

EDITORIAL

Clinical and
Experimental
Allergy

The need for a biological diagnosis of penicillin allergy

This editorial discusses the findings of the paper in this issue by Abuaf et al. [22]

V. Kvedariene^{*,†} and P. Demoly^{*}

^{*}Exploration des Allergies – Inserm U657, Hôpital Arnaud de Villeneuve, University Hospital of Montpellier, Montpellier, Cedex, France and [†]Clinic of Chest diseases, Allergology and Radiology, Vilnius University, Vilnius Lithuania ou bien Vilnius university, Vilnius, Lithuania

According to the Nomenclature Review Committee of the World Allergy Organization, drug allergies refer to drug hypersensitivity reactions where a definite immunological mechanism [1] can be demonstrated. The prevalence of self-reported penicillin allergy is high; indeed, a cross-sectional survey in Portugal found a 4.5% adult population self-reported penicillin allergy [2]. The prevalence rate of true penicillin allergy is, however, much lower [3, 4], and therefore a firm diagnosis is needed [5]. The clinical picture of drug allergy is very heterogeneous, encompassing distinct diseases such as morbilliform or bullous exanthema, urticaria and angiooedema, pustular exanthemas, fixed drug eruptions, anaphylaxis, blood cell dyscrasia, fever, interstitial lung disease, hepatitis, nephritis, vasculitis, DRESS (drug reaction with eosinophilia and systemic symptoms) and various forms of autoimmune diseases. However, it is important to distinguish between immediate and non-immediate reactions. The former occur within the first hour after the last drug administration and are manifested clinically by urticaria, angiooedema, rhinitis, bronchospasm or anaphylactic shock.

The diagnosis of drug allergy often relies on clinical histories, skin tests and a few validated *in vitro* tests, such as serum-specific IgE assays, which are available only for a few drugs. The sensitivity of these tests is not 100% and, in selected cases, provocation tests are therefore necessary. However, new diagnostic tools, such as the basophil activation test (BAT), have been developed and are still under validation. Their routine use could increase the sensitivity of diagnostic work-ups, thus reducing the need for drug provocation tests and filling the gap between the high number of non-allergic drug allergy suspicions and

the lack of specialized centres in all countries, which would enable confirmation or elimination of the diagnosis. There is evidence now that the BAT can contribute to the diagnosis of anaphylactic reactions due to several drugs, particularly muscle relaxants and β -lactams [6, 7]. At present, the most commonly used antigens in BATs are CD63 and CD203c.

CD63, the gp53 or lysosome-associated membrane protein (LAMP)-3, is one of the most reported markers for flow cytometric determination of basophil activation. CD63 is deeply anchored into the basophilic intracytoplasmic granules and is generally weakly expressed on the surface of basophils, monocytes, macrophages and platelets. In cases of allergic inflammation, as a result of fusion between the granule and the plasma membrane, CD63 is expressed with a high density on activated basophils and mirrors histamine release [8]. This up-regulated expression of basophilic CD63 can be detected by multi-colour flow cytometry using specific monoclonal antibodies [9]. As far as β -lactams are concerned, the CD63-based BAT was first evaluated in two studies [7, 10] including, respectively, 58 and 70 patients with immediate reactions to these antibiotics. BAT sensitivity was about 50% and specificity was over 90%. However, unlike the study by Sanz et al. [7], the one by Torres et al. [10] assessed not only patients with positive histories and positive skin tests and/or CAP-FEIA but also patients negative to both these tests and positive to challenges. It is interesting to note that one of the seven patients of the latter group was BAT positive. This group of patients with true allergy and negative skin tests is of crucial importance. They account for up to 33% of β -lactam-allergic patients and are only diagnosed by potentially harmful and cumbersome oral provocation tests [11].

Another more basophil-specific marker, CD203c (neural cell surface differentiation antigen E-NPP3, ecto-nucleotide pyrophosphatase/phosphodiesterase 3), is a multifunctional ectoenzyme exclusively and constitutively expressed on the surface of basophils [12]. Five years

Correspondence:

Prof. Pascal Demoly, Allergy Department, Hôpital Arnaud de Villeneuve, University Hospital of Montpellier and Inserm U657, 34295 Montpellier, Cedex 5, France.

E-mail: pascal.demoly@inserm.fr

Table 1. Comparison between CD63 and CD203c markers of basophil activation

Allergen	Number of patients	Cut-off value	CD63 expression Se/Sp (%)	CD203c expression Se/Sp (%)
Latex (13)	27	Two dilutions with > 10% positivity	50/100	75/100
Neuromuscular blocking agents (19)	21	Two dilutions with > 10% positivity	79/100	36/100
Insect venom (20)	43	Related to allergen concentration and ROC analysis (18.2–21.5%)	89/100	97/89
Cat (21)	20	Mean of control group + 2SD: 8.1% for CD63 and 1.38 SI for CD203c	100/100	95/95
Amoxicillin (22)	27	Two dilutions above the mean of control tube + 2SD	22/79	52/100

SD, standard deviation; Se, sensitivity; SI, stimulation index; Sp, specificity.

ago, the claim of an improvement of BAT sensitivity with CD203c by Boumiza et al. [13] was immediately challenged [14, 15]. The CD63 and CD203c indeed have some differences. The expression of CD63 is closely related to the phenomenon of basophil degranulation [8], while CD203c is present on all basophils and further expressed with different kinetics and partly different enzymatic regulations [16, 17]. It also seems to be more easily activated in a non-specific manner [18]. Direct comparisons between these two markers of basophil activation have been performed (Table 1) in latex [13], muscle relaxant [19], insect venom [20] and cat allergies [21]. In the paper published in this issue of the journal, Abuaf et al. [22] provide another comparison in amoxicillin allergy. Among 27 amoxicillin-positive skin test patients, four out of 20 with anaphylaxis and two out of seven with urticaria/angioedema had positive amoxicillin CD63-based BAT. The CD203c-based BAT was positive in 12 and two of these patients, respectively. In the discussion section, the authors raise appropriate technical/physiological issues, including incubation time and different activation pathways of the two markers. More issues have also been highlighted recently [18]. General recommendations regarding preference for one of the tests cannot be given at this stage because of the contradictory results, and other head-to-head comparisons are needed. Although it is still worth saying that the sensitivity of BAT is below that of skin testing for penicillin allergy, these tests are becoming an important addition to our allergy diagnostic tools. The number of European groups possessing the technology is low, but experienced researchers and clinicians are currently under a thorough process of standardisation and validation [18].

