Estudo *in vitro* dos basófilos é uma ferramenta diagnóstica e de investigação útil em alergologia

Basophil assays are useful diagnostic and research tools in allergology

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RESUMO

O mecanismo imunológico subjacente às doencas alérgicas mediadas por IgE é a hipersensibilidade do tipo I, em que os mastócitos e os basófilos são as células efectoras. Esta reacção é reproduzida *in vitro* no teste de libertação de histamina e outros mediadores e no teste de activação dos basófilos. Estas são ferramentas muito úteis, não só no diagnóstico de diversas doenças alérgicas e seguimento de doentes submetidos a imunoterapia específica, mas também ao nível da investigação dos mecanismos imunológicos de alergia. Ambas as técnicas são discutidas no presente artigo.

Palavras-chave: alergia, alergénio, basófilo, teste de activação dos basófilos, desgranulação, libertação de histamina, teste de desgranulação dos basófilos, citometria de fluxo.

ABSTRACT

The immunological mechanism of IgE-mediated allergic diseases is type I hypersensitivity, where basophils and mast cells are the main effector cells. This reaction is reproduced in vitro in basophil mediator release and basophil activation assays. These are useful tools not only for the diagnosis of various allergic diseases and follow-up of patients undergoing allergen-specific immunotherapy, but also in research into the mechanisms of allergy. Both basophil assays are discussed in this article.

Key-words: *Allergy, allergen, basophil, basophil activation test, histamine release, basophil degranulation test, flow cytometry.*

INTRODUCTION

The immunologic mechanism underlying IgE-mediated allergic diseases is type I hypersensitivity. In sensitised patients, allergen-specific IgE antibodies bind to high-affinity IgE receptors (FcεRI) on the surface of mast cells and basophils for relatively long periods of time. On subsequent exposure, allergens bind to IgE on the surface of mast cells and basophils which leads to cross-linking of FcεRI receptors and triggering of complex intracellular signalling cascades. These culminate in the release of both pre-formed mediators (e.g. histamine, proteoglycans, serine proteases) and *de novo* synthesis of cytokines (e.g. IL-3, IL-4, IL-13) as well as leukotrienes, all of which contribute to allergic inflammation[1].

The IgE-mediated allergic reaction has been reproduced *in vitro*, both as a diagnostic and as a research tool, using mast cells and basophils. Basophils have the advantage of being easily available as they can be readily isolated from peripheral blood. Traditionally, functional *in vitro* tests based on allergen-induced activation of IgE-bearing basophils have focused on the mediators released by these cells after stimulation with allergen[2]. However, in parallel with the release of vasoactive mediators, basophils upregulate the expression of different activation markers on their surface, which can be evaluated by flow cytometry – this is the so-called basophil activation test (BAT)[3].

This article aims to give an overview of the two main types of functional assays used to study IgE-mediated basophil activation *in vitro*: mediator release and basophil activation assays.

MEDIATOR RELEASE ASSAYS

When IgE-receptors on basophils are cross-linked by an allergen, the cells undergo degranulation and release bioactive mediators. Histamine is one of the most important mediators, as it is responsible for many of the symptoms in the immediate phase of the allergic response, and can be easily measured *in vitro* in the supernatants of basophils previously stimulated by allergen.

The primary source of cells in this experimental setting can be whole blood, dextran- or Ficoll-isolated leukocytes and basophils that have been further purified by negative selection using magnetic cell-sorting techniques[4]. Experimental designs using passive sensitisation[5], i.e. stripping of native membrane-bound immunoglobulins and preincubation of basophils with patients' sera before stimulation with allergen, are particularly interesting for mechanistic studies. When collecting the blood for this kind of experiment, it is recommended that the donors have not taken drugs or food a few hours before blood donation and that blood is collected to a syringe or tube containing anticoagulant. The appropriate anticoagulant to be used depends on the chosen laboratory protocol. Blood should be processed as soon as possible, preferably within 4 hours of collection.

Crude allergen extracts or purified/recombinant allergens may be used for cell stimulation. For each donor, different allergen concentrations should be tested, usually in 10-fold serial dilutions, as the sensitivity of the basophils to specific allergen stimulation varies among patients. As positive controls, anti-IgE should be used to gauge IgE--mediated cell activation and formyl-methionyl-leucyl--phenylalanine (fMLP), a chemotactic stimulus which activates basophils through an IgE-independent mechanism, as a control for functional cell viability. As a negative control, cells are stimulated with buffer alone. Degranulation is optimal at 37°C and occurs within 30 minutes[6] in the presence but not in the absence of extracellular calcium; thus a calcium-containing buffer must be used.

The histamine concentration in the supernatants can be measured using different techniques, namely radio--immunoassay (RIA), enzyme-linked immunosorbent assay (ELISA) or spectrofluorometric assays, which measure the fluorescence of an adduct formed by reacting histamine with o-phthaldehyde[6,7]. Histamine release is usually expressed as a percentage of the total basophil histamine content, which is determined by the sum of intra and extracellular histamine contents (where intracellular histamine contents are liberated by lysis of the cell pellets).

Spontaneous release, i.e. release of histamine after incubation in buffer alone, should be less than 5% of the total histamine content. However, particularly in atopic patients higher spontaneous histamine releases may be observed. Response to anti-IgE often gives rise to a bell-shaped dose–response curve[6] – Figure 1. Utilizing defined allergens, the histamine release test provides direct information concerning the reactivity and sensitivity of basophils. The reactivity is defined as the ability to release histamine in response to an IgE-dependent stimulus and is given by the maximal histamine release. Sensitivity is defined as the dose of the stimulus that is able to trigger half of the maximal histamine release. A response to an allergen is considered positive when a reaction is clearly dose-dependent and the percentage of histamine is greater than 10% (or 5% after correction for spontaneous release). Based on the magnitude of reaction after stimulation with anti-IgE donors can be categorized as good responders (histamine release greater or equal to 50%), intermediate responders (20-50%), low responders (5-19%) and non responders (less than 5%). This classification is subjective and based on arbitrary figures, and therefore should be used as a reference and may not be very useful clinically. In the minority of individuals who are non-responders to IgE-dependent stimuli, the assay is uninterpretable. Defects in spleen tyrosine kinase (Syk), present in the early phase of the intracellular signalling pathway leading to degranulation, has been described in these individuals[8]. These and other molecular mechanisms should be explored in the future as they could lead to useful findings about potential novel treatments of allergic diseases[9,10].

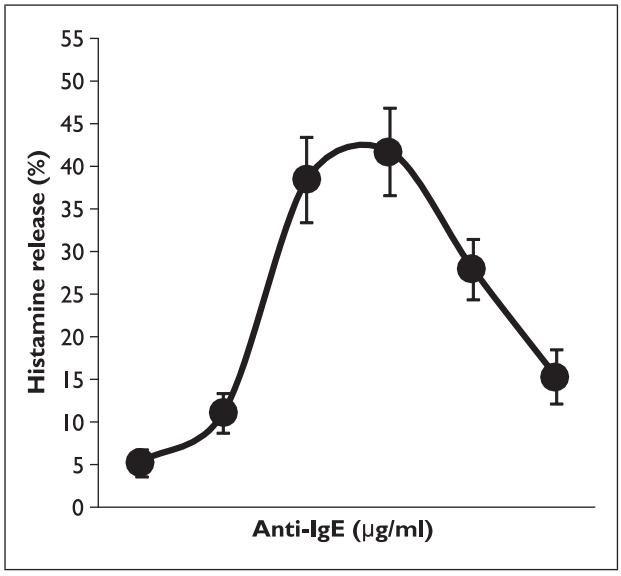

Figure 1. Dose-response curve of histamine release after basophil stimulation with anti-IgE

Following 30 min stimulation, leukotrienes may also be measured in the basophil supernatant, e.g. LTC4, usually by ELISA[11,12]. The release of various cytokines from basophils may also be detected and quantified by ELISA[6] or using more sophisticated bead-based assays. However, the optimum incubation periods for release of these mediators vary from 4 hours, for IL-4, to over 16 hours in the case of IL-13. Furthermore, basophils from some individuals

have high constitutive expressions of IL-4 (i.e. preformed and not *de novo* synthesised) which may also be released within minutes of stimulation[13].

Another mediator measured in the supernatant of mast cells and various cell lines, such as LUVA, LAD-2 and RBL cells, to detect degranulation is β-hexosaminidase[14-16]. This is a granule-stored enzyme, an exoglycosidase, with optimal activity at low pH, and is secreted in parallel with histamine. The measurement of its activity has been extensively used to monitor mast cell and basophil degranulation by adding fluorogenic β-hexosaminidase substrate at low pH and incubating at 37°C for 60 mins. This reaction is terminated by changing the pH and the colour due to the substrate hydrolysis is measured by fluorometry. The results are expressed as percentages of the total β-hexosaminidase content of the cells, which is determined by summing the extracellular release and the release after cell lysis.

BASOPHIL ACTIVATION TEST

Using a similar experimental setting, whilst the supernatant may be used for measurement of mediator release, the cells may be analysed by flow cytometry to evaluate the expression of basophil activation markers[2,3]. This type of experiment may be performed using mixed cell populations (e.g. PBMC, even whole blood) or purified basophils. In any case, identification markers have to be used to gate on basophils and detect the expression of the activation markers in that selected population.

Different cell-surface markers may be selected for identification of basophils, the most common ones being anti-IgE, anti-CD123 and anti-CCR3 – Table 1. Some authors use anti-CD203c both as an identification and an activation marker, advocating that it allows performing a single marker BAT[17]. However, CD203c can also be high-

Table 1. Main basophil identification markers[2,3,22]

Marker	IgE	CD123	CCR3	CRTH2
Synonym	–	IL-3Rα	CD193	DP2, CD294
Function	Immune response against parasites Type I hypersensitivity	Low-affinity (α) subunit of IL-3 receptor that associates with CD131, the common β-chain of the IL-3, IL-5, and GM-CSF receptor, to form the high-affinity IL-3 receptor IL-3 receptor is involved in cell signaling for cell growth and differentiation	Receptor for C-C type chemokines – e.g. eotaxin, *major* cationic protein (MCP) and RANTES	Receptor for prostaglandin D2
Cells expressing in peripheral blood	On monocytes, dendritic cells and basophils bound to FceRI On eosinophils, macrophages, B cells, and platelets bound to FceRII	High expression on plasmocytoid dendritic cells and basophils Low expression on monocytes, eosinophils, myeloid dendritic cells, and subsets of haematologic progenitor cells	High expression on eosinophils and basophils Also detected in Th1 and Th2 cells	Basophils, eosinophils, Th2 lymphocytes
Markers to be used in combination	aHLA-DR		aCD3	

ly expressed in basophils following Ficoll-mediated isolation and by priming factors such as IL-3, which by themselves do not cause substantial degranulation.

In the peripheral blood, IgE is detected on dendritic cells and basophils, which express the high affinity IgE receptor (FcεRI), and also on eosinophils, monocytes, macrophages, B cells and platelets, which express the low affinity IgE receptor (FcεRII or CD23). The expression of IgE on the surface of basophils varies with the atopic status of the patient, increasing in atopic patients. Labelling basophils with an anti-IgE antibody can further activate the cells, which can be reduced by fixing, cooling and adding EDTA--containing buffer to the cells before staining. CD123 is the low affinity subunit of the IL-3 receptor, which is expressed in high levels on plasmocytoid dendritic cells and basophils, and in low levels on monocytes, eosinophils, myeloid dendritic cells and subsets of hematologic progenitor cells. Additional staining with anti-HLA-DR discriminates between HLA-DR negative basophils and HLA--DR positive dendritic cells and monocytes. One of the advantages of identifying basophils with anti-CD123 and anti-HLA-DR is that their expression is not so much influenced by the allergic status of the donor as anti-IgE. CCR3 is the receptor for C-C type chemokines (e.g. eotaxin, MCP and RANTES). It is highly expressed on basophils and eosinophils but also on Th1 and Th2 cells. Thus, an anti-CD3 marker should be used in combination with it to exclude the CD3 positive T cells. Haussmann *et al*[18] have compared the main three basophil identification methods and concluded that CD123/HLA-DR and CCR3 are the most accurate, with CCR3 having the advantages of being most constant with the atopic background of the patient and of identifying basophils with a single marker. However, CCR3 has the disadvantage of being downregulated after basophil activation, which does not occur with CD123/HLA-DR.

After stimulation with allergen, the expression of different proteins is upregulated on the surface of basophils. Although the intracellular pathways driving the upregulation of these markers are not completely understood, they seem to form two distinct groups of markers that are upregulated concomitantly: one including CD63, CD107a and CD107b and another CD203c, CD13 and CD164[19]. The most studied and widely used are CD63[20] and CD203c[17], which are proteins expressed on the membrane of the granules that fuse with the plasmatic membrane of the basophils during degranulation, increasing their expression on the surface of the cell[21] – Table 2.

These markers behave differently in their upregulation profiles[22, 23]. The increase in their expression in response to specific activators and inhibitors follows different kinetics and seems to be directed through alternative signal transduction pathways. The expression of CD203c is low in resting basophils that have not been primed with IL-3 and increases after activation, whilst CD63 is not expressed in resting cells. The upregulation of CD63 is bimodal, with only a subpopulation of basophils expressing it, whilst CD203c expression is less prominent but often generalised to the whole cell population, even to cells that did not express CD63.

Dose-response curves with different agonists and inhibitors show dissociation between the two activation markers: CD203c is associated with the low-dose events of chemotaxis and CD63 is associated with degranulation[19]. Different studies have suggested that CD63 may reflect anaphylactic degranulation whilst CD203c reflects piecemeal degranulation. MacGlashan[24] hypothesised in a recent published study that this may be the reason why neither CD63 nor CD203c strictly reflect histamine release. Histamine release measured in the cell supernatant is an average of what occurs in a heterogeneous population of basophils, being a result of the sum between the two pathways of basophil activation. This highlights the advantage of using flow cytometry to study basophil activation as it gives more complete and detailed information about the behaviour of individual cells after stimulation with allergen.

