

The total eosinophil count in a nonatopic population

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It is concluded that the total eosinophil count of healthy nonatopic individuals is lower and exhibits a significantly narrower range than the published data for "normal" values would indicate. The data also suggest that skin reactivity to extracts of common airborne allergens might serve as a basis for reassessment of the genetic transmission of atopy.

The determination of peripheral eosinophil values is a useful adjunct in the diagnosis and management of allergic disorders. It is, therefore, of importance to define the so-called "normal" or "nonatopic" range. The "normal" range (cells per cubic millimeter) had been previously reported as 0 to 240,¹ 50 to 400,² 50 to 250,³ 40 to 550,⁴ and 50 to 550⁵; and the average was reported as 99,¹ 179,² 200,³ and 160 to 173.⁶ Such a wide range suggests heterogeneity in the populations tested, which probably included many latent- and some overt-atopic individuals. Apparently no previous attempts at skin testing were made as a means of excluding subjects in whom atopy in latent or preclinical form might have been present. In this study, the total peripheral eosinophil count was determined among normal individuals lacking skin sensitivity to common inhalant allergens, and among those with skin reactivity without symptoms. The term "atopy" as used in this study is defined as a group of related disorders including hay fever, asthma, and dermatitis having a familial incidence and characterized by a wheal-and-flare reaction occurring within 20 minutes following intracutaneous injection of the specific exciting agent.

MATERIALS AND METHODS

Employees of the Massachusetts General Hospital ranging from 20 to 60 years of age volunteered for this study. Care was taken to include only those

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who were free from any form of illness for at least a month before the study was undertaken. A questionnaire was filled out by the subjects, under the guidance of a physician. This included a detailed family history (FH) and a detailed past and present personal history, with particular emphasis on diseases which are known or are suspected to have an influence on the eosinophil blood levels, including the atopic diseases. Those persons with current skin disorders and those who had lived in the tropics at one time or another were also excluded. The severity of any atopic illness in the past was noted as well as the relationship when a positive family history was elicited.

Skin testing (ST) was performed with the use of the standard scratch and intracutaneous methods. Scratch tests were done on the left forearm using concentrated extracts of two commercial housedust preparations,* cat dander 1:10, Hormodendron and Alternaria, each 1:40, and birch, oak, timothy, and ragweed pollen extracts, all 1:20 (weight by volume). Phenolized saline was used as the diluent and as the control. The sites were read after 20 minutes. Intracutaneous tests were done with the above-mentioned allergens that gave doubtful or negative results on scratch testing. Dilutions of 1:4,000 for each of the household preparations, 1:100 for cat dander, and 1:200 for each of the two molds and for each of the pollen extracts were used. A separate disposable tuberculin syringe and a 27 gauge disposable needle were used for each test site. The sites were read after 12 minutes. The wheals that were at least 50 per cent larger than the area of the control site were termed positive. These were almost invariably accompanied by a flare. The intensity of the reactions was recorded with a freehand drawing of both the wheal and the flare.

Before skin testing was done, 5 ml. of venous blood was collected and was gently mixed in a tube containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. Blood was drawn to the 1.0 mark of a leukocyte diluting pipette, which was then filled to the 11.0 mark with an eosin stain.⁷ The pipette was shaken for several seconds in a mechanical shaker and was allowed to stand for 15 to 20 minutes to permit thorough staining of cells. It was again shaken thoroughly and, after the first three drops were expelled, both sides of a Fuchs-Rosenthal counting chamber were filled. After allowing time for settling, the eosinophils in all 32 squares of both chambers were counted and the total was multiplied by 1.56 to obtain the number of cells per cubic millimeter of blood.

RESULTS

A total of 77 symptom-free subjects were selected. As shown at the bottom of Fig. 1, they were divided into the following categories: *A*, those subjects with negative skin test(s) (*ST*), negative family history (*FH*) of allergy, and negative past personal history (*PH*) of allergy; *B*, those with positive *FH* alone; *C*, those with positive *ST* alone; *D*, those with positive *FH* and *ST*; *E*, those with positive past *PH* and *ST*; and *F*, those with positive *FH*, *PH*, and *ST*. There were two subjects with negative *ST* that were not categorized in Fig. 1. One of them has a *PH* and another had both positive *FH* and *PH*.

