

Current Methods in the Characterization of Immune Mechanisms Involved in the Elicitation of Drug Hypersensitivity Reactions.

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Abstract

Adverse drug reactions (ADRs) remain a major health problem worldwide. Incidences of ADRs in the developing world have been documented by various studies, and this highlights the need for greater understanding of the molecular basis underlying these reactions in individuals with identified drug hypersensitivity. We review the general theories of immune stimulation by small molecules and the molecular basis of immune involvement in the manifestation and pathophysiology of ADRs. We then describe the methods used in the evaluation of drug hypersensitivity and in the identification of specific antigens (immunogens) which could be implicated in the pathogenesis of ADRs. This review also highlights the problems faced by researchers in resource deprived countries, and recommends important steps to solve these problems. We are of the opinion that a concerted effort should be made to develop the infrastructures needed in developing countries to encourage scientific research in this burgeoning field of immunopharmacology.

(149 words)

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Introduction

Edwards and Aronson in their definition of an adverse reaction tried to take into account the fact that while some unpleasant reactions may be mild in nature, errors in medication, contaminants and supposedly inactive excipients may be responsible for the adverse effects being observed. They thus defined ADRs as “an appreciably harmful or unpleasant reaction, resulting from an intervention related to the use of a medicinal product, which predicts hazard from future administration and warrants prevention or specific treatment, or alteration of the dosage regimen, or withdrawal of the product.”¹ The growth in the repertoire of pharmaceutical agents used in the treatment of disease has ensured that adverse drug reactions (ADRs) remain a major health issue, with recent studies in the UK indicating that ADRs are a common clinical problem accounting for 2 - 6% of all hospital admissions.² ADRs have been found to affect the skin, liver, blood, kidney and lungs³⁻⁵ and have been shown to

contribute significantly to an increase in healthcare costs due to an extended length of hospital stay and the requirement of additional clinical investigations in more serious cases.^{2, 6, 7} In other cases the outright withdrawal of the offending agents from distribution and clinical use such as the selective serotonin reuptake inhibitor (SSRI) Zeldin withdrawn from clinical use in 1983 due to adverse neurological effects⁸, and the appetite suppressant Fenfluramine and phentermine withdrawn by regulatory agencies in 1997 due to adverse cardiovascular effects,^{9, 10} led to huge financial losses for the pharmaceutical companies involved.¹¹

The clinical impact of ADRs has thus necessitated the development of ADR monitoring and reporting systems collectively referred to as pharmacovigilance. Defined by the world health organization, pharmacovigilance is ‘The science and activities relating to the detection, assessment, understanding and prevention of adverse effects or any other

drug related problem' (WHO). ADRs are a complex disease requiring a multidisciplinary approach to understand their pathomechanisms. Basic and clinical pharmacology, chemistry, immunology, clinical medicine, toxicology, epidemiology, and pharmacogenetics have all contributed to our understanding of the disease pathogenesis.

Epidemiology and risk factors

Adverse reactions to drugs occur frequently, and in many cases are difficult to avoid. Data has shown that a sizeable proportion of hospitalized patients experience a form of ADR, which also constitutes one of the most important reasons for hospital admission (2-6%) with a substantial increase in the elderly. Drug-related hospital admissions in individuals aged over 75 years old accounts for approximately 30% of all hospitalized patients.^{12, 13} Due to the resultant significant morbidity, mortality and socioeconomic impact of ADRs, several studies have been carried out to quantify the impact of ADRs. The incidence of ADRs in hospitalized patients has been estimated to range from 1.8% in a study conducted at the LDS Hospital in Salt Lake City, which identified 731 reactions among 36,653 hospitalized patients during an eighteen month period, 6.5% in the United Kingdom, to a maximum of 15.1% in a meta-analysis of thirty three studies conducted in the USA over a thirty year period, from 1966-1996.^{2, 14, 15} Epidemiologic data regarding the prevalence of drug allergies suggested a varied prevalence among different populations, which could be attributed primarily to the different criteria for inclusion and methodologies employed.¹⁶ The apparent lack of reliable data generally obtained from respondents by self-reporting from the general population prevents the direct comparison of the prevalence between hospitalized and ambulatory patients.^{17, 18} Several classes of drugs including the beta lactams, the non-steroidal anti-inflammatory agents, and anti-seizure drugs are associated with a high incidence of ADRs. β -lactam

antibiotics have been recognized as the most frequent cause of immunological reactions.¹⁹

