Fixed drug eruption: pathogenesis and diagnostic tests

Tetsuo Shiohara

Department of Dermatology, Kyorin University School of Medicine, Tokyo, Japan

Correspondence to Tetsuo Shiohara, Department of Dermatology, Kyorin University School of Medicine, 6-20-2 Shinkawa, Mitaka, Tokyo 181-8611, Japan
Tel: +81 422 47 5511; fax: +81 422 41 4741;
e-mail: tpshio@ks.kyorin-u.ac.jp or tpshio@kyorin-u.ac.jp

Current Opinion in Allergy and Clinical Immunology 2009, 9:316–321

Purpose of review

Fixed drug eruption is a simplified disease model for elucidating the mechanism(s) of how skin inflammation is induced by skin-resident T cells. In this review, we focus on how the presence of intraepidermal $CD8^+$ T cells resident in the fixed drug eruption lesions can provide exciting new clues to our understanding of pathomechanisms of inflammatory skin diseases.

Recent findings

Intraepidermal $CD8^+$ T cells with an effector–memory phenotype resident in fixed drug eruption lesions have a major contributing role in the development of localized tissue damage. Activation of these $CD8^+$ T cells is sufficient for triggering the lesion, however, but not sufficient to cause extensive tissue damage observed in the fully evolved lesions; additional recruitment of $CD4^+$ and $CD8^+$ T cells to a specific tissue site would also contribute to the late stage of lesion development. The influx of regulatory T cells into the epidermis observed in fully evolved lesions would serve to limit harmful immune reactions. Consistent with this, positive patch test reactions are only observed at the site of previous lesions harboring significant numbers of intraepidermal $CD8^+$ T cells.

Summary

Intraepidermal $CD8^+$ T cells may represent double-edged swords of the skin immune system with protective and destructive capacity.

Keywords

fixed drug eruption, intraepidermal T cells, patch tests, regulatory T cells

Curr Opin Allergy Clin Immunol 9:316–321

1528-4050

Introduction

The epidermis has long been regarded as a passive barrier intended to protect the underlying tissues from invading pathogens and antigenic encounter. Therefore, many immunologic responses occurring in the skin or epidermis were thought to be mediated by inflammatory cells migrating from the blood vessels. Indeed, targeting these responses by modulating the skin-directed migration of inflammatory cells has been considered to be an important therapeutic option. Accumulating evidence, however, indicates that the epidermis is a site of accumulation of a distinct subset of T cells; particularly in mice, the epidermis possesses a unique dense network of T cells with a specialized protective function known as dendritic epidermal T cells (DETCs) [1]. In contrast, in humans, T cells different from murine DETCs are indigenously resident in the epidermis but at such low frequencies that their physiological function remains unknown [2–4]. Moreover, because of the difficulty in distinguishing skin-resident T cells from circulating T cells on the basis of the differential expression pattern of surface markers, a possible role for skin-resident T cells in tissue damage has long been neglected. In this regard, we previously demonstrated that intraepidermal T cells expressing the T cell receptor (TCR)-αβ are abundantly detected between basal keratinocytes in the lesions of fixed drug eruption (FDE) over prolonged periods of time after clinical resolution [4–8]. Because a complicated multistep process from the initiation of inflammatory responses to eventual tissue damage could be examined in sequence by following the evolution of individual FDE lesions after challenge with the causative drug, FDE is thought to be a simplified disease model for elucidating the mechanism(s) of how skin inflammation is caused by skin-resident T cells. In this article, we discuss recent data, mostly derived from studies on evolving FDE lesions, which help explain how FDE lesions initially occur and eventually resolve.

