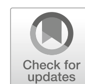

In Vitro Delayed-Type Sensitivity Testing

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Detlef Becker

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Abstract

Although patch testing is regarded to be the most standardized and reliable method for diagnosis of delayed-type hypersensitivity to contact allergens, its substitution by in vitro techniques would be advantageous for several reasons.

The lymphocyte transformation test (LTT) measures the allergen-specific proliferation or activation of T cells in vitro and has been explored as potential alternative for patch testing for decades. Beside numerous attempts to increase its sensitivity and specificity, the major drawback of a cell culture-based method is the poor solubility of many contact sensitizers and the need for chemical modification of prohaptens.

The LTT is used with regularity only for diagnosis of hypersensitivity to beryllium and adverse reactions to drugs. Its usefulness in occupational dermatology is limited and restricted to special situations as a supplement for patch testing with large panels of well-established sensitizers.

Keywords

Patch testing · Lymphocyte transformation test · Delayed-type hypersensitivity · Beryllium · Drug hypersensitivity · In vitro testing · MELISA · Nickel · Mercury · T cells · ELISpot · Cytokines · Cell proliferation · Sensitivity · Specificity

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S. M. John et al. (eds.), *Kanerva's Occupational Dermatology*,
https://doi.org/10.1007/978-3-319-68617-2_85

Abbreviations

BrdU	Bromodeoxyuridine
LTT	Lymphocyte transformation test
PBMC	Peripheral blood mononuclear cells

1 Core Messages

- Patch testing is a standardized and reliable method for diagnosis of delayed-type hypersensitivity
- In vitro testing is demanded as substitute for in vivo testing for several reasons.
- The lymphocyte transformation test (LTT) is the most methodologically sound technique for in vitro testing.
- Its specificity and sensitivity in comparison to patch testing as gold standard are dissatisfying.
- The LTT is used with regularity only for diagnosis of hypersensitivity to beryllium and drugs.
- Routine patch testing with a large panel of contact sensitizers cannot be substituted by in vitro techniques.

2 Introduction

Patch testing is regarded to be the most standardized and reliable method for diagnosis of delayed-type hypersensitivity to contact allergens. Although the method is indispensable for daily differential diagnosis of contact eczema, the development of in vitro tests for contact hypersensitivity is demanded as substitute or supplement for the in vivo exposure of patients to contact allergens. Concerns raised on patch testing can be summarized as follows:

1. The clinical signs of eczema provoked by contact to an irritating or sensitizing compound are more or less nonspecific. A doubtless differentiation between allergic and irritant contact eczema using clinical, histological, and even molecular criteria is impossible. Additional indices obtained from in vitro tests should help to categorize a questionable compound as sensitizer or irritant in general and aid for proper diagnosis in single cases.
2. The responsiveness of the skin during patch testing is augmented by inflammation and eczema even outside of the test area, critically. Therefore, patch testing is not recommended in chronic eczema or early after episodes of allergic contact dermatitis. In practice, this rule has to be broken in patients with persisting or frequently relapsing eczema. Patch test results obtained under such conditions need to be interpreted very cautiously, due to frequent false-positive reactions. In vitro testing might help to discriminate between a relevant sensitization and artifacts.
   (a) Beside factors related to the clinical situation of the patient, patch testing may be restricted by chemical or toxicological features of the allergen itself (cancerogenicity, teratogenicity, boosting of sensitization, iatrogenic sensitization). These hazards would be avoided by the use of an in vitro technique.

3 State of the Art of In Vitro Testing for Delayed-Type Hypersensitivity

Allergic contact eczema is the clinical manifestation of a delayed-type hypersensitivity reaction. The initial inflammatory signal depends on the specific activation of effector T cells, and an ideal in vitro test should spot this process with the same specificity and sensitivity as patch testing. T cell receptors recognize haptens covalently or noncovalently bound to peptides in the binding grove of MHC molecules. Every prerequisite and component necessary to build these target structures has to be provided in the in vitro situation; otherwise T cell activation fails. Any secondary process or signal specifically induced by this activation should be suitable as endpoint for an in vitro test. During an early stage of understanding the immunology of delayed-type hypersensitivity, methods like the migration inhibition test and procoagulant activity assay were explored. They have been replaced by techniques for the direct analysis of cell proliferation and cytokine production.

