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Diagnosis of Immediate Allergic Reactions to Drugs

by B. A. Baldo

For many years, our understanding of immediate allergic reactions to drugs rested almost solely on the classical studies on penicillins. It is now clear that IgE-mediated allergic reactions occur with a range of different drugs, and that small structures as different as a quaternary ammonium ion and a dimethoxybenzyl group may act as allergenic determinants, binding IgE antibodies and provoking allergic release of mediators in sensitive subjects. To maximize the chance of successfully and accurately diagnosing a drug-induced immediate allergic reaction, skin tests with both free and conjugated drug(s) and specific radioimmunoassay for the drug(s) should be employed to supplement the case history. In cases where anaphylaxis is suspected, assay of mast cell tryptase is a valuable diagnostic aid. Measurement of tryptase will, for the first time, help to define a homogeneous group of patients with type 1 drug allergy in which skin, immunoassay, and mediator tests may be evaluated.

Allergy to Small Molecules and Immunological Dogma

In his studies of artificial antigens and haptens on the one hand, and of hypersensitivity to simple chemicals on the other, Landsteiner [1] pioneered two areas of investigation that are still basic to our thinking on drug allergy. Despite the frequent lack of evidence of prior contact with an incitant chemical or drug, or its metabolite(s), Landsteiner concluded that "It could be assumed that a non-antigenic substance can sensitize after combination with protein in the animal body." Since that time, it has been generally accepted that "small" MW compounds (for example <1000 Daltons) only become immunogenic by combining with a larger carrier molecule, usually, but not always, a protein. Despite this dogma, it is often not possible to demonstrate protein binding, or even to see from the structure of the drug how protein binding could occur. This is reflected in positive skin tests with many unconjugated drugs even though conjugation onto a carrier is said to be a requirement for cross-linking of cell-bound IgE, and hence for a positive skin test to occur. With many drugs, such as neuromuscular blocking drugs (NMBDs), trimethoprim, sulfamethoxazole, thiopentone, narcotics, atropine, neostigmin, beta-lactam antibiotics, etc., skin tests are commonly performed using free, unconjugated drugs. It is clear that, in some cases at least, positive skin test responses are obtained in patients allergic to the drug, but not in control

subjects. Clearly, there is more to the presumed carrier effect than we currently understand, and the question of whether or not simple chemicals or free drugs both sensitize and provoke allergic reactions needs to be resolved.

The dogma of prior exposure to a drug or chemical also does not always appear to hold, and cases where no previous contact can be established are not uncommon. A fairly frequently seen example of this occurs in subjects (usually women) who experience an anaphylactic reaction following the administration of a NMBD prior to anesthesia. At least 60–70% of such subjects have never had a previous anesthetic, suggesting that prior sensitization with cross-reacting allergenic determinants in the subjects' environment has occurred [2, 3]. In the light of inhibition studies showing potent inhibitory activity by a variety of drugs and other chemicals containing substituted ammonium ions [3–5], the phenomenon of heterospecificity, as expressed by Van Regenmortel [6], may be relevant:

"The degree of fit between different epitope-paratope partners can vary by a factor of a million, and the best possible fit obtainable in the case of any particular paratope is not necessarily actualized with the epitope used for immunization. Each paratope is multispecific, i.e., it is able to bind to a number of different epitopes, and it may bind more strongly to other epitopes than to the one against which it was raised."

For one group of drugs at least, namely the NMBDs, the structural requirement for pharmacological action may also determine the allergenic properties of these compounds. For neuromuscular blocking activity, at least two quaternary or tertiary ammonium groups at a suitable distance are required [7]. Since the ammonium groups are also the allergenic (IgE-binding) determinants on NMBDs [8], the allergenic divalency of the compounds may allow cross-linking of adjacent cell-bound IgE, a necessary prelude to allergic release of mediators of anaphylaxis.

Anaphylactic Reactions to Drugs

Our research and applied clinical studies are chiefly concerned with anaphylactic responses to drugs, so the focus presented and discussed here is primarily on this severe form of immediate hypersensitivity. In diagnosing anaphylaxis the following criteria were used:

1. The reaction was life-threatening.
2. It had the classical features of anaphylaxis.
3. There was multi-system involvement and/or laboratory confirmation.
4. Other explanations were unlikely.