The results of BAT may be shown for each condition in dotplots or histograms and differences in comparison with controls may be determined in terms of percentage of basophils expressing the defined activation marker (usu-

Table 2. Main basophil activation markers[2, 3, 22]

Marker	aCD203c	aCD63
Synonym	neural cell surface differentiation antigen	gp53, LAMP-3
Family	ectonucleotide pyrophospha-tase/phosphodiesterases (ENPP-3)	transmembrane- 4 superfamily (tetraspanins)
Function	glycosylated type II transmembrane molecule that catalyses the hydrolysis of oligonucleotides, nucleoside phosphates, and nicotinamide adenine dinucleotide (NAD)	secretory granule-associated protein involved in vesicle fusion events
Cells expressing in peripheral blood	is exclusively and constitutively expressed by basophils	basophils, mast cells, monocytes, macrophages and platelets
Expression in resting basophils	Low expression (can also be used as an identification marker)	is anchored to the intracellular granules and barely expressed on the surface of the membrane, both in healthy subjects and in allergic patients
Expression in IgE--activated basophils	– levels of CD203c rapidly increase in a dose- and time-dependent way – generally less prominent than CD63 – unimodal – often occurs in almost all cells	– upregulated concomitantly with basophilic degranulation as a result of fusion between the granule and the membrane during exocytosis – expressed at high density (> 1 log scale) – bimodal expression – only a subpopulation of cells express CD63 with a high intensity
IL-3 priming	– Sensitive to IL-3 priming	– not sensitive to IL-3 priming
Parallel expression	– transmembrane glycoprotein sialomucin endolyn (CD164) and the ecto-enzyme CD13 (gp150) – associated with piecemeal degranulation	– CD107a (LAMP-1), CD107b (LAMP-2) – associated with anaphylactic degranulation
Kinetics of IgE mediated activation	Upregulation starts after 5 min Maximal expression = 5-15 min Plateau until 60 min	Upregulation starts after 3 min Maximal expression = 5-10 min Plateau until 60 min
Non IgE mediated stimulators and Inhibitors	– fMLP upregulates expression, less than CD63, reaching a plateau with increasing doses of fMLP – TPA upregulates expression (delayed in comparison with aIgE) – wortmannin almost completely inhibitis expression – PGD2 does not upregulate expression	– fMLP upregulates expression significantly and progressively with increasing doses of fMLP – TPA upregulates expression (earlier than aIgE) – wortmannin decreases expression in half the maximum – PGD2 upregulates expression

Abbreviations: gp – glycoprotein; LAMP – lysosomal-associated membrane glycoprotein; TPA – 12-O-tetradecanoylphorbol-13--acetate; PGD2 – prostaglandin D2; fMLP – formyl-methionyl-leucyl-phenylalanine.

ally used for CD63) or in terms of mean fluorescence intensity (MFI) by calculating the ratio between the MFI of the selected condition and the MFI of the negative control (usually used for CD203c) – Figure 2. As a reference, for most allergens 15% positive cells and SI of 2.0 are the cut--offs for positive tests, but this varies with allergens and the establishment of proper cut-offs requires receiver--operating characteristic curves to establish optimal sen-

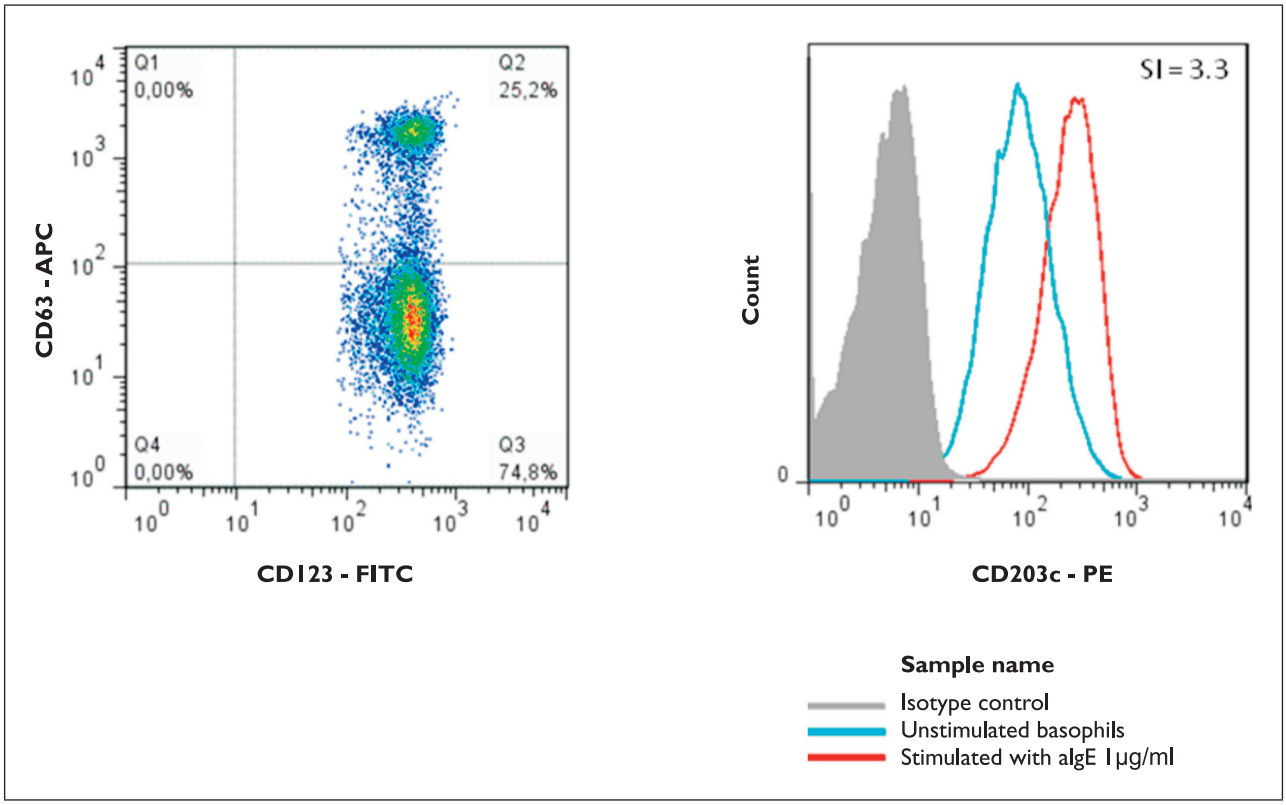

Figure 2. Basophil activation after stimulation with 1µg/ml anti-IgE results in expression of CD63 by 25.2% of basophils and in a SI CD203c of 3.3. Basophils were gated as SSClow, CD123+ and HLA-DR- cells

sitivity and specificity. The interpretation of results should always be tailored to each individual case. The response in a time-course and dose-response manner is an additional important sign of allergen-mediated basophil activation.

Short incubation with IL-3 may increase the sensitivity of the assay and has been used in some studies[25]. IL-3 causes nonspecific increase in CD203c expression but not CD63. However, it may be a cause for false positive results[26], one reason for that being the concentrations of IL-3 that are used which are much higher than the physiological ones.

The molecular mechanisms governing basophil activation are complex and not entirely clarified. Traditionally, analysis of signalling is based on western blot and ELISA techniques, which represent a mean value for the total isolated cell population[6]. Recently, a proof of concept was provided that flow cytometry may be used to quantify phosphorilation of p38-MAPK in basophils[27]. Similar methods may be used to evaluate consecutive phosphorilation of the proteins involved, as has been done for other cells and signalling pathways. Flow cytometry offers various advantages over the traditional techniques. It allows identification of cells with heterogeneity in responsiveness, it combines surface with intracellular staining and integrates immunophenotyping of individual cells. Flow cytometry enables to study the cells in their natural environment, avoiding basophil purification and potential interference from additional manipulations. Furthermore, this novel technique also significantly shortens the time of analysis from days to hours and reduces the sampling volume considerably, rendering it more accessible for clinical and research applications.

CLINICAL APPLICATIONS

Within certain limits, basophil assays reproduce IgE mediated allergic reactions *in vitro*; therefore, they may be useful for the diagnosis and monitoring of allergic diseases, namely after interventions such as allergen specific immunotherapy and anti-IgE treatment. Gober *et al*[28] studied a group of patients allergic to insect venom and collected blood before and after sting challenge to assess the expression of basophil activation markers after stimulation with insect venom and to compare activation marker expression after allergen stimulation *in vivo* and *in vitro*. Despite some methodological drawbacks[29], patient heterogeneity and the fact that allergen stimulation *in vitro* resulted in greater basophil activation compared to what happened after *in vivo* challenge, there was a general agreement between clinical presentation and the results of BAT. Basal CD63 expression and upregulation of CD69 and CD203c expression was greater in patients with a history of systemic reaction on immunotherapy. This study suggests that basophil activation markers are useful biomarkers of anaphylaxis.

The interest for BAT in the diagnosis of various allergic diseases is growing, namely of pollen, cat, food, drug and venom allergies[30-39]. This test is particularly important in cases where skin prick test and serum specific IgE determination give equivocal results discordant with the clinical history. Interestingly, Ocmant *et al*[12] showed that BAT discriminated between allergic and non-allergic subjects among patients sensitised to egg or peanut, highlighting the advantage of BAT over methods that only detect specific IgE antibodies. BAT has shown to be useful also in the diagnosis of chronic urticaria and in the detection of autoantibodies in a subgroup of these patients[40].

BAT has proven to be helpful in assessing the acquisition of tolerance to foods in food allergic children. In a recent study by Sampson and colleagues, tolerance to extensively heated milk (HM) was assessed by oral food challenge (OFC) among children with milk allergy[41]. Patients with negative OFC to extensively HM who reacted to unheated milk were considered to have "HM tolerance", an intermediate clinical phenotype between milk allergy and milk tolerance. Basophils of HM tolerant patients showed lower reactivity *in vitro* compared to HM reactive patients[42]. Basophil reactivity was recovered in the absence of autologous serum and progressively decreased with increasing concentrations of the serum from HM tolerant patients, suggesting that a serum factor was responsible for the inhibition of basophil reactivity to milk allergens[42]. BAT may also be useful in determining when to safely perform an oral food challenge to assess tolerance and reintroduce the food in the child's diet. In a recent study by Rubio *et al*[43], BAT showed a sensitivity of 91%, a specificity of 90% and positive and negative predictive values of 81 and 96% in detecting children with persistent cow's milk allergy. These values are greater than the ones of serum specific IgE and skin prick test usually used in clinical practice. Similar approaches may be used for other foods.

In patients undergoing allergen-specific immunotherapy, loss of allergic reactivity in BAT is observed in parallel to clinical improvement. Similar findings have been reported in patients undergoing immunotherapy to respiratory allergens[44,45], food allergens[46] and insect venom[47]. Some studies have suggested that BAT can predict clinical sensitivity and that the expression of CD63 on basophils may be useful in deciding when to stop venom immunotherapy[48-50]. BAT may also prove to be very useful in monitoring patients undergoing treatment with omalizumab. In a study of seven patients treated with omalizumab and 27 allergic patients not treated, Nopp *et al*[51] showed that the basophil sensitivity, given by a formula based on the allergen concentration that elicited 50% of the basophil maximal reactivity, was a good quantitative measure of efficacy of this treatment.

Recent studies have reported very interesting observations that point out the potential of BAT not only in improving the diagnosis of allergic diseases but also in unravelling some of the unsolved questions about atopic diseases and clinical reactivity in sensitised patients. Basophils of atopic when compared with non atopic patients show an activated profile as happens with patients with chronic urticaria[52]. This *in vivo* priming reflects ongoing basophil activation. Interestingly, basal expression of CD203c

has been shown to be increased in patients with uncontrolled asthma and frequent asthma exacerbations[53]. These and other studies pave new avenues in the use of BAT for research of immunological mechanisms of allergic diseases.

CONCLUSION

Basophil mediator release and basophil activation tests are assays that reproduce IgE mediated reactions *in vitro*. They have the potential of not only improving the diagnosis and follow-up of patients with various allergic diseases or undergoing allergen specific immunotherapy but also of helping with research into the immunological mechanisms of allergy.

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Basophil assays are useful diagnostic and research tools in Allergology

Estudo in vitro dos basófilos é uma ferramenta diagnóstica e de investigação útil em Alergologia

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ABSTRACT

The immunological mechanism of IgE-mediated allergic diseases is type I hypersensitivity, where basophils and mast cells are the main effector cells. This reaction is reproduced in vitro in basophil mediator release and basophil activation assays. These are useful tools not only for the diagnosis of various allergic diseases and follow-up of patients undergoing allergen-specific immunotherapy, but also in research into the mechanisms of allergy. Both basophil assays are discussed in this article.

Key-words: Allergy, allergen, basophil, basophil activation test, histamine release, basophil degranulation test, flow cytometry.

RESUMO

O mecanismo imunológico subjacente às doencas alérgicas mediadas por IgE é a hipersensibilidade do tipo I, em que os mastócitos e os basófilos são as células efectoras. Esta reacção é reproduzida *in vitro* no teste de libertação de histamina e outros mediadores e no teste de activação dos basófilos. Estas são ferramentas muito úteis não só no diagnóstico de diversas doenças alérgicas e seguimento de doentes submetidos a imunoterapia específica, mas também ao nível da investigação dos mecanismos imunológicos de alergia. Ambas as técnicas são discutidas no presente artigo.

Palavras-chave: alergia, alergeno, basófilo, teste de activação dos basófilos, desgranulação, libertação de histamina, teste de desgranulação dos basófilos, citometria de fluxo.

INTRODUCTION

The immunologic mechanism underlying IgE-mediated allergic diseases is type I hypersensitivity. In sensitised patients, allergen-specific IgE antibodies bind to high-affinity IgE receptors (FcεRI) on the surface of mast cells and basophils for relatively long periods of time. On subsequent exposure, allergens bind to IgE on the surface of mast cells and basophils which leads to cross-linking of FcεRI receptors and triggering of complex intracellular signalling cascades. These culminate in the release of both pre-formed mediators (e.g. histamine, proteoglycans, serine proteases) and *de novo* synthesis of cytokines (e.g. IL-3, IL-4, IL-13) as well as leukotrienes, all of which contribute to allergic inflammation[1].

The IgE-mediated allergic reaction has been reproduced *in vitro*, both as a diagnostic and as a research tool, using mast cells and basophils. Basophils have the advantage of being easily available as they can be readily isolated from peripheral blood. Traditionally, functional *in vitro* tests based on allergen-induced activation of IgE-bearing basophils have focused on the mediators released by these cells after stimulation with allergen[2]. However, in parallel with the release of vasoactive mediators, basophils upregulate the expression of different activation markers on their surface, which can be evaluated by flow cytometry – this is the so-called basophil activation test (BAT)[3].

This article aims to give an overview of the two main types of functional assays used to study IgE-mediated basophil activation *in vitro*: mediator release and basophil activation assays.

MEDIATOR RELEASE ASSAYS

When IgE-receptors on basophils are cross-linked by an allergen, the cells undergo degranulation and release bioactive mediators. Histamine is one of the most important mediators, as it is responsible for many of the symptoms in the immediate phase of the allergic response, and can be easily measured *in vitro* in the supernatants of basophils previously stimulated by allergen.

The primary source of cells in this experimental setting can be whole blood, dextran- or Ficoll-isolated leukocytes and basophils that have been further purified by negative selection using magnetic cell-sorting techniques[4]. Experimental designs using passive sensitisation[5], i.e. stripping of native membrane-bound immunoglobulins and preincubation of basophils with patients' sera before stimulation with allergen, are particularly interesting for mechanistic studies. When collecting the blood for this kind of experiment, it is recommended that the donors have not taken drugs or food a few hours before blood donation and that blood is collected to a syringe or tube containing anticoagulant. The appropriate anticoagulant to be used depends on the chosen laboratory protocol. Blood should be processed as soon as possible, preferably within 4 hours of collection.

Crude allergen extracts or purified/recombinant allergens may be used for cell stimulation. For each donor, different allergen concentrations should be tested, usually in 10-fold serial dilutions, as the sensitivity of the basophils to specific allergen stimulation varies among patients. As positive controls, anti-IgE should be used to gauge IgE--mediated cell activation and formyl-methionyl-leucyl--phenylalanine (fMLP), a chemotactic stimulus which activates basophils through an IgE-independent mechanism, as a control for functional cell viability. As a negative control, cells are stimulated with buffer alone. Degranulation is optimal at 37°C and occurs within 30 minutes[6] in the presence but not in the absence of extracellular calcium; thus a calcium-containing buffer must be used.

The histamine concentration in the supernatants can be measured using different techniques, namely radio--immunoassay (RIA), enzyme-linked immunosorbent assay (ELISA) or spectrofluorometric assays, which measure the fluorescence of an adduct formed by reacting histamine with o-phthaldehyde[6,7]. Histamine release is usually expressed as a percentage of the total basophil histamine content, which is determined by the sum of intra and extracellular histamine contents (where intracellular histamine contents are liberated by lysis of the cell pellets).

