

Monitoring the acute phase response in non-immediate allergic drug reactions

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Purpose of review

The aim of this article is to evaluate the advantages of monitoring the immunological response of non-immediate allergic drug reactions in parallel with the affected tissues, skin and peripheral blood, in order to improve our understanding of the immunological response.

Recent findings

Several studies have shown that in the skin and peripheral blood, the agents that take part in the development of the immunological reaction express a number of markers that parallel the evolution of the disease process. These markers include cytokines, chemokines, and cytotoxic factors, as well as many other markers involved in such mechanisms as drug metabolism and signal transduction.

Summary

Monitoring the acute phase response to a drug in the skin with parallel studies in the blood provides clues that increase our understanding of the underlying pathological mechanisms in adverse reactions to drugs with an immunological basis. This approach, together with molecular biology techniques such as microarrays and genomic studies may be useful in future, in better characterizing the clinical subtype and prognosis of nonimmediate allergic drug reactions and generating targeted treatment regimens.

Keywords

immunological response, monitoring, drug, nonimmediate reaction

Introduction

Drug allergies are included in type B adverse drug reactions. They are unpredictable, uncommon, and usually unrelated to the pharmacologic actions of the drug [1,2]. Because of the many different clinical entities of drug allergy and difficulty differentiating and evaluating the mechanisms involved, monitoring the evolution of the reaction is necessary for several reasons, explained in detail in this review.

Adverse reactions to drugs: clinical entities

Depending on the time between drug intake and the appearance of symptoms, drug allergy can be classified as immediate, accelerated or delayed [3]. Immediate reactions correspond to those occurring within 1 h of drug intake, with typical manifestations being urticaria, angioedema and anaphylaxis. Delayed reactions appear within 48–72 h or more after drug intake, the most frequent manifestations being delayed urticaria, maculopapular exanthema (MPE), erythema multiform, fixed drug eruptions (FDEs), acute generalized exanthematous pustulosis (AGEP), Stevens Johnson syndrome (SJS), toxic epidermal necrolysis (TEN), drug rash with eosinophilia and systemic symptoms (DRESS), drug hypersensitivity syndrome and organ specific reactions [4•]. Accelerated reactions are more difficult to define, but are usually considered to occur within 1–48 h. These include such manifestations as urticaria and serum sickness-like syndromes. The onset of symptoms and skin test positivity occur within hours of drug application up to 48 h or more in both accelerated and delayed reactions, indicating that a T-cell response has been produced. Thus, we propose defining these reactions as non-immediate [5]. The skin is the main organ affected in drug allergy and many drugs can be involved [4•], either alone or through the generation of metabolites [6,7]. This review will focus on nonimmediate reactions, classically included under the type IV Gell and Coombs classification of immunologically mediated hypersensitivity reactions [8].

Two diagnostic approaches are used to evaluate nonimmediate reactions: skin testing and in-vitro assays. Unlike immediate reactions, neither of these approaches has an acceptable sensitivity and clinicians often feel helpless in the clinical evaluation. Although guidelines have been proposed in position papers and reviews [5,9,10],

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Abbreviations

AGEP	acute generalized exanthematous pustulosis
CLA	cutaneous lymphocyte antigen
DRESS	drug rash with eosinophilia and systemic symptoms
MPE	maculopapular exanthema
PBMC	peripheral blood mononuclear cell
SJS	Stevens Johnson syndrome
TEN	toxic epidermal necrolysis
TNF	tumour necrosis factor

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sufficient evidence-based research in answering this question remains lacking. Different T-cell subpopulations have been observed in this type of reaction. In-vitro diagnosis has the same drawbacks as in-vivo testing.

These difficulties necessitate a deeper understanding of the underlying pathophysiology of these reactions, in order to obtain tools to discriminate the type of reaction during patient evaluation.

Mechanisms of nonimmediate reaction responses

Because of our limited understanding and the inadequacy of the diagnostic methods available, great emphasis has been placed over the last decade on the description of the underlying pathological processes. The mechanisms attributed to drugs are based mainly on clinical observations, with the assumption that the clinical entities were attributable to a particular mechanism. Often, the same mechanism is equally applicable to more than one clinical entity, be they exanthema, severe desquamative exanthema, SJS or TEN. Moreover, these clinical entities may range from mild to severe reactions.

