

Basophil Activation Tests (BAT): Degranulation, Cytometry and Chemotaxis in Drug Allergy

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Summary

Objective: To Increase the knowledge on Drugs Hypersensitivity Reactions (DHRs); activation, development of reliable diagnostic tests for better: selection of studies, diagnosis, identification of risk groups, prevention, cross-reactions, severe skin drug reactions and alternative therapeutics.

Methods: Review longitudinal and transversal studies about: immune mechanisms, hypersensitivity responses, parameters of methods and diagnostic consistency.

Results: The basophils are the most accessible cells for the study (peripheral blood 0.5%-1%); when they get activated release histamine as a response to allergens; the most used tests are the BAT (IgE- dependent); FcεRI-mediated signaling, the binding to the antigen (bivalent dimers); depends on the concentration (bell curve - ideal form); and more complex antigens present non-ideal dose-response curves (several forms). There are 4 types of evaluating BAT: 1) Secretion of granules 2) Membrane expression of activation markers: CD63, CD69, and CD203c by cytometry 3) the old technique of modified degranulation of basophils (MDB) and 4) Modified Leukocyte Migration Inhibitor Factor (MLIF). Currently there is an increase in the prevalence of DHRs: 7% in older adults, 18% in children (15% -24%) and 5%-15% in hospitalizations. The main cause of allergy are antibiotics: penicillin and β-lactams (50%); more frequently in women (40-60 years).

Conclusions: The BAT and alternative complementary tests with dilutions confirm the diagnosis and suggest the degree of sensitivity of the patient, predicting the response to treatment and reducing the risks in patients with reactions to drugs. These tests are simple, inexpensive and give great support in the diagnosis of drug reactions with coverage of several types of hypersensitivity.

Keywords: Hypersensitivity, Allergy, Adverse reactions to drugs, Antibiotics, NSAID, Anesthetic, BAT, DHRs

Background

Von Pirquet in 1905 described “the serum sickness” in patients using treatment with (diphtheria and tetanus horse serum) and in 1906, introduces the term “allergy” as a special type of defensive or immunological response to foreign substances that normally would not induce a response. In 1930, sulfonamides were also associated with more frequent reactions, which later was denominated as “drug fever” [1-4]. Since 1940, penicillin has been the most used antibiotic and currently remains as the most frequent cause of allergies [5].

The pharmaceutical industry has created new penicillins keeping the β -lactam ring, but changing the side branch without losing any antibiotic capacity; which produce cross-reactivity in allergic reactions and anaphylaxis [6-10]. During the 20th century and the beginning of the 21st century, the increase in the synthesis of drugs (Nonsteroidal anti-inflammatory drug [NSAIDs], analgesics, anesthetics, antihypertensives, steroid contraceptives, chemotherapy, antiretrovirals, etc.) caused a growth in the use of these drugs and increased allergies; some authors consider drug allergy as the epidemic of the 21st century.

The prevalence has increased in all stages of life: adult women (40%), elderly adults (7%), hospitalized patients (5%-15%) and mainly children (18% with a range of 15%-24%) [7,11,12]. Consequently, healthcare services want to improve the strategies for diagnosis, treatment,

prevention and lethality.

Classification of Adverse Drugs Reactions (ADRs)

Drugs may produce predictable ADRs (type A, 80%) associated with pharmacological activity, dose dependency, toxicity and slow metabolizers; they can also produce unpredictable adverse reactions (type B, 20%) [13-15] which are dose independent (low concentration) and associated with aggravated intolerance, pseudo-allergies and more severe and lethal allergies. Hypersensitivity is an exacerbated immune response that produces a clinical pattern with systemic and dermal disorders (skin as the most affected organ), anaphylaxis and sudden death [8]. Gell and Coombs created the classification of hypersensitivity which was modified in 1996 [2,7,13,16] to distinguish 4 types of hypersensitivity: I, II, III, IV; later on, Pichler [2,17] subdivided type IV into a, b, c and d [17]. The International Consensus on Drug Allergy (ICON, 2013) defines the drugs hypersensitivity reactions (DHRs) [17] and says that they are caused by activation of the immune adaptive system considering immune mechanisms and activation responses supporting the diagnosis, treatment, follow-up and prevention. This classification is based on the laps of time it takes for the signs and symptoms to occur after the drug administration: 1) immediate (0-1 hour), 2) accelerated (1-72 hours), 3) delayed (more than 72 hours) (Figures 1 and 2, Table 1) [17].

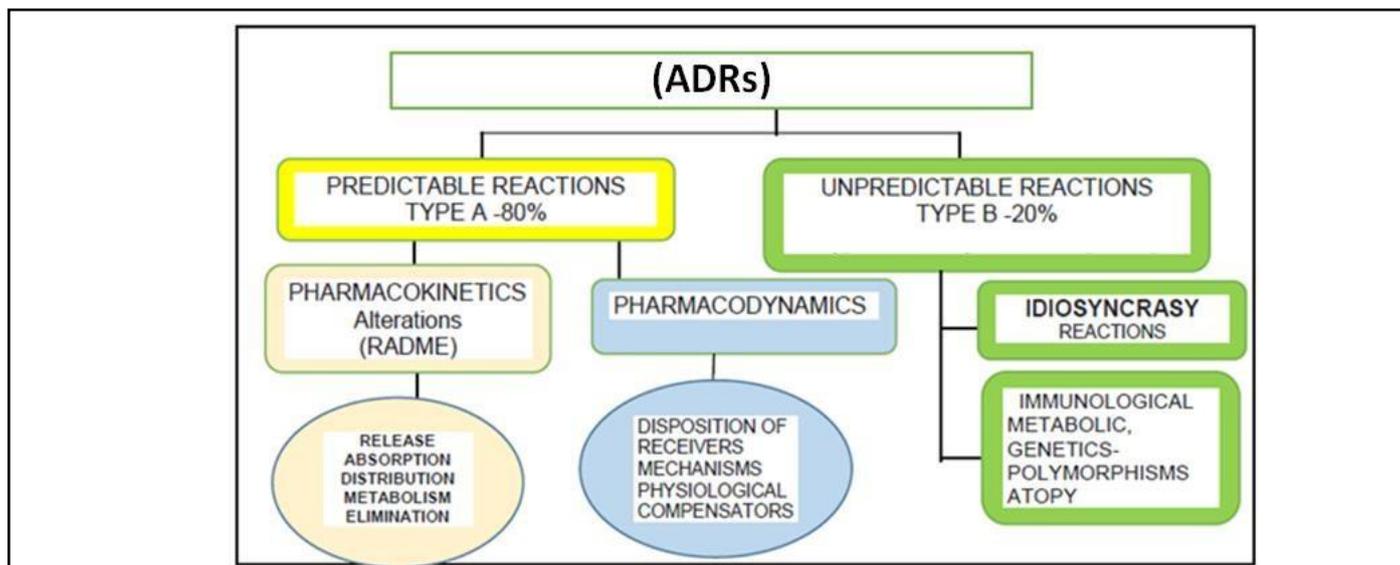
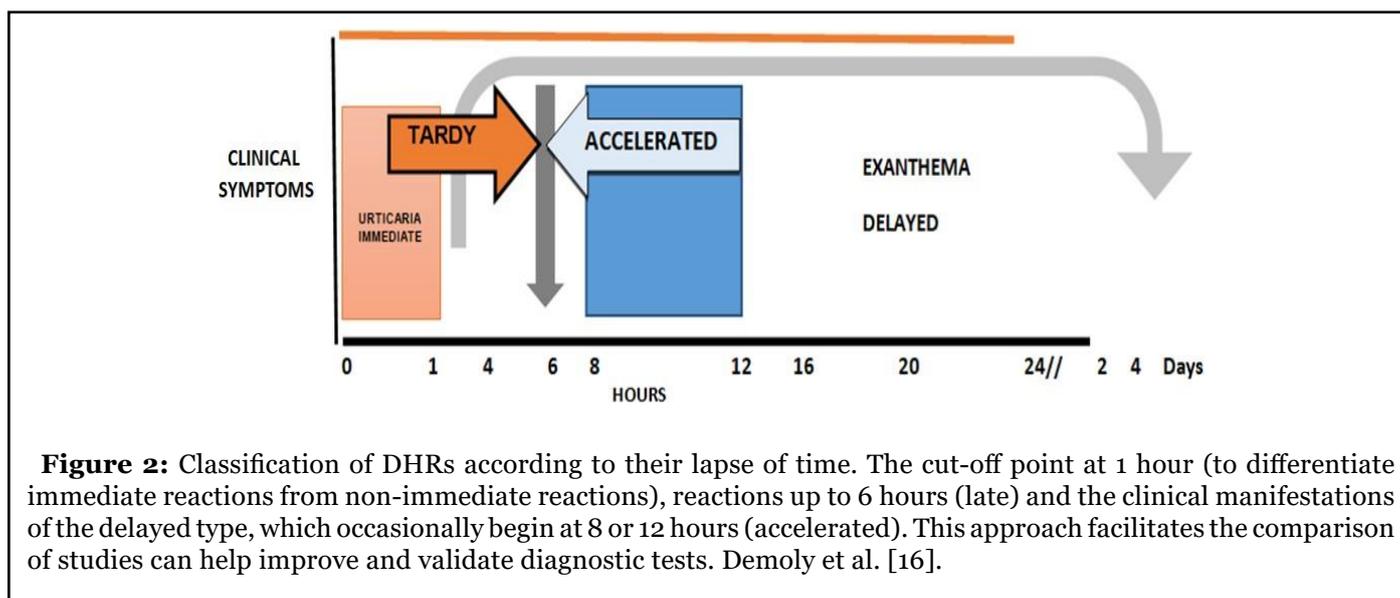


