

Recent advances in the diagnosis of drug allergy

Marie-Noël Primeau and N. Franklin Adkinson Jr

The diagnosis of immunologic drug reactions is based primarily on a detailed clinical history and historical data on relative immunogenicity of the culprit drugs. Except for a few standardized skin tests, most of the other methods for diagnosing drug allergy have unproven diagnostic or predictive clinical utility. Many tests for drug-specific immune responses are suggestive if positive, but have unknown negative predictive values. The present review addresses the most recent published literature regarding the diagnosis of drug allergy. Recent advances in the use of the lymphocyte transformation test, and delayed intradermal skin tests and patch tests for the diagnosis of delayed cutaneous reactions to penicillins suggest that these tests may have clinical utility, although confirmatory reports are still missing. For the diagnosis of acute vaccine reactions, gelatin-specific IgE as measured by radioallergosorbent test has now been shown to be reliably associated with allergic reactions to gelatin-containing vaccines.

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Division of Allergy and Clinical Immunology, Johns Hopkins Asthma and Allergy Center, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

Correspondence to N. Franklin Adkinson Jr, M.D., Johns Hopkins Asthma and Allergy Center, 5501 Hopkins Bayview Circle, Baltimore, MD 21224, USA
Tel: +1 410 550 2051; fax: +1 410 550 2055; e-mail: FADKINSO@jhmi.edu

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Abbreviations

CAST	cellular antigen stimulation test
LTT	lymphocyte transformation test
MMR	measles-mumps-rubella
NSAID	nonsteroidal anti-inflammatory drug
RAST	radioallergosorbent test

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Introduction

The classical Coombs and Gell classification can generally be applied to immunologic drug-induced reactions, while acknowledging that several types of drug hypersensitivity reactions have undefined pathophysiological mechanisms. Table 1 summarizes the Coombs and Gell classification for drug allergy, with specific underlying mechanisms and possibly useful diagnostic tests. In the present review, recent articles on immunologic testing for type I reactions are discussed. No new developments for drug-specific IgG and IgM measurements have appeared in the recent literature, and these methods are not discussed further in this review. Most of the recent advances in the field of drug allergy pathogenesis focus on the role of T cells in drug-induced exanthema. One important clinical consequence of this work is the proposed use of in-vivo and in-vitro drug-specific T cell assays for the diagnosis of such drug reactions. The review addresses the diagnostic utility of these tests. New developments in the diagnosis of nonsteroidal anti-inflammatory drug (NSAID) intolerance and adverse reactions to vaccines and are also discussed briefly.

Diagnostic tests for type I reactions

With the exception of immediate type i.d. skin testing for the evaluation of penicillin allergy, none of the tests for type I reactions listed in Table 1 is regularly used clinically. In this section, the use of immediate type intradermal (i.d.) skin tests for the evaluation of cephalosporin allergy is reviewed. The use of leukocyte histamine release, leukocyte leukotriene release, and basophil activation for different drugs is also reviewed.

Evaluation of patients with immediate reactivity to cephalosporins

The current consensus recommendation [1] for the administration of a cephalosporin to individuals with a previous allergic reaction to cephalosporins are to choose one with a different side chain and perform a graded challenge. Skin testing with the cephalosporin to be used can also be attempted, although such skin testing is not standardized and the negative predictive value is not known.

Romano *et al.* [2*] recently assessed reactivity to different penicillins and cephalosporins in a group of 30 individuals with immediate reactions to cephalosporins. Out of the 35 skin tests and radioallergosorbent tests (RASTs) of the culprit cephalosporins, 32 and 9 were positive, respectively, with no patients having a

Table 1. Gell and Coombs classification of immunologic drug reactions, with corresponding immunologic tests

Type of reaction (Gell and Coombs scheme)	Description/mechanisms	Immunologic testing
I	Immediate hypersensitivity; IgE-mediated reactions	Immediate-type skin testing; RAST; Immunoblots-IgE; leukocyte histamine release; basophil activation (surface markers); leukotriene release
II	Cytotoxic reaction; mediated by IgG/IgM and complement	Drug-specific antibody; Coombs test
III	Immune complex disease; mediated by IgG/IgM and complement	Drug-specific antibodies; circulating immune complex; C3, C4, CH50; immunohistopathology
IV	Delayed or cell-mediated hypersensitivity	Delayed-type intradermal and patch skin tests; in-vitro lymphocyte stimulation

