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APPLICATION OF THE LYMPHOCYTE TRANSFORMATION TEST (LTT) TO THE DIAGNOSIS OF POLLEN ALLERGY

By

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The elaboration of different methods for the diagnosis of allergy of immediate and delayed type is one of the major problems in modern allergology. Allergen (antigen)-induced blast transformation of sensitized lymphocytes is being now widely used. Many workers (1, 2, 4, 5, 7, 8, 9, 10, 11, 18) use this method successfully in routine practice, employing various allergens, such as bacterial, tissue, drug, food, etc. Blast lymphocyte transformation has proved advantageous in clinical allergology and in the diagnosis of pollen allergy (3, 6, 17, 19, 20).

This study was designed to assess its possibilities and to compare it with skin-allergic tests, latex agglutination, passive hemagglutination, now widely applied in the diagnosis of pollen allergy.

MATERIALS AND METHODS

Patients. Twenty-seven patients with anamnestic and clinical symptoms of hay fever during may-august were examined. All the patients were tested with mixed grass pollen by prick-test. They gave strong positive reactions. The examination preceded the specific desensitization (december, january). Parallel experiments were carried out with 15 healthy individuals.

Antigen. Mixed grass pollen (product of RIEM) was prepared by extraction with Coca III solution, without phenol to avoid the non-specific stimulating effect on the lymphocytes. It was used at a concentration of 30000 PNU/ml.

Lymphocyte Transformation Test (LTT)

1. *Isolation of small and medium size lymphocytes.* 10 ml blood samples were withdrawn from the cubital vein and 500 U heparin were then added. The samples were subjected to differential centrifugation at 500 and 1000 rpm for 10 min in a refrigerated Janetzky K-23 centrifuge at 4° C by a modified method of Kelley (14) (Petrunov & Konstantinova (16)). The sediment consisted of small and medium size lymphocytes ($30-60 \times 10^6$ cells/ml) with a purity of 95-98 per cent and vitality 98-100 per cent as determined by the method of Johanovsky (12).

2. *Cultivation of lymphocytes* was carried out in 25 ml wide flat-bottomed conical flasks containing 5 ml culture medium (3.5 ml medium 199 + 1.5 ml homologous serum + 100 Penicillin U/ml + 100 g Streptomycin/ml). To the medium were added to $1.5-2.5 \times 10^6$ cells/ml. Triplicate flasks were prepared with addition of 1) 1 ml of the antigen (30000 PNU), 2) 0.01 ml phytohemagglutinin A (Difco) per ml of the medium, and 3) 1 ml of Coca III solution. Prior to tightly plugging the flasks with rubber stoppers carbon dioxide was bubbled through the cultivation medium. Cultivation was performed at 37° C for 96 hours.

Reading of the results. Following cultivation for 96 hours the culture medium was decanted into centrifuge tubes and the cells were separated by centrifugation at 1000 rpm for 15 min. Samples were prepared from the sediment, stained after Giemsa, and the cell morphology was recorded. Four hundred cells were counted in each preparation. Cultivation flasks, where the vitality was below 50 per cent, were abandoned. LTT was assessed according to the following criteria: a) cell size above 12 microns, b) increase of the nucleus-protoplasm ratio, c) appearance of vacuoles in the protoplasm—foam-like protoplasm, d) more fine chromatin with enlightened areas, e) presence of faint perinuclear halo.

The reaction was considered positive when the transformation was at least twice as strong as that in the control.

Skin-allergic test. The prick-test was applied, using mixed pollen allergen at a standard concentration of 1000 PNU. The reaction was read 20 min later.

Passive hemagglutination was carried out by the method of Boyden (13).

Latex-agglutination was carried out by the method of Petrunov and Avramova (15) employing white and colour latex, a product of RIEM.

RESULTS AND DISCUSSION

The results of the blast transformation of sensitized lymphocytes are illustrated in table 1. Positive blast transformation was demonstrated in 18 of the individuals sensitive to pollen. The average percentage of lymphocytes transformed was 11.2 per cent, the control being 3.5 per cent. The average percent-

TABLE 1

Antigens	Percentage of Lymphocyte Transformation	
	Sensitized Individuals	Normal Individuals
Mixed Grass Pollen	11,2 %	2,5 %
Phytohemagglutinin	37,4 %	29,4 %
Coca III Solution (Control)	3,5 %	2,3 %

age of blast transformation in the control group, provoked by a mixed grass pollen allergen, amounted to 2.5 per cent, not differing from that induced by Coca III—2.3 per cent. The high degree of transformation, induced by the non-specific stimulator phytohemagglutinin provides indirect evidence for the viability and the reactivity of the isolated lymphocytes. It appears from table 1 that the percentage of transformation in the experimental group was 3 times higher than in the controls, which is indicative of the specificity of the reaction. Some workers (Amache (1), Halpern (8) are inclined to regard the reaction as positive, if the lower limit of transformation in the experimental group is 4 per cent and that of the controls is 2 per cent. In order to ensure specificity of the reaction, we raised the lower limit of the positive reactions to 6 per cent transformation in the experimental flasks and be-