Figure 1: Pathogenic mechanisms of ADRs. The World Health Organization (WHO), defines: An ADR to any predictable noxious reaction that appears at therapeutic doses, depends on the doses and is related to pharmacological actions. Within this group are other [14] unpredictable reactions [18], independent of the dose recognized as: hypersensitivity or allergies (DHRs) associated with immunological mechanisms, susceptibility (atopy) and polymorphism (pharmacogenetic, MHC-HLA). Adapted from Gibaldi [19], Lares-Asseff & Trujillo-Jimenez [14] and Giner-Munoz [6,7].



Type	Type of Immune Response	Clinical symptoms	<i>In vitro</i> Diagnostics	<i>In vivo</i> Diagnostics
I	Measured by IgE Eosinophils Mast Cells and Basophils (IMMEDIATE)	Urticaria, Angioedema, Rhinitis, Bronchospasm, Anaphylaxis	IgE specific serum, Triptase Cell stimulation test (CAST) Basophil Activation Technique (BAT): MBD, CD63	Cutaneous test (prick, intradermal), Challenge test, Proving test [Coombs]
II	Citotoxicity dependent on IgG and IGM antibodies, (NOT IMMEDIATE) and Complement	Hemolytic anemia, thrombocytopenia, Neutropenia, Autoimmunity	Coombs test Antibodies vs platelets Antibodies vs neutrophils	Only challenges to the drug can make diagnosis but are high risk [Coombs]
III	Deposit of immuno complexes [IgG and IgM], (NOT IMMEDIATE) Complement or Fc	Serum disease, Vasculitis, LES-Like by medications, Glomerulonephritis, Drug fever	C3, C4, Antinuclear Antibodies (ANA), Antineutrophil cytoplasmic antibodies (ANCA), Cyclic Citrullinated Peptide Antibodies (CCP), Anti-Thyroid, etc. Liver and kidney function tests, Pathological anatomy	Biopsies with Immunofluorescence [Coombs]
IVa	TH1 (IFN γ) TNF α , IL-12 and Macrophages (LATE)	Contact dermatitis	Lymphocyte Transformation test or Blastoid Transformation (LTT or BT), Modified Leukocyte Migration Inhibitory Factor (MLIF), Cytotoxic T lymphocyte Precursors (CTLp), Cytokines by ELISA or PCR	Patch test [Pichler]

IVb	TH2 (IL-4, IL-5, IL-13 Eosinophils)	Maculo-papular eruptions (MPE) with eosinophilia (DRESS)	Complete Blood Count (CBC) with revision eosinophil cellularity, atypical lymphocytes. MLIF, BT, LTT	Patch test [Pichler]
IVc	Cytotoxic T Lymphocytes (CTLs), CD4/CD8 (Perforin, Granzyme B, Fas L)	Contact dermatitis, Maculo-papular and bullous diseases (Steven-Johnson syndrome [SJS]), Toxic Epidermal Necrosis (TEN)	MLIF Liver Function test, CD4/CD8 (death keratinocytes) Activity of IgM vs Herpes Virus, Epstein Barr and Cytomegalovirus (CMV)	Patch test [Pichler]
IVd	T Cells, IL-8 CXCL8 in leucocytes Neutrophil inflammation	Acute generalized Exanthematous Pustulosis (AGEP), Pharmacodermias associated with neutrophilia	CBC T cells CD4/CD8	Patch test [Pichler]

Table 1: Hypersensitivity classification according to the Gell and Coombs modified by Sell, Pichler [2,17,20] and ICON [16]. Giner-Munoz [6,7], Rojas-Espinosa [2], Demoly et al. [16], Irigoyen-Coria et al. [13] and Mayorga et al. [21].

Mechanisms of Immune Response to Types of Drugs in DHRs: Haptens, Pro-Haptens, Binding p-i TCR

Prohaptent (inactive-reactive). Pharmacogenetic polymorphism

Drugs (generally non-immunogenic haptens) that are chemical substances of low molecular weight (less than 1000 Da) in the form of aromatic, heterocyclic components, -p-NH₂Cl, sulfonamides, sulfide, OH components, halogens, with high resonance and instability (β -lactam with low polarity and hydrophilicity which do not facilitate covalent bonds with autologous proteins). Drugs are eliminated through metabolism with bioactivated detoxifying enzymes (hepatic N-acetylation, oxidation of cytochrome P450-CYP); this occurs mainly in the liver (microsomal), and also in the kidneys, lungs, intestine, plasma and nervous tissue. There are variabilities or ethnic polymorphisms, especially in slow acetylators, in which drugs remain more time in circulation, haptens bind to the protein, become a Hapten-Protein carrier complex (H-P), are engulfed and introduced into the Antigen Presenting Cells (APC) for processing and presentation on the membrane with the major Histocompatibility Complex APC-MHC complex to induce a response cellular or humoral with IgE, IgG and IgM [7,13,22].

Active-reactive

Drugs with aromatic, polar groups and nitrogen, facilitate the direct binding or nucleophilic attacks to membranes in order to create a covalent bond with autologous proteins and induce an APC-MHC immune response [7,13,22]

p-I concept (pharmacological interaction with immune receptors)

Drugs without that lack hapten characteristics can bind (non covalently) to TCR and sending signals to create a hypersensitivity response. This explains a fast occurrence of Clinical symptoms without previous sensitizations and sometimes chaotic immune reaction, some cross-reactions to the drug or its metabolites [7,14,17,22-24].

HLA restriction in hypersensitivity

HLA class I, mainly HLA-B, described for several severe reactions in DHRs; for example, it has been found that abacavir is strongly associated with the HLA-B * 5701 allele in white population; carbamazepine like an inductor of Steven Johnson Syndrome (SJS) has been associated with the HLA-B * 1502 and HLA-B * 5801 alleles in Chinese patients, while allopurinol has been associated with adverse reactions in SJS and necrolysis epidemical toxic (NET) with HLA-B 5701 (Figures 3 and 4, Table 2) [7,22,25,26].

