

ALLERGY PRACTICE FORUM

Allergic reactions to drugs: current concepts and problems

The problem of drug allergy

Adverse reactions to drugs constitute a common problem in clinical practice. They can be divided broadly into those that can be accounted for by the known pharmacological and chemical properties of the drug (termed intrinsic or Type A) and those that appear to depend rather on some abnormality of host responsiveness (termed idiosyncratic or Type B). Idiosyncratic reactions are, by their nature, unpredictable, often dose-independent, and the symptoms are diverse. A large proportion of idiosyncratic reactions are labelled 'allergic' because the clinical manifestations resemble those seen in the various types (I–IV) of classical hypersensitivity, or involve some form of immunological disruption. A true figure cannot be placed on the prevalence of allergic reactions to drugs because of the subjective and non-standardized criteria (usually based on clinical observations rather than laboratory tests) that are adopted for such a definition; but most estimates suggest that 10–25% of all adverse drug reactions are allergic [1,2]. The types of symptoms seen in allergic drug reactions include skin rashes (ranging from mild morbilliform type to severe Stevens–Johnson type), fever, lymphadenopathy, and most seriously but much less frequently cytopenias (including haemolytic anaemia, agranulocytosis and thrombocytopenia) and anaphylaxis.

Laboratory diagnosis of drug allergy

The classification of an adverse drug reaction as 'allergic' infers that the reaction is mediated by the immune system, or more precisely that the immune system recognizes and responds to a drug-derived antigen. Allergic drug reactions may be directed against the drug itself or towards new antigenic determinants generated on self proteins as a result of chemical interaction with the drug [3]. Alternatively, native determinants may no longer be recognized as self as a result of chemical modification of adjacent structures leading to 'by-pass' of immune suppression [4]. It is this area of uncertainty concerning the nature of drug-derived antigens that often makes laboratory confirmation of suspected allergic drug reactions problematical. Laboratory tests for allergies to conventional foreign

protein antigens are relatively straightforward. For example, in an allergic reaction such as hay fever in which one suspects involvement of IgE antibody, one can screen serum samples for IgE antibodies directed against protein extracts of various pollens by radioallergosorbent test (RAST) or enzyme-linked immunosorbent assay (ELISA). Antigen-induced histamine release from isolated basophils also provides evidence of IgE-dependent allergic sensitization. In the case of a delayed allergic reaction that one suspects is cell-mediated, for example to skin-sensitizing chemicals, the patients blood mononuclear cells can be stimulated *in vitro* with the appropriate antigen, and the cellular response measured by lymphocyte transformation (usually uptake of [³H]thymidine into DNA) or production of lymphokines.

With regard to suspected allergic reactions to drugs, evidence of an immune response directed against the drug is harder to obtain, since an understanding of the nature of the drug-derived antigen is essential before one can synthesize the relevant test antigen for use in laboratory tests such as RAST, ELISA, lymphocyte transformation, and basophil/mast cell degranulation assays. Our ignorance concerning the chemical nature of drug-derived antigens stems in turn from our ignorance of drug metabolism, in particular of those pathways that lead to generation of drug–macromolecule conjugates that have the potential to act as functional antigens. It has long been our contention that identification of drug-specific antibodies necessitates the use of test antigens that resemble chemically those generated naturally. Hence, study of the metabolism and disposition of a drug, with special reference to conjugation reactions, must proceed alongside immunological studies.

To establish that a drug induces adverse reactions via an allergic, i.e. immunologically mediated, mechanism, evidence must be obtained for a population of antibodies or lymphocytes in the blood of affected patients that recognize the drug or a drug-derived antigen in a relatively specific way. A far more difficult problem is to establish whether the observed drug-specific antibodies or cells are a cause or an effect of the adverse reaction: evidence that immune sensitization predisposes to, or predicts a later reaction would be convincing. Another question that arises is that although a drug-derived

antigen may trigger an effector response which is responsible for an adverse reaction, the population of antibodies or sensitized cells may not necessarily have been drug-induced, but may have arisen from sensitization to some cross-reactive antigen. In other words, the drug may act as antigen but not necessarily as immunogen. To tackle this problem the temporal relationship between drug exposure and the appearance of immune sensitization must be investigated. For most drugs against which antibodies or sensitized T cells have been reported, the evidence linking immune sensitization with adverse reactions is somewhat anecdotal, but nevertheless provides a valuable lead for further investigations of the mechanisms involved in allergic reactions.

