Mechanisms in Drug Allergy

A *Glaxo* Symposium

MECHANISMS IN DRUG ALLERGY

A Glaxo Symposium

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With 36 Illustrations

CHURCHILL LIVINGSTONE
Edinburgh and London
1972

PREFACE

A SIGNIFICANT proportion of the population will at some time of their life experience a reaction to a drug, thought to be allergic in origin; all clinicians, irrespective of their speciality, will undoubtedly encounter such reactions in their professional duties; and pathologists, in their various capacities, may well be asked to investigate the pathogenesis of a lesion. The subject of drug allergy is therefore of potential interest to many people. Over the last decade the subject of immunology has seen great advances raising it to the status of a science, but relatively little effort has been devoted to the investigation of allergic reactions to drugs in the laboratory.

The intention of this Symposium held in April 1972 was to bring together scientists and clinicians, immunologists and dermatologists to discuss this one subject and in its fulfilment much that was interesting and stimulating was provided for everyone. The views expressed often differed, and the various opinions have been preserved as far as possible in the discussions in this volume, particularly in the Panel Discussion where it is obvious that many people find difficulties in conceiving that there could be a single *in vitro* technique for diagnosing drug allergy. It is probable that no solution has been found to the problem, but various possibilities have at least emerged.

The Symposium achieved its object, and the Proceedings appear in this crystallized form through the help and co-operation which we have received from a considerable number of people. We are particularly grateful to Miss Sheila Altoun for her help in organizing the meeting, David Kedgley who was responsible for recording the discussions, Mrs. Susan Howe and Miss Linda Fisher for their invaluable secretarial services and, not least, the Participants and the Chairmen for their contributions.

C.H.D.
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August, 1972

FOREWORD

To the uninitiated, there should be no great problems in drug allergy in this age when concepts and understanding of allergic reactivity are so advanced and sophisticated. But allergic drug reactions are *particular situations* and it is this very *particularity* that creates the problems.

I have been concerned with allergic drug reactions for over twenty years and although I have not made any contributions to the subject, I feel I know and appreciate the problems. The papers in this book and in the scientific and medical literature in general must be read with this *particularity* and with the following questions in mind.

(i) Are we certain that the untoward drug reaction is allergic?
(ii) Have we reliable diagnostic or confirmatory tests—both clinical and laboratory?
(iii) Are we certain of the nature of the operative allergenic determinant of the drug or its metabolite(s) and of the *in vivo* coupling which makes it antigenic?
(iv) Do we know the precise Reaction Type(s) producing the clinical syndrome and would this knowledge assist in the selection of appropriate therapy?
(v) Are we able to set up a model system in experimental animals? Often this will be quite impossible.
(vi) Are we able to desensitize and do we understand the mechanisms of desensitization involved?

If it were possible in the majority of cases to answer satisfactorily all these questions, there would be grounds for considerable satisfaction. This stage, however, is a long way off and leads for the future are still not clearly defined.

Much can be done on experimental animals especially where administration is parenteral. The determinant, whether on the drug administered or on a metabolite, has to be identified, remembering that the pathway may be quite different from that in man. Again, stressing this *particularity*, 'Sedormid', for instance, complexes only with human platelets and so it has been found impossible to set up 'Sedormid' purpura experimentally in animals. The low incidence of allergic reactions to drugs under conditions of normal administration also makes a model study in laboratory animals difficult.

For many reasons it should be best to study man himself where the low incidence of reactions in the population is overcome by the fact that the patients (positive reactors) present themselves for investigation. Unfortu-

nately, laboratories with the appropriate research facilities and professional expertise are not always at hand for the investigations. This of course will be remedied in due course, when the growing incidence of drug reactions will necessitate that such facilities should be available. The working out of successful diagnostic methods and therapeutic procedures for a particular drug hypersensitivity is likely to be dependent on there being a sufficient number of cases for investigation. But there is no final accommodation because then, as likely as not, the drug will be withdrawn from the market and a new one, with all the attendant problems will be introduced.

