

EDITORIAL

Clinical and Experimental Allergy

Facilitated antigen presentation: circumstantial evidence?

This editorial discusses the findings of the paper in this issue by Würtzen et al. [8].

L. K. Poulsen

Laboratory of Medical Allergology, Allergy Clinic, Department 7551, National University Hospital, Copenhagen O, Denmark

The Argentine essayist, writer, and philosopher, Jorge Luis Borges, who, along with Joyce, Proust, and Kafka, is renowned for NOT having received the Nobel Prize (in literature that is), wrote about three ways of postulating reality [1]. The first consists of just mentioning the important facts, while the second imagines a more complex reality than explained to the reader, who is rather told of derivations and consequences. Finally, the third and most convincing method invents the circumstances by which the reader is taken to firmly believe and accept the underlying narration. Borges points to authors like H. G. Wells (*The War of the Worlds*, *Time Machine*, etc.) and Daniel Defoe (*Robinson Crusoe*) as prestigious masters of the latter technique. In experimental medicine, data rule, but from time to time, suspicion may arise that even firm and conclusive results from well-designed *in vitro* experiments may not sufficiently prove the full story of the physiological processes they are assumed to support.

Specific antibodies may enhance an immune response up to 1000 times but may also reduce it to <1% [2], and this phenomenon has been given a variety of names including antigen focusing, antibody feedback regulation, or facilitated antigen presentation (FAP). In particular, it was demonstrated – first in mice (reviewed in [2]) and later in atopic patients [3] – that IgE may enhance antigen presentation up to 100-fold by binding to the so-called low-affinity IgE receptor, CD23, on B cells, which will then present the antigen irrespective of the cell's own specificity. Although the high-affinity IgE receptor, FcεR1, may also be involved in antigen presentation, i.e. on Langerhans and dendritic cells, the involvement of CD23 on B cells was clearly established [2].

While the CD23 molecule has been known for 20+ years, its role in immune regulation is still far from fully

elucidated. The molecule exists both in a free and a cell-bound form, the latter as two splice variants, varying only in their intracytoplasmic domain. A number of endogenous proteases may cleave the molecule from the cell surface and various proteolytic fragments have been ascribed biological effects [4]. A recent review [5] summarizes our current knowledge: surface-CD23 may occur in trimers, each of which will bind to one of the two Cε3-domains of IgE, leaving the other free to bind other CD23 molecules, thereby allowing for the possibility of forming macromolecular networks in the cell membrane. CD23 does not only bind IgE; however, another part of the molecule binds to CD21 (complement receptor 2) and even binding to MHC class II molecules and several of the integrins have been reported [5]. This may further enhance the complex formation in the cell membrane as well as engage CD23 in cell-to-cell interactions. It is beyond the scope of this discussion to present all the biological activities that have been ascribed to CD23. It suffices to say that besides the potentiation of T cell stimulation by FAP, both positive and negative effects on immunoglobulin synthesis (of IgE and other isotypes) and involvement of CD23 in gastrointestinal antigen uptake have been reported (reviewed in [4] and [5]).

If FAP is important for the allergic disease process, it may be of considerable therapeutic interest to exploit this phenomenon and three paths have been followed:

1. It has been demonstrated that the 'blocking antibodies' (predominantly IgG1 and IgG4) formed during allergen-specific immunotherapy may block FAP [6]. The original methodology was cumbersome but because the FAP was CD23-dependent, it was suggested that by using the formation of CD23–IgE–allergen complexes as a simplified read-out, the capacity and convenience of the technique could be enhanced [7]. An article in this issue of *Clinical and Experimental Allergy* [8] elegantly compares two different versions of the FAP assay with a double immunochemical assay for IgE-blocking activity and with the basophil histamine release test, the latter of which was

Correspondence:

Lars K. Poulsen, Laboratory of Medical Allergology, Allergy Clinic, Department 7551, National University Hospital, Blegdamsvej 9, DK-2100 Copenhagen O, Denmark. E-mail: lkpallgy@mail.dk

originally used in the first *in vitro* characterization of blocking antibodies as predominantly IgG [9]. The authors investigate sera from a clinical trial of allergen-specific immunotherapy and find a close correlation between the different readouts including the previously conducted biological FAP assay, where T cell activation was used as a read-out [10]. This would suggest that within the immunotherapy field, it might be sufficient to use a pure immunochemical method, avoiding the cellular work involving flow cytometry. However, as the authors mention in their discussion, there is unfortunately no predictive value of any of the tests for the individual treated patient, and we may still not know exactly what to look for, when measuring blocking antibodies.

