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# Drug Hypersensitivity

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## 1. Introduction

The in vitro diagnosis of allergic reactions to drugs is of particular interest to clinicians, as neither the clinical history nor the in vivo tests are fully conclusive. In addition, these tests may often be associated with risk, as occurs with the drug provocation test. Various different in vitro tests are available for the evaluation of drug hypersensitivity depending on the immunological mechanism involved, either IgE mediated or T cell mediated.

For IgE-mediated reactions, the determination of serum-specific IgE, antigen-specific histamine release and sulphidoleukotriene production after in vitro stimulation of effector cells, as well as analysis of the activation markers of these cells (the basophil activation test), provide greater diagnostic precision.

Serum-specific IgE assays are still the most common in vitro method for evaluating immediate reactions. However, either there is no great commercial availability of fixed drugs for this study, with the exception of antibiotics (penicillin G and V, ampicillin, amoxicillin and cefaclor), a few muscle relaxants and certain substances, like insulins and ACTH. The most validated immunoassay, ImmunoCAP (Phadia, Uppsala, Sweden), has been widely used to evaluate immediate reactions to beta-lactams, mainly penicillins, with a specificity approaching 90% and a sensitivity of up to 50% (Blanca et al., 2001; Sanz et al., 2002a)

The in vitro study of the cell response in non-immediate reactions mainly assesses the T-cell response. A cellular response involving drug-related T-cell activity may be assessed in vitro by means of both the lymphocyte transformation test (LTT) (Pichler & Tilch, 2004) and the flow cytometric lymphocyte activation test, based on upregulation of the activation marker CD69 (Beeler et al., 2008). The combination of these tests with an assay of the production of drug-specific cytokines (e.g., IFN-g, IL-2, IL-5, IL-8 and IL-12) can increase the sensitivity and specificity to 48% and 82%, respectively (Romano et al., 2011; Rozieres et al., 2009a).

## 2. Cellular tests for the diagnosis of immediate type drug hypersensitivity reactions

### 2.1 The Basophil activation test

The in vitro diagnosis of drug allergies has advanced much over recent years, mainly due to the incorporation of new technologies, such as flow cytometry, which permits the analysis

of various types of cells, even when they are poorly represented in peripheral blood, as is the case with basophils. These cells are a useful model for the study of IgE-mediated reactions, as they are effectors of the reactions (Schleimer et al., 1985).
Basophils are able to release the content of their granules (either preformed mediators, like histamine, or formed de novo, like sulphidoleukotrienes) after antigen-specific activation. In addition, after this process they express, or overexpress, activation molecules on their membranes (Fig. 1).

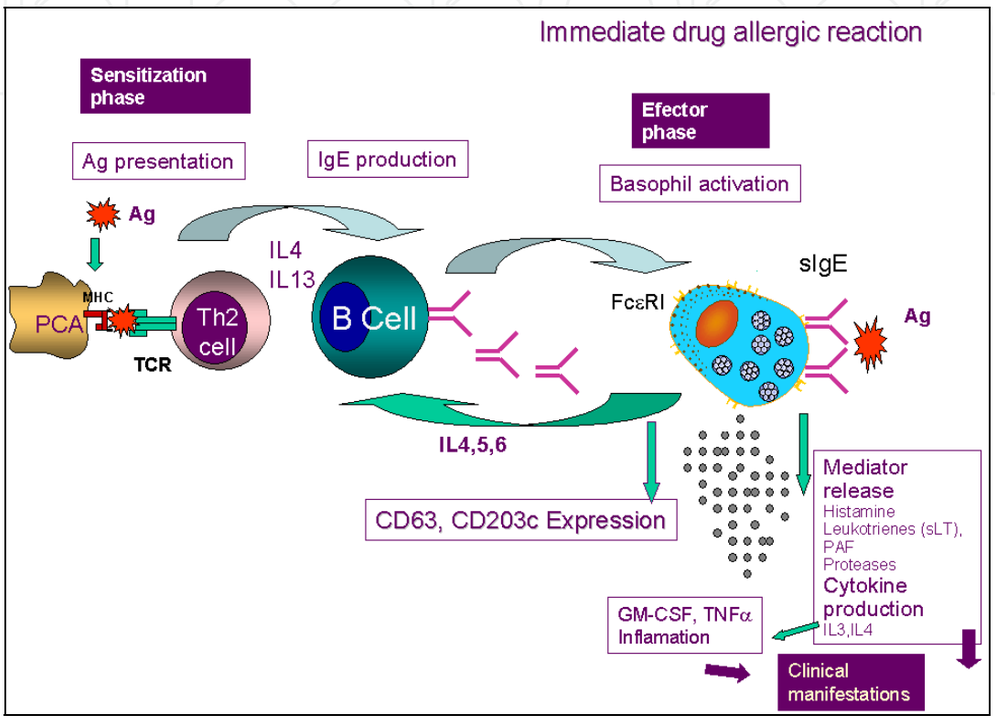


Fig. 1. Immune response in Immediate drug allergic reaction.