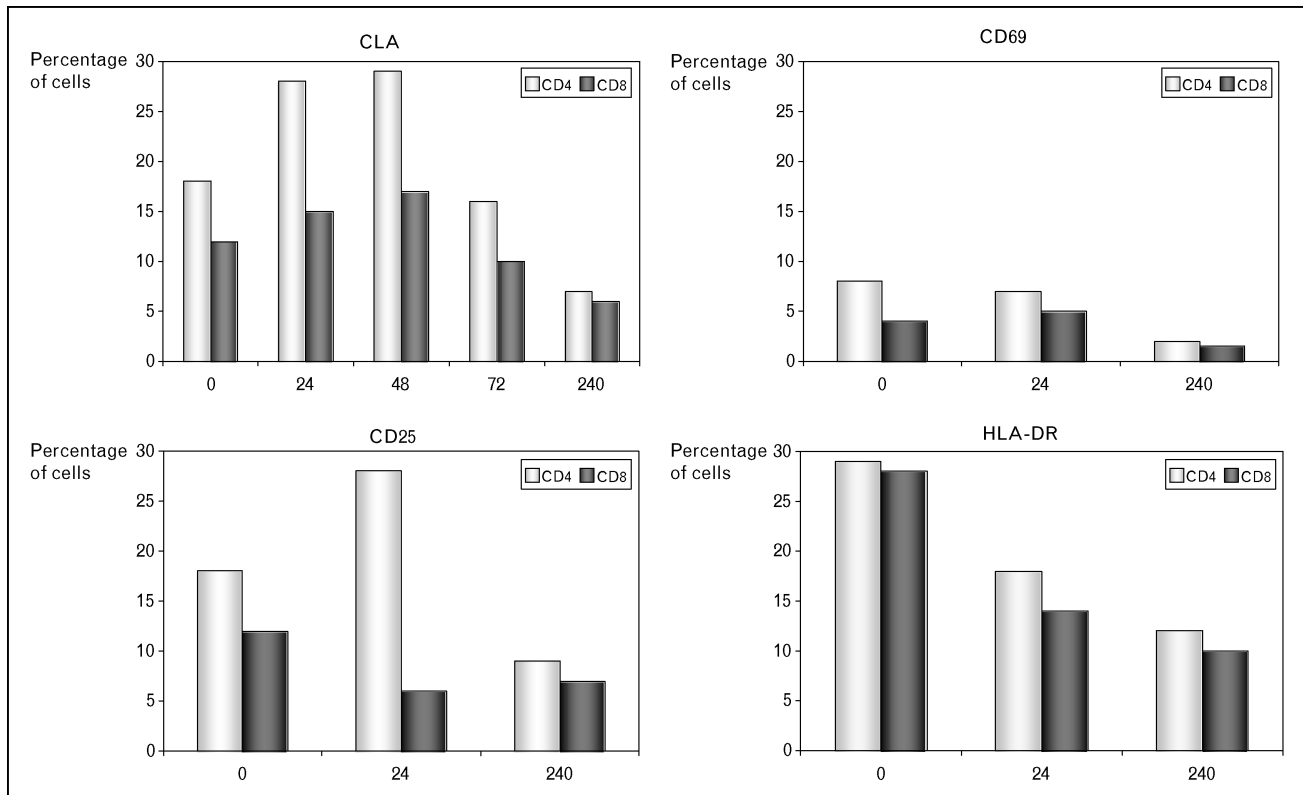
Grouping together all the recent information about clinical manifestations, skin test responses and laboratory studies shows that the key protagonist is the lymphocyte [11], though other cells, such as antigen-presenting cells (dendritic cells or Langerhans cells) or keratinocytes also play a role. The wide variety of entities attributed to these mechanisms has resulted in a new subclassification that considers their similarities and differences: type IVa, for which T cells producing IFN- γ activate macrophages, and the typical clinical manifestation is eczema; type IVb, mediated by T cells producing Th2 cytokines (IL-4 and IL-5), which induce B cells to produce antibodies and mast cell and eosinophil responses, mainly in DRESS, MPE and bullous exanthema; type IVc, induced by CD4 and CD8 T cells, which produce cytotoxic mediators that result in keratinocyte apoptosis in MPE and massive apoptosis in TEN; type IVd, characterized by neutrophil activation and recruitment induced by T cells by the production of a chemokine, CXCL8, the characteristic clinical manifestation of which is AGEP [12]. Although differences exist in the mechanisms involved in this classification, clinical or immunohistochemical overlapping is present.