Spontaneous release, i.e. release of histamine after incubation in buffer alone, should be less than 5% of the total histamine content. However, particularly in atopic patients higher spontaneous histamine releases may be observed. Response to anti-IgE often gives rise to a bell-shaped dose–response curve[6] – Figure 1. Utilizing defined allergens, the histamine release test provides direct information concerning the reactivity and sensitivity of basophils. The reactivity is defined as the ability to release histamine in response to an IgE-dependent stimulus and is given by the maximal histamine release. Sensitivity is defined as the dose of the stimulus that is able to trigger half of the maximal histamine release. A response to an allergen is considered positive when a reaction is clearly dose-dependent and the percentage of histamine is greater than 10% (or 5% after correction for spontaneous release). Based on the magnitude of reaction after stimulation with anti-IgE donors can be categorized as good responders (histamine release greater or equal to 50%), intermediate responders (20-50%), low responders (5-19%) and non responders (less than 5%). This classification is subjective and based on arbitrary figures, and therefore should be used as a reference and may not be very useful clinically. In the minority of individuals who are non-responders to IgE-dependent stimuli, the assay is uninterpretable. Defects in spleen tyrosine kinase (Syk), present in the early phase of the intracellular signalling pathway leading to degranulation, has been described in these individuals[8]. These and other molecular mechanisms should be explored in the future as they could lead to useful findings about potential novel treatments of allergic diseases[9,10].

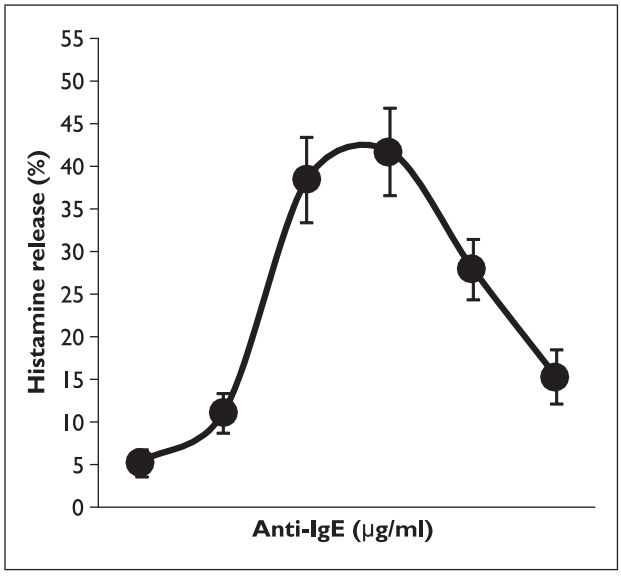

Figure 1. Dose-response curve of histamine release after basophil stimulation with anti-IgE

Following 30 min stimulation, leukotrienes may also be measured in the basophil supernatant, e.g. LTC4, usually by ELISA[11,12]. The release of various cytokines from basophils may also be detected and quantified by ELISA[6] or using more sophisticated bead-based assays. However, the optimum incubation periods for release of these mediators vary from 4 hours, for IL-4, to over 16 hours in the case of IL-13. Furthermore, basophils from some individuals

have high constitutive expressions of IL-4 (i.e. preformed and not *de novo* synthesised) which may also be released within minutes of stimulation[13].

Another mediator measured in the supernatant of mast cells and various cell lines, such as LUVA, LAD-2 and RBL cells, to detect degranulation is β-hexosaminidase[14-16]. This is a granule-stored enzyme, an exoglycosidase, with optimal activity at low pH, and is secreted in parallel with histamine. The measurement of its activity has been extensively used to monitor mast cell and basophil degranulation by adding fluorogenic β-hexosaminidase substrate at low pH and incubating at 37°C for 60mins. This reaction is terminated by changing the pH and the colour due to the substrate hydrolysis is measured by fluorometry. The results are expressed as percentages of the total β-hexosaminidase content of the cells, which is determined by summing the extracellular release and the release after cell lysis.

BASOPHIL ACTIVATION TEST

Using a similar experimental setting, whilst the supernatant may be used for measurement of mediator release, the cells may be analysed by flow cytometry to evaluate the expression of basophil activation markers[2,3]. This type of experiment may be performed using mixed cell populations (e.g. PBMC, even whole blood) or purified basophils. In any case, identification markers have to be used to gate on basophils and detect the expression of the activation markers in that selected population.

Different cell-surface markers may be selected for identification of basophils, the most common ones being anti-IgE, anti-CD123 and anti-CCR3 – Table 1. Some authors use anti-CD203c both as an identification and an activation marker, advocating that it allows performing a single marker BAT[17]. However, CD203c can also be high-

Table 1. Main basophil identification markers[2,3,22]

Marker	IgE	CD123	CCR3	CRTH2
Synonym	–	IL-3Rα	CD193	DP2, CD294
Function	Immune response against parasites Type I hypersensitivity	Low-affinity (α) subunit of IL-3 receptor that associates with CD131, the common β-chain of the IL-3, IL-5, and GM-CSF receptor, to form the high-affinity IL-3 receptor IL-3 receptor is involved in cell signaling for cell growth and differentiation	Receptor for C-C type chemokines – e.g. eotaxin, major cationic protein (MCP) and RANTES	Receptor for prostaglandin D2
Cells expressing in peripheral blood	On monocytes, dendritic cells and basophils bound to FceRI On eosinophils, macrophages, B cells, and platelets bound to FceRII	High expression on plasmocytoid dendritic cells and basophils Low expression on monocytes, eosinophils, myeloid dendritic cells, and subsets of haematologic progenitor cells	High expression on eosinophils and basophils Also detected in Th1 and Th2 cells	Basophils, eosinophils, Th2 lymphocytes
Markers to be used in combination	aHLA-DR		aCD3	

ly expressed in basophils following Ficoll-mediated isolation and by priming factors such as IL-3, which by themselves do not cause substantial degranulation.

In the peripheral blood, IgE is detected on dendritic cells and basophils, which express the high affinity IgE receptor (FcεRI), and also on eosinophils, monocytes, macrophages, B cells and platelets, which express the low affinity IgE receptor (FcεRII or CD23). The expression of IgE on the surface of basophils varies with the atopic status of the patient, increasing in atopic patients. Labelling basophils with an anti-IgE antibody can further activate the cells, which can be reduced by fixing, cooling and adding EDTA--containing buffer to the cells before staining. CD123 is the low affinity subunit of the IL-3 receptor, which is expressed in high levels on plasmocytoid dendritic cells and basophils, and in low levels on monocytes, eosinophils, myeloid dendritic cells and subsets of hematologic progenitor cells. Additional staining with anti-HLA-DR discriminates between HLA-DR negative basophils and HLA--DR positive dendritic cells and monocytes. One of the advantages of identifying basophils with anti-CD123 and anti-HLA-DR is that their expression is not so much influenced by the allergic status of the donor as anti-IgE. CCR3 is the receptor for C-C type chemokines (e.g. eotaxin, MCP and RANTES). It is highly expressed on basophils and eosinophils but also on Th1 and Th2 cells. Thus, an anti-CD3 marker should be used in combination with it to exclude the CD3 positive T cells. Haussmann et al[18] have compared the main three basophil identification methods and concluded that CD123/HLA-DR and CCR3 are the most accurate, with CCR3 having the advantages of being most constant with the atopic background of the patient and of identifying basophils with a single marker. However, CCR3 has the disadvantage of being downregulated after basophil activation, which does not occur with CD123/HLA-DR.

After stimulation with allergen, the expression of different proteins is upregulated on the surface of basophils. Although the intracellular pathways driving the upregulation of these markers are not completely understood, they seem to form two distinct groups of markers that are upregulated concomitantly: one including CD63, CD107a and CD107b and another CD203c, CD13 and CD164[19]. The most studied and widely used are CD63[20] and CD203c[17], which are proteins expressed on the membrane of the granules that fuse with the plasmatic membrane of the basophils during degranulation, increasing their expression on the surface of the cell[21] – Table 2.

These markers behave differently in their upregulation profiles[22, 23]. The increase in their expression in response to specific activators and inhibitors follows different kinetics and seems to be directed through alternative signal transduction pathways. The expression of CD203c is low in resting basophils that have not been primed with IL-3 and increases after activation, whilst CD63 is not expressed in resting cells. The upregulation of CD63 is bimodal, with only a subpopulation of basophils expressing it, whilst CD203c expression is less prominent but often generalised to the whole cell population, even to cells that did not express CD63.

Dose-response curves with different agonists and inhibitors show dissociation between the two activation markers: CD203c is associated with the low-dose events of chemotaxis and CD63 is associated with degranulation[19]. Different studies have suggested that CD63 may reflect anaphylactic degranulation whilst CD203c reflects piecemeal degranulation. MacGlashan[24] hypothesised in a recent published study that this may be the reason why neither CD63 nor CD203c strictly reflect histamine release. Histamine release measured in the cell supernatant is an average of what occurs in a heterogeneous population of basophils, being a result of the sum between the two pathways of basophil activation. This highlights the advantage of using flow cytometry to study basophil activation as it gives more complete and detailed information about the behaviour of individual cells after stimulation with allergen.

The results of BAT may be shown for each condition in dotplots or histograms and differences in comparison with controls may be determined in terms of percentage of basophils expressing the defined activation marker (usu-

Table 2. Main basophil activation markers[2, 3, 22]

Marker	aCD203c	aCD63
Synonym	neural cell surface differentiation antigen	gp53, LAMP-3
Family	ectonucleotide pyrophospha-tase/phosphodiesterases (ENPP-3)	transmembrane- 4 superfamily (tetraspanins)
Function	glycosylated type II transmembrane molecule that catalyses the hydrolysis of oligonucleotides, nucleoside phosphates, and nicotinamide adenine dinucleotide (NAD)	secretory granule-associated protein involved in vesicle fusion events
Cells expressing in peripheral blood	is exclusively and constitutively expressed by basophils	basophils, mast cells, monocytes, macrophages and platelets
Expression in resting basophils	Low expression (can also be used as an identification marker)	is anchored to the intracellular granules and barely expressed on the surface of the membrane, both in healthy subjects and in allergic patients
Expression in IgE--activated basophils	– levels of CD203c rapidly increase in a dose- and time-dependent way – generally less prominent than CD63 – unimodal – often occurs in almost all cells	– upregulated concomitantly with basophilic degranulation as a result of fusion between the granule and the membrane during exocytosis – expressed at high density (> 1 log scale) – bimodal expression – only a subpopulation of cells express CD63 with a high intensity
IL-3 priming	– Sensitive to IL-3 priming	– not sensitive to IL-3 priming
Parallel expression	– transmembrane glycoprotein sialomucin endolyn (CD164) and the ecto-enzyme CD13 (gp150) – associated with piecemeal degranulation	– CD107a (LAMP-1), CD107b (LAMP-2) – associated with anaphylactic degranulation
Kinetics of IgE mediated activation	Upregulation starts after 5 min Maximal expression = 5-15 min Plateau until 60 min	Upregulation starts after 3 min Maximal expression = 5-10 min Plateau until 60 min
Non IgE mediated stimulators and Inhibitors	– fMLP upregulates expression, less than CD63, reaching a plateau with increasing doses of fMLP – TPA upregulates expression (delayed in comparison with aIgE) – wortmannin almost completely inhibitis expression – PGD2 does not upregulate expression	– fMLP upregulates expression significantly and progressively with increasing doses of fMLP – TPA upregulates expression (earlier than aIgE) – wortmannin decreases expression in half the maximum – PGD2 upregulates expression

Abbreviations: gp – glycoprotein; LAMP – lysosomal-associated membrane glycoprotein; TPA – 12-O-tetradecanoylphorbol-13--acetate; PGD2 – prostaglandin D2; fMLP – formyl-methionyl-leucyl-phenylalanine.

ally used for CD63) or in terms of mean fluorescence intensity (MFI) by calculating the ratio between the MFI of the selected condition and the MFI of the negative control (usually used for CD203c) – Figure 2. As a reference, for most allergens 15% positive cells and SI of 2.0 are the cut--offs for positive tests, but this varies with allergens and the establishment of proper cut-offs requires receiver--operating characteristic curves to establish optimal sen-

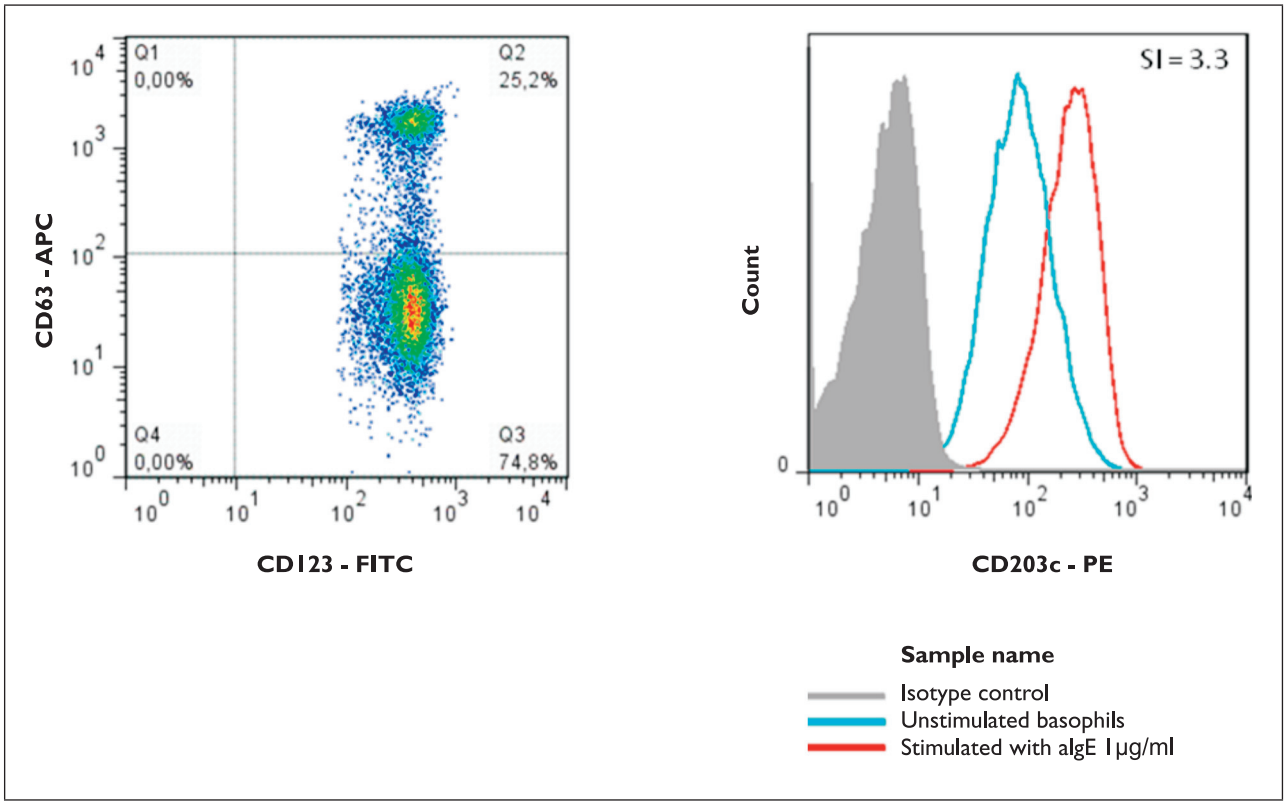

Figure 2. Basophil activation after stimulation with 1µg/ml anti-IgE results in expression of CD63 by 25.2% of basophils and in a SI CD203c of 3.3. Basophils were gated as SSClow, CD123+ and HLA-DR- cells.

sitivity and specificity. The interpretation of results should always be tailored to each individual case. The response in a time-course and dose-response manner is an additional important sign of allergen-mediated basophil activation.