These are some of the difficulties facing those investigating the mechanisms in drug allergy—the limitations of animal models, the dispersion and low incidence of cases, the shortage of suitable research centres with service commitments and good clinical liaisons, scarcity of money and lack of patronage. It is here that I should like, on behalf of members who attended the symposium, to thank Glaxo not only for financing the meeting and recording, in symposium form, some of the progress steadily being made, but also for the encouragement it gives to investigators who have chosen this difficult field in which to work.

August, 1972

R. R. A. COOMBS

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IMMUNOCHEMICAL MECHANISMS IN DRUG ALLERGY

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DURING the last ten years, considerable progress has been made in the understanding of the molecular mechanisms of immunological reactions to simple chemicals and, by extension, to drugs of low molecular weight. Considering untoward drug reactions from the clinical point of view, symptoms and syndromes following the administration of drugs can be classified in three main categories.

1. Symptoms with demonstrated immunological pathogenesis (Table 1).
2. Symptoms with possible immunological pathogenesis, but where immunological mechanisms have not yet been conclusively demonstrated (Table 2).
3. Symptoms which are not due to immunological mechanisms (Table 3).

In this paper I will be concerned with the first category which probably encompasses a majority of the untoward reactions to drugs observed in practice. When analyzing immunological mechanisms, it may seem logical to start with the antigen before considering the various forms of immunological response of the individual to that antigen.

THE ANTIGENS IN DRUG ALLERGY

Immunochemical analysis shows that different properties are required from antigens for inducing the immune response (i.e. production of antibodies and of sensitized cells) than for eliciting various types of allergic reactions in already sensitized individuals. Accordingly, let us consider first some factors involved in the sensitization phase which influence the immunogenicity of drugs.

The sensitization phase

It was considered almost a dogma in immunology that small molecular weight chemicals (i.e. molecular weight under 1,000, as is the case for most

TABLE 1

ADVERSE DRUG REACTIONS WITH DEMONSTRABLE IMMUNOLOGICAL PATHOGENESIS

Mechanism	*Clinical syndrome*	*Main causative drugs*
Antibody-mediated		
IgE	Anaphylactic shock	Penicillins
	Generalized urticaria and angioneurotic oedema	Aspirin
IgE/IgG	Haemolytic anaemia	Penicillins
		α-Methyldopa
		Quinine
	Thrombocytopenia	'Sedormid'
		Chlorothiazide
		Digitoxin
		Quinine
		Novobiocin
	Agranulocytosis	Amidopyrine
		Sulphasalazine
		Propylthiouracil
Cell-mediated	Morbilliform exanthema	Ampicillin
		Sulphonamides
	Erythroderma	Mesantoin
		Gold salts
	Drug-fever	

drugs) must bind covalently to a carrier molecule (usually a protein) in order to become immunogenic. In sensitization to small molecular weight chemicals *in vivo*, the role of the carrier molecule is assumed to be played by autologous proteins. Among the arguments which have been advanced since the time of Landsteiner to establish the conjugation theory we may briefly recall the following.

1. With several compounds and groups of analogous chemicals, there seems to be a direct relationship between the chemical reactivity (e.g. ability to form amide bonds with amino groups) and the capacity to sensitize.

2. Allergenic drugs and simple chemicals induce the formation of antibodies specific for the structure of the conjugated group, rather than for the original non-reacted drug. Frequently, the first amino acid and the neighbouring carrier areas, to which the drug has been covalently bound through conjugation, participate in the specificity of the antibodies induced. For example, antibodies induced by penicillin in man and experimental animals have a thousandfold higher affinity for the penicilloyl-amide group than for penicillin itself (de Weck, 1971a).

TABLE 2

ADVERSE DRUG REACTIONS WITH A POSSIBLE, BUT NOT ESTABLISHED, IMMUNOLOGICAL MECHANISM

Clinical syndrome	*Main causative drugs*
Fixed drug eruption	Phenolphthalein Phenazone Barbiturates Sulphonamides
Lyell, Stevens-Johnson syndromes	'Irgapyrine' Barbiturates Sulphonamides
LE syndrome (arthritis, fever, pericarditis, pleurisy, rash, leukopenia)	Hydralazine Hydantoins Troxidone (trimethadione) Isoniazid Procainamide
Fever and salivary gland enlargement	Phenylbutazone Sulphafurazole (sulphisoxazole)
Jaundice (with rash, fever and eosinophilia	Chlorpromazine Phenothiazine PAS Sulphonamides Chlordiazepoxide α-Methyldopa Halothane (?)
Pulmonary infiltration with eosinophilia	Nitrofurantoin
Lymphadenopathy (pseudolymphoma)	Phenytoin
Isolated eosinophilia	Penicillins

