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THE IMMUNOLOGIC AND METABOLIC BASIS OF DRUG HYPERSENSITIVITIES[1]

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INTRODUCTION

Drug-induced toxicities can, in general, be classified into those that are intrinsic and those that are idiosyncratic (1). The intrinsic toxicities are characterized as primarily dependent upon the intrinsic chemical properties of the drug, often host independent, dose dependent, and usually reproducible in animals. In the past, this group of toxicities was clinically very important. Today, however, intrinsic toxicities are becoming less of a medical problem because of an increased understanding of the structural features of drugs that contribute to their intrinsic toxicity, particularly that produced by reactive metabolites (2), and because such toxicities are often uncovered in preclinical animal trials.

In contrast, the idiosyncratic toxicities are of major concern to physicians because they are difficult to predict and usually are not presented until the drug has been in general use for some time. Also, they are host dependent, often apparently dose independent, difficult to reproduce in animals, and relatively uncommon. Some idiosyncratic drug reactions are due to a metabolic abnormality of the host, such as a congenital deficiency in glucose-6-phosphate dehydrogenase, which increases susceptibility of red blood cells to oxidative damage by various classes of drugs (3); but many have an immunological etiology or hypersensitivity (allergic) basis.

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It has been estimated that the hypersensitivity class of idiosyncratic reactions accounts for between 3–25% of all drug reactions (3–6). The clinical manifestations of drug allergies can be quite diverse and life-threatening and can include pathological states such as anaphylaxis, serum sickness, asthma, urticaria, dermatitis, fever, hemolytic anemia, thrombocytopenia, granulocytopenia, hepatitis, nephritis, vasculitis, pneumonitis, and lupus-erythematosus–like syndrome (3–11). Frequently, several of these conditions are presented concurrently. Whereas much is known about the mechanisms of hypersensitivities produced by therapeutic animal antisera, proteins, and peptides, which are inherently immunogenic (12–14), how most intrinsically nonimmunogenic small organic molecules cause allergic reactions remains a mystery.

This review deals specifically with the allergic reactions produced by the latter group of compounds, which represents the majority of drugs in therapeutic use today (3, 7, 15–17). First, we discuss some general immunological concepts that must be considered when studying drug hypersensitivities. Next, we consider specific examples of drug allergies and the methods that have been used to detect specific sensitizations. Third, we illustrate how current methodologies of drug metabolism and immunochemistry have been applied to study a specific drug hypersensitivity. The final section summarizes and discusses the future of research on hypersensitivity to drugs.

GENERAL IMMUNOLOGICAL CONCEPTS RELEVANT TO THE STUDY OF DRUG HYPERSENSITIVITIES

Generation of Drug-Induced Immune Response

The complexity of the immune system has slowed progress in our understanding of the molecular basis of drug hypersensitivities. During the last few years, however, many of the enigmas of this multicellular and highly regulated system have been clarified to the point where investigators can begin to make rational mechanistic proposals for the generation and regulation of drug-induced hypersensitivities. Some of these recent basic immunological findings are briefly addressed here. But it should be pointed out that virtually everything known about the function of the immune system has been derived from model studies with very defined molecules that are intrinsically immunogenic rather than from investigations in which drugs have been administered to animals or humans. Hence, most of the assertions made in this review concerning drugs and the immune system are extrapolations from these model studies.

In general, in order for an organic molecule to be recognized as nonself and to induce an immune response, it must have a minimum molecular weight of

approximately 1000 daltons (3, 15). Since most drugs are smaller than this, it is generally assumed, based upon model studies with nonimmunogenic small organic molecules, that they must become covalently bound to an endogenous carrier macromolecule to form a drug-carrier conjugate (3, 7, 15–17) before they can interact with the immune system. A recent report has suggested but not proved that there may be exceptions to this rule (18).

Drugs may form drug-carrier conjugates by means of three different general mechanisms. If the drug or its decomposition products, which may be formed in the manufacturing of the compound (19), are chemically reactive, they can react directly with tissue macromolecules. The drugs penicillin, captopril, penicillamine, and such industrial chemicals as the phthalic anhydrides, diisocyanates, diisothiocyanates, formaldehyde, and ethylene oxide are examples of this category (3, 15, 16, 20–25). Most drugs that cause hypersensitivity reactions, however, are not intrinsically reactive and therefore must be activated into reactive species within the body. The most common way this occurs is through biotransformation (3, 7, 15–17). Although it is fairly well established how this would probably happen for most drugs that cause hypersensitivities (2, 16), only in the case of halothane hepatitis, discussed in detail later, have the identity of the bound metabolite and characterization of the endogenous carrier molecules been defined. Photoactivation in the skin is another way that drugs might be activated into reactive species that can form drug-carrier conjugates (26–28). The structures of the covalently bound moieties involved in photoallergies are as ill-defined as those due to metabolic activation.