The basophil activation test (BAT) is based on the identification of basophils by labelling with monoclonal antibodies against different molecules, such as the IgE receptor, anti-CD 123 (IL-3 receptor) and fluorochrome-labelled anti-HLA-DR or anti-CCR3. This strategy, coupled with the identification of cell activation markers, such as CD63 or CD 203c, enables the comparison of cell activation before and after in vitro antigen-specific stimulation (Sanz et al., 2002b). Parallel to this process there is production of mediators, such as sulphidoleukotrienes that can be quantified simultaneously.
The CD63 molecule is a tetraspan, 53 kDa granular protein that is expressed not only on basophil granules but also on monocytes, macrophages and platelets. The expression of this marker correlates with degranulation and histamine release, which makes it an ideal marker of basophil activation (De Weck et al., 2008).
For in vitro stimulation with allergen, the peripheral blood cells are incubated with the suspected allergen for 15-40 minutes at 37ºC (Fig. 2).
After stopping the reaction, the cells are labelled with anti-CD63-PE and anti-IgE-FITC monoclonal antibodies. Two controls are used: a negative control in which the cells are incubated with the stimulation buffer used in the assay and that often contains IL-3

(negative control alias basal stimulation); as a positive control, an anti-IgE or an anti-IgE receptor antibody can be used (Sanz et al., 2008) (Fig. 3). The results are analised through flow cytometry (Fig. 4).

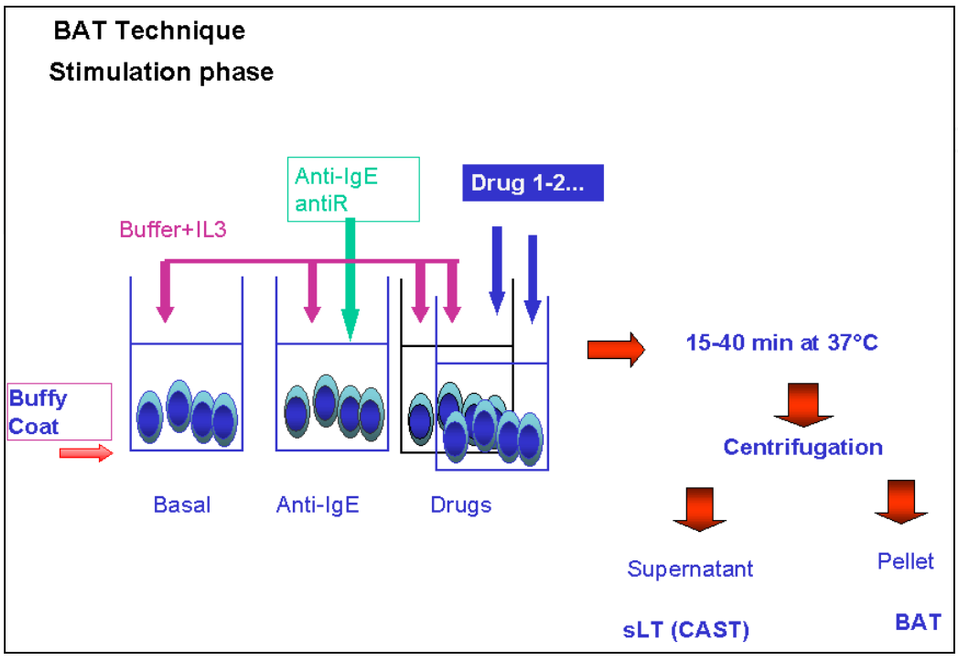


Fig. 2. Basophil activation test. Stimulation phase.

