

Immune pathomechanism of drug hypersensitivity reactions

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Drug hypersensitivity research has progressed enormously in recent years, and a greater understanding of mechanisms has contributed to improved drug safety. Progress has been made in genetics, enabling personalized medicine for certain drugs, and in understanding drug interactions with the immune system. In a recent meeting in Rome, the clinical, chemical, pharmacologic, immunologic, and genetic aspects of drug hypersensitivity were discussed, and certain aspects are briefly summarized here. Small chemicals, including drugs, can induce immune reactions by binding as a hapten to a carrier protein. Park (Liverpool, England) demonstrated (1) that drug haptens bind to protein in patients in a highly restricted manner and (2) that irreversibly modified carrier proteins are able to stimulate CD4⁺ and CD8⁺ T cells from hypersensitive patients. Drug haptens might also stimulate cells of the innate immune system, in particular dendritic cells, and thus give rise to a complex and complete immune reaction. Many drugs do not have hapten-like characteristics but might gain them on metabolism (so-called prohaptens). The group of Naisbitt found that the stimulation of dendritic cells and T cells can occur as a consequence of the transformation of a prohapten to a hapten in antigen-presenting cells and as such explain the immune-stimulatory capacity of prohaptens. The striking association between HLA-B alleles and the development of certain drug reactions was discussed in detail. Mallal (Perth, Australia) elegantly described a highly restricted HLA-B*5701-specific T-cell response in abacavir-hypersensitive patients and healthy volunteers expressing HLA-B*5701 but not closely related alleles. Expression of HLA-B*1502 is a marker known to be necessary but not sufficient to predict carbamazepine-induced Stevens-Johnson syndrome/toxic epidermal necrolysis in Han Chinese. The group of Chen and Hong (Taiwan) described the possible “missing link” because they showed that the presence of certain T-cell receptor (TCR) clonotypes was necessary to elicit T-cell responses to carbamazepine. The role of TCRs in drug binding was also emphasized by Pichler (Bern, Switzerland). Following up on their “pharmacological interactions of drugs with immune receptors” concept (p-i concept), namely that drugs can bind directly to TCRs, MHC molecules, or both and thereby stimulate T cells, they looked for drug-binding sites for the drug sulfamethoxazole in drug-specific TCRs: modeling revealed up

to 7 binding sites on the CDR3 and CDR2 regions of TCR V α and V β . Among many other presentations, the important role of regulatory T cells in drug hypersensitivity was addressed. (J Allergy Clin Immunol 2011;127:S74-S81.)

Key words: Drug hypersensitivity, hapten, prohapten, p-i concept, dendritic cells, T lymphocytes

In the modern world we are exposed to a myriad of (new) chemicals, mostly at very low concentrations. Some chemicals are used as drugs and are given consistently in comparatively high doses. They are designed to interfere with either invading pathogens or endogenous enzymes and receptors for endogenous mediators. Drug therapy is often a balance between the beneficial and harmful effects of these drugs. Particularly puzzling side effects are caused by the immune system. These so-called hypersensitivity reactions are also classified as type B reactions and not predictable drug side effects. Others prefer to call them off-target reactions of drugs. Interestingly and importantly, some reactions are now quite predictable side effects because they are highly associated with expression of specific HLA alleles.¹

Any drug is assumed to be able to elicit hypersensitivity reactions. However, the frequency differs widely. Antibiotics and antiepileptics are the most prevalent drug classes responsible. The risk of sensitization and the severity of clinical symptoms depend on the state of immune activation of the subject, dose, frequency of exposure, route of exposure (epicutaneous is more sensitizing than oral or parenteral applications), duration of exposure, sex (reactions are more frequent in female subjects), and immunogenetic predisposition (in particular HLA-B alleles), whereas a pharmacokinetic predisposition has rarely been detected in a reproducible manner.

It is critical that drug-safety scientists work from a standard set of terms and definitions to understand the pathogenesis of drug hypersensitivity. This often requires manipulation of the immunologic literature to create a “drug-specific” form. The following panel of definitions has been devised specifically for the study of drug hypersensitivity^{2,3}:

- Hapten: a low-molecular-weight chemical with the propensity to bind irreversibly to protein. A hapten might or might not stimulate an immune response.
- Costimulatory agent: a substance that interacts with dendritic cells, stimulating maturation and possibly polarization of the immune response.
- Immunogen: a substance that stimulates an immune response, having stimulatory capacity for the innate and adaptive immune system.
- Antigen: a substance that interacts with high affinity with immunologic receptors.

