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BONE-MARROW EOSINOPHILIA IN THE MOUSE

I. Eosinophil Leukocyte Levels in Untreated Mice

By

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An increase of eosinophil leukocytes in the blood has been described for many diseases. Booth (4) listed 40 conditions accompanied by increased eosinophilia, and Wintrobe (32) had given a similar list a few years before. Other authors (2) argued more critically that some clinical pictures accompanied by increased blood eosinophilia are variants of the same picture or represent overlapping. A review of the literature yields two groups of diseases in which a distinct increase in the number of blood eosinophils is regularly observed, i.e. worm infections and atopic allergy.

The course of the blood eosinophilia in worm infections has been thoroughly studied; investigators had infected themselves with infectious *Ascaris* eggs in order to follow the course of the eosinophilia and the infection (10, 13). A detailed study was also made on the victims of a dubious prank whereby infectious eggs of *Ascaris suis* were put in food (26). Blood eosinophilia in atopy was investigated by Voorhorst (31) and Varekamp & Voorhorst (29), whose results indicate that the number of eosinophil cells in the blood does not increase until the atopic patient is exposed to the relevant allergen.

Systematic investigation of the number of eosinophil cells (eos) in the bone marrow has been less frequent. Some earlier publications (8, 9, 14) indicate that this number increases in allergic diseases and/or bronchial asthma.

In atopy, an infiltration of eos is also commonly seen in the affected organs during exposure to the allergen (22, 23, 24). If it is accepted, as is generally the case at present, that eos are formed in the bone marrow and reach organs via the blood (3), it could be expected that in atopic patients—who show not only an elevated number of blood eos but also infiltration of eos into, for instance, the respiratory tract—the number of bone-marrow eos would also be increased. The previously mentioned studies (8, 9, 14) would seem to confirm this. It is conceivable that the increase in the number of eos in worm infections and atopy is brought about by the same mechanism, since in both conditions there is also an increase in the IgE level, not only the total IgE but also the specific (5, 16, 17, 26).

In recent years functions of the eos have been elucidated, e.g. phagocytosis, particularly that of antigen-antibody complexes (20, 28) and antigen-IgE complexes (15), and the detoxification of histamine, although the latter is considered controversial (Felarca & Lowell (11); see also their discussion with Archer (1). Parish (25) has provided a list of all known substances thought to be 'eosinotactic'.

When all these findings are considered together with the phenomenon of increased eos levels in worm infections and atopy as manifested clinically, it seems clear that our knowledge about the function of the eosinophil cells is still fragmentary and that the significance of the entire process is essentially just as uncertain as it was a few decades ago. We do not know what factors give rise to increased eosinophilia. This led us to try to find a model, and thus an animal species, in which an increase in the number of eos in the bone marrow and blood could be reliably reproduced. Such a model could be expected to provide information giving insight into the human pathology. Voorhorst (30) demonstrated that the mouse, infected with infectious eggs of *Ascaris suum*, develops a distinctly increased number of blood eos, and Bradbury et al. (5) obtained the same result in the rat. In a preliminary communication one of the present authors (6) reported that eos

in mouse bone marrow could also be elevated by one subcutaneous injection of an emulsion of Freund's adjuvant and allergen, the rise occurring after the 7th day. In four experiments the mean ‰ eos in bone marrow lay between 101 and 124, 10 to 14 days after a subcutaneous injection of an emulsion of 0.25 ml incomplete Freund's adjuvant and 0.25 mg grass-pollen extract (2,500 N.eq.U), whilst the group of mice subcutaneously injected with an emulsion of 0.25 ml incomplete Freund's adjuvant and 0.25 ml physiological saline showed a mean bone-marrow eosinophilia of approximately 20 ‰.

These values are arithmetical means of a number of values (usually five) for individual mice. It was found, however, that within a group of mice given the same treatment at the same time, or not treated at all, appreciable differences occurred between the values of individual mice. These differences proved to be even greater for mice treated in the same way but at different times, and this also held for the untreated mice.

On this basis it seemed necessary to perform a statistical analysis of the number of eos/1000 cells in the bone marrow of treated and untreated mice. This article, the first of two, concerns untreated mice. Experiments were designed for the investigation of three kinds of eos variability: 1) within groups of mice, 2) between groups of mice at intervals of about 3 months and 3) over a period of about 1 month.

Since the results were to serve primarily for predictive purposes, we attempted to find answers to the following questions:

- a) For an as yet uninvestigated mouse in a group under study, where can the ‰ eos be expected to lie?
- b) For a mouse in a new group, where can the ‰ eos be expected to lie?
- c) If a group of mice is kept under investigation for some time (e.g. a month) under constant conditions as to housing, diet, etc., where can the ‰ eos be expected to lie at any time in an individual mouse?