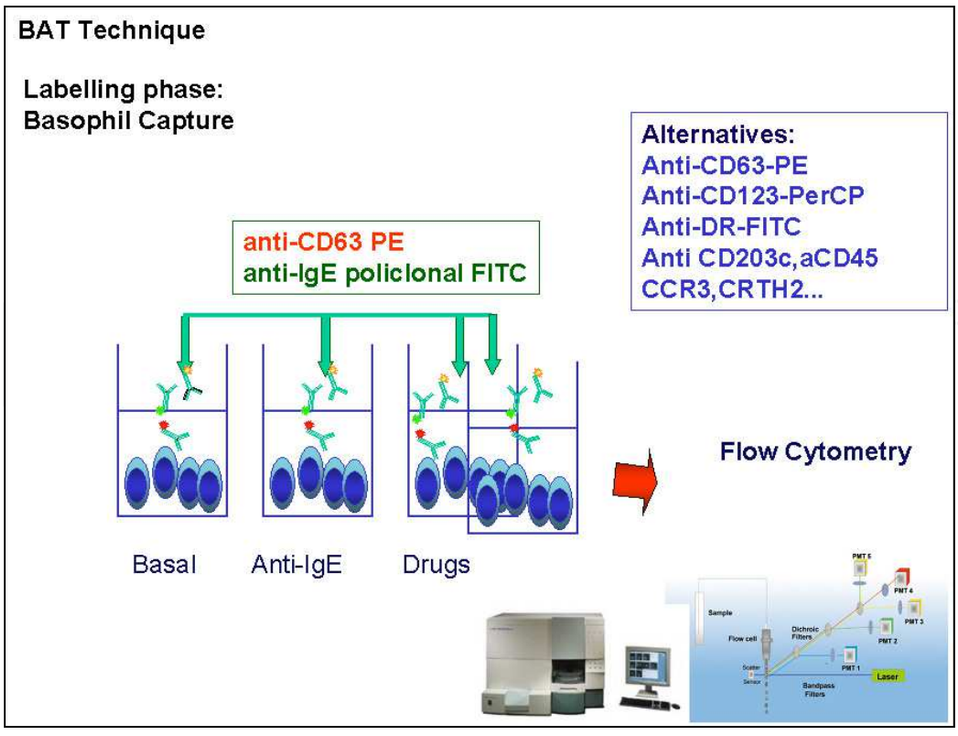


Fig. 3. Basophil activation test. Labelling phase.

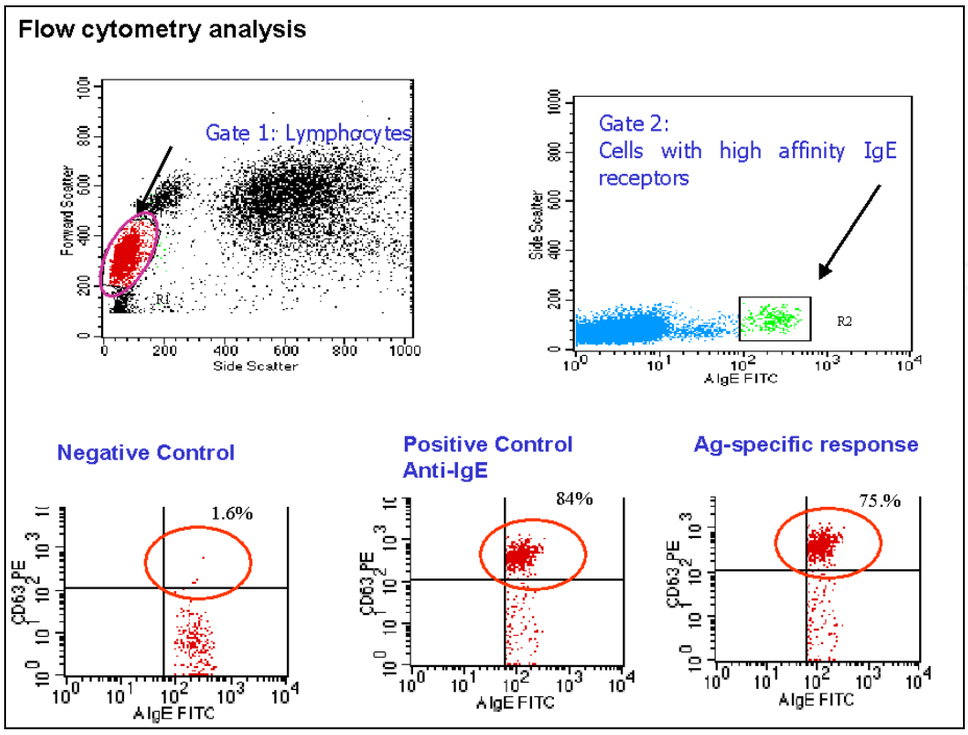


Fig. 4. Flow cytometry analysis.

Numerous studies over recent years have shown the usefulness of this technique for the in vitro diagnosis of allergic diseases (Ebo et al., 2006a, 2004a; Kleine-Tebbe et al., 2006) involving different allergens, such as inhalants (Paris-Köhler et al., 2000; Sanz et al., 2001; Saporta et al., 2001), hymenoptera venoms (Sabbah et al., 1998; Sainte-Laudy et al., 2000), or latex (Ebo et al., 2002; Sanz et al., 2003). For the diagnosis of allergy to drugs like muscle relaxants (Abuaf et al., 1999; Monneret et al., 2002), betalactam antibiotics (Sanz et al., 2001b, 2002a; Torres et al., 2004), pyrazolones (Sabbah et al., 1997; Sanz et al., 2005) and non steroidal anti-inflammatory drugs (NSAIDs) (Gamboa et al., 2004) BAT has opened up an important diagnostic pathway, either on its own or combined with other techniques (de Weck et al., 2008b; Gamboa et al., 2003a), allowing avoidance of challenge tests in an important number of cases (Hausmann et al., 2009; Romano et al., 2011; Sanz et al., 2009).