*Endo Products, Inc., and Hollister-Stier Laboratories.

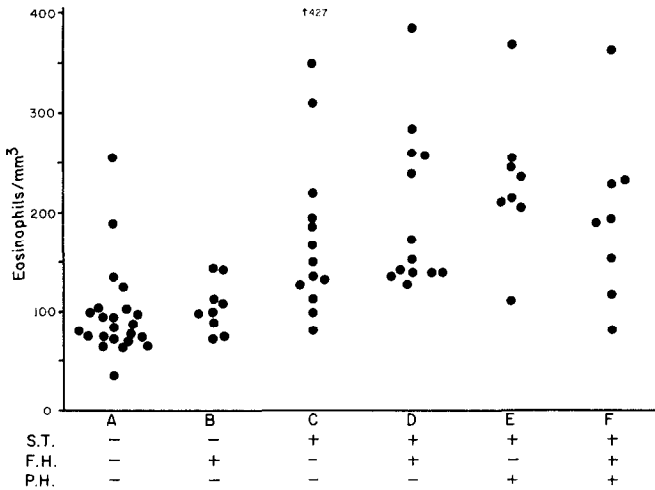


Fig. 1

These were included with the *ST*-negative group in all subsequent analysis. The mean of the total eosinophil counts (TEC) for Group *A* was 97; for *B*, 105; for *C*, 192; for *D*, 198; for *E*, 231; and for *F*, 195 per cubic millimeter. Groups *E* and *F* were the only ones (aside from the two that were not categorized) that had a *PH* of allergy and most had skin reactivity to the allergen(s) with which they were tested. On relating the TEC's with degrees of severity of the *FH*, no pattern was evident. When the TEC was related to the skin reactivity, two groups emerged, Group I comprising *A* and *B* with means of 97 and 105, respectively, and Group II comprising *C*, *D*, *E*, and *F* with means of 192, 198, 231, and 195, respectively. Frequency distributions for the TEC in Groups I and II are shown in Fig. 2. The peak of the curve of Group I (skin test negative) is about 75, and that of Group II (skin test positive) lies between 125 and 225 per cubic millimeter. The distributions (although skewed) seem to indicate that there are two populations, characterized by the presence or the absence of skin reactivity.

Table I is an analysis of the two main groups based on skin reactivity. Among Group I with negative skin tests the range was from 34 to 257, with a mean of 99.61, a median of 92, and a standard deviation of ± 40.53 . Group II with positive skin tests had a range of 81 to 427, mean of 201.63, median of 193, and a standard deviation of ± 71.88 . The difference between the two groups was highly significant, with a *p* value less than 0.01. Because of skewness in distribution, the logarithm of each value was obtained and the data thus transformed was again subjected to statistical analysis. The difference between the groups was again significant with the *p* value less than 0.01.

DISCUSSION

No relationship was evident between the level of the total eosinophil count (TEC) and a past history (PH) or family history (FH) of atopic disease.

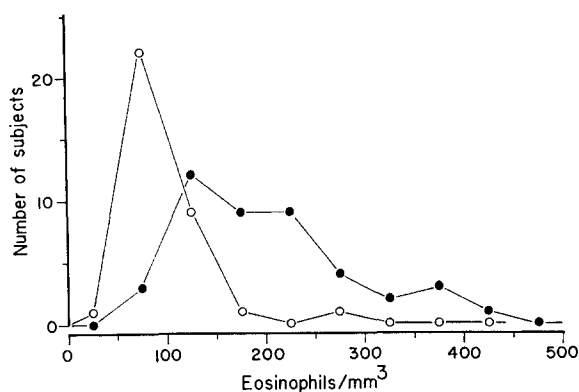


Fig. 2

Table I

Skin reactivity	No. of subjects	Total eosinophil count			
		Range	Mean	Median	Standard deviation*
Absent	34	34-257	99.61	92	± 40.53
Present	43	81-427	201.63	193	± 71.88

*The p value for the difference = <0.01 .

