Role of T cells in nonimmediate allergic drug reactions

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Purpose of review

This review presents the current knowledge of the role of T cells in drug allergy manifesting as exanthematous, pustular and bullous skin diseases, collectively referred to as nonimmediate allergic drug reactions.

Recent findings

Both CD4+ and CD8+ T cells producing type 1 and type 2 cytokines and endowed with cytotoxic properties are involved in nonimmediate allergic drug reactions. Recent studies have confirmed that CD8+ T cells play a major role in the pathophysiology of nonimmediate allergic drug reactions, and have characterized new cytotoxic molecular pathways responsible for the severity of the bullous forms of nonimmediate allergic drug reactions.

Summary

Nonimmediate allergic drug reactions are mediated by T cells and mostly affect the skin. Nonimmediate allergic drug reactions comprise several diseases ranging from the frequent and benign maculo-papular exanthema to the severe and rare toxic epidermal necrolysis. Progress in the knowledge of the pathophysiology of nonimmediate allergic drug reactions comes from a better understanding of the mechanisms of drug recognition by T cells and from a careful analysis of the phenotype and functions of CD4+ and CD8+ T cells infiltrating the skin lesions. Recent studies have confirmed that the different clinical forms of nonimmediate allergic drug reactions are associated with distinct types of T cell-mediated skin inflammation. However, CD8+ T cells appear as major effector T cells in most of the nonimmediate allergic drug reactions. Future studies to analyze the early cellular and molecular events leading to the development of the allergic skin reaction will be helpful in order to define diagnostic and therapeutic targets.

Keywords

CD8+ T cells, cytotoxicity, drug allergy, pathophysiology

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Introduction

The skin is the most frequent target of allergic drug reactions mediated by either IgE (immediate) or T cells (nonimmediate or delayed). Nonimmediate allergic drug reactions (NIADRs) comprise several diseases manifested as exanthematous, pustular and bullous eruptions, with different evolution profiles and severity. The most frequent NIADRs are usually benign and comprise maculo-papular exanthema (MPE) and fixed drug eruptions (FDEs). 'Acute generalized exanthematous pustulosis' (AGEP) and 'drug rash with eosinophilia and systemic symptoms' (DRESS) are infrequent and may be severe. Toxic epidermal necrolysis (TEN) is very rare but extremely severe. All these diseases are mediated by drug-specific T cells which are found in the blood of sensitized patients and infiltrate the skin to produce the skin inflammatory damage upon activation by drug-presenting skin cells. This review will concentrate on the role of T cells in the pathophysiology of NIADRs. Although several studies yielded important information in the field of drug allergy, the mechanisms leading to drug sensitization in predisposed patients still remain unknown. In contrast, the latest studies analyzing the kinetics of T cell activation in the skin have allowed a better understanding of the relative contribution of CD4+ and CD8+ T cells in the onset of the allergic responses.

Drug-specific T cells

Drugs are low-molecular-weight chemicals able to bind to T cell receptors (TCRs) to activate adaptive immunity.

The outcome of drug interaction with T cells depends on several parameters and leads to either tolerance (the majority of cases) or to induction of drug-specific effector T cells responsible for the onset of an inflammatory reaction in tissues in which T cells are activated.

Drug recognition by T cells

Drug interactions with TCRs usually involve a drug/peptide complex presented in the groove of major histocompatibility complex (MHC) molecules by antigen-presenting cells (APCs), defining the hapten concept. This occurs for chemically reactive drugs such as β-lactams which form covalent binding to lysin residues of proteins [1]. Other drugs, for example sulfomethoxazole, are pro-haptens and must be metabolized or bioactivated to a chemically reactive form before they are able to bind covalently to proteins and induce an immune response against the carrier/hapten complex.

Drugs can also directly interact with the TCRs without binding to the MHC/peptide of the APC as in the P-i concept (pharmacological interaction of drugs with immune receptors). Chemically inert drugs, such as lidocaine and carbamazepine, unable to bind covalently to peptides or proteins, can nevertheless associate with a low affinity to TCRs and provoke T cell activation [2,3].

