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RESEARCH LETTER

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In vitro detection of T cell sensitization by interferon- γ secretion in immediate-type drug allergy

To the Editor,

The lymphocyte transformation test (LTT) is used for the in vitro detection of drug sensitization in assumed drug allergic reactions. Due to its complexity, it is currently not part of the routine diagnostic work-up and its application is limited to respective laboratories providing the technical requirements for its conductance. In the LTT, peripheral blood mononuclear cells (PBMCs) of the drug allergic patient (and a healthy control person) are incubated in vitro for 6 days with the culprit drug. Following antigen (drug-) specific activation, the T cells transform into blasts (giving the name for the LTT) and start to proliferate. In the classical LTT, T cell *proliferation* is measured by means of incorporated radioactivity following the addition of a radio-labelled DNA-base (3H-thymidine). Nowadays, the detection of selected cytokines by ELISA is a commonly used read-out method, which in addition allows the characterization of the in vitro T cell reactivity, e.g. in terms of a Th1 or Th2 reaction.^{1–4}

The LTT is recommended in particular for *delayed-type* (type IV) reactions, in which the distal effector phase is also mediated by T cells. However, it was also successfully applied in immediate-type (type I) reactions, where the distal effector phase is typically mediated by IgE antibodies, with frequently positive reactions in anaphylaxis and occasionally positive reactions in urticaria.^{2,3} In most studies, the results of the LTT in immediate-type reactions were reported for single patients but it was not the primary aim of the study to investigate its sensitivity for immediate-type reactions. In contrast, Luque et al.³ analysed its sensitivity (64.5%) for a larger set of patients with immediate-type reactions (29 anaphylaxis, 2 urticaria) to beta-lactam antibiotics. As a read-out parameter, drug-specific proliferation measured by incorporation of radio-labelled thymidine was used.

To the best of our knowledge, no study has been published that assessed the sensitivity of the LTT in *immediate-type* reactions in a larger set of patients using *ELISA-based determination of cytokine secretion* as a read-out method. Here, we report on 12 LTTs in 12 patients with a stringent history of immediate-type reactions (2 urticaria, 10 anaphylactic reactions) to antibiotics (9 beta-lactams, 1 sulfamethoxazole) and other drugs (1 gelatine, 1 rocuronium) (Table 1). From all patients, an informed consent was obtained.

PBMCs from 7/12 patients gave a positive result with an IFN- γ read-out (=stimulation index (SI): IFN- γ secretion stimulated/unstimulated cultures ≥ 2 (or ≥ 3 for beta-lactams); drugs were tested in

three different concentrations) resulting in a sensitivity of 58%. The specificity was 75% based on 3/12 healthy controls without known allergy to the drugs also giving a positive result (see Table 1). If related to the number of suspected drugs tested ($n = 14$), the sensitivity and specificity were 57% and 71% based on eight positive results in patients and four positive results in healthy controls, respectively. Additionally, we calculated the positive predictive value (PPV) and negative predictive value (NPV) resulting in 70% for the PPV and 65% for the NPV.

In contrast, PBMCs from only two patients secreted the Th2 cytokine IL-5 after drug stimulation, although all patients did so for the positive control tetanus toxoid. Additionally, PBMCs from all controls gave a negative result for IL-5 but were positive, if stimulated with tetanus toxoid.

The sensitivity in our analysis based on the detection of Th1 cytokine IFN- γ by ELISA (58%) was lower than that reported by Luque (64.5%) who used proliferation as a read-out.³ In a recent meta-analysis, the reported sensitivity (80%) for the ELISA based cytokine read-out—irrespective of the phenotype—was clearly higher.⁴

Notably, in our study the specificity was low (ca. 75%) compared to reported specificities for the ELISA-based cytokine read-out in the LTT ranging from 62%–100%.⁴ All (false) positive reactions (4/4) in PBMCs of healthy controls referred to beta-lactams which may reflect that 9/12 tested drugs were beta-lactams.

