according to the virus, agents, or regimens and suggest that patients receiving systemic corticosteroids are more likely to develop CMV disease and HHV-6-associated clinical symptoms. Thus, although patients with DIHS/DRESS are usually treated with corticosteroids, it remains to be determined which therapy can significantly reduce the degree and duration of virus reactivations and prevent long-term sequelae. In this regard, we have recently demonstrated that long-term sequelae such as autoimmune disease and autoantibody production were much more common in patients with DIHS/DRESS not treated with corticosteroids than in those treated with corticosteroids (29). These findings may indicate that systemic corticosteroid therapy during the acute stage may have served to reduce the risk of subsequently developing autoimmune disease through the beneficial effect of corticosteroids on EBV loads demonstrated here.

In conclusion, our findings justify the frequent monitoring of herpesvirus reactivation, particularly EBV reactivation, in patients with severe drug eruptions, to predict and improve the short-term or long-term outcome, and our findings should be further explored for their variability and validity in a study with a larger sample size.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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Crucial Role of Viral Reactivation in the Development of Severe Drug Eruptions: a Comprehensive Review

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Abstract A growing number of cells, mediators, and pathways have been implicated in severe drug eruptions. Fifteen years ago, we published landmark studies that sparked the current advances in our understanding of the role of viral reactivations in severe drug eruptions. Viral reactivations then became critically important as diagnostic tools, but how precisely they participated in the pathogenesis remained less well-defined. The question of whether viral reactivations are pathogenic or are instead as epiphenomenon of severe tissue damage has plagued the field of drug allergy for some decades. Recent evidence points to a crucial role for tissue-resident memory T (TRM) cells in immune protection against viral infections. Yet immune protection against viral infections is but one side of a coin, the other side of which comprises effector cells capable of mediating severe immunopathology: Once drug antigen is cross-recognized by these T cells, they could be activated to kill surrounding epidermal cells, resulting in drug-induced tissue damage. Such TRM cells could persistently reside in the skin lesions of fixed drug eruptions (FDE) and are most likely a major cell type responsible for the development of FDE. We also discuss the role of regulatory T (Treg) cells in the setting of drug allergy, in which herpesviruses are reactivated in sequence. Although many details of the complicated interactions among viruses, anti-viral immune responses, TRM cells, and Treg cells remain to be elucidated, we review the current status of this rapidly advancing field.

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Introduction

The long-standing question of why drug allergy develops in limited numbers of susceptible individuals who take drugs is still largely unresolved. Many studies have addressed this question: Clinical observations have indicated that drug allergy is often precipitated by viral infections [1, 2]. According to the viral hypothesis, viral infections could predispose genetically susceptible individuals to the subsequent development of drug allergy [3, 4]. The list of viruses triggering or exacerbating drug allergy in susceptible individuals is constantly growing and includes Epstein–Barr virus (EBV), herpes simplex virus (HSV), human herpesvirus 6 (HHV-6), cytomegalovirus (CMV), and varicella-zoster virus (VZV) [5–7]. Fifteen years ago, we [7] and Dr. Hashimoto's group [8] independently published landmark studies that sparked the current advances in our understanding of the role of viral infections in drug allergy. These initial studies have detected HHV-6 DNA by polymerase chain reaction (PCR) in blood and skin specimens from patients with a certain type of drug allergy over a predictable time course, namely 2–3 weeks after onset. However, because HHV-6 detection by PCR was limited to convenience blood samples obtained 2–3 weeks after onset of the drug allergy, there are difficulties in assigning the causality of drug allergy to the virus. What, then, is the meaning of the virus detection at 2–3 weeks after onset of the drug allergy? These findings could be interpreted as indicating the possibility that viruses are involved in acute exacerbations of drug allergy but not in the induction. On the other hand, there is also the growing body of evidence that drug allergy can be

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profoundly influenced by viral infections that occurs before onset of drug allergy, as exemplified by ampicillin rashes in infectious mononucleosis (IM) [1]. Alternatively, virus infection may be additional event that is required for drug sensitization to progress to drug allergy. Thus, the complexity of assigning a pathogenic role to any virus in the development of drug allergy is underscored by the available evidence that the severity and clinical course of drug allergy can be influenced by viral infections that occur before, concurrent with, or subsequent to drug allergy. In this review, we focus primarily on how viral infections and virus-driven immune responses can evoke drug-specific immune responses that are presumably capable of eliciting cell and tissue damage.

Tissue Localization of Resident Memory T Cells After Infection with HSV

Elucidation of the events leading to clearance of infected viruses from skin could be key to our understanding of how a drug-specific immune response can develop after viral infection. Recent studies have clearly shown that after the clearance of viral infection such as HSV, a small fraction of memory T cells persist as a stable population to confer protection upon reencountering the same virus in peripheral tissues such as skin [9–12]. These HSV-specific T cells persist in the skin for at least 6 months after infection with HSV and express CD8, VLA-1, and CD103, molecules important for epithelial localization. These CD8$^+$ T cells, defined as tissue-resident memory T (TRM) cells, are different from CD8$^+$ T cells of the central memory phenotype (TCM) that largely recirculate between the secondary lymphoid organs, in that TRM cells are resident in the epidermis and are confined largely to the original site of infection [9, 10]. These CD8$^+$ TRM cells are phenotypically distinct from TCM cells with low expression of CD62L and CD122 but high expression of CD69 [9]. According to a recent report [11], they show a steady-state crawling behavior in between keratinocytes, and their migratory dendritic behavior allows the detection of antigen-expressing target cells in physiologically relevant time frames of minutes to hours. Interestingly, these CD8$^+$ TRM cells in distant skin sites markedly have been shown to reduce viral loads to levels comparable to those observed at the actual site of previous infection [12]. These CD8$^+$ TRM cells produce effector cytokines such as IFN-γ, persist at the site of infection for many months, and are highly effective at rapidly eliminating virus from the skin. After viral infection through the skin, these CD8$^+$ T cells distribute not only to the site of infection but also throughout the entire skin surface [12], providing long-lived protective T cell immunity against re-infection of the virus. Although these virus-specific CD8$^+$ TRM cells are also found in sensory ganglia, brain, intestinal mucosa, and salivary glands, common features of these

differentially localized TRM cells are the expression of CD103 and of CD69. The salivary glands as well as the skin also harbor virus-specific CD8$^+$ TRM cells uniquely expressing E-cadherin at surprisingly high frequencies after systemic virus infection [13].

Thus, virus-specific CD8$^+$ TRM cells resident in the skin site are thought to regulate whether skin infection with the virus could result in viral control, asymptomatic persistence, or severe pathology. As demonstrated by Jiang et al. and Mackay et al. [12, 14], these skin-resident CD8 TRM cells are long-lived and non-recirculating and are superior to circulating TCM cells at providing rapid long-term protection against cutaneous viral infections ever in the absence of persisting local antigen presentation. Depending on the viral loads in the skin site, virus-specific TRM cells resident in the skin site could have either a beneficial or detrimental role in controlling virus-associated morbidity: At a medium dose of virus, TRM cell-mediated lysis of virus-infected cells contribute to sufficient control of viral burden, while, at a high dose of virus, TRM cells would act detrimentally by severely damaging virus-infected epidermal cells, ultimately resulting in severe T-cell-dependent immunopathology.

CD8$^+$ TRM Cells in the Lesions of Fixed Drug Eruption

The classic fixed drug eruption (FDE) lesions are characterized by a solitary or small number of well-circumscribed, round, and/or oval erythematous macules and plaques with dusky centers on the skin and/or mucous membrane: These lesions usually start abruptly an exactly the same site with each administration of the causative drug [15, 16]. Although the individual FDE lesions are 1–4 cm in diameter and rarely exceed 10 cm, these lesions become more numerous and more severe unless the causative drug is withdrawn. New FDE lesions often develop at the site of viral infection such as HSV and previously traumatized or inflamed skin such as insect bites, burn, and venipuncture sites [17]. A peculiar linear pattern of FDE lesions suggestive of previous herpes zoster (HZ) have been also reported, although it is unclear whether the patient had preceding HZ before onset of FDE [18]. An unusual cellulitis-like FDE has also been reported: An erythematous and edematous plaque with undetermined borders mimicking cellulitis was elicited by the subsequent administration of the causative drug at the same sites [19]. These findings, together with our previous report describing the development of typical FDE lesions at exactly the same site as the patient's previous HSV lesion, suggested to us the possibility that cells with "protective" function may be recruited from the circulation, either nonspecifically or specifically, upon primary insults, such as trauma and viral infections, and they could persist at relatively high frequencies in the lesional

skin and be responsible for the subsequent induction of FDE lesions (Fig. 1). Indeed, innate immune cells such as dendritic cells or $\gamma\delta^+$ T cells and antigen-specific CD4$^+$ and CD8$^+$ T cells are shown to be recruited from the circulation to the inflammatory site such as skin and persist in the epithelium in a number of diverse physiological and pathological settings.