According to the hapten hypothesis, covalent conjugation of the drug to a body protein is required to induce an immune response to drugs [13]. Some are able to conjugate spontaneously to body proteins (e.g. β -lactams), but many others require transformation to become reactive (protohaptens). This process is complex and depends on endogenous enzymes, mainly cytochrome P450. The possibility of direct interaction of drugs with the immune

system without previous processing has also been proposed for certain drugs (pharmacologic interaction hypothesis) [12] but although the capacity for being recognized by specific clones has been shown, the ability to induce them has not.

The initial steps in drug metabolism, processing and presentation by dendritic cells to T cells are common. The signalling and inflammatory markers produced by dendritic cells during the presentation may contribute to the differences in each type of clinical reaction. Once the hapten-carrier conjugate formed is internalized by antigen-presenting cells and processed into peptide fragments for presentation in the lymph nodes to specific T-lymphocytes, these are activated and proliferate by clonal expansion producing memory T cells [13]. The functional characteristics of these lymphocytes are determined by their distinct cytokine secretion profiles: Th1 (IFN- γ producing) and Th2 (IL-4, IL-5, IL-10, IL-13 producing) cells [14]. Activation of dendritic cells, with chemical allergens and upregulation of costimulatory molecules and cytokines, contributes to the polarization to either Th1 or Th2 pattern of cytokine production [15]. Although the hapten-carrier is recognized by the immune system, with the generation of antibodies or T cells, it is currently believed that a danger signal is required to trigger the pathological response [16].

Great heterogeneity exists in mechanisms of drug allergy. In erythema multiform, MPE, urticaria and fixed drug eruptions, there is a predominance of CD4 T cells in the perivascular dermis, and similar levels of CD4 and CD8 T cells have been found in the dermoepidermal layer and epidermis [17]. These cells are activated and express high levels of the skin homing receptor, cutaneous lymphocyte antigen (CLA) (Fig. 1), and produce perforin and granzyme B [17,18]. A certain heterogeneity has been found with the cytokine pattern [19,20]. In the case of fixed drug eruptions, a resident CD8 population with an effector phenotype results in rapid IFN- γ production, with the swift appearance of clinical symptoms [21]. In other important entities, such as AGEP, T cells and neutrophils are involved [22,23]. Here T cells have a double function, inducing apoptosis by the production of perforin and Fas ligand [24], and attracting neutrophils by the production of chemokine CXCL8 (IL-8), which interacts with the specific receptor CXCR2 in neutrophils [25]. SJS and TEN, considered the same disease but with different degrees of skin lesions, are the most severe drug allergy [4]. Their pathogenesis has been related to T cells inducing massive apoptosis of epidermal keratinocytes, produced via two pathways, Fas/Fas ligand [26] or perforin-granzyme B [27]. Immunohistological studies in drug-induced TEN and SJS have shown that the dermal infiltrate is mainly composed of CD4 T lymphocytes, whereas CD8 cells are located along the epidermal

Figure 1 Monitoring of different cellular markers during the course of non-immediate reactions

Markers monitored were skin homing receptor (cutaneous lymphocyte antigen; CLA) and activation markers (CD69, CD25 and human leukocyte antigen (HLA)-DR) during the course of non-immediate reactions.

junction and migrate into the epidermis [11] and the blister fluids [28,29]. There are high concentrations of IFN- γ , IL10 (which is a chemotactic for peripheral blood CD8) and IL-18, which is the principal IFN- γ -inducing factor produced by human keratinocytes, and also inflammatory cytokines, such as tumour necrosis factor (TNF)- α and IL-15, as well as Fas-L in blister fluids [30]. A high expression of CLA has been observed during the acute phase, which enables T cell migration to skin [31,32]. In this process, the CLA⁺ T cells are long-lasting, probably because of a continuous traffic to skin [14,33]. The pathophysiology of DRESS overlaps two type IV subtypes [34]. There is production of eotaxin and IL-5, which is important for eosinophil recruitment (corresponding to type IVb), but there is also a high production of perforin and granzyme B by T cells (corresponding to type IVc) [35,36]. The most common organ-specific reaction is immunological hepatitis, in which hepatocytes produce reactive drug metabolites during drug biotransformation. These metabolites can be processed and presented by hepatic Kupffer cells to drug-specific CD4 T lymphocytes. Moreover, these reactive metabolites are able to bind covalently to cytochrome P450 proteins. This complex can bind to human leukocyte antigen

(HLA) class I molecules expressed in hepatocytes and be recognized by drug-specific CD8 T cells [37,38].

