

Delayed-type hypersensitivity and circulating basophils

A population study

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The factors related to delayed-type hypersensitivity (DTH) have been investigated in two samples of elderly non-hospitalized ($n = 375$) and hospitalized subjects ($n = 409$). Percent basophils was found to be consistently related to the number of positive responses to seven common antigens in the different samples or subsamples considered. The mean number of responses, after adjustment for age, sex and sample was 1.55 below 0.5% basophils, 1.87 between 0.5 and 1.5% and 1.96 over 1.5% ($P < 0.01$). No relation was observed between percent basophils and the mean diameter of the positive responses, suggesting that basophils might be related to the early phase of DTH reactions. As previously reported, the number of positive responses was found to be lower in women than in men and to be inversely related to age.

Key words: delayed-type hypersensitivity; basophils.

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Delayed-type hypersensitivity (DTH) is sometimes used to assess cell-mediated immunity in various categories of patients. A multiple puncture device has been designed to standardize the simultaneous application of seven recall antigens, but few population studies have taken advantage of its ease of use to investigate factors which could influence individual responses (3, 4, 5, 6, 7, 8). Age, sex, social class and area of application (arm or back) have been studied. Among other factors potentially related to DTH, we focused our attention on circulating basophils. These cells have been identified on the site of DTH (1, 2, 9), and they play a key role in another delayed-onset reaction, cutaneous basophil reaction.

SUBJECTS AND METHODS

Two samples of subjects were studied. The first consisted of 375 non-hospitalized individuals,

over 60 years of age, who came for a free health check-up at the Nancy Center for Preventive Medicine (eastern France). The second consisted of 409 subjects, representing all patients over 60 years of age hospitalized for 1 year in a step-down unit at Hôpital Charles Foix (Ivry-Seine, Paris near suburb).

In both samples, total leukocyte counts were obtained with a Coulter Counter S+ (Coulter Electronics, Hialeah, FL) and basophil percentages were estimated on 200 cells using a Hematrack 360 (Geometric data Corporation). Delayed-type hypersensitivity (DTH) was assessed by multi-puncture, using multitest (Institut Mérieux, Lyon, France). Responses to the seven antigens (tetanus, diphtheria, streptococcus, tuberculin, candida, trichophyton and proteus) were read 48 h after application on the volar face of the forearm. In the non-hospitalized sample, the multitest was applied by different nurses but read by a single

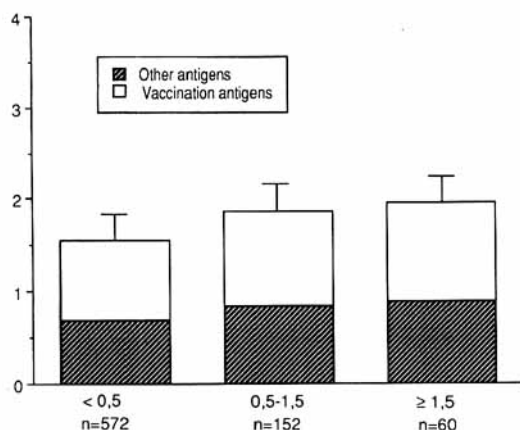


Fig. 1. Number of positive responses, adjusted for sex sample and age, according to percent basophils. Vaccination antigens $P < 0.05$; other antigens $P < 0.05$.

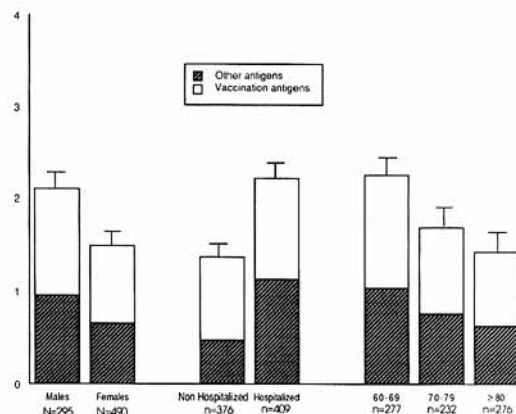


Fig. 2. Number of positive responses, adjusted for percent basophils and for the two other factors, according to sex sample and age. Vaccination antigens, effect of sex $P < 10^{-4}$, sample $P < 0.05$, age $P < 10^{-4}$; other antigens, effect of sex $P < 10^{-4}$, sample $P < 10^{-4}$, age $P < 10^{-4}$.

physician. In the hospitalized sample, the multi-test was applied and read by a single physician. The diameter of induration was defined as the mean of two perpendicular diameters measured with a calliper. A response was considered positive if the diameter of induration was at least 2 mm. The mean diameter of responses was defined as the sum of the diameters of positive responses divided by the number of positive responses.