Laboratory confirmation tests used were:

1. Raised hemoglobin levels
2. Positive tryptase test
3. Positive skin test
4. Positive radioimmunoassay (RIA) for specific IgE antibodies

Over the period 1974–1992, 867 subjects were studied, most of them being referred following a reaction suspected of being anaphylactic. True anaphylaxis was diagnosed in 474 subjects who experienced general anesthesia, but only in three subjects

TABLE 1
DRUGS RESPONSIBLE FOR 476 CASES OF ANAPHYLAXIS

Drug	Number
NMBDs	
Alcuronium	120
Suxamethonium	79
Atracurium	25
<i>d</i> -Tubocurarine	20
Gallamine	16
Pancuronium	10
Vecuronium	5
Decamethonium	1
Suxamethonium and 1 other NMBD ^a	7
Induction agents	
Thiopentone	43
Alfathesin	29
Propanidid	6
Methohexitone	1
Midazolam	1
Propofol	1
Induction agent and NMBD	
Thiopentone and 1 other NMBD ^b	3
Other drugs	
Colloid solutions	26
Antibiotics	11
Narcotics	7
Protamine	9
Contrast media	4
Local anesthetics	3
Atropine	2
Neostigmine	1
Platelets	1
Cause not found	46

^aAlcuronium, atracurium, gallamine, or *d*-tubocurarine.

^bAlcuronium, atracurium, or suxamethonium.

receiving a local anesthetic. The latter result agrees with the findings of Snidel and de Shazo [9], who concluded that anaphylaxis to local anesthetics is rare. The drugs responsible in the 477 cases of clinical anaphylaxis are listed in Table 1.

The clinical features observed in 315 patients with anaphylaxis caused by anesthetic drugs are listed in Table 2. It should be noted that the complete set of symptoms and signs did not occur in all patients, and that cardiovascular collapse was the most common, and the worst, feature.

Skin Testing for Drug Allergy

Skin testing [10] by prick or intradermal testing is generally carried out 4–6 weeks after a reaction and may be positive for many years (see below). The great advantage of the skin test is its simplicity. The method is quick, sensitive, reproducible, inexpensive, and can be performed by almost anybody. Its disadvantages include difficulties related to standardization of the method and the solutions used for testing, and variations that may be seen in results from different operators. Skin testing is usually used in two forms:

- Intradermal testing, in which about 10–20 µl of the drug is injected beneath the dermal layer.
- Prick testing, in which a droplet of a solution of drug, usually at a higher concentration than used intradermally, is placed on the skin and the dermis pricked through the droplet with a needle or lancet.

In testing for anesthetic allergy, the methods agree on the detection of the responsible drug in over 95% of cases. A wheal of 2 mm diameter or larger is usually taken as positive, although Pepys (personal communication) and Moneret-Vautrin and Laxenaire [11] believe that false positives are so rare that even smaller wheals should generally be regarded as positive.

Skin testing for diagnosis of drug allergy works well with some free drugs such as NMBDs and protamine, occasionally with other drugs such as beta-lactam antibiotics [12, 13], trimethoprim, and sulfamethoxazole [14–16], and not at all or rarely

TABLE 2
CLINICAL SIGNS AND SYMPTOMS IN 315 PATIENTS WITH ANAPHYLAXIS DUE TO ANESTHETIC DRUGS

Signs and Symptoms	Number
Cardiovascular collapse	283
Bronchospasm	
Severe	52
Transient	50
Rash	42
Urticaria	43
Erythema	151
Angioedema	73
Pulmonary edema	11
GI symptoms	30

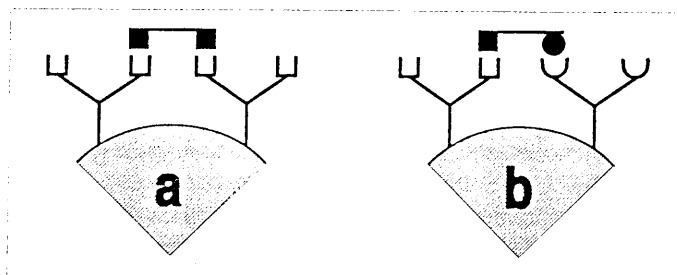


Figure 1. Types of cross-linking or bridging of adjacent cell-bound IgE molecules suggested to occur in allergic release of mediators by unconjugated drug molecules. (a) Bridging via an allergenically divalent unconjugated drug molecule. This is thought to occur in subjects who have an anaphylactic reaction to NMBDs. (b) Bridging via a free drug molecule containing two (or more) different allergenic determinants.

with others such as colloids and contrast media. In stating this, however, we should be aware that careful and comprehensive skin test studies using drugs with clearly defined drug-allergic patient groups have generally not been undertaken, so it is, as yet, impossible to confidently conclude whether a particular drug does or does not produce positive skin test responses. Why, for example, one subject allergic to trimethoprim should respond with a clearly positive prick test to the free drug, while another subject, with apparently the same diagnosis and symptoms, shows a negative response is not understood. In such cases, the question of the patients' responses to drug-protein conjugates often remains. Cases where subjects show a positive skin test to the free drug should be compared to cases where only drug conjugates provoke reactions. Such studies may provide important insights into the nature of the allergenic structure eliciting the reaction and the mechanism leading to allergic mediator release. Such a study should also include an examination of carrier effects and the effect of different points of covalent attachment of the drug to the carrier. Apart from the penicillins [17-19], little information is available on these questions for other drugs implicated in allergic reactions.