The detection of cell proliferation in cultures of mononuclear blood-derived cells is now the mostly accepted and well-referenced method of choice and named as lymphocyte transformation test (LTT), or the synonyms lymphocyte proliferation test (LPT) and lymphocyte activation test (LAT).

The LTT is poorly standardized, and several modifications have been suggested and explored to improve its sensitivity and specificity. Main technical aspects are summarized in Fig. 1.

Peripheral blood mononuclear cells (PBMC) are prepared from heparinized blood by density gradient centrifugation. Although few groups use separation techniques for enrichment of antigen-presenting cells or T cells (Räsänen et al. 1991), most often, PBMC are stimulated with haptens, directly. One modification of the LTT is called MELISA (Stejskal et al. 1994). The technique avoids the use of heparin and utilizes a preculture of PBMC to minimize the amount of monocytes. Whether this technique is superior to the common LTT protocol is being controversially discussed (Cederbrant et al. 1997; Valentine-Thon et al. 2006).

To support the proliferation of activated T cells, cytokines like IL-4, IL-7, and IL-12 have been added. This results in a substantial improvement of sensitivity not even for nickel sulfate, which has become a model allergen in many studies, but also for other common allergens of the standard patch test series (Moed et al. 2005; Spiewak et al. 2007).

Most authors recommend the titration of haptens, some remove the antigen after short-term stimulation, but most ensure its presence during short-term culture for 3–7 days under sterile cell culture conditions. The most common method to quantify cell proliferation is the incorporation of [^{3}H]thymidine or bromodeoxyuridine (BrdU), alternatively. If the fluorescence marker carboxyfluorescein diacetate succinimidyl ester (CFSE) is added at the beginning of cell culture, its dilution by cell division can be quantified using flow cytometry. In addition, a selective analysis of surface markers of these dividing cells helps to identify subgroups of responding T cells (Milovanova et al. 2004). A similar analysis of T cell subgroups can be achieved by selective flow cytometric quantification of BrdU incorporation into the DNA of proliferating cells (Farris et al. 2000) or screening for the proliferation marker Ki-67 (Popple et al. 2016).

Beside cell proliferation, secretion of cytokines like IL-5 and interferon γ may be quantified by MELISA or following a modified protocol by the use of the ELISpot technique (Jakobson et al. 2002; Lindemann et al. 2003; Masjedi et al. 2003; Bordignon et al. 2008). Positive control mitogens or recall antigens like tetanus toxoid or candida antigens are used.

The results of the proliferation assays are presented as stimulation indexes calculated from the quotient of stimulated versus control cells. Cytokine levels are depicted as absolute values and ELISpot data as number of cytokine-secreting cells.

The LTT is validated by comparison to patch test results as gold standard. By this measure, sensitivity and specificity vary as a function of the hapten, method, and investigator. False-negative as well as false-positive results,

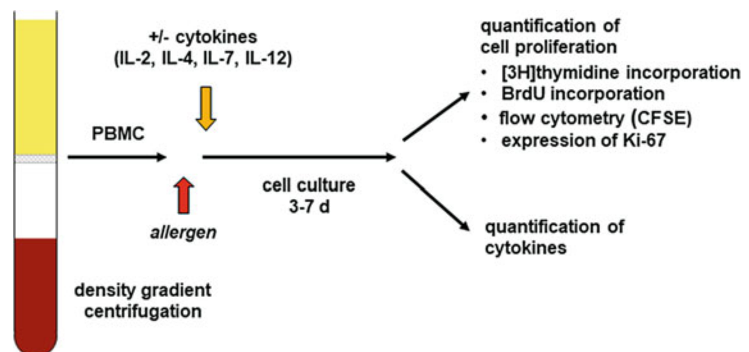

Fig. 1 Basic principle and modifications of the lymphocyte transformation test. *PBMC* peripheral blood mononuclear cells, *BrdU* bromodeoxyuridine, *CFSE* carboxyfluorescein diacetate succinimidyl ester

especially those seen for metal salts, cannot be controlled without patch test results for comparison. Because in vitro testing has its indication when patch testing is impossible or produces doubtful reactions, its results should be rated as part of the complete diagnostic procedure but never as ultimate proof or exclusion of a clinically relevant sensitization.