Statistical comparisons were performed by chi-square test of independence, Student's *t*, unbalanced variance and covariance analysis, using SAS statistical package (SAS Inst., Cary, NC).

RESULTS

As could be expected, hospitalized subjects were significantly older and included a higher proportion of women (72%) than non-hospitalized (52% $P < 10^{-4}$). The mean ages of the 114 hospitalized and the 181 non-hospitalized men were 78.2 and 68.5, respectively ($P < 10^{-4}$) and of the 295 hospitalized and the 195 non-hospitalized women were 82.8 and 67.7 ($P < 10^{-4}$). Percent basophils (mean 0.36, SD 0.67) and basophil count (mean 22.7, SD 43.9) did not differ significantly according to sex, age or population studied.

The number of positive responses (mean 1.59, SD 1.42) increased with basophil percent-

ages (respectively 1.49, 1.84 and 1.92 for $< 0.5\%$, 0.5 to 1.5% and $> 1.5\%$ basophils, $P < 0.001$). The same increasing trend was observed for both men and women, as well as in each age group or among the hospitalized subjects (data not shown). When the seven antigens were considered separately, a significant trend was observed for four of them: tuberculin, candida, streptococcus, proteus (data not shown).

The number of positive responses was inversely related to age among non-hospitalized (1.66, 1.15 and 0.62, respectively, for subjects in their sixties, seventies or eighties and over, $P < 0.001$) and among hospitalized subjects (respectively 2.55, 1.87 and 1.54, $P < 0.01$). Women had a lower mean number of positive responses than men among the non-hospitalized (1.14 against 1.82, $P < 10^{-4}$) as well as among the hospitalized (1.53 against 2.18, $P < 10^{-4}$).

To take into account the relative influence of these factors, a multifactorial variance analysis was performed on the number of positive responses and on the mean diameter of positive responses to the seven antigens. Thus, we could test the independent effect of percent basophils,

sex, sample and age and we could estimate adjusted means. To find the consistency of the observations, separate analyses were performed on the number of positive responses to vaccination antigens (tetanus, diphtheria, tuberculin) and on the number of positive responses to other antigens (streptococcus, candida, trichophyton and proteus). The four factors: percent basophils, sex, sample and age, had a significant effect on both numbers of positive responses considered. As seen in Fig. 1, after adjustment for sex, age and sample, the number of positive responses to vaccination antigens increased with percent basophils (respectively 0.86, 1.02 and 1.07 for $< 0.5\%$, 0.5 to 1.5% , and $> 1.5\%$ basophils, $P < 0.01$) as did the number of responses to other antigens (respectively 0.69, 0.85, 0.89, $P < 0.05$). As seen in Fig. 2, the numbers of responses to vaccination antigens and other antigens were lower for women than for men (resp. $P < 10^{-4}$ and $P < 10^{-4}$), they were lower in the non-hospitalized than in the hospitalized sample ($P < 0.05$ and $P < 10^{-4}$), and they decreased with age ($P < 10^{-4}$ and $P < 10^{-4}$). Results were essentially not modified if a square root transformation was performed on percent basophils, as is recommended for Poisson distribution. However, the mean diameter of the positive responses was not significantly related to percent basophils, age, or sex (data not shown). The mean diameter of positive responses from non-hospitalized subjects (4.5 mm) was larger than that of hospitalized subjects (3.7 mm, $P < 0.01$).

The relation of the number of responses with basophil count was less significant than its relation with percent basophils; in fact it did not reach significance ($P = 0.07$).

DISCUSSION

In two samples of elderly subjects, we have observed a consistent positive relation between positive DTH responses to seven antigens and percent basophils. Previous observations of lower responses in women than in men and of a decrease of responses with ageing have been confirmed.

Three limitations of this survey must be mentioned. None of the two samples was repre-

sentative. The non-hospitalized subjects were volunteers who accepted a free medical check-up. The hospitalized subjects were investigated systematically on admission in one unit. However, it is not likely that the relation between percent basophils and number of positive responses was biased, on the contrary the same bias should have affected the two samples independently since the same kind of relation was observed in each of them. Counting basophils on 200 cells will obviously be imprecise, but such imprecision can only bias associations with other factors towards zero and can be compensated by studying more subjects than would have been necessary with a more accurate measure of percent basophils. The evaluation of DTH by multitest is also imprecise since it depends not only on the immune functions of the subject but also on the history of his exposures to the seven antigens, the application of the multipuncture device, and the reading. It should be noted that some of the differences observed between the two samples might not come from the populations studied. One can expect that hospitalized subjects would have smaller mean diameters of responses than non-hospitalized who are obviously in better health; the opposite difference observed for the number of positive responses might be related to the fact that the readings in the two groups were performed by a different physician. Again, errors of measurement can only mask associations with other factors.

