Factors Influencing the Manifestation of Skin Allergic Reaction to Microorganism Antigens in Healthy Subjects

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The patterns of variation in skin allergic reactions to antigens of *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Escherichia coli,* and tuberculin were studied in healthy subjects. The extent of skin reaction was found to vary within a wide range in different populations, with no relationship to genotypic factors. The results of the skin test were related to the type of antigen and did not correlate with the sex, age, or blood group of examinees or with the results of the leukocyte migration inhibition test. Effects of "familial" or climatic and geographic factors were also negligible.

Key Words: *skin test; variable factors; hereditary reaction*

The skin test, or skin allergic test, is one of the immunological tests that has been used in practical immunological diagnosis of infectious and allergic diseases for almost a century. The results of this test are still assessed purely by external manifestations, such as the local status of the skin at the site of injection. This obviously makes evaluation of the test results subjective. Nevertheless, the skin allergic test is still one of the most easily available, informative, and integral methods for assessing cell-mediated immunity.

The totality of the immune system cells contributing to the recognition and elimination of foreign substances and to the formation of delayed-type hypersensitivity underlies the mechanism of this test. It is carried out in a test organism where all the natural immunomodulators are present, from interleukins to hormones. A skin test permits assessment of the reactivity of the cellular component of immunity to antigens of virtually any structure, including artificially synthesized compounds which have no natural analogs.

The interest of scientists in skin tests has noticeably declined of late. A retrospective analysis of the records of immunologic studies of humans showed, however, that the skin's reaction to injected antigens varies within a wide range. This prompted us to study the factors determining the variability of the test results in a population of clinically healthy subjects.

MATERIALS AND METHODS

Thirty-eight clinically healthy subjects without apparent symptoms of acute infection were examined: members of 12 families aged 10 to 69 (mean age 40.3±2.7). Of these, 44.7% were women, 18 subjects were residents of the city of Yakutsk (5 families), and 20 were Muscovites (7 families).

Standard antigens for skin tests from *Staphylococcus aureus, Staphylococcus epidermidis, and Escherischia coli* (manufactured by the Research Institute of Epidemiology and Immunology, Kazan), and tuberculin (St. Petersburg) were used. Standard sterile isotonic NaCl solution (Research Institute of Epidemiology and Immunology, Kazan) was the negative control. Antigen preparations were injected intracutaneously under sterile conditions in a dose of 0.1 ml according to the manufacturer's recommendations. The test results were assessed

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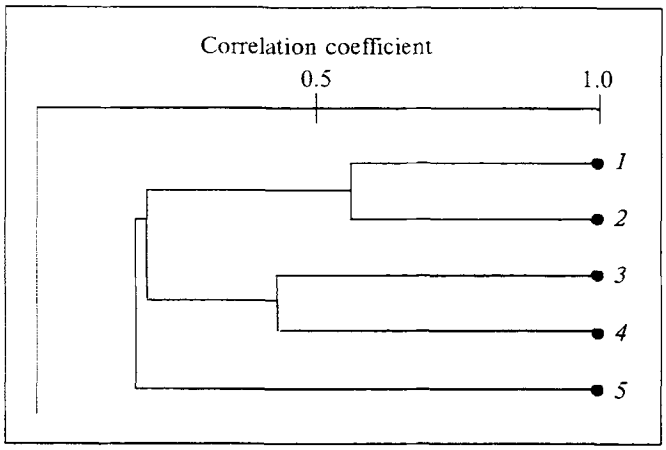

Fig. 1. Correlations between the levels of skin reactivity to bacterial antigens (cluster analysis). *1*) *S. pyogenes*; *2*) tuberculin; *3*) *S. epidermidis*; *4*) *S. aureus*; *5*) *E. coli*.

after 24 h by measuring the zone of local hyperthermia and edema (in mm).

The data were statistically processed [7] using the methods of variational, correlation, variance, and cluster analyses [2].

Familial analysis was carried out as described previously [6]. Relationships within the groups of compared individuals: parent-child, parent-parent, and parent-outsider were estimated using the intrapair correlation coefficient [4]. The contribution of the genotypic (G) and paratypic components of the general phenotypic variance of marker values (E) was assessed. The phenotypic variance was differentiated to distinguish the random component and the effect of residence in the same area, or the "familial" factor. The genotypic variance of the variability of the studied parameters was calculated after Holtzinger's formula [6]. The effect of paratypic factors was estimated as $E=1-G$, and of the "familial" factor according to a previously described method [1].