### 2.1.1 Basophil activation test and sulphidoleukotriene production in immediate type allergic reactions to beta-lactams

We studied the reliability of BAT, the determination of specific IgE by CAP (CAP-FEIA, Phadia, Uppsala, Sweden) and the production of leukotrienes after antigen-specific stimulation using the Cellular Antigen Stimulation Test (CAST ELISA (Bühlmann Laboratories, Allschwill, Switzerland) for the in vitro diagnosis of immediate allergy to betalactams in a series of 81 patients (58 patients with positive and 23 with negative skin tests) and in 30 healthy controls (Gamboa et al., 2004b; Sanz et al., 2001b, 2002a). Our results, as well as those found by Torres et al (Torres et al., 2004), showed that the sensitivity of BAT in the diagnosis of immediate hypersensitivity reactions to beta-lactams was 50%, and the specificity 93.3%, although there were certain differences depending on the technique applied (e.g., use of total blood or isolated cells) (de Weck et al., 2008a).

In our study (Sanz et al., 2002a) the best predictive values were obtained with BAT, in comparison with immunoassay or CAST. The positive predictive value of BAT was 18.9% and the negative predictive value 97.7%, with amoxicillin being the antibiotic with the highest predictive values. The joint use of CAP (Phadia) and BAT allowed identification of 65.2% of the betalactam allergic patients, with a specificity of 83.3% (Sanz et al., 2008).
In betalactam allergic patients with negative skin tests BAT was positive in 39.1%, CAST was positive in 22.7% with a specificity of 83.3%, and CAP showed a sensitivity of 21.7% with a specificity of 83.3%. The association of all three techniques allowed diagnosis in 60.9% of the patients of this group, with a specificity of 70% (Gamboa et al., 2004b).
Torres et al (Torres et al., 2004) studied the reliability of BAT in 70 patients with immediate allergic reactions. Like us, these authors found a greater positivity to amoxicillin (28.6%), followed by benzylpenicillin (BP) (21.7%), penicilloyl polylysine (PPL) (20%), ampicillin (12.5%) and MDM (2.2%).
They, too, reported a BAT sensitivity for cephalosporins of 77.7%. These results have since been confirmed in a multicentre study involving 181 betalactam allergic patients and 80 controls. This study concluded that the association of this technique with the determination of the production of antigen-specific sulphidoleukotrienes increases the sensitivity to 62% (de Weck et al., 2008b).
A certain lack of association exists between clinical and BAT cross-reactivity, which restricts the usefulness of this method for predicting cross-reactive responses in beta-lactam reactions. With BAT it is possible to find positive results when BP is used as the hapten in patients who are selective responders to amoxicillin (Torres et al., 2004).

### 2.1.2 BAT in IgE-mediated hypersensitivity reactions to quinolones

IgE-mediated hypersensitivity reactions to quinolones are a particularly difficult situation for the clinician as skin testing may induce false positive results. A recent report confirms that BAT is a useful method for diagnosing patients with confirmed immediate allergic reactions to quinolones (Aranda et al., 2011). In this study, involving 38 patients, the Sepharose-RIA technique was positive in 12 cases (31.57%), BAT in 27 (71.05%) and the combination of both tests showed positive results in 28 (73.68%). The drug most frequently involved in the reaction was moxifloxacin (63.2%), followed by ciprofloxacin (28.9%). The authors conclude that the BAT is a useful method for diagnosing patients, especially in those with severe reactions where drug provocation testing may increase the risk.

### 2.1.3 Basophil activation test in muscle relaxant allergic reactions

Muscle relaxants are an important group of drugs involved in 60% of adverse reactions during anaesthesia. Relevant studies have found that BAT sensitivity and specificity for muscle relaxants varied from 36% to 92% and 93% to 100%, respectively (Sudheer et al., 2005). Most of these studies reported a high specificity and a sensitivity above 50%, reaching 91% (Abuaf et al., 1999; Kvedariene et al., 2006; Laxenaire et al., 1999; Monneret et al., 2002; Moss, 1995; Stellato et al., 1991).
This sensitivity is lower (36%) when the marker CD203c is used (Sudheer et al., 2005). However, results are discordant; Kvedarine et al. (Kvedariene et al., 2006) observed in 47 patients a sensitivity of 36% and a specificity of 93% performing BAT (CD63 in whole blood). On the other hand, Ebo et al. (Ebo et al., 2006b) reported the sensitivity of a similar BAT technique to be 91.7% and the specificity 100% for rocuronium in a study including 14

patients with an anaphylactic reaction to drugs and with a positive skin test to rocuronium. However, the sensitivity apparently increases up to 80% when BAT is performed shortly after the clinical reaction (Kvedariene et al., 2006); this technique allows evaluation of the cross-reactivity between different muscle relaxants (Ebo et al., 2006b).
Leysen et al (Leysen et al., 2011) recently reported that BAT is complementary to skin testing in the assessment of cross-reactivity between rocuronium and vecuronium. In patients with negative skin tests and positive sIgE results, BAT helps in interpreting the clinical significance of a positive sIgE result.
These authors found a cross-reactivity with vecuronium in 69% of 104 curarized patients with a history of profound hypotension and severe bronchospasm immediately after induction of anaesthesia