The distinction between an immunogen and an antigen is important for our discussion (29). An immunogen is a substance that can elicit a specific immune response, whereas *antigen* refers to a substance that is recognized by the product of a previous immune response. For example, a hapten is defined as a small molecule, such as a drug, that is not immunogenic alone, but that may elicit the formation of antibodies able to bind to the free hapten when covalently bound to a carrier macromolecule. In this case, the free hapten is antigenic without being immunogenic. In contrast, any compound that is immunogenic is always antigenic.

Once a drug-carrier conjugate is formed, it may act as an immunogen and elicit a specific humoral (antibody) response, a specific cellular (T lymphocyte) response, or both responses (3, 15). Model immunization studies with small nonimmunogenic organic molecules covalently attached to self (autologous) proteins suggest that immune responses could be elicited against three general classes of antigenic determinants (epitopes) of the drug-carrier conjugate (30, 31). First, these can include the bound derivative of the drug (haptenic epitopes), or at least portions of it. Second, they can represent novel epitopes of the carrier molecule (also known as new antigenic determinants or

NAD) that result from the covalent binding of the hapten to the carrier; these could represent new structures corresponding to that region of the carrier bound directly to the hapten and may include in some cases a portion of the hapten as a part of the epitope. Alternatively, the NAD may consist of conformational epitopes created by the binding of the hapten to the carrier. Third, the immune response may be elicited against native carrier epitopes (autoantigenic determinants), ordinarily seen as self, that as a consequence of hapten binding are able to bypass self tolerance and induce an immune response (30–33).

Investigations with synthetic hapten-carrier conjugates have additionally shown that when the conjugate contains more than 20 hapten residues per carrier molecule, immune responses may be elicited exclusively against haptenic epitopes (30). Recent studies have indicated that such high levels of hapten density are probably not reached after the administration of most drugs that cause hypersensitivities (16). If exceptions to this finding do occur, the drug-carrier conjugates with high hapten density are probably formed near the site of administration of a drug that is intrinsically reactive or at a subcellular region where a highly reactive metabolite of the drug is formed. Consequently, the immune responses elicited by most drug-carrier conjugates would probably not be directed solely against the bound drug, but instead against epitopes consisting of the bound drug, NAD, native carrier, or a mixture of these antigenic determinants.

Model studies have also established that the carrier molecule should play a crucial role in the overall expression of the immune response against a drug-carrier conjugate (16, 30, 34, 35). How the carrier molecule effects this function at the molecular level, at least in part, has been discovered recently. Since these findings may prove to be of fundamental importance to future drug allergy research, we summarize them in the following discussion of the induction of specific B lymphocyte (antibody) and specific T lymphocyte (cellular) responses by a hypothetical drug-carrier conjugate.

Specific B Lymphocyte Response

Each B lymphocyte contains on its surface unique immunoglobulin molecules, with antigenic specificity, which serve as receptors for immunogens (36). When an epitope of a drug-carrier conjugate binds to these surface receptors, the B cell is stimulated to divide and differentiate either into a clone of effector plasma cells that secrete antibody molecules with a specificity identical to that of the surface receptor antibodies, or into specific memory cells (37). If an appropriate repertoire of B cells exists, a polyclonal antibody response may be directed against other epitopes of the drug-carrier conjugate. It has been known for many years that in the case of most immunogens, the immune response elicited by the interaction of an immunogen with B cells

alone is usually weak. In order for a strong response to occur, the activated B cells must be further stimulated by specific helper T lymphocyte (T_H) accessory cells (35, 38), and the specific T_H cells must themselves be activated by interaction with the immunogen. The epitope recognized by the T_H cells, however, is distinct from the epitope of the immunogen that stimulated the specific B cells. The process is even more complex because the receptors on the surface of the T_H cells that interact with the immunogen do not recognize the epitope on the immunogen directly (39–48). Instead, they can only bind to the immunogen when it is complexed to a specific class of cell surface glycoproteins found on macrophages, Langerhans cells of the skin, B cells, activated T cells, and certain cells of other tissues—these accessory cells are collectively termed antigen-presenting cells (APC).

