

Chemistry of drug allergenicity

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Of the very large number and variety of drugs used in medicine, those that are frequently implicated in immediate allergic reactions are relatively small in number and include neuromuscular blocking drugs used in anaesthesia, β -lactam antibiotics, some other antibacterial agents including broad-spectrum antibiotics and quinolones and, less often, some narcotics. Structure-activity and immunochemical investigations have been most numerous and detailed for neuromuscular blocking drugs and β -lactams, particularly penicillins. For the former group of drugs, morphine is proving to be a useful agent for the in-vitro detection of clinically relevant neuromuscular blocking drug- as well as morphine- and fentanyl-reactive IgE antibodies. The employment of so-called 'major' and 'minor' determinants for a range of different penicillins and cephalosporins has revealed previously unsuspected heterogeneity in patient recognition responses, and has reinforced findings on the allergenic importance of side-chain groups. Many reports have been published on anaphylaxis to chlorhexidine, and progress in identifying allergenic determinants is reviewed together with the still inadequately understood subject of IgE antibody recognition of quinolone antibacterial agents. *Curr Opin Allergy Clin Immunol* 1:327–335. © 2001

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Abbreviations

MDM minor determinant mix
NMBD neuromuscular blocking drugs

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Introduction

Apart from early studies on β -lactam antibiotics, particularly the penicillins and with heavy emphasis on benzylpenicillin [1–4], there has been a dearth of immunochemical and structural studies on drug allergens. In recent years, predominant contributions to knowledge in this field have come from ongoing research in our laboratory, where insights into structure-reactivity relationships have been pursued for allergies to neuromuscular blocking drugs (NMBD), narcotics, penicillins, cephalosporins and some other antibacterial agents [5,6,7,8*]. Such studies have led to two important conclusions: that patient IgE antibody responses are heterogeneous, reflecting the structural heterogeneity of allergenic determinants on individual drugs and the often fine structural specificity differences distinguished by antibodies from allergic individuals. Important distinguishing features on allergenic determinants may, for example, be as small as a single methylene, methoxy or hydroxyl group [9–11] (Z. Zhao, B.A. Baldo, J. Rimmer, unpublished data).

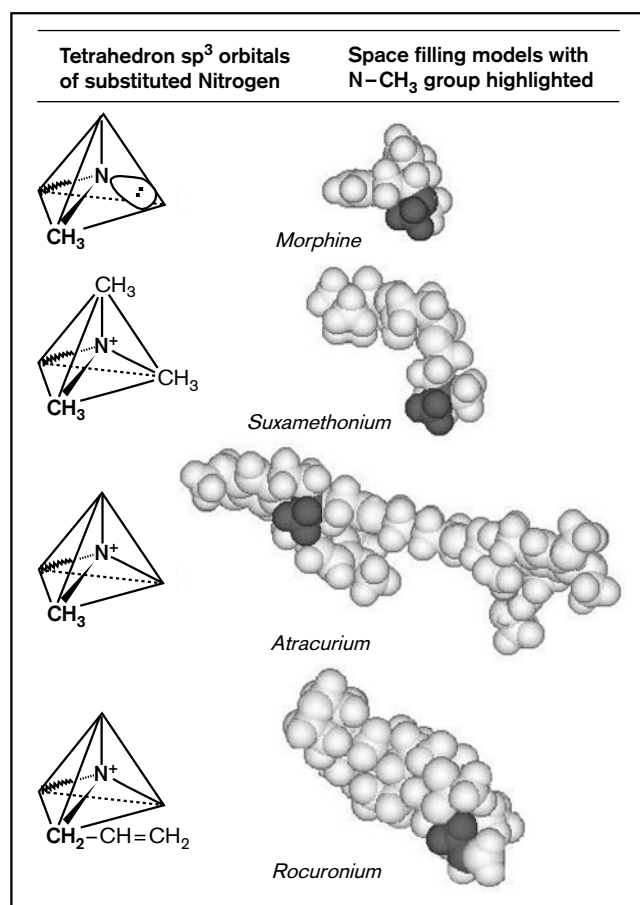
Recent contributions to our knowledge of the chemistry of drug allergenicity and structure–activity relationships in some important drug allergies are presented below in the form of summarized findings on some of the more important drugs known to provoke allergic reactions in man.