To investigate the possibility that CD8$^+$ T$_{RM}$ cells could persist in FDE lesions, we immunohistochemically character-ized resting FDE lesions long after clinical resolution. FDE lesions typically resolve after discontinuation of the causative drug, leaving hyperpigmentation localized to the sites of pre-vious flare. Such resolved FDE lesions are characterized by a small number of CD3$^+$ CD8$^+$ T cells aligned along the epi-dermal site of the dermoepidermal junction: These T cells persist for a long time in the lesion, referred to as resting FDE lesions, after resolution as a phenotypically homoge-nous, stable population of T cells that constitutively express TCR-$\alpha\beta$, CD45RA, CD103, CLA, CD11b, CD69 but not CD27 and CD56 [15]. In contrast, these T cells are rarely found in the uninvolved epidermis of FDE patients and healthy individuals. This phenotype of T cells most closely resembles that of T$_{RM}$ cells. Our previous studies demon-strated that the CD8$^+$ T cells isolated from the resting FDE lesions and subsequently expanded in vitro displayed cytolyt-ic activity against NK-sensitive or NK-resistant tumor cells and cultured keratinocytes when stimulated in an Ag-nonspecific fashion via CD3/TCR complex [20]. Nevertheless, they are not constitutively cytolytic, unlike NK cells and murine $\gamma\delta^+$ dendritic epidermal T cells (DETC). The intracellular cytokine assay with the use of CD8$^+$ T cells freshly isolated from the resting FDE lesions showed that the great majority (>80 %) of these CD8$^+$ T cells produced IFN-γ and TNF-α upon stimulation while the pro-portion of these T cells producing IL-4 was very low (<1 %). Our in situ PCR studies using FDE lesions obtained 3 h after challenge demonstrated that these CD8$^+$ T$_{RM}$ cells could be induced to express IFN-γ mRNA and protein upon clinical challenge with the causative drug [21]. Their induction of

IFN-γ mRNA was much faster than that of their dermal and peripheral counterparts. Because their rapid production of large amounts of IFN-γ mRNA and protein upon stimulation with the causative drug in vivo was clearly followed by localized epidermal damage, these CD8$^+$ T cells residing in resting FDE lesions are most likely a major cell type respon-sible for the development of FDE [16, 21].

Despite our expectation, however, no convincing evidence is presently available to indicate that the ligands for these CD8$^+$ T$_{RM}$ cells resident in the resting FDE lesions are drug antigens or viral antigens. In this regard, our previous studies demonstrated that some of these CD8$^+$ T$_{RM}$ cells can recog-nize self-proteins [16] but not drug antigens either in a totally major histocompatibility complex (MHC)-dependent or MHC-independent fashion. However, in view of our previous quantitative PCR analysis demonstrating that these CD8$^+$ T$_{RM}$ cells utilized a very limited range of TCR Vα and Vβ gene families as compared with peripheral blood T cells obtained from the same patients [20], we can assume that they can recognize a limited Ag presented by MHC molecules. In this regard, it is noteworthy that a recent report indicates that heterologous virus infections of mice result in a narrow oligoclonal TCR repertoire specific to highly cross-reactive epitopes of different viruses [22]. What causes narrowing of the TCR repertoire in mice following heterologous virus infections remains poorly understood, but the profound narrowing of the TCR repertoire diversity after heterologous virus infections is likely a consequence of expansions of the highly cross-reactive T cell population. Thus, cross-reactivity of CD8$^+$ T cells generated after heterologous virus infections may explain why FDE lesions can be induced at exactly the same site as the patient's previous HSV infection or trauma. Such cross-reactivity of CD8$^+$ T$_{RM}$ cells resident in the epi-thelium may help to control a variety of pathogens early in infection. The most likely explanation for why CD8$^+$ T$_{RM}$ cells originally distributed to the site of infections can be activated by totally unrelated drug antigens is that these CD8$^+$ T$_{RM}$ cells could be broadly cross-reactive with some

Herpetic lesions

FDE lesions

Fig. 1 HSV lesions evolve into FDE lesions. After infection with HSV, a small fraction of CD8$^+$ T$_{RM}$ cells specific for HSV persist as a stable population with antigen-presenting cells at the skin site of HSV infection to confer protection against the same virus. These T cells, once activated with cross-reactive drug antigen, can expand and become effector cells responsible for the induction of FDE

▲ HSV Ag   ＜ Receptor for HSV

Expansion of cross-reactive
CD8+ T$_{RM}$ cells by drug Ag
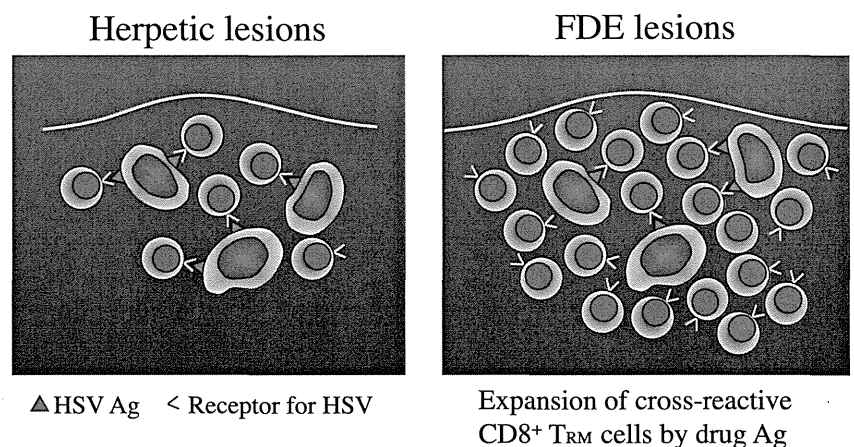
of drug antigens while preserving the fine specificity for a self-MHC-bound peptide such as viral antigen. In support of this possibility, there is now sufficient evidence to indicate that the specificity of a large proportion of antigen-specific self-HLA restricted T cells is also directed toward infectious agents, particularly herpesviruses [23–25]. Additional mechanisms for how cross-reactivity of T cells can be maintained in vivo have been reported in recent studies [26, 27]: These cross-reactive T cells can recognize self- and nonself HLA molecules while maintaining a strong antiviral immune response by recruiting non-cross-reactive T cells to control the virus. Thus, CD8$^+$ T$_{RM}$ cells enriched in resting FDE lesions could have originally evolved to protect epidermal tissue integrity from invading pathogens such as herpesviruses, and once drug antigen is cross-recognized by these T cells because of their broad cross-reactivity, they can be activated to kill surrounding keratinocytes, resulting in localized epidermal damage [15].

Role of Regulatory T Cells in FDE Lesions

The clinical spectrum of FDE is highly variable, ranging from the classic form to a generalized bullous variant with systemic symptoms initially indistinguishable from Stevens–Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN). Despite such clinical similarities between a generalized form of FDE and SJS/TEN, subsequent evolution of the two conditions is quite different: The former resolves spontaneously upon discontinuation of the causative drug, while the latter often results in full-thickness epidermal detachment, rapidly spreading to the whole body. However, less is known about critical events that are needed for preventing further disease progression to SJS/TEN. In this regard, our previous studies demonstrated that recruitment of FoxP3$^+$ regulatory T (Treg) cells into the FDE lesions is crucial for preventing CD8$^+$ T$_{RM}$ and T$_{CM}$ cells from excessively activating at the inflammatory site [28]. These observations suggested that the defect in regulatory mechanisms for preventing further progression to SJS/TEN may reside either within the cutaneous milieu in the inflammatory site, particularly in the border of the lesion, or within migrating Treg cells themselves; this is because the individual erythematous lesions of FDE have well-defined border while the SJS/TEN lesions form poorly defined macules rapidly extending to the perilesional skin.

Our recent unpublished study has demonstrated that FoxP3$^+$ Treg cells obtained from FDE patients at the acute stage retain the suppressive capacity to inhibit proliferation of CD8$^+$ T$_{RM}$ and T$_{CM}$ cells while their function in SJS/TEN patients at the corresponding stage is severely impaired [29], indicating that Treg cells in FDE are fully functional and constitute an important component of protective immunity. We have further demonstrated that FoxP3$^+$ Treg cells

preferentially accumulate beneath the epidermis and at the mid part of the dermis in the periphery of the FDE lesions while those are sparsely distributed in the upper part of the dermis of the periphery of SJS/TEN lesions. These results indicate that timely and selective accumulation of Treg cells in the periphery of FDE lesions could be crucial for preventing excessive activation and recruitment of CD8$^+$ T$_{RM}$ and T$_{CM}$ cells. Indeed, the frequency of Treg cells in the periphery of FDE and TEN lesions correlated well with the degree of protection conferred. These findings emphasize the importance of Treg cell recruitment to the extending edge of the inflammatory site for establishing the Treg response to the greater load of infiltrating Teff cells. We also provide evidence to indicate that mast cells accumulating in the FDE lesions may facilitate the rapid recruitment of Treg cells to the inflammatory sites thereby limiting tissue damage mediated by activation of CD8$^+$ T$_{RM}$ and T$_{CM}$ cells. Consistent with these data, mast cells and Treg cells have been shown to exhibit substantial colocalization in tissues and lymph nodes. Because IL-16 able to attract Treg cells was much more intensely expressed in mast cells detected in the FDE lesions and IL-16 was the only cytokine that increased rapidly in the serum of patients with FDE after clinical challenge (Y Mizukawa et al., unpublished data), we conclude that a timely and proper localization of Treg cells into the specific inflammatory site induced by mast cell-derived IL-16 in the FDE lesions could serve to limit excessive activation of potentially destructive CD8$^+$ T$_{RM}$ and T$_{CM}$ cells, resulting in spontaneous resolution of the FDE lesions.