Short incubation with IL-3 may increase the sensitivity of the assay and has been used in some studies[25]. IL-3 causes nonspecific increase in CD203c expression but not CD63. However, it may be a cause for false positive results[26], one reason for that being the concentrations of IL-3 that are used which are much higher than the physiological ones.

The molecular mechanisms governing basophil activation are complex and not entirely clarified. Traditionally, analysis of signalling is based on western blot and ELISA techniques, which represent a mean value for the total isolated cell population[6]. Recently, a proof of concept was provided that flow cytometry may be used to quantify phosphorilation of p38-MAPK in basophils[27]. Similar methods may be used to evaluate consecutive phosphorilation of the proteins involved, as has been done for other cells and signalling pathways. Flow cytometry offers various advantages over the traditional techniques. It allows identification of cells with heterogeneity in responsiveness, it combines surface with intracellular staining and integrates immunophenotyping of individual cells. Flow cytometry enables to study the cells in their natural environment, avoiding basophil purification and potential interference from additional manipulations. Furthermore, this novel technique also significantly shortens the time of analysis from days to hours and reduces the sampling volume considerably, rendering it more accessible for clinical and research applications.

CLINICAL APPLICATIONS

Within certain limits, basophil assays reproduce IgE mediated allergic reactions *in vitro*; therefore, they may be useful for the diagnosis and monitoring of allergic diseases, namely after interventions such as allergen specific immunotherapy and anti-IgE treatment. Gober et al[28] studied a group of patients allergic to insect venom and collected blood before and after sting challenge to assess the expression of basophil activation markers after stimulation with insect venom and to compare activation marker expression after allergen stimulation *in vivo* and *in vitro*. Despite some methodological drawbacks[29], patient heterogeneity and the fact that allergen stimulation *in vitro* resulted in greater basophil activation compared to what happened after *in vivo* challenge, there was a general agreement between clinical presentation and the results of BAT. Basal CD63 expression and upregulation of CD69 and CD203c expression was greater in patients with a history of systemic reaction on immunotherapy. This study suggests that basophil activation markers are useful biomarkers of anaphylaxis.

The interest for BAT in the diagnosis of various allergic diseases is growing, namely of pollen, cat, food, drug and venom allergies[30-39]. This test is particularly important in cases where skin prick test and serum specific IgE determination give equivocal results discordant with the clinical history. Interestingly, Ocmant et al[12] showed that BAT discriminated between allergic and non-allergic subjects among patients sensitised to egg or peanut, highlighting the advantage of BAT over methods that only detect specific IgE antibodies. BAT has shown to be useful also in the diagnosis of chronic urticaria and in the detection of autoantibodies in a subgroup of these patients[40].

BAT has proven to be helpful in assessing the acquisition of tolerance to foods in food allergic children. In a recent study by Sampson and colleagues, tolerance to extensively heated milk (HM) was assessed by oral food challenge (OFC) among children with milk allergy[41]. Patients with negative OFC to extensively HM who reacted to unheated milk were considered to have "HM tolerance", an intermediate clinical phenotype between milk allergy and milk tolerance. Basophils of HM tolerant patients showed lower reactivity *in vitro* compared to HM reactive patients[42]. Basophil reactivity was recovered in the absence of autologous serum and progressively decreased with increasing concentrations of the serum from HM tolerant patients, suggesting that a serum factor was responsible for the inhibition of basophil reactivity to milk allergens[42]. BAT may also be useful in determining when to safely perform an oral food challenge to assess tolerance and reintroduce the food in the child's diet. In a recent study by Rubio et al[43], BAT showed a sensitivity of 91%, a specificity of 90% and positive and negative predictive values of 81 and 96% in detecting children with persistent cow's milk allergy. These values are greater than the ones of serum specific IgE and skin prick test usually used in clinical practice. Similar approaches may be used for other foods.

In patients undergoing allergen-specific immunotherapy, loss of allergic reactivity in BAT is observed in parallel to clinical improvement. Similar findings have been reported in patients undergoing immunotherapy to respiratory allergens[44,45], food allergens[46] and insect venom[47]. Some studies have suggested that BAT can predict clinical sensitivity and that the expression of CD63 on basophils may be useful in deciding when to stop venom immunotherapy [48-50]. BAT may also prove to be very useful in monitoring patients undergoing treatment with omalizumab. In a study of seven patients treated with omalizumab and 27 allergic patients not treated, Nopp et al[51] showed that the basophil sensitivity, given by a formula based on the allergen concentration that elicited 50% of the basophil maximal reactivity, was a good quantitative measure of efficacy of this treatment.

Recent studies have reported very interesting observations that point out the potential of BAT not only in improving the diagnosis of allergic diseases but also in unravelling some of the unsolved questions about atopic diseases and clinical reactivity in sensitised patients. Basophils of atopic when compared with non atopic patients show an activated profile as happens with patients with chronic urticaria[52]. This *in vivo* priming reflects ongoing basophil activation. Interestingly, basal expression of CD203c

has shown to be increased in patients with uncontrolled asthma and frequent asthma exacerbations[53]. These and other studies pave new avenues in the use of BAT for research of immunological mechanisms of allergic diseases.

CONCLUSION

Basophil mediator release and basophil activation tests are assays that reproduce IgE mediated reactions *in vitro*. They have the potential of not only improving the diagnosis and follow-up of patients with various allergic diseases or undergoing allergen specific immunotherapy but also of helping with research into the immunological mechanisms of allergy.

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PORTUGUESA

Patch testing with corticosteroids during a ten-year period

Testes epicutâneos a corticosteróides num período de 10 anos

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ABSTRACT

Background: *Contact allergy to corticosteroids is rare and requires a high index of suspicion and clinical experience in interpreting the results of path testing.* **Aim:** *To characterise patients sensitised to corticosteroids on patch testing.* **Methods:** *We conducted a ten-year retrospective study (May 1999-April 2009) on 2323 patients (715 males/1588 females) patch tested for suspected contact allergy. All patients were tested with a Baseline Series including budesonide 0.1%pet (BUD), hydrocortisone-17--butyrate 0.1%pet (HCB) and tixocortol-21-pivalate 1%pet (TIX) (Chemotechnique Diagnostics). In addition, 136 patients were also tested with 12 other corticosteroid molecules. Readings were performed on second and fourth days, with open referral for late reactions on day 7. Patients reacting to at least one corticosteroid were evaluated regarding demographic and clinical data, and patch test results.* **Results:** *35 patients (1.5%), mean age 53±16 years, 71% female, reacted to at least one corticosteroid within the Baseline Series, 28 to BUD, 14 to HCB and 5 to TIX. No additional patient was detected with the corticosteroid series, most reactions to new molecules occurring to alclometasone, amcinonide and hydrocortisone. Among the 35 reactive patients, 57%, 28%, 9% and 6% reacted respectively to 1, 2, 3 or more corticosteroid molecules, mainly to group B (49%), D2 (25%) and A (16%), with 15 patients reacting to corticosteroids from different groups. Positive results were considered clinically relevant in 55% of cases. Relevance was not found in 47% of patients reacting to BUD.* **Conclusions:** *A Baseline Series including BUD, HCB and TIX was efficient in detecting corticosteroid-sensitised patients. Aimed testing revealed sensitisation to new molecules and allowed improving diagnosis and patient counselling. The pattern of sensitisation extended beyond the usually considered groups of corticosteroids.*

Key-words: allergic contact dermatitis, contact dermatitis, topical corticosteroids, patch tests, skin tests

RESUMO

Introdução:*A alergia de contacto a corticosteróides é rara, exigindo elevado nível de suspeição e experiência clínica na interpretação dos resultados dos testes epicutâneos no seu diagnóstico.* ***Objectivo:****Caracterizar doentes sensibilizados a corticosteróides em testes epicutâneos.* ***Métodos:****Analisaram-se retrospectivamente resultados dos testes epicutâneos de 2323 doentes com suspeita de dermatite de contacto alérgica, realizados entre Maio 1999 e Abril 2009. Todos os doentes foram testados com uma Série Básica, incluindo budesonido 0,1% vas(BUD), 17-butirato de hidrocortisona 0,1% vas(BHC) e 21-pivalato de tixocortol 1% vas(TIX)-Chemotechnique Diagnostics. Em 136 doentes foram testadas outras 12 moléculas de corticosteróides. As leituras realizaram-se em D2 e D4 e em D7 se reacções tardias. Para os doentes que reagiram a pelo menos um corticosteróide, analisaram-se os dados demográficos e clínicos e os resultados dos testes epicutâneos.* ***Resultados:****35 doentes(1,5%), 53±16 anos, 71% sexo feminino, reagiram a pelo menos um corticosteróide na Série Básica: 28 ao BUD, 14 ao BHC e 5 ao TIX. A série de corticosteróides não permitiu identificar mais nenhum doente sensibilizado a corticosteróides, tendo a maioria das reacções a outras moléculas ocorrido a alclometasona, amcinonido e hidrocortisona. Dos 35 doentes, 57%, 28%, 9% e 6% reagiram respectivamente a 1, 2, 3 ou mais moléculas, maioritariamente do grupo B(49%), D2(25%) e A(16%). Em 55% dos doentes, os resultados positivos foram considerados relevantes para a dermatite actual ou passada. Entre estes, as lesões eram maioritariamente de eczema de contacto e as patologias concomitantes mais frequentes eczema crónico, úlcera de perna, asma e rinite. Em 37% dos doentes sensibilizados ao BUD, não se encontrou qualquer relevância clínica.* ***Conclusões:****A Série Básica incluindo BUD, BHC e TIX foi adequada na identificação de doentes sensibilizados a corticosteróides. A série de corticosteróides revelou sensibilização a novas moléculas e permitiu melhorar o diagnóstico e aconselhamento dos doentes. O padrão de sensibilização estendeu-se além dos grupos previamente estabelecidos.*

Palavras-chave: *dermatite de contacto, dermatite de contacto alérgica, corticosteróides tópicos, testes cutâneos, testes epicutâneos*

INTRODUCTION

Contact allergy to corticosteroids is rare and is usually a side effect of the topical treatment of various dermatoses. It can occur either early or late during the course of the cutaneous disease and corticosteroid treatment. In the study of contact allergy, corticosteroids are usually classified, according to their chemical structure, into four groups and two subgroups (Table I), to make identification of possible cross-reactivity easier[1,2]. A correct diagnosis of contact allergy to corticosteroids demands a high index of suspicion and clinical experience in interpreting the results of patch tests. Performing patch tests to corticosteroids should be particularly considered in cases where a reduced effectiveness of topical corticosteroids or a worsening of the underlying dermatosis is seen with the treatment. However, and to obviate a low index of suspicion, recommendations are to include two or three corticosteroids able to detect the greater part of cases of contact allergy to topical corticosteroids in both the **European Basic** Standard **Series** and the **Portuguese Baseline Series** of contact allergens. Clinical experience is important in interpreting the results of patch tests to corticosteroids. Late readings, that is, a week later, should not be neglected, as these molecules' anti-inflammatory effect means a contact hypersensitivity reaction tends to have a late onset. Further, reactions presenting on the second or third day only with erythema and which are the result of drug activity on vascular tonicity should not be taken as positive.

Although contact allergy to corticosteroids is a recognised complication of topical corticosteroid treatment, there are only a few systematic series and analyses published, particularly in Portugal, which makes defining and mapping this clinical entity difficult. Our aim in this study was to characterise patients sensitised to corticosteroids on patch testing over a ten-year period.

METHODS

The sample consisted of 2323 patients, 715 males and 1588 females, who underwent patch testing at the Dermatology Department of the Hospitais da Universidade de Coimbra for suspected allergic contact dermatitis over the ten-year period from the 1st of May 1999 to the 30th of April 2009. All patients received testing with the Portuguese Baseline Contact Dermatitis Study Series, which included budesonide 0.1%pet (BUD), hydrocortisone-17-butyrate 0.1%pet (HCB) and tixocortol-21-pivalate 1%pet (TIX). In 136 patients with suspected contact allergy to corticosteroids, a complementary series of corticosteroids was also tested. These included 12 corticosteroid molecules, namely: prednisolone 1%pet, hydrocortisone 1%pet, dexamethasone-2-phosphate 1%pet, triamcinolone-2-acetonide 1% pet, clobetasol propionate 0.25%pet, alclometasone dipropionate 1%pet, amcinonide 0.1%pet, desoximethasone 2.5%pet, diflucortolone valerate 1%pet, hydrocortisone acetate 1%pet, betamethasone-12-valerate 0.12%pet and betamethasone dipropionate 1%pet. In some cases, commercial preparations in cream or pomade form used by the patients were also tested.

Table I. Classification of topical corticosteroids used in this study[1,2]

Group	Allergen
A	Hydrocortisone Hydrocortisone acetate Prednisolone Tixocortol pivalate
B	Amcinonide Triamcinolone acetonide Budesonide
C	Dexamethasone sodium phosphate Dexamethasone Diflucortolone valerate
D1	Betamethasone-17-valerate Betamethasone dipropionate Alclometasone dipropionate Clobetasol proprionate Mometasone furoate
D2	Hydrocortisone-17-butyrate

Table II. Number of positive patch tests to the corticosteroids tested

Corticosteroids tested	Positive patch tests
Budesonide 0.1%pet*	28
Hydrocortisone-17-butyrate 0.1%pet*	14
Tixocortol-21-pivalate 1%pet*	5
Alclometasone dipropionate 1%pet*	4
Amcinonide 0.1%pet**	2
Betamethasone-17-valerate 0.12%pet*	1
Betamethasone dipropionate1%pet**	1
Clobetasol proprionate 0.25%pet*	0
Dexamethasone-21-sodium phosphate1%pet*	1
Difluorocortolone valerate 1%pet**	0
Hydrocortisone 1%pet**	2
Hydrocortisone acetate 1%pet**	1
Prednisolone 1%pet**	1
Triamcinolone-21-acetonide 1%pet*	0
Desoximethasone 2.5%pet*	0

* Chemotechnique Diagnostics; ** Bial Aristegui.

In the patch tests, the allergens available from Chemotechnique Diagnostics and Bial Aristegui (as shown in Table II) were applied during two days in 8 mm Finn chambers using Scanpor® adhesive tape. Readings were taken on the second and fourth days and patients were also advised to return on the seventh day, to detect any possible late-onset reactions. Test results were evaluated in line with the International Contact Dermatitis Research Group (ICDRG) recommendations. Reactions of or over 1+ (at least with erythema and wheals or infiltration covering the test application area) were taken as positive.

Patients who reacted to at least one corticosteroid in the patch tests were analysed in terms of demographic data (sex and age) clinical data (location and characterisation of the lesions and underlying pathologies) and the patch test results (number of tests positive to corticosteroids per patient, corticosteroids involved and group they belong to, and clinical importance of the patch tests.

