Severe delayed-type drug hypersensitivity reactions

Immunological background and accelerated diagnostic work-up

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T cell-mediated, delayed-type drug hypersensitivity is an iatrogenic condition that requires special medical attention. It often presents a frustrating challenge for most practicing physicians and their patients, especially in the ambulant care setting. This overview provides recommendations for accelerating the diagnosis of drug allergy through a process called 'AiDA'- Accelerated diagnosis in Drug Allergy. It also shows how low-threshold access to specialized immunological testing can be successfully implemented. By reliably identifying the culprit drug causing hypersensitivity reactions, the risk of future drug reactions can be minimized without unnecessarily limiting further medically indicated treatment.

Introduction

Adverse drug reactions (ADRs) can occur with all pharmacological therapies. Drug hypersensitivity (DH) is an important subgroup of ADR that usually affects the skin. Still, organ involvement (hepatitis, nephritis) and blood eosinophilia are common and point towards a potentially more severe course. The terms *immediate* or *delayed-type* reaction are used to distinguish mast cell (immediate) from T cell and sometimes antibody-mediated (delayed appearing) symptoms. [1] Here, we will focus on the T cell-mediated, delayed-type reactions, typically starting 7 to 10 days after treatment initiation, and especially on the severe forms such as toxic epidermal necrolysis (TEN), Stevens-Johnson syndrome (SJS) or drug reaction with eosinophilia and systemic symptoms (DRESS).
In general, delayed-type reactions are more frequent than immediate-type reactions. The dogma that immunogenic complexes can only form after binding to an endogenous protein (haptenization) which then elicits a complex immune response with B and T cell responses, has been challenged in the last two to three decades. Most, if not all, of the severe

Table 1. Examples for HLA class I associations with different forms of delayed-type drug hypersensitivity in association with a certain ethnic background (modified according to [4])

Causative drug	HLA allele	Hypersensitivity reactions	Ethnicity	Odds ratio (95% CI)
Abacavir	B*57:01	Abacavir hypersensitivity	Caucasians	117 (29–481)
Allopurinol	B*58:01	SJS/TEN/DRESS	Asians	74.18 (26.95–204.14)
			Non-Asians	101.45 (44.98–228.82)
Carbamazepine	B*15:02	SJS/TEN	Han Chinese	115.32 (18.17–732.13)
			Thai	54.43 (16.28–181.96)
			Malaysians	221.00 (3.85–12694.65)
			Indians	54.60 (2.25–1326.20)
	A*31:01	DRESS	Han Chinese	23.0 (4.2–125)
			Europeans	57.6 (11.0–340)
		SJS/TEN	Europeans	25.93 (4.93–116.18)
		MPE	Europeans	8.33 (3.59–19.36)
		SJS/TEN/DRESS	Japanese	10.8 (5.9–19.6)
	B*57:01	SJS/TEN	Europeans	9.0 (4.2-19.4)
Flucloxacillin	B*57:01	Hepatitis	Europeans/Caucasians	80,6 (22.8–284.9)
	B*57:03	Hepatitis	Europeans/Caucasians	79.2 (13.6–462.4)

1. ADR-AC GmbH, Adverse Drug Reactions, Analysis and Consulting, Bern, Switzerland (www.adr-ac.ch)
2. Loewenpraxis und Klinik St. Anna Lucerne, Switzerland

Table 2. Warning signs (red flags) for progression to a severe form of delayed type drug hypersensitivity, e.g. DRESS, SJS/TEN

Signs & symptoms	Lab Tests
confluent infiltrative exanthema with progression to erythroderma	blood eosinophilia (> 10% and/or > 1G/l)
facial swelling	presence of atypical lymphocytes ('virocytes') in peripheral blood
bullous or pustulous lesions	hepatitis (elevated liver enzymes)
painful skin lesions	nephritis (creatinin, urine sediment)
mucosal involvement	
positive Nikolsky sign (epidermal detachment upon lateral traction of the skin)	
'B symptoms' (lymphadenopathy, fever, malaise)	