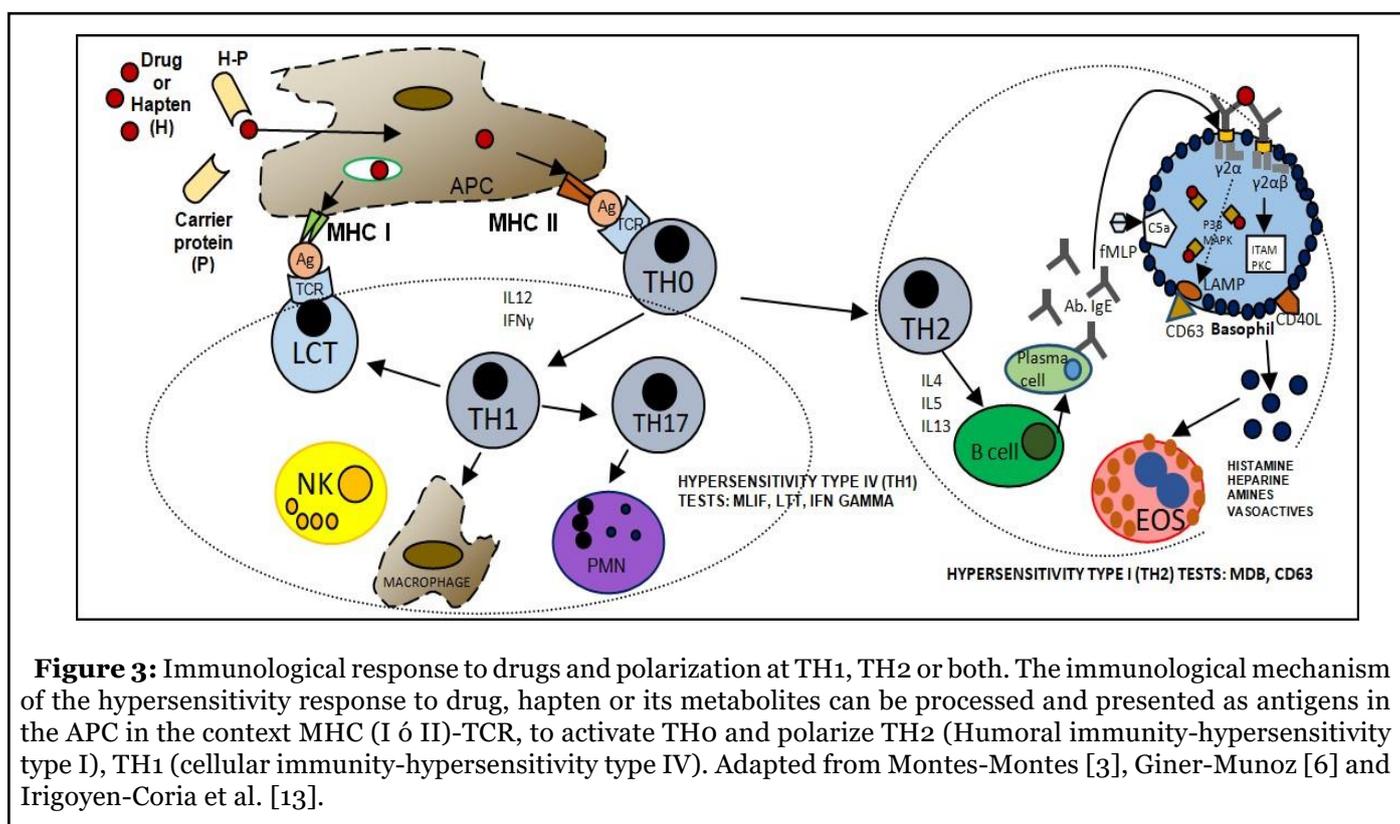


Figure 3: Immunological response to drugs and polarization at TH1, TH2 or both. The immunological mechanism of the hypersensitivity response to drug, hapten or its metabolites can be processed and presented as antigens in the APC in the context MHC (I ó II)-TCR, to activate TH0 and polarize TH2 (Humoral immunity-hypersensitivity type I), TH1 (cellular immunity-hypersensitivity type IV). Adapted from Montes-Montes [3], Giner-Munoz [6] and Irigoyen-Coria et al. [13].

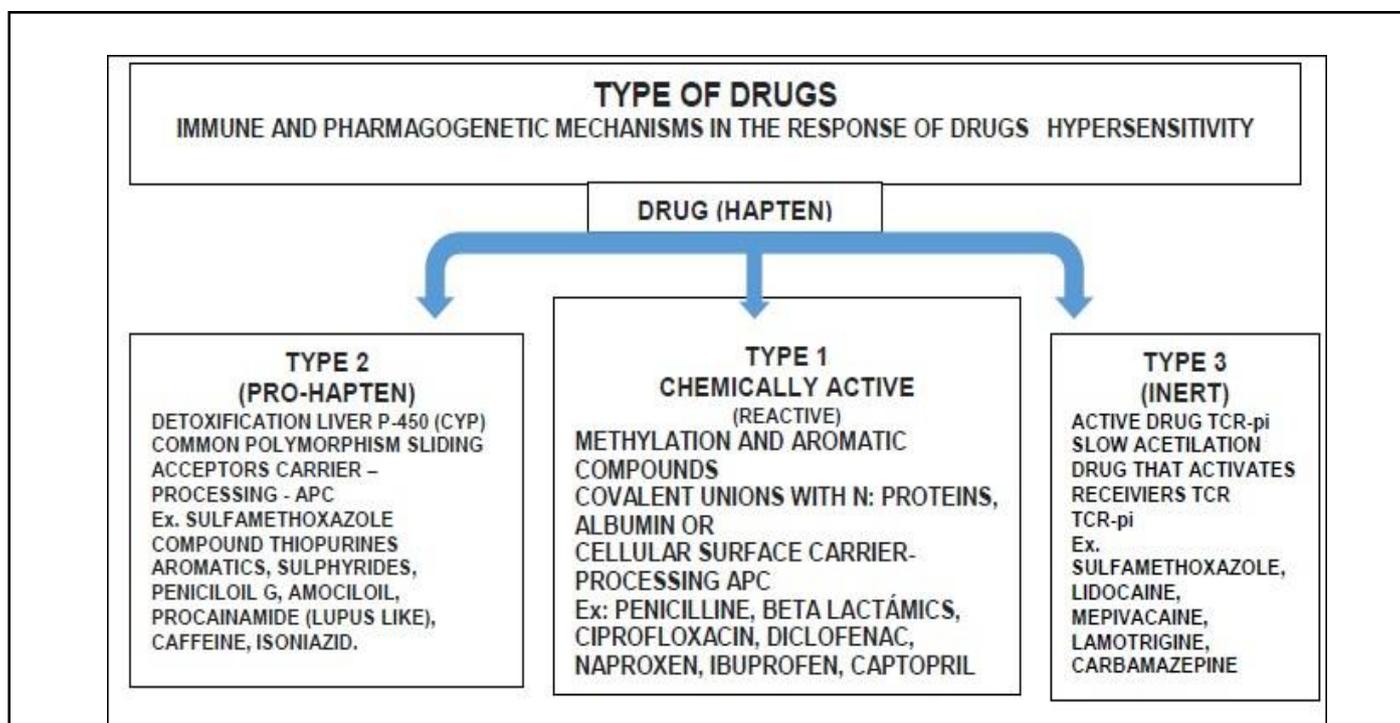


Figure 4: Classification types of drugs (haptens) based on immune and pharmacogenetic mechanisms and immunogen training for the immune response in context MHC in DHRs. Adapted from Giner-Munoz [7], Irigoyen-Coria et al. [13], Lares-Asseff & Trujillo-Jimenez [14], and Hewitt [22].

Antibiotics	Cross Reaction	NSAID	Cross Reaction	Others	Cross Reaction
Penicillin (Beta Lactam)	Penicillin derivatives BPO Beta Lactam	Diclofenac	ASA Salicylate Pyrazolones	Anesthetic Lidocaine	Mepivacaine Sulfonamide Allopurinol
Cephalosporine	Penicillin derivatives	Naproxen	ASA Salicylate Pyrazolones	Antiretroviral Abacavir (1)	Sulfonamide
Sulfonamide	Furozemide Lidocaine Benzocaine Carbamazepine	Ibuprofen	Salicylate Pyrazolones	Tenofovir (2)	Sulfonamide
Ciprofloxacin	Metronidazole	Acetyl Salicylic acid (ASA)	Salicylate Pyrazolones	Antihypertensives	PDN Prednisolone
Clarithromycin	Lamotrigine Mepivacaine			Vitamins, Steroids, etc	

Table 2: Cross Reactions of medicines that cause allergy to Drugs. Adapted from Irigoyen-Coria et al [13], Mallal et al [25], Guzman et al. [27], Torres et al. [28], Allende-Bandres et al. [29] and Gall et al. [30].

Atopy

It refers to an individual's genetic predisposition or susceptibility to develop sensitivity towards an allergen; it requires previous contact (intermittent or continuous) and depends on factors such as environment, temperature, sex (steroidal hormones) and age. This exaggerated immune response is mediated primarily by IgE antibodies, this antibody binds to FCεR1, increasing the half-life of IgE (2 - 60 days). We can find IgE synthesis from fetal stage to end of life. Higher concentrations of high and low affinity receptors have been reported in mast cells and basophils associated with severity, IL-4 inhibits the development of TH1 cells and activating TH2 cells, IL-3 favors the change of the isotype of B lymphocytes for the production of IgE, and the induction of the inflammatory process in DHR (Figure 3) [2,7,31-35].

Diagnosis of Drug Allergy and Diagnostic Tests

Medical history

It is the cornerstone of the diagnosis and has a better predictive value. It is based on clinical criteria, anamnesis, diagnostic algorithm, records of clinical history (signs and symptoms), followed by provocation and skin tests,

as well as laboratory tests suggested by physicians in some healthcare systems (Figure 5) [7,16,36].

Natural history

IgE antibodies may persist for years. Memory T lymphocytes (T_H1) are more intense during non-immediate DHRs: consequently, it is recommended to avoid the use of drug for long time [16,23].

