Is there an *in vitro* test for type IV allergy discriminating between sensitization and allergic disease?

This editorial discusses the findings of the paper in this issue by Lindemann et al. [4] pp. 1468–75

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Contact dermatitis is defined as an inflammatory response of the upper layers of the skin – namely epidermis and dermis – that occurs as a result of contact with exogenous substances. It accounts for approximately 80% of environment/occupation-based dermatoses and 30% of all occupational diseases [1]. Contact dermatitis includes irritant contact dermatitis and allergic contact dermatitis – irritant contact dermatitis being the most common form. Irritant contact dermatitis is induced by either an obligatory irritant inducing an inflammatory response in every individual or is the result of a cumulative toxic process in susceptible individuals. Because of the fact that irritative contact dermatitis is *per definitionem* a non-immunologic mechanism, no immunologic sensitization can be detected *in vitro*. Thus, the only tool for diagnosis – in case of irritative contact dermatitis – is a thorough history, perhaps accompanied by tests for individual disturbed barrier function (e.g. alkali resistance method, nitrazine yellow [2]).

Allergic contact dermatitis is a delayed hyper-sensitivity (type IV) reaction and involves sensitization before the development of clinical symptoms. Since its introduction into medicine in 1895 by Joseph Jadassohn, the patch test is the method of choice in the diagnosis of contact sensitization [3]. However, considering the more than 100 years bygone since then, it seems to be time to come up with an objective tool for diagnosis of contact sensitization with predictive value for clinical relevance. Until today, the combination of clinical history and patch test results constitutes the two cornerstones in the diagnosis of type IV allergy. Simple, safe and accurate *in vitro* methods identifying contact allergy would offer both theoretical and practical advantages. At present, the only substantial *in vitro* test – although primarily in the experimental setting – is the lymphocyte transformation test (LTT). Currently, research focuses in many areas on the identification of biomarkers for disease or progression of diseases – whereas the necessity of an *in vitro* diagnostic tool for contact dermatitis is largely neglected.

This editorial aims to discern – on the basis of the publication in this issue of Lindemann et al. [4] – current and given up methods for *in vitro* diagnosis of allergic contact dermatitis.

Contact dermatitis – clinical background and immunologic response

Development of contact allergy is the result of an interplay between environmental exposure and individual susceptibility because only a fraction of exposed individuals become sensitized. Epidemiologic studies concluded that contact allergy is influenced by sociodemographic parameters [5], a not clearly defined genetic susceptibility [6–8] and plays an important role in the general population. The most frequent elicitor of contact sensitization is fragrance mix, followed by nickel, thimerosal and balsam of Peru [1, 9], with women being more often sensitized than men [5].

Chemicals that possess the capacity to cause skin sensitization have been recognized to be reactive (electrophilic) or at least the precursor of an electrophile. Contact allergens are almost exclusively haptens – i.e. they need binding to a protein in order to become a full antigen. Hapten-induced immune reactions are classic examples of adaptive immunity. However, for this process to occur, it has been noted that in addition to haptenation of skin proteins, secondary stimuli 'danger signals' are required [10]. Such signals might be derived from keratinocytes and/or Langerhans cells perturbed by a chemical sensitizer. Thus, a substance that induces allergic contact

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dermatitis in susceptible individuals mostly shares both irritant and sensitization potential – this characteristic reflects the set of problems we are confronted with in practice regarding both *in vitro* and *in vivo* test techniques.

The current paradigm of contact sensitization follows a two-step mechanism: a sensitization and an effector phase [11]. During sensitization, the hapten penetrates the skin, binds to a protein to become a full antigen and is taken up by local dendritic cells (DCs). Antigen-loaded DCs migrate while undergoing a maturation process to regional lymph nodes, where they present the antigen to naïve T cells that consequently expand clonally. By expressing skin-homing factors such as CLA [12] or CCR4 [13], they are enabled to recirculate into the skin. At the second and every subsequent encounter of susceptible individuals with the allergen, the CLA/CCR4+T cells migrate into the skin. Here, they induce a cascade of cellular reactions, leading finally to the eczematous inflammation characterized by the formation of spongiosis and apoptosis of keratinocytes [11, 14, 15].