RESULTS

Thirty-five patients, 1.5% of the population studied, reacted to at least one corticosteroid in the Contact Dermatitis Baseline Series. This patient group had a mean age of 53±16 years and 71% were female. The most frequently seen sensitisations were to the corticosteroids included in the Baseline Series: 28 patients were sensitised to BUD, 14 to BHC and five to TIX. Performing testing with the complementary corticosteroid series did not identify any other patient sensitised to corticosteroids. The majority of the reactions to molecules not integrating the Baseline Series were to alclometasone (four patients), amcinonide (two patients) and hydrocortisone (two patients) (Table II). The majority of patients (94%) were sensitised to other contact allergens.

Of the 35 patients sensitised to corticosteroids, 57% reacted to only one molecule, 28% to two, 9% to three and 6% to more than three molecules. Group B molecules were in the main involved (49%), as were D2 (25%) and A (16%), with 15 patients (43%) reacting to corticosteroids of different groups (Figure 1).

In terms of the importance of positive skin tests, 55% of patients had results considered clinically relevant to the current or prior dermatitis (Table III). Of these, the lesions were mostly contact eczema, often complicating a pre-existing stasis eczema and/or a leg ulcer. There was also a case of oral pemphigus lesions and another of a late-onset reaction following parenteral corticosteroid administration. The contact eczema lesions, excluding the cases of leg ulcer and stasis dermatitis, were on the hands (eight patients), upper limbs (six patients), face (four patients), feet (four patients), lower limbs (one) and/or trunk (one). The most frequently

Table III. Clinical relevance of the sensitisations detected to the Baseline Series corticosteroids tested

Corticosteroids	Clinical relevance		
	Present	Past	Not relevant or unknown
Budesonide 0.1%pet*	10	5	13
Hydrocortisone-17-butyrate 0.1% pet*	10	3	2
Tixocortol-21-pivalate - 1%pet*	1	0	4

* Chemotechnique Diagnostics.

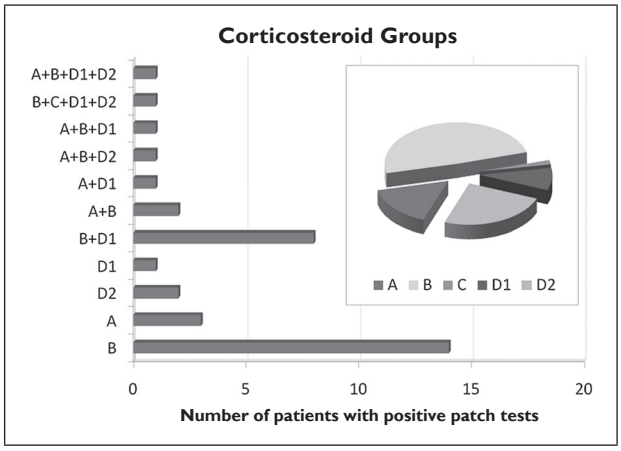
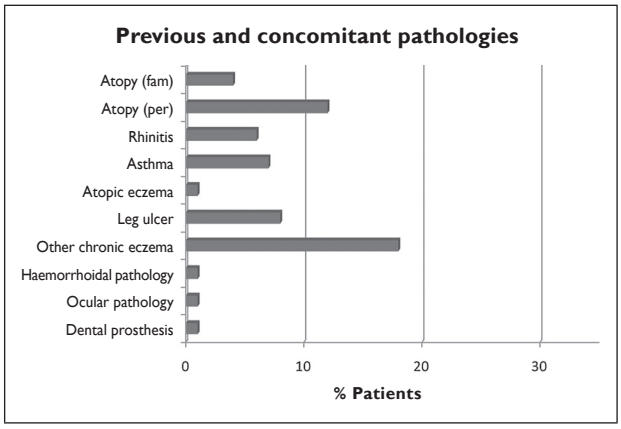


Figure 1. Groups of topical corticosteroids involved in the positive patch tests



AF – family history; AP – personal history; Patol. – pathology.

Figure 2. Concomitant pathologies in patients with positive patch tests to corticosteroids

seen concomitant conditions were chronic eczema, leg ulcer, asthma and rhinitis (figure 2). The greater part of sensitisations not deemed clinically relevant were to BUD (47%).

DISCUSSION

Contact allergy to corticosteroids is uncommon, but has been the subject of a growing number of publications aiming to better define this clinical entity. Our study characterises a patient population sensitised to corticosteroids and concludes that the Baseline Series was suitable for identifying sensitised patients. Performing a complementary series of tests of corticosteroids revealed sensitisation to new molecules and allowed improvement in diagnosis and patient follow-up as the pattern of sensitisation to corticosteroids extended beyond the established groups.

The prevalence of sensitisation to corticosteroids seen in this patient population with suspected contact dermatitis was of 1.5%, similar to the GPEDC study[3], which in 1992 found reactivity in 1.8% of about 6000 patients also with suspected contact dermatitis tested that year in Portugal with a mix of 3 corticosteroids included in the Baseline Series of allergens. Contact allergy to corticosteroids detected was considered clinically relevant in 55% of patients, which corresponded to 0.86% of the total patients who underwent patch testing. In the literature, the prevalence of sensitisation to corticosteroids ranges from 0.2% to 5.8%[4-7]. In a US study which included patients undergoing patch tests to a series of corticosteroids, the prevalence of contact allergy to corticosteroids was 10.69%[8]. Possible explanations for the differences seen in the rates reported at different centres are the index of suspicion of contact allergy to corticosteroids, the decision to perform patch testing and the patient population studied, namely if the prevalence was calculated for the patients undergoing patch tests overall or for patients with suspected corticosteroid allergy. Other reasons for the differences seen concern the selection of molecules to be tested, performing or not performing a specific corticosteroid series, the vehicles selected, the drug concentrations and the test methods used and the consideration of macular erythema without wheals or infiltration as a positive result, as was the case in the abovementioned US series[8]. Differences in

the prevalence of sensitisation to corticosteroids at different geographical locations may also be connected to the different molecules available on the market and prescription habits in the region[9], in that fluorinated corticosteroids seem to be less allergenic[10], and the underlying pathologies in the patient population, namely the prevalence of patients with leg ulcer and stasis dermatitis[11].

In effect, contact allergy to topical corticosteroids seems to be more frequent in patients with leg ulcer and stasis dermatitis, something also seen in the patient group we studied. Probably due to the chronicity of the inflammatory dermo-epidermal lesions and increasing hydration of the stratum corneum, which increases the penetration of corticosteroid molecules, their presentation as antigens by the Langerhans cells is highly facilitated. Other underlying pathologies seen were asthma and rhinitis, particularly connected to the sensitisation considered clinically irrelevant to BUD, which in the majority of cases only signifies exposure to the drug. However, a possible airborne exposure to BUD drops in aerosols containing this drug in a workplace environment or via proximity to family members who use them was then not investigated[12,13].

The Baseline Series, which includes TIX, BUD and BHC was adequate to identify the patients sensitised to corticosteroids. In 1989, Dooms-Goossens identified TIX as a good marker of sensitisation to corticosteroids[14]. In a study by Boffa *et al.*[15] TIX and BUD allowed identification of over 90% of the patients sensitised to corticosteroids. Other later studies showed the value of the association of TIX, BUD and BHC in detecting patients sensitised to these drugs[3, 16, 17]. This reflects the fact that TIX is a good marker of group A corticosteroids, BUD of group B and BHC of group D and that sensitisation to group C corticosteroids is very rare. The mechanism by which this last group of corticosteroids rarely induces sensitisation remains to be elucidated and might be related to differences in the allergenicity of the molecules or with the routes of administration commonly used for drugs of this group (such as, for example, via the eyes, nose or mouth), which could promote tolerance induction[18]. Although it did not identify new patients sensitised to corticosteroids, the performance of a series of tests to corticosteroids revealed sensitisation to new molecules and allowed for improved diagnosis and recommendations to patients about the alternatives to the drugs involved in contact allergy. Also relevant is the use of products used by the patient him/herself, namely in establishing the clinical relevance of the sensitisations discovered[19].

In an attempt to make it easier to identify possible cross-reactivities with the specific corticosteroid responsible for the contact allergy in each individual case studied, the topical corticosteroids were classified in line with their chemical structure based on a literature review and a descriptive study of 15 cases of contact allergy to these drugs[1]. However, this classification has been contested, seeing as cross-reactivities depend on more factors than the chemical structure of the drug applied to the skin. The degradation and metabolisation of these steroid molecules can generate new molecules with different chemical structure and new potentials in terms of immunologic reactivity, in so far as the new molecules can present cross-reactivity with other corticosteroids which the molecule whence they derive did not present. We found the pattern of sensitisation to corticosteroids extended beyond the groups previously established. This reflects the fact that while dividing corticosteroid molecules into groups is useful, it cannot be considered watertight, as cross-reactivity between drugs of different groups could exist, namely by isomers, as happens between the B and A groups with the D2 group. Further, the likelihood of a patient being cosen-

sitised to different drugs must also be considered. It is thus vital to test molecules of different groups when sensitisation to corticosteroids is detected to better define which drugs can be used in the future as an alternative to those which cause contact allergy.

CONCLUSION

Contact allergy to corticosteroids is infrequent and demands a high index of suspicion. The possibility of clinically irrelevant sensitisation demands a detailed clinical history and careful establishing of the clinical importance of the sensitisations found, meaning that testing with the patient's own products can be useful. In patients sensitised to the corticosteroids in the Baseline Series, it is important to test a specific series of corticosteroids to improve diagnostic rigour and identify alternative drugs that the patient can use in the future.

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PORTUGUESA

Accidental exposures in food allergy

Exposições acidentais na alergia alimentar

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ABSTRACT

Background: The usual recommendation in food allergy treatment is strict eviction until tolerance is established. It is important to know how an eviction diet fails in order to improve the information we give to food-allergic patients. **Objective:** *To identify the frequency and to characterise accidental food exposures in a group of food-allergic patients.* **Material and methods:** Children aged ten years or less, allergic to cow's milk proteins, egg, fish, peanut or nuts were selected from the files of patients followed up at the Allergology and Clinical Immunology outpatient clinic of Hospital Dona Estefânia. Parents/caregivers answered a questionnaire regarding the culprit food, symptoms and characterisation of accidental exposures. **Results:** We selected a group of 65 patients, with a median age of 4.3 years (63% males), corresponding to 69 cases of food allergy – 42 cases of cow's milk protein allergy, 11 cases of fish allergy, 10 cases of egg allergy, five cases of nut allergy and one case of peanut allergy. The first reaction occurred, in most cases, by ingestion (95.6%) and was immediate (78.3%). Symptoms were mucocutaneous in 75.4%, gastrointestinal in 33.3% and respiratory in 23.2%. Anaphylaxis occurred in 17%. The eviction diet failed in 68.1% cases, which corresponded to 68 accidental exposure occurrences, most with symptoms (87.1%). Of these 68 accidental exposures, the culprit food was cow's milk in 69.1% (n = 47), egg in 14.7% (n = 10), fish in 13.2% (n = 9) and nuts in 2.9% (n = 2). Mucocutaneous manifestations were the most frequent (55.9%), followed by respiratory symptoms (25%) and gastrointestinal symptoms (23.5%). Anaphylaxis occurred in 20.5%. Most accidental exposures were at home (36.8%) and at school (29.4%). After the reaction, parents/caregivers administered medication in 41.2% of cases, waited until spontaneous resolution took place in 38.2% and went to Emergency Room Departments in 20.6%. **Conclusion:** Eviction diet failures were frequent, most of them with symptoms. Most accidental exposures occurred at home and at school. This may indicate gaps in parents'/care givers' knowledge. The characterisation of accidental exposures in

food-allergic patients may help to improve the information transmitted to parents/caregivers to help them identify risk factors and be aware of avoidance measures.

Key-words: *contact, food allergy, exposure, ingestion, accidental reaction*

RESUMO

***Introdução**: A recomendação habitual no tratamento da alergia alimentar é a evicção completa, até à aquisição de tolerância. É importante perceber em que situações ocorrem falhas na evicção, de forma a orientar o melhor possível o doente com alergia alimentar. **Objectivo**: Conhecer a frequência e caracterizar as exposições acidentais, num grupo de doentes com alergia alimentar. **Material e Métodos**: A partir dos registos do Serviço de Imunoalergologia do Hospital Dona Estefânia, foram seleccionados doentes com idade ≤ 10 anos, com alergia às proteínas do leite de vaca, ovo, peixe, amendoim ou frutos secos. Os pais/prestadores de cuidados, responderam a um inquérito telefónico, referente ao alimento implicado, falhas na dieta e sintomas. **Resultados**: Contactou-se um grupo de 65 doentes com idade média de 4,3 anos (63% do sexo masculino), totalizando 69 casos de alergia alimentar – cerca de 42 casos de alergia ao leite, 11 casos de alergia ao peixe, 10 de alergia ao ovo, 5 de alergia aos frutos secos e 1 de alergia ao amendoim. Na maioria dos casos a 1.ª reacção foi desencadeada por ingestão (95,6%) e foi imediata (78,3%), manifestando-se por sintomas mucocutâneos (MC) em 75,4%, gastrointestinais em 33,3% e respiratórios em 23,2%. Ocorreu anafilaxia em 17%. Houve falhas na dieta em 68,1% dos casos, que contabilizaram um total de 68 eventos de exposição acidental, na maioria (87,1%) com sintomas. Destes 68 eventos de exposição acidental, em 69,1% (n=47) o leite foi o alimento implicado, em 14,7% (n=10) foi o ovo, em 13,2% (n=9) o peixe e em 2,9% (n=2) os frutos secos. As manifestações clínicas mais frequentes foram MC (55,9,9%), seguindo-se as do tracto respiratório (25%) e as do tracto gastrointestinal (23,5%). Em 20,5% dos eventos de exposição acidental, ocorreu reacção anafiláctica. A maior parte das ingestões / exposições acidentais ocorreram em casa (36,8%) e na escola (29,4%). Perante a reacção foi administrada terapêutica em 41,2%, aguardaram resolução espontânea 38,2% e recorreram ao Serviço de Urgência 20,6% dos casos. **Conclusões**: As falhas na dieta de evicção foram frequentes, a maioria com sintomas. Aconteceram maioritariamente em casa e na escola, o que pode sugerir lacunas no conhecimento dos pais/prestadores de cuidados. A caracterização das exposições acidentais nos doentes com alergia alimentar poderá ajudar a optimizar a transmissão de informação, a estes e aos seus responsáveis, relativamente à prevenção de situações de risco.*

***Palavras-chave:** alergia, alimentar, contacto, exposição, ingestão, reacção acidental*

INTRODUCTION

The prevalence of food allergy has been increasing in western countries, namely in paediatric age individuals[1]. It is estimated at being around 6-8% in childhood[2]. The foodstuffs most frequently involved are cow's milk, egg, fish, soya, wheat, shellfish, peanut and nuts. Currently, the only safe recommendation in treating food allergy is complete eviction until tolerance is aquired[3,4].

Accidental ingestion and exposure are a constant source of worry for patients and their families, given that they can lead to potentially fatal reactions, which can bring with it a

heavy emotional burden[5,6]. Challenges such as reading foodstuff labels or the care involved in avoiding cross-contamination occur daily, as do the potential limitations to social activities which involve food, such as school, eating out, visiting friends and family. This has a negative impact on these patients' quality of life[7,8]. Further, patients and their parents/caregivers have to be instructed about what to do in case of accidental exposure[3,9]. It is important to understand in which situations accidental exposure can occur, to better guide the daily life of a patient with food allergy. Health professionals' knowledge of patients' day-to-day reality can improve the information transmitted to parents/caregivers to help them identify risk factors and be aware of avoidance measures.