So far as the PH was concerned, selection of the subjects eliminated those with current or chronic atopic manifestations, so that this negative finding was not unexpected. Little relation was seen between the TEC and the intensity of skin reactivity. Considering Group I and II together, the correlation coefficient calculated for the relation between the TEC and the intensity of skin reactivity gave a low value (0.44). When those subjects lacking skin reactivity (namely Group I) were omitted from the analysis calculation gave a very much lower correlation coefficient (0.043), a finding which is again consistent with the presence of two distinct groups. Because a close correlation has been observed among selected atopic subjects between exposure to an allergen and elevation of the TEC,⁸ the lack of relation between the TEC and level of skin reactivity observed in the present study suggests that if exposure at some level to one or more reacting allergens is the reason for the higher TEC in the skin-reactive group, the stimulus to eosinophilia would appear to be independent of the level of skin reactivity. It has been observed that although eosinophilia is in general proportional to the severity of atopic disease,⁸ the intensity of skin reactivity and the intensity of atopic disease vary independently. Skin reactivity, therefore, rather than being a continuum from none to the most intense, may be either present at some definable level or absent. In this case, the capacity to become skin reactive might be genetically determined, whereas the intensity of the skin reactivity might be attributable to factors other than those accounting for its mere presence.

The work of Uhrbrand,⁴ subsequently cited by Wintrobe,⁵ showed a very wide range of the eosinophil count, and the subjects included constituted a very heterogeneous group, many of whom were suffering from various disorders that were thought to have no influence on the TEC. This study may well have included some latently and some overtly atopic individuals. Had skin testing been omitted in the present study, the so-called "normal" range would have exhibited a similar range, namely 34 to 427 per cubic millimeter, quite close to that reported.^{4, 5} As it turned out, however, the great majority of those individuals with no skin reactivity had counts below 200 per cubic millimeter.

Discombe,¹ having attempted to exclude atopic subjects by history, obtained a mean TEC that was closely similar to that obtained by us. The mean was 98.6 per cubic millimeter, with 5 per cent exceeding 190. The fact that his mean came very close to that of our skin test-negative group, in spite of the probable inclusion of some latently atopic individuals, may be explained by the fact that his blood specimens were obtained by pricking the ear lobes rather than by venipuncture. Tissue juices conceivably may have diluted the blood to some extent which could have resulted in lower counts. In neither his study nor ours was a significant difference observed between the sexes. Uhrbrand⁴ attempted to determine the effect of the time of the day on the TEC. A fall of between 15 to 20 per cent from the 8:00 A.M. base line was observed between the hours of 10 in the morning and 2 in the afternoon, whereupon there was a rise above the base line reaching a peak at around midnight. This was considered to reflect changing levels of endogenous steroids. In the present study, the blood samples were drawn between the hours of 9 in the morning and 5:30 in the afternoon. Though looked for, no pattern suggesting an effect of the time of the day on the TEC was observed.

REFERENCES

1. Discombe, G.: Criteria for Eosinophilia, *Lancet* 1: 195, 1946.
2. Brown, G. T.: Eosinophilia in Asthma, Hay Fever, and Allied Conditions, *J. Lab. & Clin. Med.* 12: 1145, 1927.
3. Wintrobe, M. M.: *Clinical Hematology*, ed. 4, Philadelphia, 1961, Lea & Febiger, Publishers, p. 232.
4. Uhrbrand, H.: The Number of Circulating Eosinophils, *Acta med. scandinav.* 160: 99, 1958.
5. Wintrobe, M. M.: *Clinical Hematology*, ed. 5, Philadelphia, 1961, Lea & Febiger, Publishers, p. 249.
6. Acland, J. D., and Gould, A. H.: Normal Variation of the Count of Eosinophils in Man, *J. Physiol.* 733: 456, 1956.
7. Dunger, R.: Eine einfache Methode der Zählung der eosinophilen Leukozyten und der praktische Wert dieser Untersuchung, *München med. Wehnschr.* 57: 1942, 1910.
8. Lowell, F. C.: Unpublished data.