The in-vitro response

In addition to the lymphocyte transformation test, we will outline the generation of T cell lines, T cell clones, cytotoxic studies and other studies that provide information about the participation of specific T cells. The detection of these peripheral lymphocytes is only possible in rare cases and cloning drug-reactive T cells has proven difficult. Although the lymphocyte transformation test has been used, it has not been sufficiently validated. The series reported lack adequate numbers of cases, many drugs are involved, and the clinical entities are rather heterogeneous [39]. These studies have been done not only in terms of specific cell proliferation but also in terms of the mediators involved. In general, the reactions have been defined as Th1 or Th2, depending on the production of several cytokines, chemokines and even transcription factors [14]. As occurs with other immunological responses, these reactions also follow this paradigm, although sometimes not so clearly defined [40]. These discrepancies probably arise because research has been hindered by technical problems. In peripheral blood

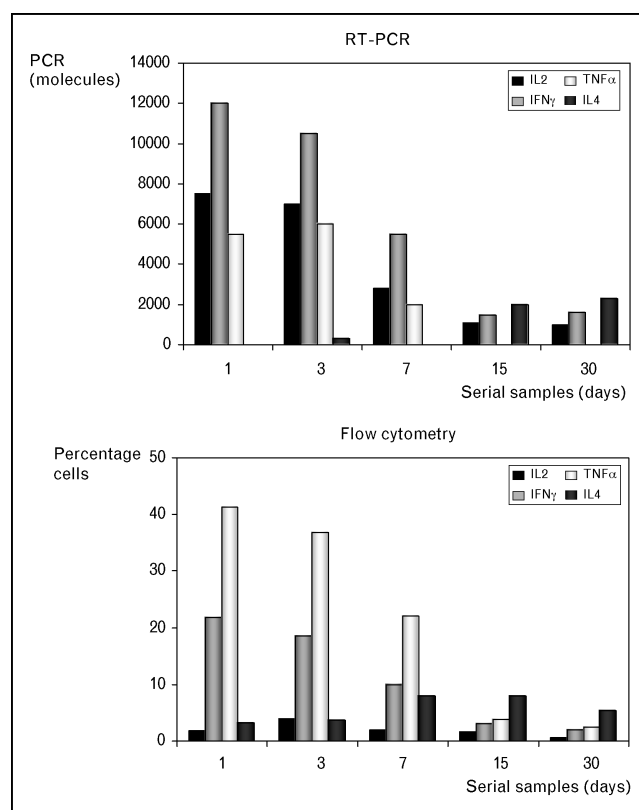
mononuclear cells (PBMCs) and T-cell clones from patients allergic to β -lactams, polarization depended on the patient's reaction [41,42], with a good correlation between cytokine levels and disease severity [41]. This is not, however, always so evident [43–45]. In patients with nonimmediate reactions, incubation of cells with the drug showed a surprising overproduction of IL-5, although a slight Th1 pattern existed [46]. Other authors studied patients with immediate and nonimmediate reactions to β -lactams by flow cytometry and found that independently of the reaction, specific T-cell lines to penicillin G mainly produced IL-4 alone or together with IFN- γ synthesis [47]. Many explanations exist for these different results. Not all the studies were done in the same cell types, some were made in a mixture of cells, as occurs in PBMCs (T and B lymphocytes, monocytes, natural killer) [41,45], whereas others used T-cell clones [42–44,47]. Moreover, the studies involved different methodologies, some requiring long incubation periods that could alter the phenotype of the original cells. Accordingly, for many years our group and others have approached the problem by monitoring the immunological response during the acute episode and the resolution period.

Monitoring the in-vivo response

A rational strategy is to monitor the clinical response during the acute episode by obtaining sequential blood and skin samples in order to detect the underlying process. A wide range of markers can be used in both peripheral blood and affected tissue. This strategy, followed by our group in the last 5 years, not only provides information about the development and progress of the reaction but also about the degree of response to treatment [29]. Because several clinical entities exist, it is reasonable to assume that many markers, including cytokines, chemokines, adhesion molecules and others, will participate. More important is the determination of changes in these markers rather than their quantification *per se*. Available methods fall into three groups: immunoassays for secreted proteins, flow cytometry for cell surface and intracellular marker production and molecular biology techniques for determining gene expression by mRNA production (Fig. 2).