Clinical findings relevant to understanding pathogenesis

FDE usually appears as a solitary or a small number of pruritic, well circumscribed, erythematous macules that evolve into edematous plaques; these lesions typically recur at exactly the same sites with each administration of the causative drug, but upon the discontinuation resolve spontaneously, leaving hyperpigmentation. After clinical resolution, the lesions remain quiescent and typically present as gray–brown macules or plaques on the skin, mucous membranes, or on both for prolonged periods

 DOI:10.1097/ACI.0b013e32832cda4c

unless the causative drug is given. The lesions usually flare within 30 min to 8 h after drug intake; mean length of time from drug intake to the onset of symptoms is approximately 2 h. Sensation of burning often precedes the appearance of these lesions. Although FDE can occur anywhere on the skin or mucous membrane, the most commonly affected sites are the lip, palms, soles, glans penis, and groin areas; interestingly, herpes simplex virus (HSV) is frequently reactivated in these areas of healthy individuals. In some cases, the lesions become more widespread with bullous lesions and systemic manifestations, such as high fever and arthralgia, mimicking Stevens–Johnson syndrome (SJS) or toxic epidermal necrolysis (TEN). These FDE lesions become more numerous, and new lesions also develop on the previously uninvolved areas unless the causative drug is withdrawn.

FDE lesions initially appear when susceptible patients are sensitized to a particular drug. Such sensitization occurs more rapidly in patients intermittently receiving the causative drugs rather than those continuously receiving them. Thus, the period required for sensitization is highly variable depending on patients, ranging from a few weeks to several years. The previously involved sites do not necessarily flare with each exposure, which is known as the refractory period [8]. The duration of this period is also variable, lasting from a few weeks to several months. Guin *et al.* [9] reported that FDE lesions appeared to wander because some of the previously involved sites did not flare with each exposure whereas others flared.

There have been many reports describing patients with typical FDE but who had no significant history of drug intake preceding the eruptions. Some cases of recurrent exacerbations of FDE lesions without significant history of drug intake might be attributable to nonspecific exogenous factors. Nonmedical factors, such as food and ultraviolet irradiation, have been reported to precipitate exacerbations of FDE lesions [10–12].

In some patients, despite the continued administration of the causative drug, the pigmented lesions eventually disappear; such patients would be spontaneously desensitized to the causative drug. Kelso and Keating [13] have reported successful desensitization for treatment of FDE to allopurinol; interestingly, desensitization to the causative drug in FDE has exclusively been reported to occur with allopurinol [14–16].

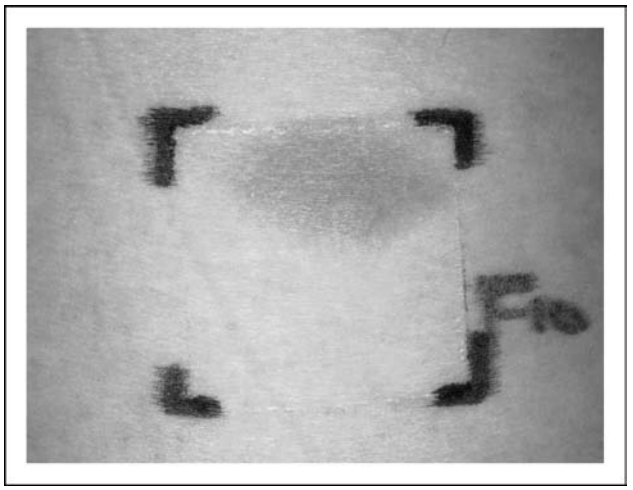
Diagnosis

The diagnosis of FDE is generally thought to be easy for many dermatologists even after clinical resolution. However, because the clinical spectrum is quite varied, the diagnosis of FDE is not as straightforward as generally thought. FDE often presents with a wide spectrum of clinical manifestations indistinguishable from those of other skin diseases, such as erythema multiforme [17], SJS or TEN, cellulitis [18], paronychia, neutrophilic dermatosis [19], lichen planus, and parapsoriasis en plaques [20]. Such unusual forms of FDE may be easily overlooked unless clinicians take special care to recognize the presence of such variants [17]. As blister formation often occurs at an advanced stage of FDE reactions in association with systemic symptoms such as fever, clinicians often find a great deal of difficulty in distinguishing between a multiple, bullous variant of FDE and TEN, particularly when bullous lesions become more widespread with systemic manifestations. In addition, this variant does not leave typical hyperpigmentation after clinical resolution as is typically seen in nonpigmenting FDE [21], thus often leading to misdiagnosis as TEN or bullous pemphigoid. Careful history taking about drug intake and a prior history of recurrent lesions in the same sites are essential for the precise diagnosis of FDE.