Various factors may play a role in the development of adverse drug reactions, with a majority typically due to an extension of the expected pharmacological activities of these pharmaceuticals. While this may be the case in a large proportion of reactions, inter-patient pharmacokinetic and pharmacodynamic variability, pharmacological, immunological and genetic factors are also involved in the development of ADRs. These factors could be related to the patient age or sex, the drug employed, and environmental or social related issues, as well as disease related factors,²⁰ and genetic predisposition.^{21, 22}

Patients at the extremes of age have been shown to be predisposed to the development of certain forms of ADRs. Elderly patients that have multiple disease states are exposed to multiple drugs and thus are particularly susceptible to the development of ADRs. Infants and very young children are also at risk due to their low level of organ and tissue development such as immature renal tubular function, physiologic hypoalbuminemia, low body fat, and immature blood brain barrier in neonates below the age of 8 weeks.²³⁻²⁶ This leads to an inability to readily metabolise a host of drugs. Like in infants, with elderly patients the mechanism of drug absorption, distribution, metabolism, and elimination is less likely to be studied widely.^{13, 27}

Gender differences play a role in the action of many drugs and thus affect the way the body deals with the drugs, altering drug pharmacokinetics and pharmacodynamics in the process. Sex differences have been implicated in the severity and frequency of ADRs to antiretroviral agents.²⁸ Moreover hepatic enzyme activity, specifically CYP3A4 leading to the enhanced metabolism of midazolam in Chinese women when compared to men,^{29, 30} and in the effect of colchicine which may selectively affect fertility in males but not in females³¹ are poignant examples.

Pregnancy, menopause and menstruation which are issues confined specifically to females could also have profound drug effects.³²

Alcohol consumption and smoking affects the metabolism of many drugs, and in turn are capable of altering susceptibility to ADRs. They activate enzymes that transform drugs, altering the predicted action of these agents hence producing effects capable of causing damage to the liver and other organs.^{20, 33}

There is evidence to suggest that ethnicity controlled by genetic factors exerts a substantial influence on drug response and action. In the evaluation of risk factors for ADRs in patients on angiotensin converting enzyme (ACE) inhibitors, ACE related angioedema was found to be more prevalent in African Americans when compared with other ethnic groups.³⁴ In another study the risk of cough with blood pressure lowering drugs was three times greater in black patients than non-black patients.³⁵ Recently, the human leukocyte antigen (HLA) genes have been identified as genomic markers of predisposition to several forms of ADRs; for example abacavir hypersensitivity is associated with HLA-B*57:01.³⁶ Further associations include allopurinol and HLA-B*58:01 in Hong Kong Han Chinese and Portuguese,³⁷⁻³⁹ carbamazepine and HLA-B*15:02,⁴⁰ or HLA-A*31:01 in the Han Chinese and European populations respectively.⁴¹⁻⁴³

The documentation of ADRs in the developing countries has been a mixed bag. It has been observed that risk factors for immune reconstitution syndrome (IRS), which leads to a worsening of the clinical status in HIV patients on Highly active anti-retroviral therapy (HAART) are higher in developing countries⁴⁴. Another study carried out over a period of 12 months, documented adverse drug reactions in 78.7% of patients on the HAART regimen in South Africa. This study implicated the combination therapy in 84.7% of the serious adverse reactions, with stavudine specifically implicated as the possible

causative agent in 93% of cases.⁴⁵ The fact that these, and majority of the drug hypersensitivity studies carried out in the developing world have been restricted to collection of data using questionnaires⁴⁶⁻⁴⁹ means that while comparison of epidemiological data will be possible, equally important molecular aspects of these reactions will not be unraveled. The importance of this cannot be understated considering the individual predispositions involved in these reactions especially as it concerns the more serious type B ADRs. The possibility therefore exists of serious hypersensitivity reactions escaping investigation due to issues of underreporting and the lack of the know how to interpret purely visible signs.