In addition to immediate, IgE-mediated reactions that conform to the text-book definition of true 'allergies', it should be pointed out that there are a number of adverse drug sensitivities that fall under the heading of cell-mediated reactions or delayed hypersensitivities. In these reactions, T cells recognize drugs and may recognize the same, or different, structural features on the molecules than B cells, which are involved in IgE-mediated sensitivities [12–14].

Morphine and the diagnosis of allergy to neuromuscular blocking drugs

Morphine, by virtue of its single tertiary ammonium group, is a potent inhibitor of the binding to NMBD of IgE antibodies in the sera of individuals who experience an anaphylactic reaction after exposure to a NMBD during anaesthesia. An examination and comparison of the molecular orbitals and tetrahedron structures of the substituted nitrogens in morphine and some NMBD, and the accessibility of substituted ammonium groups seen on space-filling models (Fig. 1), supports the antibody recognition findings.

Figure 1. Structural relationship between morphine and some neuromuscular blocking drugs



Structures of substituted nitrogens in morphine and some neuromuscular blocking drugs and space-filling models showing the accessibility of cross-reactive N -methyl groups.

In a study of 347 patients with suspected anaphylaxis to a NMBD, a morphine solid phase immunoassay proved to be a more sensitive and efficient test for the detection of IgE antibodies to NMBD than the specific immunoassays for NMBD. In a homogeneous group of 118 subjects specified by an evaluation of serum trypsin [15–17], the morphine radioimmunoassay for drug-reactive IgE antibodies detected 67 out of 69 (~97%), nine out of 10 and 15 out of 15 reactors to suxamethonium, vecuronium and rocuronium, respectively [18••]. The highest detection rate for IgE antibodies to a NMBD was 70% for suxamethonium (Table 1).

Morphine thus offers an improvement over a battery of individual immunoassays for the detection of NMBD-reactive IgE antibodies, and provides a valuable adjunct to skin testing and trypsin determinations in the diagnosis of allergic reaction to NMBD. In addition, the morphine immunoassay reduces the time, labour and cost of in-vitro testing in cases of suspected NMBD-

induced anaphylaxis. The test is particularly useful when a diagnosis is needed in individuals who are skin-test negative or before skin testing is performed.

Allergy to narcotics

IgE antibodies that react with morphine and codeine have been identified and studied in the sera of individuals allergic to narcotics [19] (N.H. Pham, B.A. Baldo, unpublished data). Structural features of the morphine allergenic determinant were shown to make up the cyclohexenyl ring, with a hydroxyl group at C-6 and a methyl substituent attached to the nitrogen atom. The inhibition of morphine-reactive IgE antibodies by meperidine (pethidine) and methadone, compounds with opioid activity, was somewhat unexpected because examination of the two-dimensional structures of these compounds revealed no readily obvious similarity to morphine. From an immunochemical perspective, however, the presence of an N -methyl group in particular in each compound, and the shared sequence of an aromatic ring separated from the nitrogen by three carbon atoms, was seen as the basis of the cross-reactive recognition [19].

Clear-cut cross-reactivity was not seen with fentanyl, a phenylpiperidine analgesic like meperidine. Fentanyl is usually used only for anaesthesia and, as an analgesic is approximately 80 times as potent as morphine. Despite numerous cases of suspected allergic reactions to the drug, the involvement of fentanyl-reactive IgE antibodies has been difficult to demonstrate. Recently, however, IgE antibodies to both morphine and fentanyl have been demonstrated, making feasible a test for the diagnosis of fentanyl-specific allergic responses. Table 2 shows comparative inhibitory potencies, that is, the extent of antigenic complementarity between the narcotic inhibitors and the combining sites of IgE antibodies in the sera of two different subjects. Antibodies from one of the subjects recognized morphine alone, whereas IgE from the second subject recognized both morphine and fentanyl. The fine structural features of fentanyl that are complementary to the IgE antibodies have not yet been identified.