In-vivo and in-vitro testing in determining the causative drug

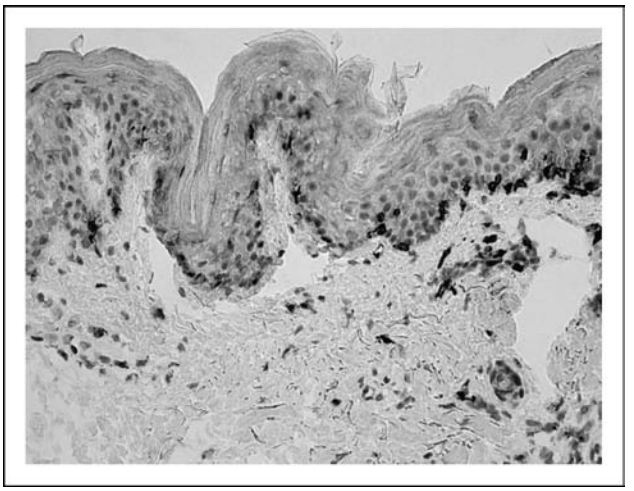
Systemic oral challenge and topical provocation tests are usually performed to identify the drug responsible for the FDE. Oral challenge tests with a single therapeutic dose of the suspected drug, starting at one-tenth of the therapeutic dose, can be done with relative safety with a low risk of inducing systemic adverse reactions. Although patch tests are usually performed with the commercialized form of the drug used by the patient on the upper back of the patients in other drug eruptions, this may give misleading negative results in FDE. There are several reasons for the negative results. First and most importantly, patch tests should be done at the sites of previous lesions in FDE because patch tests at the nonlesional sites usually yield a negative response [22]. Secondly, the timing of patch testing may also influence the result. To avoid its refractory period, patch tests at the site of previous FDE lesions should be performed at least 2 weeks after resolution of the lesions. Thirdly, patients may not be sensitized to the original drug but to its metabolites. The patch tests using the commercialized form may give false-negative results because these metabolites are usually neither known nor available for patch tests. Fourthly, false-negative results may be attributed to low concentrations of the drug used in patch testing. In most cases, patch tests should be performed with the drug mixed in petrolatum to 10–20% or diluted in water at the same concentration. Although positive patch test reactions can be more easily obtained with the drug diluted to 50%, this should not be done because stronger delayed reactions would occur on days 3–7, indicating patch test sensitization. Finally, false-negative results may result from the limited penetration properties

Figure 1 A positive patch test reaction for the causative drug 10% in petrolatum



Note the positive reaction confined to the previously involved pigmented area.

Figure 2 Intraepidermal $CD8^+$ T cells resident in the resting fixed drug eruption lesions



Immunoperoxidase stain, ×66.

of the drug. When multiple drugs are suspected, patch tests will be particularly useful as a screening test. Thus, a positive patch test reaction confined to the previously involved sites (Fig. 1) is generally regarded as strong evidence that the FDE lesions are caused by the drug, although it must be kept in mind that a negative test does not exclude it.

Although the lymphocyte transformation test (LTT) is a reliable method to identify the causative drug in many types of drug eruptions, such as maculopapular eruptions, acute generalized exanthematous pustulosis, and drug-induced hypersensitivity syndrome [23], positive LTT reactions are rarely obtained in patients with FDE. Nevertheless, a positive LTT reaction may be obtained in cases of generalized variant of FDE indistinguishable from TEN. In summary, oral challenge remains the most reliable method for establishing the causative drug in FDE.