Current concepts of immune recognition with reference to drugs acting as antigens

It is a long-standing doctrine in immunology that chemical substances with a molecular weight of less than a few thousand (into which category most drugs fall) are non-immunogenic, and must covalently conjugate to a protein 'carrier' molecule to become a target for the antibody response. Precursor cells of the B-lymphocyte lineage that recognize the low molecular weight constituent of the chemical-protein conjugate proliferate and differentiate into mature antibody-producing cells. The antibodies they produce bind to the small molecule in free (unconjugated) form. Small molecules which are immunogenic only in carrier-conjugated form, but in unconjugated form can be recognized as antigen by antibody, are called 'haptens'. The anti-hapten antibody response requires co-operation between a population of cells that recognizes the hapten and a second population of cells that recognizes the carrier [5]. The cells expressing hapten specificity are antibody-producing B cells, while those expressing carrier specificity are T cells. The latter provide 'help' for antibody production by B cells. In practice, a distinction in chemical terms between determinants recognized by B cells and T cells cannot readily be made, because any exposed region of a protein molecule has the potential to be recognized by B cells (hence antibody) [6], while simple chemical substituents on macromolecules, for example the trinitrophenyl (TNP) moiety, can be recognized not only by B cells but also by T cells [7,8].

B cells and T cells recognize antigen in a quite different way. B cells recognize and bind soluble free antigen, while T cells recognize antigen only when presented in association with self cell surface molecules coded by genes of the major histocompatibility complex (MHC)—a phenomenon known as 'MHC-restriction'. As a general rule helper T cells (those which co-operate with B cells in antibody production) recognize antigen in association with MHC

molecules of class II which are present on specialized antigen-presenting cells—either macrophage-like cells such as dendritic cells, or B cells [9]. Cytotoxic T cells, whose usual function is killing of virus-infected cells, but which also respond to the products of minor histocompatibility loci, tumour antigens and chemically modified cell surfaces, recognize antigen in association with MHC molecules of class I which are present on most somatic cells [10]. In both MHC class I and MHC class II-restricted recognition of antigen by T cells, the T cell recognizes a peptide fragment of antigen which is generated by cellular proteolytic degradation of the parent antigen. The peptide cleavage fragment (10–20 residues in length) occupies a groove in the MHC molecule, and it is this complex of peptide plus MHC molecule that is recognized by the T-cell antigen receptor [11–14].

Our current understanding of the cellular co-operation between helper T cells and B cells in the antibody response is based on the experiments of Lanzavecchia [15]. He showed that antigen-specific B cells captured antigen via their immunoglobulin receptors, and accumulate it intracellularly. The antigen is processed and then presented by B cells in association with class II MHC molecules—in which form it is recognized by, and triggers proliferation of, T cells specific for a determinant on the same parent antigen. The clonally expanded population of T cells provide help for B cells presenting the appropriate processed antigen. B-cell proliferation and differentiation is subsequently induced by soluble T-cell-derived factors (interleukins 4, 5, 6). This mechanism satisfies the requirement for hapten-carrier linkage [5] and also accounts for the fact that a single receptor on T cells recognizes antigen in conjunction with MHC molecule. The implications of this model to immune responses to drugs are that for a drug to be immunogenic it must become chemically bound to a protein in such a way as to generate a determinant that is recognized by B cells, and a determinant that, after processing and presentation in peptide form in association with MHC molecules, can be recognized by T cells. Unlike earlier models of T-B-cell co-operation, in which the hapten-carrier conjugate formed a 'bridge' between B and T cells, there is no longer an obligatory requirement that T and B cells simultaneously recognize different parts of the antigen molecule: B cells that process and present antigen can receive help from any T cell that recognizes a processed form of the antigen [16]. Presumably therefore a drug-derived determinant on self proteins can satisfy the requirements for recognition by both T and B cells. A further important implication of processing of antigen by B lymphocytes is that very low external concentrations of antigen (down to 10^{-12} M) are sufficient to trigger T cells after capture and concentration by B cells [15,16]. This is relevant to drug immunogenicity