Penicillins and cephalosporins

Penicillins and cephalosporins have a backbone structure consisting of a β -lactam ring joined to an S-containing ring structure, the latter being a thiazolidine ring for penicillins and a dihydrothiazine ring for cephalosporins (Fig. 2). Individual penicillins and cephalosporins are distinguished by a side-chain (R1) attached to the β -lactam ring by an amide linkage, whereas for cephalosporins, the R2 substituent at the 3-position on the dihydrothiazine ring provides an additional distinguishing point for individual members of the family [20].

Table 1. Detection of drug-reactive IgE antibodies in tryptase-positive sera from skin prick test-positive subjects^a who experienced an anaphylactoid reaction during anaesthesia

Skin prick test-positive to:	Number ^b	No. positive for IgE antibodies to:	
		Morphine	NMBD
One or more NMBD	118	100/118	56/118
Breakdown for individual NMBD:			
Suxamethonium	69	67/69	47/69
Vecuronium	10	9/10	2/10
Rocuronium	15	15/15	3/13
Pancuronium	2	0/2	0/2
Alcuronium	8	4/8	2/8
Atracurium	12	3/12	2/6
Gallamine	1	1/1	0/0
Mivacurium	1	1/1	0/0

^aPositive skin-prick test for neuromuscular blocking drugs (NMBD).

^bRemaining subjects in this study of 347 patients made up of: serum tryptase elevated, positive skin test for drug other than a NMBD – 45 subjects; serum tryptase not elevated, skin test negative – 56 subjects; serum tryptase not elevated, no skin test performed – 128 subjects.

Adapted from Fisher and Baldo [18••].

Penicillins and cephalosporins are reactive substances, and for the penicillins at least, metabolic transformations and chemical alterations giving rise to different antigenic determinants have been extensively studied [1–4]. Findings from this large body of research, together with more recent serological and immunological results identifying allergenic determinants at the fine structural level, have demonstrated heterogeneity both of β -lactam allergenic determinants and patient antibody recognition patterns [7•,8•].

The employment of different structural conjugates, in particular penicilloyl and cephalosporoyl ('major') determinants, and penicillanyl and cephalosporanyl ('minor') determinants, has revealed an unsuspected degree of selective recognition, for example IgE antibodies reactive with '-oyl' but not the corresponding '-anyl' determinants, and vice versa [7•,8•,21•]; or reactivity with only one penicillin, e.g. flucloxacillin [22–24]. Antibodies reactive with β -lactams have been shown to detect a wide range of specificities, including structures as small as a portion of a side-chain, such as the methylene group in benzylpenicillin and cephalothin [9] (Z. Zhao, B.A. Baldo, J. Rimmer, unpublished data), a complete side-chain [22–24], and an entire penicillin or cephalosporin molecule (Fig. 2) [6,23,25].

A remarkable demonstration of fine structural specificity differences on penicillins distinguished by IgE antibodies from allergic patients was recently studied. Antibodies from one subject reacted only with the amoxicilloyl determinant, whereas IgE from another allergic patient recognized the amoxicillanyl but not the amoxicilloyl specificity (Z. Zhao, B.A. Baldo, K. Baumgart, D. Mallon, unpublished data). Quantitative hapten inhibition studies showed that in the former case, IgE antibodies recognized both the hydroxyaminobenzyl side-chain and the thiazolidine ring carboxyl, and these

requirements were satisfied when amoxicillin was presented in the '-oyl' but not the '-anyl' form. With the second serum, the proposed explanation is more subtle. Linkage to the polylysine carrier via the 2-carboxyl in the '-anyl' linkage makes close association of the amoxicillin side-chain and the peptide carrier impossible because both are at opposite ends of a relatively inflexible molecule. Antibody access to the side-chain is thus possible with the amoxicillanyl determinant. This is not the case with the amoxicilloyl determinant, in which opening of the β -lactam ring (that is, the '-oyl' form) allows increased rotation around C-6 and C-7, and this makes possible the close proximity of the hydroxyl group on the side-chain and the peptide carrier. Such close proximity allows the possibility of hydrogen bonding, which prevents free unhindered access of antibody to the complementary hydroxybenzyl antigen (Fig. 3).