The cell surface glycoproteins involved are encoded by genes of the major histocompatibility complex (MHC) and are termed class 2 molecules. In mice, the MHC is referred as H-2 and is located on chromosome 17, whereas in humans the gene cluster is called HLA and is found on chromosome 6. The class 2 molecules of the APC may bind to the immunogen (drug-carrier conjugate) either in its native state or after it has been internalized and processed to an unfolded state or a hydrolyzed form. Which state of the immunogen is actually presented on the surface of the APC and is recognized by specific T_H cells is determined by the relative binding affinities of the various forms of the immunogen for the class 2 molecules.

The involvement of class 2 molecules in the interaction of T lymphocytes with immunogen effectively means that class 2 molecules restrict which epitopes of an immunogen can be presented to the repertoire of T_H cells for their activation and for subsequent B cell stimulation. This sequence of events explains, at least in part, why the immunogenic potential of a drug-carrier conjugate is likely to be highly dependent upon the structure of the carrier molecule (16, 30, 34, 35). Moreover, since the MHC is highly polymorphic, and each locus has several alleles, every individual will have a different complement of the MHC genes.

Specific T Lymphocyte Response

Two general categories of T lymphocyte cells, the delayed hypersensitivity class (T_{DH}) and the cytotoxic class (T_C), may be activated into specific effector cells when their epitope specific cell surface receptors bind drug-carrier conjugates (36, 49, 50). Both cell types, however, only bind and become activated by an immunogen when it is presented to them either in association with class 2 molecules by an APC (50, 51) or in association with another specific class of cell surface glycoproteins (52–55). These other glycoproteins, present on surfaces of all nucleated cells of the body, are known as class 1 molecules; they are also encoded by the MHC genes and are

highly polymorphic. The class 1 proteins, like the class 2 molecules, may restrict which hapten-carrier conjugates become immunogenic, since not all processed hapten-carrier conjugates would be expected to bind effectively to a particular structural variant of class I molecules. In addition, T_C cell activity may be regulated by specific T_H cells (56). Therefore, specific T cell responses, like B cell responses, are determined by multiple factors, which can include, at least in part, the repertoire of specific T_{DH} and T_C cells, the MHC haplotype, and the structure of carrier component of the drug-carrier conjugate.

Other Regulatory Processes

Many other immune regulatory processes and risk factors may also influence the sensitization potential of a drug. A complete discussion of these factors is beyond the scope of this review, and they have been discussed elsewhere. For our purposes it suffices to say that they include idiotypic regulation (57); other regulatory cell types, such as suppressor T lymphocytes (58, 59) and suppressor-inducer T lymphocytes (59–61); cytokines (such as the interleukins and interferons) (62); and other influences, among which are age, sex, and the physiological status of the patient, as well as drug dosage and duration and route of administration (3).

Allergic Mechanisms of Cytotoxicity

Once a drug-carrier conjugate has induced the formation of specific antibodies or specific sensitized T lymphocytes, their subsequent interaction with the drug-carrier conjugate or even the drug and its stable metabolites may, depending upon the specificity of the immune response, cause tissue damage through four general immunological mechanisms of hypersensitivity (63). In Type I hypersensitivity, both mast cells, which line blood vessels of most tissues, and blood basophils bind molecules of IgE and to a lesser extent IgG_4 (in humans) via their Fc receptors (63, 64). When adjacently bound immunoglobulin molecules become cross-linked by binding to specific antigens, the mast cells degranulate and release pharmacological mediators, such as histamine, prostaglandins, leukotrienes, and neutrophil and eosinophil chemoattractants. These mediators cause vasodilation, increased capillary permeability, bronchoconstriction, and inflammation. Drug-induced urticaria, asthma, and anaphylaxis may arise through this immunological pathway. Since the cross-linking of surface bound immunoglobulins is required to produce this type of hypersensitivity, it should only be observed with those drugs that form multivalent drug-carrier conjugates (3, 16) or are inherently divalent because they have identical structural features in different regions of the molecule, as in the case of several of the muscle relaxant drugs and the antiseptic chlorhexidine (Table 1).

