The immunological basis of adverse drug reactions

A report on a Symposium held in Liverpool on 6th April 1988

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A symposium entitled 'The Immunological Basis of Adverse Drug Reactions' was held recently (6th April 1988) as part of the British Pharmacological Society meeting at the University of Liverpool. The aim of the symposium was to present recent advances in our understanding of the ways in which drugs interact with the immune system to induce adverse reactions. The programme was intended to reflect the diversity of mechanisms by which drugs may induce immunologically mediated adverse reactions.

Drugs may be recognised as specific targets by the immune system, leading to the generation of drug-specific antibodies (covered by B. K. Park and J. Neuberger) or sensitized T cells (E. Gleichmann). Alternatively, drugs may interfere with the normally finely balanced tuning of the immune system, and hence disrupt normal immune functions, such as clearance of circulating immune complexes (E. Sim).

The symposium was opened by Professor A. Breckenridge (Liverpool) who outlined the clinical problem of adverse drug reactions. A useful nomenclature of types of adverse reactions has emerged over the years. The most common adverse reactions are those associated with an augmented effect of the drug (hence called Type A). These are usually predictable, the effect is related both to the pharmacological action of the drug and to the dose administered, and by and large, they are not serious. Type B (bizarre) reactions are unpredictable from a knowledge of drug action and, moreover, there is no obvious dose-response relationship. Their timing in relation to drug exposure varies, but there is usually a delay, and the illness caused is often recognised to have an immunological basis. Whereas these reactions are much rarer than Type A adverse reactions, they are more serious and most fatalities from drug adverse effects usually fall into this group. Type C adverse reactions are those associated with long term therapy e.g. analgesic nephropathy and Type D are delayed effects such as carcinogenicity or teratogenesis. It is Type B adverse reactions, according to this nomenclature, that the symposium addressed.

The nature of reactions that lead clinicians to suspect involvement of the immune system in such reactions can be summarised according to the Gell and Coombs classification of hypersensitivity. These four types: Type I, IgE-dependent immediate reactions; Type II, antibody-dependent cell cytotoxicity; Type III, immune complex disease; and Type IV, antibody-independent T cell-mediated reactions. Although such a classification is recognised as an over-simplification, it nevertheless provides a useful framework for consideration of the various mechanisms of drug hypersensitivity reactions as covered by the speakers at the symposium.

Dr Kevin Park (Liverpool) discussed the various ways in which drugs may act as immunogens and considered the role of disposition in this process. Our current understanding of chemical and drug hypersensitivity is based on the assumption that low molecular weight compounds must become covalently bound to a macromolecular carrier to elicit an immune response, and thereby act as haptens (Park *et al.*, 1987). Dinitrofluorobenzene has been used widely as a model hapten, and it has been shown that intravenous, intramuscular and topical administration of this substance can elicit specific humoral and cellular anti-hapten responses in experimental animals. Metabolic studies have shown that approximately 5% of an intravenous dose of dinitrofluorobenzene becomes covalently bound to plasma proteins and that such conjugates are degraded in the liver and the hapten is excreted as an *N*-acetyl-lysine conjugate (Maggs *et al.*, 1986).

Much of our present understanding of drug hypersensitivity in man is based on experience with penicillin. Penicillin may induce a wide range of immunological reactions including anaphylaxis, skin rashes and blood dyscrasias. It is now well established that the immunogenicity of penicillin is a consequence of the ability of the drug to conjugate directly to nucleophilic groups in proteins, and thus form the penicilloyl hapten. Antibodies detected in the serum of patients are not directed towards penicillin itself, but towards

the penicilloyl hapten. There is, however, still considerable debate as to the nature of the carrier protein, and in particular whether the ultimate immunogen is formed *in vivo* (from autologous proteins) or *ex vivo* from protein contaminants present in drug preparations. Recent clinical and laboratory studies have shown that penicillin is unstable in solution, and undergoes degradation to products which are more immunogenic and chemically reactive towards protein, than the drug itself (Neftel *et al.*, 1982; Christie *et al.*, 1987). Captopril and penicillamine are two drugs which become extensively and covalently bound to cysteine residues in plasma proteins. However, disposition studies have shown that the disulphide linkage is biochemically labile and can be readily dissociated by endogenous thiols such as glutathione, which possibly explains why captopril is only very weakly immunogenic and penicillamine is non-immunogenic in both man and experimental animals (Coleman *et al.*, 1988).