2. Therapeutic humanized antibodies to IgE must have specificity to the epitope involved in binding to the subunit of the high-affinity IgE receptor, FcεR1, but at least one such antibody [11] also seems to block the interaction between IgE and CD23, when added to FAP assays [12]. A highly interesting study has been published recently where FAP (or rather its surrogate, the formation of CD23–IgE–allergen complexes) were compared in patients receiving anti-IgE treatment (Omalizumab) and specific immunotherapy in a double-dummy, placebo-controlled design with four arms [13]. While sera from patients treated only with specific immunotherapy induced a significant inhibition in the surrogate FAP assays, this was considerably surpassed by sera from both groups that had received anti-IgE. A smaller additive effect was detectable and, surprisingly, the inhibition of the FAP assay was discernible up to 30 weeks after cessation of the treatment [13], suggesting a strong reduction of anti-IgE treatment on FAP.

3. Direct therapeutic targeting of CD23 may be obtained by humanized antibodies that bind to the receptor and block IgE binding [4]. Such an antibody, named idec-152 or Lumiliximab, has been tested in a single-dose phase 1 trial and, interestingly, total IgE levels in the highest dosages were reduced by up to 30% for up to 85 days [14]. Similarly, the number of B cells reduced (but still within normal ranges) in the higher dosages. The antibody has been tested *in vitro* and shows the suppression of T-cell stimulation, but whereas the suppression seems less than that of anti-CD86 [15], no studies have been reported allowing a direct comparison with anti-IgE or blocking antibodies induced by allergen-specific immunotherapy. Later studies have, however, suggested that all CD23-expressing B cells are killed by the antibody [16], and it seems that the present indication for this antibody may be chronic lymphatic leukemia for CD23-expressing tumour cells.

Thus, treatment modalities exist that are likely to inhibit FAP strongly when such an activity is measured by the available *in vitro* assays. Nevertheless, there is still limited evidence to suggest that inhibiting FAP would lead

to reduced pathology or symptom reduction. As for all three types of treatment, there are several other pathways that could explain the observed clinical effects of the treatment, such as formation of regulatory T cells in immunotherapy, removal of IgE from effector cells in anti-IgE-treatment, and a general B-cell killing in anti-CD23 treatment. Moreover, if FAP amplifies an immune response, it could potentially amplify both the allergic/Th2 arm and presumably counter-acting responses such as Th1/Treg/IgG-blocking antibodies. It may seem surprising that IgE, which normally constitutes a minor part of the immune system, can have such a deep impact on many other types of immune response, but perhaps it is significant – as remarked by Gould and Sutton [5] – that the CD23–CD21 interaction may have been the primary one during evolution.

In spite of species differences, support for the clinical relevance of FAP in humans could be provided by animal experiments, where it is possible to control individual factors, such as specific IgE and antigen that can be administered separately. It is puzzling that when a transgenic mouse with up-regulated B-cell CD23 was compared with a wild-type animal, FAP could indeed be demonstrated *in vitro*, but when IgE+, the relevant antigen, was used for immunization, the wild-type animals with lower CD23 produced a *higher* antibody response [17]. In both CD23-transgenic and wild-type animals, specific IgE increased the number of immunization-induced antigen-specific T cells, but to the same extent. The authors concluded that whereas CD23 is likely to enhance antigen presentation, it has negative regulatory properties, which, at the same time, may reduce or balance the final immune response [17].

FAP is a biological process that can be demonstrated *in vitro* and in animal models. Promising methods are now being developed to determine FAP and its inhibition by anti-allergic treatments, and this will allow new important studies to be conducted. Because of the pleiotropic nature of the central molecule, CD23, and the fact that different parts of the immune system may be enhanced by FAP, it still seems premature to reason for a causal relationship between FAP and clinical efficacy.