4 The Limits of In Vitro Test Systems

The activation of effector T cells in a highly sensitive in vitro test system is a proof for the existence of these cells but not for clinically relevant contact hypersensitivity. Nowadays, a missing reaction to a contact allergen is rated not only as exclusion of a sensitization but also as possible reflection of a state of tolerance. The immune system might react to the hapten upon contact but does not allow the development of allergic contact dermatitis. An efficient in vitro test has to model this complex and as yet only incompletely understood process in detail; otherwise false-positive results are produced, inevitably. And indeed, for nickel sulfate and mercury, it has been demonstrated that many patients without nickel allergy (Lisby et al. 1999) or patch test reaction to mercury (Cederbrant et al. 1999) showed a positive response in the LTT.

The immunogenic hapten-peptide complex necessary for the restimulation of effector T cells is formed in the skin. Whether this process takes place in an aqueous cell culture system needs to be shown for every single allergen. Problems may arise for hydrophobic or completely water-insoluble compounds as well as for prohaptens (Chipinda et al. 2011). The latter are precursors of the actual allergen and formed by chemical modification in the skin but not necessarily in a cell culture system (Krasteva et al. 1993). An insufficient formation of the relevant hapten-peptide structure may be the main reason for false-negative results of the LTT.

5 Occupational Contact Sensitizers Studied with LTT Techniques

The LTT is not used routinely for diagnostics in occupational dermatology. But it is an important tool for early diagnosis of the granulomatous lung disorder chronic beryllium disease (CBD) (Mroz et al. 2009; Middleton and Kowalski 2010; Fireman et al. 2016). Due to the lack of standardized in vivo tests for drug hypersensitivity, the method is used in cases of an expected delayed-type mechanism (Pichler and Tilch 2004; Merk 2005; Kano et al. 2007; Doña et al. 2017) and has been explored for diagnosis of suspected occupational hypersensitivity to drugs in workers of pharmaceutical plants (Stejskal et al. 1986; Riviera et al. 1995; Ghatan et al. 2014). Most studies for evaluation of the LTT have been performed using a very common contact sensitizer like nickel (Ständer et al. 2017). In contrast, the number of studies describing a valuable contribution of in vitro testing for the diagnostic procedure in occupational dermatology is limited.

Table 1 lists occupational contact sensitizers which have been examined in the LTT as well as further studies covering allergens with relevance for occupational dermatology.

Table 1 Occupational contact sensitizers studied with LTT techniques

Chloroacetophenone	Brand et al. (1995)
Chromium	Räsänen et al. (1991)
	Martins et al. (2008)
Cobalt	Moed et al. (2005)
Fragrance mix	Moed et al. (2005)
Isothiazolinones	Stejskal et al. (1990)
Methylisothiazolinones	Masjedi et al. (2003)
	Popple et al. (2016)
Nickel	Räsänen and Tuomi (1992)
	Spiewak et al. (2007)
Omeprazole	Ghatan et al. (2014)
Paraphenylenediamine	Kneiling et al. (2010)
	Bordignon et al. (2015)
Persulfates	Yawalkar et al. (1999)
Urushiol	Byers et al. (1979)

6 Conclusive Remarks on the Role of In Vitro Tests for Delayed-Type Sensitivity in Occupational Dermatology

Due to an inacceptable sensitivity and specificity, high costs, and the need for laboratory infrastructure, the LTT, including its modifications as well as any other in vitro technique, is not suitable as substitute or confirmation for routine patch testing with a large panel of contact sensitizers.

On the other side, if in vivo methods are inapplicable or generate doubtful results, the LTT may be useful for specialized centers as a supplement for diagnosis.

Irrespectively of the limited usefulness for clinical purpose, in vitro techniques offer fascinating opportunities for scientific studies and should help to further uncover basic molecular mechanism of delayed-type hypersensitivity.

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