In summary, our results confirm published data that the LTT may also prove helpful in patients with immediate-type drug reactions. However, due to the low sensitivity (58%), the results should be considered in the context of a stringent history and other allergy test results. It may be less useful than in delayed-type reactions. From a clinical point of view, it can thus help to confirm a drug hypersensitivity in these patients, but cannot exclude it. Its application may be helpful to widen the diagnostic options for patients with severe anaphylactic reactions (e.g. grade IV), in whom physicians do not want to perform skin tests or for patients in whom skin testing may be inappropriate/contraindicated (e.g. pregnancy, factious urticaria). Furthermore, one might prefer performing an in vitro test prior to skin testing in patients where a strong allergic reaction is expected. In this context, the basophil activation test (BAT) is another important in vitro test system to detect drug-induced immediate type reactions. To our knowledge, there are

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only very few data regarding a comprehensive direct head-to-head comparison between the LTT and BAT in terms of specificity and sensitivity in immediate type drug hypersensitivities.⁵ However, for the BAT studies reported a sensitivity ranging from 36.1% to 91.7% and a specificity ranging from 79% to 100% depending on the drug.⁶ The time to testing (2 weeks for the BAT) is comparable to the earliest time to testing period when the LTT should be performed. The BAT and its application in immediate type drug hypersensitivity are summarized elsewhere.^{6,7}

Key messages list

- IFN- γ secretion can be used as a read-out in the LTT in patients with Th2 phenotypes.
- Due to low sensitivity, results should be considered in context with history and other results.
- It may widen diagnostic options for patients in whom skin testing may be inappropriate/contraindicated.

TABLE 1 Characteristics and test results of the patients

Number	Age, gender	Clinical phenotype	Suspected drug	Prick test	Intracutaneous test (no delayed readings after 24 or 48 h)	Specific IgE/total IgE/BAT (if tested)	LTT result IFN- γ (SI)	LTT result of healthy control	Time to testing
1	38 years female	anaphylactic reaction grade II	cefuroxime	not determined	not determined	not determined/total IgE 17.0 kU/I BAT positive	positive cefuroxime 5 μ g/ml: SI 36.7 50 μ g/ml: SI 54.2 200 μ g/ml: SI 44.5	negative	9 months
2	25 years female	anaphylactic reaction grade I	cefuroxime	negative	positive	not determined/total IgE 34.5 kU/I	positive ceftriaxone 50 μ g/ml: SI 3.0 200 μ g/ml: SI 13.8	negative	3 months
			not applicable	penicillin	negative	positive	not determined/total IgE 34.5 kU/I BAT positive	negative penicillin	10 years
The patient had a stringent history for a cefuroxime induced anaphylactic reaction but was also tested positive intracutaneously for ceftriaxone. We tested ceftriaxone in the LTT, which was positive. Additionally, the patient reported a reaction against penicillin in childhood, which was confirmed by a positive intracutaneous test and BAT. However, the LTT gave a negative result which was included in our sensitivity calculation, if all tested drugs were considered.									
3	74 years female	anaphylactic reaction grade III	gelatine	not determined	not determined	positive gelatine (CAP class: 0; 0.22 kU/I)/total IgE 12.2 kU/I/ BAT: positive	positive gelatine 1 μ g/ml: SI 2.2	negative	7 months
4	59 years female	anaphylactic reaction grade III	amoxicillin	negative	positive	negative (amoxicillin)/total IgE 121 kU/I	positive amoxicillin 250 μ g/ml: SI 5.3 500 μ g/ml: SI 30.0	negative	27 months
5	56 years female	anaphylactic reaction grade III	cefuroxime	positive	not determined	not determined/total IgE 54.8 kU/I	positive cefuroxime first LTT: 50 μ g/ml: SI 7.5 second LTT: 50 μ g/ml: SI 3.3	first: positive cefuroxime 50 μ g/ml: SI 15.9 second: negative (different control person as in the first LTT)	First: 2 months Second: 4 months
The Patient was tested two times and reacted both times to cefuroxime. For the calculation of the overall sensitivity, the number of patients was considered. However, if the number of LTTs is considered the sensitivity would be result in 61.5% and the specificity in 76.9%.									