Pathogenesis of fixed drug eruption

Intraepidermal $CD8^+$ T cells resident in the FDE lesions clearly have a major contributory role in the development of localized tissue damage [4–8]. Resting FDE lesions long after clinical resolution are characterized by significant numbers of $CD8^+$ T cells with an effector–memory phenotype aligning along the epidermal side of the dermoepidermal junction (Fig. 2). These T cells are composed of a phenotypically homogeneous population that expresses TCR-αβ, CD3, CD8, CD45RA, and CD11β but not CD27 and CD56 [4,6,7]. This phenotype of T cells most closely resembles that of effector–memory T cells [24–26]. These T cells are also found in intact epidermis at an exceedingly low level and are phenotypically more heterogeneous in nature [3]. Such accumulation of T cells with an effector–memory phenotype has also been found at the site of repeated pathogen entry, such as the lung [27], suggesting that these T cells may confer protective immunity [28,29]. These T cells are also consistently found at significantly higher levels following infection in such tissues [27,30,31]. In support of their protective immune effector function, T cells with an effector–memory phenotype preferentially migrate into the sites of infection, such as mucosal sites, and persist for long periods of time following infection [27,30–33,34••, 35•], a finding consistent with FDE lesions initially appearing at sites of previously traumatized skin, such as burn scars and insect bites [33]. Thus, the immune functions of intraepidermal $CD8^+$ T cells found in the FDE lesions may be protective in nature and not always destructive. These findings reinforce the notion that intraepidermal $CD8^+$ T cells resident in the FDE lesions are critical in the initiation of destructive immune responses while protecting the epidermis from repeated infections. In this regard, we have recently noted that the vast majority of patients with FDE are asymptomatic HSV-seropositive individuals without a previous history of clinical herpetic lesions [28]. In view of the finding that anti-HSV IgG titers were much higher in these FDE patients than those with a history of HSV recurrences, these intraepidermal $CD8^+$ T cells resident in the FDE lesions may represent effector–memory T cells that are originally recruited from the circulation into the site of repeated infections to mediate protective immunity.

Intraepidermal $CD8^+$ T cells are not constitutively cytolytic but, once activated via the CD3–TCR complex,

display a cytolytic activity against natural killer (NK)-sensitive or NK-resistant tumor cells and cultured keratinocytes [5]. They produce large amounts of IFNγ without proliferation, when activated *in vivo* and *in vitro* [5–8]. The lack of an antigen-induced proliferative response by these T cells makes it difficult to demonstrate their antigen specificity [5], although some of these T cells may be self-reactive [5,8]. Although our quantitative PCR analysis of intraepidermal $CD8^+$ T cells isolated from resting FDE lesions demonstrated that they utilized a very limited range of TCR *Vα* and *Vβ* gene families as compared with peripheral blood T cells obtained from the same patients [5], neither has the antigen specificity of these $CD8^+$ T cells nor has the nature of the self-antigens unmasked after drug intake has been determined.

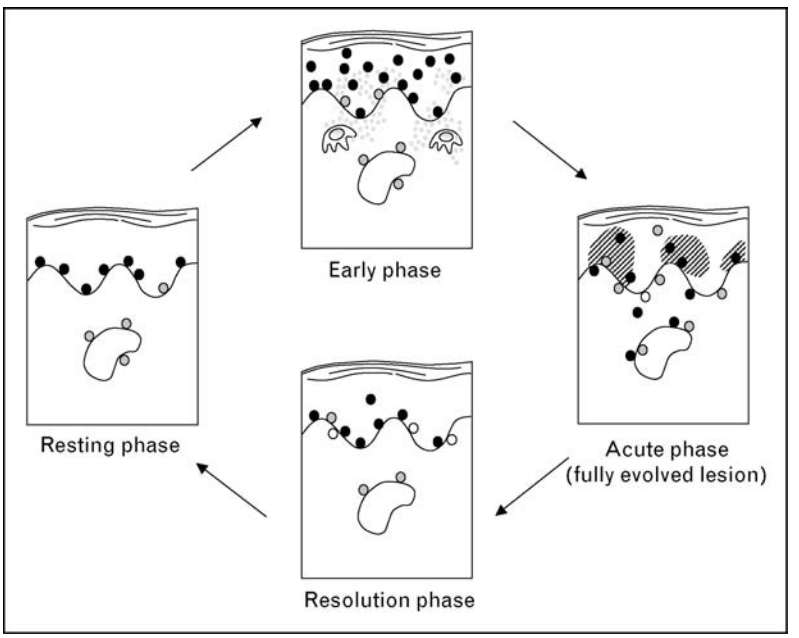
Tissue damage results when intraepidermal $CD8^+$ T cells are activated to directly kill surrounding keratinocytes and release large amounts of cytokines such as IFNγ into the local microenvironment (Fig. 3). The main effector function of intraepidermal $CD8^+$ T cells is mediated by IFNγ, although the effector function also involves direct cytolysis by perforin or Fas L. Probably, activation of intraepidermal T cells is sufficient for triggering the lesion but not sufficient to cause extensive tissue damage observed in fully evolved lesions. Cytokine or adhesion molecule-mediated nonspecific recruitment of $CD4^+$, $CD8^+$ T cells and neutrophils to a specific tissue site without recognition of their cognate antigen would serve to enhance tissue damage, thereby contributing to the late stages of development of FDE lesions. These intraepidermal $CD8^+$ T cells that have participated in the early phase of the inflammatory cascade may have been diluted by the influx of such nonspecific recruitment of $CD4^+$ and $CD8^+$ T cells. Thus, it is difficult to differentiate the effects of intraepidermal T cells from those secondarily recruited from the circulation in a biopsy specimen obtained at a given time.