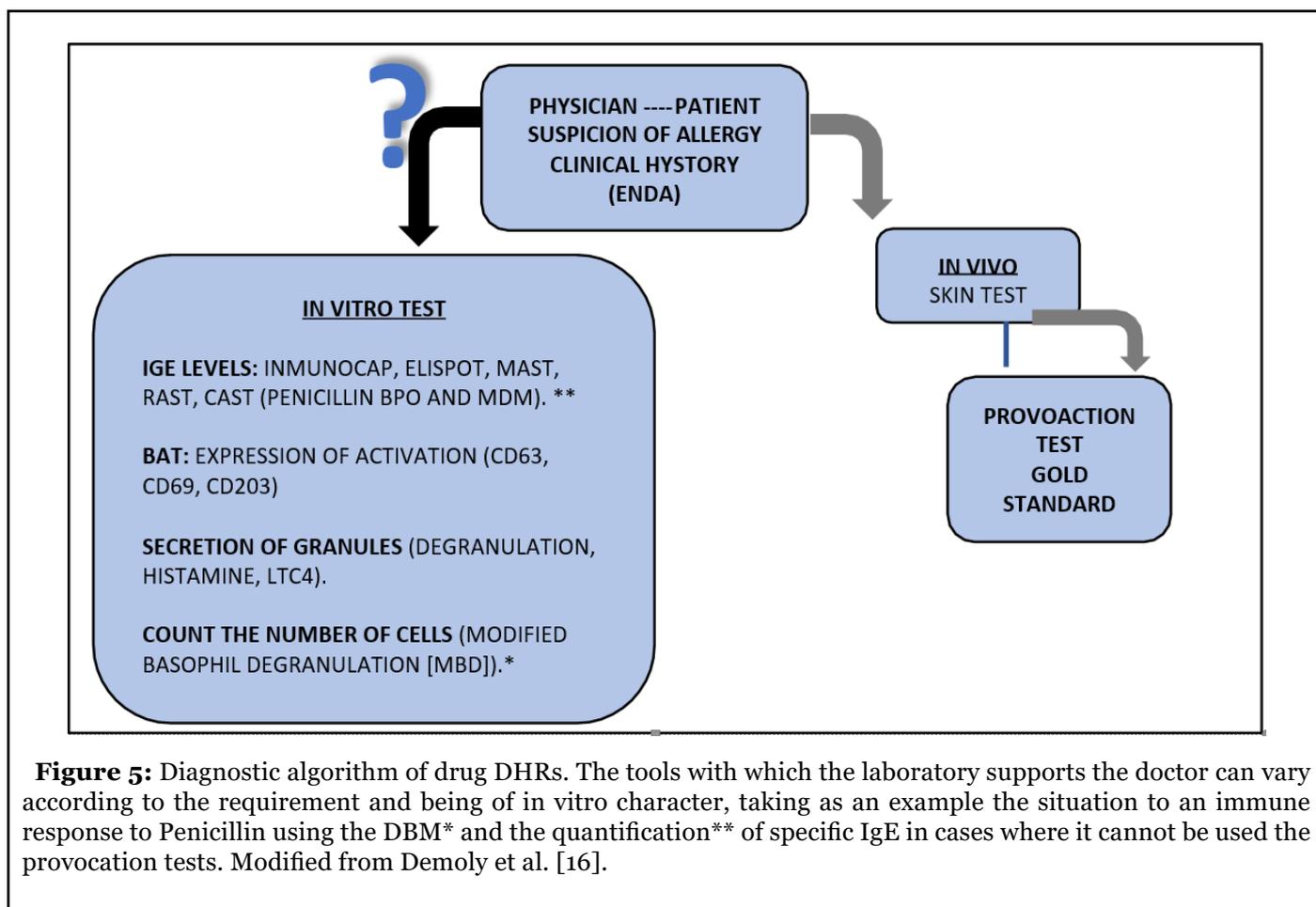
In vivo tests (provocation tests)

They are considered the gold standard to confirm or rule out a drug as the main inductor of hypersensitivity reactions.

Skin tests is the most frequently used, for better results is used with different dilutions and with controls negative and positive (histamine). Medical monitoring is necessary to avoid risks of anaphylaxis [7,16,18,31,37].

In vitro tests

IgE levels: Most of the DHRs diagnostic methods assess the type I hypersensitivity mediated by IgE and can be demonstrated by measure of peripheral blood levels of this antibody by different techniques like Immunocap, MAST, RAST, CAST, Chemo-luminescence, etc.



Basophil activation tests (BAT):

- Evaluating the secretion of granules (degranulation), histamine, LTC4 heparin, vasoactive amines.
- Expression of activation markers (CD63, CD69, CD203c, etc.) in the membrane of basophils.
- Count the number of cells can degranulate in exposition to the antigen (drug) (Figure 6).

Secretion methods: In the last five decades of the twentieth century, it was demonstrated that activated leukocytes released histamine and other vasoactive amine molecules as a response to *in vivo* and *in vitro* allergens; the fast or slow release by cytokines was also demonstrated, as well as the disappearance of basophils, all evaluated through electronic microscopy [18,31,38].

There are 2 types of participating cells: mast cells and basophils; the latter are more accessible for functional studies, since they are found circulating in peripheral blood (mononuclear cells of 7-9 μm that constitute 0.5%-1% of the leukocyte total) [7,13,18].

- **Histamine:** It is a premade mediator immediately released as a response to allergens or drugs. It is among the firstly used elements for in vitro and in vivo tests (erythema evaluation), for fluorometry, spectrophotometry and ELISA. Nevertheless, not all donors or patients release the total amount of histamine, and it may vary between 80% and 95%; therefore, basophil population needs to be spiked. There is also spontaneous release without stimuli that might be associated with higher sensitivity, reactivity and lack of specificity [10,13,18].
- **Leukotriene C4 (LTC4):** They are other slowly secreted mediators. They are lipid metabolites determined by ELISA and RIA methods [18,10,39].
- **Cytokines IL4:** They are recently formed, slow release molecules; IL3: they are determined by ELISA [13,18].

Modified basophil degranulation (MBD): It refers to the disappearance of basophils (count of cells) after activating and releasing their granulations; therefore, losing their morphology. It is determined by

microscopy method and specific staining. Basophils are incubated *in vitro* with the suspected drug, which causes the degranulation and releasing of their content (100% of specificity [Negative Predictive Value, -PV], 84% of sensitivity [Positive Predictive Value, +PV]). The modification consists in increasing the concentration of basophils 3 to 5 times the physiological value in peripheral blood (<1%), cell adjustment (# of basophils 56-100 x 10³ μL), standardization (dose of 0.1 to 1.0 mg/mL and response time of 30 min at 37°C with drugs-bell curve), basophil staining (toluidine blue, Wright, methyl red), negative control (physiological saline solution), positive control (formyl-methyl-leucyl-phenylalanine [fMLP]), with a minimum reading of 2000 leukocytes and a minimum value of 7 basophils (execution time <24 h). The reference values (RV) for MBD are 0%-30% of degranulation obtained in non-allergic population (Figures 6 -10) [7, 10,13,18,25,40].

BAT: Mechanisms, Standardize and Improve Test Reliability

Dose-response time curves of basophils in murines and humans with different allergens

The most common stimulus for basophil studies depends

on the activation through antigen-specific IgE (allergen or drug) of the cell surface connected to FcεRI (high affinity) which consists of an α chain and β and γ chains associated with the α chain as αγ2 and αβγ2. The role of β (chromosome 11q and 5q) is to increase the expression of the receptor by promoting the maturation and intracellular transit of the α chain, as well as the survival of IgE. The FcεRI-mediated signaling requires the binding of this receptor to the antigen (simple bivalent dimers inducing signaling); these binding depends on the concentration and affinity, which increases up to an optimal level and later returning to the basal state (bell curve as an ideal form); however, in human and murine models, more complex antigens (drugs) with higher affinity presented non-ideal dose-response curves (several forms). The basophil-IgE-epitope-antigen (drug) reaction can be considered as an antigen-antibody precipitation reaction; this results in the reduction of the receptor's mobility and RceI immobilization. The β and γ chains contain ITAM sequences that initiate the activation signaling of MAP kinase. The other receptor for IgE is low affinity Fcε II (CD63), which controls the growth and differentiation of B cells, increases the synthesis of IgE and also blocks the binding between IgE and eosinophils (Figures 3, 6-9) [7,18,39,41-44].