As mentioned above for NMBDs, a single unconjugated drug molecule may have the capacity to cross-link or bridge adjacent cell-bound complementary IgE molecules, thus triggering release of mediators of allergy. In theory this might occur via two or more identical or similar allergenic determinants on the drug, or via the involvement of different allergenic determinants on the molecule. These two types of cross-linking are illustrated in Figure 1. NMBDs are examples of the former drugs, while thiopentone, trimethoprim, and beta-lactam antibiotics, all of which carry more than one IgE-binding determinant [17, 19, 20], are examples of the latter type. Types of cross-linking or bridging for mediator release are discussed in more detail elsewhere [21, 22].

Detection of Drug-Reactive IgE Antibodies

Immunoassays for detecting drug-reactive IgE antibodies are undertaken on the assumption that the detection of the antibody in serum reflects IgE bound to mast cells. Radioimmunoassays (RIAs) for detecting IgE antibodies to an expanding range of drugs, including NMBDs, anesthetic induction agents, narcotics, beta-lactams, and other antibacterials, have been de-

veloped, and their use has contributed greatly to our understanding of the mechanisms and to improving diagnosis [20, 23].

In our studies carried out over more than a decade we have found that serum tests detected the drug responsible for an allergic reaction with about the same frequency as skin tests. In addition, a combination of the RIA and skin test usually detects the drug responsible better than either test alone. Allergic cross-reactivity detected by RIA is often greater than that detected by skin tests [21, 23]. Two limiting features of the RIA tests are false negatives that may result from the detection of circulating IgE, which underestimates the amount of cell-bound antibody, and the possible concealment of allergenic determinants following covalent coupling of the drug to the carrier solid phase.

Inhibition of IgE binding using the drug in question together with analogs is a valuable investigation for verifying the results of direct binding studies. These quantitative tests may be carried out to demonstrate a true dose response, as well as to determine and compare the amounts of drugs required to achieve a predetermined degree of inhibition (often 50%) or the degree of inhibition obtained with a fixed amount of drug. Quantitative inhibition studies provide an efficient and precise method for studying allergenic cross-reactivity between drugs [20].

Clinical Relevance of *In Vitro* Cross-Reactivity Findings

In interpreting which, if any, drug (in addition to the one that provoked the reaction) a patient may be sensitive to, the following points need to be considered:

1. The origin of the drug-reactive IgE antibodies in the patient's serum. Some subjects react to a drug without apparent prior exposure, for example, many reactors to NMBDs, where the precise allergenic stimulus for the formation of the IgE antibodies, and their specificity, are unknown. In these cases, the drug showing the highest uptake of IgE in direct binding studies and/or the best inhibitor *in vitro* is not necessarily the drug or chemical that provoked the allergic sensitization.
2. Inhibitory activity *in vitro* may not always parallel the *in vivo* allergenic activity of a drug. The former is a reflection of the complementarity of the drug to the IgE antibody combining sites, while *in vivo* activity reflects cross-linking of cell-bound IgE and the release of the mediators responsible for the signs and symptoms of allergy.
3. The nature of the drug solid phase used *in vitro* in direct binding and inhibition studies. Not only will different drugs influence the result, but different points of attachment of a drug will determine the nature of the determinant groups available for reaction with antibody.

Difficulties in Interpreting Skin and Serum IgE Tests

Skin test results for drug allergy do not always correlate closely with the presence of drug-reactive IgE antibody.

ies in the serum, and the incidence of cross-reactions between drugs detected by IgE immunoassay is often greater than that detected by skin testing [2, 3]. This is also often the case with inhalant and food allergens, where the RAST may reveal reactive extracts that do not parallel the skin test results or clinical picture. The reason for this is probably that the two tests measure different things: *in vitro* reactivity with antibody combining sites in the case of the RIA, and *in vivo* reactivity at the mast cell level with the skin test.

In attempting to select a safe drug for a drug-allergic patient, the wisest approach is to carefully consider the history and skin tests, IgE direct binding, and inhibition of antibody binding results. Some examples where this was done with subjects allergic to NMBDs are shown in Tables 3-5: The RIA results are expressed as multiples of the uptake of ^{125}I -anti-human IgE obtained with the negative controls (3 $\times$ taken as positive); RIA inhibition results are expressed as nanomoles of drug per tube needed for 50% inhibition of binding of IgE.

In case 1 (Table 3), suxamethonium was obviously responsible for the reaction, but there was cross-sensitivity to the other NMBDs, although pancuronium was only relatively weakly reactive. Pancuronium was given subsequently without any adverse effect despite its clear inhibitory activity.