Factors predisposing to T cell-mediated drug allergy

Genetic factors

Genetic factors predisposing individuals to severe drug reactions have been intensively investigated. Interestingly, the strongest associations between drug allergy (DRESS and TEN) and human leukocyte antigen (HLA) haplotypes concerned MHC class I molecules suggesting that CD8+ T cells are the main effector T cells in these diseases. Because genetic factors were found in some ethnic groups, and for only some drugs it is likely that other predisposing factors may be associated with the development of drug allergy [4–7].

Environmental factors

Activation of an adaptative immune response requires the presence of danger signals, for example those borne by microorganisms which bind to toll-like receptors of host cells. The nature of the danger signals involved in drug allergy is still poorly defined and may comprise different forms of stress, for example chemical, physical and viral inflammatory signals [8,9]. In this respect, drug allergy more commonly develops in patients with concomitant viral infections. For example, infection with the Epstein–Barr virus (EBV), human herpes virus 6 (HHV-6) and HIV increases the frequency of drug hypersensitivity reactions [10,11].

Involvement of T cells in drug allergy

That T cells are involved in drug allergy comes from several observations. Positive skin patch tests to drugs demonstrate a specific sensitization [12] to medications and biopsies of positive tests have been used for isolation of drug-specific T cells in patients with cutaneous adverse drug reaction (CADR). Still, skin tests remain negative in a large number of patients, in most cases of severe diseases such as TEN, and for some drugs which may not penetrate the skin upon epidermal contact or may need metabolic activation to become antigenic.

Phenotype and functions of drug-specific T cells

Immunolabeling of skin biopsies of acute lesions and/or patch tests demonstrated the presence in the lesions of activated T cells, usually with a predominance of CD4+ lymphocytes in the dermis and a majority of CD8+ cells in the epidermis [13]. Drug-specific T cells can be found in the blood of patients using both secondary proliferation assays and cytokine release tests [14–17,18•]. Recent advances in the immunobiological diagnosis of drug allergy include the use of Elispot assays able to enumerate the frequency of specific T cells [19•,20]. Human T cell clones have been derived from the blood or from skin lesions of patients with a variety of drug reactions [21,22]. Both CD4+ and CD8+ T cell clones have been obtained with most medications that induce allergic reactions (penicillins, cephalosporins, sulfamethoxazole, phenobarbital, carbamazepine, lamotrigine) with a majority of CD4+ clones. Some clones produced a Th0 profile of cytokines (simultaneous release of IL-4 and IFN-γ). A Th2 orientation (production of IL-4) was frequent in CD4+ clones, whereas CD8+ clones were usually Th1 (production of IFN-γ) and often cytotoxic. Presence of Th17 cells (producing IL-17) was recently reported [18•]. Drug presentation to T cell was MHC restricted, usually as expected by HLA class II for CD4+ cells and by HLA class I for CD8 [23–25].

Correlation between T cell functions and the clinical phenotype of nonimmediate allergic drug reactions

Analysis of lesional and circulating T cells in the various NIADRs has revealed that each particular disease was associated with a predominant, if not unique, T cell profile demonstrating that the clinical presentation and the severity of NIADR depend on distinct immune mechanisms, mediated by different effector T cells and leukocytes [26]. The current paradigm is that CD8+ cytotoxic T cells mediate the bullous diseases, that is TEN and FDE, when they induce keratinocyte apoptosis, epidermal necrosis and bulla formation through both Fas/Fas-L interaction and the production of perforin/granzyme/granulysin lytic molecules [27,28,29••]. In contrast, the skin inflammation of nonbullous forms of CADR, such as MPE, DRESS and AGEP, is thought to be provoked by CD4+ T cell

cytokines and cytotoxicity mediating the recruitment of distinct subsets of leukocytes [22,23,26]. However, the picture is probably not so clear-cut since CD8+ T cells are consistently found in the epidermis of nonbullous NIADR including MPE and nonbullous forms of FDE.