TABLE 2
Positive Reactions Demonstrated by Different Methods
Applicable in Diagnosis of Pollen Allergy

Methods	Number of Patients		Positive Reactions			
	sensitized	normal	Number in		Percentage in	
			sensitized	normal	sensitized	normal
Lymphoblast						
Transformation	27	15	18	1	67	6,7
Skin Test	27	15	27	0	100	0
Latex						
Agglutination	27	15	23	0	85,2	0
Passive						
Hemagglutination	27	15	12	1	44,5	6,7

low 2 per cent in the controls. The advantages of LTT were ascertained by parallel investigations by the above methods (table 2). Latex-agglutination yielded the highest percentage—85.2 per cent, excluding the skin tests—all positive, followed by LTT—67 per cent and passive hemagglutination—44.5 per cent. Only one of the healthy individuals (6.6 per cent) gave positive reactions in the LTT and PHA. This fact, together with the higher percentage of positive reactions in the sensitized individuals, especially when LTT was applied, shows the high sensitivity of this method. It should be emphasized, however, that the skin tests and LTT, on the one hand, and passive hemagglutination and latex-agglutination, on the other, differ substantially. Whereas the former detect effector antibodies in allergic immediate type reactions—reagins which are cell-bound—the latter reveal only accessory antibodies. That is why the results obtained with the skin-allergic tests and LTT are far more important in the diagnosis of allergy.

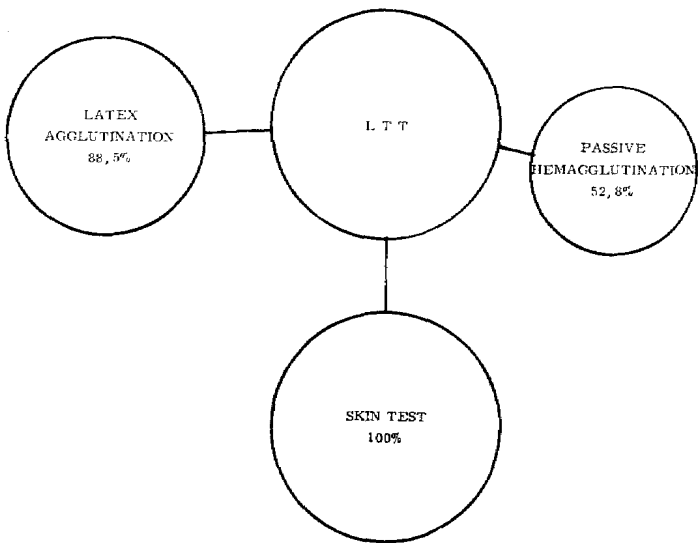


Fig. 1 shows the coincidence of the results obtained, using the four methods. Here again latex-agglutination gave a higher percentage of coincidence—88.5 per cent, compared to passive hemagglutination—52.5 per cent, which, owing to the ease of performance, could be applied in clinical allergology.

The diagnosis of pollen allergy is very efficiently achieved by skin-allergic tests, which are highly specific. In case of contra-indications, LTT is the only method of choice of the same value and can be successfully applied.

The LTT is superior to the skin test in the case of drug, food and even bacterial allergy. Then LTT may be indispensable in putting the correct diagnosis.

SUMMARY

The authors have employed the LTT for the diagnosis of sensitization to grass pollen in patients suffering from hay-fever. Preparation of viable pure lymphocytes and their cultivation with the respective allergen was achieved in accordance with the Kelley method as modified by the authors.

The LTT was carried out along with the skin test on 27 patients suffering from hay-fever. Determination of circulating antibodies to pollen was achieved by passive hemagglutination after Boyden and latex agglutination. From the 27 patients examined by LTT, 18 gave positive results, the average transformation percentage being over 11 per cent, while that of the controls amounted to 3.5 per cent. Comparing these results with those obtained by passive hemagglutination showed a coincidence in 52.5 per cent, while in the case of latex agglutination it amounted to 85 per cent.

As accompanying antibodies of the IgG class are detected by both the passive hemagglutination and the latex agglutination, whereas cell-bound reagins of the IgE class are demonstrated with the skin test and the LTT, the authors recommend the latter two methods as most reliable in the diagnosis of pollen allergy.

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