Several investigators have studied the relations between DTH, measured by multitest, and age or sex. Fewer positive reactions in elderly subjects than in young adults have been reported among French (5), British (6) and Danish (7) people. In this study, we observed a significant decrease in the total number of responses with increasing age of elderly subjects. As for the sex factor, conflicting observations according to the antigen considered have been reported (3), but other investigators observed, as in this survey, fewer positive responses in women than in men (5, 6, 7).

The number of positive responses consistently increased with percent basophils. When non-hospitalized or hospitalized subjects were ana-

lysed separately, or when subsamples were compiled according to sex or age, the same tendency was observed. When all subjects were considered simultaneously, the effect of percent basophils on the number of positive responses to vaccination antigens, other antigens, or all antigens was significant (resp. $P < 0.05$, $P < 0.05$, $P < 0.01$). When the seven antigens were considered separately, a significant trend was observed for four of them. By contrast, no significant difference was observed for the mean diameter of positive responses. Thus percent basophils appear to be related to the triggering of DTH, but not to the intensity of the reaction. The relation of the number of responses with basophil count was less significant than its relation with percent basophils; in fact it did not reach significance, probably because measurement errors on percent basophils and total leukocyte count are multiplied in the calculation of basophil count.

Morphometric studies have noted the involvement of basophils in DTH of humans (1, 2, 9), but as far as we know, their role is still unclear. The adjusted increase of the number of positive responses seemed larger from 0–0.5% to 0.5–1.5% (0.32) than from 0.5–1.5% to 1.5% and over (0.09), but a small proportion (7.7%) of the subjects had at least 1.5% basophils and the test of a deviation from a linear trend would have lacked statistical power. Two hypotheses can be put forward to explain our findings. A causal relation might exist and basophils might play a limiting role in DTH, probably in its triggering, or this relation might come from an unidentified factor influencing both basophil count and DTH reactions.

REFERENCES

1. Askenase PW, Atwood JE. Basophils in tuberculin and "Jones-Mote" delayed reactions of humans. *J Clin Invest* 58, 1145–1154, 1976.
2. Dvorak HF, Mihm MC, Dvorak A M et al. Morphology of delayed-type hypersensitivity reactions in man. I. Quantitative description of the inflammatory response. *Lab Invest* 31, 111–130, 1974.
3. Kniker WT, Anderson CT, McBryde JL, Roumiantzeff M, Lesourd B. Multitest CMI for standardized measurements of delayed cutaneous hypersensitivity. Normal values and proposed scoring system for healthy adults in the U.S.A. *Ann Allergy* 52, 75–82, 1984.
4. Lesourd B, Corriel RN, McBryde JL, Kniker WT. Cell-mediated immunity in school children assessed by multitest CMI skin testing II. Epidemiologic factors affecting immune responsiveness. *Ann Allergy* 54, 446–452, 1985.
5. Lesourd B, Roumiantzeff M, Wisniack J, Biron G, Loeper J, Moulias R. Un test simple d'immunité cellulaire au lit du malade— "la multipuncture". Définition d'une population française de référence. *Nouv Presse Med* 9, 3435–3442, 1980.
6. Maxwell AP, McCluskey DR. Assessment of cell-mediated immunity in a British population using multiple skin test antigens. *Clin Allergy* 16, 365–369, 1986.
7. Moesgaard F, Nielsen ML, Larsen PN, Christophersen S, Mosbech H. Cell-mediated immunity assessed by skin testing (Multitest®). I. Normal values in healthy Danish adults. *Allergy* 42, 591–596, 1987.
8. Moesgaard F, Nielsen ML, Larsen PN, Christophersen S, Mosbech H. Cell-mediated immunity assessed by skin testing (Multitest®). II. Correlation between responses from arm and back. *Allergy* 43, 109–112, 1988.
9. Wolf-Jürgensen P. Basophilic leukocytes in experimental tuberculin reactions studied by the skin-window technique. *Acta Allergol* 20, 38–46, 1965.

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