#### 2.1.4 Basophil activation test as a diagnostic tool for immediate radiocontrast media (RCM) hypersensitivity

A study involving 26 patients with immediate RCM reactions and 43 specimens from healthy volunteers found that the specificity of BAT ranged from 88.4% to 100%, with a sensitivity around 50% (most of the patients in this study experienced only mild skin symptoms) (Pinnobphun et al., 2011). The authors demonstrated the potential of BAT as a diagnostic tool for immediate RCM hypersensitivity, particularly as a confirmation test, with significantly higher activated basophil percentages in BAT in patients with a history of immediate RCM reactions than in normal controls. The time between the RCM reaction and the BAT in their study was rather long (1–4 years), and this time lag may have lowered BAT reactivity.

#### 2.1.5 Basophil activation test in IgE-mediated selective reactions to NSAIDs

Until now only two articles have been published on selective NSAID reactors, patients reacting exclusively to only one NSAID family through an IgE-mediated mechanism and tolerating other NSAID groups (Gamboa et al., 2003a; Gómez et al., 2009). The first study comprised 26 patients with exclusive IgE-mediated allergy to methamizole. BAT sensitivity was 42.3% and specificity 100%. Upon adding the leukotriene release test, the sensitivity increased to 52% in the overall patient group, with a specificity of 92% (Gamboa et al., 2003a). In the second study, involving 51 patients with immediate reactions also to methamizole, the sensitivity was 54.9% but with a lower specificity, 85.71% (Gómez et al., 2009). The differences in the sensitivities and specificities may have been due to differences in the time interval between the in vitro test being performed and the reaction (16.9 versus 8 months, respectively), and the number of cases with anaphylaxis (57.5% versus 74.5%). Importantly, the BAT was positive in 38.5% of patients with negative skin tests but even more importantly, the BAT was positive in 75% of patients with anaphylaxis and negative skin tests. (Gómez et al., 2009).
BAT is the only in vitro technique currently available which allows the evaluation, with sufficiently high sensitivity and specificity values, of patients with selective IgE-mediated reactions to methamizole without the use of drug provocation tests and their associated dangers for patients. Negative BAT testing is associated with the delay to the test after the occurrence of the reaction.

#### 2.1.6 Basophil activation test in NSAID hypersensitivity reactions

The mechanism involved in these type of reactions is not well known although it is clear that it is a non immunological mechanism.

In this first study (Gamboa et al., 2004a) in 60 patients with this syndrome the use of only 2 drugs (aspirin and diclofenac) allowed a diagnosis to be reached in 58% of these patients, with a specificity of 90%. In similar studies carried out in 43 patients with NSAID hypersensitivity (Rodríguez-Trabado et al., 2008), BAT achieved a sensitivity of 43% with a specificity of 100%.
Subsequently, the European Network for Drug Allergy began a multi-centre study to validate the real usefulness of this technique (de Weck et al., 2010). However, a notable heterogeneous response in the results from each participating group in both sensitivity and specificity was observed that can be attributed to two main factors: different clinical characteristics in the study patients and technical variation between the different groups. The specificity value was notably better in those groups that used buffy coat than in those using plasma leukocytes. The lower values in sensitivity and specificity found by others increase the need for further studies. In this paper (de Weck et al., 2010), we further analyzed basophil activation in vitro in apparently normal individuals tolerating the administration of NSAIDs, in particular the specificity of these reactions and the role of technical conditions, such as the cell isolation technique. Different clinical and technical conditions may give rise to discordant results relating to NSAID hypersensitivity reactions (de Weck et al., 2009).

#### 2.1.7 Basophil activation test in various drug-allergic reactions

Other drugs are also of interest since evaluation of allergy to drugs that cannot be studied by other in vitro techniques is interesting. BAT has proven useful for the in vitro diagnosis of such allergy-causing drugs as corticosteroids (Lehmann Ott, 2008), heparins (Caballero & Fernández-Benítez, 2003; Ebo et al., 2004b), omeprazole (Gamboa et al., 2003b), cyclosporine (Ebo et al., 2001), Gelofusine (Apostolou et al., 2006), dexchlorpheniramine (Caceres Calle & Fernández-Benítez, 2004), chlorhexidine (Ebo et al., 2006c), iodinated povidone (Le Pabic et al., 2003), hyaluronidase (Ebo et al., 2005), or bovine serum albumin (Orta et al., 2003).

