

Use of *in vitro* release of interferon- γ in the diagnosis of contact allergy to potassium dichromate – a controlled study

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The use of *in vitro* release of interferon- γ (IFN- γ) in the diagnosis of contact allergy to potassium dichromate was studied in 20 patients who had positive patch tests to chromate and in 30 control subjects (10 patients with contact dermatitis, allergic to other allergens, 10 patients with other dermatologic diseases and 10 healthy subjects). The release of IFN- γ in the supernatants of the peripheral blood lymphocytes was significantly higher in the patients with proven allergy to chromate ($P=0.001$). Further studies are needed to determine if IFN- γ release may serve as an additional diagnostic tool in contact dermatitis.

Key words: allergic contact dermatitis; contact allergy; delayed-type hypersensitivity; interferon- γ ; peripheral blood lymphocytes, potassium dichromate. © Blackwell Munksgaard, 2003.

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Introduction

Allergic contact dermatitis (ACD) is a consequence of T-cell-mediated response to hapten sensitization of the epidermis. It is a good example of the mechanism known as delayed-type hypersensitivity (DTH) (1). Interferon- γ (IFN- γ), a major physiologic macrophage-activating factor, is an important cytokine in the effector phase of DTH. It also plays a role in many other immunologic reactions: it upregulates class I and II complex antigens, increases the efficiency of macrophage-mediated killing of intracellular parasites and influences the class of antibody produced by B cells (2).

Patch testing is still considered the gold standard for the diagnosis of ACD. However, it has some major disadvantages (3–5): The interpretation of results is subjective and dependent on the observer's experience. The need to dilute the allergen may lead to false-negative results. The test is inconvenient to the patient and may cause 'angry back' phenomenon. There are different series (from different manufacturers) that do not always yield the same results. Although much of our knowledge and understanding of the pathogenic mechanisms in contact allergy is derived from *in vitro* tests, these have not yet gained widespread acceptance in the clinical field.

The aim of this study was to develop an *in vitro* technique for the diagnosis of ACD from chromium, a relatively common allergy often associated with exposure to cement, leather or household products. The test is based on the quantitative measurement of the IFN- γ released from cultured lymphocytes.

Materials and Methods

Patients

The study sample consisted of 50 subjects treated in the outpatient clinic of the Department of Dermatology at Rabin Medical Center. There were 29 men and 21 women aged 19–65 years (mean 45). The patients were divided into 4 groups: 20 patients with proven allergy to potassium dichromate by European standard patch tests series (Chemotechnique Diagnostics AB, Malmö, Sweden) (group A, study group), who had active eruption and the relevant test result; 10 patients with patch-test-proven allergy to allergens other than potassium dichromate (group B, control); 10 patients with dermatologic disease other than ACD (group C, control); and 10 healthy subjects with no skin manifestations (group D, control).

IFN- γ test

The IFN- γ assay is based on specific *in vitro* cell-mediated immunity (6, 7). Lymphocytes from heparinized venous blood were separated by Ficoll-Hypaque (Uni-sep Maxi, Novamed, Jerusalem, Israel) gradient centrifugation, washed with RPMI-1640 medium (Biological Industries, Kibbutz Beit Ha'emek, Israel) containing penicillin and streptomycin to remove all heparin and suspended in medium containing 15% fetal calf serum and 1% glutamine. They were then counted and divided into test tubes (0.5×10^6 cells per culture) containing potassium dichromate in 2 concentrations (0.5 and 1 μcg , the maximal concentration not toxic to the lymphocytes) or potassium-free medium (controls); 10 μcg of phytohaemagglutinin was added to each tube, to a total volume of 1 ml. The cells were incubated for 24 hr at 37° celsius and then centrifuged at 2500 r.p.m. (625 g) for 25 min. The supernatants were collected and stored at -70° celsius until assayed. Enzyme-linked immunosorbent assay was performed with the Quantikine Kit (CR&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. The IFN- γ released in the supernatants of the peripheral blood lymphocytes was expressed as a percentage. The results were calculated by the following formula:

$$\% \text{IFN-}\gamma \text{ increase} = \frac{\text{IFN-}\gamma \text{ (pg/ml) with the allergen} - \text{IFN-}\gamma \text{ with medium alone}}{\text{IFN-}\gamma \text{ with medium alone}},$$

reaction as a percentage up to 20% referred to as negative, between 20% and 30% borderline and above 30% positive.