Table 3. Drugs eliciting severe cutaneous or systemic delayed type reactions

Stevens-Johnson Syndrome (SJS) and toxic epidermal necrolysis (TEN)	Drug reaction with eosinophilia and systemic symptoms (DRESS)°
allopurinol*	**carbamazepine***
phenytoin	**phenytoin**
carbamazepine*	**lamotrigine**
lamotrigine	**Betalactam antibiotics**
cotrimoxazole (SMX)	minocyclin
nevirapine	allopurinol*
NSAID (oxicams)	dapsone*
	sulfasalazin
	cotrimoxazole (SMX)
	vancomycin

List incomplete: the most frequent elicitors are given in bold.
** The type of reaction might be determined by the presence of a certain HLA-allele*

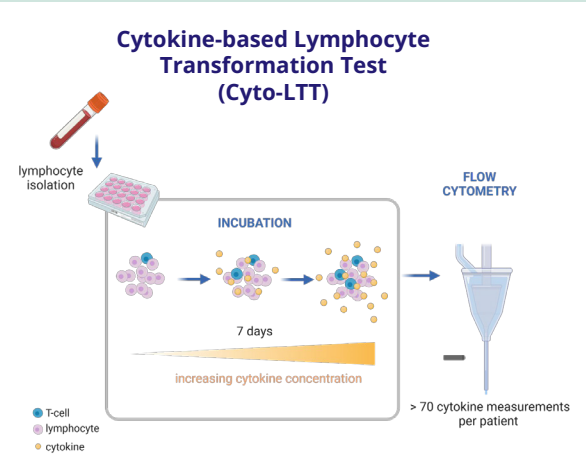
delayed-type reactions, are due to direct T-cell stimulations. They are caused by non-covalent binding of the drug to immune receptors, mainly the human leukocyte antigen (HLA) or the T cell receptor (TCR), summarized under the term *p-i concept* (*p*harmacological interaction with *i*mmune receptors). [2,3]
Epidemiological studies have found a strong genetic link between specific HLA alleles and severe forms of DH, confirming this concept (table 1). [4] As a result, routine HLA screening before prescribing certain drugs such as abacavir and its avoidance in HLA B*57:01-positive individuals has nearly abrogated severe courses of hypersensitivity reactions to this particular anti-HIV drug. [5,6]

Pathogenesis and etiology

Delayed-type drug hypersensitivity is mostly T cell-mediated and appears delayed because the number of drug-reactive T cells is low at the beginning. Symptoms of the skin and other organs occur as soon as the pool of these effector T cells have expanded and a sufficient number has migrated into the skin/organ. [2] This explains the typical time lag between the treatment initiation and the appearance of symptoms 7 to 10 days after the start of the treatment. On the other hand, clinicians should be aware that symptoms caused by an already expanded T cell pool may appear much faster (within a few hours) upon re-exposure. In addition, symptoms may even manifest during the first treatment cycle with the respective drug if the treatment lasts long enough.
T-cell stimulation is central to delayed-type hypersensitivity reactions. In hapten reactions (e.g., beta-lactams), it depends

Box 1. Cytokine-based Lymphocyte Transformation Test (Cyto-LTT)

PBMCs containing T cells and monocytes (as antigen presenting cells, APC) are isolated from whole peripheral blood and cultured for seven days with the addition of the suspected elicitor and potential alternatives. Read-out based on proliferation (e.g. radiolabeled thymidine incorporation on day 7) has been replaced by the measurement of various cytokines such as IFN γ. To further increase sensitivity, a set of specific mediators in the cell culture supernatant (for Th2 reactions: IL- 5, IL- 13; for Th1: IFN γ, and for cytotoxic reactions: Granzyme B and Granulysin) are measured simultaneously [15]. The method is known as cytokine-based lymphocyte transformation test (Cyto-LTT) and can be performed independently of and even before skin testing. For details on the pre-analytic requirements and test methodology see www.adr-ac.ch/cellular-in-vitro-tests/). With a combination of skin and laboratory testing the elicitor as well as safe alternatives can be determined in most of the cases (> 80% in betalactams).