#### 2.1.8 The sensitivity of BAT for evaluating immediate allergic reactions to drugs may decrease over time

The diagnostic capacity of BAT has been shown to fall as the time increases between the clinical reaction and the test, both for allergic reactions to betalactams (where the sensitivity of BAT decreases to 12.5% one year after the reaction) (Blanca et al., 1999), and for reactions to muscle relaxants, where the sensitivity fell from 85.7% to 47.6% 4-8 years after the reaction (Abuaf et al., 1999) as well as for allergy to dipyrone, where 60% of the patients had a negative BAT 6 months after the reaction (Gómez et al., 2009), or expressed another way, if the tests are carried out within 6 months from the initial drug reaction, the efficacy of the BAT increases to 56% (Gamboa et al., 2003a). Thus, it is generally recommended to perform BAT at least two weeks after the allergic reaction but within no more than 6 months (Rodríguez Trabado et al., 2006), given that negativization of BAT does not correlate with tolerance to the drug (Antúnez et al, 2006).
In summary, BAT is a useful technique for the diagnosis of allergy to drugs, though additional validation studies are warranted.

## 3. Cellular tests in the diagnosis of non-immediate type drug hypersensitivity reactions

Although it has been shown that delayed reactions to drugs are mediated by effector T lymphocytes (Fernández et al, 2010) with a Th1 profile, based on the transcription factors

involved and with cytokine and chemokine production (Cornejo-Garcia et al., 2007; Fernández et al., 2009; Mullen et al., 2001; Rengarajan et al., 2000), an important number of heterogeneous clinical manifestations nevertheless indicate that different mechanisms with different cell subsets and mediators may be involved (Posadas et al., 2002) (Fig. 5).

### 3.1 Lymphocyte transformation test

Among the in vitro tests proposed for delayed hypersensitivity reactions to drugs, the lymphocyte transformation test (LTT) was one of the earliest. However, for some years now its role in the evaluation of these reactions has been questioned. This is owing to the existence of studies reporting series with a low number of cases where many drugs were involved and the clinical entities experienced by the patients were very heterogeneous (Mayorga et al., 2006; Luque et al., 2001). However, promising results together with the need for an in vitro test are now revitalizing its use.

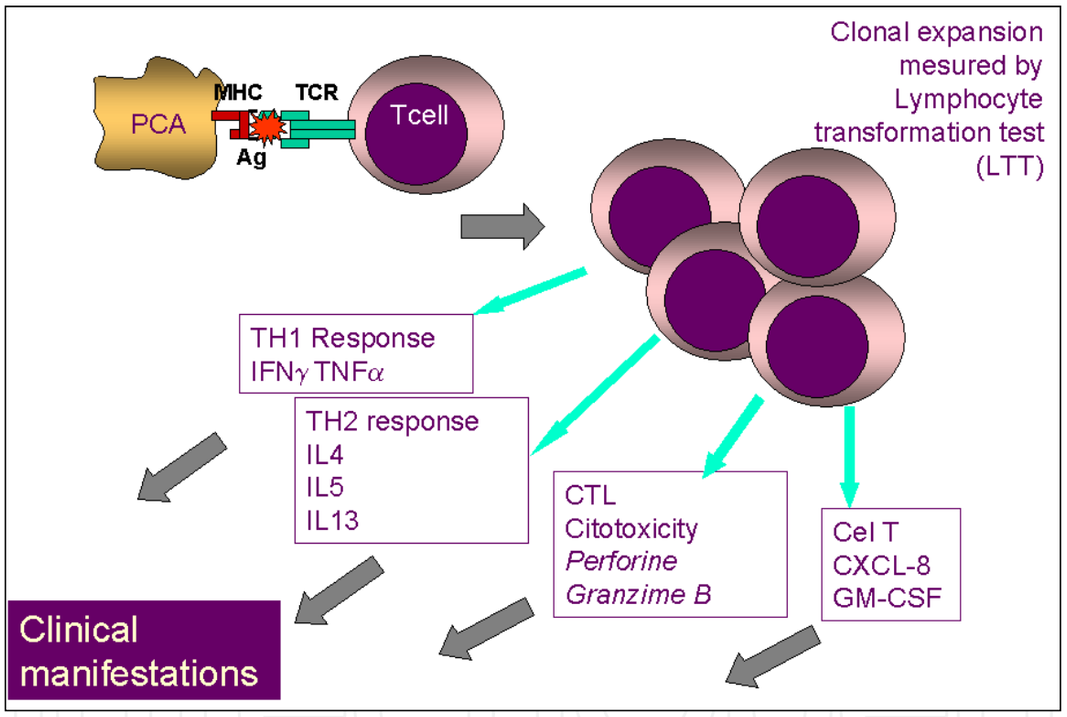


Fig. 5. Non immediate drug allergic reactions. Different profiles of T response.

The LTT enables identification of the drug involved and cross-reactivities, but it is important to take into account that a negative LTT does not always indicate the absence of a delayed reaction to the drug.