Most drugs do not react directly with proteins and alternative mechanisms for the formation of drug-protein conjugates have been advanced. For most drugs it is assumed that a chemically reactive metabolite, generated by drug oxidation, will react spontaneously with autologous macromolecules. However, direct experimental evidence for such a mechanism is sparse. It has been demonstrated that a number of drugs, which are associated with occasional immunological reactions, may undergo activation by human liver microsomes to chemically reactive metabolites *in vivo*. However, parallel *in vivo* metabolic studies have shown that phase II biotransformations, which also occur in the liver, normally preclude the formation of, or rapidly detoxify chemically reactive metabolites, and thus prevent (or reduce) the release of circulating haptens which would interact with the immune system.

Chemical activation of drugs may also be mediated by cells of the immune system. It has been demonstrated that stimulated human polymorphonuclear leucocytes oxidise the antimalarial drug amodiaquine to a quinoneimine. This process can also occur *in vivo*, since antibodies directed against the quinoneimine have been detected in sera of patients and experimental animals given amodiaquine (Christie & Park, 1988).

Studies with model compounds have shown that the immune system can respond to very small quantities of hapten ($> \text{nmol}$) and that for autologous protein conjugates epitope density (number of drug molecules conjugated per molecule of protein) is an important determinant of the intensity of the immune response. There-

fore, the degree of haptenation and immunogenic potential of a particular drug will depend upon the balance between drug activation and drug detoxication in a particular tissue or cell type. Individuals with a deficiency of a particular enzyme may be susceptible to drug hypersensitivity. Spielberg and co-workers (1981) have shown that patients who presented with a severe hypersensitivity reaction to phenytoin, have a genetically determined deficiency in the enzyme epoxide hydrolase in their lymphocytes. It was postulated that phenytoin undergoes bioactivation to an arene oxide which produces toxicity by either direct cytotoxic mechanisms, or indirectly by haptenation of autologous proteins and a subsequent immune response.

Dr James Neuberger (Birmingham) provided an overview of recent attempts to understand the mechanisms involved in halothane hepatitis. The work neatly illustrates the importance of close co-operation between clinical and experimental work in studies of this kind. Halothane hepatitis is defined as 'otherwise unexplained liver damage occurring within 28 days of halothane exposure in a person with a previously normal liver'. This will therefore exclude those minor forms of liver damage which may occur in up to 30% to 40% following halothane anaesthesia and those with pre-existing liver disease such as alcoholic liver disease (Neuberger & Kenna, 1987). Since the large National Halothane Study of 1966, a number of features of halothane hepatitis have suggested that the disease represents an idiosyncratic drug reaction with an underlying immune mediated basis. These features include the very high incidence of adverse reactions after multiple halothane exposure, eosinophilia, autoantibodies and the possible association with allergy to other drugs (Neuberger & Kenna, 1987).

Early attempts to define immune sensitization to halothane, and in particular to detect specific anti-drug antibodies, produced conflicting results. One reason for this is that it is difficult to predict the nature of the antigenic determinant that might be formed from halothane. The molecule is small, and reactive metabolites derived from the drug may react with cellular macromolecules to generate several novel antigens. In order to circumvent the problems of selecting the appropriate antigen for *in vitro* testing Vergani *et al.* (1978) designed a set of experiments in which rabbits were exposed to halothane and allowed to recover. Eighteen hours later the animals were killed and the liver removed and liver homogenates were used as antigens for antibody testing in man. Using this method sensitization was detected in eight out of twelve patients with

the presumed diagnosis of halothane hepatitis. Initial studies were performed using indirect immunofluorescence and antibody cell mediated cytotoxicity techniques, and the results were confirmed by use of an enzyme-linked immunosorbent assay. The circulating antibodies which react with halothane altered (rabbit) liver membranes, were *not* detectable in the serum of patients with halothane exposure but no liver damage, or indeed with any other form of liver disease. Sensitization to halothane could also be demonstrated by testing lymphocytes *in vitro*. In some sero-negative cases, lymphocytes from patients were shown to secrete halothane antibody into the supernatant.