Nevertheless, the precise phenotype and functions of T cell responsible for the onset of the allergic responses still remain unknown for the most frequent diseases (MPE) and for one of the most severe (DRESS). Knowledge of the regulatory mechanisms that control the effector T cells is still limited.

Clinical presentation and immunopathology of nonimmediate allergic drug reactions

T cell-mediated delayed drug allergy may present as MPE, pustular exanthema, epidermal necrolysis or as a drug-induced reaction with systemic symptoms (DRESS), the two latter being the most severe NIADR.

Toxic epidermal necrolysis

Toxic epidermal necrolysis may present with clinical features of Stevens–Johnson syndrome (SJS) and Lyell's syndrome, with the main differentiating feature being the percentage of skin denudation/epidermal detachment in terms of body surface area of involvement (<10 and >30%, respectively), and the predominance of lesions at mucosal orificial sites in SJS. TEN involves a massive apoptosis of keratinocytes evolving into epidermal detachment and bullae formation. These two diseases are fatal in approximately 34% of cases, especially when epidermal necrosis is widespread, in aged patients and in those with comorbidities [30,31]. The drugs most frequently associated with TEN are sulfonamides, anticonvulsants (carbamazepine, phenytoin, lamotrigine), allopurinol and oxicams.

Drug-specific CD8+ T cells mediate keratinocyte apoptosis. In TEN, blisters contain activated CD8+ CD56+ T cells with natural killer (NK)-like features [27,28,29••,32] that kill autologous lymphocytes and keratinocytes and induce both apoptosis and necrosis in a drug-specific, HLA class I-restricted and perforin/granzyme B-mediated pathway [27,28]. Keratinocyte death is provoked both by direct CD8+ cytotoxicity and by the production of death mediators among which are TNF-α, Fas-ligand, Tweak and TRAIL [28]. More recently, granulysin has been proposed as a major cytotoxic molecule responsible for extended keratinocyte necrosis in TEN [29••]. A role for regulatory T cells (Treg) in controlling the expression of TEN was suggested from studies in a mouse model of TEN in which anti-OVA cytotoxic T-cells killed OVA-expressing keratinocytes only in a context of a deficiency in CD4+ regulatory T cells [33].

Drug rash with eosinophilia and systemic symptoms

Drug rash with eosinophilia and systemic symptoms is also known as 'drug-induced hypersensititivity syndrome' (DIHS). DRESS expresses as a severe eruption with edema, lymphadenopathy, fever, hepatitis and hematological abnormalities among which eosinophilia is the most characteristic feature [34]. Eosinophils infiltrate the skin and other affected organs and are thought to be responsible for tissue damage. Visceral involvement differentiates DRESS from other more frequent exanthematous eruptions and may comprise arthralgias, pulmonary infiltrates and interstitial nephritis. Frequently eliciting drugs are anticonvulsants (carbamazepine, phenytoin, lamotrigine), sulfonamides, allopurinol and minocycline. The histopathological analysis shows edema and perivascular lymphocytic infiltration with eosinophils.

The pathophysiology of DRESS is still not precisely known. Although IL-5 is the major cytokine involved in eosinophil chemotaxis and activation, predominance of IL-5-producing T cells is not a feature of DRESS. Activated CD4+ and CD8+ T cells, producing type 1 cytokines (IFN-γ), are found in the blood of patients during the acute phase [11–15]. Drug-specific CD4+, CD8+ and CD4+CD8+ T cells displaying different effector functions (cytotoxicity and cytokine production) and homing characteristics have been cloned from DRESS patients' blood many years after the resolution of clinical symptoms [35]. CD4+ and CD8+ cells expressed high levels of the activation marker HLA-DR, and a high level of IFN-γ was detected in the supernatant of carbamazepine-stimulated T cell clones. Recent studies have found a reactivation of herpes virus (cytomegalovirus and EBV) infections during the onset of DRESS suggesting that viral propagation could play a role in development of the symptoms [36]. However, whether the strong systemic immune activation observed in DRESS is the cause or the consequence of reactivation of herpes viruses remains to be established [37].