Flow cytometry has proved valuable for quantifying many markers, including the subpopulations involved [48,49]. Ever more studies are using this technology in drug allergy [18,21,23,26,27,29,33,50]. This enables us to see the parallel evolution of cells involved in the two compartments: the skin (in both biopsies and blister fluid cells) and blood (PBMCs), showing an increase at the very early stage in the reaction in the cells from the blister fluid, with CLA+ cells appearing later in peripheral blood, probably due to recirculation from the skin (Fig. 3) [29,33]. These cells also show a Th1 cytokine

Figure 2 Measurement of mRNA expression (by real time reverse transcription-polymerase chain reaction) and intracellular production (by flow cytometry) of different cytokines during the monitoring of a toxic epidermal necrolysis induced by lamotrigine



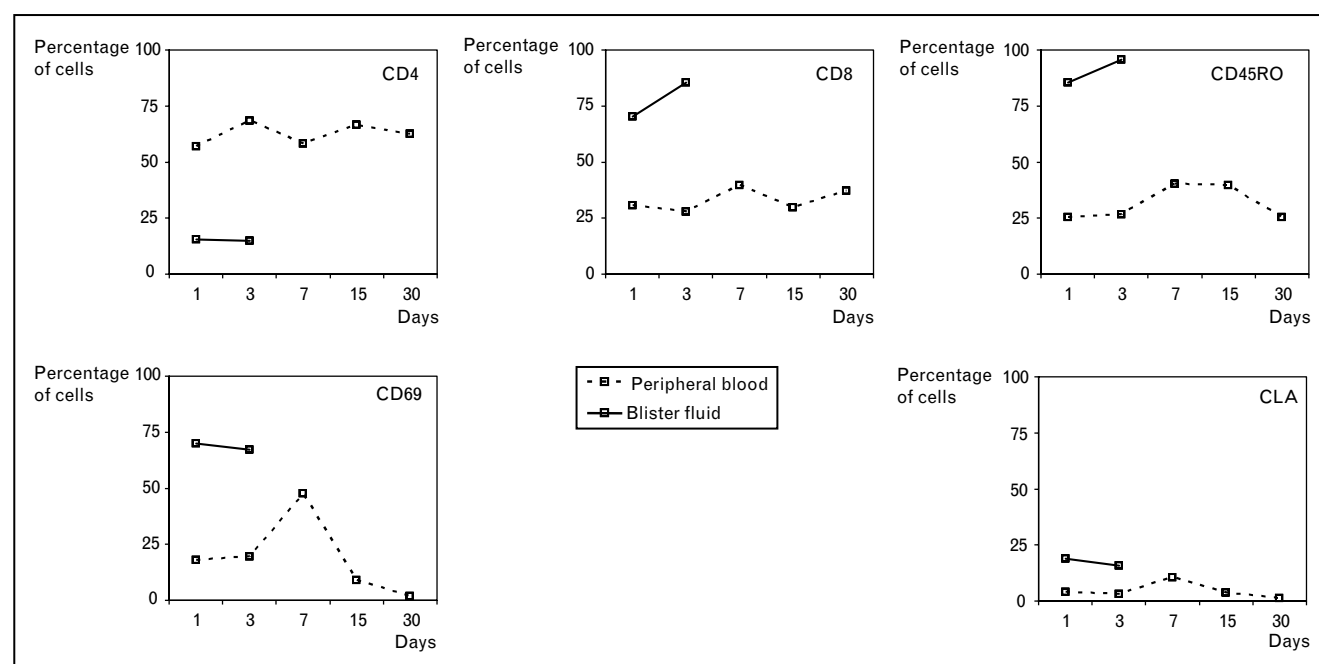
Despite differences in the levels of each cytokine depending on the methodology used, the same tendency can be observed. RT, reverse transcription; PCR, polymerase chain reaction; TNF, tumour necrosis factor.

pattern with production of cytotoxic markers such as perforin and granzyme B in parallel with the reaction and its severity [27,30].

The quantitative reverse transcription-polymerase chain reaction (RT-PCR) provides high sensitivity and specificity to measure low quantities of mRNA in small amounts of tissue, as in biopsy samples [51]. The high sensitivity is very important to detect those cytokines expressed in very low levels, such as IL-10, IL-4 or IL-2. Many studies have used this method to analyse drug allergy [21,22,29,50] and to monitor cytokine and cytotoxic marker production [29,33].