RAST, radioallergosorbent test.

positive RAST/negative skin test. The skin test concentration was 2 mg/ml for all cephalosporins. Only 13% also had a positive skin test or RAST result in response to penicillin determinants. The majority of individuals had positive tests only to the culprit cephalosporin when tested with a panel of cephalosporins. That study re-enforces the suggestion that specific skin testing be included in the evaluation of patients with a history of primary cephalosporin allergy. It also emphasizes the relative insensitivity of RAST testing for IgE-mediated drug allergy. In that study, no challenges were performed with cephalosporins that gave negative skin test responses. Therefore, the question regarding the usefulness of skin testing in the choice of an alternative cephalosporin remains. Also, the specificity of such skin testing was not evaluated in the study, because only those patients with positive histories were evaluated.

Measurement of leukotriene synthesis

The cellular antigen stimulation test (CAST; Bühlmann Laboratories AG, Allschwil, Switzerland) is based on stimulation of isolated human leukocytes with an allergen and measurement of the generated sulphido-leukotrienes by an enzyme immunoassay. Its use has been promoted mainly for the diagnosis NSAID intolerance (see below). One study [3] examined the measurement of sulphido-leukotriene production in IgE-mediated β -lactam allergy. Five out of seven patients with a history of IgE-mediated allergy to β -lactam and none of five healthy nonatopic control individuals had antigen-specific sulphido-leukotriene production. The interpretation of the results is limited by the arbitrary threshold value for positivity. Furthermore, the variability of results for the negative control individuals was not shown. The diagnosis of IgE-mediated drug sensitivity was not confirmed by drug challenge, and only two out of five patients had positive skin testing with penicillin or amoxicillin. More extensive experience is needed to validate the use of in-vitro leukotriene production for the diagnosis of drug allergy, including a direct comparison with basophil histamine release and RAST.

Basophil activation to evaluate allergy to muscle relaxants and other drugs

The evaluation of allergic reactions to muscle-relaxant drugs is normally based on the results of skin testing or serologic detection of specific IgE. Some investigators are now proposing in-vitro basophil activation as an alternative [4]. The assay is based on the detection by flow cytometry of altered expression of CD45, CD63, and surface IgE on basophils after allergen activation. When used to evaluate 33 patients with a compatible history of allergy to muscle relaxants and 29 control individuals, the test had a 100% specificity but a low sensitivity (55%) when two of the three markers were significantly altered. With the same subjects, the sensitivity and specificity of intradermal skin testing were 100 and 63%, respectively.

Monneret *et al.* [5] reported the detection of activated basophils in four cases of severe allergic reactions to muscle relaxants. Basophil activation was detected by upregulation of CD63 and was positive in all cases. The same group evaluated basophil CD63 upregulation in a total of 23 drug reactions seen in 17 patients [6]. The reactions were classified as either truly drug allergic ($n=18$) or not ($n=5$), on the basis of history and skin testing. The drugs more frequently implicated were muscle relaxants (52%) and β -lactam antibiotics (21%). The sensitivity of detecting CD3 upregulation was 71% (60% for β -lactams and 80% for muscle relaxants). None of 10 nonreactive control volunteers recently exposed to muscle relaxants had a positive response. This test is labor intensive and requires sophisticated equipment. In the case of muscle relaxant allergy it has few advantages over skin testing, which is more sensitive. Therefore, it remains an investigational tool.

The basophil histamine release test

The histamine release test is also based on the role of basophils in allergic reactions. Its predictive capacity was assessed in a prospective study of 35 drug allergic and 73 control individuals [7]. The diagnosis of drug allergy was confirmed by positive history and skin testing for all, and with provocation challenges for some individuals. The

histamine release test had 51% sensitivity, 63% specificity, 29% positive predictive value, and 81% negative predictive value. In the CD63 upregulation study reported by Monneret *et al.* [5], histamine release was also evaluated and had a sensitivity of 24%. These poor diagnostic results are evidence against the use of this test for the diagnosis of drug allergy when skin testing can be performed.

Diagnostic tests for type IV reactions

Historically, little attention has been devoted to the role of T cells in drug allergy as the mechanisms of antigen processing leading to T cell activation have not been well understood. In recent years, identification and proliferation of drug-specific T cell lines and clones have provided useful models for clarifying the active role of drug-specific T cells in the pathogenesis of delayed cutaneous drug allergy. The proposed diagnostic use of *in vivo* and *in vitro* drug-specific T cell assays for T cell mediated drug reactions is reviewed in this section.