TABLE 3

ADVERSE DRUG REACTIONS NOT DUE TO IMMUNOLOGICAL MECHANISMS

Type of effect	*Clinical symptoms*	*Main causative drugs*
Enzyme deficiencies or pharmacological abnormalities	Haemolytic anaemia (G-6-PD)*	Primaquine
	Peripheral neuropathy	Isoniazid
	Paralysis (pseudo-cholinesterase)	Succinylcholine
	Asthma, rhinitis, nasal polyposis	Aspirin
Toxic effects	Agranulocytosis	Chloramphenicol Cyclophosphamide
	Hepatitis	Chlorpromazine Thiouracil Halothane Mono-amine oxidase inhibitors Testosterones Anabolic steroids

*G-6-PD = glucose-6-phosphate dehydrogenase.

3. Several non-conjugating molecules are apparently non-immunogenic, although they may be strongly but reversibly bound to proteins such as serum albumin.

4. Investigations on the minimal size required for oligopeptides and oligosaccharides in order to induce an immune response have usually shown that a molecule of minimal size (e.g. seven amino acids) is required (Sela, 1969).

However, in recent years experiments on the immunogenicity of small molecules, such as nucleic acids or hapten-oligopeptides, have also indicated that binding through non-covalent bonds to an immunogenic protein (designated as 'Schlepper') may suffice to induce an immune response against the haptenic group. Indeed 'Schlepper' molecules such as denatured proteins (e.g. methylated albumin) or immunogenic bacteria (mycobacteria) have been found to be efficient (Plescia *et al.*, 1964; Stupp *et al.*, 1971). There is no evidence up to now that normal autologous proteins function as 'Schleppers'. Nevertheless, the possibility should be kept in mind especially for those drugs and chemicals which appear to be sensitizing from a clinical point of view, but which from known metabolism do not give a reactive conjugating derivative. The interesting possibility that mere adsorption to cell membranes may favour immunogenicity (see below) is also worth investigating.

The main interest of immunochemical studies in drug allergy has usually been directed towards the reaction potential of the drug itself and towards its reactive derivatives or metabolites. From a practical point of view, it seems important to make a distinction between reactive derivatives occurring spontaneously through the intrinsic chemical reactivity of the drug itself (e.g. the degradation of penicillin to penicilloic acid) and, on the other hand, the production of them through interaction with the organism and metabolic pathways of reactive metabolites. In the first case, care will have to be taken through appropriate conditioning and storage of drug preparations that the degradation of the drug to reactive derivatives is kept to a minimum. Aqueous penicillin solutions, for example, should be kept at neutral pH and low temperature and should be sufficiently buffered, then used as soon as possible after preparation of the solution. Sufficient attention has not always been paid to such factors by penicillin manufacturers in the past. In the case of metabolites, and when the development of a potentially allergenic substance apparently requires interaction with the body's enzymatic systems, individual and genetic factors may considerably influence the actual amount of sensitizing metabolites being formed. This appears, for example, to be the case with phenacetin (unpublished experiments).

In recent years, the problem of impurities in drug preparations and their role in drug allergy has received increasing attention. Among substances present as impurities in drug preparations, one may distinguish:

1. substances added as preservatives or additives and usually considered as more or less inert from an immunological point of view,

2. side products of drug synthesis which may be reactive chemicals, and

3. side products of biological origin in drugs prepared by biological means (e.g. protein impurities).

Although known sensitizers will certainly not willingly be added to drug formulations, some unexpected pitfalls have been encountered: it is only after several years of research on the chemistry and immunological effects of penicillin that I learned, by pure chance, that carboxymethylcellulose (CMC) is frequently present in drug formulations containing penicillin. Since we had shown that penicillin not only reacts and binds covalently to amino groups but also to hydroxyl groups (Schneider and de Weck, 1968), the possibility that CMC could serve as a carrier for the penicilloyl group became obvious. In fact, we have shown that penicillin binds to CMC and forms conjugates which are very efficient in eliciting allergic reactions in sensitized patients (Schneider *et al.*, 1971).