The Effect of Viral Infections on the Subsequent Development of Drug Allergy

Available evidence strongly suggests that viral infections create a favorable milieu for the initiation and progression of adverse drug reactions [4]. It remains unknown, however, how preceding viral infections induce or contribute to the subsequent development of adverse drug reactions. When considering a complex interaction between viral infection and drug allergy, it is noteworthy that there must be mechanisms that protect the host from excessive immune responses to viruses, which could in themselves lead to greater pathological consequences than the invading viruses. Evidence is recently accumulating that CD4$^+$FoxP3$^+$ Treg cells, either natural or inducible, can inhibit the function of effecter T (Teff) cells at the site of viral infections, thereby inhibiting severe immunopathology. On the other hand, the Treg response may be potentially harmful to the host in terms of infection control because their activation and expansion secure survival of invading viruses for an extended period of time, thereby causing chronic infectious diseases. Numbers and function of Treg cells, therefore, should be controlled depending on the stage of viral

infections. During the early stage of infection, dampening Treg function would result in vigorous anti-viral responses that control infections. Some studies have demonstrated that Treg cells lose their suppressive capacity in response to engagement of virus-sensing mechanisms such as TLR signaling [30]. Alternatively, it has been proposed that, during viral infection, TCM and TRM cells responding to infection would become resistant to Treg-mediated suppression as a result of exposure to proinflammatory cytokines and increased costimulatory signals [31]. At later time points in infection, however, expansion of functional Treg cells is likely to occur to protect overstimulation of the immune system. Thus, a time-dependent balanced, rather than biased, Treg responses would be necessary for host protection and the resolution of infection. One must appreciate, however, the fact that most of previous studies on the role of Treg cells in the setting of viral infections were not extended beyond the acute period of infection to determine how Treg cells were involved in the pathogenesis of virus-induced diseases.

To investigate the role of Treg cells in the context of viral infections, we initially evaluated the frequencies of $CD4^+CD25^+FoxP3^+$ Treg cells in total PBMC of patients with viral infections, such as VZV and parvovirus B19. Although recent studies demonstrated an increase in Treg frequencies in acute dengue [32] and measles infection [33], our results showed no significant alterations in Treg frequencies and their absolute numbers in the setting of these viral infections. These apparently conflicting results suggest that there is more to be learned about the frequency of Treg cells during acute infections: The number of Treg cells during viral infections would be different depending on the virus, virulence, or dose. More importantly, we demonstrated that Treg cells obtained from patients with these viral infections, VZV and parvovirus B19, exhibited a significantly impaired capacity to suppress CD3-driven Teff cell proliferation, as compared with those from healthy controls. The degree of functional defect in patients at the acute stage of these viral infections was comparable to that in patients with TEN, which was previously described by us [29]. Their impaired capacity at the acute stage of these viral infections, however, had returned to a presumed baseline, which was indistinguishable from that of healthy controls, upon clinical resolution. The defect during the acute stage was not due to increased resistance of Teff cells obtained from these patients to Treg-mediated suppression. In contrast, functional activity of the Treg cells obtained from patients with Mycoplasma pneumoniae (MP) remained defective even 1 year after clinical resolution (R Takahashi et al., manuscript submitted). These results indicate that defective Treg function observed during the acute stage of the viral infections and both the acute and resolution stages of MP infections would serve to lower the activation threshold of drug-specific T cells or pathogen-specific T cells, thus facilitating the development of drug allergy. In these viral infections, a loss of Treg function

was transient and the defective Treg cells regained their functional competence upon resolution, while MP infection persistently abrogated Treg functions even after clinical resolution. These results provide an explanation for why patients with MP-associated SJS displayed polysensitivity to multiple drugs with different structures that cannot be easily explained by drug antigen-driven T cell activation [34]. Thus, viral or MP infections are likely to be prime candidates for subsequently developing drug allergy in susceptible individuals, probably through a transient or persistent loss of Treg functions.

Viral Reactivation in Drug-Induced Hypersensitivity Syndrome

Several drug eruptions encompass several distinct clinical entities, the most serious being TEN/SJS. Drug-induced hypersensitivity syndrome (DiHS), also referred to as drug reaction with eosinophilia with systemic symptoms (DRESS), represents the opposite end of a spectrum of severe drug eruptions. DiHS/DERSS offers a unique opportunity to link between viral infections and the development of severe drug eruptions, due to its strong association with HHV-6 infection [7, 8]. This syndrome has several unique features that cannot be solely explained by a drug Ag-driven, oligoclonal T cell activation: The delayed onset in relation to the introduction of the causative drug is one of the important features of this syndrome that can be distinguished from other types of drug eruptions, which usually start 1–2 weeks after starting therapy. This syndrome typically occurs with fever and cutaneous lesions 3 weeks to 3 months after starting therapy with a limited numbers of drugs, mainly anticonvulsants. Importantly, more severe reactions often occur 3–4 days after withdrawal of the causative drugs: This paradoxical worsening is also characteristic of DiHS and may be mistaken for severe infectious diseases. Patients with DiHS often show unexplained cross-reactivity to multiple drugs with different chemical structures, including those starting after onset of symptoms. In addition, variable clinical symptoms, such as renal and liver symptoms, continue to deteriorate one after another even for weeks after stopping the causative drug. Although maculopapular or erythematous eruptions are initially observed on the face, upper trunk, and upper extremities, most erythematous macules do not evolve into blisters and no mucous membrane involvement is usually seen [15, 35, 36].

The peripheral blood usually shows marked leukocytosis with atypical lymphocytosis or eosinophilia of various degrees in most of cases, although in some cases leucopenia or lymphopenia may precede the leukocytosis. A dramatic decrease in serum IgG, IgA, and IgM levels is typically observed at onset, and the lowest levels are usually seen a week after withdrawal of the causative drug. Despite such variable

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clinical presentations and courses, HHV-6 reactivations can be detected at a particular time point, 2–3 weeks after onset of rash in the vast majority of patients regardless of treatment [15, 36]: A strong association between HHV-6 reactivations and this syndrome has been supported by a large number of independent groups over the years in Japan [36, 37]. This is the reason why HHV-6 reactivations as evidenced by the rise in anti-HHV-6 IgG titers and HHV-6 DNA levels can be used to confirm a clinical diagnosis of DiHS [38]. Although HHV-6 was initially thought to be the only virus reactivated during the course of DiHS, recent studies of real-time measurements for viral loads have demonstrated that other herpesviruses, such as EBV, HHV-7, and CMV, are also reactivated in sequence during the course of the disease as demonstrated in graft-versus-host diseases (GVHD) [35, 39] (Fig. 2). According to our sequential analysis of viral loads in patients with DiHS, the cascade of reactivation events initiated by HHV-6 or EBV would extend, with some delay, to HHV-7 as well and eventually to CMV [39] (Figs. 2 and 3). Consistent with the previous observations that the severity of GVHD was correlated with the levels of HHV-6 DNA [40], the magnitude of HHV-6 reactivation as evidenced by the increase in HHV-6 DNA levels was correlated well with the severity of inflammatory responses that occur in vivo in patients with DiHS [15, 41]. These findings provide strong evidence to suggest the role of HHV-6 or other herpesviruses in the etiology of the disease, rather than a mere bystander, although reactivation of these viruses as a result of a transient immune dysfunction cannot be definitely excluded. Because of the unique biological properties of herpesviruses, particularly their "immunotropic" nature, and their possible interactions with other herpesviruses, they may have detrimental effects on the immune system once reactivated in the course of the disease. Investigators have been hampered by difficulty in assigning a pathogenic role to any herpesvirus in patients with DiHS who manifest clinically variable symptoms in different organs. Thus, despite rapid advances in the biology and genetics of herpesviruses, progress in understanding the pathogenic role of these herpesviruses has not come easily.

How, then, can the etiological role of herpesviruses be confirmed? One relevant observation from years of research on the role of immune responses against EBV is that cutaneous and visceral symptoms of DiHS/DRESS are mediated by activated CD8$^+$ Teff cells which are largely directed against herpesviruses, such as EBV, and that the causative drug can reactivate herpesviruses in vitro [42]. The result of this study indicates the possibility that herpesvirus reactivations triggered by the causative drug could have the immunopathogenic role in DiHS/DRESS but not a mere epiphenomenon of the underlying immunodeficiency.

Role of Treg Cells in DiHS

Our recent study clearly demonstrates that the acute stage of DiHS/DRESS is characterized by dramatic expansions of fully functional CD4$^+$FoxP3$^+$ Treg cells while their suppressive capacity is profoundly impaired in the acute stage of SJS/TEN [43]. Although it is difficult to determine when Treg expansions occur before the development of DiHS, this expansions of Treg cells would occur far before onset of DiHS, which would contribute to not only the delayed onset but also to viral reactivations [36]. In order to counterbalance activating Teff cells, expansions of Treg cells are likely to be key for maintaining a healthy balance between protection and immunopathology. However, once the balance has been disturbed toward activation of Teff cells, DiHS ensues (Fig. 4). Thus, the expanded Treg cells would also limit the severity of Teff cell-mediated immunopathology, which is reflected by the observation that epidermal damage can be rarely detected in the