Although drugs have the potential to act as costimulatory agents and provide maturation signals to dendritic cells, dendritic cells might also receive maturation signals from non-drug-related sources (disease, stress, and trauma).

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Abbreviations used

p-i concept: Pharmacological interactions of drugs with immune receptors concept
SJS: Stevens-Johnson syndrome
TCR: T-cell receptor
TEN: Toxic epidermal necrolysis
Treg: Regulatory T

In this short review we summarize some new developments in drug hypersensitivity, which were discussed at the 4th Drug Hypersensitivity Meeting held in Rome, April 22 to 25, 2010. We briefly address how small molecules interact with the immune system and focus on pathogenetic aspects of drug hypersensitivity, which are clinically important and might explain some side effects.

Specific questions that we cover include the following:

1. Will characterization of physiologically relevant drug antigens lead to the development of improved chemical “tools” for biological tests?
2. Do prohaptens, when they are metabolized, stimulate dendritic cells by providing the “danger signal” and form cellular antigens for T cells?
3. For drugs that interact directly with immunologic receptors, does the drug bind to the HLA or T-cell receptor (TCR) to elicit a reaction?
4. What is the missing link in genetically predisposed subjects who have the genetic risk factor but do not react to the drug?

Fig 1 summarizes the interplay between different systems in patients susceptible to drug hypersensitivity. We are aware that we omit many interesting contributions, in particular those about immediate reactions to drugs, but in the interest of brevity, we focus mainly on interactions of T cells with dendritic cells in drug hypersensitivity.

HOW DO SMALL MOLECULES STIMULATE THE IMMUNE SYSTEM?

Hapten concept

The origin of the hapten concept lies in early studies by Landsteiner and Jacobs.⁴ They identified a relationship between the reactivity of chemical allergens toward protein and sensitization potential. The hapten concept has been revised and refined to encompass drug hypersensitivity largely through studies with β -lactam antibiotics. With reference to T cell-mediated reactions, the hapten-protein conjugate is thought to be “recognized” by dendritic cells, taken up and processed, or broken down into peptide fragments. The derived peptides associate with MHC molecules for presentation to specific TCRs.

The recent development of advanced protein mass spectrometers has allowed the analytic chemist to begin to probe the way in which drugs modify protein. Park (Liverpool, United Kingdom) used β -lactam hypersensitivity as a model to describe an integrated approach to relate the chemistry of drug antigen formation in patients to the stimulation of antigen-specific T cells. Although it is well established that the formation of a stable covalent bond between β -lactam antibiotics and lysine residues on protein is an obligatory step in immune sensitization,⁵⁻⁷ the site and number of

drug modifications required to elicit an immune response is ill defined. Using human serum albumin, which accounts for the majority (approximately 90%) of serum-bound penicilloyl groups, Park demonstrated that β -lactams actually modify lysine groups in a highly restricted pattern. Fewer than 20% of available lysine residues on albumin were modified with different β -lactams, even when analyses were conducted under forced chemical conditions. Importantly, similar profiles of modifications were detected when albumin binding in cell-culture supernatants and patients' plasma was analyzed.⁸ Thus it is possible to generate physiologically relevant antigens in the laboratory in cell-culture systems. A synthetic β -lactam albumin conjugate was synthesized and shown to stimulate lymphocytes and the majority of T-cell clones from β -lactam-hypersensitive patients.

Chemelle and Terreux (Lyon, France) have pioneered a novel computer-based approach for the prediction of potential sites of protein modification by β -lactams. Clearly, any integration of *ab initio in silico* techniques and advanced mass spectrometry will provide the framework required to define antigen processing of drug-protein hapten conjugates. With an improved understanding of drug-specific amino acid modifications, it might be possible in the near future to design and synthesize novel protein and peptide antigens for use in tests to diagnose drug hypersensitivity.

For well-characterized drug haptens, such as the β -lactam antibiotics, it is possible to define relevant antigenic determinants and immunogens.^{9,10} However, our mechanistic understanding is far from complete. It is vital to establish prospective studies to investigate the time course, quality, and intensity of the drug-specific immune response in patients. Within this translational research environment, it might be possible to define the relationship between antigen formation and various immunologic parameters, such as B-cell antibody production and effector and regulatory T (Treg) cells, which determine clinical outcome.