Therefore in addition to the simple questionnaires and already established clinical tests for the confirmation of suspected allergies which include skin tests, RAST, CAST (sulphido leukotriene release), mast cell tryptase and patch testing,⁵⁰ further *in-vitro* analysis tailored towards understanding the molecular basis of these reactions should be considered following the confirmation of drug hypersensitivity. These analyses could unravel individual idiosyncrasies and pharmacogenetic differences that could answer pertinent questions concerning ADRs that have remained unanswered.

Classification of ADRs

ADRs are usually classified into two types A and B based on their perceived dose dependence and predictability. These classifications are not all encompassing thus more categories have been developed. These include type C (continuing), type D (delayed use), and type E (end of use) reactions. In addition to those above, further classifications have been proposed to include a combination of dose and time related reactions associated with cumulative dose, time-related reactions alone, withdrawal-induced reactions and reactions attributable to the failure of drug therapy usually due to inadequate dosing and drug

interactions.^{1, 51}

i. **Type A ADRs:** are due to exaggerations in the drugs known pharmacological effect and are usually dose dependent, predictable and account for a majority of ADRs. Type A reactions make up approximately 85% of all ADRs and include reactions like GIT bleeding after treatment with NSAIDs, and interactions with other drugs and/or any underlying illnesses. Generally type A ADRs are dose-dependent and are related to the pharmacokinetic properties of the drug. A reduction in the dose or withdrawal of the offending drug usually leads to a resolution of the ADR.⁵²

ii. **Type B ADRs:** These reactions are usually idiosyncratic and unrelated to the drugs known pharmacology. They are less common and tend to be more serious than type A reactions, comprising about 10-15% of all ADR.⁵³ Type B reactions were previously thought to be unrelated to the dose and unpredictable as the mechanism was ill-defined. Many type B reactions provoke the host immune system as a part of

the disease profile. The involvement of drug-specific immunoglobulins, T-lymphocytes and other components of the adaptive immune system have been identified as important mediators in the pathogenesis of the ADRs.⁵⁴

Drug Hypersensitivity Reactions

Drug hypersensitivity reactions make up about one sixth of all ADRs.⁵⁵ These reactions have been earlier classified pathophysiologically by Gell and Coombs into four types namely; Type I, Type II, Type III, and Type IV. This classification did not take into account the large body of drug reactions that have now been defined clinically such as pseudo-allergic reactions, primarily antibody-mediated reactions and T cell mediated reactions which serve as a basis for more recently proposed classifications. The most recent classification utilizes the basic structure proposed by Gell and Coombs, and also goes further to include those that were excluded earlier^{56, 57}.