Fixed drug eruptions

Fixed drug eruption usually appears as a solitary or a small number of pruritic, well circumscribed, erythematous macules that evolve into edematous plaques. These lesions typically resolve after discontinuation of the offending drug, leaving hyperpigmentation of the lesional site in patients with pigmenting FDE [38,39]. Lesions recur in exactly the same sites when rechallenged with the same offending drugs. Nonpigmenting FDE is more common in patients with fair skin. Histology shows an interface dermatitis manifested by degeneration of basal keratinocytes associated with lymphocytic infiltrate of the dermis and invasion of the epidermis by T cells. Several drugs are responsible for FDE, for example tetracyclines, sulfonamides, carbazepine.

Fixed drug eruption is a disease mediated by activation of intraepidermal CD8+ T cells [40]. Resting FDE lesions are characterized by the presence of large numbers of CD8+ T cells [phenotype of effector memory cells (CD45RA+ CD27−)] along the epidermal basal layer [40,41•,42]. Upon activation, these CD8+ T cells have the capacity to produce large amounts of IFN-γ. IL-15 derived from the lesional epidermis could maintain the survival of the intraepidermal CD8+ T cells even in the absence of antigenic stimulus over a prolonged period of time (>4 years) [40,41•,42,43]. As the frequency of intraepidermal CD4+ T cells is inversely correlated to the severity of FDE, CD4+ T cells are thought to act as regulatory cells able to ameliorate the tissue-damaging effect of intraepidermal CD8+ T cells.

Acute generalized exathematous pustulosis

Acute generalized exathematous pustulosis is characterized by fever and the acute eruption of nonfollicular pustules overlying erythrodermic skin [38]. Histopathology shows subcorneal pustules, spongiosis with a background of dermal edema and spongiosis, leukocytoclastic vasculitis, and focal necrosis of keratinocytes. Immunohistology in AGEP reveals an intraepidermal infiltration of HLA-DR-expressing CD4+ and CD8+ T cells [38].

Drug-specific cytotoxic T cells expressing perforin/granzyme and Fas ligand are thought to induce vesicle formation by provoking keratinocyte death [44]. CD8+ T cells express CCR6 as skin homing receptor and synthesize high amounts of the neutrophil-attracting chemokine IL-8 (CXCL-8)[45,46]. The recruitment of neutrophils is observed in the late phase of development of the lesion. In addition, drug-specific T cells produce large amounts of granulocyte-macrophage colony-stimulating factor (GM-CSF), and the majority release IFN-γ and TNF-α [46].

Maculo-papular exanthema

Maculo-papular exanthema is the most common pattern of drug-induced skin eruption that can be induced by a very wide range of drugs [26]. Clinical presentation of MPE is diverse from the very frequent skin erythematous rash mimicking viral eruption to localized or generalized maculo-papular plaques. Histopathological examination shows a superficial, mainly perivascular, mononuclear infiltrate composed of CD3+ T cells, neutrophils and some eosinophils [47]. CD4+ T cells are predominantly noted perivascularly in the dermis, whereas both CD4+ and CD8+ T cells are equally distributed at the dermoepidermal junction zone adjacent to basal keratinocytes [48–50].

The T cell subset responsible for MPE is still a matter of debate. Drug-specific CD4+ and CD8+ T cells are found in the skin and blood [22,25,51,52]. These cells produce both type 1 and 2 cytokines and may exert cytotoxicity. Analysis of acute lesions demonstrated the predominance of CD8+ T cells in the epidermis [13,53]. Recent studies confirmed that CD8+ T cells are a major player in MPE since drug-specific CD8+ cytotoxic T cells were found in the blood of most of patients who have developed a penicillin-induced MPE [19•]. More importantly, analysis of the kinetics of T cell infiltration in the skin of MPE patients during drug-induced skin patch tests demonstrated that CD8+ T cells were recruited very rapidly after drug skin contact, hours before the infiltration of other leukocytes including that of CD4+ T cells [54].