when the amount of drug-protein conjugate generated *in vivo* that is immunologically accessible may be very low.

Another point worth considering is that the nature of any drug-macromolecule conjugate generated *in vivo* may determine the nature of the immune response to that conjugate. If the drug conjugates to a protein in free solution, for example in plasma, that conjugate may be captured only by those cells that are specialized for this function, namely macrophages (non-specifically) and B cells (specifically), which may then process the antigen for presentation to T cells that are predominantly MHC class II-restricted (helper cells). MHC class I-restricted cells (most somatic cells) are not specialized for antigen capture, but process and present antigens generated from within the cell, such as viral and tumour antigens. Therefore, for a drug to induce a class I-restricted cytotoxic T-cell response a prime requirement would be

for it to produce haptens on cellular rather than extra-cellular proteins.

Antibody responses to drugs

Drug-specific antibodies have been detected in patients experiencing adverse reactions to a wide range of drugs (Table 1). However, these reports often do not establish unambiguously the specificity of the antibody for a defined naturally generated drug-derived antigen. Furthermore, evidence for a causal relationship between antibody response and adverse drug reaction is usually circumstantial at best, and for most of the anti-drug antibodies listed in Table 1 we must await confirmatory reports and more detailed longitudinal studies before we can answer questions regarding their role in suspected allergic reactions to drugs. In this review I will not

Table 1. Drugs against which antibodies have been detected in man

Drug	Type of antibody	Assay	Reference
Amodiaquine	IgG	ELISA	34
Anaesthetics and muscle relaxants (alcuronium, gallamine, succinyl choline, thiopentone, tubocurarine)	IgE	SPB	35,36
Captopril	IgG	ELISA	25
Cephalosporins	IgG, IgM	HA	37
Chlorhexidine	IgG, IgE	ELISA	38
Cyclophosphamide	IgG, IgE	RBA	39
Dapsone	IgG	ELISA	40
Elliptinium	IgM	RBA	41
Ethanol	IgG	IF	42
Ethynyloestradiol	NK	RBA	43
Halothane	IgG	ELISA	44
Methyldopa	IgG	IF	45
Mianserin	IgG	IF, CT	46
Morphine	IgG	RBA	47
Nomifensine	IgG, IgM	HA	48
Penicillamine	IgG	SPB	28
Penicillins	IgG, IgE, IgM	ELISA	33,49
Phenytoin	IgG	HA	50
Practolol	NK	RBA	51
Salicylates	IgG	HA	52

CT, antibody-dependent cytotoxicity; ELISA, enzyme-linked immunosorbent assay; HA, haemagglutination; IF, immunofluorescent staining of cell-associated antibody; RBA, radiobinding assay; SPB, solid phase assay employing drug chemically linked to beads; NK, not known.

attempt a comprehensive coverage of anti-drug antibodies but rather will focus on three drugs that have been subject to extensive investigation in recent years in our laboratory, namely: captopril, D-penicillamine and benzylpenicillin. All three drugs have been associated with adverse reactions that appear to be of the allergic type. We selected these three drugs for the simple reason that they all conjugate spontaneously by covalent linkage to proteins, whereas the majority of drugs (including most of those listed in Table 1) are chemically unreactive and must become metabolically activated to generate antigens. The mechanisms by which drugs become metabolically activated to generate potentially antigenic conjugates have recently been reviewed elsewhere [17–19] and will not be considered further here.