Fig. 2 Sequential reactivation of various herpesviruses during the courses of DiHS and GVHD. Importantly, herpesviruses are reactivated in DiHS in the fundamentally same order as in GVHD
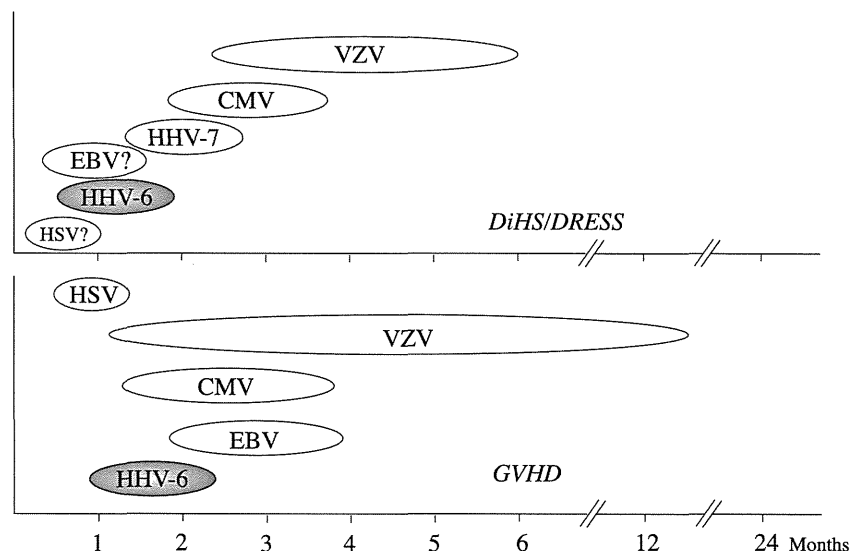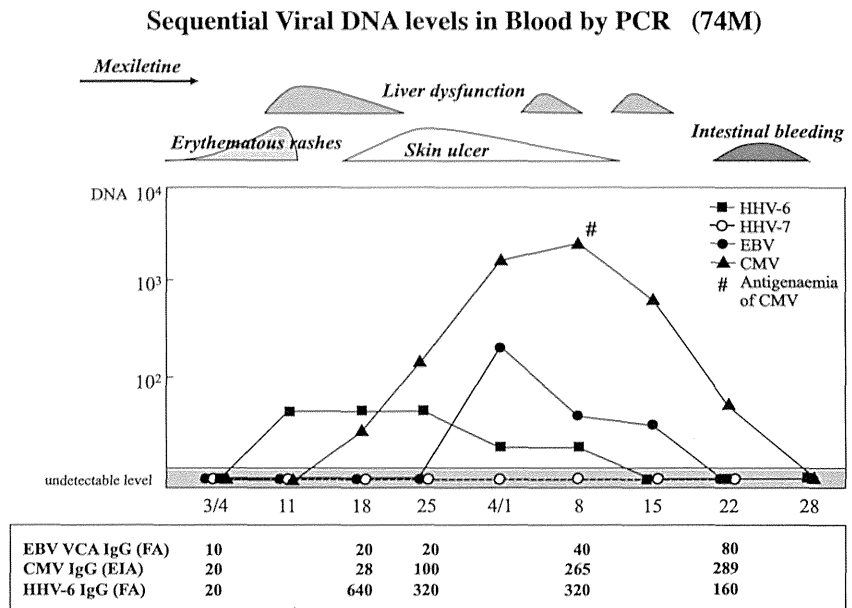
Fig. 3 Relation between clinical symptoms and herpesvirus viral DNA loads and anti-viral antibody titers in a representative case with DiHS due to phenobarbital

Sequential Viral DNA levels in Blood by PCR (74M)

	3/4	11	18	25	4/1	8	15	22	28
EBV VCA IgG (FA)	10		20	20		40		80	
CMV IgG (EIA)	20		28	100		265		289	
HHV-6 IgG (FA)	20		640	320		320		160	

skin lesions of DiHS. The expanded population of Treg cells in the peripheral blood of DiHS patients during the acute stage is likely the inducible Treg (iTreg) cells that are induced in the periphery under specific conditions of cytokine and antigen [43]. iTreg cells can be produced from CD4$^+$CD25$^-$ T cells by culture with antigen and TGF-β or IL-10 and TGF-β, while IL-6 inhibits iTreg induction and promotes Th17 [44, 45]. Consistent with this view, our preliminary study shows that in vitro culture with the causative drug of peripheral blood lymphocytes from DiHS patients after resolution results in expansions of Treg cells (unpublished observation). Importantly, a gradual loss of Treg-cell function occurs after the resolution of DiHS, although it remains unknown when and how it occurs: Expanded Treg cells, upon their contraction, may become functionally exhausted and loss their

essential functional activity necessary for immune protection. Such functional exhaustion is likely to result from repeated activation by Treg cells frequently occurring during the courses of DiHS and is a way of limiting the magnitude of Treg cell responses, which may compromise effective immunity against infections agents. Indeed, reflecting a loss of Treg-cell function after resolution, several autoimmune diseases such as type 1 diabetes mellitus, thyroiditis, SLE, and sclerodermoid GVHD-like disease [46] have been reported to develop at intervals of several months to years after clinical resolution of DiHS [35, 47] (Fig. 5). In view of the finding that the imbalance between the Treg and Teff-cell compartments has been shown to trigger the development of autoimmune disease, resolution of DiHS may be accompanied by a shift away from Treg differentiation and toward IL-17-
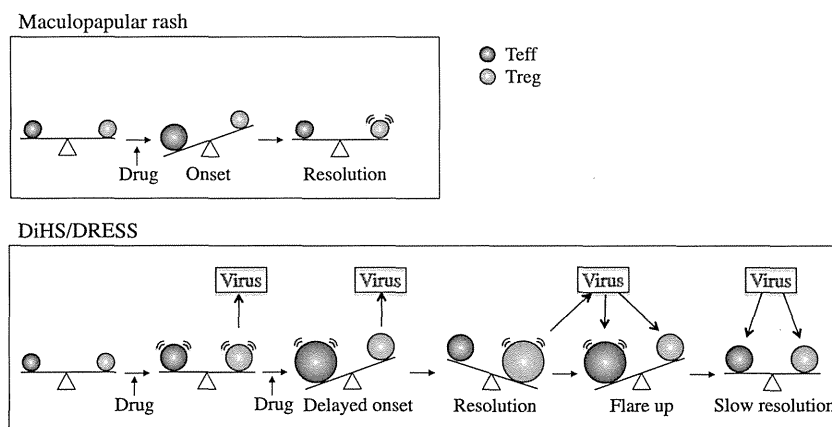

Fig. 4 A hypothetical model for the development of DiHS and maculopapular type of drug eruption. In maculopapular rash, Treg cells are overwhelmed with expansions of Teff cells. In DiHS, protracted use of the causative drug results in expansions of not only Teff but also Treg cells, thereby delaying onset and causing viral reactivation. Eventually,

however, the delicate balance between Teff and Treg is disturbed toward activation of Teff cells, leading to onset of DiHS. Systemic corticosteroids can improve clinical symptoms, probably by potentiating Treg-cell function

Fig. 5 Acute and resolution
stages of DiHS. After resolution
of clinical symptoms, Treg cells
expanded during the acute stage
are contracted and become
exhausted in function, thereby
triggering autoimmune diseases

DiHS/DRESS
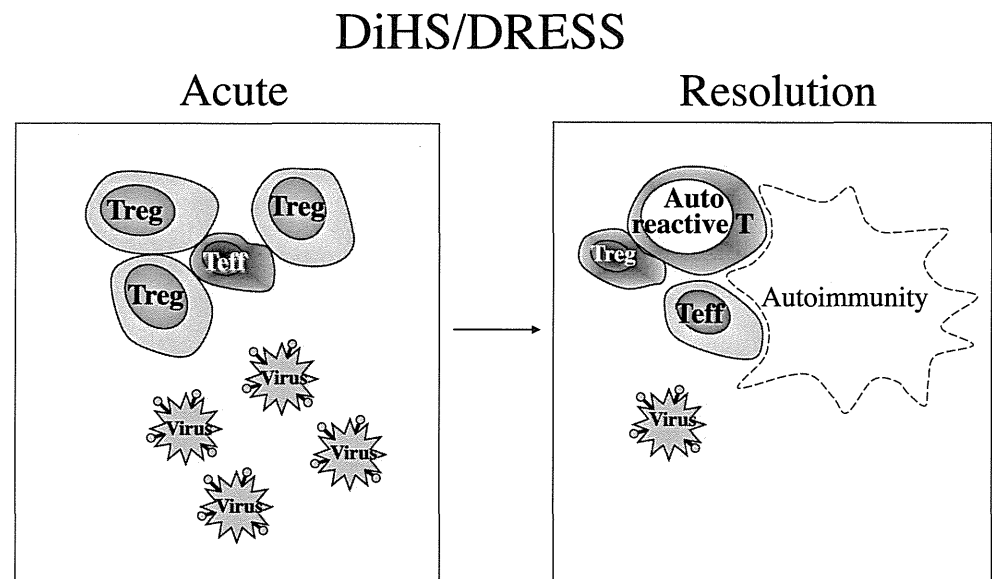
Acute

Resolution

producing (Th17) cell differentiation. We therefore measured the frequencies of Treg cells and Th17 cells within circulating $CD4^+$ T cells during the acute stage and again long after resolution. We found that Th17 cells were increased in frequency coincident with the decrease in Treg cell frequency upon resolution in DiHS. A significant increase in various autoantibody titers such as anti-nuclear antibody (ANA) and anti-thyroglobulin antibody was specifically observed in patients with DiHS after resolution, which likely reflects a shift to Th17 cell differentiation (unpublished data).