RESULTS

The findings of our experiments characterize the status of skin allergic reactivity of examinees. Table 1 shows that the size of the hyperemia zone in response to injected antigen varied within a wide range in different individuals. In none of the cases was a reaction to sterile normal saline discernible.

The form of distribution of markers in the examined population was continuous and, except for the reaction to *E. coli,* approached normal. According to some authorities [3,5], this indicates a multifactorial nature of changes in the above parameters, provided that the effects of the determining factors are equivalent.

The diameter of the hyperemia zone and edema was the greatest in response to *S. aureus* and the smallest in response to *S. epidermidis*. The mean values for these parameters differed 3.2-fold ($p<0.001$) and of variances 1.6-fold ($p<0.001$). The widely used Mantoux test occupied an intermediate position: its results reliably differed only from those of the skin test with *S. epidermidis*, being 2.1 times more, and from the test with *S. aureus* (1.5 times less).

When studying the nature of interindividual differences in the examined skin allergic reactivity, we paid special attention to the significance of an individual genotype, the sex, age, and place of residence of the examinees, as well as the type of antigen. The stages of statistical processing of the results were determined by the significance of the factor itself and by the necessity of taking it into consideration in subsequent analysis.

The calculations demonstrated an appreciable contribution of the climatic and geographic factor to the variability of skin reactivity to *S. epidermidis* and *E. coli* antigens. In the residents of Yakutsk the studied parameters were, respectively, 5.5 and 2 times lower than in Muscovites ($p<0.001$). The share of this factor in the total phenotypic variance of marker values was as high as 23.7 ($p<0.001$) and 11.0% ($p<0.05$), respectively. In subsequent stages we therefore took the said territorial effects into consideration. Skin reactivity to other antigens virtually did not depend on the place of residence of the examinees.

Additional analysis of the effects of the climatic and geographic factor was carried out separately in groups of men and women with due consideration for sex heterogeneity of the sampling. The Yakut men were found to differ from Muscovites only in the reaction to *S. epidermidis* antigens: the mean group values were 3.6 times lower ($p<0.01$) and the effect of the factor 12.9% lower in them in comparison with Muscovites. At the same time, Moscow women differed from Yakut women for a much wider range of markers. Skin allergic reaction to *S. epidermidis* antigen was 8 times higher ($p<0.01$), to *S. aureus* 1.8 times higher ($p<0.05$), and to *E. coli* 2.8 times higher ($p<0.05$). The contribution of the climatic and geographic factor to individual differences in the extent of the observed reactivity in women was no less appreciable: 40.1 ($p<0.01$), 28.3 ($p<0.05$), and 23.3% ($p<0.05$), respectively.

However, despite the described differences in the reactivity of men and women, the results of skin tests virtually did not correlate with the sex of examinees either in the total sample or within

TABLE 1. Analysis of Variations of Skin Allergic Reactivity

Antigen	Diameter of zone of hyperemia and edema, mm ($M\pm m$)	Asymmetry	Excess	K
S. epidermidis	4.7±1.1	1.15	−0.10	1.00
S. aureus	14.9±1.7	0.74	0.53	3.17***
S. pyogenes	11.0±1.4	0.37	−0.86	2.34***
E. coli	8.9±1.5	0.73	−0.65	1.89*
Tuberculin	9.8±1.1	0.46	−0.51	2.09**

Note. K: coefficient of comparison with reaction to *S. epidermidis*: one asterisk shows $p<0.05$, two asterisks $p<0.01$, three asterisks $p<0.001$.

territorial groups. A similar conclusion was drawn as regards the effects of age, blood group, and the results of the leukocyte migration inhibition test with the same antigens.

The study demonstrated that the extent of skin infiltration depends to a certain measure on the type of antigen injected. Since it was impossible to differentiate the antigens by molecular composition, we confined ourselves to their taxonomic classification. The results of analysis of variance indicate that the effect of the type of antigen in determination of individual variability of the levels of skin allergic reactivity amounted to as much as 13.8% ($p<0.01$) in the total sampling. It is noteworthy that this parameter was 21% ($p<0.01$) for the residents of Yakutsk, but for Muscovites only 10% ($p<0.05$). This fact once more confirmed the significance of territorial and climatic differences.