Although these techniques have all proven useful, they, nevertheless, have their limitations. Not all the mRNA detected will be reflected in the production of the encoded protein [52] because of the posttranscriptional and posttranslational processes [53], as seen in TNF- α and IL-15 expression, which could explain the

Figure 3 Flow cytometry measurements of antigen surface markers in peripheral blood and blister fluid from skin during the course of a toxic epidermal necrolysis induced by lamotrigine



In the first two samples, higher percentages of CD8, memory (CD45RO), activated (CD69) and the skin homing receptor (cutaneous lymphocyte antigen; CLA) were observed in blister fluids. By day 7, an increase in these markers was observed in peripheral blood, probably because of cells from skin. This could not be compared in the skin because no further blisters appeared.

discrepancies in the results obtained with RT-PCR and enzyme-linked immunosorbent assay [54,55]. This is a particularly important control mechanism in molecules with potent activities, such as chemokines or cytokines, which have to be synthesized at a precise moment, in a low quantity, and in a specific cell, and posttranscriptional dysregulation could result in a pathological process [53]. Another important control process of the immunological response is chemokine and cytokine degradation induced by proteases [55,56]. This can produce an underestimation of molecule levels [56], being particularly problematic in those markers like IL-4 whose concentrations are normally very low.

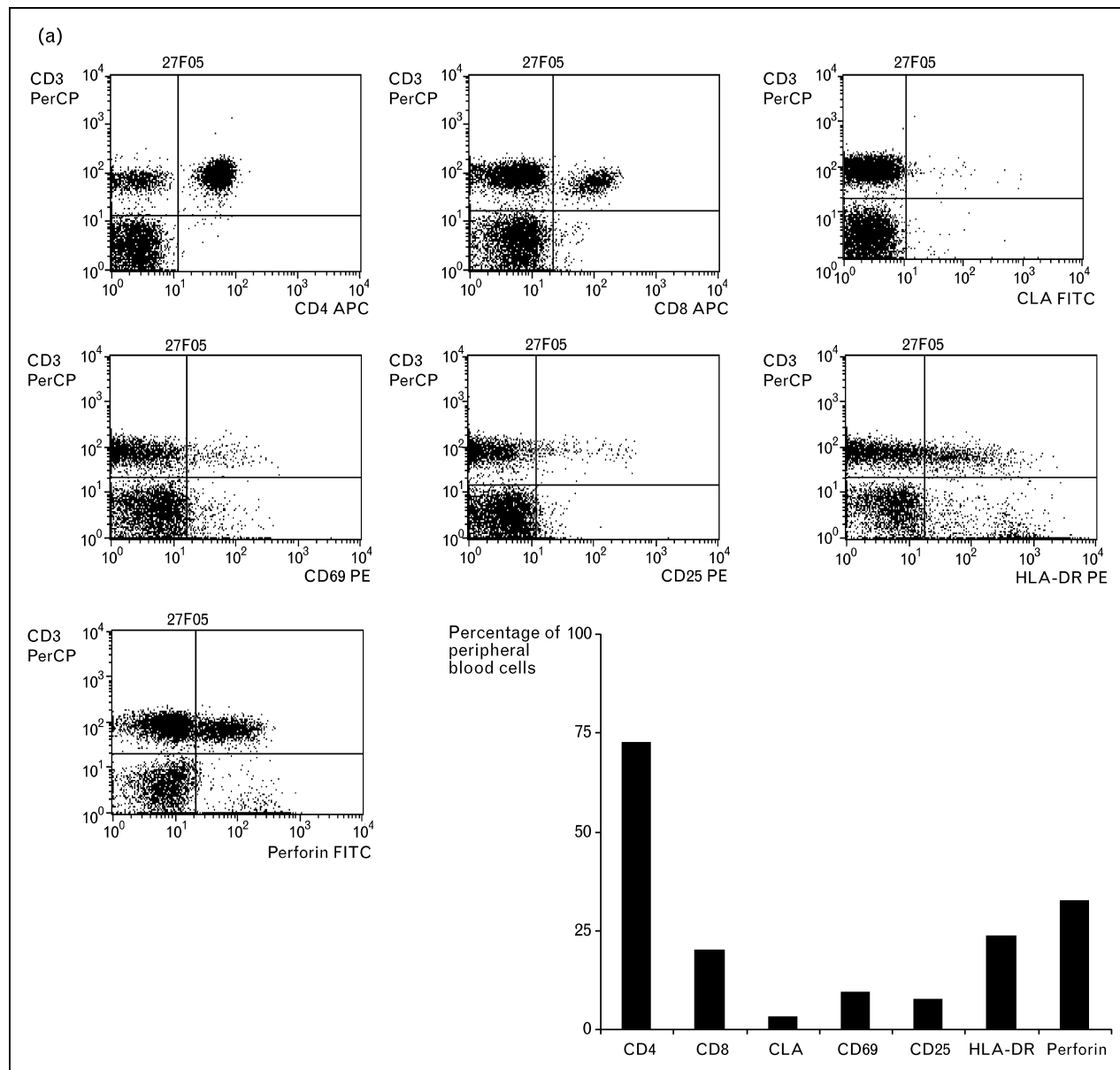
Many mediators are secreted in brief, transitory peaks, so that the timing of sample collection is critical. Reports have shown that different cytokines are produced in peaks at different times [49,57–61], with variations in the maintenance of detectable levels [48,49]. Th2 cytokines seem to have lower detectable levels than Th1 cytokines, so different incubation times have to be assumed [49,57]. The kinetics of the different markers also depend on the producer of the cell subset, thus, some inflammatory markers are much more rapidly secreted and in higher amounts by effector and memory T cells than by naive T cells [58,62]. Moreover, the kinetics of cytokine expression can also depend on the stimuli [49].