Table 1 Drug toxicities in which specific humoral and cellular immunity have been implicated

Drug	Toxicity[a]	Immunity		References
		Humoral	Cellular	
Acetaminophen	T	+		131
Salicylates	A,As,U	+		79,80
Muscle Relaxants:	A	+		84,86,87
Alcuronium, Gallamine, Decamethonium, Succinylcholine, Tubocurarine				
Thiopentone	A	+		85
Floxacillin	H		+	102
Erythromycin	H		+	103
Diphenylhydantoin	H,SS	+		81
Chlorpropamide	HA	+		94
Allopurinol	D,U		+	104
Practolol	O	+		109
Amoxicillin	HA	+		88
Penicillins	As,SS,D,U,A	+	+	21,91,105
Ethinyloestradiol	Th	+		132
Alprenolol	D		+	106
Mianserin	T,N	+		99
Cyclophosphamide	A,U	+		92,93
Cloxacillin	H	+		108
Tolbutamide	HA	+		96
Captopril	U,D,Ne	+	+	90,107
Cephamandole	T	+		100
Quinine, Quinidine	T	+		133–135
Nomifensine	HA	+		18,98
Methyldopa	HA,N,H	+		18,101,136
Cianidanol	HA	+		18
Amodiaquin	N	+		137
Probenecid	HA	+		97
Penicillamine	N	+		82
Chlorhexidine	A	+		89
Tienilic acid	H	+		110
Procainamide	DLE	+		9,11
Hydralazine	DLE	+		9
Halothane	H	+	+	See below

[a]A, anaphylaxis; As, asthma; D, dermatitis; H, hepatitis; HA, hemolytic anemia; Ne, nephritis; N, neutropenia; O, oculomucocutaneous syndrome; SS, serum sickness; T, thrombocytopenia; Th, thrombosis; U, urticaria; DLE, drug-induced lupus erythematosus.

Type II hypersensitivity is initiated when antibodies bind to specific tissue antigens (65). This may be followed by cellular or complement effector cytotoxic processes. For example, certain cells that have Fc receptors, such as killer (K) cells and certain macrophages (49, 65–67), bind to the Fc portion of the bound antibodies and as a consequence are activated and lyse the target cell. This process is known as antibody-dependent cell cytotoxicity (ADCC). Similarly, the complement system is activated when it is cross-linked through

its binding to the Fc portion of two molecules of IgG (isotypes IgG1, IgG2 and IgG3 in humans) or to one molecule of bound IgM, either of which is bound to the surface of a cell (68, 69). This may result not only in the direct lysis of the cell, but also in secondary processes mediated by various complement factors. One mediator is C3b, which can covalently bind to the surface of cells. Phagocytic cells, such as macrophages and neutrophils, which have both C3b and Fc receptors, can attach to the surface of cells containing bound IgG and C3b molecules and cause cellular damage either by phagocytosis or exocytosis. Another important complement factor is C5a. It can cause a localized inflammatory response and subsequent nonspecific cellular damage by increasing vascular permeability, neutrophil chemotaxis and activation, and mast cell degranulation. These Type II processes could account for a variety of drug allergies involving cytotoxicity to specific organs and cells of the hematological system.

Immune complex disease is the Type III group of hypersensitivity reactions (70). Immune complexes can be formed whenever antibodies meet a multivalent antigen (3, 71, 72). They may be formed at discrete locations where antigen is produced and released from cells, or produced in the blood, where they are generally removed effectively by cells of the reticuloendothelial system. If the immune complexes become deposited in tissues, or onto surfaces of cells, they may bind and activate complement, leading to cell lysis directly or indirectly by the inflammatory reactions outlined for Type II allergies. Drug induced serum-sickness–like syndrome, characterized by urticaria, fever, arthritis, glomerulonephritis, and vasculitis, as well as lupus-erythematosus–like syndrome and other cytotoxicities could be produced by this immunological mechanism.

Type IV allergy (73), unlike the other forms of drug hypersensitivity, is a consequence of the interaction of antigens with specific lymphocytes and not with specific antibodies (36, 49, 50, 74, 75). The specific effector lymphocytes, which appear to be T_C and T_{DH} cells, cause tissue damage, in general, through two different mechanisms. For example, the T_C cells can kill cells directly by lysis, when they are activated after binding antigen in association with MHC class 1 or class 2 molecules on the surface of target cells (49, 74). In contrast, the T_{DH} cells do not directly kill cells when they are presented antigen by APC. They instead cause damage indirectly through the release of various factors including lymphokines, which cause a localized inflammatory response with an influx and activation of mononuclear phagocytic cells (36, 50, 75). Drug-induced dermatitis and other organ-specific cytotoxicities could be mediated by either one or both of these mechanisms.