Theories of Cellular Immune Stimulation by Small Molecules

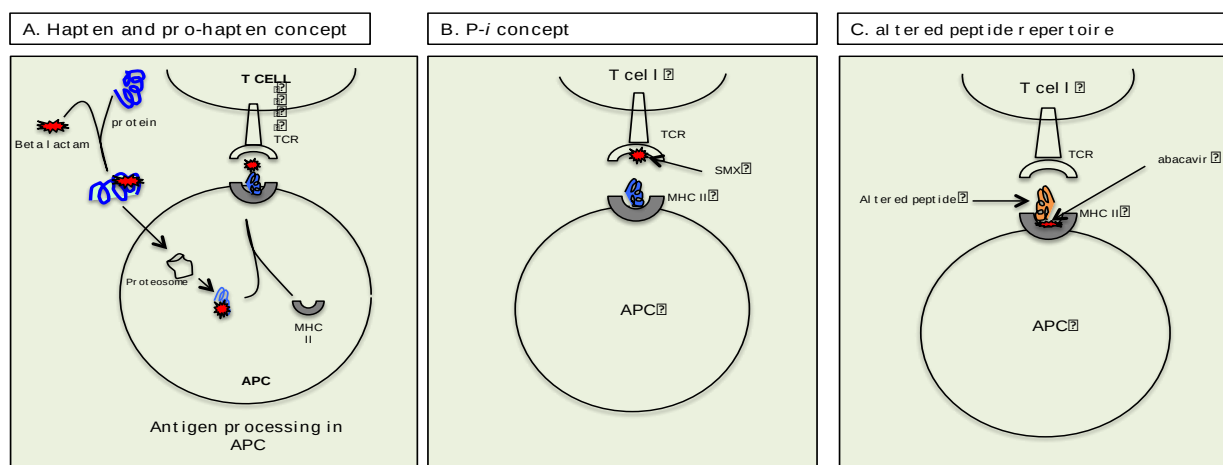


Figure 1. Some beta lactams and their derivatives bind to proteins which are processed internally by APCs and the products (peptides) bound to MHC molecules and presented to TCRs on the cell surface of the APC molecule (A) Some drugs may not require initial sensitization from initial exposure and may bind directly to some of the variable antigen specific T cell receptors (TCRs) (B) Some drugs like Abacavir may bind specifically to the antigen binding cleft of some HLA allotypes and thereby influence a change in the pool of presented self-peptides (C).

The recognition of conventional antigens by

T cells is made possible due to specialized

antigen presenting cells (APCs), such as dendritic cells, macrophages, and B lymphocytes, which capture extracellular protein antigens, internalize and process them, then display the processed antigen on the surface of major histocompatibility complex (MHC) molecules. CD4⁺ helper T lymphocytes are thought to recognize antigens in association with MHC class II molecules (MHC class II restricted recognition), and CD8⁺ cytotoxic lymphocytes (CTLs) recognize antigens in association with class I molecules (MHC class I restricted recognition). Dendritic cells are the most efficient APCs for initiating primary responses by activating naive T-cells. Macrophages and B lymphocytes present antigens to differentiated helper T-cells in the effector phase of cell-mediated immunity and in humoral immune responses, respectively. The mechanisms by which a chemical or drug acts as an antigen and elicits immune activation has not been fully elucidated.⁵⁸ Most pharmaceutical agents cannot bind directly to MHC molecules due to their low molecular weight and therefore cannot be directly presented to T cells by APCs⁵⁹, but drug-specific T-cells do exist. The hapten and pro-hapten concept, P-I concept, and the altered self-peptide repertoire (figure 1) are theories that have been proposed to try and explain how drugs can act as antigens for T cells.⁶⁰

Hapten Concept/Pro-Hapten Concept:

Haptens are low molecular weight compounds (MW < 1000 Daltons), that may only elicit an immune response when complexed covalently to a large carrier such as a protein. "Hapten" is a term coined by Landsteiner and Jacobs to explain both humoral and cellular immune responses to low molecular weight chemicals observed in their research⁶¹⁻⁶³. Most drugs being of low molecular weight were thought to be unable to stimulate immune responses due to their size, and thus undergo haptenation⁶⁴⁻⁶⁶ by binding to endogenous/self-proteins. This leads to modification of the proteins, and