Two pathways must be triggered to initiate an immune response, namely the antigenic signal (signal 1), sensed by specific TCRs, and the maturation signal (signal 2), sensed by dendritic cells, which subsequently provide costimulatory signals to T cells on activation. Maturation signaling after drug exposure can occur through oxidative stress, the irreversible modification of critical “stress sensor” proteins, or both.^{11,12} Thus dendritic cells might receive maturation signals from other cells (keratinocytes, natural killer cells, and B cells) rather than the drug *per se*.^{13,14} Furthermore, it has been hypothesized that the provision of drug-related maturation signals might reactivate latent virus-specific CD8⁺ T cells, which are thought to play a role in certain forms of cutaneous hypersensitivity.¹⁵ The provision of maturation signals for dendritic cells is well documented for chemical sensitizers, and Martin (Freiburg, Germany) elegantly described stress responses and innate immune pathways triggered by contact allergens before the development of an adaptive T-cell response.¹⁶

Knowledge relating to drug-dendritic cell interactions and the role of bystander cells in the provision of dendritic cell maturation signaling is in its infancy. Amoxicillin has been shown to drive dendritic cells from hypersensitive patients into a semimature state, whereby they can induce a T-cell response.¹⁷ Increased expression of the maturation marker CD40 is seen on dendritic cells exposed to sulfamethoxazole and sulfamethoxazole metabolites,¹⁸ and abacavir increases the number of cells from hypersensitive patients expressing CD40.¹⁹ Despite this, the key steps in dendritic cell activation in drug-hypersensitive patients and whether this is important in adverse reactions is not known.

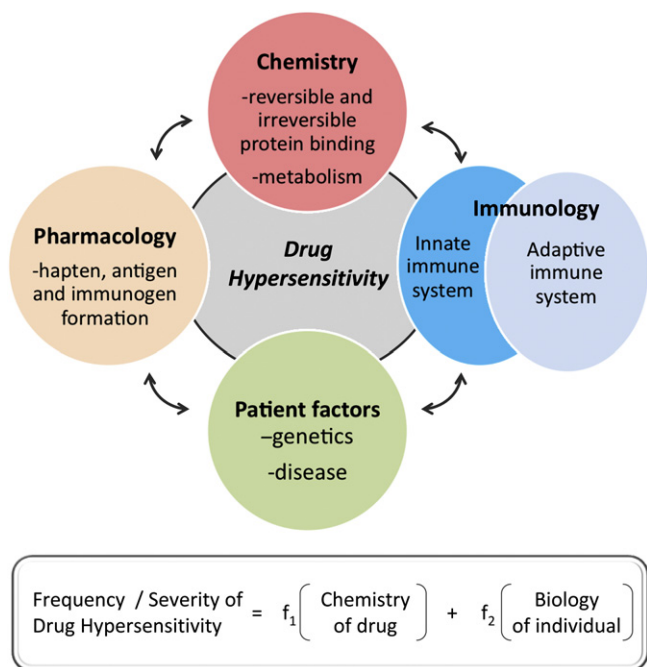


FIG 1. Systems involved in drug hypersensitivity.

Prohaptent concept and activation of dendritic cells and T cells

The prohaptent hypothesis tries to reconcile this phenomenon with the hapten hypothesis by stating that a chemically inert drug can be “bioactivated” through the normal processes of metabolism to chemical (protein)–reactive intermediates or haptens.

The pharmaceutical industry has attempted to tackle the problem of bioactivation by testing molecules for their propensity to form chemically reactive intermediates. The chemical basis of drug bioactivation can often be rationalized, and synthetic strategies can be put in place to prevent such bioactivation without significant loss of primary pharmacology.^{20,21} However, there is no simple correlation between drug bioactivation in the test tube and drug hypersensitivity reactions in the clinic. In this respect it is likely that different chemically reactive metabolites differ in terms of their electrophilicity, intracellular targets, stress signaling, detoxification pathways, reversibility in protein adduct formation, and immunologic recognition of the protein antigens to which they give rise.