Important conclusions for the diagnosis of β -lactam allergies [7•] from the emerging picture provided by our immunochemical and serological findings with the antibiotics are: (i) β -lactam antigens used for skin testing and in-vitro assays for IgE should permit the detection of sensitivity to side-chain determinants as well as the core structures of penicillins and cephalosporins; (ii) tests should include determinants for '-oyl' determinants and a 'minor' determinant such as the '-anyl' specificity; and (iii) diagnostic conclusions should not be based on results from only a single test antigen. A selected battery of different determinants should be employed because, for example, some individuals react to one or more penicillins but not others, or to '-oyl' but not '-anyl' (or vice versa) specificities.

In a study of 59 patients with penicillin-induced urticaria or anaphylaxis [26], skin tests were performed with the benzylpenicilloyl determinant, minor determinant mix

Table 2. IgE antibody recognition of morphine and fentanyl. Comparison of reactivities with morphine, fentanyl and related narcotics

Inhibitor	Structure (>N-R)	Amount (nmol) of compound needed for 50% inhibition in subject:		Inhibition (%) of IgE antibody binding to morphine solid-phase with 200 nmol of compound in subject:	
		(1)	(2)	(1)	(2)
Morphine	>N-CH ₃	24	< 1	72	96
Meperidine	>N-CH ₃	830	22	31	74
Fentanyl	>N-CH ₂ CH ₂ -C ₆ H ₅	> 2000	43	3	62
Nalorphine	>N-CH ₂ CH=CH ₂	240	58	46	57
Naltrexone	>N-CH ₂ -□	> 2000	~ 800	3	17

Figure 2. General structures and allergenic determinants of β -lactam antibiotics

General structures of penicillins, cephalosporins and '-oyl' and '-anyl' antigens employed for the detection of IgE antibodies in diagnostic tests and the spectra of β -lactam allergenic determinants recognized by IgE antibodies in the sera of allergic subjects.