Schematic depiction of Cyto-LTT

Box 2. Clinical example for the use of the Cyto-LTT)

45 y/o female patient with undifferentiated connective tissue disease (UCTD). Two weeks after starting immunomodulatory treatment with hydroxychloroquine 200mg daily, she developed a pruritic confluent maculopapular exanthema with an almost steroid-resistant course for weeks. After discontinuation of the drug, recovery lasted 8 to 9 weeks with scaling over the entire body surface, including ears, toes and soles. There is no injectable form for hydroxychloroquine and no standardized skin test procedure. Five months after the resolution, a Cyto-LTT was performed, which showed a clear sensitization to hydroxychloroquine. It was recommended to avoid the culprit drug and chloroquine as the parent compound in the future. No further episode occurred.

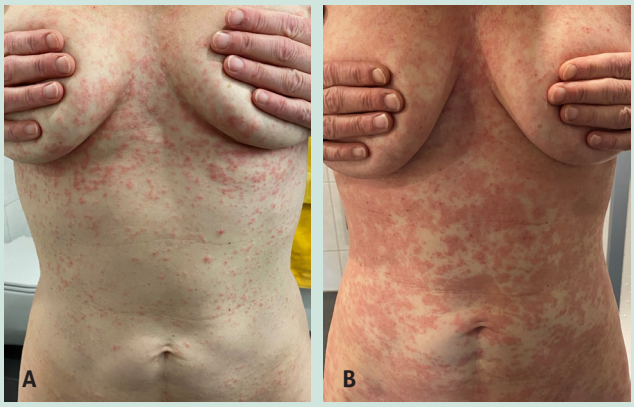


Figure 1: A - early exanthema (2 days after symptom onset); B - late stage exanthema (7 days after symptom onset)

Pure Substances	IL-5	IL-13	IFNg	GzB	GL
Chloroquine Diphosphate	Negative	Negative	Negative	Negative	Negative
0.01 µg/ml	8,2	10,1	8,3	2,2	1,1
0.05 µg/ml	1,1	1,0	1,0	0,8	0,8
0.1 µg/ml	1,0	1,0	0,3	0,1	0,6
0.5 µg/ml	1,0	1,0	0,3	0,2	0,5
2 µg/ml	1,3	1,0	0,3	0,1	0,1
Hydroxychloroquine Sulfate	**POSITIVE**	**POSITIVE**	**POSITIVE**	**POSITIVE**	Negative
0.01 µg/ml	1,0	1,0	0,6	0,4	0,1
0.05 µg/ml	53,7	17,1	49,8	4,2	1,3
0.1 µg/ml	33,0	11,1	41,9	4,3	1,3
0.5 µg/ml	6,0	4,1	6,6	0,4	1,3
Positive Controls	**POSITIVE**	**POSITIVE**	**POSITIVE**	**POSITIVE**	Negative
Pokeweed Mitogen	41,1	138,7	1106,8	18,8	1,3
Tetanus Toxide	14,1	20,1	132,2	18,8	1,3

Cytokine results depicted as stimulation index SI = [cytokine conc. with drug] / [cytokine conc. w/o drug]

on the presentation of haptenized peptides by antigen-presenting cells (APC) on their HLA molecule and engagement of the corresponding T cell receptor (TCR) on CD4+ or CD8+ T cells. [2,3]. But this 'classical' stimulation is the exception in severe forms of DH. Direct binding of the drug to one of the naturally highly polymorphic immune receptors (HLA or TCR), especially low molecular weight chemicals, designed to directly bind to receptors or enzyme pockets, elicit an unintended and uncontrolled immune stimulation without prior haptenization and processing of a hapten-modified protein. [2,3] This non-covalent, direct binding of drugs to immune receptors is an *off-target activity of drugs on immune receptors with extraordinary immunological consequences* and may render the HLA-TCR complex in a way that it looks like an allo-allele, causing *extreme symptoms* similar to e.g. graft-versus-host disease (GvHD). [7]

Therefore, *p-i* stimulations are often massive immune stimulations, and the activated T cells in DRESS, for example, are subsequently highly susceptible to otherwise subthreshold stimuli. 'Flare-up' reactions may occur, or additional DH reactions may be acquired to structurally unrelated but concomitantly given drugs, leading to so-called multiple drug hypersensitivity (MDH). [8-10] This is why the eliciting drug and *all non-vital co-medications* should be stopped or avoided in DRESS until the disease has completely subsided.