The prototype prohaptent sulfamethoxazole was used to discuss the prohaptent concept. Sulfamethoxazole itself is not protein reactive but gains reactivity in the liver through intracellular metabolism by using a 2-step process. Cytochrome P450–dependent reactions lead to the formation of sulfamethoxazole hydroxylamine.²² Sulfamethoxazole hydroxylamine does not bind to protein and can be found in the circulation and is even excreted in the urine. However, it is easily auto-oxidized in aqueous solution to a nitroso intermediate,^{23,24} which modifies thiol groups on cellular and serum protein, generating a panel of chemically diverse antigenic determinants.^{25–27} Skin is the major target for sulfamethoxazole-associated hypersensitivity reactions; the liver is targeted infrequently, despite exposure to high quantities of metabolites. The liver is rich in regulatory immunologic mechanisms that might prevent the development of liver-specific immune responses and scavenger molecules (glutathione and antioxidants)

that detoxify reactive sulfamethoxazole metabolites before they can form antigenic determinants. Thus although the liver is exposed to high levels of hapten, antigens might only be formed in levels that do not surmount the organ’s significant regulatory immune mechanisms.

It is well established that blood- and skin-derived T cells from hypersensitive patients are stimulated by sulfamethoxazole and nitroso sulfamethoxazole.^{28–30} In a recent study Castrejon et al³¹ demonstrated that almost 90% of T cells were stimulated through a (pro-)hapten mechanism involving the irreversible modification of protein by nitroso sulfamethoxazole. These data indicate that nitroso metabolites form potent antigenic determinants for T cells from hypersensitive patients and suggest that T-cell responses directed against drugs bound directly to MHC molecules can occur through cross-reactivity with the haptenic antigen.

Despite these extensive studies, the potential role of immune cell metabolism in the generation of highly localized drug haptens that form more relevant antigens at sites of immune activation and whether metabolite formation is associated with dendritic cell maturation signaling, the provision of antigens for T cells, or both have not been defined, largely because the analytic methods are not readily available to relate metabolism and antigen formation to immune function. Using an anti-drug antibody to quantify antigen formation, Laverne et al³² presented data showing that sulfamethoxazole is metabolized by immune cells. Adduct formation in dendritic cells was significantly enhanced in the presence of various pathological factors (eg, bacterial endotoxins, viral proteins, and cytokines), some of which might be present in patients receiving the drug. Sulfamethoxazole metabolism was time dependent; high levels of protein antigens were detected after 16 hours. Antigen-presenting cells pulsed with sulfamethoxazole for 16 hours stimulated the majority of T cells cloned from hypersensitive patients. The response was blocked by inhibition of drug-metabolizing enzymes, which confirmed that metabolism in antigen-presenting cells and subsequent protein binding represents an important pathway for antigen formation in hypersensitive patients.

These studies showing metabolic activity in antigen-presenting cells might also provide a rational explanation for the detection of specific T-cell responses with abacavir-pulsed antigen-presenting cells (Park and Naisbitt, Liverpool, England; Adam and Yerly, Bern, Switzerland; and Mallal and Philips, Perth, Australia).

As discussed above, a partial maturation of dendritic cells is observed after exposure to therapeutic concentrations of sulfamethoxazole. In Rome Naisbitt also presented data showing that bystander cells cultured with higher toxic sulfamethoxazole concentrations provided a potent dendritic cell maturation signal characterized by increased costimulatory receptor (CD40, CD80, and CD86) expression and increased MHC class II expression and cytokine secretion. A strong positive correlation was observed between the expression of costimulatory receptors and drug metabolite–specific killing of bystander cells. These data indicate that drug haptens provide 2 distinct signals to dendritic cells. First, exposure to low levels of hapten results in partial activation and a semimature dendritic cell phenotype. Second, higher levels of hapten exposure, which can occur with certain disease states (eg, HIV and cystic fibrosis), causes bystander cell death and the provision of a potent signal for the full maturation of dendritic cells.