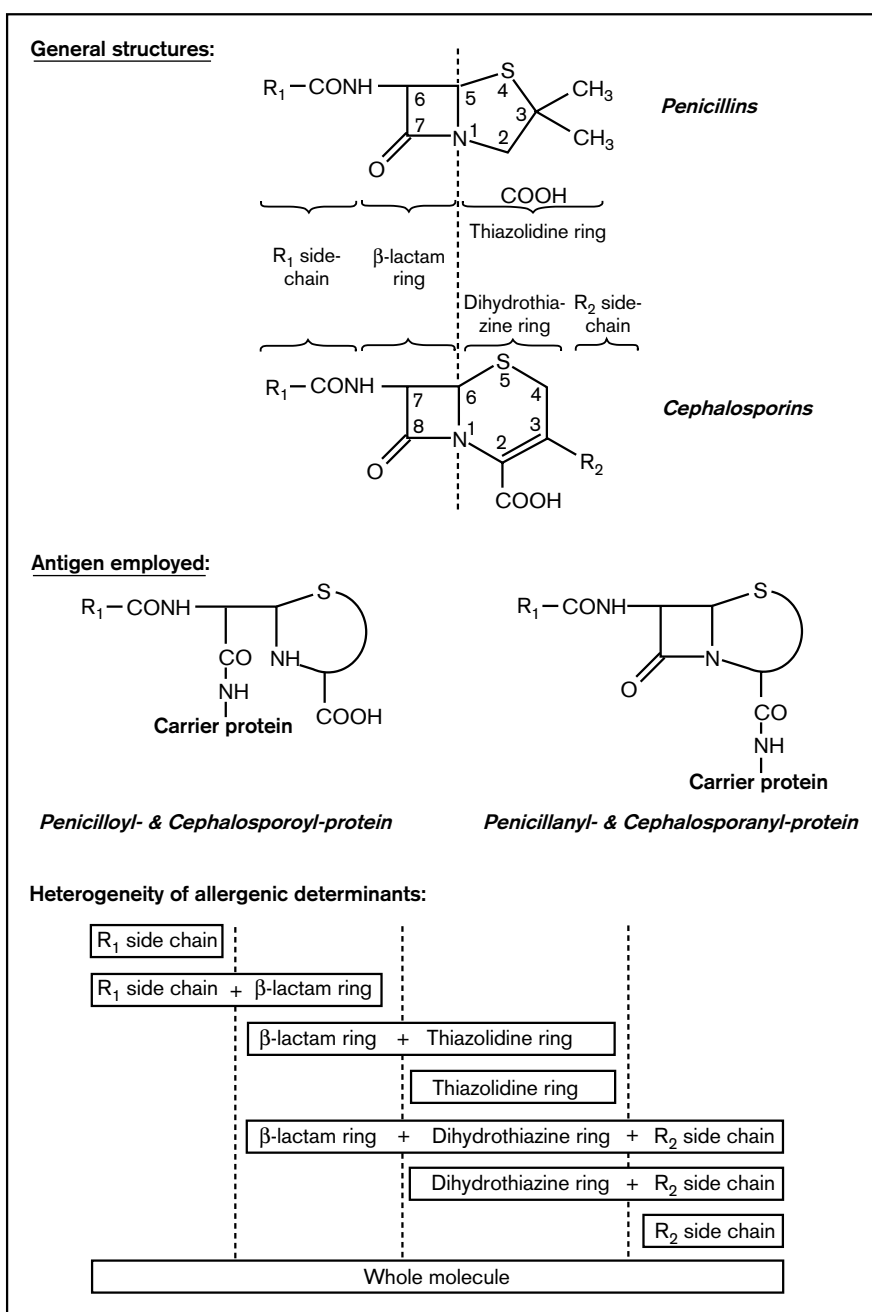
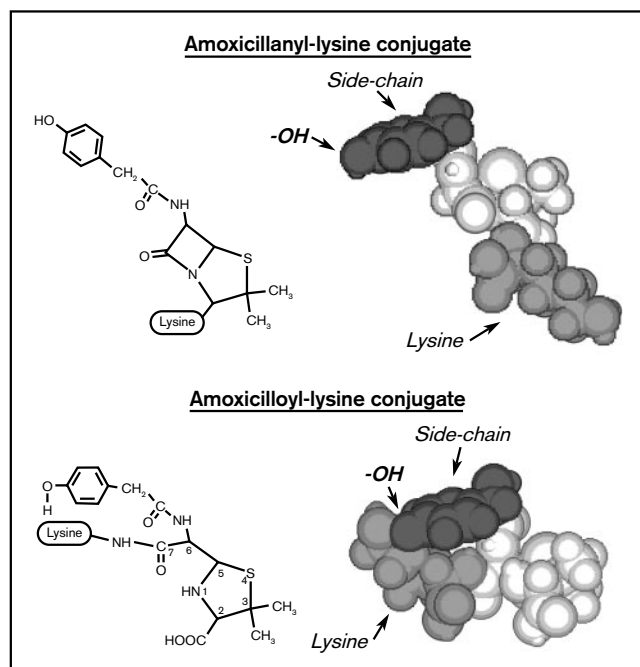


Figure 3. Structures and space-filling models showing the likely configurations of amoxicillanyl- and amoxicilloyl-lysine conjugates in solution



Close proximity with the resultant H-bonding between the hydroxyl of the side-chain and the carrier is possible with the '-oyl' but not with the '-anyl' conjugate.

(MDM), amoxicillin and ampicillin. Skin-test positivity to the benzylpenicilloyl determinant was associated with urticaria, whereas positive reactions to MDM, amoxicillin and ampicillin were associated with anaphylactic shock. The latter finding with MDM has been reported before [2].