Our aim was to study the frequency of accidental exposure (ingestion/contact/inhalation) in a group of patients with food allergy and characterise accidental food exposure, what led to it and reactions to it, in order to improve the information we give to food-allergic patients and tailor it to their reality.

MATERIAL AND METHODS

Children aged ten years or less, allergic to cow's milk proteins, egg, fish, peanut or nuts were selected from the files of patients followed up at the Allergology and Clinical Immunology outpatient clinic in Hospital Dona Estefânia. Parents/caregivers were contacted by phone and asked to answer a questionnaire. The questionnaire (Table I) mapped out demographic data, the trigger foodstuff(s), personal and family history of allergic disease, characterisation of the first reaction (how it was triggered, its clinical manifestations, time lag between exposure and reaction) and failure in the diet, characterisation of accidental ingestions in terms of number, the place and manner in which they occurred, how the food was presented (obviously or hidden) and parents'/caregivers' attitudes to the reaction.

Some patients were allergic to more than one foodstuff simultaneously. In order to analyse the results, we defined as a "case" each food allergy in each patient, meaning the number of cases is higher than the number of patients. Unlike demographic data, which is based on the number of patients, all the remaining analysis of results is based on the number of cases of food allergy or the number of occurrences of accidental exposure.

RESULTS

We contacted the parents of 65 patients, 62 of which had allergy to only one foodstuff, two to two foodstuffs and one to three. Hence the analysis deals with a total of 69 cases of food allergy.

Of the demographic data (Table II), we highlight the predominance of males and that over half the patients had personal and family histories of another allergic disease, although only 9.2% had family history of food allergy.

The 69 cases of food allergy consisted of 42 cases of cow's milk allergy, 11 cases of fish allergy, 10 of egg allergy, five of nut allergy and one of peanut allergy. The first reaction was triggered by ingestion in 95.6% (66/69) of cases and by contact in 4.4% (3/69) of cases. In the majority of cases (78.3%), the reaction was immediate, occurring within less than 30 minutes in 60.9% (42/69) and within 30 minutes to two hours in 17.4% (12/69). In the remaining 21.7% (15/69) cases, the reaction manifested after two or more hours. In terms of the initial clinical manifestation, mucocutaneous (MC) symptoms were predominant (75.4%; 52/69), followed by gastrointestinal (GI) (33.3%; 23/69) and respiratory (R) symptoms (23.2%; 16/69). Mucocutaneous symptoms were

Table I. Questionnaire

Clinical File:_____ Date of Birth:___/___/_____ Sex: M F	
Father Mother Other _____ Reliability: Good Reasonable Poor	
Personal History of Allergy: No Eczema Rhinitis/Conjunctivitis Asthma	
Family history: Atopy: Yes No Food allergy: Yes No	
FOODSTUFF	
First reaction	Symptoms
Age	
Triggered by: Ingestion _____ Contact _____ Inhalation_____ Other _____	Mucocutaneous (MC) _____ Gastrointestinal (GI) _____ Respiratory (R) _____ Cardiovascular (CV) _____ Other _____
Exposure – reaction interval:_____	
Failure of eviction diet: Yes ☐ With symptoms ☐ No symptoms ☐ No ☐	

ACCIDENTAL EXPOSURES

	Age	Place	Trigger	Symptoms	Foodstuff identified	Hidden Foodstuff	Quantity	Attitude
1.ª								
2.ª								
3.ª								
4.ª								
5.ª								
6.ª								

Place: Home (H), Restaurant (R), School (S), House of family member/friend (HF/F), Other (O)
Triggered by: Ingestion (Ing), Contact (C), Inhalation (Inha), Other (O)
Symptoms: Mucocutaneous (MC) – pruritus, erythema, urticaria, angiooedema, eczema, oral allergy syndrome; Gastrointestinal (GI) – nauseas, vomiting, diarrhoea, colic, haematochezia, poor ponderal progression; Respiratory (R) – rhinitis, conjunctivitis, cough, wheeze, stridor, oedema of the glottis, dyspnoea; Cardiovascular (CV) – sweating, palidity, cyanosis, tachycardia, palpitations, hypotension, shock.

Table II. Sample characterisation (n = 65 patients)

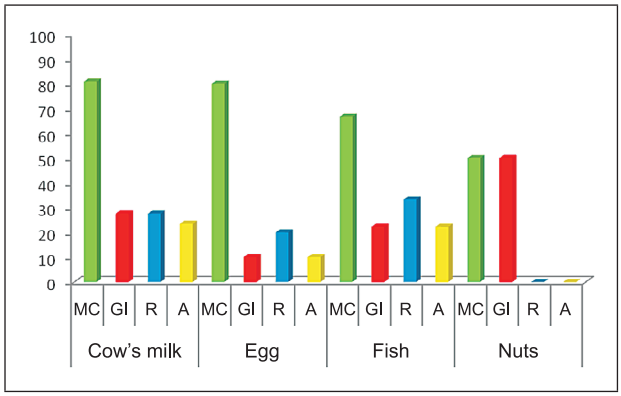
Age	Mean age: 4.3 years (minimum: 9.5 months; maximum: 10 years)
Gender	Male: *n* = 41 (63%) / Female: *n* = 24 (37%)
Personal history of allergic disease	Yes: *n* = 41 (63%) / No: *n* = 24 (37%)
Family history of allergic disease	Yes: *n* = 42 (64,6%) / No: *n* = 23 (35,4%)
Family history of food allergy	Yes: *n* = 6 (9,2%) / No: *n* = 59 (90,8%)

mainly urticaria and/or angio-oedema (36/52 cases), followed by atopic eczema in only 10 cases and cutaneous erythema in six. Around 17.4% (12/69) of cases were anaphylaxis, with mucocutaneous and respiratory involvement in seven cases, mucocutaneous and gastrointestinal in three and mucocutaneous, respiratory and gastrointestinal in two. No case had involvement of the cardiovascular system.

Failures in the eviction diet that had been started when the food allergy was diagnosed occurred in 68.1% of cases (47/69) and in the majority of these cases symptoms occurred (87.1%; 41/47). We highlight that in only around one-third of cases (31.9%; 22/69) was the eviction diet maintained with no apparent failures. In the cases of cow's milk allergy, there were failures in the eviction diet in 73.8% (31/42) cases; in the cases of fish allergy in 81.9% (9/11); in egg allergy there were failures in 50% (5/10) cases; in the cases of peanut/nut allergy there were failures in 33.3% (2/5) cases.

In the 47 cases of food allergy in which there were failures in the eviction diet, it was possible to count a total of 68 accidental exposures to the foodstuff in question. In terms of the origin of these accidental exposures, cow's milk was the trigger foodstuff in 69.1% (47/68), egg in 14.7% (10/68), fish in 13.2% (9/68) and nuts in 2.9% (2/68). These accidental exposures (*n* = 68) occurred mainly by ingestion (77.9%; 53/68), less by cutaneous contact (20.6%; 14/68) and rarely by inhalation (1.5%; 1/68). The majority of cases in which accidental exposure was by ingestion suffered only 1 episode (*n* = 22); in eight cases there were 2 episodes and in five cases there were 3 episodes of accidental ingestion.

The most frequent clinical manifestations in accidental exposure occurrences were, just as in the initial reaction, 55.9% (38/68) MC, followed by respiratory tract reactions (25%; 17/68) and gastrointestinal tract reactions (23.5%; 16/68). Figure 1 shows the type of clinical reaction for each foodstuff. In 14 cases (20.5%) there were anaphylactic reactions. Of these, there was mucocutaneous and respiratory involvement in five cases; mucocutaneous and gastrointestinal in four; mucocutaneous, respiratory and gastrointestinal involvement in three; and gastrointestinal and respiratory involvement in two. No case had involvement of the cardiovascular system. Eight of the 14 accidental exposure events with anaphylaxis occurred in patients whose initial manifestation was anaphylaxis.



Legend: MC – mucocutaneous; GI – gastrointestinal; R – respiratory; A – anaphylaxis

Figure 1. Clinical manifestations in accidental exposure

We investigated whether the foodstuff was identified or hidden, in the accidental exposures. In the case of cow's milk and fish, the foodstuff was visually identifiable in around half of the cases (cow's milk 51%, fish 56%). Egg and nuts were mostly hidden (egg 70%, nuts 100%), meaning they had been used as an ingredient but their physical presence was not evident. Figure 2 shows these data.

The majority of the 68 accidental exposures occurred at home (36.8%; 25/68) or at school (29.4%; 20/68). They occurred less often in the house of a family member or friend (17.6%; 12/68), and rarely in restaurants (7.4%; 5/68) or other public places (8.8%; 6/68). In terms of their circumstances, there was some variability as to the foodstuff in question, as Table III shows.

Table III. Circumstances in which accidental exposure occurred

Foodstuff	Cause of accidental exposure	n
Cow's milk (*n* = 47)	Hidden use	23
	Sweets (cakes, biscuits, desserts, ice creams)	12
	Savouries (sauces, rissoles)	9
	Soya yoghurt with milk	1
	Soothing balm for first teeth	1
	Swaps (baby cereal, milk, yoghurt)	10
	Milk derivatives	7
	Cutaneous contact	5
	Others	2
Egg (*n* = 10)	Sweets (chocolate, biscuit, croissant, desserts)	5
	Cutaneous contact	3
	Dishes made with egg	2
Fish (*n* = 9)	Ingestion of fish (lack of information to third party)	4
	Foodstuffs cooked together with fish	3
	Cutaneous contact	1
	Inhalation of steam during cooking	1
Nuts (*n* = 2)	Biscuits	2

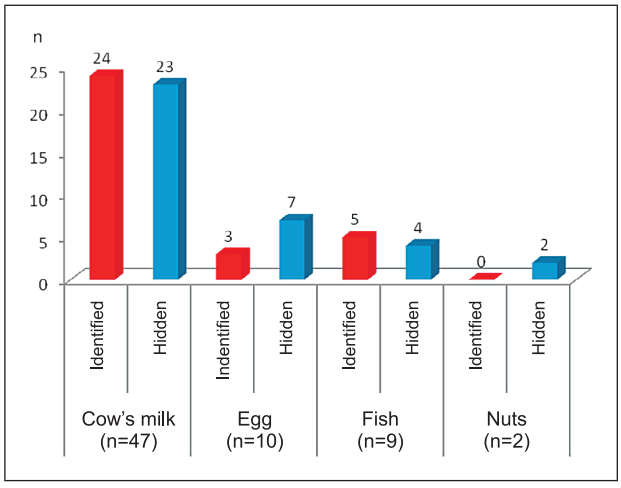


Figure 2. Identification of the trigger foodstuff

The hidden use of cow's milk or its derivatives, mainly in sweets and savouries, was the most frequent cause of exposure to this foodstuff, and was responsible for 23 occurrences (Table III). The swapping with foodstuffs containing cow's milk proteins, such as milk, baby cereals and yoghurts, which look identical to those which do not contain cow's milk proteins (for example, similar packaging) and the non-recognition (or forgetting) that cow's milk derivatives must also be excluded from these patients' diets was also frequent. Other more particular situations are labelled *Others* and include: the case of a child whose dummy was washed in a steam heater usually used to heat the milk in espresso coffee machines and which triggered peri-bucal urticaria due to probable contamination; the case of a child who developed urticaria symptoms after being in a swimming pool in which another child had vomited after ingesting milk.

In cases of egg allergy, the reasons given for the accidental exposure incidents were the use of egg in sweet or savoury dishes and accidental contact with egg in the kitchen during the preparation of meals.

Concerning fish, the most frequently reported symptoms were ingestion of fish due to a failure to transmit information to people taking care of the child, in addition

to the ingestion of food cooked together with fish. Table III shows other more particular cases. The situation triggered by cutaneous contact occurred at Lisbon Zoo and was caused by a sea lion giving a "kiss" to a child during the sea lions' feeding time (the sea lions are fed with fish). The reaction seen in this case was contact urticaria.

Reactions to nuts were triggered by ingestion of biscuits containing nuts.

The attitude in response to the reaction was to administer emergency treatment in 41.2% (28/68) of cases, wait for spontaneous resolution in 38.2% (26/68) and Emergency Room (ER) visits in 20.6% (14/68). The most frequently used medicine was H1-antihistamine (25%; 17/68), followed by oral corticosteroids (17.6%; 12/68), while there was less frequent recourse to short-acting bronchodilator (13.2%; 9/68). There was only one case of adrenaline being administered (1.5%; 1/68) by healthcare professionals at the ER.

DISCUSSION

This study aimed to identify the frequency of and to characterise accidental food exposures in a group of food-allergic paediatric patients. The results show that in our patients, accidental foodstuff ingestion is common and very often accompanied by symptoms of greater or lesser severity.

There were failures in the eviction diet in 68.1% of cases. We found no similar studies in the literature, meaning it was difficult to compare our data. A Canadian study, however, specifically analysing the role of foodstuff labels, found that 47.8% of patients had experienced accidental exposures[10]. These were mainly patients with peanut allergy and around half the sample was recruited from food-allergy patients' associations, which could explain the lower percentage of eviction diet failure. Additionally, the same study reported that patients allergic to peanut, nuts, fish or shellfish had a lower percentage of accidental exposure than those allergic to other foodstuffs. This difference can be explained by the fact that those foodstuffs give rise to more severe reactions, leading to greater care being taken in their identification by both the food industry and the patient[10].

In our patients, the majority of the accidental exposures led to a clinical reaction (87.2%) and, unlike what is usually reported[9], they occurred more frequently at home or at school, that is, as part of the child's daily routine. They occurred more rarely in restaurants or at friends' or family members' houses. It could be that vigilance is greater in situations typically seen and taught as being those containing risk, thus effectively reducing the occurrence of accidental exposure episodes at these places. A day-to-day domestic environment is probably more conducive to relaxing one's vigilance.

As regards the form of exposure, in addition to the situations usually reported in the literature – the hidden presence of the foodstuff and failures in reading product labels[3,9,10] – we identified some others which often occur. We highlight the swapping for foodstuffs of the same type and with similar packaging (baby cereal, milk, yoghurts), the lack of knowledge (or forgetting) that foodstuffs of the same family and derivatives contain the trigger allergen (for example, cow's milk derivatives) and finally, failures to pass on the information to third parties (family members, members of school staff, nannies). The remaining cases, which illustrate more unusual forms, are also important as knowledge of these and dissemination of that knowledge could help prevent similar situations in other patients.

The data we obtained seem to suggest that the information transmitted to parents/caregivers might not be sufficient and that there is a need to pass on more and better information and duly educate parents/caregivers to avoid the trigger foodstuff in food-allergy cases.

A multidisciplinary approach is important in at least some cases of children with food allergy, with support from a nutritionist/dietician, to improve the information given to parents/caregivers. In addition, and as is the case in some chronic diseases, patients and parents/caregivers can benefit from support groups with whom to share experiences of concrete situations[11]. There are as yet no food-allergy support groups in Portugal.

In terms of the attitude taken to the reaction, only a fifth of patients needed to go to the ER. Of the remaining, around half had spontaneous resolution – most likely the cases with less severe reactions – and the other half were resolved with antihistamines, corticosteroids and/or bronchodilators, which could mean that the majority of parents/caregivers are well informed as to how to deal with an acute reaction. There is some evidence that patients with a history of more severe reactions take greater care in avoiding accidental exposures[10]. This could also contribute to the seemingly low frequency of severe reactions in the sample studied.