The involvement of halothane metabolism in the generation of drug related antigens has been demonstrated in a series of experiments in which rabbits were exposed to halothane under different oxygen tensions, after enzyme induction. It was found that metabolism via the oxidative route was necessary for the expression of antigens on liver cell membranes (Neuberger *et al.*, 1981). Immuno-blotting techniques revealed the presence of at least three different polypeptide antigens which were localised to the microsomal subfraction of liver homogenates (Kenna *et al.*, 1985). Studies in both animals and humans have suggested that all individuals exposed to halothane generate the antigen and that therefore if the disease is immune mediated the defect in susceptible patients is more likely in the immune response to antigen, rather than in antigen generation.

Dr Edith Sim (Oxford) presented evidence that certain drugs inhibit clearance of circulating immune complexes by binding to components of the complement system, hence leading to immune complex disease, exemplified by drug-induced systemic lupus erythematosus (SLE) of which vasculitis is a common component. The complement system is involved in immune complex handling and it promotes both solubilisation of immune complexes and opsonisation of immune complexes through covalent binding of C3, the most abundant component in the cascade. In order for C3 to become covalently bound to immune complexes via the classical pathway, C1 and C2 must become associated with the complex, and covalent binding of C4 is also necessary. The covalent binding of C4 and C3 to immune complexes involves a transesterification reaction or a trans-amination from an internal thiol ester which becomes exposed and reactive once the proteins C3 and C4 are activated in the complement cascade by proteolysis. Any event which inhibits binding of C4 to immune complexes in turn inhibits C3 binding, since activated C4 on the surface of an immune complex is part of the

enzyme involved in C3 activation. Furthermore a decrease in C3 binding to immune complexes will inhibit solubilisation (*in vivo* more likely to induce precipitation) of immune complexes and opsonisation and so lead to immune complex deposition at sites such as venules as in SLE. Dr Sim and colleagues have shown that hydralazine and isoniazid (Sim *et al.*, 1984), penicillamine (unpublished) and hydroxylamine metabolites of procainamide and practolol (Sim *et al.*, 1988) inhibit the covalent binding reaction of C4 by competing with amino and hydroxyl groups on the surface of the immune complex to which C4 becomes bound. Hydralazine and isoniazid themselves become covalently bound to C4 by an amide bond with the active site thiolester carbonyl group. Acetylated metabolites of hydralazine and isoniazid are poor inhibitors of C4 binding to complement activating surfaces. This is consistent with the parent drug itself being the toxic agent.

C4 is encoded at two highly polymorphic loci (C4A and C4B) within the major histocompatibility complex. The purified gene product of C4A is at least four-fold more sensitive to inhibition by hydralazine and isoniazid than C4B (Sim & Law, 1985). With penicillamine, C4A is at least ten-fold more sensitive than C4B (50% inhibition of C4A occurs at 100 μM). Inhibition of C4A would be expected at the therapeutic level of penicillamine (65 μM) and it was suggested that this may represent its mechanism of action as an antirheumatic. The development of proteinuria and glomerulonephritis with penicillamine could result from impaired clearance of immune complexes in a particular sub-group of patients. It has been shown that there is an association between penicillamine immunotoxicity and the HLA DR3 antigen. DR3 is in turn associated with individuals who have only one half the amount of C4A compared with normal individuals. The overall picture presented by Dr Sim was of a range of factors which predispose to drug-induced immune complex diseases, including ability to metabolise the drug and histocompatibility type, for which a biochemical rationale is available. The numerical level of the red blood cell immune adherence receptor (CR1 and CD35) which is partly under genetic control is also likely to be important (Mitchell *et al.*, 1987).

Dr Ernst Gleichmann (Düsseldorf) pointed out the close similarities between the symptoms associated with many immunotoxic drug reactions and those seen in chronic graft-versus-host (GVH) disease. GVH disease is induced when foreign immunocompetent cells are administered into an immunosuppressed individual, or when parental strain lymphocytes are injected into adult F_1 hybrid mice. In both cases the donor