According to the hapten hypothesis, drugs must bind irreversibly to skin cells to form antigens that will be targeted by

skin-infiltrating T-cell clones. For a prohaptent to form an antigen in skin, skin cells would need to express significant quantities of drug-metabolizing enzymes. Merk (Aachen, Germany) presented data illustrating that human skin cells do indeed express significant levels of certain enzymes.³³ A CYP cocktail representative of human skin^{34,35} that contains a relevant panel of drug-metabolizing enzymes was devised and used in mechanistic studies to characterize prohaptent metabolism. Based on these findings, Blanca and co-workers (Malaga, Spain) described an interesting modification of the lymphocyte transformation test in which prohaptens were incubated with skin cell lines to generate metabolites that are then transferred in serum-containing medium to lymphocytes from hypersensitive patients. This approach seems to be generating encouraging results with cells from carbamazepine-hypersensitive patients and might represent an important breakthrough in the development of biological diagnostic tests for prohaptens; however, it is critical that the authors confirm that the system does actually transfer drug metabolites in either soluble or albumin-conjugated form and rule out an allogeneic effect.

Pharmacologic interaction with immune receptors

The haptent-protein interaction model leads to presentation of a haptent-modified peptide by MHC molecules. This involves processing and presentation of haptent-protein adducts and requires the formation of an irreversible, often covalent bond between the drug and the peptide, whereas weaker bonds (eg, van der Waals forces, hydrophobic interactions, electrostatic interactions, and hydrogen bonding) occur between the drug-peptide conjugate and immune receptors.

However, there are other means by which nonreactive drugs can stimulate immune cells. This additional concept to explain the stimulatory feature of a drug is quite simple: drugs are actually often designed to bind selectively to certain protein pockets of receptors or enzymes. This binding interaction is based on the ability of specific drug moieties to interact in a reversible fashion with protein. The term "pharmacophore" was originally defined by Paul Ehrlich in 1909 as the molecular framework of the drug that carries the essential features responsible for its mechanism of action. More recently, the pharmacophore has gained an International Union of Pure and Applied Chemistry definition: an ensemble of steric and electronic features that is necessary to ensure the optimal supramolecular interactions with a specific biological target and to trigger (or block) its biological response.³⁶ From this definition, it is clear that the pharmacophore of a drug might interact with immune receptors, such as the TCR or MHC molecules, directly and provide a sufficiently strong signal to stimulate a response. Immune receptors are extremely heterogeneous, and some might have "protein pockets" similar to the receptor/enzyme for which the drug was designed and fits well. Because these molecules are polymorphic, binding of drugs to these particular proteins differs in subjects. Such an interaction of drugs with immune receptors would imitate the drug interaction with its normal ligand. Thus Pichler³⁷ came up with the "pharmacological interactions of drugs with immune receptors concept" (p-i concept).³⁷ In contrast to the haptent concept, which induces a complete and complex immune response, the ensuing p-i response might be restricted to T cells only, probably only to preactivated T cells, with a low threshold for activation. Costimulation might be unnecessary. However, p-i responses might also be enhanced from triggering drug-induced stress pathways, drug-induced bystander cell death, or viral infections.

This p-i concept complements the original thought that the immune-stimulatory capacity of most chemicals and drugs is based on covalent binding and might be predicted by their protein reactivity. Evidence for the p-i mechanism lies in various experimental data and has been reviewed repeatedly.³⁷⁻³⁹

- Aldehyde-fixed antigen-presenting cells (unable to process antigen or to make a prohaptent to a haptent) are still able to activate specific T-cell clones if incubated together with the (inert) drug.
- Drug binding to proteins is much more labile than the covalent interactions of haptens and can even be washed away.
- Calcium influx in T-cell clones occurs within seconds after the addition of the drug and before drug uptake, metabolism, and processing can occur.
- Elution of peptides from HLA-B*1502, which presents carbamazepine to reactive T-cell clones, were not carrying a covalently bound carbamazepine/carbamazepine metabolite.⁴⁰
- Some peculiar clinical data do also support the p-i concept: the p-i concept is a single-receptor stimulation concept and consequently would only stimulate a single cell lineage (T cells). Indeed, carbamazepine and many other drugs (or nickel!) only elicit a strong T-cell response but no antibodies. This differentiates the p-i concept from the haptent model, in which a complete immune response with T and B cells often occurs, as for other protein antigens.

PRIMARY BINDING SITES FOR DRUG ANTIGENS

It is important to note that full T-cell activation by drug antigens (measured based on immediate Ca^{2+} influx into specific T cells, cytokine synthesis, or proliferation) requires the interaction of the TCR with MHC on antigen-presenting cells.^{37,38} This finding raises the question of whether the antigen binds first to the MHC molecule, modifying its structure, which is sensed by the TCR and thus leads to specific TCR activation, or whether the drug binds primarily to a specific TCR, rendering the MHC interaction only a supplementing signal?