These factors require the methodology to be very sensitive, even when we have a good understanding of the kinetics of the different markers. These limitations again necessitate monitoring the reaction by collection of sequential samples.

Researchers are often faced with the problem of what the physiological values are in order to interpret the results in allergic responses. This can be difficult to establish because of interindividual variations in some markers. In these cases, monitoring by obtaining sequential samples during the development of the reaction or the progression of the disease can be useful.

Skin and blood

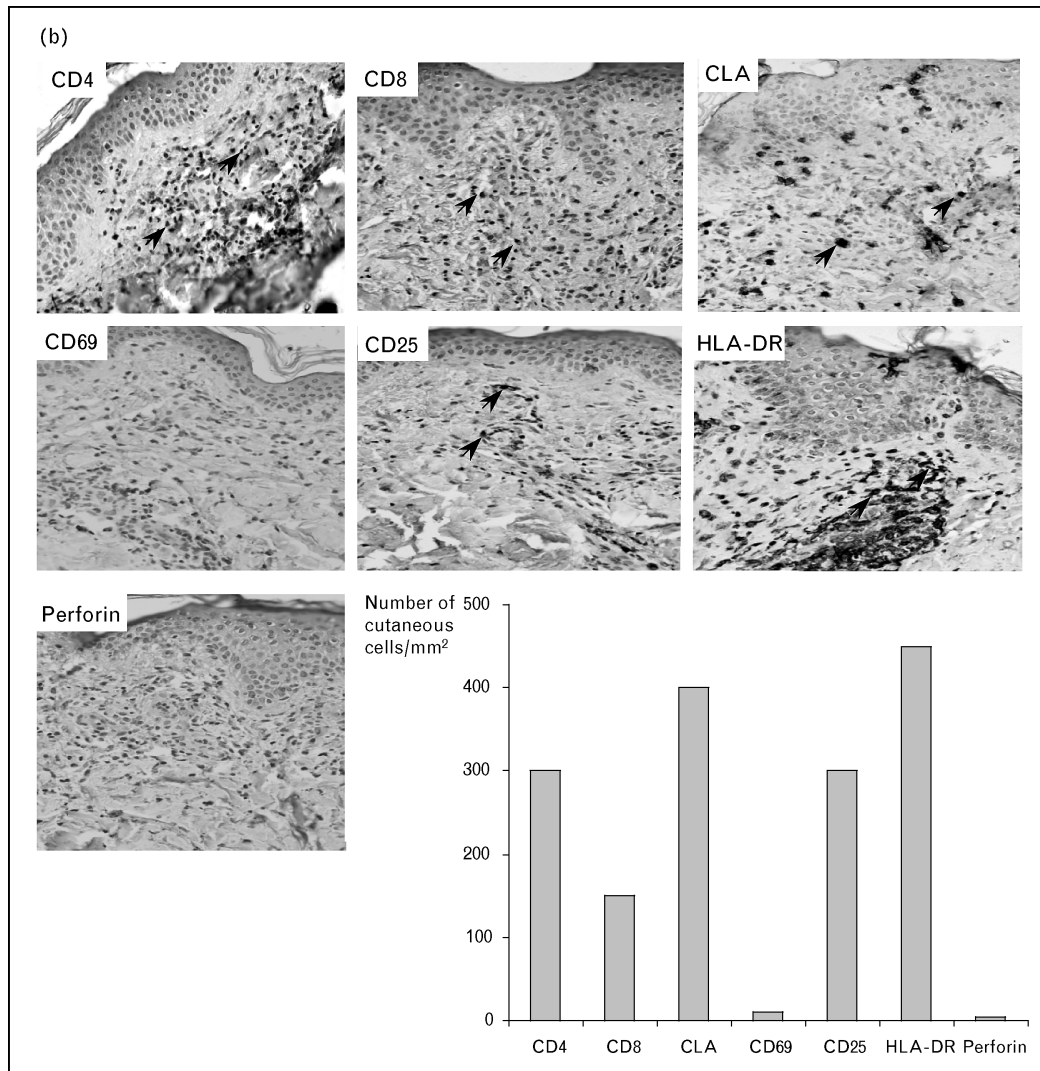
Why the skin is so frequently affected in drug hypersensitivity is not fully understood. Investigation on leukocyte trafficking to the skin has provided insights into the pathophysiological mechanism. It is likely that trafficking of antigen-specific T cells, which is regulated by differential cell-surface expression of chemokine and tissue homing receptors to the skin, plays a key role in different skin diseases [63]. Increased levels of CLA have been detected on activated T cells in drug allergy in parallel with disease severity in both peripheral blood and skin cells from biopsies and blister fluid (Fig. 4) [29,33]. When immediate and nonimmediate reactions were compared