Other types of impurities, the participation of which in immunological reactions is more immediately obvious, are protein impurities present in those drugs which are either extracted from a biological source, such as hormones (e.g. insulin) or prepared by biological means (e.g. preparations of 6-amino-penicillanic acid by enzymatic degradation of benzylpenicillin). In the case of insulin, allergic reactions can develop which may be quite disturbing for the patients and have frequently made it necessary to interrupt treatment. These reactions, apparently, have decreased considerably with better purification methods and recrystallization of the insulin. Despite the frequently expressed opinion that allergic reactions to insulin no longer present a problem and have become relatively seldom, precise investigations reveal that commercial insulins still contain appreciable amounts of highly immunogenic impurities. The investigation of 47 patients with clinical allergy to insulin has shown us, for example, that the great majority of these patients are not allergic to the pure insulin molecule itself but react to side-products related to insulin such as proinsulin, partially split insulin-proinsulin molecules, insulin dimers and insulin molecules modified by the extraction procedure. Proteins are largely excluded from insulin which has been recrystallized several times. On the other hand, recrystallization does not eliminate insulin side-products which may only be separated by chromatography and polyacrylamide gel electro-

phoresis. It has been shown by Schlichtkrull (1970) in experimental animals and by us (in co-operation with Dr. Fankhauser) in groups of patients systematically treated with insulin of variable and controlled degrees of purity, that current immunogenicity is directly related to the purity of the preparation used. However, even very pure preparations differ in their degree of immunogenicity according to their physico-chemical form: a very pure depot (amorphous) preparation appears to be more immunogenic than the corresponding crystalline and fast-acting preparation.

Another famous example of allergenicity due to protein impurities has been the demonstration of such material in penicillin preparations by Feinberg and the Beecham group (Batchelor *et al.*, 1967). Since then, different opinions have been expressed at various times and by several authors about the importance and significance of these impurities in clinical penicillin allergy. Whereas protein impurities play little if any role in modern commercial preparations as a cause of allergic reactions to benzylpenicillin or penicillin V, the matter may be entirely different for some semi-synthetic penicillins prepared from 6-aminopenicillanic acid obtained through enzymatic degradation of benzylpenicillin.

Among patients clinically sensitive to ampicillin, we observe a clear-cut group hypersensitive to the penicillin nucleus, who will cross-react in various immunological tests to other penicillins and also to entirely chemically synthesized ampicillin. Another group, despite obvious clinical hypersensitivity, does not react to other penicillins and penicillin derivatives. Whether their reactions are due to sensitization to contaminating proteins, to peculiar polymers which form very easily in ampicillin solutions or whether their reactions are of non-immunological nature is, in my opinion, not yet satisfactorily clarified.

Another source of impurities should not be forgotten, namely the possibility that side-products occurring during synthesis of a drug may be carried over, even in traces, in the final preparation. Since drugs are like any other chemical compound synthesized by interaction of reactive chemicals and since chemical reactions respond to the law of mass action and since purification procedures are always somewhat relative, it is not astonishing that reactive chemicals may still be present in traces in some drug preparations. We have reported such an example concerning hypersensitivity to aspirin (de Weck, 1971b; de Weck and Lazary, 1972).

Elicitation of allergic reactions to drugs

One of the most consistent findings in investigations on the mechanisms of elicitation of immediate-type allergic reactions has been that plurivalent conjugates (i.e. carrying several antigenic determinants per molecule) are most efficient in eliciting immediate-type allergic reactions due to specific

immunoglobulins, and in forming antigen-antibody complexes. The formation of eliciting antigen-immunoglobulin complexes on the membrane of mast cells carrying a special type of immunoglobulin (IgE), and the activation of enzymatic mechanisms leading to histamine release, have been shown by a series of arguments to be due to the bridging of immunoglobulin molecules by an antigen which should be at least bivalent. Levine, Ovary and our own group have demonstrated independently and, I believe, convincingly that even very small bivalent antigens are capable of eliciting anaphylactic reactions, whereas monovalent antigen determinants almost always inhibit such reactions (de Weck and Schneider, 1969).