Longitudinal Analyses of Herpesvirus Loads in Severe Drug Eruptions

Although sequential reactivations of several herpesviruses have exclusively been demonstrated during the acute stage of DiHS, no previous studies were extended beyond the acute stage of the stage. We therefore sought to investigate whether herpesvirus reactivations could be observed in SJS/TEN and beyond the acute stage of both diseases. EBV, HHV-6, and CMV DNA loads were sequentially determined during a 2-year period after onset. Our quantitative PCR analysis revealed persistently elevated EBV loads in patients with SJS during the acute stage and long after clinical resolution [48]. In contrast, only a fraction of patients with DiHS/DRESS had increased levels of EBV DNA in the blood at onset. In many patients with SJS, increased EBV DNA persisted for up to 2 years after resolution. These results suggested that patients with high EBV DNA loads may be at risk of subsequently developing SJS, although we could not totally exclude the alternative possibility that the aggressive clinical course observed during the acute stage of SJS may be responsible for EBV reactivations. However, this alternative possibility is unlikely because the degree of the EBV loads in

patients with SJS did not correlate with the severity of clinical symptoms and laboratory abnormalities. Surprisingly, we noted that no patients with TEN demonstrated elevated EBV loads during the acute stage and after clinical resolution [48]. In view of clinical similarities between SJS and TEN, differences in the pattern of the viral loads between them were surprising and could be interpreted as indicating the possibility that these two diseases may be distinct in the pattern of persistent viral infections although they may share important common pathophysiologic processes [49].

Increased EBV, CMV, and HHV-6 loads only occurred during the acute stage and a post 100-day period in patients with DiHS/DRESS [48]. Nevertheless, the dynamics of EBV, CMV, and HHV-6 reactivation varied considerably in these patients according to the use of systemic corticosteroids. Although CMV and HHV-6 DNA loads were higher in those receiving systemic corticosteroids than those not receiving them, EBV DNA loads were significantly higher in those without them [48]. Interestingly, the increase in various autoantibody titers, which was detected 1 year after the resolution of DiHS/DRESS, was associated with the elevated EBV loads during the acute stage of DiHS/DRESS and preferentially observed in patients not receiving systemic corticosteroids [50]. These results could be interpreted as indicating that the use of systemic corticosteroids during the acute stage of DiHS may serve to prevent the progression to autoimmune disease as long-term sequelae of DiHS/DRESS, probably through the decrease in EBV DNA loads. Similar observations have been also noted in the generation of autoantibodies to epidermal proteins, periplakin (unpublished data). Consistent with the results of autoantibodies such as ANA, the generation of autoantibodies to periplakin was preferentially observed in patients with DiHS/DRESS who were not treated with systemic corticosteroids. These findings suggest that immune responses

preventable with systemic corticosteroids and/or increased EBV DNA loads could trigger the subsequent generation of autoantibodies to periplakin and that early resolution by systemic corticosteroids may lead to better long-term outcomes for patients at risk of subsequently developing autoimmune disease.

Management of Patients with Severe Drug Eruptions Associated with Viral Reactivations

Physicians, when treated patients with severe drug eruptions, need to be aware of underlying viral infections, particularly herpesvirus infections, as one of the most important aspects of management of these patients. Because those patients often receive immunosuppressive agents either early or later in the course of their illness, a wealth of information on the interaction between herpesviruses and immune responses should be gathered to better manage those patients.

When we consider how to better manage those patients, one must appreciate the concept of immune reconstitution syndrome (IRS) [51–53]. IRS is an increasingly recognized disease concept and is observed with a broad spectrum of immunosuppressive therapy-related opportunistic infectious diseases and severe drug eruptions complicated by viral reactivations. Increased occurrence of opportunistic infections associated with defects in the immune system was generally recognized as a result of microbial damage afflicted by these pathogens. Contrary to this belief, an intriguing aspect that has received little attention so far is that restoration of hose immunity may also have adverse sequelae, particularly when it occurs abruptly and rapidly. Indeed, when the timing of onset of an adverse event was carefully assessed in patients infected with HIV, the onset of this event was concentrated within 6–14 days of starting antiretroviral therapy (ART) [54], coincident with restoration of host $CD4^+$ T cell number and reactivity. This clinical deterioration observed after starting HAART therapy was originally called IRS. This syndrome develops not only in patients with HIV infection but also in non-HIV immunocompetent hosts, such as patients with severe drug eruptions and those on immunosuppressive therapy, upon reduction or withdrawal of immunosuppressive agents or chemotherapy. Recently, IRS has also been reported to develop in lymphopenic and neutropenic patients [49] and patients receiving tumor necrosis factor (TNF) α inhibitors [55–58]. Clinical illness consistent with IRS includes tuberculosis, herpes zoster, herpes simplex, CMV infections, and sarcoidosis [53]. The manifestations of IRS are diverse and depend on the tissue burden of the preexisting infectious agents during the immunosuppressive state and the nature of the immune system being restored. Because in some cases IRS is self-limited within a week without any therapy while others are fatal or life-threatening, management of this syndrome should be decided on an individual basis (Table 1).

The clinical characteristics of IRS modified from criteria proposed by Shelburne et al. [59] are as follows: (1) paradoxical deterioration of preexisting infectious disease a attributable to the recovery of the immune system; (2) a decrease in the dose of pathogens, e.g., viral loads, with or without an increase in $CD4^+$ T cell counts; (3) clinical symptoms not explained by a newly acquired infection, by the expected clinical course of a previously recognized infectious agents, or by side effects of therapy; and (4) any event occurring after initiation of ART or after withdrawal or reduction of immunosuppressive agents including biologics, regardless of whether patients are HIV-positive or HIV-negative. In view of the observations that paradoxical worsening of clinical symptoms associated with reduction in viral loads is typically observed after withdrawal of the causative drug at onset of DiHS [15, 16, 35, 36], DiHS is likely a manifestation of the newly observed IRS [35, 51]. Various clinical observations in DiHS/DRESS could be explained by assuming that rapid restoration of pathogen-specific immunity after withdrawal of the causative drug with immunosuppressive properties, as described previously [15, 16, 53], would serve to reduce viral loads at onset, thereby rendering them undetectable in the blood. This consideration could explain why any herpesvirus DNA can be hardly detected at onset of DiHS/DRESS.

Systemic corticosteroids have been the mainstay of treatment for IRS and are the only treatment for which clinical trial data exist [53]. However, there have been no clear guidelines for how patients with IRS are treated with systemic corticosteroids. Because a mild form of IRS can respond to specific treatment for the underlying pathogens, immunosuppressive therapy is not generally needed, and the management is predominantly supportive. In patients with severe forms of IRS, however, immunosuppressive therapies in addition to antimicrobial therapies are necessary to ameliorate clinical symptoms [53]. In case of DiHS/DRESS, anti-microbial therapies should be avoided because they may increase the risk of

Table 1 Clinical illness consistent with IRS

Mycobacterium avium complex infection
Tuberculosis
Cryptococcosis
Herpes simplex
Herpes zoster
Hepatitis C virus infection
Hepatitis B virus infection
Cytomegalovirus infection
Sarcoidosis
Graves disease
Hashimoto thyroiditis
Drug-induced hypersensitivity syndrome

Modified from [53]

developing additional drug rashes due to cross-reactivity to multiple drugs, which has been reported to occur. During the course of DiHS/DRESS, systemic corticosteroids gave promising results in terms of ameliorating vigorous restoration of immune responses to pathogens, which is reflected in the clinical manifestations. Nevertheless, once systemic corticosteroids have started, drug dose should be reduced gradually upon resolution of clinical manifestation. We have to recognize that patients under immunosuppressive therapy, particularly those with DiHS/DRESS, are at greater risk of subsequently developing the wide spectrum of IRS ranging from herpes zoster to fatal CMV disease [41]. Our frequent monitoring of viral loads in the course of DiHS/DRESS revealed that the increase in CMV DNA loads coincided with a tapering of corticosteroid dose. This finding indicates that tapering corticosteroids more gradually over a prolonged period of time may help to limit the severity of IRS. The usual dose for the treatment of DiHS/DRESS is prednisolone 40–60 mg/kg. This dose needs to be tapered over 8–12 weeks to prevent the relapse of various symptoms as manifestation of IRS. The pattern of viral reactivations enhanced upon immune restoration would be different depending on the virus, immunosuppressive agents, or regimens. Given the ability of corticosteroids to reduce the EBV loads in patients with DiHS/DRESS, patients who are at risk of subsequently developing EBV-associated autoimmune disease may benefit from systemic corticosteroids.

Conclusion

Although we know that sequential reactivations of herpesviruses occur in many patients with DiHS/DRESS and that increased EBV loads during the course of the disease may lead to the generation of autoantibodies, the causal role of herpesviruses in the development of severe drug eruptions, if any, remains to be defined. Together with the current knowledge of anti-viral immune responses, we are now in a position to dissect the relative contribution of these responses to protective immunity and immunopathology.

