

Analysis of IgE down regulation induced by basophil activation. Application to the diagnosis of muscle relaxant allergic hypersensitivity by flow cytometry

J. Sainte-Laudy¹, A. Boumediene¹, F. Touraine², I. Orsel³ and M. Cogné¹

¹ Laboratoire d'Immunologie, Hôpital universitaire Dupuytren, 2, avenue Martin-Luther-King, 87042 Limoges Cedex, France,

Fax: ++33 0555058054, e-mail: jean.sainte-laudy@chu-limoges.fr

² Département de pathologies respiratoires et d'Allergologie, CHU Dupuytren, Limoges, France

³ Département d'Anesthésie, CHU Dupuytren, Limoges, France

Introduction

Mast cells and basophils are the main cells bearing the high affinity receptor for IgE. Their responses can be investigated respectively by skin tests and in vitro cellular tests, mainly histamine release, flow cytometry and, more recently, leukotriene release. Flow cytometry seems to be particularly promising [1], as data obtained over the past 10 years has shown high sensitivities and high specificities for proteins (aero-allergens, foods, latex and venoms). In contrast, results obtained with drugs (mainly muscle relaxants, beta-lactams and NSAIDs) showed similar specificities but much lower sensitivities [2] which are not suitable for routine allergy diagnosis. Drug allergy is one of the most important and difficult topics in allergy diagnosis as drugs can induce life-threatening reactions. The mechanisms involved are often unclear, with IgE-dependent reactions representing only part of drug-induced hypersensitivity reactions.

Among the different drugs used within the anaesthetic cocktail, muscle relaxants (MR) are particularly important as they are responsible for more than 60 % of reactions. Our aim was to increase the sensitivity of flow cytometry for drug allergy diagnosis by setting up a new protocol based on the use of IgE as an early activation marker [3, 4].

Material and methods

Thirteen patients who had experienced a perianaesthetic shock were selected on the basis of the clinical history, skin tests (prick and intra-dermal reaction (IDR) in case of negativity), quaternary ammonium specific IgE [5], plasma histamine and tryptase. Ten had experienced a clear MR induced shock and the 3 others beta-lactam induced shocks (amoxicillin (2) and cefuroxime).

In brief, buffy coat was prepared from total blood taken on EDTA, and leukocyte suspensions were challenged (37 °C, 30 min) with the al-

lergen dilutions (MR were diluted as for IDR) in the presence of IL3 (2 ng/ml) [4]. An anti-IgE (polyclonal Caltag, 0.2 µg/ml) positive control was run systematically in parallel. Basophil activation was stopped by addition of EDTA (5 mM) and leukocytes were labelled by a mixture of anti-IgE FITC and anti-CD63 PE (final dilution 1/10). Erythrocytes were lysed and leukocytes were resuspended in the sheath buffer. A minimum of 500 basophils were counted and IgE mean intensity of fluorescence (IgE-MFI) and percentage of CD63 positive basophils were given by the cytometer (XL Coulter). Results were expressed as an index according to the following formula: Index = (% CD63 + (test) × 10^{MFI-IgE (neg control) / %CD63 + (neg control) × 10^{MFI-IgE (test)} × 10 / MFI(neg control).}

Results and discussion

Two patients were non-anti-IgE responders (one was controlled one month later with the same results) and were excluded from the study as non-interpretable. Twenty-one tests performed on the 3 non MR induced shocks allowed the calculation of the positivity threshold which was set at index = 5 for 2 successive allergen dilutions (2 standard errors).

Among the 8 patients (56 skin tests and 56 flow cytometric tests) for whom we had arguments in favour of an IgE dependent shock (rocuronium (1), succamethonium (3), vecuronium (2) and cis-atracurium (2)); 1 patient (vecuronium), who had experienced a grade III shock 13 years previously, was skin test and flow cytometry negative in spite of positive quaternary ammonium IgEs and a positive inhibition by vecuronium. One patient who had experienced a recent succamethonium induced shock with high plasma histamine and tryptase (respectively 17 nmol/l and 81 µg/l) and positive quaternary ammonium specific IgEs (7.4 (expressed as a ratio versus a negative serum pool, a ratio higher than 2 being considered as positive)) was only positive (index = 71) by flow cytometry for the culprit drug, and skin tests were negative. Skin tests and flow cytometry were positive for at least the culprit MR for all the other 7 patients and correla-

tion between skin tests and flow cytometry was significant (chi-squared, $p < 0.001$).

A CD63 upregulation was observed in 57 % of the cases (in agreement with the literature), the other positive tests being due to the IgE MFI down regulation alone.

These results highlight the interest in the use of an early activation marker, such as IgE. The confirmation of these results, in a larger series of experiments, may lead to the use of flow cytometry as an efficient tool for MR allergy diagnosis.

References

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