The patch test – pros and cons

The patch test today is still the gold standard in the diagnosis of allergic contact dermatitis. On the basis of almost 100 years of practice, this test is well standardized and – although with a subjective readout – a reliable test. It has a predictive value with regard to clinical relevance [16]. However, we have to be aware of the fact that patch testing harbours several caveats and risks. First of all, it bears the risk of iatrogenic sensitization due to improper testing or testing of unknown material. Furthermore, the problem of local reactions to some allergens, e.g. paraphenylenediamine, exists. Patch test reactions to this contact allergen are known to be often strong and sometimes may even lead to scarring processes. As mentioned above, a contact-sensitizing agent exhibits both irritative and sensitizing potential. For example, in the case of chromium – when used at a standard concentration of 0.5% chromium dichromate – one out of two reactions is considered to be irritant. In this case, both sensitization and clinical relevance can be difficult to interpret. Another disadvantage of the epicutantest is the fact that it is often not possible to be performed in individuals at the most prominent need, namely, in the case of generalized skin reactions. Furthermore, false-positive reactions are observed in the context of 'angry back syndrome' or 'excited skin syndrome'. False-negative results can occur at low concentrations of the substance, systemic or local therapy with corticosteroids or after sun exposure [17]. Thus, the need for a reliable *in vitro* test for sensitization and prediction of clinical relevance becomes obvious.

In vitro tests for evaluating contact sensitization

The probably first *in vitro* assay for a delayed-type hypersensitivity reaction was established as early as in 1932 when Rich and Lewis showed that an antigen (tuberculin) inhibited migration of cells from tissue explants taken from sensitized animals [18]. Leucocyte migration inhibition in metal-allergic patients was first described by Mirza et al. (1995) [19]. In the 1970s and early 80s, this test was kind of *en vogue* [20] in the diagnosis of contact sensitization.

Today, however, this test belongs to the not entirely understood and – presumably therefore – forgotten *in vitro* tests. In the history of *in vitro* diagnosis of contact allergy the leucocyte pro-coagulant activity also has to be mentioned. This test, described by Aldridge et al. in 1985 [21], was a unique application in the *in vitro* diagnosis of contact allergy with promising results, however, still awaiting confirmation.

The basis for the LTT was given in 1960 when Nowell [22] first described that lymphocytes cultured in the presence of phytohaemaglutinin (PHA) transform into blasts. The finding that uptake of radio-labelled thymidine by lymphocyte's nucleic acids in culture correlates well with lymphocyte stimulation has led to this as a standard method in cellular immunology [23].

In the 60s and 70s of the last century, the LTT was established in order to determine metal allergy *in vitro* – first described by Aspegren and Rorsman [24]. However, the problem of non-specific proliferation of lymphocytes in the presence of nickel became evident already at that time [25] and still remains a major problem nowadays [26].

Valentine-Thon et al. [27] aimed at evaluating LTT in a cohort of 700 patients with suspicion for metal allergy and found a good reproducibility and sensitivity to detect allergy – however, the negative controls, i.e. non-sensitized individuals in this large study were lacking. The question remains whether the LTT is a predictive test for clinically relevant sensitization to metals. Furthermore, the timing of the LTT in relation to epicutaneous testing or accidental exposure seems to be important, adding to the problem of a predictive test with clinical relevance.

A proposed solution for the low specificity of the test is the combination of antigen-specific proliferation and cytokine release. McKimm-Breschkin et al. were the first researchers to describe a hapten (Oxazolone)-specific production of IFN-γ after lymphocyte stimulation. Notably, at that time, IFN-γ was measured by means of its ability to inhibit the growth of viral plaques [28]. Cedebrant et al. aimed at analysing whether the secretion of cytokines, especially IL-10 and IL-17, or the use of T cell receptor (TCR) Vβ families in Ni-stimulated primary peripheral blood mononuclear cell (PBMC) cultures might be more useful for discriminating between allergic and

non-allergic subjects [29]. They concluded that the determination of IL-10 production in primary PBMC cultures is a potentially promising *in vitro* method for discrimination of Ni allergy in females, as compared with cell proliferation. Despite the unexplained gender-specification, these results are in line with the observation of Cavani et al. They describe that nickel-allergic patients exhibited primarily a Th1 outcome while non-allergic patients showed a predominance of so-called T-regulatory 1 (Tr1) cells with high IL-10 production [30–32]. Thus, an IFN-γ/IL-10 quotient combined with classical LTT could be a promising endpoint to distinguish sensitized vs. non-sensitized individuals. Spiewak et al. [33] aimed at strengthening the *in vitro* test by undertaking it with polarizing *in vitro* conditions either for Th1 or Th2. They found significant differences between patients with Ni-allergic contact dermatitis and controls for 'type 2' cytokines IL-13 and IL-5, with further increase of allergen-specific responses occurring when cultures were supplemented with IL-7 and IL-4. These results are intriguing and expand our knowledge on the nature of T cell responses towards metals; however, it is – to date – not suitable for routine applications. An important further caveat is that the immune systems may disparately react to different antigens [34]; thus, a general ratio of cytokine secretions might be predictive for one but not the other antigen/allergen.

Unlike allergic contact dermatitis to Ni, where the *in vitro* cytokine responses by PBMCs or specific T cell clones have been described extensively [11, 35], few studies have addressed the immunological cytokine profile induced *in vitro* by other metal sensitizers.