References

- 1 Borges JL. The postulation of reality. In: Weinberger E, ed. *Jorge Luis Borges: selected non-fiction*. New York: Penguin Books, 1999; 59–64.
- 2 Hjelm F, Carlsson F, Getahun A, Heyman B. Antibody-mediated regulation of the immune response. *Scand J Immunol* 2006; **64**:177–84.
- 3 van der Heijden FL, van Neerven RJ, Kapsenberg ML. Relationship between facilitated allergen presentation and the presence of allergen-specific IgE in serum of atopic patients. *Clin Exp Immunol* 1995; **99**:289–93.

- 4 Rosenwasser LJ, Meng J. Anti-CD23. *Clin Rev Allergy Immunol* 2005; 29:61–72.
- 5 Gould HJ, Sutton BJ. IgE in allergy and asthma today. *Nat Rev Immunol* 2008; 8:205–17.
- 6 van Neerven RJ, Wikborg T, Lund G *et al.* Blocking antibodies induced by specific allergy vaccination prevent the activation of CD4+ T cells by inhibiting serum-IgE-facilitated allergen presentation. *J Immunol* 1999; 163:2944–52.
- 7 Shamji MH, Wilcock LK, Wachholz PA *et al.* The IgE-facilitated allergen binding (FAB) assay: validation of a novel flow-cytometric based method for the detection of inhibitory antibody responses. *J Immunol Methods* 2006; 317:71–9.
- 8 Würtzen PA, Lund G, Lund K, Arvidsson M, Rak S, Ipsen H. A double blind-placebo controlled birch allergy vaccination study II: correlation between inhibition of IgE binding, histamine release and facilitated allergen presentation. *Clin Exp Allergy* 2008, in press.
- 9 Lichtenstein LM, Holtzman NA, Burnett LS. A quantitative in vitro study of the chromatographic distribution and immunoglobulin characteristics of human blocking antibody. *J Immunol* 1968; 101:317–24.
- 10 van Neerven RJ, Arvidsson M, Ipsen H, Sparholt SH, Rak S, Würtzen PA. A double-blind, placebo-controlled birch allergy vaccination study: inhibition of CD23-mediated serum-immunoglobulin E-facilitated allergen presentation. *Clin Exp Allergy* 2004; 34:420–8.
- 11 Chang TW. The pharmacological basis of anti-IgE therapy. *Nat Biotechnol* 2000; 18:157–62.
- 12 van Neerven RJ, van Roomen CP, Thomas WR, de BM, Knol EF, Davis FM. Humanized anti-IgE mAb Hu-901 prevents the activation of allergen-specific T cells. *Int Arch Allergy Immunol* 2001; 124:400–2.
- 13 Klunker S, Saggar LR, Seyfert-Margolis V *et al.* Combination treatment with omalizumab and rush immunotherapy for ragweed-induced allergic rhinitis: inhibition of IgE-facilitated allergen binding. *J Allergy Clin Immunol* 2007; 120:688–95.
- 14 Rosenwasser LJ, Busse WW, Lizambri RG, Olejnik TA, Totoritis MC. Allergic asthma and an anti-CD23 mAb (IDEC-152): results of a phase I, single-dose, dose-escalating clinical trial. *J Allergy Clin Immunol* 2003; 112:563–70.
- 15 Poole JA, Meng J, Reff M, Spellman MC, Rosenwasser LJ. Anti-CD23 monoclonal antibody, lumiliximab, inhibited allergen-induced responses in antigen-presenting cells and T cells from atopic subjects. *J Allergy Clin Immunol* 2005; 116:780–8.
- 16 Pathan NI, Chu P, Hariharan K, Cheney C, Molina A, Byrd J. Mediation of apoptosis by and antitumor activity of lumiliximab in chronic lymphocytic leukemia cells and CD23+ lymphoma cell lines. *Blood* 2008; 111:1594–602.
- 17 Carlsson F, Hjelm F, Conrad DH, Heyman B. IgE enhances specific antibody and T-cell responses in mice overexpressing CD23. *Scand J Immunol* 2007; 66:261–70.