The almost nil recourse to adrenaline, despite 20.5% of the accidental reactions reported being anaphylactic, is in line with data seen in other studies, which also found a lower than desirable use of this drug in emergency situations[12]. That said, we admit that there could have been some use of adrenaline in the children who went to the ER, with their parents being unaware of this fact.

CONCLUSIONS

There were frequent failures in the eviction diets, the majority with symptoms. Accidental exposures occurred mainly as part of a child's everyday routine, which could indicate gaps in parents'/caregivers' knowledge.

A mapping of accidental exposures in a sample of patients with food allergy can help to improve the information transmitted to patients and their parents/caregivers to help them identify risk factors and be aware of avoidance measures.

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Toxic epidermal necrolysis – sodium valproate and vancomycin?

Necrólise epidérmica tóxica – Valproato de sódio e vancomicina?

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ABSTRACT

The prevalence of adverse drug reactions (ADR) in hospitalised patients is estimated at 10-20% and can be potentially life-threatening. Toxic epidermal necrolysis (TEN) is one of the most severe forms of ADR, with low incidence but high mortality. The authors present the case of a 79-year-old female, with severe haemorrhagic cerebrovascular disease, due to head injury. The patient was admitted to an intensive care unit and in the course of treatment with meropenem, vancomycin and sodium valproate developed a TEN reaction. Lymphocyte transformation test (LLT) was performed in order to identify the eliciting drug. Stimulation indices were < 2.0 for meropenem, 7.4 for vancomycin and 6.4 for sodium valproate, with a cut-off value >3. Although still a research tool, LLT was decisive to prove the immunologic basis of the reaction. Vancomycin and sodium valproate are strictly contraindicated in this patient.

Key-words: Lymphocyte transformation test; sodium valproate; toxic epidermal necrolysis; vancomycin.

RESUMO

A prevalência de reacções adversas a medicamentos (RAM) em doentes hospitalizados é estimada em 10-20% e podem ser potencialmente fatais. A necrólise epidérmica tóxica (NET) é uma das apresentações de RAMs mais severa, com baixa incidência mas mortalidade elevada. Os autores apresentam o caso de uma mulher de 79 anos, com doença cerebrovascular hemorrágica grave, pós-traumática, com necessidade de internamento em Cuidados Intensivos que, sob terapêutica com meropenem, vancomicina e valproato de sódio, desenvolveu um quadro de NET. Para identificação do fármaco responsável realizou-se teste de transformação linfoblástica (TTL). Os índices de estimulação obtidos foram < 2,0 para o meropenem, 7,4 para vancomicina e 6,4 para o valproato de sódio; a sua valorização foi efectuada com cut-off >3. Apesar de ser ainda um instrumento de investigação, o TTL foi decisivo na confirmação da base imunológica da reacção. Vancomicina e valproato de sódio estão totalmente contra-indicados nesta doente.

Palavras-chave: *Necrólise epidérmica tóxica; teste de transformação linfoblástica; valproato de sódio; vancomicina.*

INTRODUCTION

The majority of allergy-caused adverse drug reactions (ADR) present with cutaneous involvement and can manifest as maculopapular exanthema, urticaria, erythema multiforme, drug rash or more severe clinical features —severe adverse cutaneous reactions[1]. These include acute exanthematous pustulosis, drug rash with eosinophilia and systemic symptoms (DRESS)/drug-induced hypersensitivity syndrome, Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) or Lyell's syndrome[2].

The latter two are considered nosologically close conditions, with an increasing severity spectrum and mortality ranging from 1-5% to 25-70%, and an annual incidence of 6 and 2 cases per million people, respectively[3]. The leading cause of SJS/TEN is drug related, particularly with prolonged use and in the first few weeks of treatment. The role concomitant infections or drug interactions play in the aetiopathogenesis of these reactions remains to be elucidated, however. The most frequent causes of death are secondary sepsis and haemodynamic failure[4].

Treatment strategy for these patients requires a high index of clinical suspicion, with immediate suspension of the drugs used and hospital stay in Intensive Care/Burns Units with wide-ranging multidisciplinary involvement[4,5].

CASE REPORT

Female patient, 79 years old, no relevant personal or family history, admitted to the neurological intensive care unit of the Hospital de Santo António dos Capuchos, with a Glasgow coma scale score of 4 due to post-traumatic haematoma tetraventricular hydrocephalus and subarachnoid haemorrhage. There was no indication for surgery, but the patient needed mechanical ventilation.

There was favourable neurological evolution with mechanical ventilation after two weeks. In terms of nosocomial infections, the patient developed bacteremia with methicillin-resistant *Staphylococcus aureus* (MRSA), four weeks after admission, needing antibiotic therapy with vancomyn. As fever persisted and there was increased systemic infection/inflammation, meropenem was added after 13 days of vancomycin. Four days later (4th day of meropenem, 17th of vancomycin and 29th of sodium valproate, administered to prevent epileptogenic activity),

the patient developed enanthem of the lips and fever restarted, leading to suspension of meropenem for suspected ADR. The next day **erythemato-violaceous** lesions and flaccid blisters with extremely fragile skin (positive Nilkolski's sign) appeared on the face, trunk and upper limbs (approximately 30% of body surface). Aggravated oral mucous membrane lesions were also seen, particularly all over the lip border, with **erosions** and **haemorrhagic scabs and** mucositis. There was no oedema or laryngeal lesions. There was involvement of the vaginal mucosa and punctiform keratitis of the right eye.

As TEN was suspected, vancomycin and sodium valproate were also suspended, and the severity of the case led to the patient being transferred to the emergency unit of Hospital São José (polyvalent intensive care) where he received sedoanalgesia **and mechanical ventilation**. In addition to general measures of restoring adequate hydroelectrolyte balance, high doses of n-acetylcysteine and effective cutaneous protection, three plasmapheresis sessions were performed.

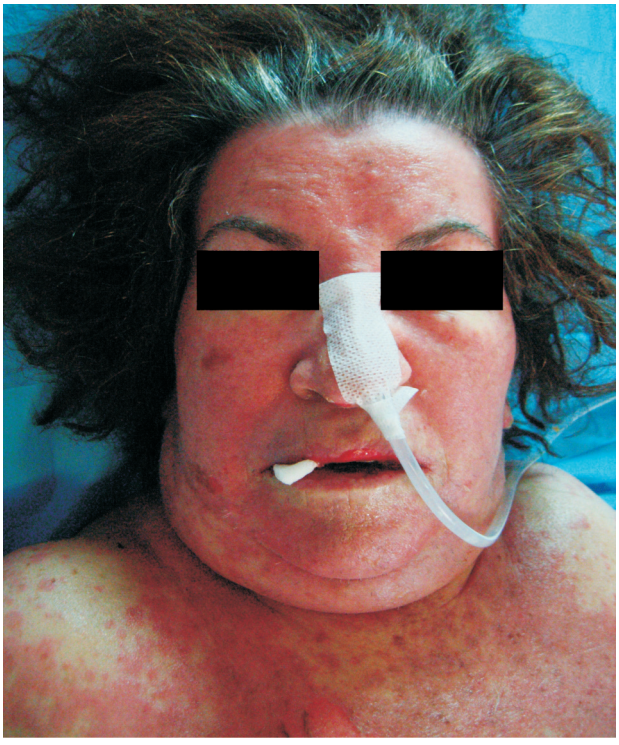

Figure 1. Toxic epidermal necrolyses – initial cutaneous lesions.

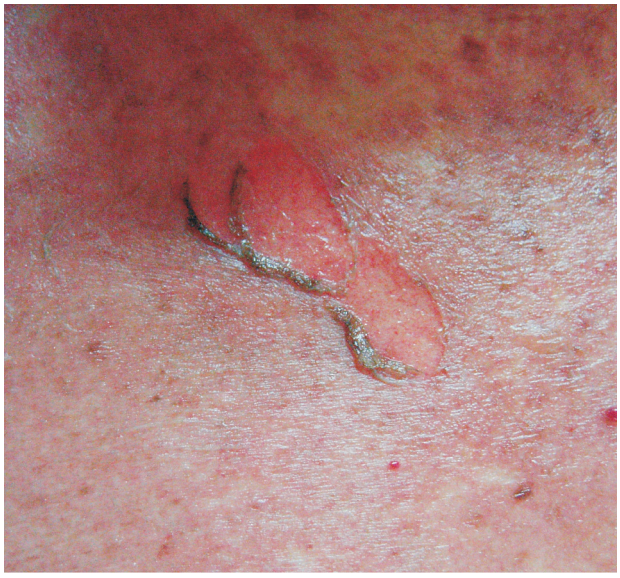

Figure 2. Nikolsky's Sign.

After a week of significant aggravation of the erythemato-bullous lesions (80-90% of the body surface), there was progressive clinical improvement and, three weeks later, only cicatricial lesions remained. The patient received midazolam, propofol, alfentanil, fluconazole and ceftazidime, with no ADR.

The allergology and clinical immunology work-up led to a lymphocyte transformation test (LLT) being performed around four weeks following the acute stage. Sodium valproate, vancomycin and meropenem were tested, with stimulation indices 6.4; 7.4 and < 2, respectively, with a positive cut-off value >3.

DISCUSSION

The occurrence of ADR is estimated at 10-20% in in-hospital patients and 7% in the population at large, making it a major cause of in-hospital mortality[6].

Toxic epidermal necrolysis, more common in females, is clinically characterised by fever, systemic toxicity and genera-

lised epidermal necrosis with cutaneous exfoliation and mucosal erosion, reaching over 30% of the body surface, (more than 10% in SJS). Differential diagnosis with other conditions can be difficult , namely with scalded skin syndrome caused by a staphylococcal toxin (usually with no pain or enanthema) or toxic shock syndrome caused by *Staphylococcus aureus* (with skin desquamation, mainly of the palms of the hands and the soles of the feet, fever and rapid progress to shock).

Diagnosis is essentially made clinically, but It can be confirmed by skin biopsy identifying subepidermal phlyctena, basal membrane vacuolisation and keratinocyte necrosis[4,5]. A skin biopsy was not performed in our patient as there were no diagnostic doubts and as polyvalent intensive care was urgently needed.

Several evolution stages are described. The prodromic stage lasts 2-3 days and is characterised by non-specific symptoms which begin 1-45 days after exposure. The acute stage reaches its peak in 2-3 days and the mucosal erosions usually precede the skin lesions. If there are no complications, the recovery stage lasts 1-3 weeks[4,5].

The aethiopathogenic mechanisms leading to keratinocyte apoptosis remain to be fully elucidated, but seem to involve immunologic and metabolic processes, in particular errors in the xenobiotic detoxification pathways, with accumulation of immunogenic, metabolites or with a direct cytotoxic effect[2].

There are reports of SJS attributed to herpes and *Mycoplasma* infections, neoplasms, radiotherapy, but mainly drugs. On the other hand, TEN cases are almost always drug induced[4].

The interaction between drugs and viruses (HIV, EBV or HHV-6, among others) seems to play a facilitator role in hypersensitivity phenomena. Viral infections, auto-immune diseases or other xenobiotics can interfere with drug metabolism or with their recognition by the immune system and, thus, induce sensitisation[2].

Over a hundred TEN-associated drugs have been reported. Sulphonamides are the most frequently implicated (in around one-third of adults), but also anti-epileptic drugs, alopurinol, oral penicillins and NSAIDs (namely those derived from pirazolone or oxicam, with longer half-lives)[4]. Beta-lactams can also trigger TEN. Meropenem has been reported in some cases which mention cross-reactivity with cephalosporins in patients with TEN, due to probable involvement of the beta-lactam ring. While this was one of the drugs initially suspected, this was not confirmed *in vitro*. We highlight in addition the reintroduction of beta-lactams (ceftazidime) during the hospital stay, without recurrence of the lesions.

Vancomycin is also implicated in these cases, although more rarely[4]. This is a glycopeptide used in MRSA infections and coagulase-negative staphylococci. The most common adverse reaction is "red man syndrome" (**erythema and pruritus, sometimes** with hypotension), resulting from mast cell degranulation by non-immunological mechanisms. Other ADRs associated with vancomycin include neutropenia, oto- and nephrotoxicity, interstitial nephritis and SJS/TEN. Similar cases of NET have emerged, but these have been associated with linear IgA deposits. This is an important differential diagnosis in suspected SJS/TEN[7].

As an alternative, some authors have suggested linezolid or streptogramins. Teicoplanin and vancomycin belong to the same class of drugs, meaning there could be a higher risk of cross-reactivity. Other alternative drugs could be suggested after sensitivity tests[7].

Hypersensitivity to anti-epileptic drugs could be specific to the molecule or class. These drugs are divided into phenytoins, barbiturates (phenobarbital, primidone), iminostilbenes (carbamazepine), succinimides (etosuximide), sodium valproate, oxazolidinediones (trimetadione), benzodiazepines (diazepam, clonazepam, clorazepate dipotassium and parenteral lorazepam) and other (gabapentine, lamotrigine and vigabatrin)[8].

Rzany and colleagues[9] identified 352 cases of SJS/TEN and 21% of these were attributed to anti-epileptic drugs. The most frequently implicated were phenobarbital, carbamazepine, phenytoins, valproate (13 patients) and, in only three patients, lamotrigine. The association between valproate and SJS/TEN is somewhat biased by the concomitant use of other drugs, namely NSAIDs, in several patients studied. The majority of ADRs occurred within the first eight weeks of treatment[9].

Reactions to anti-epileptic drugs, namely in patients with DRESS syndrome, were initially described to aromatic drugs (i.e., phenytoin, phenobarbital and carbamazepine), with an estimated 40-80% cross-reactivity between them [8]. However, there are also reports of severe ADR to non-aromatic anti-epileptic drugs, (i.e.,, valproate, gabapentine or lamotrigine). Thus, in cases of hypersensitivity to valproate, alternative treatments between classes with lesser risk of cross-reactivity should be sought, namely benzodiazepines[8].

The role of *in vitro* tests in diagnosing ADR, namely LLT and the basophil activation test, has received a great deal of attention over the last thirty years, due to their safety and comfort for the patient, since these can avoid possible sensitisation with re-exposure to the drug and allow the mechanisms involved in the reaction to be elucidated[2].

Several studies have been developed with this technique and their results have been encouraging, suggesting a sensitivity of 60-70% and a specificity of 85-100%[10]. The main limitations and methodological difficulties of LLTs' are the use of stimulation indices (SI) as sensitisation indicators, and the timing of carrying out the test. This is as the T memory response can vary depending on the allergen in question and the type of reaction. An interval of 4-8 weeks to 3-6 months after the acute stage is recommended. In patients with SJS/TEN, LLT tests are positive in less than 10% of cases, and an interval less than six weeks is suggested to increase the test's sensitivity[10]. False positive results can occur, particularly to vancomycin and contrast products[10]. However, the increased SI obtained and the severity of the reaction make this test contraindicated in our patient.

Skin tests in patients with SJS/TEN are not recommended by several authors due to the risk of re-exposure to the trigger drug. However, these can be considered in studying hypersensitivity to beta-lactams as there is little likelihood of a reaction to meropenem.

CONCLUSION

This was a rare and severe case of TEN caused by reaction to drugs.