confers on them a form of immune recognition. Chemical haptens or drugs also have the tendency to bind covalently to particular amino acid residues with some β -lactams and their derivatives as examples, binding covalently to lysine residues of human serum albumin.^{67, 68} The pro-hapten concept would require bio-activation/metabolism of drugs or chemical agents to intermediates that can react with endogenous constituents to initiate a chain of chemical and biochemical events which could lead to cellular damage. Nitroso sulfamethoxazole (SMX-NO), the protein reactive metabolite of sulfamethoxazole (SMX) has been found to selectively modify cysteine residues on proteins. The abiotic activation of drugs and chemical agents without the requirement of a particular enzymatic system is also possible. Following exposure to air, various non-reactive molecules such as abietic acid (a colophony resin), terpenes and p-phenylenediamine (PPD) are rapidly transformed into hapten reactive derivatives. Thus, in addition to the hapten and pro-hapten mechanisms of activation a further extension of the nomenclature was proposed. The term 'pre-hapten' was therefore coined to include nonreactive sensitizing chemicals that undergo activation as described above.^{69, 70}

Previous studies have also established a relationship between the nature and magnitude of the T-cell response and the degree of covalent hapten binding to proteins. In that study dinitrochlorobenzene (DNCB) and dinitrofluorobenzene (DNFB), chemicals that exhibited a Type 1 cytokine secretory profile were shown to bind selectively to cellular proteins while trimellitic anhydride⁷¹, fluorescein isothiocyanate³⁶ and dinitrobenzenesulfonyl chloride (DNBS-Cl) that exhibited type 2 secretory profiles bound to serum. Notably, the reactions were not associated with the chemical structures or known amino acid specificity.⁷²

The P-I Concept: The direct pharmacological interaction of drugs with

the immune receptors referred to as the P-i concept proposes that some drugs may bind non-covalently to some of the variable antigen specific T cell receptors (TCR's) and MHC molecules.⁷³ This concept tries to explain the induction of a primary immune response without the initial sensitization from previous exposure by the drug allergen. Though the exact mechanism of this TCR dependent T-cell activation by drugs has not been elucidated. Three ideal properties which a T cell must possess for activation to take place have been defined which include the expression of a TCR that may bind the drug, a low threshold of activation and an additional interaction of the TCR with the MHC on the APC which must occur to enhance the response of the drug.⁵⁸

Altered Self-Peptide Repertoire: MHC molecules are highly polymorphic proteins encoded by Human leukocyte antigens (HLAs) that initiate immunity by presenting pathogen-derived peptides to T cells. Abacavir and carbamazepine are two drugs that mediate significant immune hypersensitivity reactions that are strongly associated with the HLA alleles B*57:01 and B*15:02 respectively.^{74, 75} These reactions are not explained simply by either the hapten and pro-hapten or the P-i concepts of drug binding. What we now know is that abacavir and carbamazepine (to a lesser extent) are able to bind specifically to the HLA alleles, altering the shape and chemistry of the antigen-binding cleft, and are thus able to influence a change in the pool of presented self-peptides capable of

binding the HLA alleles B*57:01 and B*15:02.⁷⁴ It is this guided alteration of endogenous peptides that is assumed to be responsible for the significant incidence of hypersensitivity reactions in patients bearing the HLA risk alleles. More recently a similar association has been reported with acyclovir and HLA-B*57:01 but without the induction of hypersensitivity.⁷⁶

Humoral Involvement in Hypersensitivity Reactions

The immune system has been found to be involved in the development of ADRs, with effects ranging from those due to immunosuppression^{77, 78} to severe hypersensitive reactions discussed earlier in this text.

The characterisation of drug-specific CD4+ T-cell clones coupled with the identification of secreted cytokine products from these cells highlights their role in the elicitation of certain forms of hypersensitivity reactions.^{54,67,79,80} The identification of anti-drug antibodies,^{81,82} immunoglobulin subclasses and their secretions indicates that the humoral immune system contributes to the effector and regulatory immune process in the disease pathogenesis.^{83, 84}

Antidrug antibodies have been identified previously with studies from the 50's and 60's to the present day focussing on the detection and characterization, their hypersensitivity mechanisms, clinical implications and the development of improved detection techniques. Several studies are listed below (Table 1).