Another increasingly important reason to employ skin testing with β -lactam antibiotics is to attempt to decrease the use of broad-spectrum antibiotics in patients said or thought to be allergic to penicillins or cephalosporins. A proportion of such patients may lack β -lactam-reactive IgE antibodies [27•].

Chlorhexidine

The antiseptic agent chlorhexidine, a chlorophenyl diguanido derivative of hexane, has been implicated in allergic reactions, including anaphylaxis, after contact with skin, eyes, other mucous membranes and with catheters treated with the agent [28–30,31•]. An early study on an IgE-mediated shock reaction [28] showed that chlorhexidine and chloguanide (two molecules of which make up the symmetrical molecule of chlorhexidine) share the same allergenic determinant. Antibodies with the same specificity were detected in a recent case of anaphylaxis to the antiseptic (Table 3). In another

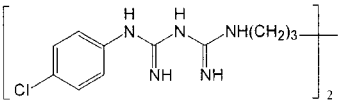
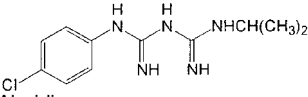
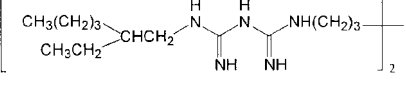
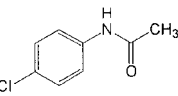
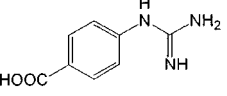
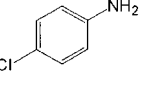
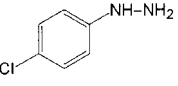
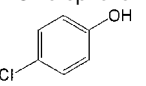
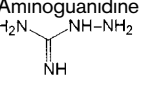
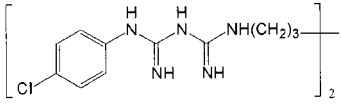
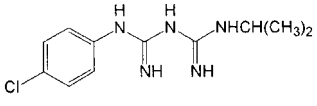
recent study of anaphylaxis to chlorhexidine [31•], the allergenic determinant complementary to the drug-reactive IgE antibodies was shown to be the entire chlorhexidine molecule. This conclusion was supported by the clear-cut finding of antibody recognition of alexidine, demonstrating that the antibody combining sites extend beyond the chlorguanide portions of the chlorhexidine molecule and includes the hexamethylene central bridge present in both alexidine and chlorhexidine (Table 3). In identifying both specificities, the lack of inhibitory activity by 4-chlorophenol and other selected substituted phenyl compounds showed that the terminal portions of the chlorhexidine molecule did not alone account for IgE antibody recognition of the antiseptic (Table 3).

Immunochemical aspects of antibody recognition of quinolone antibacterial agents

In a preliminary study of suspected allergic reactions to quinolone antibacterial agents [32–34], quinolone-reactive IgE antibodies were detected in the sera of some patients with allergic symptoms induced by a quinolone, but also in the sera from some 'control' subjects with no suspected allergies to these drugs. No reaction was detected with cord sera. Drug conjugates for use in immunoassays were prepared by utilizing functional groups at opposite ends of the quinolone molecules (Fig. 4). Conjugates prepared by coupling through the piperazine side-chain, but not through the carboxyl group, were reactive with IgE antibodies. This suggested that the allergenic determinant structure complementary to the antibody combining sites on reactive IgE molecules was the 3-carboxy-4-quinolone ring structure (N.H. Pham, P. Campi, R. Zerboni, B.A. Baldo, unpublished data).

Although IgE antibodies in the sera from individuals with true type I allergy to quinolones could be inhibited by preincubation with free drug, this was also found with some of the 'control' sera. In an effort to investigate further the specificity of the observed antibody binding and the importance of the substituted 4-quinolone molecules, compounds representing different, and increasing, portions of the pipemidic acid molecule were coupled to Sepharose and tested for IgE antibody binding using sera from quinolone-allergic patients and 'control' sera that reacted with pipemidic acid-sepharose complex. Little or no reactivity was observed with the four simplest compounds, piperazine, 4-phenylpiperazine, 1-(2-pyridyl)piperazine, and 1-(2-pyrimidyl)piperazine (Fig. 4). One serum from a subject allergic to quinolones and one serum from the apparently non-allergic 'control' group reacted with 1-(2-pyridyl)piperazine. With 4-piperazinoacetophenol, however, the analogue with a relatively close structural similarity to pipemidic acid, clear-cut IgE binding was observed,