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POSITION PAPER

International Consensus on drug allergy

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Abstract

When drug reactions resembling allergy occur, they are called drug hypersensitivity reactions (DHRs) before showing the evidence of either drug-specific antibodies or T cells. DHRs may be allergic or nonallergic in nature, with drug allergies being immunologically mediated DHRs. These reactions are typically unpredictable. They can be life-threatening, may require or prolong hospitalization, and may necessitate changes in subsequent therapy. Both underdiagnosis (due to under-reporting) and overdiagnosis (due to an overuse of the term 'allergy') are common. A definitive diagnosis of such reactions is required in order to institute adequate treatment options and proper preventive measures. Misclassification based solely on the DHR history without further testing may affect treatment options, result in adverse consequences, and lead to the use of more-expensive or less-effective drugs, in contrast to patients who had undergone a complete drug allergy workup. Several guidelines and/or consensus documents on general or specific drug class-induced DHRs are available to support the medical decision process. The use of standardized systematic approaches for the diagnosis and management of DHRs carries the potential to improve outcomes and should thus be disseminated and implemented. Consequently, the International Collaboration in Asthma, Allergy and Immunology (iCAALL), formed by the European Academy of Allergy and Clinical Immunology (EAACI), the American Academy of Allergy, Asthma and Immunology (AAAAI), the American College of Allergy, Asthma and Immunology (ACAAI), and the World Allergy Organization (WAO), has decided to issue an International CONsensus (ICON) on drug allergy. The purpose of this document is to highlight the key messages that are common to many of the existing guidelines, while critically reviewing and commenting on any differences and deficiencies of evidence, thus providing a comprehensive reference document for the diagnosis and management of DHRs.

Drugs can induce several different types of immunological reactions that, together with nonallergic drug hypersensitivity reactions (DHRs), comprise 15% of all adverse drug reactions (1). Nonallergic DHRs resemble allergy, but without any proven immunological mechanism.

Drug hypersensitivity reactions are of significant concern for clinicians and patients and are also a significant cause of the postmarketing withdrawal of drugs (2). Even though urticarial and maculopapular eruptions are the most frequent manifestations, there are many other clinical presentations (1). DHRs affect more than 7% of the general population and therefore represent an important public health problem (3). Both underdiagnosis (due to under-reporting (3, 4)) and overdiagnosis (due to an overuse of the term 'allergy', for example, in the presence of symptoms due to co-existing factors such as infections (3, 5)) are potential problems. Misclassification based on the DHR history alone may limit therapeutic options and can lead to the use of more-expensive and potentially less-effective drugs (6). Moreover, one drug allergy may lead to the misconception that the patient is allergic to all drugs.

Few guidelines and/or consensus documents are available to support medical decision making on all aspects of DHR. These documents vary in scope and methodology: They are national (6–10), regional, or international (11–22); concern one specific drug class (7, 8, 14–16, 18, 20, 21, 23); focus specifically on evaluation tools/management (11–13, 17, 19, 23); or are more general (6, 8, 24, 25). Although there is no doubt that the use of common systematic approaches for the diagnosis and management of DHRs can considerably improve outcomes, worldwide dissemination and implementation remain major challenges. For these reasons, the International Collaboration in Asthma, Allergy and Immunology (iCAALL) (26), recently formed by the European Academy of Allergy and Clinical Immunology (EAACI), the American Academy of Allergy, Asthma and Immunology (AAAAI), the American College of Allergy, Asthma and Immunology (ACAAI), and the World Allergy Organization (WAO), has decided to proceed with the compilation of an International CONsensus (ICON) on drug allergy. The purpose of this document is to highlight the key messages that are common to the existing guidelines, while critically reviewing and commenting on any differences, thus providing a comprehensive reference to be disseminated more widely. As for the ICON on pediatric asthma (27), unmet needs, research, and guideline update recommendations are generated.

Methodology

A working committee was formed and approved by the current board of iCAALL and the participating organizations. The criteria used for the formation of the committee were as follows: regional representation, relevance to the field, and previous participation in drug allergy guidelines. The members of the committee proposed relevant documents for appraisal. These included (i) the AAAAI/ACAAI/Joint Council of Allergy, Asthma and Immunology drug allergy updated practice parameters (6, 7), (ii) the WAO drug allergy initiatives (24, 25), (iii) the British Society of Allergy and Clinical Immunology (BSACI) guidelines (8, 9), and (iv) the many task force reports and consensus documents of the EAACI Drug Allergy Interest Group (DAIG) as well as its core group, the European Network of Drug Allergy (ENDA) (11–21, 23). Each member was responsible for the preparation of text and relevant tables comparing the included documents in a specific domain. A draft was subsequently compiled and circulated (in September 2012) among the authors for comments and corrections. The revised document was then sent (in April 2013) to an independent reviewing committee, selected on the basis of their publications over the past 5 years in top peer-reviewed journals as first/last authors. Their comments were taken into account in the final draft, which was then approved by the governing boards of the participating organizations. Recommendations were extrapolated from the reference documents and presented using levels of Evidence A–D (28) (Table 1).

Definition and classifications of drug hypersensitivity reactions

Definition

Drug hypersensitivity reactions (DHRs) are the adverse effects of pharmaceutical formulations (including active drugs and excipients) that clinically resemble allergy (29) (Box 1). DHRs belong to type B adverse drug reactions, which are defined by the World Health Organization as the dose-independent, unpredictable, noxious, and unintended response to a drug taken at a dose normally used in humans (30, 31). A-type reactions, including overdoses and pharmacological reactions, are dose dependent and predictable. However, some dose dependence has been shown repeatedly in DHRs (e.g., for nonsteroidal anti-inflammatory drugs (NSAIDs), antiepileptic drugs) and some are

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Abbreviations

AAAAI, American Academy of Allergy, Asthma and Immunology; ACAAI, American College of Allergy, Asthma and Immunology; AGEP, acute generalized exanthematous pustulosis; AIDS, acquired immunodeficiency syndrome; BSACI, British Society of Allergy and Clinical Immunology; CD, cluster of differentiation; DAIG, Drug Allergy Interest Group; DHR(s), drug hypersensitivity reaction(s); DPT(s), drug provocation test(s); EAACI, European Academy of Allergy and Clinical Immunology; EBV, Epstein–Barr virus; FcεRI, high-affinity IgE receptor; FDE, fixed drug eruption; HHV, human herpes virus; HIV, human immunodeficiency virus; HLA, human leukocyte antigen; HSS/DRESS/DiHS, hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms/drug-induced hypersensitivity syndrome; iCAALL, International Collaboration in Asthma, Allergy and Immunology; ICON, International CONsensus; IgE, immunoglobulin E; MCH, major histocompatibility complex; MDH, multiple drug hypersensitivity; NMBA, neuromuscular-blocking agents; NPV, negative predictive value; NSAID(s), nonsteroidal anti-inflammatory drug(s); RCM, radiocontrast media; SDRIFE, symmetrical drug-related intertriginous and flexural exanthema; SJS, Stevens–Johnson syndrome; TEN, toxic epidermal necrolysis; TNF-α, tumor necrosis factor alpha; WAO, World Allergy Organization.

Table 1 Recommendations for DHR diagnosis and management

No.	Statement	Levels of evidence	Grade of recommendation	References
R1	Lifelong avoidance of the drug and cross-reactive drugs is recommended when drug-induced anaphylaxis has occurred	4	D	(6, 102)
R2	The specific allergy work-up should be carried out 4–6 weeks after complete resolution of all clinical symptoms and signs of a suspected DHR	4	D	(12, 13)
R3	Sensitivity and predictive values of skin tests vary among drug classes: from 'good' for immediate DHRs to β-lactam antibiotics, muscle relaxants, platin salts and heparins, to moderate to low for most other drugs	2++	B	(22)
R4	Skin testing is helpful for diagnosis of immediate DHRs to iodinated RCM	2+	C	(16, 22)
R5	A DPT is the gold standard for the identification of the drug eliciting a DHR	2+	C	(6, 8, 13)
R6	For DPT, the oral route is preferred whenever possible	3	D	(6, 8, 13)
R7	Contraindications must be observed before performing DPT, and immediate treatment available allowing complete and rapid recovery	4	D	(6, 8, 13)
R8	Patients who suffered severe immediate reactions to β-lactams and who displayed negative results at the first evaluation, which included a DPT, can be considered for retesting 2–4 weeks after initial evaluation	2–	D	(6, 18)
R9	For currently available biological methods to diagnose drug allergy, a negative test does not exclude the imputability of the drug, whilst a positive result shows sensitivity to the drug but does not reliably confirm causality	2+ for ß-lactams 2– to 3 for others	C	(6, 8)
R10	HLA-B*5701 screening reduces the risk of DHR to abacavir and is mandatory before starting treatment	1++	A	(47) Not rated in previous consensuses (6–23)
R11	An indicative, regularly updated list of drugs to avoid and the list of possible alternatives should be given to patients with a DHR	4	D	(8)
R12	The search for safe alternatives may require DPTs in a hospital setting when the alternatives belong to the same drug class	2+	C	(6, 8, 13)
R13	Specific questioning for a history of drug allergy by every clinician prior to issuing a prescription is essential from both a medical and a medico-legal view-point	4	D	(6, 8, 20)
R14	Preventive measures by pre-medication (e.g. slow injection and pre-treatment with glucocorticosteroids and H1-antihistamines) are useful mainly for non-allergic DHRs, but corticosteroids and H1-antihistamines may not reliably prevent IgE-dependent anaphylaxis	2+	C	(6, 8, 102)
R15	In the absence of generally accepted protocols for drug desensitization in cases of immediate DHRs, reference to successfully applied existing protocols is recommended	2+	C	(6, 19)
R16	Desensitization to aspirin as a therapeutic intervention may be considered in selected asthmatic patients with aspirin exacerbated respiratory disease or nasal polyps	2–	D	(6, 19)

DHR(s), drug hypersensitivity reaction(s); RCM, radiocontrast media; DPT(s), drug provocation test(s); HLA, human leukocyte antigen.

predictable due to the disease state (e.g., human immunodeficiency virus (HIV) infection/acquired immunodeficiency syndrome (AIDS), Epstein–Barr virus (EBV) infection) or a similar previous reaction to the same drug or drug class.