(Continues)

TABLE 1 (Continued)

Number	Age, gender	Clinical phenotype	Suspected drug	Prick test	Intracutaneous test (no delayed readings after 24 or 48 h)	Specific IgE/total IgE/BAT (if tested)	LTT result IFN- γ (SI)	LTT result of healthy control	Time to testing
6	48 years female	anaphylactic reaction grade IV	cefuroxime	questionable/unclear	positive	not determined/total IgE 274 kU/l	positive cefuroxime 50 μ g/ml: SI 6.7	positive cefuroxime 50 μ g/ml: SI 3.0 200 μ g/ml: SI 4.6	25 months
7	57 years female	urticaria	rocuronium	negative	positive	not determined/total IgE: 3.27 kU/l	positive rocuronium 1 μ g/ml: SI 697.0 10 μ g/ml: SI 3.0 100 μ g/ml: SI 3.0	positive rocuronium 100 μ g/ml: SI 2.3	30 months
8	50 years male	anaphylactic reaction grade III	amoxicillin	not determined	not determined	positive (CAP3)/total IgE 604 kU/l	negative amoxicillin	negative amoxicillin	4 months
		not applicable	penicillin G	not determined	not determined	positive (CAP3)/total IgE 604 kU/l	positive penicillin 500 μ g/ml: SI 14.2	positive penicillin 500 μ g/ml: SI 4.4	not applicable
		The patient had a stringent history of an amoxicillin-induced anaphylactic reaction. The LTT gave a negative result (SI: 2.7). However, the patient showed drug-specific IgE for penicillin and a positive LTT for penicillin (SI: 14.2). Since the LTT was negative for amoxicillin (although positive for penicillin), this LTT was considered negative for the calculation of the overall sensitivity.							
9	52 years female	urticaria	cefuroxime	negative	positive only after 8 h	not determined/total IgE 97.5 kU/l	negative cefuroxim	negative cefuroxim	5 months
10	34 years male	anaphylactic reaction grade III	amoxicillin	positive	not determined	negative/total IgE 956 kU/l	negative amoxicillin	negative amoxicillin	5 months
11	35 years female	anaphylactic reaction grade III	ampicillin	not determined	not determined	ampicillin 8.49 kU/l/total IgE 26 kU/l	negative ampicillin	negative ampicillin	5 months
12	53 years female	anaphylactic reaction grade II	sulfamethoxazol	not determined	not determined	not determined/not determined/BAT positiv	negative sulfameth-oxazole	negative sulfameth-oxazole	3 months

Note: The different grey shades refers to:

- white: positive LTT (patient) and negative LTT (control person)
- light grey: positive LTT (patient) and positive LTT (control person)
- grey: negative LTT (patient) and negative LTT (control person).

All concentrations (three tested) with a positive SI are shown.

As a new finding, the data suggest that the detection of IFN- γ secretion (a Th1 cytokine) can be used as a read-out parameter in the LTT in patients with Th2 phenotypes (e.g. anaphylactic reactions). This finding may reflect that the effector memory T cells—which are the target cells in the LTT—were in contrast to the clinical phenotype not of a Th2 phenotype. Further studies are necessary to characterize these memory cells in the LTT in a sufficient number of different clinical phenotypes.

IL-5, although representing a Th2 cytokine (which has been proven helpful in delayed-type reactions⁸), had a very low sensitivity for the in vitro detection of drug sensitization in immediate-type (Th2) reactions in our analysis. Taken together these findings also underline that the in vitro reaction pattern in the LTT (Th1 or Th2) does not necessarily have to correspond with the clinical reaction pattern (Th1 or Th2) as also demonstrated in other studies.⁹

AUTHOR CONTRIBUTION

AG, GW, SR, AY and BS contributed to the conception and design of the study. The experiments were performed by AG. The evaluation and discussion of the results were performed by AG, GW, AY and BS. The first draft of the manuscript was written by AG and BS. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

KEYWORDS

drug allergy, IFN- γ , IL-5, immediate type reaction, lymphocyte transformation test

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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DISCLAIMER

The information and views set out in this manuscript are those of the authors and do not necessarily reflect the official opinion of the Federal Institute for Drugs and Medical Devices.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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