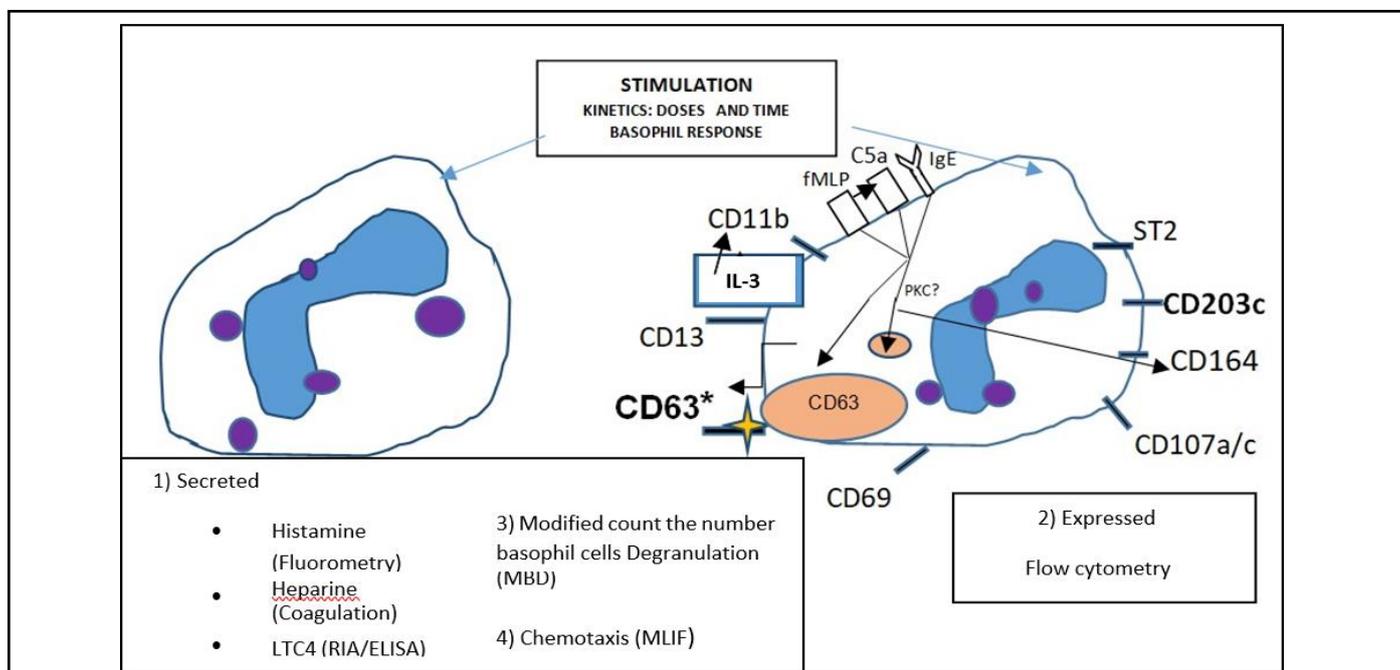


Figure 6: Predominant methods to evaluate BAT. In 2000, two predominant methodological ways were reported to evaluate the activation of basophils. 1) By measuring the release of secreted mediators: (Histamine 80-95% release-fluorometry; 2) Expression by Flow Cytometry mainly CD63 being the most common (fast responders, 2- 3 times more with signals of transduction and correlation with anaphylactic degranulation) and CD203c.3) Modified Count of the number of basophil cells (MBD) and 4) Chemotaxis MLIF. Taken from Mac Glashan Jr [18] modified by us.

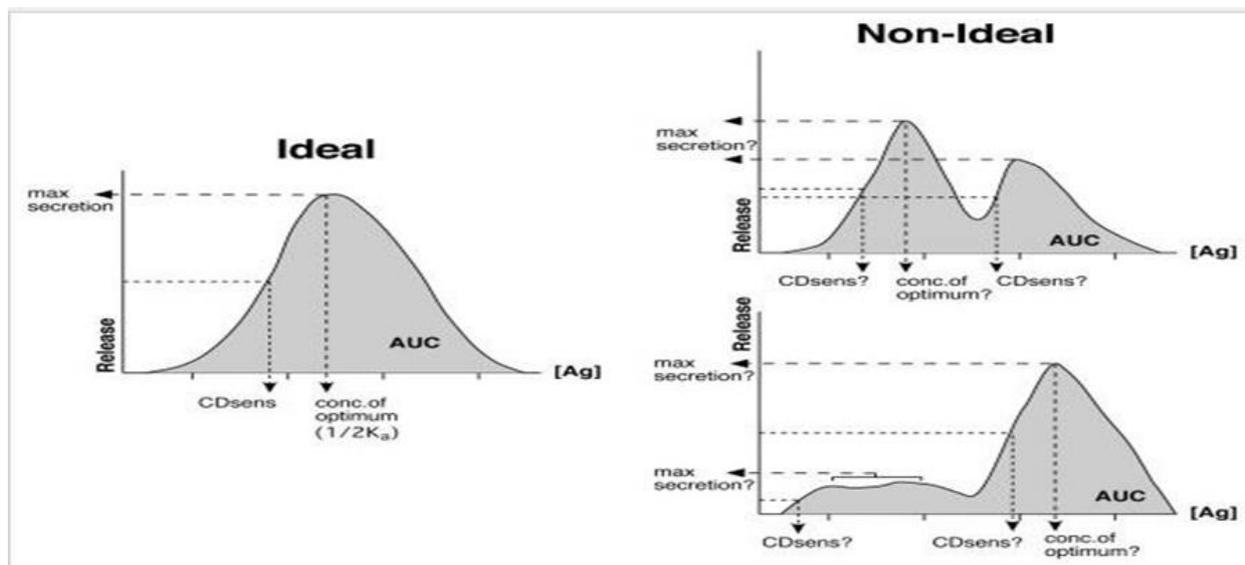


Figure 7: Dose response curves of allergens in murine basophils. Ideal (divalent single dimers) as typical (Non-Ideal). There are 4 metrics in common (1) 50% maximum response CD sens (2) maximum Histamine release response concentration (bivalent hapten that reflects affinity in the Ag-Ab binding, (3) maximum amount of secretion (4) Area under the dose response complete curve is not ideal, it is more difficult to obtain and it is due to the complexity of the allergens, source of preparation and the individual distribution of the specific antibodies of the epitope, affinity for the IgE. Taken from MacGlashan Jr. [18].

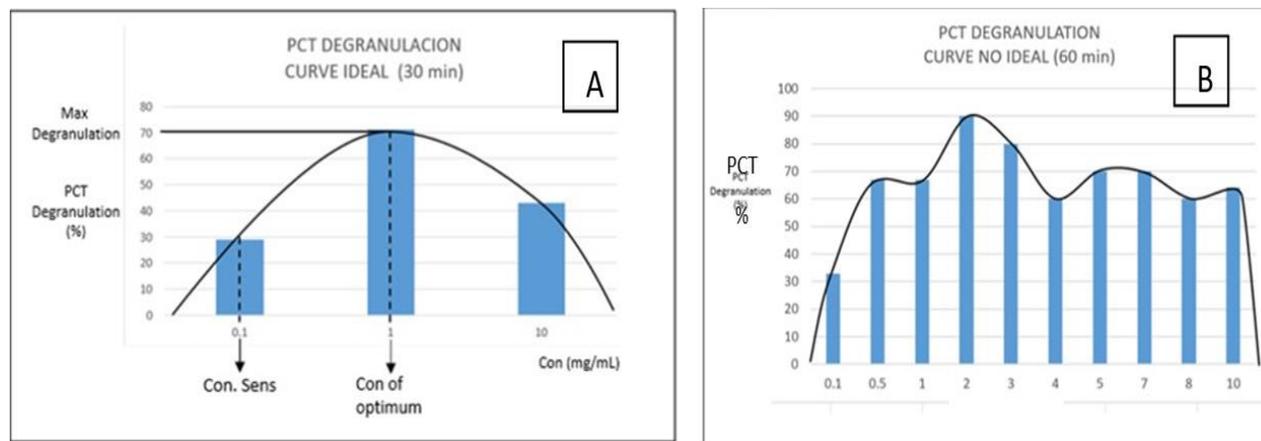


Figure 8: Curve dose response of allergens-drugs in the human (allergic) basophils. We observed a typical curve at 30 min (A. ideal in bell) and B. not ideal curve in 60 min that implies complexity, different affinities according to the IgE of allergic individuals with atopy, difference in age and sex. Therefore, it is suggested to perform the MDB test at 2 different concentrations ranging from 0.1 to 1 mg/mL. Own elaboration and only mentioned in Irigoyen-Coria et al. [13].

In essence, the binding of the paratope (2 IgE molecules) to the epitope induces intermembrane chain crosslinking and signaling transduction through kinase proteins (PKC), which provokes degranulation and release of

histamine, heparin, proteoglycans and vasoactive amines that amplify the allergic reactions (Figures 9,10 and Table 3) [11].