Clinical features (phenotypes)

P-i stimulation can result in a heterogeneous clinic. [2,3] In some reactions, there is massive T-cell expansion and persistent effector T-cell hyperreactivity (MPE, DRESS). In other cases, the reactive T cells lead to a predominant cytotoxic effect (keratinocyte killing) and no longer proliferate, and there is no intolerance to other drugs (SJS/TEN).

In maculopapular exanthema (MPE), the epidermis is affected by virus-like skin rashes with either scaling or blister formation and, depending on the extent of tissue infiltration, other skin sensations such as warmth or pain occur in addition to itching. In the acute phase, the effector T cells in the skin may only be

the tip of the iceberg. Therefore, the involvement of internal organs (liver, lungs, kidney) and the extent of blood eosinophilia (>1.0 G/l as an indicator of general tissue infiltration) should be investigated. [11] Possible warning signs, so-called 'red flags' for a potentially more severe course at initial evaluation, are summarized in table 2.

Any drug may be involved in a delayed-type hypersensitivity reaction. Therefore, a list of typical culprits for severe forms of DH (with slight variations for the different reaction types) may help clinicians to identify a skin reaction as a possible hypersensitivity reaction, see table 3.

Practical clinical considerations

In the acute phase of a delayed-type DH reaction, it is recommended to test for organ involvement beyond the skin to determine the severity of a suspected T cell-mediated delayed-type reaction.

After resolution, skin tests (e.g., intradermal and epicutaneous/patch test with delayed reading) with the suspected elicitor, potentially cross-reacting, and alternative drugs are the mainstay of standard allergological work-up. [12] Access to such sophisticated testing procedures that follow international standards [13] is limited in most countries and waiting lists are long. In recent decades, additional laboratory tests, such as the lymphocyte transformation test (LTT), have become available and accessible to complement this work-up. [14] With some modifications of the original method, especially using a cytokine-based read-out, the test characteristics (sensitivity/specificity) for putative drug hypersensitivity reactions could be optimized. [15] Timing is crucial, as test sensitivity decreases over time and can lead to false-negative test results when the waiting period for an appointment with the specialist offering such a test is too long. LTT is based on living T cells isolated together with monocytes from whole peripheral blood (PBMCs) and cultured for seven days with the addition of the suspected elicitor and potential alternatives. See box 1 for the schematic overview of the lymphocyte transformation test.

Especially in severe forms of delayed-type DH, where both skin testing and challenge testing might be contraindicated due to the risk of possibly triggering another allergic episode, or in cases where the suspected drug is not available in a form suitable for skin testing, a laboratory test may be the only available test (see box 2 for clinical example).

Referral and accelerated allergy work-up

After the patient's convalescence, especially in severe immunologic reactions, plans should be made to obtain a reliable diagnosis and identify the culprit drug. Unfortunately, most patients have to wait months for their appointment with a specialized center.

Alternatively, we suggest conducting an early laboratory test with a (specialized) practitioner closely collaborating with an laboratory experienced in DH diagnostics and with the patient's family physician to obtain the relevant information about the patient's medical history. This way, the time between the hypersensitivity reaction and the subsequent work-up can be shortened, at least for the laboratory tests. In the best case, it could be aligned with the subsequent skin and/or provocation tests to maintain optimal test sensitivity. Otherwise, especially in rural areas, the patient and their family physician might refrain from a seemingly complicated further investigation, including testing. This could be the case