However, some exceptions have been reported and some univalent or apparently univalent molecules have been used for eliciting anaphylactic reactions, mainly passive cutaneous anaphylaxis in guinea-pigs. In some cases, the apparently monovalent antigen may undoubtedly be qualified as 'pseudomonovalent' since it carries on its 'tail' chemical groupings and electrostatic charges which undoubtedly permit aggregation and/or binding to a 'Schlepper' molecule. In some cases, the binding possibly takes place to the cell membrane itself, providing the anchor required for inducing allosteric modification of immunoglobulins fixed on the cell surface. It is to be noticed that in all instances where univalent antigens have been reported to be capable of eliciting anaphylactic reactions, antibody of high affinity had to be used and always a markedly higher dose of univalent antigen (sometimes up to 25,000-fold more than that of a plurivalent elicitor). Since a large number of univalent haptens have been shown to be specific inhibitors in many immunological systems, it seems logical to assume that the exceptional eliciting activity of some apparently univalent haptens may be due to special properties not directly related with their interactions with the antibody's combining site.

The fact that plurivalent antigens are good elicitors of antibody-mediated reactions and especially of anaphylactic reactions emphasizes the possible role of polymers and of high molecular weight impurities or additives as potential carriers in drug preparations. In penicillin allergy, one has long been puzzled by the speed with which sensitized patients sometimes develop anaphylactic reactions to the administration of small amounts of penicillin. From the point of view of the chemist, and considering the relatively slow rate of conjugation of penicillin with proteins *in vitro*, it is somewhat difficult to visualize that plurivalent eliciting conjugates are formed *in vivo* within seconds. We may now assume that eliciting conjugates are already preformed and present in the bottle in the form of polymers, or of conjugates with protein impurities, or with carboxymethylcellulose.

Complexes of antibodies in solution with bivalent antigens only activate complement when, and if, ring structures of relatively large size are formed. There is no evidence that the IgE-dependent mast cell reaction is complement-

dependent. On the other hand, the formation of complement-activating immune complexes, which are responsible for the development of Arthus reactions, seems to require antigens which are at least trivalent. Upon injection of conjugating chemicals *in vivo*, the large excess of carrier groups available for reaction probably favours the formation of monovalent conjugates. Accordingly, the formation *in vivo* of highly substituted conjugates is probably an exception. This might explain why Arthus reactions, which are frequent upon injection of multideterminant foreign proteins, are a relatively seldom event in drug allergy.

Cellular reactions

Whereas the specific mechanisms of antibody-dependent allergic reactions, although still requiring further investigations, nevertheless appear somewhat clarified, this is by no means the case for allergic reactions depending upon interaction of antigens with sensitized lymphocytes (cellular immunity). This gap in our knowledge is the more regrettable since the great majority of allergic reactions to drugs takes the form of generalized rashes and morbilliform exanthemata which are, in my opinion, a manifestation of cellular immunity and delayed-type hypersensitivity. We still know very little about the molecular mechanisms of interaction of drugs with sensitized lymphocytes and the way in which sensitized lymphocytes are stimulated by simple chemicals. We have therefore been interested in investigating the mechanisms by which penicillin is able to stimulate lymphocytes from sensitized patients and animals *in vitro*. Several authors have proposed schemes of interaction by which plurivalent antigens or antigens presented as a multimolecular matrix would cause modifications analogous to bridging on immunoglobulin receptors of sensitized lymphoctes (Mitchison, 1971). In this context, our observation that the optimally bridging penicilloyl-polylysine molecule is consistently a poor lymphocyte stimulator when compared with penicillin itself, and that small bivalent anaphylactic elicitors never stimulate penicillin-sensitized lymphocytes *in vitro*, obviously suggest that different mechanisms other than bridging are involved. Detailed investigations which are to be reported elsewhere (Spengler *et al.*, 1972) have shown the following.

1. The intact penicillin molecule is a better stimulator of penicillin-sensitive lymphocytes than any preformed conjugates, including plurivalent conjugates formed with various types of peptide and protein carriers.

2. Penicillin exerts its stimulating effect on sensitized lymphocytes only upon conjugation, since prior hydrolysis to penicilloic acid by various means completely prevents stimulation.

3. Conjugation to cell membranes during culture is more stimulating than