Table 1. Detection of antidrug antibodies (studies from 1961 – 2015).

Emphasis is on the detection and characterization, mechanisms, clinical implications and experimental detection of antidrug antibodies.

Study focus	Reference(s)
1. Detection and characterization	114, 115, 135
2. Mechanisms of hypersensitivity	136, 137
3. Clinical implications	138-140
4. Assay development for antidrug antibody detection	141-144

An increase in the numbers of B and T-cells coincides with the protection against re-infection with blood borne pathogens, but it is also dependent to a large extent on pre-existing titres of neutralizing antibodies. It has been hypothesized that antigen dependence via various cellular mechanisms, and the repetitive stimulation of B cells which may occur via a chronic low-grade infection or repeated exposure to external virus, bacteria or toxins is required for the differentiation of memory B cells to short-lived plasma cells with the ability to maintain high antibody titres.⁸⁵

Covalent binding in drug-specific activation

The covalent binding of drugs to proteins leads to modifications that result in the production of major and minor antigenic determinants. Major determinants include penicilloyl and nitroso metabolites from penicillin, and sulfamethoxazole respectively, of which multiple experimental data exists to show their interactions with the cellular adaptive system.^{64,65,86,87} The minor antigenic determinants on the other hand may contribute to the induction of IgE mediated allergic responses.⁸⁸ Mass spectrometry initially developed in the early 20th century has significantly aided the

qualitative and quantitative analysis of these peptides, proteins, reactive metabolites and conjugates. Plasma desorption, fast atom bombardment (FAB), and thermospray ionisation initial steps in the identification of proteins were faced with the disadvantages of using high concentrations of small proteins and the fact they didn't work well with larger proteins.⁸⁹⁻⁹⁴ Later development of matrix assisted laser desorption ionisation (MALDI) and electrospray ionisation (ESI) techniques with enhanced utility were the keys required to unlock the doors enabling the full characterization of these proteins and peptides⁹⁵⁻⁹⁷. Thus it was suggested that these methods possess the capability to define the role of covalent binding of drugs and other low molecular weight chemical compounds to proteins in immunogenicity.⁸⁸ Current applications have involved the use of Multidimensional liquid chromatography with ESI (MudPIT) to characterize cisplatin binding sites,⁹⁸ liquid chromatography-mass spectrometry (LC-MS) analysis to identify 2,4-dinitro-1-chlorobenzene covalent adducts in the skin and LC-MS/MS to detect the presence of acyl glucuronide metabolites in diclofenac patients.^{99,100} Other applications involved defining protein haptation mechanisms in contact and delayed-type drug hypersensitivity.¹⁰¹⁻¹⁰³