Clearly, the type of hypersensitivity reaction seen with a drug that must be metabolized to produce drug-carrier conjugates should be dependent on the

location of its metabolism and on the chemical properties of its reactive metabolites. If the drug is predominantly activated by metabolism within a specific organ, and if its metabolites are highly reactive and short-lived (76), the formation of drug-carrier conjugates and subsequent allergic reactions should be quite organ specific, as appears to be the case in halothane hepatitis (see discussion of halothane hepatitis below). How the immune system would come into contact with such immunogens to produce initial sensitization and subsequent cellular damage is not known, but such contact could occur via several possible pathways: (*a*) their formation directly in the plasma membrane; (*b*) their translocation to the plasma membrane from other subcellular regions of the cell during normal membrane processing and MHC class 1 and class 2 processing and presenting pathways; or (*c*) their release from cells that have been killed by the intrinsic toxic effects of the drug (54, 55, 77). In contrast, if the drug is metabolized into highly reactive metabolites in more than one organ, or into metabolites that are reactive but long-lived and therefore able to escape from the site of metabolism (76), multiple organ damage or systemic reactions might be presented, such as those produced by Type I and Type III hypersensitivities.

DRUG HYPERSENSITIVITIES

Clinical Criteria

The diagnosis of an allergic reaction to a drug relies on several clinical criteria (3). For example, the reaction usually occurs only after repeated exposures or at least 8–9 days after the first exposure, which suggests that a period of sensitization is required. Further, the reaction often appears to be dose independent and frequently is accompanied by blood and tissue eosinophilia and fever. If the patient is rechallenged with the drug, the same pathological condition should be observed. In most cases, the symptoms usually subside promptly when treatment with the drug is discontinued, unless a drug-induced autoimmune reaction has been initiated. The most convincing evidence of a drug allergy is demonstration that specific antibodies or sensitized T lymphocytes are reacting with the drug or its metabolites (Table 1). In most cases, however, this last criterion has not been satisfied, either because the clinical tests were not performed, or because they were found to be negative.

Finally, definitive proof that a toxicity has an allergic basis requires that it be produced in animals, where the immunological mechanisms of cellular damage can be thoroughly studied, since not all drug-induced antibodies or sensitized T lymphocytes will necessarily cause tissue damage (77). This criterion, to the best of our knowledge, has not yet been met for any drug that must be metabolically activated to elicit an immune response.

Methods for Detecting Specific Antibody and Specific T Lymphocyte Responses

SPECIFIC ANTIBODY RESPONSES One of the earliest procedures developed for detecting drug-related antibodies is based upon the passive hemagglutination reaction (PHA). In general, drugs are first covalently bonded to an exogenous carrier protein, such as bovine serum albumin, and this bonding is followed by the irreversible attachment of the drug-carrier conjugate to the surface of rabbit red blood cells (RBC). Serum samples from the patients are then added to the sensitized RBC and hemagglutination titers are determined (78–82). Recent, more sensitive modifications of this procedure involve either the initial adsorption of a drug-protein carrier complex onto a solid support or the direct covalent attachment of the drug or one of its derivatives to an activated solid support, such as epoxy-activated Sepharose 6B or cyanogen bromide activated paper discs. The serum samples are added to the bound drug-carriers, followed by anti-human antibodies that have either a covalently bound iodine-125 radioisotope for the radioallergosorbent test (RAST) (23, 83–89), or a covalently bound enzyme, usually alkaline phosphatase or horseradish peroxidase for the enzyme-linked immunosorbent assay (ELISA) (90, 91). The RAST and ELISA procedures can be used to determine the isotype (IgG, IgA, IgM, IgD, or IgE) of the specific human antibodies by using isotype specific secondary anti-human antibodies. Evidence for the presence of drug-related IgE antibodies has also been obtained through use of less specific procedures, which include intradermal skin testing (92) and passive cutaneous anaphylaxis (89, 93).

Typical procedures for detecting drug-related antibodies associated with hemolytic anemia, thrombocytopenia, and neutropenia involve incubating normal RBC, platelets, or granulocytes with drug and serum samples. In the case of RBC, the end point is either hemagglutination or lysis after the addition of complement (18, 88, 94–98). The end point with platelets or neutrophils is often the binding of a secondary anti-human antibody containing a covalently bound fluorescent label (99–101).