cells are tolerated (either because the host is immunosuppressed or because the parental strain mice lymphocytes are not recognised as foreign) but the transplanted cells recognise allogeneic histocompatibility antigens on the hosts own cells, and mount an immune response directed against the host, leading to a wide spectrum of immunological disturbances. Gleichmann and colleagues have studied extensively the mouse model of GVH disease and shown that in F_1 recipients of parental strain lymphocytes the disease may take one or other of two basic forms (Gleichmann *et al.*, 1984). One is characterised by symptoms indicative of immune stimulation, including lymphoid hyperplasia, hypergammaglobulinaemia and the formation of autoantibodies particularly against nuclear antigens, and severe immune complex glomerulonephritis, all of which are features of SLE-like disorders. The other basic form of GVH disease in the mouse model is characterised by immunosuppression, seen as severe hypoplasia of the lymphopoietic system, leading to aplastic anaemia and hypogammaglobulinaemia. The immunostimulatory form of GVH disease in the mouse model is triggered by donor $\text{Lyt } 1^{+2-}$ T helper cells which recognise class II MHC structures of the F_1 recipient, whereas induction of the suppressive GVH disease requires both helper and cytotoxic/suppressor T cells and incompatibility in the F_1 recipients at both class I and class II MHC structures. Gleichmann suggested that a number of drugs, including phenytoin, hydralazine and penicillamine, which are known to induce in some individuals SLE-like disorders, do so by a mechanism analogous to that involved in immunostimulatory GVH disease. To induce a stimulatory GVH reaction, a drug must be recognised in association with class II MHC structures (which are present on antigen-presenting B lymphocytes and macrophages) on autologous (self) cells. T helper cells that recognise modified self in this way may provide help for antibody production by B cells, particularly those which recognise antigens incorporating repeating sub-units, such as DNA, which can provide more readily a B cell signal by cross-linking of IgG receptors. Gleichmann presented the popliteal lymph node (PLN) assay as a useful and simple test for compounds with lymphocyte-activating activity. Either free chemical or chemically modified syngeneic spleen cells are injected into one hind footpad of mice. A positive response is seen as enlargement of the draining PLN. The enlarged PLN shows increased total cell numbers and increased IgG-secreting cells (Gleichmann, 1981; Gleichmann *et al.*, 1983). Solvent vehicle is injected into the other hind footpad, and the contralateral PLN serves as

control. Drugs and chemicals that have given positive PLN responses include phenytoin, captopril, enalapril, penicillamine and mercuric chloride. The question of how drugs such as phenytoin might conjugate to lymphoid cells was raised during discussion—it appears probable that some degree of metabolic activation to form a reactive intermediate takes place at a peripheral site, perhaps in the lymphoid tissue itself. Rather more sophisticated assays for demonstrating T lymphocyte sensitization to drugs and chemicals were also presented by Gleichmann, which involved elegant adoptive cell transfer experiments, and also restimulation of isolated draining lymph node cells with drug-haptenated cells. Draining local lymph node cells from mice injected with penicillamine-haptenated spleen cells can be restimulated *in vitro* with haptenated spleen cells. The proliferating responder cells were identified as $\text{Lyt } 1^{+2-}$ helper T cells that were highly specific for penicillamine-haptenated cells, and could even distinguish between D- and L-stereoisomers of the drug (Nagata *et al.*, 1986).

The round table discussion at the end of the symposium was concerned largely with the question of screening procedures for hypersensitivity during drug development. It was felt that one obstacle in such an endeavour is the presently accepted classification of hypersensitivity reactions, which is probably too simplistic from a mechanistic point of view and is difficult to apply in the clinical situation.

It is obviously desirable that screening procedures should be developed with which to predict which drugs are likely to provoke hypersensitivity reactions and also to identify those individuals who may be susceptible to drug-induced hypersensitivity, but at the present time no such simple tests are universally recognised. In the past 20 years advances have been made in terms of chemical and immunochemical mechanisms by which drugs may perturb the immune system and thus produce immunotoxic reactions. Drugs which are likely to be immunogenic, can be recognised with a reasonable degree of confidence, but it must be remembered that there exist very marked species differences in immune response to any immunogen. A much more difficult question is why only certain individuals are susceptible to drug-induced immunotoxicity. At present, it is not possible to identify the normally very small number of individuals who will suffer an immunologically mediated adverse reaction to a particular drug. However, the experimental approach of analysis of genetic polymorphism, both with respect to immune function and drug metabolism, may provide some clues in our attempt to unravel this intriguing problem.

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