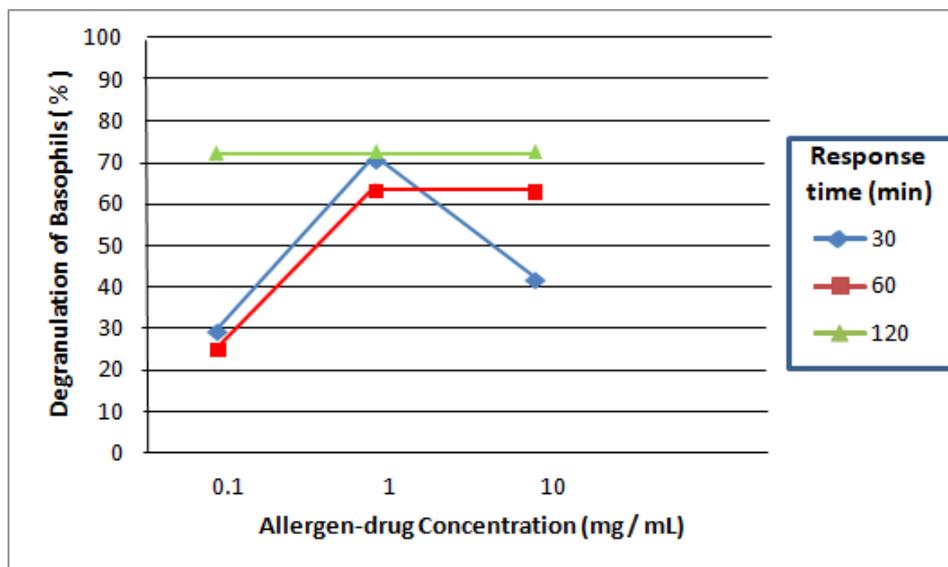


Figure 9: Time curve response of allergens-drugs in the human basophils in MDB. We observed that according to the range 0.1 to 1 mg/mL in the anaphylactic basophil degranulation curve; the optimal incubation time is 30 minutes where there is maximum sensitivity to detect fast responders, sensitive, very sensitive with greater affinity and greater degranulation. Own elaboration and only mentioned in Irigoyen-Coria et al. [13]

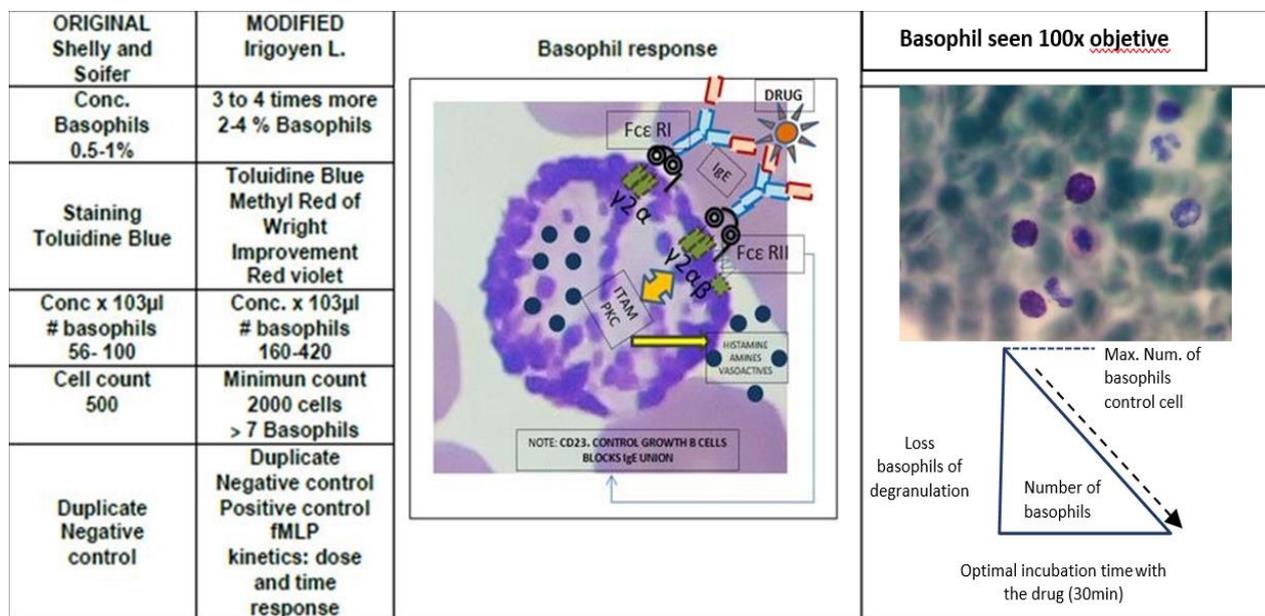


Figure 10: Alternative Test BAT: MDB. It consists of activating the basophils, causing degranulation (100% Specificity, 84% Sensitivity), the reference values (RV) for DBM are 0-30% degranulation obtained from the non-allergic population. Own elaboration and only mentioned in Irigoyen-Coria et al. [13].

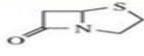
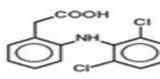
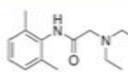
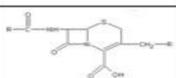
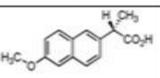
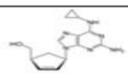
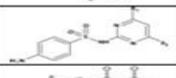
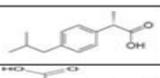
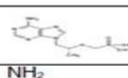
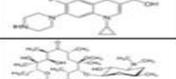
Allergy response from highest to lowest (%)	Antibiotics 50%	Chemical structure	NSAID 40%	Chemical structure	Others 10%	Chemical structure
	Penicillin (Beta Lactam)		Diclofenac		Anesthetic Lidocaine	
	Cephalosporins		Naproxen		Antiretroviral Abacavir (1)	
	Sulfonamide		Ibuprofen		Tenofovir (2)	
	Ciprofloxacin		Acetyl salicylic acid (ASA)		Antihypertensives	NH ₂ COOH SO ₂ , F, CL
	Clarithromycin				Vitamins, Steroids, etc	

Table 3: Frequency and chemical structure of drugs that cause allergy to medicines in the population of the Mexico City. Adapted from Irigoyen-Coria et al. [13], Kumar et al. [45]

Methods of activation markers expression

Since the last decade of the twentieth century and especially at the beginning of the twenty-first, a wide variety of techniques have been developed at research and commercial kits levels to identify the expression of basophil activation markers that use flow cytometry and monoclonal antibodies. The main clinical experience is with CD63, CD203c and CD69. Pharmacology bases BAT specificity on sign transductions through PKC, which stimulates the expression of gp53 receptor (CD63) (transmembrane lysosomal protein tetraspanin LAMP-31) on the basophil surface, as CD63 may or may not require IL-3 and its specificity depends more on the

epitope (drug)-paratope-IgE complementarity, affinity, avidity, atopy. The other three reported mechanisms are: a) proteins constituting the membrane and that express themselves with fast release of CD11c vesicles, b) recently synthesized proteins which express in higher time frames of hours and require transcription, translation and transportation to the plasma membrane, and c) expression on the basophil surface through the C5a receptor, releasing histamine when activated by a positive formyl-methyl-leucyl-phenylalanine (fMLP) which can be used as positive control for the determination of CD63. Other markers include CD69, CD203c, CD13, CD11b, etc. (Figure 6 and Table 4) [7,9,13,18,38,46,47].

Activity marker	Expression	Stimulated	Speed	Location	It depends	Specificity
CD69	Weak	IL-3	Slow (h)	Does not form part of granule membrane	ARN m	Low
CD203c	Moderate	Several including IL-3	Slow	It can be fused with membrane		Low
CD11b		Various	Fast			Low
CD63	High	Associated with Anafilatic degranulation	Fast (min)	It is part of the membrane LAMP3	PKC fMLP	High Prevent fMLP Activated platelets
CD123	High	Receptor IL-3	Fast	It is part of the membrane		Non-Specific Avoid dendritic cells

Table 4: Characteristics of the Activation Markers for their election in BAT. They are used in research and development of commercial kits or implemented and standardized in clinical laboratories and human allergy as well as murine homologs for the purpose of diagnosis of DHRs; the activation marker is determined by the needs of the study. The most common one used is CD63 (associated with degranulation) some kits use IL-3, in others not (our group does not use it), negative and positive controls are included; RV are variable according to the study population. Adapted from MacGlashan Jr [18].