SPECIFIC T LYMPHOCYTE RESPONSES The most common way that drug-related specific T lymphocyte responses have been detected is by means of the lymphocyte transformation test (102–107). This is performed by incubating lymphocytes of patients in the presence of drug and tritiated thymidine. When sensitized lymphocytes are cultured in the presence of appropriate antigen, they respond by undergoing transformation into blast cells, and this change is accompanied by DNA synthesis and cell division. The uptake of tritiated thymidine is used as a measure of this transformation process. Positive lymphocyte transformation tests are usually regarded as evidence for the existence of specific effector (T_C or T_{DH}) T cell activity. This, however, may

not be correct because other subsets of T lymphocytes, such as helper, suppressor-inducer, or suppressor T cells, may actually be responding in the assay. In fact, in the only study that was found where subsets were determined, the major group of activated lymphocytes measured were in fact suppressor-inducer T lymphocytes (105). Another test that has been used to measure the presence of drug-related T cell activity is the macrophage migration inhibition test (108). In this test, normal macrophages are cultured in the presence of test lymphocytes from patients and the drug. Sensitized lymphocytes, particularly T_{DH} cells, produce a lymphokine, which inhibits the normal migration of macrophages.

METHODOLOGICAL PROBLEMS The major deficiency with most of the specific antibody and specific T cell tests that have been used in drug hypersensitivity studies is their reliance on antigens which are either the parent drug or a derivative that is bound either to a pure protein or a synthetic solid support. Consequently, an immune response directed against a covalently bound metabolite, which has a structural feature not represented in the antigens used in the assay, might not be detected. In an attempt to circumvent this potential problem, some investigators have incorporated either known metabolites or serum and urine samples, containing mixtures of metabolites, as a source of antigen in their assays (18, 98, 102–104). Although these approaches have met with some success, they still would miss immune responses directed against epitopes that consist of the bound metabolite and portions of the carrier molecule, NAD of the carrier, or the native carrier molecule.

One approach to detecting immune responses directed against covalently bound metabolites was employed in the study of the beta-blocking agent practolol (109). In this case, practolol was incubated with hamster liver microsomes in the presence of I-125 labeled human serum albumin (HSA). The reactive metabolites generated then bound covalently to the HSA, and the resulting complex was used as a source of haptenic antigen in an antibody assay. More general procedures that can be used to detect antibody responses against epitopes of the entire drug-carrier conjugate molecule are ELISA and electrophoretic immunoblotting procedures utilizing tissues from humans or animals treated with the drug of interest, or immunoprecipitation methods involving the culturing of radiolabelled cells in the presence of the pertinent drug, followed by immunoprecipitation with human antibodies and protein-A, SDS/PAGE electrophoresis and autoradiography. Employing these methods, antibodies in the serum of patients exposed to tienilic acid (110) and procainamide (11) have been found to be directed on the one hand against native epitopes of liver microsomal cytochromes P-450, and on the other hand against histones, ribosomal RNA, and a 40 kd protein. Whether or not the

production of these autoantibodies is initiated by a loss of tolerance arising from the covalent binding of the given drug to these macromolecules (30–33) remains to be determined.

Only in the case of halothane, however, have carrier molecules of drug-carrier conjugates been identified. Indeed, studies with halothane illustrate how a blend of modern drug metabolism and immunochemical techniques have been applied to investigate the mechanism of a drug hypersensitivity.

Halothane Hepatitis

Immunochemical studies have demonstrated that sera from the majority of halothane hepatitis patients contain antibodies of the IgG isotype that react with halothane-carrier conjugates expressed in the livers of animals or humans treated with halothane (for reviews of the immunological basis of halothane hepatitis see references 77, 111–113). The antibodies are thought to play a role in the pathogenesis of this drug reaction inasmuch as they mediate ADCC killing of hepatocytes from halothane treated rabbits in vitro and are not detectable in sera from normal individuals, from patients exposed to halothane without sustaining liver damage, or from patients with hepatitis due to viral infection or to intrinsic hepatotoxic compounds such as acetaminophen.