Figure 4 Simultaneous determination of different cell markers

(a) In peripheral blood by flow cytometry. (b) In skin biopsies by immunohistochemical analysis. CLA, cutaneous lymphocyte antigen; HLA, human leukocyte antigen; PerCP, peridinin chlorophyll protein; FITC, fluorescein isothiocyanate.

by analysing the two compartments it was found that in delayed reactions there is a parallel between results found in skin and peripheral blood, with a higher participation of CD4+ cells the more severe the reaction [64]. Other reports have shown that most CLA-positive T cells express skin-homing chemokine receptors such as CCR4 and CCR10 [50,65,66] and an increase in chemokine CCL27 production in keratinocytes, which correlates with the CCR10 expression in memory T lymphocytes and also with severity [21].

Although chemokines are secreted constitutively to allow effective skin immunosurveillance [67], most are produced in response to environmental signals such as inflammatory cytokines, pathogens and physical–chemical stressors [68]. Other activities of the chemokines include the modulation of leukocyte effector functions by cytotoxicity, mediator release, and cytokine production. Apart from trafficking, some chemokine receptors can act as markers of the type of reaction (Th1/Th2), similar to the different cytokines, since CCR4 and CCR8

Figure 4. (Continued).

are mainly expressed in Th2 lymphocytes which mainly produce IL-4, IL-5, IL-10, and IL-13, and CXCR3 in Th1 lymphocytes producing IFN- γ , TNF- α and IL-12. Subsequently, studies of TEN blister fluid identified cytotoxic CD8+, HLA-DR and CD56+ lymphocytes that are able to kill autologous keratinocytes. The blister fluid lymphocytes stained for granzyme B, results that are consistent with earlier findings of markedly increased expression of TNF- α , perforin, and granzyme B from blister fluid in SJS and TEN which mediated necrosis of keratinocytes [27].

In drug allergy, when the same parameters are measured in the two compartments involved, skin and blood, differences can be observed. To understand the kinetics, it is useful to envisage the underlying mechanical process. Initially, although not fully confirmed, the hapten generated in skin is presented by a mature dendritic cell

to a specific naive T cell in the local lymph nodes. The process then continues with release of a wide number of markers that attract by chemotaxis cells from other organs, including bone marrow and peripheral blood. A certain number of cells are trapped in the affected tissue with a variety of functions, many of them contributing to the lesions and others controlling the progression of drug allergy. Certain cells migrate to peripheral blood and moreover the dilution factor produced a low concentration in peripheral blood and for this the determinations are not totally correspondent. In any case, in severe lesions with large skin extension, the stimuli are so great that even in peripheral blood we can observe these changes [29,33].

Conclusion

No one single methodology is currently available with sufficient accuracy to diagnose allergic nonimmediate

reactions to drugs. Monitoring the acute response and resolution period may be just the beginning, despite all the work done so far. This approach may be done not only in skin diseases but also in acute and chronic organ transplantation, bronchial asthma, gastrointestinal diseases, as well as many other entities. The vast number of mediators that can be determined and the multigenetic control of these diseases suggest that the number of genes involved is greater than those mentioned. For example, those participating in drug metabolism, inflammation, acute phase protein induction, and the metalloproteinase system will provide additional information. This is possible thanks to microarray technology, where relevant markers can be chosen to identify a specific process. Finally, sorting cells from peripheral blood or tissue in combination with these sophisticated systems will enable functional studies to be performed and also improve our understanding of the underlying mechanism of non-immediate reactions.

Acknowledgements

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References and recommended reading

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- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 303).

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