The answers to these questions will of course be probabilistic

in nature because of the many possible sources of variation between observation, both biological and technical.

MATERIAL AND METHODS

The Animals

We chose the mouse, i.e., specified pathogen-free (SPF) Swiss random-bred mice (Central Institute for the Breeding of Laboratory Animals; T.N.O., Zeist, The Netherlands; strain symbol Cpb:SE), because it was known that reproducible increased bone-marrow eosinophilia could be obtained from it (6). Females weighing 18–20 g at the beginning of the experiments were used. They were kept in round metal pans (radius 12 cm) which could hold maximally 11 mice (12) and were placed in a virtually dark room at a temperature of 21–22° C. The bottom of the pan was covered with a mixture of sawdust and peat; the lid was made of perforated metal. The animals had access to water and mouse pellets with cod-liver oil (H. S. Bach, Oss, The Netherlands) *ad libitum*.

Preparation of Bone Marrow

The mice were killed with a mixture of ether and chloroform, and the sternum was removed. The marrow could be seen through the sternum wall as four to five superposed, sharply bordered, red compartments, the upper two of which were opened with a pin. A smear was made from the marrow by carefully loosening it with saline from a Pasteur pipette and drawing some into the pipette. The marrow within the first compartment was placed onto the right half of a slide, the marrow from the second onto the left half. The preparations were allowed to dry in the air and were stained according to Lendrum (18) with slight modifications. In both smears the number of eos in 500 nucleated cells was determined. Thus, a total of 1000 cells were counted to obtain the $\%_{500}$ value. These counts were invariably made first in the left-hand smear and then in the right-hand smear, called the first and second 500, respectively.

Statistical Analysis

As already mentioned, we were interested in predictions concerning the $\%_{500}$ eos of new mice. In our case these predictions were made by the calculation of a so-called predictive probability distribution for new mice. (For a general introduction to the predictive statistical theory, see Roberts (27), for the techniques of predictive analysis of variance, as used in this paper, see Lindley (19).)

RESULTS

Variation and Prediction in Groups of Untreated mice

The results were obtained from four groups of mice, 15 in each. The groups were sampled at intervals of about 3 months. All 15 mice in a group were prepared on the same day, group I on March 23, 1973; group II on June 8, 1973; group III on September 21, 1973; group IV on January 18, 1974. Table 1 gives the numbers of eos in these animals.

TABLE 1

Number of Eos in a Count of 1000 Nucleated Bone-Marrow Cells from Four Groups of 15 Untreated Mice, Sampled at Intervals of about 3 Months.

Group I	Group II	Group III	Group IV
25	35	18	31
18	47	6	25
31	60	15	32
32	53	15	15
29	56	19	29
25	55	18	25
29	49	12	23
43	37	26	30
41	30	19	38
19	42	9	29
24	35	12	24
37	39	19	24
25	55	18	13
42	94	14	13
11	40	18	33

As can be seen from this Table, there are important differences between the four groups.

To characterize the four groups as a whole, the means and standard deviations were computed for each group (Table 2). As was to be expected, the standard deviations increase with increasing mean. The coefficient of variation, i.e. the standard deviation divided by the mean, can be used as a rather stable measure of variation for observations like these (see Table 2).

The first question we have to answer concerns the prediction of new observations from a partially investigated group of mice. The probability distribution, from which probabilistic predictions on the ‰ of new mice from the same group will be derived, appears to be a Student distribution, assuming that the original data are normally distributed. The Student distribution is characterized by the means and standard deviations given in Table 2. The degrees of freedom are in our case, 15. (Tables with percentage points of the so-called standardized Student distribution, can be found in all standard textbooks on statistics (21).)

TABLE 2
Means, Standard Deviations, and Coefficients of Variation for the Data in Table 1.

	Group I	Group II	Group III	Group IV
Mean	28.7	48.5	15.9	25.6
S.D.	9.3	15.6	4.9	7.4
Var. coefficient	0.32	0.32	0.31	0.29

Some relevant percentage points are given in the last column of Table 3. These values, which are used to find the corresponding percentage points for the predictive distributions for a new observation in each of the groups, are easily obtained by multiplying the standard deviation of the group by the Student value, and adding the result to the mean of the group.

Table 3 can now be used for making all kinds of predictions about new observations in one of the four groups. Two examples of this kind of prediction are the following:

1. With 90 per cent probability, a new ‰ observation made in group I will fall between 12 and 45, in group II between 21 and 76, in group III between 7 and 24, and in group IV between 13 and 39.

2. With 75 per cent probability a new observation made in group I will fall below 35, in group II below 59, in group III below 19, and in group IV below 31.