Different studies have shown the good sensitivity and specificity of the LTT, with heterogeneity in the drugs and clinical entities (Luque et al., 2001; Naisbitt et al., 2003a, 2003b; Nyfeler & Pichler, 1997; Schnyder & Pichler 2000). The most frequent drugs studied are beta-lactam antibiotics and anti-epileptics, particularly carbamazepine (Hertl et al., 1993; Luque et al., 2001; Mauri-Hellweg et al., 1995; Nyfeler & Pichler, 1997; Tapia et al., 2004; Yawalkar et al., 2000). These studies have shown an overall sensitivity of 60-70% for LTT (Lerch et al., 2007; Luque et al., 2001; Mauri-Hellweg et al., 1995; Pichler & Tilch, 2004). In

the case of beta-lactams, for immediate and non immediate reactions, the sensitivity was 64.5% and 57.9%, respectively and the specificity was 92.8% (Luque et al., 2001; Pichler & Tilch, 2004). In a retrospective analysis of patients with adverse drug reactions, the LTT sensitivity was seen to depend also on the drug involved in the reaction (Nyfeler & Pichler, 1997; Roujeau et al., 1985) and the sensitivity might significantly improve when LTT is performed at the optimal time (Kano et al., 2007). This timing could vary depending on the clinical manifestations, since it has been shown that LTT should be performed within one week for patients with MPE and SJS/TEN whereas 5-8 weeks is optimal for patients with DRESS (Kano et al., 2007).

Recently, a modification of the LTT with inclusion of monocyte derived dendritic cells as antigen presenting cells has shown promising results, with higher sensitivity and specificity in delayed reactions to amoxicillin, heparins, glucocorticosteroids and contrast media, as well as providing the possibility of detecting a response over a longer period of time, preserving sensitivity to the culprit drug (Lopez S et al., 2009, 2010; Nyfeler & Pichler, 1997; Rodríguez-Pena et al., 2006).

### 3.2 ELISPOT

The Elispot assay is based on the detection of a cytokine caught by an immobilized antibody and revealed by a secondary antibody (Czerkinsky et al., 1988). With this method it is possible to detect different cytokines as well as cytotoxic markers released by antigen or drug stimulated cells (Beeler et al., 2006, 2008; Rozieres et al., 2009a; Zawodniak et al., 2010). This test has a high sensitivity, and is able to detect fewer than 25 secreting cells per million PBMC (Schmittel et al., 1997, 2001). The results are expressed as spot forming cells obtained from the rate between the cytokine secretion produced in the presence of the drug compared to the absence of stimulus. The Elispot assay has detected lymphocytes secreting cytokines such as IFN-γ, IL-5, IL-13 from allergic patients in the presence of the culprit drug. Recently, this test has been used to determine the release of the granule content (granzymes and perforin) by cytotoxic cells after activation with the culprit drug (Zawodniak et al, 2010). The test showed a high sensitivity and specificity, although in some cases no correlation with LTT results was found, probably because cytotoxicity-based tests measure effector cell function, which is distinct from proliferative response.

The secretion of cytokines (IFN-γ) or cytotoxic granules (Granzyme B) has been detected in vitro in response to the culprit drug in allergic patients but not in tolerant subjects (Rozieres et al., 2009a; Zawodniak et al., 2010), demonstrating that the Elispot can be a complementary method to evaluate delayed reactions to drugs.

### 3.3 Monitoring delayed reaction to drugs

Another approach for the in vitro evaluation of delayed reactions to drugs is the follow-up of the immunopathological response by performing serial determinations in peripheral blood and the affected skin, from the acute phase to basal conditions (Mayorga et al., 2006). This enables an idea of the underlying process to be built up, not only in the development and progress of the reaction but also related to the degree of response to treatment (Mayorga et al., 2003). These reactions involve not only different cell populations but also activation markers, signalling molecules, cytokines, chemokines and transcription factors. These are all assessed and it is important to determine the changes in the different markers rather than their quantification *per se*.

Among the available methods the most widely used are flow cytometry and mRNA gene expression by real time PCR. These methodologies provide important information about the immunological mechanisms involved, since different clinical manifestations like urticaria, MPE, DRESS, AGEP, FDE or TEN are produced by different cell subsets contributing to each particular entity. Different authors have shown that $CD4^+$ T lymphocytes are mainly involved in MPE, DRESS, and AGEP (Fernandez et al., 2009; 1995; Hertl et al., 1993; Mauri-Hellweg et al., 1995; Pichler, 2003; Schnyder et al., 2000; Shiora & Muzukawa, 2007; Torres et al., 2006, 2009; Whittam et al., 2000; Zedlitz et al., 2002), whereas $CD8^+$ T lymphocytes are the effector cells in FDE and TEN (Alanko et al., 1987; Chung et al., 2008; Nassif et al., 2002, 2004a). Although other studies indicate that both $CD4^+$ and $CD8^+$ T lymphocytes may be involved in TEN (Torres et al., 2006) and $CD8^+$ T cells have been found in the epidermis of non bullous lesions including MPE (Rozieres et al., 2009b).