Table 3. Inhibition of chlorhexidine-reactive IgE antibodies by chlorhexidine, chloguanide and some structurally related compounds

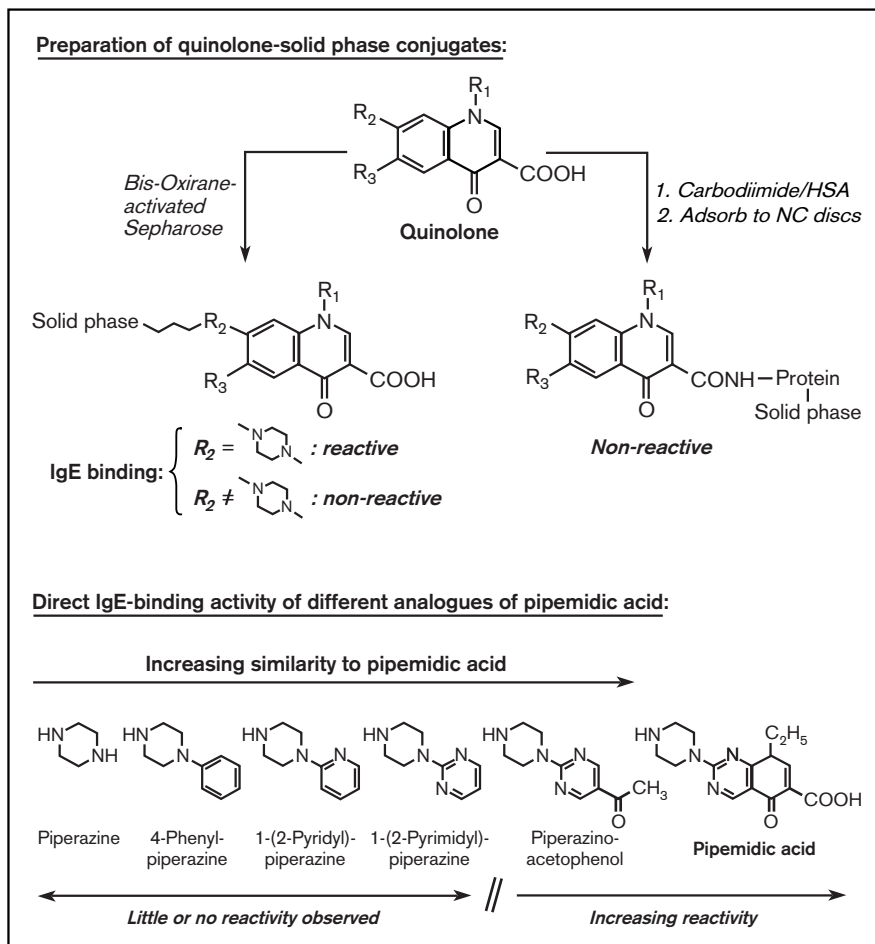
Compound/structure	% Inhibition of IgE antibody binding to chlorhexidine-sepharose					
	Subject Ma		Subject Ra		Subject Du	
	10 nmol	100 nmol	10 nmol	100 nmol	10 nmol	100 nmol
Chlorhexidine 	81.3	85.9	27.4	76.5	36.1	81.9
Chloguanide 	34.5	64.0	34.2	74.2	42.5	87.6
Alexidine 	40.5	53.2	0	0	0	0
4-Chloroacetanilide 	0	0	0	0	0	0
4-Guanidinobenzoic acid 	0	0	0	0	0	0
4-Chloroaniline 	0	0	0	0	0	0
4-Chlorophenylhydrazine 	0	0	0	0	0	0
4-Chlorophenol 	0	0	0	0	0	0
Aminoguanidine 	0	0	0	0	0	0
Allergenic determinants identified:  Entire chlorhexidine molecule  Half a chlorhexidine molecule (entire chloguanide molecule)						

although antibody reactivity was weaker than that obtained with pipemidic acid. Again, both allergic sera and some 'control' sera were found to react, confirming that quinolone-reactive IgE antibodies occur in some

individuals with no suspected or apparent allergic sensitivity to these drugs. The origin of these IgE antibodies and their clinical significance remain obscure and need further investigation.