Only when a definite immunological mechanism (either drug-specific antibody or T cell) is demonstrated, these reactions should be classified as drug allergy. For general communication, when an allergic drug reaction is suspected,

DHR is the preferred term, because true drug allergy and nonallergic DHR (29) may be difficult to differentiate based on the clinical presentation alone, especially in cases of acute severe DHR.

Box 1: Definition of drug hypersensitivity reactions

1 Drug hypersensitivity reactions (DHRs) are adverse effects of drugs that clinically resemble allergic reactions.
2 Drug allergies are DHRs for which a definite immunological mechanism (either drug-specific antibody or T cell) is demonstrated.
3 For general communication, when a drug allergic reaction is suspected, DHR is the preferred term.

Classifications

The classification of DHRs is challenging because, for many drugs and clinical presentations, the underlying mechanism is poorly understood (Box 2). A generally accepted classification should facilitate the comparison of studies and help to enhance and validate diagnostic techniques.

Box 2: Classification of drug hypersensitivity reactions

1 Drug hypersensitivity reactions (DHRs) are heterogeneous.
2 Clinically, DHRs can be classified as:
  a Immediate DHRs (urticaria, angioedema, rhinitis, conjunctivitis, bronchospasm, gastrointestinal symptoms [nausea, vomiting, diarrhea, abdominal pain], anaphylaxis, anaphylactic shock); they typically occur within 1–6 h after the last drug administration.
  b Nonimmediate DHRs (delayed urticaria, maculopapular eruptions, fixed drug eruptions, vasculitis, toxic epidermal necrolysis, and Stevens–Johnson syndrome, drug reaction with eosinophilia and systemic symptoms (DRESS), acute generalized exanthematous pustulosis and symmetrical drug-related intertriginous and flexural exanthemas; internal organs can be affected either alone or with cutaneous symptoms (DRESS, vasculitis) and include hepatitis, renal failure, pneumonitis, anemia, neutropenia, thrombocytopenia); they may occur at any time as from 1 h after from the initial drug administration.
3 Mechanistically, DHRs can be defined as allergic (Table 2) and nonallergic.

Clinically, DHRs are commonly classified as immediate or nonimmediate/delayed depending on their onset during treatment (18). Immediate DHRs are possibly induced by an IgE-mediated mechanism and occur within 1–6 h after the last drug administration (32) (Fig. 1). Typically, they occur within the first hour following the first administration of a new course of treatment. They usually manifest as isolated symptoms such as urticaria, angioedema, conjunctivitis, rhinitis, bronchospasm, gastrointestinal symptoms (nausea, vomiting, diarrhea, abdominal pain), or as anaphylaxis or

anaphylactic shock. In certain guidelines, when DHR symptoms are systemic, non-IgE-dependent, and mimicking anaphylaxis, they are designated as 'anaphylactoid' reactions (6). This is no longer the case in EAACI and WAO (29) guidelines, where the term 'nonallergic DHRs' is preferred. Nonimmediate DHRs may occur any time as from 1 h after the initial drug administration. They commonly occur after many days of treatment and are often associated with a delayed T-cell-dependent type of allergic mechanism. Maculopapular exanthemas and delayed urticaria are the most common clinical presentations of nonimmediate DHRs. Although artificial, this classification is very important in clinical practice for workup planning. In any case, a precise description of the morphology and chronology of the reaction is mandatory. But there are still limitations, because other factors such as the route of administration, the role of drug metabolites, and the presence of co-factors or co-prescribed drugs may accelerate or slow down the onset or progression of a reaction (32) (Fig. 1).

Mechanistically, drugs are capable of inducing all of the types of immunological reactions described by Gell and Coombs (33), but the most common are IgE- and T-cell-mediated reactions (Table 2). Certain drugs, such as antiepileptic drugs and allopurinol, cause mainly T-cell-mediated reactions, while others, such as neuromuscular-blocking agents (NMBA), provoke mainly IgE-mediated reactions. Some of the others (e.g., β-lactams) may lead to both types of reaction.

Pathogenesis and pathophysiology

Immune/allergic and nonimmune/nonallergic DHRs

Drug allergies are adverse reactions whereby antibodies and/or activated T cells are directed against the drugs or against
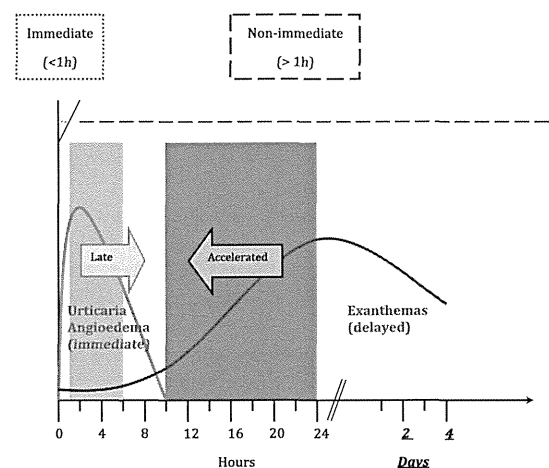

Figure 1 Chronology of DHRs. The separation at 1 h into immediate or nonimmediate reactions although it may not sufficiently reflect the extension of the pathophysiologically determined immediate-type reactions up to 6 h (Late) and the delayed-type clinical manifestations occasionally starting as early as 8–12 h (Accelerated) facilitates the comparison of studies and should help to enhance and validate diagnostic techniques (adapted from (32) with permission).

Table 2 Classification of drug allergies (adapted from (33))

Type	Type of immune response	Pathophysiology	Clinical symptoms	Typical chronology of the reaction
I	IgE	Mast cell and basophil degranulation	Anaphylactic shock Angioedema Urticaria Bronchospasm	Within 1 to 6 h after the last intake of the drug
II	IgG and complement	IgG and complement-dependent cytotoxicity	Cytopenia	5–15 days after the start of the eliciting drug
III	IgM or IgG and complement or FcR	Deposition of immune complexes	Serum sickness Urticaria Vasculitis	7–8 days for serum sickness/urticaria 7–21 days after the start of the eliciting drug for vasculitis
IVa	Th1 (IFN-γ)	Monocytic inflammation	Eczema	1–21 days after the start of the eliciting drug
IVb	Th2 (IL-4 and IL-5)	Eosinophilic inflammation	Maculopapular exanthema, DRESS	1 to several days after the start of the eliciting drug for MPE 2–6 weeks after the start of the eliciting drug for DRESS
IVc	Cytotoxic T cells (perforin, granzyme B, FasL)	Keratinocyte death mediated by CD4 or CD8	Maculopapular exanthema, SJS/TEN, pustular exanthema	1–2 days after the start of the eliciting drug for fixed drug eruption 4–28 days after the start of the eliciting drug for SJS/TEN
IVd	T cells (IL-8/CXCL8)	Neutrophilic inflammation	Acute generalized exanthematous pustulosis	Typically 1–2 days after the start of the eliciting drug (but could be longer)

one of its metabolites. Numerous reactions with symptoms suggestive of allergy are often erroneously considered to be real drug allergies. The suggested pathomechanisms of these reactions include the following: (i) nonspecific mast cell or basophil histamine release (e.g., opiates, radiocontrast media, and vancomycin), (ii) bradykinin accumulation (angiotensin-converting enzyme inhibitors), (iii) complement activation (e.g., protamine), (iv) possibly an alteration in arachidonate metabolism (e.g., aspirin and nonsteroidal anti-inflammatory drugs), and (v) the pharmacological action of certain substances inducing bronchospasm (e.g., β-blockers, sulfur dioxide [SO_2] released by pharmaceutical formulations containing sulfites).

Immediate allergic DHRs
Immediate allergic DHRs develop as a result of IgE production by antigen-specific B lymphocytes after sensitization. IgE antibodies bind to the high-affinity Fc RI receptors on the surface of mast cells and basophils, creating a multivalent binding site for the drug antigen (34). Following subsequent drug exposure, the antigen – presumably a hapten–protein complex – cross-links bound IgE, stimulating the release of preformed mediators (e.g., histamine, tryptase, some cytokines such as TNF-α) and the production of new mediators (e.g., leukotrienes, prostaglandins, kinins, other cytokines). The preformed mediators stimulate a response within minutes, whereas the cytokine inflammatory component develops after several hours, the time required for protein synthesis and the recruitment of immune cells. β-Lactam-mediated anaphylaxis is the best defined immediate allergic DHR (18).

Nonimmediate/delayed allergic DHRs
Most nonimmediate/delayed allergic DHRs are mediated through the actions of T lymphocytes (34). The skin is the most commonly targeted organ by drug-responsive T cells, but any organ can be involved. Diclofenac, for example, as well as several other carboxylic acid nonsteroidal anti-inflammatory drugs, can cause immune-mediated liver injury, which may be explained by hepatic metabolism and selective modification of hepatic proteins (35). It is important to note that the same drug might produce different clinical symptoms and signs in different individuals, despite the drug being administered at the same dose via the same route. We are lacking data regarding specific drug processing, but, based on peptide immune recognition, the following scenario is possible. To stimulate naive T cells, dendritic cells first process the drug antigen. The antigen is then internalized and transported to the regional lymph nodes. To develop an effective immune response, the innate immune system needs to be activated, providing important maturation signals, often referred to as 'danger signals' (36) which include direct drug or disease-related stress. On arrival at the lymph nodes, the antigen is presented to naive T cells. Alternatively, some drug antigens might directly stimulate pathogen-specific T cells, thus avoiding the requirement for dendritic cell priming of T cells. However, for some authors, this hypothesis is difficult to reconcile with the time between initial drug exposure and the development of clinical signs (34). Antigen-specific T cells migrate to target organs and, once re-exposed to the antigen, they are activated to secrete cytokines that regulate the response and cytotoxins (e.g., perforin, granzymes, and granulysins) that produce tissue damage.