References

- Johansson SG, Bieber T, Dahl R *et al.* Revised nomenclature for allergy for global use: report of the Nomenclature Review Committee of the World Allergy Organization, October 2003. *J Allergy Clin Immunol* 2004; 113:832–6.
- Gomes E, Cardoso MF, Praca F, Gomes L, Marino E, Demoly P. Self-reported drug allergy in a general adult Portuguese population. *Clin Exp Allergy* 2004; 34:1597–601.
- Rebello Gomes E, Fonseca J, Araujo L, Demoly P. Drug allergy claims in children: from self-reporting to confirmed diagnosis. *Clin Exp Allergy* 2008; 38:191–8.
- Messaad D, Sahla H, Benahmed S *et al.* Drug provocation tests in patients with a history suggesting an immediate drug hypersensitivity reaction. *Ann Intern Med* 2004; 140:1001–6.
- Romano A, Demoly P. Recent advances in the diagnosis of drug allergy. *Curr Opin Allergy Clin Immunol* 2007; 7:299–303.
- Kvedariene V, Kamey S, Ryckwaert Y *et al.* Diagnosis of neuromuscular blocking agent hypersensitivity reactions using cytofluorimetric analysis of basophils. *Allergy* 2006; 61:311–5.
- Sanz ML, Gamboa PM, Antépara I *et al.* Flow cytometric basophil activation test by detection of CD63 expression in patients with immediate-type reactions to betalactam antibiotics. *Clin Exp Allergy* 2002; 32:277–86.
- Knol EF, Mul FP, Jansen H, Calafat J, Roos D. Monitoring human basophil activation via CD63 monoclonal antibody 435. *J Allergy Clin Immunol* 1991; 88:328–38.
- Ebo DG, Hagendorens MM, Bridts CH, Schuerwegh AJ, De Clerck LS, Stevens WJ. In vitro allergy diagnosis: should we follow the flow? *Clin Exp Allergy* 2004; 34:332–9.
- Torres MJ, Padial A, Mayorga C *et al.* The diagnostic interpretation of basophil activation test in immediate allergic reactions to betalactams. *Clin Exp Allergy* 2004; 34:1768–75.
- Bousquet PJ, Kvedariene V, Co-Minh HB *et al.* Clinical presentation and time course in hypersensitivity reactions to betalactams. *Allergy* 2007; 62:872–6.
- Ghannadan M, Hauswirth AW, Scherthaner GH *et al.* Detection of novel CD antigens on the surface of human mast cells and basophils. *Int Arch Allergy Immunol* 2002; 127:299–307.
- Boumiza R, Monneret G, Forissier MF *et al.* Marked improvement of the basophil activation test by detecting CD203c instead of CD63. *Clin Exp Allergy* 2003; 33:259–65.
- Ebo DG, Lechkar B, Schuerwegh AJ, Bridts CH, De Clerck LS. Comments regarding 'marked improvement of the basophil activation test by detecting CD203c instead of CD63'. *Clin Exp Allergy* 2003; 33:849.
- De Weck AL, Sanz ML. For allergy diagnostic flow cytometry, detection of CD203c instead of CD63 is not at all an improvement in other hands (correspondence). *Clin Exp Allergy* 2003; 33:849–52.
- Hennessdorf F, Florian S, Jakob A *et al.* Identification of CD13, CD107a and CD164 as novel basophil-activation markers and dissection of two response patterns in time kinetics of IgE-dependent upregulation. *Cell Res* 2005; 15:325–35.

- 17 Monneret G, Boumiza R, Gravel S *et al.* Effect of prostaglandin D(2) and 5-lipoxygenase product on the expression of CD203c and CD11b by basophils. *J Pharmacol Exp Ther* 2005; **312**:627–34.
- 18 de Weck AL, Sanz ML, Gamboa PM *et al.* Diagnostic tests based on human basophils: more potentials and perspectives than pitfalls. *Int Arch Allergy Immunol* 2008; **146**:177–89.
- 19 Sudheer PS, Hall JE, Read GF, Rowbottom AW, Williams PE. Flowcytometric investigation of peri-anaesthetic anaphylaxis using CD63 and CD203c. *Anaesthesia* 2005; **60**:251–6.
- 20 Eberlein-König B, Varga R, Mempel M, Darsow U, Behrendt H, Ring J. Comparison of basophil activation tests using CD63 or CD203c expression in patients with insect venom allergy. *Allergy* 2006; **61**:1084–5.
- 21 Ocmant A, Peignois Y, Mulier S, Hanssens L, Michils A, Schandené L. Flow cytometry for basophil activation markers: the measurement of CD203c up-regulation is as reliable as CD63 expression in the diagnosis of cat allergy. *J Immunol Methods* 2007; **320**:40–8.
- 22 Abuaf N, Rostane H, Rajoely B *et al.* Comparison of two basophil activation markers CD63 and CD203c in the diagnosis of amoxicillin allergy. *Clin Exp Allergy* 2008; **38**, this issue.