Although a severe form of FDE clinically and histologically mimics TEN, subsequent evolution of the two diseases is quite different. The former resolves spontaneously upon discontinuation of the causative drug, whereas the latter often results in a potentially fatal outcome even after withdrawal of the drug. Our recent studies have important implications for populations that serve to prevent disease progression to TEN; $CD4^+FoxP3^+$ regulatory T (Treg) cells were abundantly detected in the fully evolved FDE lesions (24 h-old lesions), which eventually resolved [36]. The influx into the epidermis of $CD4^+$ T cells, in particular Treg cells, during the evolution of drug reactions could reflect an appropriate response that may contribute to homeostatic control of potentially harmful immune reactions mediated by intraepidermal $CD8^+$ T cells.

These findings suggest that FDE is a form of classic delayed-type hypersensitivity (DTH) mediated by $CD8^+$ T cells. However, FDE lesions usually appear within 2 h of clinical challenge with the causative drug, inconsistent with typical DTH reactions. In this regard,

Figure 3 The cascade of events triggered by drug intake

In the resting state, intraepidermal $CD8^+$ T cells remain quiescent but in a primed state, as evidenced by the expression of CD69. Upon drug intake, they are activated to release IFNγ and cytotoxic granules into the local microenvironment. Mast cells localized in the vicinity of the epidermis also contribute to the activation of intraepidermal $CD8^+$ T cells via the induction of cell adhesion molecules on surrounding keratinocytes through the action of TNFα. In fully evolved lesions, keratinocytes are killed by the direct action of intraepidermal $CD8^+$ T cells in concert with $CD4^+$ T cells recruited later on from the circulation. At the end of the immune response, Treg cells are recruited into the lesions and serve to inhibit severe immune responses mediated by intraepidermal $CD8^+$ T cells and other infiltrating T cells; the majority of the expanded or activated population is removed by apoptosis. A proportion of intraepidermal $CD8^+$ T cells prevented from undergoing apoptosis by IL-15 provided from keratinocytes leads to the persistence of a memory T cell population. Treg, regulatory T cells.



we previously demonstrated that mast cells localized in the vicinity of the epidermis in FDE lesions could be readily activated upon skin exposure to the causative drug [36]. During the initial phase of FDE reactions, mast cells are thought to contribute to the activation of intraepidermal $CD8^+$ T cells through the induction of cell adhesion molecules on keratinocytes. Furthermore, studies on in-vitro models showed that mast cells, which accumulate at sites of previous FDE lesions, could accomplish this task by producing TNFα. Our sequential studies of developing FDE lesions have demonstrated that the immediate wheal-and-flare-like reaction is followed by activation of intraepidermal $CD8^+$ T cells.

Conclusion

Given the remarkable similarity in the phenotype and function between intraepidermal $CD8^+$ T cells resident in FDE lesions and virus-specific effector–memory $CD8^+$ T cells, we hypothesize that intraepidermal $CD8^+$ T cells represent those strategically seeded to the epidermis upon contact with infection or trauma to protect tissue integrity from external insults. If so, clinicians involved in the treatment of these patients need to be aware of the risk that loss of intraepidermal $CD8^+$ T cells may manifest in an increased risk of viral infections, thereby leading to the threat of systemic viral infections.

Acknowledgements

This work was supported in part by a research grant-in-aid for scientific research from the Ministry of Education, Culture, Sports, Science and Technology (Tetsuo Shiohara) and a health and labor science research grant from the Ministry of Health, Labor and Welfare of Japan (Tetsuo Shiohara). This work was also supported by the Japanese Research Committee on Severe Cutaneous Adverse Reaction.

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