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Chemical basis of drug allergies

According to the hapten hypothesis, in order to stimulate a reaction, a drug should act as a hapten and bind irreversibly to proteins (34), generating antigens. This theory is relevant for chemical compounds, but not for proteic or carbohydrate compounds of drugs such as insulin, enzymes, monoclonal antibodies, and recombinant proteins. This is also especially relevant for oral drugs that preferentially bind to proteins such as albumin in gastric stomach fluid (37). However, in most cases, the gastric peptic function digests and inactivates the hapten–protein complex. Several drug modifications of the same protein are possible, generating a multivalent antigen for eliciting IgE-mediated immediate DHRs. For the elicitation of delayed-type T-cell-mediated reactions, the role of the carrier protein and/or the hapten has not always been fully defined. Furthermore, it is not known as to whether there is a threshold level of modification that needs to be surmounted to stimulate a T-cell response. The majority of drugs, however, are not directly protein reactive (33), and in such cases, hapten formation is thought to occur as a consequence of metabolic activation (e.g., sulfonamides) (the prohapten hypothesis). By generating a reactive metabolite, it is also feasible that activation of the innate immune system occurs, which is a prerequisite for a classical immune response.

An alternative hypothesis (the pharmacological interaction with immune receptor (p-i) concept) has evolved from analysis of the response of T-cell clones to drug stimulation, suggesting that drugs, although smaller than traditional antigens, might also interact directly with immunological receptors through a reversible interaction with the immune receptors (33). According to this hypothesis, a drug can directly bind and activate T cells (providing MHC binding as well) or bind to HLA molecules, which then activate T cells indirectly, by altering the MHC–peptide groove. This latter concept was recently further extended by showing that some drugs, when they bind to HLA molecules, promote an exchange of embedded peptides (38). However, the functional consequence of this peptide exchange is still unclear. Abacavir binds at the F pocket antigen-binding site of HLA-B*5701, selecting an array of novel self-peptides that induce the activation of CD8-positive T cells, inducing a severe DHR similar to graft-vs-host disease without eosinophilia (38). This recently uncovered mechanism of DHRs may be applicable to other small molecules with HLA allotype preferences.

Pharmaco- and immunogenetic basis of drug allergies

Drug hypersensitivity reactions involve both immune- and nonimmune-mediated mechanisms, with strong genetic interplay in some severe nonimmediate/delayed allergic DHRs. Indeed, a strong association between carbamazepine-induced Stevens–Johnson syndrome/toxic epidermal necrolysis (SJS/TEN) has been described for HLA-B*1502 in a Han Chinese population (39) and subsequently in Indian (40) and Thai (41), but not in European and Japanese patients (42–45). The association seems to be phenotype specific (SJS, but not hypersensitivity syndrome/drug

reaction with eosinophilia and systemic symptoms/drug-induced hypersensitivity syndrome (HSS/DRESS/DiHS)) (46). In contrast, HLA-A*3101 has been shown to be associated, in northern Europeans, with a spectrum of carbamazepine-induced reactions including maculopapular exanthemas, DRESS/DIHS, and SJS/TEN (42). For the drug abacavir, an association between HLA-B*5701 expression and severe DHRs in Caucasians has been shown (47). The incidence of this allele in abacavir-hypersensitive patients is high (94.4%) (48) in the Australian cohort, but lower (22.2%) in other studies (49), although still significantly higher than in the average population prevalence. Other genetic variants have been associated with DHRs (50) (Table 3). In immediate DHRs, some cytokine gene polymorphisms have been weakly associated with β-lactam-induced anaphylaxis (51, 52).

Role of viruses in the pathogenesis of DHRs

Viral infections can lead to skin eruptions and mimic DHRs if a drug (mostly an antibiotic) is taken at the same time (53). Although they are the leading cause of skin eruptions, viral infections can also interact with drugs, leading to mild eruptions in the case of the 'ampicillin rash' linked to the EBV infection (54) and severe reaction during DRESS (55). The first virus shown to be re-activated in DRESS patients was the human herpesvirus (HHV)-6 (56), but all herpesviruses can be involved (55). Strikingly, it was shown that HHV-6 replication can be induced in vitro by amoxicillin (57).

Clinical presentations

Acute and delayed manifestations of DHRs

Immediate DHRs usually present in the form of isolated urticaria, angioedema, rhinitis, conjunctivitis, bronchospasm, gastrointestinal symptoms (nausea, vomiting, diarrhea), or anaphylaxis, which can lead to cardiovascular collapse (anaphylactic shock) (58). Nonimmediate DHRs often affect the skin with variable cutaneous symptoms (59–61) such as late-occurring or delayed urticaria, maculopapular eruptions, fixed drug eruptions (FDE), vasculitis, blistering diseases (such as TEN, SJS, and generalized bullous fixed drug eruptions), HSS, acute generalized exanthematous pustulosis (AGEP), and symmetrical drug-related intertriginous and flexural exanthemas (SDRIFE). Internal organs can be affected either alone or with cutaneous symptoms (HSS/DRESS/DiHS, vasculitis, SJS/TEN) and include hepatitis, renal failure, pneumonitis, anemia, neutropenia, and thrombocytopenia.

Danger/severity signs of DHRs

The approach to the patient with a presumed DHR in the acute phase involves the following steps: (i) a complete history of the drugs taken (types, doses, duration), (ii) a detailed description of the symptoms and signs (types, onset, localization, and evolution), with (iii) a complete examination of the skin and the mucous membranes (including the mouth, eyes, and genitals), and (iv) the

Table 3 Pharmacogenomic biomarkers as predictors of severe DHRs (adapted from (50))

Gene or allele	HLA carriage rate	% of patients with an ADR	% of association between patients and controls	Relevant drug	Relevant ADR and ethnicity	Odds ratio	Negative predictive value %	Positive predictive value %
HLA-B*5701	6–8% Caucasians <1% African-Asian 2.5% African-American	5–8	100	Abacavir	SCAR	960	100 if patch test is negative	55
HLA-B*1502	10–15% Han Chinese <0.1% Caucasians and Japanese		100–8.6	Carbamazepine	SJS–Han Chinese	2504	100 in Han Chinese	3
HLA-B*5801	9–11% Han Chinese 1–6% Caucasians		100–15 55	Allopurinol	SCAR–Han Chinese SJS–Caucasians	580 50	100 in Han Chinese	3
HLA-A*3101	2–5% in northern Europeans	1–6/10 000	60.7–12.5 37/41.7–2 to 5	Carbamazepine	SCAR–Japanese DRESS/SJS–Caucasians	10.8 12.4/25.9		

search for danger/severity signs, which include clinical symptoms as well as some laboratory parameters (Fig. 2) (62). This approach will lead to the correct diagnosis, an appropriate choice of allergy tests later on and, during the acute phase, will facilitate the decision as to whether the drug should be stopped or not. If danger/severity signs are present, the suspected drugs should be stopped immediately.

Multiple drug hypersensitivity syndrome

About one-third of patients consulting in a drug allergy unit report more than one 'drug allergy' (63). First described (64) as drug allergies to two or more chemically different drugs, multiple drug hypersensitivity (MDH) differs from (i) cross-reactivity (due to structural similarities, common metabolic pathways, or pharmacologic mechanisms), (ii) flare-up reactions (exacerbation of an existing drug allergy by the early switch of therapy to a novel drug) (65), and (iii) multiple drug intolerance syndrome (66). Multiple drug intolerance syndrome includes patients with intolerance to three or more neither structurally nor pharmacologically related drugs, with no confirmation after evaluation (67) and possibly driven by patient anxiety (68). In documented DHRs, the prevalence of MDH ranges from 1% to 10% (69) and may relate to moderate and severe DHRs (65).

T-cell activation by different compounds has been clearly demonstrated in MDH (70–72). In these patients, T cells do not appear to have any deficiency in T-reg function or number (73), but the fact that the drug-reactive T cells belong to an *in vivo* preactivated cell fraction ($CD4^+$ $CD25^{dim}$, may be due to *in vivo* occurring T-cell activation) makes them more susceptible to T-cell stimulation via the p-i concept (65).

Natural history of DHRs

The IgE antibody response is not permanent over time, and decreased antibody levels may occur months to years after the occurrence of a DHR, as shown for penicillin allergy (74). However, IgE sensitization may persist for years, as shown for NMBA (75). Experts therefore recommend (R1, Evidence D) lifelong avoidance of the drug and cross-reactive drugs when drug-induced anaphylaxis has occurred (6, 9, 20). T-cell memory seems to be even stronger for nonimmediate/delayed DHRs (76).

In selective responders to amoxicillin, patients are able to tolerate other penicillins and are not at increased risk of allergies upon exposure to closely related penicillins (77). Finally, resensitization studies indicate that some patients with a previous positive history and negatively tested may become positive after therapeutic administration (18). Even if this topic remains debatable, with regard to the time lapse between the tests, the normal sensitization incidence, or the number of subsequent tests, both the EAACI-DAIG/ENDA guideline (18) and the Practice Parameters experts (6) agree that consideration may be given to retesting individuals with particularly severe previous reactions to a β-lactam.