The halothane-induced neoantigens were first observed by indirect immunofluorescence on the surface of hepatocytes from rabbits exposed to halothane. The neoantigens were later found to be concentrated in the microsomal fraction of the cell by an ELISA method, in which subcellular fractions of rabbit liver from halothane treated rabbits were first adsorbed onto the wells of microtiter plates, followed by the sequential additions of human sera and horseradish peroxidase conjugated anti-human IgG. Further characterization of the neoantigens by immunoblotting with sera from several halothane hepatitis patients has revealed that they correspond to five polypeptides (100 kd, 76kd, 59kd, 57kd, and 54kd) that are expressed predominantly in the microsomal fraction of the liver. Although the same neoantigens are recognized by a given sample of serum, whether immunoblotting is done with liver microsomes from rabbits (114), rats (115) or humans (116), the sera do differ in patterns of antibody specificity; the 100 kd and 76kd neoantigens are the ones most commonly recognized (Table 2). Typical immunoblots using sera from two patients with halothane hepatitis and liver microsomes from rabbits and rats treated with halothane are shown in Figure 1.

IDENTIFICATION OF THE HALOTHANE-INDUCED NEOANTIGENS AS TRIFLUOROACETYL (TFA)-CARRIER PROTEINS Two reactive intermediates are produced by cytochromes P-450 mediated metabolism of halothane ($CF_3CHClBr$) in liver microsomes; they are an oxidative TFA-halide

(CF_3COX), and a reductive 1-chloro-2,2,2-trifluoroethyl radical. Since both of these products can covalently bind to microsomal proteins, either one or both might potentially be responsible for the formation of halothane-carrier conjugates. Nevertheless, TFA-halide has recently been found to be the sole source of the halothane-carrier conjugates in liver microsomes from halothane treated rats (115). For example, each of the halothane-induced neoantigens recognized by immunoblotting with the patient's antibodies (Table 2; Figure 1) also appeared to contain bound TFA groups, since the proteins reacted with a specific anti-TFA antibody (117, 118). This finding was confirmed by observations that generation of the neoantigens, as measured by immunoblotting with the human sera and anti-TFA antibodies, was greatly reduced in liver microsomes from rats treated with deuterated halothane, as compared with halothane (117, 118). Further, the hapten derivative N-epsilon-TFA-L-lysine partially blocked the binding of the human antibodies to the neoantigens, but nearly abolished the binding of the anti-TFA antibodies. 1M piperidine treatment of the rat microsomes, which selectively removes TFA groups from proteins (119), abolished most of the interactions of patients' antibodies and anti-TFA with the microsomal neoantigens, but caused negligible protein degradation or aggregation. This finding established conclusively that the TFA hapten was responsible for the generation of the halothane-carrier conjugates that were recognized by the human antibodies. Moreover, it is clear that the patients' antibodies do not recognize the bound TFA hapten alone, but rather epitopes that consist of the TFA hapten and portions of the specific carrier proteins. This was so because the inhibition of binding of the human antibodies to the halothane-carrier conjugates by the hapten derivative

Table 2 Halothane-induced neoantigens recognized in rat liver microsomes by immunoblotting with sera from halothane hepatitis patients[a]

Antigens recognized (kd)	Number of sera
100	12
76	3
57	3
100+76	18
100+57	3
100+76+59+54	2

[a]Rats were administered halothane, and after 12 hr, liver microsomes were prepared. Immunoblotting with human sera was performed as outlined in Figure 1. Of 68 sera tested, 41 (60%) contained antibodies to halothane-induced neoantigens, which were defined as antigens expressed only in microsomes from halothane treated animals. Data taken with permission from Ref. 115.

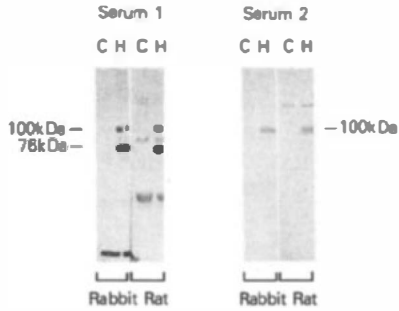

Figure 1 Detection of halothane-induced neoantigens in rabbit and rat microsomes by immunoblotting with sera from halothane hepatitis patients. Rabbits or rats were administered halothane, and after approximately 18 hr, liver microsomes were prepared. Constituent polypeptides were resolved by SDS-PAGE and transferred electrophoretically to nitrocellulose. The blots were developed by incubating them sequentially with patient's serum, horseradish peroxidase conjugated goat anti-human IgG, and 4-chloro-1-naphthol substrate reaction mixture. The experimental procedures have been described in detail elsewhere (114, 118). C and H represent microsomes from untreated and halothane treated animals respectively.