Throughout these studies we have found the following criteria useful for identification of drug-specific antibodies in immunoassays employing drug–protein conjugates as the test antigen [20,21]: the putative anti-drug antibody binds to the drug–protein conjugate but not to the unconjugated protein alone, and antibody binding to antigen is inhibitable by the free drug or a simple derivative in free solution. By testing a range of substances for blocking of antibody binding it is also possible to define in chemical terms the specificity of antibody.

Captopril

Captopril (CP; D-3-mercapto-2-methyl-propanoyl-L-proline) is a simple amino acid-derivative which inhibits angiotensin-converting enzyme, and is therefore used in the treatment of hypertension. Because it incorporates a free sulphhydryl (-SH) group, the drug readily conjugates by disulphide linkage to other thiols, including proteins. *In vivo*, 35% of circulating CP was covalently bound by disulphide bridging to plasma proteins, representing 3% of the dose, 3 hr after administration to rats [22]. Although CP–protein conjugates dissociate readily *in vivo*, with a half-life of only 1–2 hr [22], we showed that CP–protein conjugates are sufficiently stable to induce an IgG anti-CP antibody response in rabbits [23]. Chronic administration of free CP to rats, either by the intraperitoneal or intramuscular routes led to an IgG anti-CP antibody response detected by ELISA, employing disulphide linked CP–protein conjugates as test antigen [24]. The drug-induced anti-CP antibody was demonstrated to be specific for an antigen that incorporated the CP moiety, as antibody binding was inhibited by free CP and CP–disulphide [24]. These experiments show clearly that sulphhydryl drugs are potentially immunogenic: the strength of the immune response probably depends upon the relative rate and extent of conjugation and dissociation of drug *in vivo*. In a survey of 45 patients receiving CP

therapy, five of whom had experienced skin reactions, we detected CP-specific IgG antibody in two patients. Again the antibody was blocked by free CP–disulphide. Only one of the five patients with skin rashes was antibody positive. No anti-CP antibodies were detected in a control group of healthy volunteers [25].

D-penicillamine

The anti-arthritis drug D-penicillamine (D-PEN; dimethylcysteine), like CP, is a simple amino acid-derivative which incorporates a free sulphhydryl group. Also like CP, D-PEN conjugates extensively to plasma proteins. *In vivo*, 100% of circulating drug was covalently conjugated by disulphide linkage to plasma proteins 3 hr after administration to rats, equivalent to 3.5% of the dose [26]. Although D-PEN–protein conjugates are sufficiently stable to be immunogenic in the rabbit when administered with Freund's complete adjuvant [27], after chronic administration of D-PEN to rats we failed to detect either IgG or IgM antibodies directed against the drug, although interestingly an IgG anti-albumin response was induced, perhaps reflecting a breakdown of tolerance to this protein [26]. In serum samples from 40 patients receiving D-PEN, six of whom had experienced adverse reactions, we were unable to detect IgG, IgM or IgE antibodies directed against the disulphide-linked antigen [Foster and Coleman, unpublished data]. In contrast, Storch and colleagues [28] have detected antibodies directed against D-PEN in human patients using thiazolidine ring-linked D-PEN conjugated to Sephadex beads as their test antigen: elevated levels of antibodies were found in patients who had experienced adverse reactions compared to those who had not. It will be interesting to see whether these antibodies to D-PEN are drug-induced, or whether they represent some type of crossreactivity.