In-vitro T cell assay: lymphocyte transformation test

The lymphocyte transformation test (LTT) measures *in vitro* T cell proliferative responses to antigens or mitogens. It is widely used in clinical immunology to evaluate T cell-dependent immunodeficiencies. For the diagnosis of drug allergy, the proliferation of unstimulated T cells is compared with the proliferation of T cells incubated with various concentrations of the offending drugs. The LTT for drug allergy diagnosis was abandoned in earlier decades because positive tests were frequently seen for nonreactive but recently drug-exposed patients [8]. Furthermore, the diagnostic sensitivity and specificity have not been defined for most drugs. Nyfeler and Pichler [9] resuscitated the debate regarding the usefulness of the LTT for drug allergy diagnosis. In a large retrospective study of 923 patients referred for a wide variety of possible drug allergies, those investigators described an overall sensitivity of 78% and a specificity of 85% when all diagnoses were considered together. An important limitation of that study was that the results of the LTT were correlated with a clinical diagnosis of likely or unlikely drug allergy, made by retrospective chart review. Also, there were no controls for the effect of recent drug-exposure without adverse reaction.

More recently, Schnyder and Pichler [10**] reported a sensitivity for LTT of 83% and a specificity of 100% in a study of 12 patients with delayed maculopapular exanthema in response to penicillin or amoxicillin. Six control individuals were tested before and after an uneventful exposure to a 2-week course of amoxicillin, which did not increase the drug-induced proliferative response. These results look promising, but are at odds with the earlier literature. The technique is labor

intensive, cumbersome, and expensive. It necessitates the use of radioisotopes and many days of sterile lymphocyte culture. Additional study is warranted.

Orasch *et al.* [11] studied 20 patients with acute or delayed generalized or local cutaneous reactions to local anesthetics, and found 60% had a positive lymphocyte transformation test. None of six recently exposed control individuals had a positive test. These results are surprising and difficult to interpret, because individuals reporting adverse reactions to local anesthetics usually tolerate drug challenges and subsequent re-exposures [12]. The application of the LTT to some delayed cutaneous symptoms in response to local anesthetics may be useful in examining contact sensitivity to local anesthetics, but is unlikely to add much to the current approach to diagnosing acute reactions [1].

The lymphocyte transformation test has also been reported to be useful in a case report of a severe hypersensitivity syndrome developing in a man taking both lamotrigine and sodium valproate [13]. The test identified lamotrigine as the offending agent, and sodium valproate was reintroduced and tolerated.

In-vivo T cell assays: patch test and delayed intradermal skin testing

In-vivo T cell assays, like delayed intradermal skin tests and patch tests, are attractive because they can easily be performed in a clinical setting. Romano and coworkers [14–16] reported a series of articles to define the validity of patch testing and delayed reading (>48 h) of intradermal skin tests in patients with histories of maculopapular eruptions in response to aminopenicillins. All 69 patients with a history of delayed maculopapular rash in response to aminopenicillins, and negative patch testing and delayed intradermal responses had a negative single-dose drug challenge. All 18 persons with positive patch test or delayed intradermal responses had a positive single-dose drug challenge. We have not been able to reproduce this very high sensitivity and specificity in a small group of 12 patients with a recent delayed maculopapular rash in response to amoxicillin.

Another Italian group [17] evaluated patch testing to identify cross-sensitivity among β -lactams in a group of 30 β -lactam-allergic patients with negative IgE tests (immediate skin test and RAST), but with a positive patch test to at least one β -lactam. Out of 252 oral challenges undertaken with nonincriminated and patch-test-negative β -lactam drugs, only 5% were positive. All 13 challenges done with the culprit patch-test-positive β -lactam were positive. Schnyder and Pichler [10**], in the aforementioned study of the LTT, also performed patch testing on patients with delayed exanthema in response to penicillin or amoxicillin. Their technique was slightly different,

with scarification of the skin before drug application. They reported a sensitivity of 75% and a specificity of 100% when compared with historical diagnosis.

These studies have shown that, overall, patch testing has a very good positive predictive value for delayed cutaneous rashes due to drugs. The interpretation of negative results still needs further clarification. Also, populations with different ethnic backgrounds need to be studied before this high sensitivity and specificity can be considered generalizable. In the meantime, sufficient evidence has been accumulated from these studies that the use of a drug giving a positive patch test or delayed intradermal skin test should be avoided when alternatives are available.