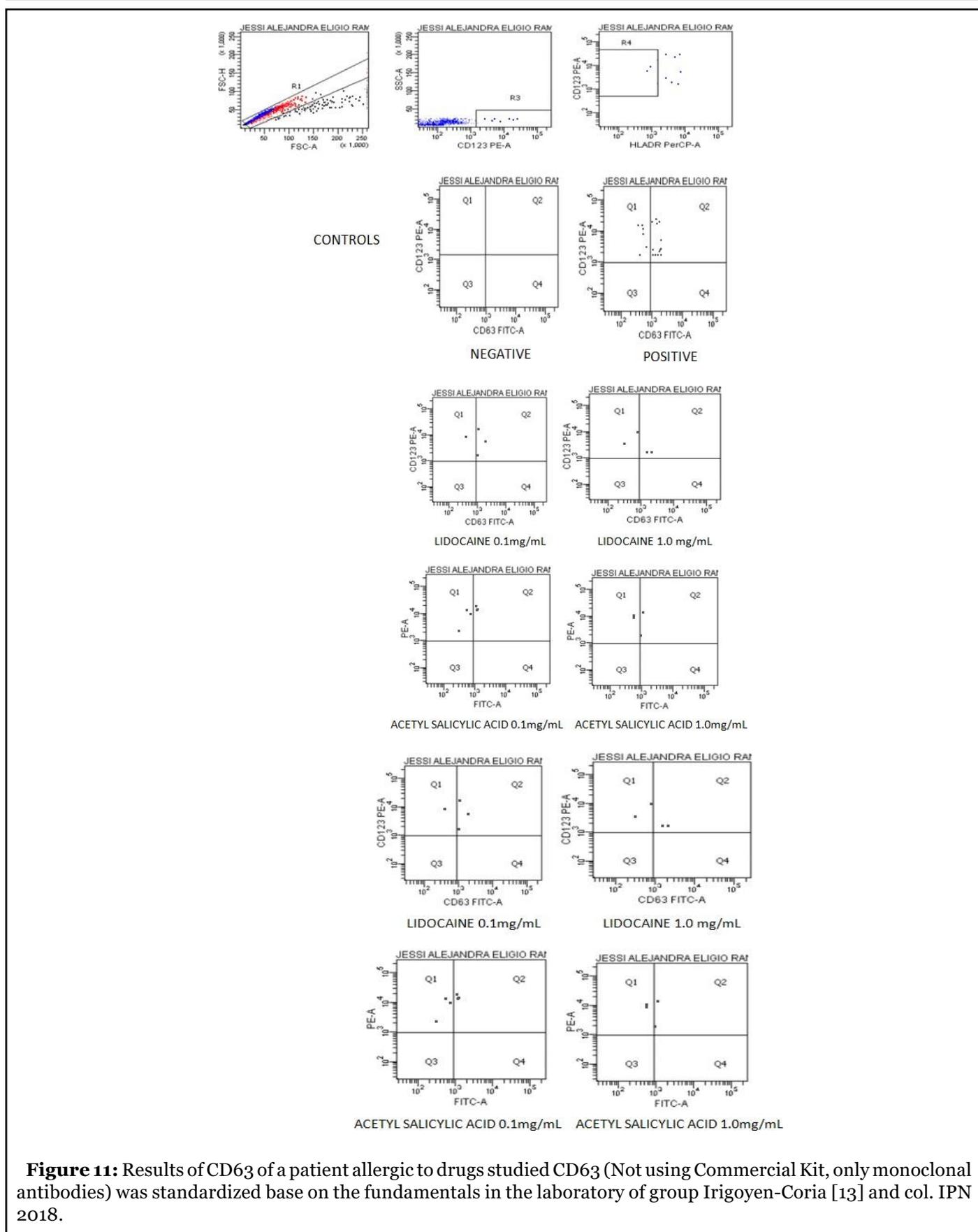


Figure 11: Results of CD63 of a patient allergic to drugs studied CD63 (Not using Commercial Kit, only monoclonal antibodies) was standardized base on the fundamentals in the laboratory of group Irigoyen-Coria [13] and col. IPN 2018.

CD63-Flow cytometry: The determination is possible by staining of monoclonal antibodies marked with fluorochromes CD63-FITC, CD123-PE and PerCP HLA-DR with reading by flow cytometry, a negative control of PBS/Ca-albumin, pH 7.2 and a positive control of fMLP (joined to the C5a receptor). Reliability is determined by specificity and sensitivity ($\geq 85\%$ - 99.4%); it is reported as CD63 %, as well as the stimulation index (SI); the

RV vary depending on the author with CD63 % = 0-5, 0-30, SI or activation $>2\%$ - 4% . Factors can also vary depending on the ethnicity, C5a receptors, IgE-drug affinity, IgE concentration, LAMP-31 distribution and titration of monoclonal antibodies marked with two-color fluorochrome: green (fluorescein isothiocyanate-FITC) and red (phycoerythrin-PE) (Tables 4,5, and Figure 11) [18,44].

Activation of Basophils CD63				
Results	%CD63	MFI	%AI	%SI
CONTROL (-)	0	0	0	0
CONTROL (+)	68.4	1,648	112.7	100
LIDOCAINE 0.1 mg/mL	75	1,284	96.3	85.4
LIDOCAINE 1.0 mg/mL	50	1,832	91.6	81.3
ACETYL SALICYLIC ACID 0.1 mg/mL	50	1,188	59.4	52.7
ACETYL SALICYLIC ACID 1.0 mg/mL	33.3	1,054	35.1	31.1

Table 5: Results of BAT-CD63. Report of allergic patient to 2 drugs (our group patient), the most sensitive was the anesthetic lidocaine at lower concentration and greater, parameters: %CD63 (Expression of activation markers), MFI (Medium Immunofluorescence), %SI (Stimulation Index), %IA (Activation Index) the negative control (PBS+Ca+Albumine pH 7.2) was 0 and the positive control (fMLP) was 68.4 percent correspond to 100% of SI. Own elaboration (The Histogram Cytometry corresponds to the Figure 11).

Functional tests as an alternative to BAT: chemotaxis and type IV hypersensitivity

A) Modified Leukocyte Migration Inhibition Factor (MLIF) Type IV a, b and c, associated with anaphylactic degranulation: It has been reported that leukocytes, including basophils (BAT-Chemotaxis) also play a directional chemotaxis role; therefore, when

microhematocrits are incubated in Bloom chambers with drugs in two dilutions (1 mg/mL and 0.1 mg/mL) in an RPMI medium, with negative and positive controls, at 37°C, the early (20 min to 2 hrs) and delayed (4, 6 and 18 hrs) migration can be measured; the % of MLIF can also be calculated vs the negative control, as well as the reference values (RV) for MLIF (0%-25% of leukocytes migration inhibition) (Figure 12) [13,37,48,49].

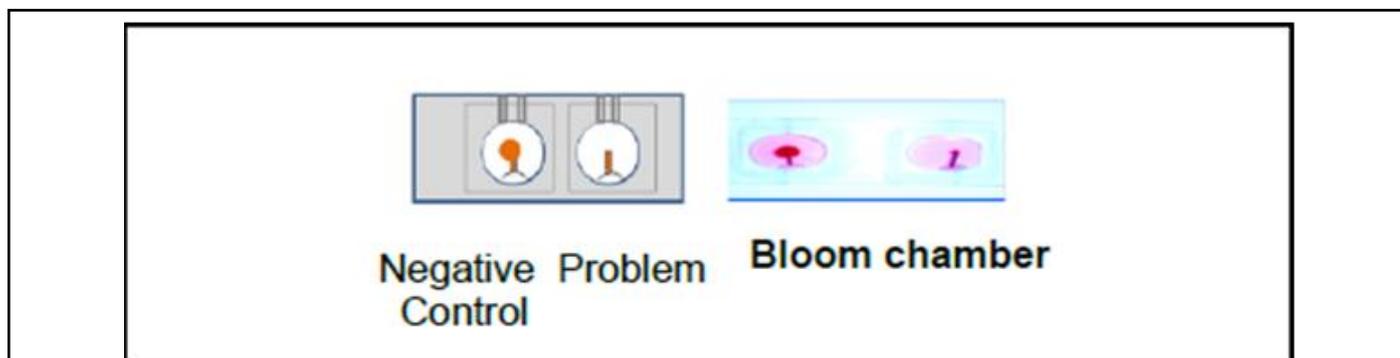


Figure 12: Example of a MLIF assembly to assess late hypersensitivity in addition to having a correlation with anaphylactic degranulation. Own elaboration.

B) Blastoid transformation of lymphocytes (BT): It refers to cultures of incubated lymphocytes (6 days) which were stimulated with drugs at different dilutions, marking the DNA with tritiated thymidine to report the stimulation index in relation to a positive control (PHA) in 48 hrs with a RV (0.83-1.89) (Pathologic Values: 2.1-6.7) [48-50] and Lymphocyte transformation test (LTT) : It is done with lymphocyte incubation with drugs and DNA concentration with dyes to avoid radioactivity [2,13].