In-Vitro Methods for the Evaluation of an Immunogenic Response

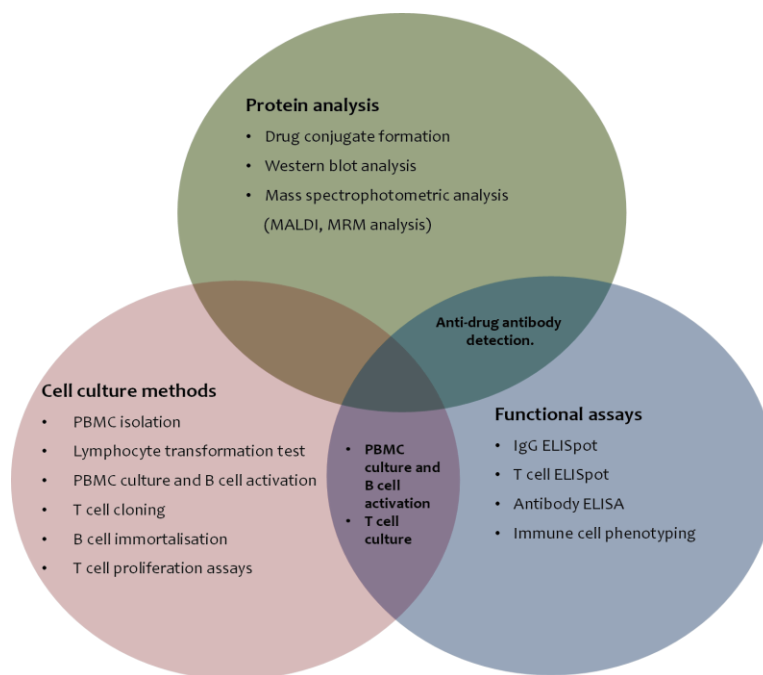


Figure 2. Interplay between currently available molecular tools to enable the full characterization to facilitate the understanding of adverse drug reactions.

Utilizing basic immunological principles, researchers have developed various methods that have been employed in the detection of the immune response in drug hypersensitivity. Hence the fact an infection gives rise to a significant increase in the number of lymphocytes specific for the target pathogen,¹⁰⁴ has led to the use of proliferation assays specifically the lymphocyte transformation test for the confirmation of hypersensitivity to chemical agent/drugs molecules/haptens. The generation of T-cell clones,^{54,105,106} the use of flow cytometry¹⁰⁷ and the application of the ELISpot and enzyme linked assays have all contributed to enhance our understanding of the molecular processes which underlie drug hypersensitivity.^{67, 108}

Evaluation of Lymphocyte Proliferation

During an infection there is a significant increase in the number of lymphocytes specific for the target pathogen.¹⁰⁴ This principle of an increase in proliferating lymphocytes as an indicator of an immune response has been applied to in-vitro studies. Proliferation is instituted in-vitro using already established methods in which cells are cultured in the presence of the test

antigen and proliferation determined using a variety of methods including those elucidated below.

Lymphocyte Transformation Test (LTT):

Isolated PBMCs are cultured in 96-well U-bottom plates under specified culture conditions for 5 days with tritiated thymidine [³H] added for the final 16 hr of incubation.^{54, 67, 106} At the end of the incubation period the plates are harvested on filter mats using a microplate harvester, and the extent of thymidine incorporation measured as counts per minute (cpm) using a beta counter (Perkin ELMER microbeta Trilux). Analysis of cell proliferation is denoted by the stimulation index (SI), of which any SI value ≥ 2 is accepted as a positive result.^{108,109}

$$\text{Stimulation index (SI)} = \frac{\text{Proliferation due to antigen}}{\text{Proliferation due to medium}}$$

While some authors were of the opinion that diagnostic tests are not helpful in the diagnosis of ADRs¹¹⁰, tests such as the lymphocyte transformation test have proven to be useful in the diagnosis and understanding of drug hypersensitivity

reactions.^{67, 106,111}

Flow cytometry: The use of carboxyfluorescein diacetate succinimidyl ester (CFSE) to measure lymphocyte proliferation by flow cytometry has become one of the most widely utilized assays for assessing lymphocyte responses.¹⁰⁷ CFSE flow cytometry methods are capable of identifying the individual cells that have undergone multiple rounds of cell division.¹¹² These studies demonstrated that the reduction in CFSE fluorescence was due to cell division. The use of CFSE proliferation in the tracking of lymphocytes is still relevant even in the face of recognized limitations with respect to the narrow concentration range of CFSE.¹¹³

Generation of clones as a tool for studying ADRs

T cell cloning has become an important tool in the study of the characterization of the immune response. Drug specific T lymphocytes have previously been characterized in carbamazepine, lamotrigine and phenindione^{54,66,105} where T cell clones were generated using specified protocols and tested for drug specificity, phenotype and functionality. The use of T cell clones in antigen proliferation assays is necessary to elucidate the nature of antigen-specific responses obtained.