Both concepts are theoretically possible: drugs that show a strikingly high HLA allele association⁴¹⁻⁴⁶ might interact primarily with the HLA molecule. In the case of abacavir, a drug hypersensitivity syndrome strongly associated with the HLA-B*5701 allele,⁴¹ key interacting residues in the HLA-B*5701 peptide-binding cleft could be identified,⁴⁷ which allow the formation of noncovalent interactions with the drug abacavir or a drug-modified peptide derived from a haptent-protein conjugate. The conclusion of Chessman et al⁴⁷ was that the ultimate antigen is not yet defined.

On the other hand, analysis of drug-specific CD4^+ T-cell clones stimulated through a p-i mechanism suggested that the interaction of the drug happens first with the TCR because the MHC-bound peptide could be exchanged or removed without affecting CD4^+ T-cell activation.⁴⁸ Moreover, some T-cell clones reacted to the drug, even if presented by allogeneic MHC molecules, indicating that no strict HLA restriction for drug presentation exists.⁴⁹ Full stimulation of these CD4^+ cells would still require an interaction with the MHC class II molecules, however, probably just by binding to common determinants of the MHC structure because various MHC class II molecules appear to be sufficient to provide T-cell stimulation.

Pichler et al addressed this question by modeling drug-TCR interactions in 11 sulfamethoxazole-specific TCRs: in 10 of 11 they found 5 to 7 hydrogen bonds to these sulfamethoxazole-specific TCRs. These interaction sites were located on the CDR2 and CDR3 regions of TCR V α and V β , suggesting that TCRs might have drug-binding sites. Based on these new findings, 2 types of p-i mechanisms were proposed by Pichler. First is one in which the drug binds first and preferentially to MHC molecules and preferentially some HLA-B alleles: this modified HLA molecule is recognized by the TCR similar to recognition of alloantigens. Second, drugs might associate with TCRs and provide some initial signal; on additional MHC interaction (often MHC class II), the T cells are fully activated and proliferate (often CD4⁺).⁵⁰

Interestingly, Rozieres et al⁵¹ (Lyon, France) have developed a murine model of drug allergy induced by skin sensitization to amoxicillin and sulfamethoxazole. CD4⁺ T-cell deficient mice have drug-specific allergic skin reactions mediated by antigen-specific IFN- γ -producing CD8⁺ T cells. It will be interesting to explore mechanisms of drug-specific T-cell activation in this model and compare the findings with those observed with human cells.

Chung et al⁵² (Taipei, Taiwan) presented data on the important role of the cytotoxic molecule granulysin in severe cutaneous drug reactions, such as Stevens-Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN). Secretory granulysin is produced by drug-specific CD8⁺ T cells and natural killer cells and is a key molecule responsible for the disseminated keratinocyte death seen in patients with SJS-TEN. It is present in high concentrations in blister fluid, and sera of affected patients and antibodies to granulysin might neutralize it. Abe et al⁵³ (Sapporo, Japan) already presented a fast bedside assay for granulysin determination in serum, which might allow an early diagnosis in this severe and often deadly disease.

WHAT IS THE MISSING LINK IN GENETICALLY PREDISPOSED SUBJECTS WHO HAVE THE GENETIC RISK FACTOR BUT DO NOT REACT TO THE DRUG?

Predisposition to drug hypersensitivity is clearly dependent on the subject, the dose, and the route of application. However, the major susceptibility factors seem to relate to the restriction of the fit of an antigen into particular immunologic receptors in an appropriate chemical form to stimulate a response in an environment rich in dendritic cell maturation signals.