Benzylpenicillin

It is well established that the antibiotic benzylpenicillin (BP) conjugates spontaneously to proteins by amide linkage via a chemical rearrangement of the molecule to generate the benzylpenicilloyl (BPO) determinant predominantly (reviewed in [29]). In studies with rats we have shown, by radiometric binding analysis, that only 5% of circulating drug is covalently bound to plasma proteins 3 hr after administration to rats, representing <0.05% of the dose [30]. Chronic administration of freshly dissolved BP to rats failed to induce IgG or IgM anti-BPO antibodies [30] whereas BP solutions that have been stored for 24 hr were immunogenic [31]. Interestingly, the reactive rearrangement product of BP, benzylpenicillenic

acid, was detected in stored but not fresh BP solutions, implicating this as the intermediate responsible for immunogen formation [31]. Benzylpenicillenic acid itself was readily immunogenic in rats. In the same study we developed an ELISA for monitoring levels of plasma BPO antigen, employing a BPO-specific rabbit antiserum. Somewhat surprisingly, although free benzylpenicillenic acid was considerably more immunogenic than either fresh or stored BP (measured as IgG anti-BPO response), circulating levels of plasma BPO antigen were higher after administration of the parent drug than the putative intermediate [31]. We are led to conclude that circulating levels of antigen may not necessarily reflect immunogenic activity. The much faster conjugation of benzylpenicillenic acid may lead to a higher hapten density on selected carrier proteins, which may then be localized to lymphoid tissues.

Studies with rabbits have shown that in this species free BP is cleared very rapidly from the circulation, and 3 hr after intravenous administration 100% of circulating drug is covalently bound to plasma proteins [32] (c.f. 5% in that rat [30]). This represents an average hapten density (molar ratio of drug to protein) on circulating plasma proteins of 0.2 compared to only 0.03 in the rat after similar doses of the drug [32]. Interestingly, fresh BP without adjuvant does induce an IgG anti-BPO antibody response in the rabbit [32] although this was not the case in the rat (see above). This species difference in drug immunogenicity may in part reflect the greater extent of conjugation to proteins seen in the rabbit, but undoubtedly genetic differences in immunological responsiveness are also important.

In a clinical survey of the prevalence of IgG, IgM and IgE antibodies in patients who claimed to be allergic to penicillin, we found IgG anti-BPO in 4/100 of the patients' sera, IgM anti-BPO in 7/100, and IgE anti-BPO in 5/100 [33].

Conclusions

According to current immunological theory, for drugs and chemicals to be recognized as immunogens by the immune system they must be processed and presented in peptide form on the surface of specialized cells in conjunction with MHC-coded molecules. Hence, potentially immunogenic drugs must have the capacity to conjugate to self proteins, and at the same time to generate 'non-self' determinants to act as targets for a system that is specialized for capture, recognition and response to foreign proteins. In antibody responses to drugs, the drug in question must generate determinants that can be recognized by immunoglobulin receptors on B cells, and after processing by these cells, by antigen

receptors on T cells (in conjunction with MHC molecules). For selected drugs we have made some progress in defining the drug-derived determinant that is recognized by antibodies (and therefore by B cells) but we are still largely ignorant concerning the nature of drug-derived determinants recognized by T cells.

By a combination of radiometric and immunochemical approaches we have shown that CP, D-PEN and BP generate haptenic groups on plasma proteins *in vivo*. In the case of CP and D-PEN the drug-derived antigen is formed by disulphide linkage, whereas for BP the major antigen formed is the amide-linked benzylpenicilloyl hapten. Administration of CP and BP (in the free form without adjuvant) to experimental animals induces antibody responses directed against the naturally formed drug-derived antigen, and antibodies against CP and BP have been detected in the sera of human patients. Animal and human antibodies directed against CP are blocked by free CP and CP-disulphide, thus demonstrating specific recognition of the drug moiety; animal and human antibodies directed against BP are blocked by benzylpenicilloylaminocaproate, thus demonstrating specific recognition of the BPO moiety.

While it is clear that adverse reactions to CP and BP may be antibody-mediated in some patients, in others we have failed to detect antibodies against these drugs.

Although D-PEN generates a haptenic group *in vivo*, we have found no evidence yet of antibodies against the drug-derived antigen in animals and patients receiving this drug. We suspect therefore that adverse reactions to this drug are either not immunologically mediated, or may involve the cellular (T cell only) rather than humoral (T + B cell) arm of the immune system.

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