Statistical analysis

The differences between groups were analysed by Fisher's exact test. $P < 0.05$ was chosen as the level of significance. Sensitivity and specificity were calculated.

Results

The results of the IFN- γ test are summarized in Table 1. Findings were positive for 14 of the

20 patients (70%) in group A, compared to only 3 patients (30%) in group B, 2 (20%) in group C and 1 (10%) in group D. The release of IFN- γ was significantly higher in the patients with proven allergy to chromate ($P = 0.001$). There was no statistically significant difference in IFN- γ release between groups B, C and D.

Sensitivity of the test was 73.7% and specificity 71.4% against the patch test as a standard.

Discussion

This study describes an *in vitro* technique for the diagnosis of potassium dichromate allergy based on the amount of IFN- γ released from peripheral blood lymphocytes after potassium dichromate challenge. We found a significantly greater IFN- γ release from the cells taken from patients with a positive patch test for potassium dichromate than from cells of patients with proven allergy to other allergens and patients without allergy.

Recent years have witnessed a growing interest in the role of cytokines in ACD, including IFN- γ , which is known to be involved in the development of DTH and atopic dermatitis (8). IFN- γ release was demonstrated a decade ago in mice during contact sensitization to several antigens, such as trinitrophenol or oxazolone (9, 10). Although the antigenic sensitization was performed *in vivo*, the calculation of the release of IFN- γ from lymphocytes was performed *in vitro*, in blood drawn after antigenic challenge.

Comparable studies in humans, however, have yielded discrepant results. Lisby et al. (11) noted no difference in IFN- γ release from cells obtained from patients with a positive patch test to nickel and nonallergic individuals. Loftenius et al. (12) analysed blood lymphocytes from 20 patients with oral contact lesions from dental amalgam and 10 healthy individuals for HgCl_2 -induced proliferation *in vitro*. They found that IFN- γ release was detectable in the cell supernatants from some of the patients but also in those from some of the controls, therefore, concluding that the test was not indicative of mercury allergy. Kubota et al. (13, 14) reported similar results in patients allergic to dust mites. Other researchers,

Table 1. Results of interferon- γ (IFN- γ) test in 50 patients

Group	Negative	Positive	Total
A Allergic to potassium dichromate	14 (70%)	6 (30%)	20
B Allergic to other allergens	3 (30%)	7 (70%)	10
C Skin diseases other than dermatitis	2 (20%)	8 (80%)	10*
D No skin diseases	1 (10%)	9 (90%)	10**

* $P = 0.004$ – comparison between group A and groups B and C.

** $P = 0.001$ – comparison between group A and groups B, C and D.

however, reported findings closer to those of the present study. Vamnes et al. (15) added gold sodium thiosulfate and gold chloride to cultures of lymphocytes from 8 patch-tested patients with proven gold allergy and from 8 nonallergic individuals. The lymphocytes from the allergic patients produced significantly more IFN- γ than the controls. Higher levels were also reported in the control study of Van den Broeke et al. (16), testing in patients with contact allergy to nickel and cobalt.

Our technique has also been tested in drug-induced reactions. Livni et al. (6), in a study of patients with drug allergy, noted comparable findings between the IFN- γ test and the macrophage migration inhibition factor (MIF) test, routinely used as a diagnostic tool in these cases. Since this study and others, our centre has replaced the MIF test with the IFN- γ test for patients with suspected drug eruptions.

In conclusion, IFN- γ production was found to be significantly higher in patients with contact allergy to potassium dichromate ($P=0.001$). Using patch tests as the gold standard, the sensitivity and specificity of the IFN- γ test were 73.7% and 71.4%, respectively. The positive correlation of the findings in the IFN- γ and patch tests warrants further studies to determine whether the *in vitro* technique may serve as an additional diagnostic tool in ACD. This test could be helpful in patients in whom patch tests are not appropriate, as in those who have a disseminated eruption or angry back syndrome. Because type 1 and type 2 cytokines are now thought to mediate ACD (9, 10), measurements of T helper 2 cytokines will be made in our studies in the future.

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