A cellular matrix rich in perpetual dendritic cell maturation signals is an unavoidable complication for the management of patients with certain underlying chronic diseases. In these patients it is possible that drug-specific dendritic cell signaling is not required for drug hypersensitivity or the threshold to be surmounted is significantly lowered. Thus drug hypersensitivity reactions might evolve in subjects expressing appropriate immunologic receptors that accommodate the drug antigen. The prevalence of reactions in these patients might simply relate to the chemical reactivity of the drug hapten, the number of different drug-modified peptides formed that associate with different affinities with MHC, or both. In this respect Whitaker (Leeds, England) discussed the problem of antibiotic use for recurrent respiratory tract infections in patients with cystic fibrosis. Immunologic reactions develop in 25% to 50% of exposed

patients with cystic fibrosis compared with 1% to 10% in the general population.^{54,55} Differences in the incidence in patients with cystic fibrosis might be related to the frequency of exposure (patients receive 3-6 courses per year), route of exposure (drugs are administered by intravenous injection), duration of exposure, or dose (patients receive up to 12 g/d). However, perturbed immunologic status associated with the development of recurrent infections is likely to be the most important susceptibility factor, decreasing the costimulatory threshold required to stimulate a response. Other disease models include infectious mononucleosis and HIV, both of which are associated with an increased risk of hypersensitivity reactions.

One of the most important areas of progress in the field of drug hypersensitivity was the finding of astonishingly strong associations of certain severe drug hypersensitivity reactions with HLA-B alleles 17 to 20 (Mallal and Phillips, Perth, Australia; Chen, Taipei, Taiwan). These associations were described for carbamazepine-induced SJS (HLA-B*1502 in Han Chinese),⁴² severe hypersensitivity reactions to abacavir (HLA-B*5701),⁴¹ severe reactions to allopurinol (HLA-B*5801),⁴⁵ and flucloxacillin hepatotoxicity (HLA-B*5701).⁴³ These strong associations must also imply some direct effect of the gene product on the side effect. Somehow these alleles must relate to the presentation of a drug antigen in a more appropriate fashion than other HLA alleles. For abacavir, Mallal discussed the data of Chessman et al,⁴⁷ who provided the first experimental evidence to relate the genetic association to mechanisms of disease by describing abacavir-specific CD8⁺ T-cell responses in hypersensitive patients and volunteers expressing HLAB*5701 but not closely related alleles.

Interestingly, for most associations, not all carriers of these risk alleles have hypersensitivity. The group of Hung and Chen (Taiwan), who originally described the association between HLA-B*1502 and carbamazepine-induced SJS,⁴² presented data to address this issue. They asked the following: Does the use of certain TCR genes and generation of specific clonotypes contribute to the difference between HLA-B*1502-positive subjects who will have hypersensitivity and those who remained tolerant? In their study, using cells from HLA-B*1502-positive subjects, they found a striking association with activation of certain TCR V β s (eg, TCR V β ₁₁) and even skewed use of specific clonotypes. Only patients expressing this TCR phenotype reacted to carbamazepine *in vitro*, whereas carbamazepine-tolerant subjects did not have these TCRs. Because they even succeeded in inducing a primary immune response in healthy HLA-B*1502 subjects if they had the respective TCR clonotype present in the circulation, they concluded that they found the missing link between HLA-B*1502-positive tolerant and diseased subjects.

TOLERANCE MECHANISMS

Most patients can take drugs without immune-mediated side effects. One could argue that they lack precursor cells able to interact with the drug. However, the great heterogeneity of the immune response to drugs, a high precursor frequency in sensitized patients,⁵⁶ and the finding that 2% to 4% of the healthy population but 30% to 50% of HIV-infected patients might react to sulfamethoxazole suggests that it is not a lack of precursor cells but rather other factors, such as the underlying immune status (preactivation of memory T cells) and "regulatory" mechanisms, that might be important.