Selection of BAT Diagnostic Methodology

The decision is made based on simple, inexpensive and analysis (manual and automated complexity, equipment, cost and time) [9,18,31]

Advantages and disadvantages of BAT [18,31]

Advantages:

- *In vitro* methods reduce the risk in hypersensitive patients or patients with severe pharmacodermis, which may lead to anaphylactic events or sudden death.
- Basophils and mast cells have a similar response to crosslinkings for FcεRI and FcεRII activation when increasing the half-life and FcεRI receptors.
- CD63 has a positive regulation in basophils and mast cells, which corresponds to an anaphylactic response.
- The activation of basophils is a fast functional test as a response to an allergen or drug.
- It is possible to separate the intrinsic factors (chemical structure of the drug) from the extrinsic factors (additives, stain, etc.).
- The study may suggest therapeutic alternatives to avoid cross-reactions.
- They have a high predictive value for drugs, allergens (food and inhaled), anesthetics and occupational toxic elements.
- They can determine the degree of sensitivity, reactivity and reading of a thousand events in the flow cytometer in allergic patients.
- There is a follow-up and post-treatment monitoring.

Disadvantages:

- It requires an implementation and standardization

process for reliability.

- The concentration of basophils must be increased by separation methods with gradients of Percoll, sedimentation with dextran and only centrifugation.
- There are specific conditions, such as type of anticoagulant, preservation, transportation (5-8°C) in horizontal position, neutral pH of the solvent (pH of 3.7 allows the IgE to dissociate from the FcεRI in 10 to 30 seconds), drug stability.
- Reaction kinetics must be performed (concentration and response time), which include viability and functionality response time.
- The determination is by secreted substances (histamine, LTC₄ and cytokines) or by fluorometry, spectrophotometry, ELISA (20,000 to 50,000 basophils).
- The requirement of: sampling, anticoagulant, viability (preservation and transport) to perform the BAT should not be greater than 48 hours.
- There are non-responding or secretagogue individuals.
- There is methodology complexity (equipment, reagents [expensive], laboratory staff's experience and interpretation).
- There is a need for standardization, precision, coefficient of variation (CV) and reference values (RV) obtained in normal population (without infection), as well as for age groups and preventive culture.
- The skin provocation test and BAT may not correspond to the diagnosis (some cases of chronic spontaneous urticaria and neurodermatitis).

Discussion

Currently the synthesis and consumption of drugs has increased; in a collateral manner also an increase prevalence DHRs (urticaria, anaphylaxis, SJS, TEN, AGEP) [4,7,13,51], as well as to autoimmunity and malignancy processes [25] since postnatal stage to the old age (7% in elderly adults, 18% in children with an interval of 15%-24%, 5%-15% in hospitalized patients); more frequent in women between 40 and 60 years age. Trigger drugs include, in first place: antibiotics like penicillin, beta-lactams and cephalosporins (50%), followed by NSAIDs and analgesics (40%), and finally anesthetics, antihypertensive, hormones, antiretrovirals, among

others (10%). Therefore, it is necessary to select a reliable methodology (BAT and complementary alternatives test with dilution, controls negative and positive) for better improvement in the diagnosis and public health [7,12,13,18,16,52,53]. The selection of studies according to the type of DHRs, associated with the activation of the adaptative immune system; facilitated by Gell and Coombs classification using a diagnostic algorithm [16].

Due to all of the above and since discovery in 1906 of "Allergy" and their association with mast cells and basophils: during several decades of the 20th century and the beginning of the 21st century, have been an important focus of study and research models of human species, murine and other; for the development and implementation of *in vivo* and *in vitro* tests. Based on mechanisms of adaptive immune response that provoke the release of histamine, heparin, vasoactive amines and activation markers (CD63, CD203c), Mac Glashan Jr. [18] has focused on the BAT studies in murine models; these models represent a good strategy to acquire knowledge about the functions of the effects, mechanisms of molecular and immunological activation, the development of techniques that they can be applied in humans. Several researchers, including Hoffman et al. and others [7,13,18,31,38,46,47] propose that, at present time, the most developed and widely used tests at clinical and research level are the IgE-dependent BAT; there are two principal types: 1) secretion of granules (degranulation), histamine, heparin, vasoactive amines, and 2) activation markers expression (CD63, CD69, CD203c, etc.) [18]; when comparing the essays in murine and human models, similar responses to the stimulus with allergens and drugs, two more were found during the development and standardization of BAT methods for anaphylactic degranulation (3.-MDB-Microscopy and 4.-MLIF-chemotaxis) in our laboratory (Figures 6 -10) [18].

Mac Glashan Jr reflects: What do we know? 1) If flow cytometry methods and Kits provide us with a reasonable alternative to traditional tests, especially CD63 (is more closely associated with degranulation) [18] over CD203c. We think there may be a problem with target because the basophil has a c5a receptor which can be activated via the complement (infectious or autoimmune inflammation); consequently, it loses some specificity? Although our article does not consider it. 2) Is intrinsic sensitivity of basophils a useful measure to know the sensitivity, we probably think that it is in accordance with the sensitivity and reactivity of the basophils since some patients show greater degranulation, a higher %CD63 and a %SI (responders, atopy) of the allergic patient, in addition to the reactivity and severity increase; 3) How is BAT diagnostic methodology selected? This decision is based

on the clinical study requested by the physician (DHRs situation), these tests are simple, inexpensive and give great support in the diagnosis of drug reactions with coverage of several types of hypersensitivity, time, as well as basophil viability and report urgency) [13,18].

Giner-Munoz is especially concerned about children and other age group; because the first worldwide cause of allergy is infections and this also with antibiotics (50%), mainly penicillin and β -lactams, prescribed by physicians due to their wide spectrum and low toxicity, therefore it is considered that BAT and alternative tests are important to avoid cross reactions and give future therapeutic options [6,7,54].

At present there are several groups of patients that require special attention in order to promote the implementation and development of BAT and other alternatives, like chemotaxis (MLIF- Type IV) [13] that may help to reduce the prevalence, risk factors, severity, lethality, and assist in solving a public health issue:

- 1) The prevention of allergies to penicillin and β -lactams: improve during the diagnostic algorithm, the epidemiology, history of previous anaphylactic allergic reactions, atopic family, as well as microbiological cultures with antibiograms as a preventive measure before prescribing antibiotics in attention primary services and hospitals (public and private); in this manner, 65% of allergic patients would be identified and avoid the use of lidocaine as a solvent in lyophilized antibiotic [13,55];
- 2) Patients with surgeries, accidents, autoimmune diseases or traumatological events require concomitant prescription of antibiotics and NSAIDs, which may cause DHRs in many cases; for example, diclofenac (TH1 and TH2) (39%) [13,56], and anesthetics [57] causing perioperative and operative anaphylaxis (60%) [13] hypnotic drugs (2 -10%);
- 3) Patients with HIV [58] make frequent use of antiretroviral treatment in the form of abacavir dose (nucleoside analogue that inhibits retrotranscription) and sulfonamide antibiotics which, in severe DHRs, activate TH1 and TH2 (80%), especially in slow acetylators [53,57,59-62];
- 4) For symptoms associated with SJS (such as erythema, general discomfort, fever, nausea and vomiting), 78% of the patients had the HLA-B*57:01 allele [25]; hypersensitivity to sulfonamides and carbamazepine was also found [31,63,64];
- 5) Neurological patients with epilepsy and bipolar disorder treated with carbamazepine developed SJS and had the HLA-B*15:02 allele [17,55,65].
- 6) Patients with gout, persistent renal lithiasis, even leishmaniasis, as well as patients with chemotherapy and control therapy for high uric acid levels, treated with alopurinol, had the HLA-B*58:01 allele [17,24,54,63,64,66].

Conclusions

The selection of studies according to the type of DHRs is important. Nowadays, BATs are the most used tests at clinical level; however, other alternatives based on the specific stimulation of IgE-dependent basophils should be considered. The BATs (CD63-cytometry with correlation of degranulation (MBD by Microscopy) and unidirectional chemotaxis against a stimulus (MLIF-IV) read at 2 hours and 18 hours to determine hypersensitivity I and IV, this would improve diagnostic value, treatment, monitoring, prevention, identification of risk groups, prevent cross-reactions and severe pharmacodermis, creating therapeutic alternatives. The future proposed studies would be Fcε I, IFN γ, IgE in umbilical cord blood, genogram, HLA, and acetylator phenotype, all of which would help to improve the diagnosis, prevention and treatment; as well as the education for the population in this area, quality of life and health.

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