sansig G, Eibel H, Le Gros G,

- Van der Putten H. Mice deficient in CD23 reveal its modulatory role in IgE production but no role in T and B cell development. *J Immunol*, 1994; 152:3378–90.
- 20 Fujiwara H, Kikutani H, Suetmasu T, Naka T, Yoshida K, Yoshida K, Tanaka T, Suemura M, Matsumoto N, Kojima S, Kishimoto T, Yoshida N. The absence of IgE antibody-mediated augmentation of immune responses in CD23-deficient mice. *Proc Natl Acad Sci*, 1994; 91:6835–9.
 - 21 Dasic G, Juillard P, Graber P, Herren S, Angell T, Knowles R, Bonnefoy JY, Kosco-Vilbois MH, Chvatchko Y. Critical role of CD23 in allergen-induced bronchoconstriction in a murine model of allergic asthma. *Eur J Immunol*, 1999; 29:2957–67.
 - 22 Cernadas M, De Sanctis GT, Krinzman SJ, Mark DA, Donovan CE, Listman JA, Kobzik L, Kikutani H, Christiani DC, Perkins DL, Finn PW. CD23 and allergic pulmonary inflammation: potential role as an inhibitor. *Am J Respir Cell Mol Biol*, 1999; 20:1–8.
 - 23 Truong MJ, Gruart V, Liu FT, Prin L, Capron A, Capron M. IgE-binding molecules (Mac-2/epsilonBP) expressed by human eosinophils. Implication in IgE-dependent eosinophil cytotoxicity. *Eur J Immunol*, 1993; 23:3230–5.
 - 24 Joseph M, Gounni AS, Kusnierz JP, Vorng H, Sarfati M, Kinet JP, Tonnel AB, Capron A, Capron M. Expression and functions of the high-affinity IgE receptor on human platelets and megakaryocyte precursors. *Eur J Immunol*, 1997; 27:2212–8.
 - 25 Texido G, Eibel H, Le Gros G, Van der Putten H. Transgene CD23 expression on lymphoid cells modulates IgE and IgG1 responses. *J Immunol*, 1994; 153:3028–42.
 - 26 Payet ME, Woodward EC, Conrad DH. Humoral response suppression observed with CD23 transgenics. *J Immunol*, 1999; 163:217–23.
 - 27 Bartlett WC, Kelly AE, Johnson CM, Conrad DH. Analysis of murine soluble Fc epsilon RII sites of cleavage and requirements for dual-affinity interaction with IgE. *J Immunol*, 1995; 154:4240–6.
 - 28 Richards ML, Katz DH, Liu FT. Complete genomic sequence of the murine low affinity Fc receptor for IgE. Demonstration of alternative transcripts and conserved sequence elements. *J Immunol*, 1991; 147:1067–74.
 - 29 Conrad DH, Kozak CA, Vernachio J, Squire CM, Rao M, Eicher EM. Chromosomal location and isoform analysis of mouse Fc epsilon RII/CD23. *Mol Immunol*, 1993; 30:27–33.
 - 30 Flores-Romo L, Shields J, Humbert Y, Graber P, Aubry JP, Gauchat JF, Ayala G, Allet B, Chavez M, Bazin H, Capron M, Bonnefoy JY. Inhibition of an in vivo antigen-specific IgE response by antibodies to CD23. *Science*, 1993; 261:1038–41.
 - 31 Joseph M, Tsicopoulos A, Tonnel AB, Capron A. Modulation by nedocromil sodium of immunological and non immunological activation of monocytes, macrophages and platelets. *J Allergy Clin Immunol*, 1993; 92:165–70.
 - 32 Hertl M, Asada H, Katz SI. Murine epidermal Langerhans cells do not express the low-affinity receptor for IgE, Fc epsilon RII (CD23). *J Invest Dermatol*, 1996; 106:221–4.
 - 33 De Andres B, Rakasz E, Hagen M, McCormik ML, Mueller AL, Elliot D, Metwali A, Sandor M, Britigan BE, Weinstock JV, Lynch RG. Lack of Fc epsilon receptors on murine eosinophils: implication for the significance of elevated IgE and eosinophils in parasitic infections. *Blood*, 1997; 89:3829–36.
 - 34 Mayer R, Bolognese BJ, Al-Mahdi N, Cook RM, Flamberg PL, Hansbury MJ, Khandekar S, Appelbaum E, Faller A, Marshall LA. Inhibition of CD23 processing correlates with inhibition of IL-4 stimulated IgE production in human PBL and hu-PBL-reconstituted SCID mice. *Clin Exp Allergy*, 1999; 30:719–727.
 - 35 Duez C, Tsicopoulos A, Janin A, Tillie-Leblond I, Thyphronitis G, Marquillies P, Hamid Q, Wallaert B, Tonnel AB, Pestel J. An in vivo model of allergic inflammation: Pulmonary human cell infiltrate in allergen-challenged allergic Hu-SCID mice. *Eur J Immunol*, 1996; 26:1088–93.
 - 36 Fahy JV, Fleming HE, Wong HH, Liu JT, Su JQ, Reimann J, Fick RB, Boushey HA. The effect of an anti-IgE monoclonal antibody on the early- and late-phase responses to allergen inhalation in asthmatic subjects. *Am J Respir Crit Care Med*, 1997; 155:1828–34.

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