These cells become activated and express high levels of CD25, CD69, CD71 and HLA-DR (Leyva et al., 2000; Mayorga et al., 2003; Nishio et al., 2007; Shiohara & Mizukawa, 2007; Torres et al., 2008). Apart from activation, different chemokine and chemokine receptors involved in cell recruitment to the affected organ, mainly the skin, can also be analyzed. Thus, an increase in the expression of cutaneous lymphocyte-associated antigen (CLA), a skin homing receptor in delayed reactions to drugs, correlates with disease severity (Blanca et al., 2000; Mullen et al., 2001; Torres et al., 2006). In a study in patients with drug-induced MPE, an increase in the expression of Th1 chemokines (CXCL9 and CXCL10) and their specific receptor CXCR3, as well as in the expression of the cutaneous homing chemokines (CCL17, CCL20, and CCL27) and their receptors (CCR4, CCR6, CCR10), with high production of TNF-α and IFN-γ, was found at the acute phase of the reaction (Fernandez et al., 2008). In MPE or DRESS an increase has also been found in IL-5 and chemokines such as RANTES and eotaxins, which explains the involvement of eosinophils expressing CCR3 (Hertl et al., 1993). In AGEP, another severe delayed drug-induced reaction, lymphocytes have a dual role as cytotoxic agents and inducing migration of neutrophils to the skin by producing IL-8 (Britschgi & Pichler, 2002; Hertl et al., 1993; Padial et al., 2004; Yawalkar et al., 2000). At the acute phase of TEN, T cells are recruited to the skin, producing cytotoxic markers such as perforin and granzyme B (Fernandez et al., 2010; Mayorga et al., 2003; Pichler, 2003; Posadas et al., 2002; Torres et al., 2006; Zawodniak et al., 2010), and they express high levels of CCR10 and their chemokine CCL27 (Tapia et al., 2004).

As mentioned, this monitoring is useful in order to understand not only the underlying mechanisms but also to assess the response to treatment. This latter was analyzed during the follow-up of TEN, where the administration of gamma globulin was associated with a rapid decrease in the cytotoxic markers (Mayorga et al., 2003).

A Th1 pattern with expression of IFN-γ, IL-12, and TNF-α and down-regulation of IL-4 has been found in delayed reactions to drugs (Cornejo-Garcia et al., 2007; Posadas et al., 2000). The polarizations also extend to the expression of transcription factors, such as T-bet in Th1 and inhibition of c-maf and GATA3 in Th2 (Cornejo-Garcia et al., 2007).

### 3.4 Trafficking between different organs

Since the skin is the organ most frequently affected in delayed reactions to drugs, it is possible to assess the parallel course in the two compartments, the skin (in both biopsies and blister fluid cells) and the peripheral blood. At the very early stage of the reaction, there is an increase in the blister fluid of CLA+ cells which later appear in peripheral blood,

probably due to recirculation from the skin (Leyva et al., 2000; Mayorga et al., 2003). These cells also show a Th1 profile, with high production of perforin and granzyme B that correlates with the reaction and its severity (Nassif et al., 2004a; 2004b).
In patients with MPE (Fernandez et al., 2008) an increase in the expression of Th1 chemokines, including CXCL9 and CXCL10, as well as cutaneous homing chemokines such as CCL17, CCL20, and CCL27, was found only in the skin. In this study they also found a high production of TNF-α and IFN-γ as well as the presence of Th1 cytotoxic T lymphocytes in the skin. However, in the peripheral blood T lymphocytes expressing only the chemokine receptors CXCR3, CCR4, CCR6, and CCR10 were found. These data indicate that during the reaction there is recruitment of Th1 cytotoxic T lymphocytes to the skin induced by different chemokines produced by cutaneous cells (Fernandez et al., 2008).

## 4. Acknowledgments

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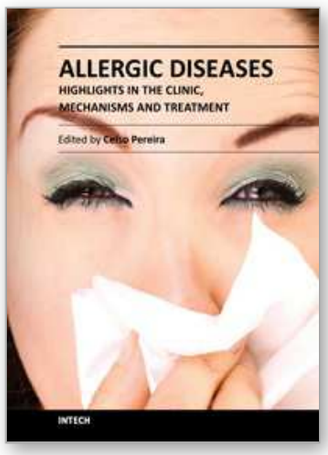


**Allergic Diseases - Highlights in the Clinic, Mechanisms and Treatment**

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The present Edition "Allergic diseases - highlights in the clinic, mechanisms and treatment" aims to present some recent aspects related to one of the most prevalent daily clinical expression disease. The effort of a group of outstanding experts from many countries reflects a set of scientific studies very promising for a better clinical care and also to the treatment and control of the allergy. This book provides a valuable reference text in several topics of the clinical allergy and basic issues related to the immune system response. The inflammatory reaction understanding in allergic disease is clearly evidenced, as well as new strategies for further researches.

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