Also, in this regard the publication of Lindemann et al. in this issue [4] investigating the *in vitro* response to chromium adds significantly to our understanding of *in vitro* response to metals. In contrast to the results seen for nickel [29], Lindemann et al. found that the ELISpot assay was of minor value for the prediction of an allergy against chromium. Volunteers with sensitization with and without allergy did not differ significantly in terms of IFN-γ, IL-2, IL-4, IL-10 and IL-12 production following stimulation with tri- and hexavalent chromium compounds. These findings correspond with the observation of Minang et al., who found a mixed Th1- and Th2-type cytokine profile in response to Cr thus, making it difficult to take cytokine-endpoints as predictive values for sensitization and disease [36]. In contrast, Lindemann et al. concluded that the LTT was able to better discriminate between sensitization without symptoms and sensitization with allergic disease and gave additional information on the patch test.

At the end of the day, it turns out that we need specific tests or combinatory endpoints for different allergens. However, introducing laboratory tests such as the LTT into routine clinical practice may be challenging for practicing dermatologists/allergists – especially in view of decreasing resources in the health care system in many countries.

Lindemann et al. conclude that the diagnosis of an allergy against chromium appears likely if the following three criteria are fulfilled: (1) a positive patch test (verification of sensitization), (2) a positive reaction in the LTT and (3) actual exposure to chromium. This conclusion reflects where we are standing with regard to *in vitro* methods in type IV allergy. They are for sure – to date – no 'stand-alone' diagnoses. However, they may be suitable in combination with clinical findings and a detailed history.

Concluding remarks

To date, none of the *in vitro* tests for contact allergy fulfils the requirements of an easy, accurate and safe test with sufficient specificity and sensitivity. However, studies such as that reported by Lindemann et al. [4] are certainly helpful in the development of a routine *in vitro* diagnostic test of contact allergy.

References

1 Diepgen TL, Weisshaar E. Contact dermatitis: epidemiology and frequent sensitizers to cosmetics. *J Eur Acad Dermatol Venereol* 2007; **2**:9–13.

2 Kolbe L, Kligman AM, Stoudemayer T. The sodium hydroxide erosion assay: a revision of the alkali resistance test. *Arch Dermatol Res* 1998; **290**:382–7.

3 Jadassohn J. Verhandl. Dtsch. Derm. Gesellschaft 1896;5. Kongress.

4 Lindemann M, Rietschel F, Zabel M, Grosse-Wilde H. Detection of chromium allergy by cellular in vitro methods. *Clin Exp Allergy* 2008; **38**:1468–75.

5 Schafer T, Bohler E, Ruhdorfer S *et al.* Epidemiology of contact allergy in adults. *Allergy* 2001; **56**:1192–6.

6 Novak N, Baurecht H, Schäfer T *et al.* Loss-of-function mutations in the Filaggrin gene and allergic contact sensitization to nickel. *J Invest Dermatol* 2008; **128**:1430–5.

7 Westphal GA, Reich K, Schulz TG, Neumann C, Hallier E, Schnuch A. N-acetyltransferase 1 and 2 polymorphisms in para-substituted arylamine-induced contact allergy. *Br J Dermatol* 2000; **142**:1121–7.

8 Westphal GA, Schnuch A, Moessner R *et al.* Cytokine gene polymorphisms in allergic contact dermatitis. *Contact Dermat* 2003; **48**:93–8.

9 Uter W, Ludwig A, Balda BR *et al.* The prevalence of contact allergy differed between population-based and clinic-based data. *J Clin Epidemiol* 2004; **57**:627–32.

10 Gallucci S, Matzinger P. Danger signals: SOS to the immune system. *Curr Opin Immunol* 2001; **13**:114–9.

11 Cavani A, Albanesi C, Traidl C, Sebastiani S, Girolomoni G. Effector and regulatory T cells in allergic contact dermatitis. *Trends Immunol* 2001; **22**:118–20.

12 Fuhlbrigge RC, Kieffer JD, Armerding D, Kupper TS. Cutaneous lymphocyte antigen is a specialized form of PSGL-1 expressed on skin-homing T cells. *Nature* 1997; **389**:978–81.

13 Campbell JJ, Haraldsen G, Pan J *et al.* The chemokine receptor CCR4 in vascular recognition by cutaneous but not intestinal memory T cells. *Nature* 1999; **400**:776–80.