Although still a research tool, LLT was decisive to confirm the immunological basis of the reaction. Even though identification of the implicated drugs is not conclusive, in view of the inherent limitations of the *in vitro* study, the use of vancomycin and sodium valproate was contraindicated in this patient.

Finally, the role of concomitant infections, drug interactions and genetic susceptibility factors in the immunopathogenic mechanisms remains to be elucidated.

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Sweet's syndrome – An unexpected diagnosis?

Síndrome de Sweet – Um diagnóstico inesperado?

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ABSTRACT

Background: Sweet's syndrome, also known as acute febrile neutrophilic dermatosis, is characterised by fever, neutrophilia and erythematous skin lesions. **Case report**: The authors describe a case of a 43-year-old female with a history of vitiligo, breast carcinoma in situ, iron deficiency anaemia and peripheral venous insufficiency, referred to the Outpatient Clinic of Allergology and Clinical Immunology for suspected toxicodermia. She had several episodes of fever, which preceded the appearance of skin lesions in plaques with inflammatory signs, associated with leukocytosis, neutrophilia and elevated values of sedimentation rate (SR) and C-reactive protein (CRP). The skin biopsy revealed dermal oedema and inflammatory infiltrate of polynuclear neutrophils in superficial perivascular location, consistent with Sweet's syndrome. **Conclusion**: Although this is a rare condition and a multiplicity of clinical features may mimic this disease, it is important to consider Sweet's syndrome in the differential diagnosis of skin lesions.

Key-words: Diagnosis, neutrophilic dermatosis, skin biopsy, Sweet's syndrome, treatment..

RESUMO

Introdução: *O Síndrome de Sweet, também designado por dermatose neutrofílica febril aguda, é caracterizado por febre, neutrofilia e lesões cutâneas eritematosas.* ***Caso clínico:*** *Os autores descrevem o caso de uma doente de 43 anos, com antecedentes de vitiligo, carcinoma da mama in situ, anemia ferropénica e insuficiência venosa periférica, referenciada à Consulta de Imunoalergologia por suspeita de toxicodermia. A doente apresentou vários episódios de febre, a preceder o aparecimento de lesões cutâneas em placa com sinais inflamatórios, associada a leucocitose com neutrofilia e elevação dos valores de velocidade de sedimentação (VS) e proteína C reactiva (PCR). A biópsia cutânea revelou edema da derme e infiltrado inflamatório de polinucleares neutrófilos em localização perivascular superficial, compatível com Síndrome de Sweet.* ***Conclusão:*** *Apesar de ser uma patologia rara e de existir uma multiplicidade de situações clínicas que o podem simular, é importante ter sempre presente o Síndrome de Sweet no diagnóstico diferencial de lesões cutâneas.*

Palavras-chave: *Biópsia cutânea, dermatose neutrofílica, diagnóstico, Síndrome de Sweet, tratamento.*

INTRODUCTION

Sweet's syndrome was first described, in 1964, by Robert Douglas Sweet. Since then hundreds of cases of this disease have been published[1,2]. Clinically, it is characterised by fever (the most frequent sign) and skin lesions (erythemato-violaceous papules/nodules/plaques), which can develop simultaneously with fever or days to weeks after[3]. These lesions are characteristically painful, asymmetrically distributed (with the most frequent locations being the upper extremities, face and neck) and clear up with no residual lesion. Extra-cutaneous manifestations have been described, such as muscle pain, joint pain, headaches, general malaise, conjunctivitis and ulcers of the oral mucosa[4]. There are three different forms of this syndrome: classic/idiopathic (Classical Sweet's Syndrome – CSS), associated with neoplastic diseases (Malignancy-Associated Sweet's Syndrome – MASS) and drug induced (Drug-Induced Sweet's Syndrome – DISS)[5]. The most frequently involved malignancies are haematological (acute myeloid leukaemia), but cases associated to solid tumours (breast, gastrointestinal and genitourinary) have also been described[4].

Sweet's syndrome can precede, accompany or appear after a diagnosis of malignant disease. It should, thus, raise a red flag, whether for a malignancy as yet unknown, or for a relapse in a cancer patient. Granulocyte-colony stimulating factor (G-CSF) is the drug most frequently associated with Sweet's syndrome, but many others can be involved, such as antibiotics, anti-epileptic drugs, antiretroviral drugs, anti-hypertensive drugs, chemotherapy drugs, anti-psychotic drugs, oral contraceptives, diuretics and non-steroidal anti-inflammatory drugs – NSAIDs). Diagnosis is made based on the criteria listed in Table I[6]. In laboratory terms, the changes seen are increased inflammatory markers, namely the sedimentation rate and the presence of leukocytosis with neutrophilia.

Given the plethora of clinical situations that can simulate this pathology, diagnosis is not always easy. The skin biopsy can reveal a diffuse infiltrate of mature neutrophils located in the upper dermis, with no evidence of leukocytoclastic vasculitis. These histopathological features are not pathognomonic of the disease. They can be seen in other neutrophilic dermatoses and in lesions caused by infectious agents. Its pathogenesis is not yet fully understood, but is probably multifactorial. Systemic corticosteroids, potassium iodate and colchicine are the

Table I. Diagnostic criteria of Sweet's syndrome.

Criteria	CSS/MASS	DISS
1	Abrupt onset of painful erythematous lesions (plaques/nodules)	
2	Histopathological evidence of dense neutrophilic infiltrate (without leukocytoclastic vasculitis	
3	Pyrexia (> 38 °C)	
4	Association with pregnancy, an underlying haematological or visceral malignancy, or inflammatory disease, or preceded by an upper respiratory or gastrointestinal infection, or vaccination	Time relationship between drug administration and onset of clinical picture or recurrence of picture after oral challenge test
5	Excellent response to treatment with systemic corticosteroids or potassium iodide	Resolution of lesions after suspension of drug or treatment with systemic corticosteroids
6	Abnormal laboratory values at presentation (≥ 3): SR > 20 mm/h; positive CRP; leucocytes...; neutrophils...	
Diagnosis	**2 major criteria (1 – 2) + ≥ 2 minor criteria (3 – 6)**	**All 5 criteria**

(adapted from Joe EK. Sweet Syndrome. Dermatology Online Journal. 2003;9(4):28).

Table II. Clinical characteristics of the different forms of Sweet's syndrome.

Characteristics	Clinical forms			
	CSS	Haemataologic malignancy	Solid malignancy	DISS
Epidemiology				
	80	50	59	71
Preceding upper airway infection or gastrointestinal infection	75-90	16	20	21
Recurrence	30	69	41	67
Symptoms/Signs				
Pyrexia>38°C	80-90	88	79	100
Musculoskeletal involvement	12-56	26	34	21
Ocular involvement	17-72	7	15	21
Lesion location				
Upper extremities	80	89	97	71
Head and neck	50	63	52	43
Trunk	30	42	33	50
Lower extremities	Infreq	49	48	36
Oral mucosa	2	12	3	7
Laboratory findings				
Neutrophils>6000/µL	80	47	60	38
SR>20mm/h	90	100	95	100
Anaemia (Hb<13g/dL; <12g/dL)	Infreq	82	83	100
Plt<150000/µL or >500000/µL	Infreq	68	50	50
RF, haematuria, proteinuria	11-50	15	7	0

(adapted from Cohen PR. Sweet's syndrome – a comprehensive review of an acute febrile neutrophilic dermatosis. Orphanet Journal of Rare Diseases. 2007;2:34).

CSS: Classical Sweet's Syndrome; DISS: Drug-Induced Sweet's Syndrome; Plt – platelets; RF – renal failure; SR – sedimentation rate

first-line drugs for treatment[7]. The prognosis varies, in line with the form of disease involved. This means that in the case of MASS and DISS it is related to the evolution of the underlying malignancy and the ingestion/suspension of the drug, respectively. In the case of CSS, it can resolve spontaneously or take months to weeks to resolve, with recurrence seen in one-third of cases. Table II summarises the clinical characteristics of the different forms of Sweet's syndrome.

CASE REPORT

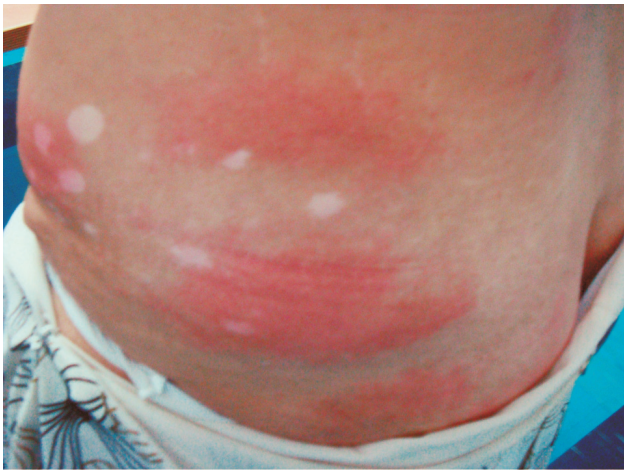
A 43-year-old female patient was referred to our Department of Allergology and Clinical Immunology for suspected toxicodermia. She had a history of vitiligo, diagnosed when she was 16 years old, breast carcinoma *in situ* (for which she had undergone radiotherapy and hormone therapy), iron deficiency anaemia, since the age of 39, and superficial venous thrombosis of the great saphenous vein at the age of 42. She had no known allergy to drugs.

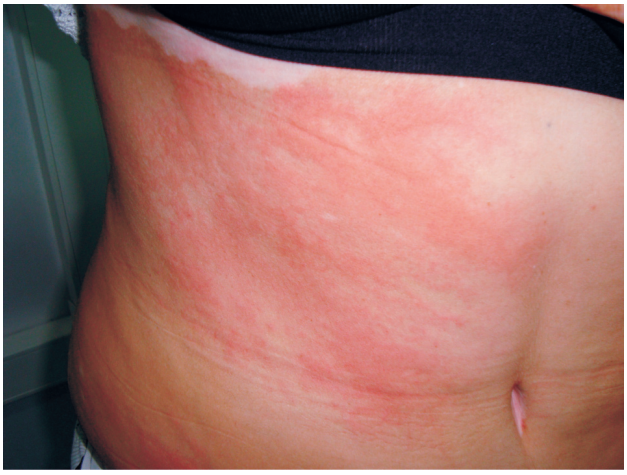
The patient was admitted to the Emergency Room (ER) with a 48-hour picture of fever (maximum temperature of 38.5 .°C), headaches, nausea and a vomiting episode. There were no other complaints, namely skin complaints. Examination revealed the patient to be subfeverish and with reddened oropharynx (with no discharge or exudate), with no other abnormalities. The analytical study revealed hypochromic microcytic anaemia (with no aggravation of the known status), leukocytosis 15470/uL, with relative neutrophilia of 89.5%, increased SR (49 mm) and slightly raised CRP (0.7 mg/dL). The patient was medicated with 10 mg IV metoclopramide and 1000 mg oral paracetamol. Symptoms improved, and the patient was discharged, with the recommendation to be vigilant and was medicated with paracetamol.

The patient returned to the ER around 24hrs afterwards, for continued headaches and nausea, with recent onset of skin lesions on the trunk and signs of inflammation, with no associated pruritus. She had no fever and had papular erythematous lesions, painful to the touch, located on the abdomen and dorsum. Analyses revealed no leukocytosis or neutrophilia, but there was a CRP increase to 12.07 mg/dL. The patient was medicated with 1800 mg IV lysine acetylsalicylate, 100 mg oral doxycycline and 25 mg oral hydroxizine, and admitted to the Internal Medicine Unit for a diagnostic work-up. This showed decreased IgA (< 6.34 mg/dL), with no other immunological changes. Renal, liver and thyroid function, ionogram, beta 2-microglobulin and protein electrophoresis showed no relevant changes. Urinalysis, chest X-ray, study of viral markers, blood culture panel, urocultures and serology exams for several pathogenic agents did not reveal infectious foci for the clinical picture described.

During the hospital stay, the patient remained apyretic, with gradual regression of the lesions. These became more localized to the right quadrants of the abdomen, maintaining the inflammatory characteristics. The patient was referred to a dermatology appointment, which considered the cause to be a viral infection or an NSAID-induced maculopapular morbilliform toxicoderma. The patient was discharged with the recommendation to avoid NSAIDs, was medicated with 100 mg doxycycline, and referred to the Outpatient Clinic of Allergology and Clinical Immunology for suspected toxicodermia.

By the date of examination, the patient had had four new episodes similar to that already described: fever (with no apparent cause) with generalized malaise and asthenia preceding the onset of skin lesions with non-pruriginous signs of inflammation on the trunk, with no triggering factor identified. These lasted approximately three to five days and had spontaneous resolution, leaving no residual lesion. During one of these episodes the patient resorted to an





Figures 1 and 2. Cutaneous lesions with characteristics of inflammation, spread over back; vitiligo lesions also visible – patient already presented vitiligo.

ER, where she was medicated with 1800 mg lysine acetylsalicylate, with good tolerance.

The question of this being a case of Sweet's syndrome was raised, and a skin biopsy scheduled should the lesions recur, which happened a month later (Figures 1 and 2). The biopsy revealed "oedema of the dermis and slight inflammatory infiltrate of polynuclear neutrophils with superficial perivascular location, that is, neutrophilic dermatitis lesions, compatible with Sweet's syndrome".

The patient was medicated with 60mg/day oral prednisolone for six days, at the end of which she was re-evaluated. As the lesions had resolved, treatment was suspended and surveillance continued. The patient has not experienced further episodes since, wherefore there has been no need for a new cycle of corticosteroids.

DISCUSSION

When the patient was first observed at our Allergology and Clinical Immunology Clinic, she had already undergone extensive tests to identify the causes of her clinical condition. Given her fever (with no apparent cause) associated with the analytical inflammatory parameters (leukocytosis with neutrophilia, increased SR and CRP), the diagnosis initially suggested was an infectious disease, namely caused by *Chlamydia*, *Rickettsia*, *Brucella* or *Borrelia burgdorferi*, which warranted treatment with doxycycline. The non-identification of a pathogenic agent and the evolution of the disease in recurrent episodes (with the patient completely asymptomatic between those episodes) did not suggest an infectious condition. That the patient had a drug-triggered (NSAIDs) cutaneous reaction was also suggested and this led to the patient being referred to us. However, the skin lesions were already present when the patient was medicated with lysine acetylsalicylate and, in addition, the subsequent administration of this drug with good tolerance ruled out a drug-related hypersensitivity mechanism. Sweet's syndrome was suggested as the most likely diagnostic probability and this was confirmed by histology.

A skin biopsy is essential in diagnosing Sweet's syndrome and this and the characteristic skin lesions are major criteria. In terms of minor criteria, the patient had fever

(temperature > 38 °C), laboratory abnormalities and good response to corticotherapy[6].

CONCLUSION

Given the severity of the pathologies that can be associated, Sweet's syndrome should primarily be considered as a systemic manifestation of an underlying disease. Histological confirmation of this syndrome should, thus, not be taken to be the end of the diagnostic workup[8]. Given our patient's history of breast cancer, a diagnosis of Sweet's syndrome could represent the first sign of a recurrence of malignancy. Since there were no new episodes after administration of corticotherapy, we can consider this as an idiopathic form of the disease. However, we stress the importance of a rigorous follow-up and multidisciplinary approach in these cases.

Although it is a rare condition and a multiplicity of clinical conditions may mimic this disease, it is important to consider Sweet's syndrome in the differential diagnosis of skin lesions.

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