In case 2 (Table 4), skin tests indicated reactions to *d*-tubocurarine and alcuronium. RIAs showed strong reactions to alcuronium and clear cross-reactions with other NMBDs. Inhibition studies confirmed this and suggested a single IgE antibody cross-reactive with alcuronium and *d*-tubocurarine. Note that thiopentone was used on both occasions and could easily have been blamed.

Case 3 (Table 5) is a clear-cut situation, since all three tests agreed that alcuronium was the cause of the reaction. Subsequent exposure to suxamethonium and pancuronium was uneventful. RIA and inhibition results suggest that quaternary ammonium groups were probably not solely responsible for binding of the drug to IgE antibodies.

These three examples illustrate the value of considering a number of tests before deciding on the diagnosis. Results of a single test often require a broad-based interpretation, and one should be cautious in providing and interpreting RIA results without the clinical history and skin test results.

Persistence of Allergy to Drugs

Little information seems to be available on the persistence of allergy to drugs. Sullivan et al. [24] found that 93%

of penicillin-allergic subjects were skin test positive 7-12 months after reactions, and 22% were still positive after 10 years or more. These findings do not support the often-stated opinion that skin test results do not persist in subjects who are allergic to the drugs. Didier et al. [25] found that skin tests, histamine release, and RIAs to suxamethonium persisted for 1-4 years in patients who experienced an anaphylactic reaction to the NMBD, and we showed positive skin and IgE tests to NMBDs 4-29 years after a reaction [26]. In our view, it would be prudent for these subjects to avoid the drug(s) for the rest of their lives. There appears to be little or no information available on the persistence of allergic sensitivity to other drugs.

TABLE 3
TEST RESULTS IN CASE 1

Case 1: The patient reacted after first exposure to thiopentone and suxamethonium. The investigations revealed:

Test	Drug					
	Thiopentone	Suxamethonium	Gallamine	Pancuronium	<i>d</i> -Tubocurarine	Alcuronium
Skin	-	+	+	-	+	+
RIA	2.4	20	6	1.7	5	23
RIA inhibition, choline		7	17	14	17	1.5

TABLE 4
TEST RESULTS IN CASE 2

Case 2: The patient reacted to both alcuronium and *d*-tubocurarine on separate occasions. Investigations showed:

Test	Drug					
	Thiopentone	Suxamethonium	Gallamine	Pancuronium	<i>d</i> -Tubocurarine	Alcuronium
Skin	-	-	-	-	+	+
RIA	2.8	14	11	4	12	24
RIA inhibition, alcuronium		160	42	15	6	2.4
RIA inhibition, <i>d</i> -tubocurarine		240	19	11	16	16

TABLE 5
TEST RESULTS IN CASE 3

Case 3: The patient reacted on first experience after thiopentone and alcuronium. Test results were:

Test	Drug					
	Thiopentone	Suxamethonium	Gallamine	Pancuronium	<i>d</i> -Tubocurarine	Alcuronium
Skin	-	-	-	-	-	+
RIA	1.2	0.8	0.4	1.5	1.5	6
RIA inhibition, alcuronium		>600	130	50	>600	4

Release of Mast Cell Tryptase

In addition to the patient's history and the results of skin and specific IgE tests, we find that the measurement in serum of tryptase released from mast cells is an extremely valuable diagnostic aid [27]. This relatively stable enzyme is elevated in anaphylactic but not other forms of shock. A positive result for tryptase together with negative skin and RIA tests suggests that the reaction does not have an immunological basis. The current test, in the form of an RIA [28], is robust, sensitive, and specific, and can employ stored serum samples, although some drop in tryptase content occurs with time. The enzyme is elevated for approximately 30 min to 6–8 hours in serum following its release from mast cells. This means that tryptase may not be found in anaphylactic subjects who die within about 30 min [29, 30; Baldo and Fisher, unpublished]. The test is also negative in reactions solely involving the lung, and when the reaction is due to the release of mediators from basophils.

We believe that the tryptase test is a very valuable diagnostic aid in the diagnosis of anaphylactic reactions to drugs, since the method is specific and, for the first time, its application will define a homogeneous patient group in which other tests may be evaluated.

Conclusions

Skin tests, even with free, unconjugated drugs may be valuable in attempts to establish the diagnosis of a suspected drug allergy. Ideally, however, the availability of suitable drug-carrier complexes would undoubtedly increase the chances of a specific diagnosis, and this is an area of research where the involvement of immunochemical expertise and progress are urgently needed. Suitable immunoassays for drug-reactive IgE antibodies, together with intelligently planned inhibition of IgE binding studies, frequently enhance the chances of an accurate diagnosis by providing molecular insights unobtainable from the history and skin tests alone. The addition of a test for serum tryptase, which is released from mast cells either by direct drug action or via a true drug-induced anaphylactic reaction, provides the most complete practical combination of methods currently available for diagnosing allergic drug reactions and gaining some insights into their mechanisms.

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