Diagnosis of nonsteroidal anti-inflammatory drug intolerance

The cellular antigen stimulation test (see above) for measurement of sulphido-leukotrienes has been evaluated for use in the diagnosis of aspirin-sensitive asthma, aspirin-induced urticaria, and other NSAID hypersensitivity [18,19]. One recent study [20] measured the production of leukotrienes by isolated peripheral leukocytes from challenge-confirmed aspirin-sensitive asthmatic persons ($n=26$) compared with aspirin-tolerant asthmatic persons ($n=32$, with 14 out of the 32 confirmed by challenge). There was no difference in the production of sulphido-leukotrienes between the two groups, either from unstimulated cells or after aspirin stimulation. Thus, for the time being, oral aspirin challenge or aspirin-lysine inhalation challenge remain the only valid diagnostic techniques for aspirin/NSAID-sensitive asthma.

The role of the CAST assay has also been evaluated for the diagnosis of aspirin-intolerant patients with chronic urticaria [21]. De-novo production of sulphido-leukotrienes stimulated by leukocyte agonists (C5a, platelet-activating factor, and f-Met-Leu-Phe) was measured in 84 patients with suspected aspirin-sensitive chronic urticaria who had either a positive ($n=18$) or a negative ($n=66$) oral aspirin provocation test. Persons with positive oral aspirin provocation tests had significantly higher levels of spontaneous sulphido-leukotriene production than did individuals with negative challenges. Although this finding suggests a pathophysiologic role of leukotrienes in aspirin-intolerant chronic urticaria, the overlap in leukocyte leukotriene production in reactors and nonreactors precludes this test as an accurate predictor of oral provocation results.

The evaluation of allergic reactions to vaccines

The importance of egg allergy in reactions to measles-mumps-rubella (MMR) vaccination has been discounted by previous studies [22,23]. The role of other compo-

nents of the vaccine has now been investigated. Sensitization to gelatin accounted for most of the reactions in a series of articles reported by Japanese investigators [24,25]. More recently, Sakaguchi and Inouye [26•] reported a study that evaluated the role of previous vaccination with gelatin-containing vaccines as the sensitizing event for reactions to a first MMR vaccination. That study found that gelatin RASTs were positive in 79 out of 87 (91%) children with allergic reactions to mumps, measles or rubella vaccines, compared with none of 200 exposed nonreactors. Those investigators also found a significant relationship between histories of prior vaccination with gelatin-containing DTaP vaccine and subsequent antigelatin IgE production. Gelatin-specific IgE, as measured by RAST, is strongly associated with allergic reactions to gelatin-containing vaccines.

Patja *et al.* [27] investigated sensitization to gelatin and other MMR vaccine components in 36 individuals with presumed allergic reactions to MMR vaccination (18 anaphylaxis, 13 urticaria/angioedema, 4 asthmatic symptoms, 1 Henoch-Schönlein purpura). A positive gelatin RAST was found in 14% of all reactors and in 25% of those with immediate (<1 h onset) reactions. They also performed immunospot-IgE assays with gelatin and other vaccine components (egg, neomycin sulfate, and human albumin), and with potential cross-reactive allergens (chicken meat, feathers, and cow's milk). The immunospot-IgE was positive for gelatin in 28% of all reactors, for eggs in 25%, for chicken meat in 22%, for cow's milk in 47%, and for chicken feathers in 14%. Immunospot-IgE was negative for the whole vaccine, neomycin, and human albumin in all persons studied. Control groups of atopic and nonatopic persons all had negative immunospot-IgE assays for gelatin, but results for other tested allergens were not reported. The rate of positive immunospot-IgE to egg, chicken, and milk was surprisingly high, and was not correlated with a clinical history of food allergies. Three patients were subsequently revaccinated with MMR. Two had a positive gelatin immunospot-IgE and tolerated the vaccine. One patient with a positive gelatin RAST experienced cough and sneezing soon after vaccination. The clinical usefulness of the immunospot-IgE assay for gelatin and other vaccine components or food allergen needs further investigation.

Conclusion

The diagnosis of drug allergy often relies on the clinical history only, because standardized validated diagnostic tests are few. Hopefully, new research into the pathophysiology of a variety of drug allergies will lead to development of new diagnostic tools. When evaluating studies to validate such tests, the importance of testing recently exposed but nonreactive control individuals should be kept in mind.

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