Figure 4. Preparation of quinolone-solid phase conjugates for the detection of complementary IgE antibodies and direct IgE-binding activities of piperazine and some piperazine derivatives relative to pipemidic acid

HSA human serum albumin; NC, nitrocellulose



Conclusion

The morphine-solid phase covalent complex prepared with bis-oxirane [19] appears to be superior to any of the NMBD for the in-vitro detection of IgE antibodies to these drugs. Although this may, at first sight, be unexpected, it should be remembered that the origin of NMBD-reactive antibodies in allergic patients' sera is unknown – in most cases studied the provoking antigen was almost certainly not an NMBD [5,6].

Morphine-solid phase complex may also be employed directly or in inhibition studies to detect antibodies to other narcotics, for example, meperidine and fentanyl. Antibody recognition of different narcotics for the differential diagnosis of allergic sensitivities is still inadequately studied, and precise fine-structural recognition differences between, for example, morphine/codeine, nalorphine, naloxone, naltrexone, fentanyl, meperidine and methadone need to be defined so that specific immunoassays for the individual drugs can be employed.

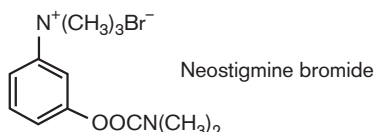
In the light of the heterogeneity of patient responses to β -lactam antibiotics [6,7*,8*], a wide range of tests both *in vitro* and *in vivo* should be developed and employed to cover side-chain differences and to detect so-called 'major' and 'minor' determinants of the clinically important penicillins and cephalosporins. Suitable test reagents for the latter in particular are lacking. As a minimum, penicillin test reagents for benzylpenicillin, phenoxymethylpenicillin, ampicillin, amoxicillin and flucloxacillin should be available. For cephalosporins, the list should include cefaclor, cephalexin, cephalothin, ceftriaxone and cefazolin. The clinically important β -lactam antibiotics will vary a little with location.

Two allergenic determinants of chlorhexidine have been defined; in one case the whole molecule is recognized whereas chlorguanide constitutes the IgE-binding determinant for other individuals. It will be interesting to see if smaller determinants, perhaps encompassing the chlorinated phenyl terminal group, are recognized by

some allergic individuals. Testing for allergy, particularly anaphylaxis, to this frequently used antibacterial agent should be considered in cases in which the cause of anaphylaxis is obscure and the agent may have been employed.

Apparent allergic sensitivity to quinolone antibacterial agents is not uncommon, but attempts to develop diagnostic tests and confidently establish specificity have been difficult. The relevance of quinolone-reactive IgE found in the sera from individuals defined as clinically allergic to a quinolone and in sera from some other allergic and non-allergic individuals remains in question. Results suggest that the IgE-binding reactivity observed is not simply 'non-specific', and the subject of adverse reactions to quinolones remains an intriguing and important clinical and immunochemical challenge.

A wide range of findings and observations point to the importance of substituted ammonium groups as allergenic determinants on many drugs and chemicals [5,6,11,18•,19]. With this in mind, the recent report, for example, by Seed and Ewan [35•] of anaphylaxis to neostigmine is most interesting because we predict that allergic recognition will be shown to involve the tertiary ammonium groups on this drug (see structure).



Specific immunoassays for the detection of IgE antibodies to each of the different amines, quaternary, tertiary, secondary [5,6,11,19] and primary, may prove valuable for distinguishing sensitivities to a variety of chemicals and pharmacologically different drugs. The recent demonstration of the recognition of primary amino groups by IgE antibodies [36•] may be relevant to some drug allergies.

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