Enzyme linked immunosorbent assays (ELISA) for the detection of Circulating drug specific-antibodies, cytokines and Antigen Secreting Cells

Alterations in levels of circulating antibodies and cytokines may be measured in serum, plasma and cell culture supernatants to determine their roles in observed hypersensitivity states. The ELISA is a sensitive and simple method for the determination of antibodies in which wells are coated with antigen and incubated with antisera, which can be detected by specific antibodies and substrates.^{114, 115} The ELISA is also used to detect the presence of

cytokines implicated in the onset of hypersensitivity reactions and mediators of inflammation such as interferon gamma (IFN- γ) and interleukin-6.¹¹⁶⁻¹¹⁸

The *in vitro* cell culture of PBMCs or cell lines incorporating co-incubation with immunogens or mitogens leads to the production and secretion of antibodies and cytokines. The number of cells expressing these antibodies and cytokines may be detected using an enzyme linked immunospot (ELISpot) assay.¹¹⁹⁻¹²¹ T lymphocytes via their specific cytokine secreted and also B lymphocytes may be determined in this manner. The ELISpot is presumed by some to be the gold standard for the enumeration of antigen specific B cells.^{122,123}

Identification of Anti-Drug Antibodies

The identification of drug-specific antibodies has been attempted using a modified form of a simple sandwich ELISA.^{82,115,124} Antigenic presentation of the immunogen (drug molecule) is made possible by employing the use of drug adducts prepared by the incubation of the drug with a protein and then precipitation with an appropriate alcoholic precipitant.¹²⁵ Flat bottomed 96 well microplates are pre-coated with modified and unmodified protein-drug adducts, and the difference in the levels of antibodies detected in serum. This is referred to as the amount of anti-drug antibody specific to the particular drug of interest. This may be confirmed by the pre-incubation of the samples with known quantities of the drug before addition into the microplate to see if there will be an inhibition in the expression of circulating anti-drug antibodies.^{82, 115, 126, 127}

Genetic determinants of drug hypersensitivity

The field of pharmacogenomics studies the link between genetic variation in humans and individual susceptibility to ADRs¹²⁸⁻¹³⁰. Various studies have identified specific genotypes responsible for ADRs including those coding for more than 20 drug

metabolizing enzymes, drug receptors and drug transport systems. Genetic variations may alter the nature of a drug's action thus affecting their pharmacokinetic and pharmacodynamic effects. Single nucleotide polymorphisms (SNPs) play a large role in but these variations may be non-SNP in nature though to a lesser extent. Alteration of drug activity of drugs such as mephenytoin, warfarin and clopidogrel due to variations in the cytochrome enzymes CYP450, CYP2D6 and also CYP2C19 identified as the main drivers of specific drug metabolism are a few examples of the role genetics may play in defining drug action.^{131,132}

Associations may also be phenotype, ethnic and drug specific in nature. Typical examples include the association of HLA-B*5701 with abacavir hypersensitivity³⁶ in Han Chinese and HLA-B*1502 and HLA-A*3101 with carbamazepine-induced SJS/TEN and in Thai, Japanese or European populations.^{41-43, 133}

Understanding the molecular events in individuals suspected or confirmed with drug hypersensitivity reactions will be

critical in the development of proper therapeutic management for these conditions. Most of the research into ADRs has been conducted in the Western world and given the existing genetic differences between the different racial populations it is a disservice to this field of research to neglect the large pool of individuals with drug hypersensitivities in other developing parts of the world. The apparent lack of training and infrastructure is a major limitation in the conduct of these studies particularly in the developing world [134]. In addition to the above, the clinical application is limited due to the amount of time required to obtain data from patients. This is especially important with type I reactions and the more serious type IV hypersensitivity reactions in which clinical interventions have to be immediate. Resolution of these issues should involve the expansion of existing pharmacovigilance programs to include a molecular drug monitoring component, thus ensuring that the necessary training and infrastructure becomes a priority.

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