In order to confirm the significance of the type of antigen in the expression of the skin allergic test, we carried out correlation analysis. This demonstrated a clear-cut relationship between the reaction to *S. pyogenes* antigen and tuberculin ($r=0.562$) and to *S. aureus* and *S. epidermidis* antigens ($r=0.429$). Cluster analysis (its results are presented as a dendrogram, Fig. 1) detected three groups of markers with a low level of branching. Group 1 consisted of *S. pyogenes* and tuberculin, group 2 of *S. aureus* and *S. epidermidis,* and group 3 included *E. coli.* Hence, the facts presented once more reinforced the importance of the type of antigen.

The total role of the individual genotype in the genesis of changes in skin allergic reactivity was assessed by methods of population genetics: familial analysis. The estimated coefficients of correlation and derivatives are presented in Table 2. The data indicate that phenotypic variations in the manifestation of the skin reaction to the test antigens are in no way liable to genotypic factors; in other words, the variability of the results of the test in the population examined depends completely on fluctuations in paratypic factors.

Further differentiation of the composition of the total phenotypic variance revealed that the random variance predominated in the environmental component. Its share was as high as 79.2±5.8%, on average, which is 3.8 times higher than the effect of the familial component ($p<0.01$). We should remember, however, that with *S. epidermidis* the value of the familial component amounted to as much as one-third of the total phenotypic variance.

Analysis of the material as a whole permits us to draw the conclusion that the skin allergic reaction is an entirely individual event depending on numerous factors of nonhereditary variability. The relationship between the skin test results and the origin of the antigen, as well as the impact of the place of residence, were fairly easy to predict. On

TABLE 2. Components of Total Phenotypic Variability of Skin Allergic Reactivity

Antigen	Inrapair correlation			Components of variance			
	parent-child	parent-parent	parent-outsider	genotypic component	paratypic component	random component	"familial" factor
S. epidermidis	0.472	0.608	0.402	0.0	1.0	0.325	0.675
S. aureus	0.531	0.558	0.550	0.0	1.0	0.018	0.982
S. pyogenes	0.362	0.564	0.459	0.0	1.0	0.194	0.806
E. coli	0.445	0.528	0.372	0.0	1.0	0.250	0.750
Tuberculin	0.491	0.572	0.428	0.0	1.0	0.252	0.748

the other hand, the results of estimation of the general principle of formation of population variability of this test with regard to individual representatives of normal human microflora were clearly unexpected. In fact, the variability of the reaction in question in different individuals represents the product of a multitude of effects equivalent in force but different in direction. This ties in well with Sergeev's theory [5]. Moreover, it should be noted that the discussed element of immunological phenotype, as has been shown in this study, is well balanced in a population of healthy subjects and functions in the self-regulation mode.

On the other hand, the results of cluster analysis make us doubt the possibility of a universal mechanism responsible for the regulation of skin allergic reactivity. Such a conclusion is readily understandable, because infectious agents, besides their antigenic composition, differ appreciably in their set of pathogenicity factors, which are known to possess a potent immunoregulatory activity.

Hence, genetic analysis helped us to theoretically validate the possibility of phenotypic correction of skin allergic reactivity in human beings. On the other hand, the results do not preclude that territorial norms of this test may have to be defined.

REFERENCES

1. A. A. Vorob'ev, Yu. V. Nesvizhskii, E. V. Budanova, and L. O. Inozemtseva, *Byull. Eksp. Biol. Med.*, **117**, № 1, 62-65 (1994).
2. B. S. Duran and P. L. Odell, *Cluster Analysis: A Survey*, Springer Verlag (1974).
3. E. T. Lil'in, V. I. Trubnikov, and M. M. Vanyukov, *Introduction to Modern Pharmacogenetics* [in Russian], Moscow (1984).
4. Yu. V. Nesvizhskii, D. V. Korogodin, E. T. Lil'in, *et al.*, *Genetika*, **23**, № 1, 180-183 (1987).
5. A. S. Sergeev, *Vestn. Akad. Med. Nauk SSSR*, № 6, 81-87 (1982).
6. E. I. Sokolov, P. B. Gofman-Kadochnikov, and E. T. Lil'in, *Essays on Twin Studies (in Clinical Medicine)* [in Russian], Moscow (1980).
7. V. Yu. Urbakh, *Biometrical Methods* [in Russian], Moscow (1964).