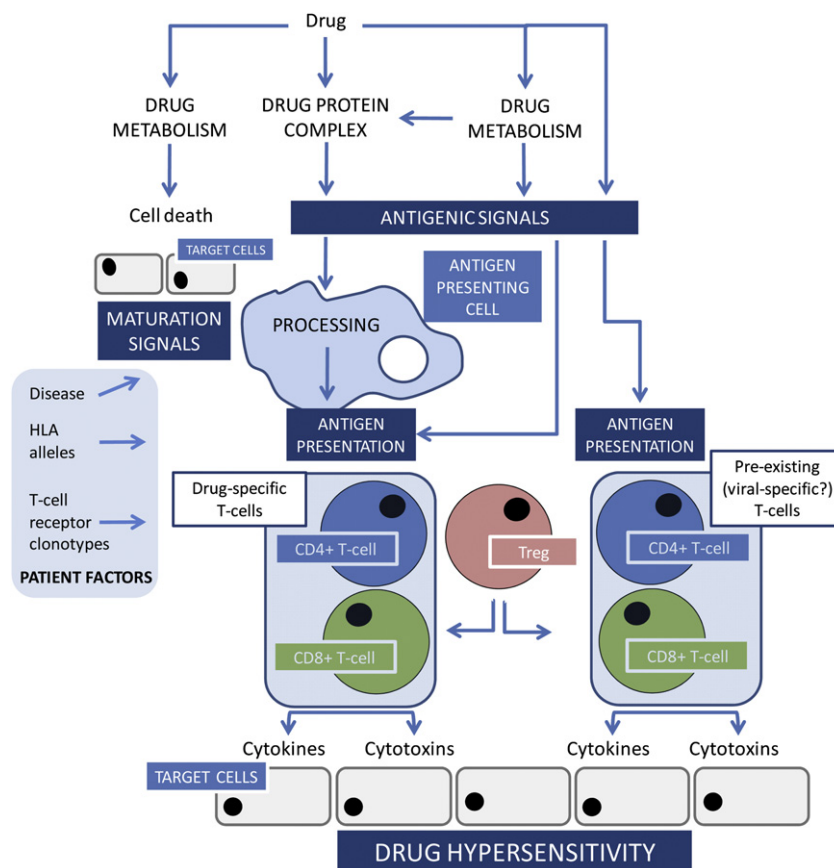


FIG 2. Drug and patient factors involved in the development of drug hypersensitivity. The main areas, stimulation of dendritic cells and their drug-induced maturation, formation of antigenic sites, preferential presentation of drugs/haptens by certain HLA alleles and reaction of a hapten-peptide complex or of the drug directly with certain TCRs, participation of Treg cells, and mechanisms of cytotoxicity, were presented at the 4th Drug Hypersensitivity Meeting and are discussed in the text.

Treg cells are believed to regulate allergic diseases through inducing immune tolerance. However, little is known about the role of Treg cells in drug hypersensitivity reactions. Takahashi et al⁵⁷ (Tokyo, Japan) have shown an increased frequency and activity of Treg cells in the blood and skin of patients with drug reaction with eosinophilia and systemic symptoms. Treg cell populations decreased after resolution of symptoms, leading to enhanced responses to additional drugs and autoimmunity. In contrast, in patients with TEN, the functional defects of Treg cells were restored on recovery. Several populations of T cells have been described that possess similar regulatory activity but differ in terms of cell-surface marker expression and cytokine secretion. Naturally occurring CD4⁺CD25⁺ forkhead box protein 3–positive CD127^{low} Treg cells represent a small portion of peripheral CD4⁺ T cells and exert suppressor function in an antigen-nonspecific manner. Acquired Treg cells (classified according to cytokine [IL-10 and TGF- β] secretion) gain suppressor function after exposure to antigen and thus have the potential to specifically regulate immune responses.

Daubner and Pichler (Bern, Switzerland) presented data on a series of studies exploring Treg cells in drug-exposed nonhypersensitive subjects in patients with hypersensitivity to one drug and in patients with multiple-drug hypersensitivity syndromes. No defect in Treg cells could be identified either functionally or

quantitatively. Removal of Treg cells did not enhance the immune response to potential antigenic drugs, such as amoxicillin. Based on these findings, the authors concluded that other mechanisms might play a role in patients with multiple drug hypersensitivity.

CONCLUSION AND FUTURE DIRECTIONS

Although the ash cloud grounded many flights throughout Europe in April 2010, the 4th Drug Hypersensitivity Meeting was a great success. More than 300 delegates with backgrounds in fields as diverse as allergy, immunology, toxicology, pharmacology, genetics, and clinical medicine came together to discuss concepts relevant to the field of drug hypersensitivity, presented data, and formulated ideas on how the field should move forward. As the sky cleared over Rome, the delegates emerged with a greater understanding of how and why drugs cause hypersensitivity in certain subjects.

The frequency and severity of drug hypersensitivity is undoubtedly a function of the chemistry of the drug and the biology of the subject (Fig 2), and as such, future research must qualify and quantify drug disposition, metabolism, and protein binding (reversible and irreversible) in all relevant biological systems. Because of the restriction of some drug reactions to only certain HLA haplotypes, *in vitro* models with human cells need to be

expanded, and animal models might mainly serve to prove a concept but are less suitable for risk assessment.

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