14 Traidl C, Sebastiani S, Albanesi C *et al*. Disparate cytotoxic activity of nickel-specific CD8+and CD4+T cell subsets against keratinocytes. *J Immunol* 2000; **165**:3058–64.
15 Trautmann A, Akdis M, Klunker S, Blaser K, Akdis CA. Role of apoptosis in atopic dermatitis. *Int Arch Allergy Immunol* 2001; **124**:230–2.
16 Ho SG, Basketter DA, Jefferies D, Rycroft RJ, White IR, McFadden JP. Analysis of para-phenylenediamine allergic patients in relation to strength of patch test reaction. *Br J Dermatol* 2005; **153**:364–7.
17 Ring J. *Allergy in practice*. Berlin: Springer, 2005.
18 Rich AR, Lewis MR. The nature of allergy in tuberculosis as revealed by tissue culture studies. *Bull Johns Hopkins Hosp* 1932; **50**:115.
19 Mirza AM, Perera MG, Maccia CA, Dziubynskyj OG, Bernstein IL. Leukocyte migration inhibition in nickel dermatitis. *Int Arch Allergy Appl Immunol* 1975; **49**:782–8.
20 von Blomberg-van der Flier M, van der Burg CK, Pos O *et al*. In vitro studies in nickel allergy: diagnostic value of a dual parameter analysis. *J Invest Dermatol* 1987; **88**:362–8.
21 Aldridge RD, Milton JI, Thomson AW. Leukocyte procoagulant activity as an in vitro index of nickel contact hypersensitivity. *Int Arch Allergy Appl Immunol* 1985; **76**:350–3.
22 Nowell P. Phytohaemagglutin as initiator of mitosis in cultures of normal human leucocytes. *Cancer Res* 1960; **20**:462.
23 Caron GA, Sarkany I, Williams HS, Todd AP. Radioactive method of the measurement of lymphocyte transformation in vitro. *Lancet* 1965; **2**:1266.
24 Aspegren S, Rorsman H. Short term culture of leucocytes in nickel hypersensitivity. *Acta Derm Venerol* 1962; **42**: 412–7.
25 Pappas A, Orfanos CE, Bertram R. Non-specific lymphocyte transformation in vitro by nickel acetate. A possible source of errors in lymphocyte transformation test (LLT). *J Invest Dermatol* 1970; **55**:198–200.
26 Schöpf E, Schulz KH. Lymphocyte transformation test (LTT) for the diagnosis of drug allergy. *Arch Klin Exp Dermatol* 1970; **237**:177–80.
27 Valentine-Thon E, Müller K, Guzzi G, Kreisel S, Ohnsorge P, Sandkamp M. LTT-MELISA is clinically relevant for detecting and monitoring metal sensitivity. *Neuro Endocrinol Lett* 2006; **28**:17–24.
28 McKimm-Breschkin JL, Mottram PL, Thomas WR, Miller JF. Antigen-specific production of immune interferon by T Cells lines. *J Exp Med* 1982; **155**:1204–9.
29 Cederbrant K, Anderson C, Andersson T, Marcusson-Ståhl M, Hultman P. Cytokine production, lymphocyte proliferation and T-cell receptor Vbeta expression in primary peripheral blood mononuclear cell cultures from nickel-allergic individuals. *Int Arch Allergy Immunol* 2003; **132**:373–9.
30 Cavani A, Mei D, Guerra E *et al*. Patients with allergic contact dermatitis to nickel and nonallergic individuals display different nickel-specific T cell responses. Evidence for the presence of effector CD8+and regulatory CD4+ T cells. *J Invest Dermatol* 1998; **111**:621–8.
31 Cavani A, Nasorri F, Ottaviani C, Sebastiani S, De Pita O, Girolomoni G. Human CD25+ regulatory T cells maintain immune tolerance to nickel in healthy, nonallergic individuals. *J Immunol* 2003; **171**:5760–8.
32 Sebastiani S, Allavena P, Albanesi C *et al*. Chemokine receptor expression and function in CD4+ T lymphocytes with regulatory activity. *J Immunol* 2001; **166**:996–1002.
33 Spiewak R, Moed H, von Blomberg BM *et al*. Allergic contact dermatitis to nickel: modified in vitro test protocols for better detection of allergen-specific response. *Contact Dermat* 2007; **56**:63–9.
34 Traidl C, Jugert F, Krieg T, Merk H, Hunzelmann N. Inhibition of allergic contact dermatitis to DNCB but not to oxazolone in interleukin-4-deficient mice. *J Invest Dermatol* 1999; **112**:476–82.
35 Büdinger L, Hertl M. Immunologic mechanisms in hypersensitivity reactions to metal ions: an overview. *Allergy* 2000; **55**:108–15.
36 Minang JT, Areström I, Troye-Blomberg M, Lundeberg L, Ahlborg N. Nickel, cobalt, chromium, palladium and gold induce a mixed Th1- and Th2-type cytokine response in vitro in subjects with contact allergy to the respective metals. *Clin Exp Immunol* 2006; **146**:417–26.