N-epsilon-TFA-lysine was weak, even at very high concentrations, and patients' sera differed in patterns of polypeptide neoantigen recognition (Table 2; Figure 1).

The results of these studies further suggest that similar TFA-carrier conjugates are the immunogens responsible for the induction of the halothane-induced antibodies and possibly even hepatitis. These possibilities will be tested in the future after the TFA-protein carrier conjugates have been purified, perhaps by anti-TFA affinity chromatography (120). Animals will be immunized with the TFA-proteins, prior to the administration of halothane, in an attempt to develop an immunological animal model of the hepatotoxicity (77, 121, 122).

The pure neoantigens might also be used to develop convenient immunoassays for the detection of sensitized individuals who, if exposed to the drug, would be at risk of developing hepatitis. In this regard, it has been suggested that hepatitis due to the inhalation of anesthetic enflurane (CHF_2OCF_2CHFCl) might be due at least in some cases to a cross-sensitization to halothane (123, 124). This possibility was recently tested, and it has been found by ELISA and immunoblotting techniques that microsomal acyl adducts of enflurane (CHF_2OCF_2CO-) not only cross-react with anti-TFA antibodies (125) but also with sera from the halothane hepatitis patients (D. D. Christ, J. G. Kenna, W. Kammerer, H. Satoh, and L. R. Pohl, manuscript submitted for publication).

Approaches and methods similar to those used in the study of halothane hepatotoxicity should be of general utility for the identification of hapten-carrier conjugates that may mediate other drug hypersensitivities.

CONCLUSIONS

Current evidence indicates that drug hypersensitivity reactions arise as a consequence of the covalent binding of drugs, their metabolites, or their degradation products to tissue carrier macromolecules, and that the type of toxicity produced by a specific drug is likely to be a function of the relative tissue distribution of its drug-carrier conjugates. An important feature of this group of toxicities, which makes them difficult to study in both humans and animals, is their relatively low frequency of occurrence. This is probably a consequence of the complex genetic basis of these toxicities, involving numerous processes that are independently regulated. For example, an individual must first have the correct types and adequate levels of cytochromes P-450 or other activating enzymes in order to metabolize a drug into its reactive metabolites (126). Once the metabolites bind to and alter cellular proteins or other macromolecules, hydrolytic enzymes may catabolize the drug-carrier conjugates before they can activate the immune system. In this regard, some drug-carrier conjugates may be less susceptible to catabolism than others and as a consequence be more immunogenic.

The most important factor contributing to the complex genetic basis of drug hypersensitivities, however, is probably the immune system, which consists of a highly regulated network of multicellular components. In this review, the importance of MHC antigens has been especially emphasized because recent discoveries have indicated the essential role they are likely to play in determining the immunogenicity of drug-carrier conjugates. Reports of associations between certain MHC haplotypes and autoimmune diseases (127) or even drug allergies (10, 128) further support the probable importance of this contributing factor. Another potentially important factor, which could have a profound influence on the pathogenic effects of an immune response elicited against drug-carrier conjugates, is the regulation of antibody isotype expression (129), inasmuch as different isotypes have different complement fixing activities (68, 69) and apparently different activities in ADCC responses (130). Other relevant modulators of the immune system, probably important in determining whether or not a drug causes a hypersensitivity reaction, are the suppressor T lymphocytes, suppressor-inducer T lymphocytes, idiotypic interactions, and cytokines.

Even with our present state of knowledge, major advances in drug hypersensitivity research should be possible in the near future. In particular, the identification and characterization of drug-carrier conjugates involved in some drug hypersensitivities is now technically possible, as outlined in the studies of halothane hepatitis. Once they have been purified, the drug-carrier conjugates can serve as antigens for identifying sensitized individuals who have experienced a hypersensitivity reaction to the drug. This information

could be used to prevent not only another allergic reaction to the drug, but possibly also cross-reactions to other drugs that are structurally related. The purified drug-carrier conjugates can also be used to immunize animals prior to the administration of challenging doses of the drug, in an attempt to develop an animal model of the hypersensitivity. Once animal models become available, we can start to understand the detailed cellular and molecular processes of drug allergies for the first time.

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