Pathophysiology of T cell-mediated drug allergy

That T cells may recognize small-molecular-weight chemicals has been known since 1935 and the original description of T cell immunity to haptens. Considerable progress in the understanding of the mechanisms by which haptens can prime T cells and induce inflammation has arisen from studies on human allergic contact dermatitis (ACD) and its mouse model, referred to as contact hypersensitivity [55]. In contrast, knowledge of the mechanisms of T cell-mediated allergies to orally or parenterally administered drugs is still only limited because of the variety of the clinical presentations of drug-induced diseases and of the lack of appropriate experimental models. Therefore, the mechanisms by which haptens provoke ACD after epicutaneous administration have served as a paradigm of how similar reactions to haptens may occur after systemic routes [56].

The current paradigm of the pathophysiology of T cell-mediated drug allergy postulates that clinical symptoms develop in sensitized individuals upon drug exposure. Two phases, sensitization and elicitation, are necessary to induce a drug-specific immune response.

The sensitization phase takes place in lymphoid organs

Sensitization of predisposed individuals takes place during oral or systemic administration of a drug. Sensitization requires the uptake of the drug by immature dendritic cells which are activated, process the drug compound and engage a differentiation program to become mature dendritic cells. Processing of the drug by dendritic cells results in its association with amino-acid residues of self-proteins and expression of the drug/peptide complex in the groove of MHC class I and II molecules at the surface of dendritic cells for presentation to CD8+ and CD4+ T cells, respectively. Specific T cell precursors are activated and expand clonally giving rise to several types of T cells with effector and memory functions. As a result, type 1, type 2 or type 17 CD4+ or CD8+ T cells may be generated alone or in combination. Drug-specific T cells

emigrate outside the lymphoid organs through efferent lymphatic vessels and enter the blood via the thoracic duct. At the end of the sensitization phase, specific T cells have gained access to the skin and recirculate between the skin, draining lymph nodes and the blood.

The elicitation phase takes place in the skin and other tissues and is associated with the onset of drug allergic skin symptoms

Following drug administration, free drugs or drugs bound to carrier proteins diffuse into all tissues and in particular the skin. Here they are taken up by skin cells and associated to MHC molecules at the cell surface. They may also bind directly to peptides already in the groove of MHC molecules. All skin cells, especially keratinocytes, express MHC class I molecules in the steady state and can therefore present the drug to specific CD8+ T cells. Skin macrophages and dendritic cells express both MHC class I and MHC class II molecules and can activate both CD8 and CD4+ T cells. Activation of skin trafficking T cells is the first step of the allergic reaction. Depending on the type of specific T cell which has been generated during the sensitization phase, T cell activation may lead to the production of one or several cytokines (type 1, type 2 and/or type 17) by both CD4+ and CD8+ T cells and CD8+ and CD4+ T cell-mediated cytotoxicity of drug-presenting skin cells through either Fas/Fas-L interaction or perforin/granzymeB/granulysin production. The inflammatory products released by specific T cells activate skin cells which in turn contribute to the skin inflammation by producing cytokines and chemokines able to induce additional waves of leukocyte recruitment into the skin, including T cells, dendritic cells/macrophages, neutrophils and eosinophils. Although activation of skin trafficking specific T cells is the first step of the allergic reaction, it is likely that the final histological and clinical picture of the reaction may rather depend on the type of leukocytes which have been recruited during the second step of the drug-induced skin inflammation. For example, the clinical picture of DRESS associated with skin recruitment of eosinophils is very different to that of MPE lesions mainly infiltrated by neutrophils.

Conclusion

Progress in understanding of the pathophysiology of drug allergy will need a careful analysis of the early molecular and cellular events occurring in developing skin inflammatory lesions. Indeed, recent studies of the pathophysiology of T cell-mediated skin allergic reactions have shown that development of a skin lesion is a multistep process involving several T cell subsets which are recruited at different times and act sequentially to induce the clinical lesion [54]. The recent availability of animal models of drug allergy will undoubtedly help towards understanding the mechanisms involved in sensitization to drugs [54].

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