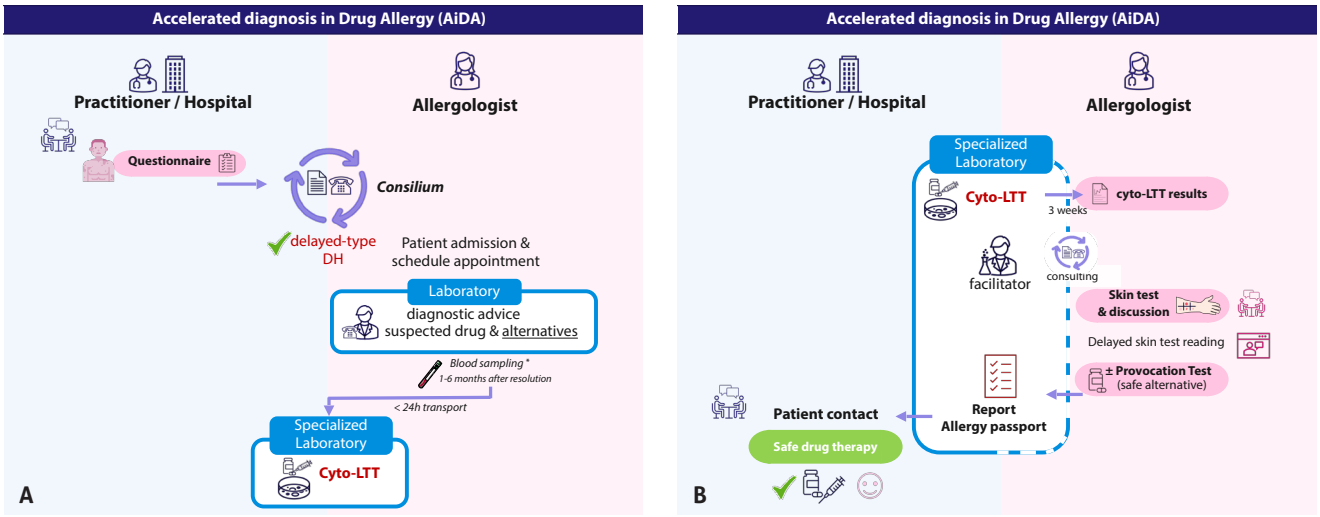


Figure 1 A/B. Accelerated diagnosis in drug allergy (AiDA). Schematic diagnostic journey for a single patient with suspected drug hypersensitivity (DH). The treating physician provides the specialist the DH-specific information in advance, which allows to estimate pre-test probability. During this first contact between specialist and treating physician, optimal management during the acute phase can also be discussed. If further work-up is pursued, laboratory testing is coordinated by the specialist (Fig. 1A). After the results are available, they can either be discussed with the laboratory if uncertainty prevails (e.g., equivocal results, additional test needed, planning of skin test procedure, etc.) or they can be directly discussed with the patient at the first in-person visit at the specialist, ideally at the time of the scheduled skin testing. If needed, additional provocation tests can be performed. A report details all DH-specific information, such as culprit, cross-reactive drugs and safe alternatives, and can be sent to the patient and treating physician (Fig 1B).

if a diagnosis's burden seems to outweigh the burden of the disease because the meanwhile fully recovered patient might have to travel "so far" and "so often" to the next, specialized center.
Therefore we propose a streamlined process, visualized in Figure 1 A/B, called AiDA (Accelerated diagnosis in Drug Allergy). It is a process that we have practiced with good results for the last years. It represents a low-threshold option for a conclusive DH work-up and can encourage more patients to embark on this diagnostic journey. The specialized laboratory can act as a facilitator in identifying and stratifying cases. On the other hand, all referring physicians can benefit from the experience of a national laboratory specialized in drug hypersensitivity with cases from all over the country.

Conclusions

Drug hypersensitivity is an iatrogenic condition that requires medical attention, but it often presents a frustrating challenge for most practicing physicians. Immunodiagnostic test possibilities for DH are not always routinely available, and indeed only in some countries. Therefore, many patients are left with the recommendation to permanently and completely avoid the putative eliciting drugs and structurally related compounds. Consequently, we advocate that low-threshold access to specialized allergological testing should be a goal for national health care. Especially since a surprisingly large number of patients experiencing DH could be safely medicated in the future once a reliable diagnosis is established.

The risk of further drug reactions can be minimized without unnecessary restrictions on future treatment. This is particularly important:

1. in severe delayed type drug hypersensitivity, e.g., DRESS, SJS/TEN (with peculiarities in their pathogenesis, namely direct, mostly uncontrolled T cell activation by direct binding to immune receptors)
2. when multiple and/or medically necessary drugs are involved
3. to clarify cross-reactivity patterns and to establish safe alternatives if re-exposure is expected (e.g., antibiotics, neuromuscular blockers, contrast media, etc.).

If the culprit of the DH reaction is reliably identified, using wallet cards, identification jewelry, and registry services should be recommended for patients with documented severe DH reactions.

Keywords

drug allergy - drug hypersensitivity - delayed-type - T cell - lymphocyte transformation test - LTT "- cytokine

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