TABLE 3

Percentage Points of the Predictive Distributions for New %₀₀ Observations Made in Each of the Four Groups, and of the Student Distribution with $V = 15$, on which They are Based.

Per cent points	Group I	Group II	Group III	Group IV	Student $V = 15$
1	5	8	3	6	— 2.60
2.5	9	15	5	10	— 2.13
5	12	21	7	13	— 1.75
10	16	28	9	16	— 1.34
25	22	38	13	20	— 0.69
50	29	49	16	26	0
75	35	59	19	31	0.69
90	41	69	22	36	1.34
95	45	76	24	39	1.75
97.5	49	82	26	41	2.13
99	53	89	29	45	2.60

It must be stressed that the new observation must fulfil the same conditions as the other observations in the same sample, because otherwise new sources of variation will enter in, and more caution will have to be used in prediction. For example, in this case the new mice must be investigated on the same day as the other mice.

Prediction for a New Group of Mice

The statistical results in the foregoing were based on the assumption that the %₀₀ eos was approximately normally distributed for each group. This assumption is reasonably well supported by the data (Table 1). For predictions about the number of eos for a mouse from a new group, the data from all four groups must be pooled. This leads to the loss of the

approximate normality, and we get a right-tailed distribution of the data. To obtain a more symmetric distribution, we used a logarithmic transformation and calculated the predictive percentage points for these logarithms of the ‰, and then transformed these percentage points. The resulting percentage points for the ‰ are given in Table 4. It should be stressed that these percentage points are the ones which interest the investigator in many research conditions; a number of experiments are done with a special breed of mice, and a new experiment is set up. What are the expectations for this new group of mice on the basis of the results obtained in past experiments?

TABLE 4

Percentage Points of the Predictive Distribution for New ‰ Observations in a New Group of Mice.

Percentage points	1	2.5	5	10	25	50	75	90	95	97.5	99
New group	5	6	8	11	16	26	42	65	84	106	139

Prediction of Variation in Time within a Group of Mice

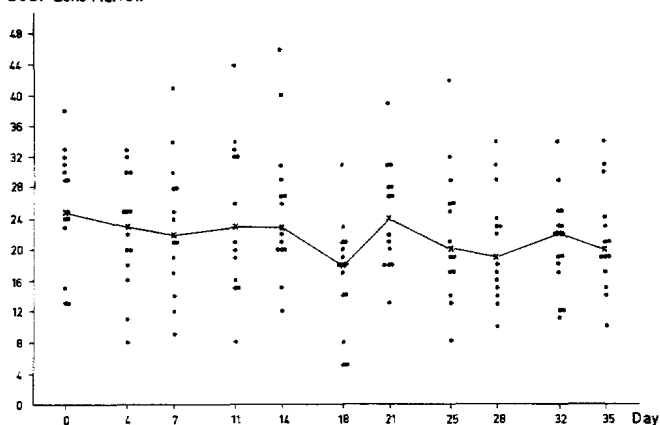
A total of 165 mice were divided non-selectively between 15 pans, giving 11 mice per pan. On days 0, 4, 7, 11, 14, 18, 21, 25, 28, 32, and 35, one mouse from each pan was killed, 15 in all. A thousand nucleated cells from the bone marrow of the sternum were counted and the ‰ eos determined. The results are shown graphically in Fig. 1 (both individual and median values). The means and standard deviations for each of the indicated days are given in Table 5. Analysis of variance led to the conclusion that the observations support the assumption that a trend in time does not exist ($P > 0.05$).

TABLE 5

Means and Standard Deviations of the ‰ Eos for Each of the Sampling Days.

Day	0	4	7	11	14	18	21	25	28	32	35
Mean	25.6	22.5	23.0	24.1	25.3	16.8	24.3	21.9	20.5	19.9	21.1
S.D.	7.4	7.3	8.5	9.5	8.9	6.9	6.7	8.5	6.9	5.2	6.5

% EOS. Bone Marrow

*Fig. 1.*

Number of bone-marrow eosinophils, expressed in ‰, in 165 mice investigated in groups of 15 animals each on 11 separate days in a 35-day period (individual and median values).

From the data in Table 5, predictions can be derived for a new mouse in this group at a new time point, as shown in Table 6.

TABLE 6

Percentage Points of the Predictive Distribution of the Group of Mice Followed for 35 Days.

Percentage points	1	2.5	5	10	25	50	75	90	95	97.5	99
‰ eos	4	7	9	12	17	23	28	33	35	38	41

DISCUSSION

Three main conclusions can be drawn from the present results:

1. In experimental work, the number of eos found at different time points cannot be directly compared, because the mean values and standard deviations vary widely in untreated mice (Table 2).

2. When a group of mice is followed over a given period (in the present study 35 days) the eosinophil level, expressed as ‰, in untreated mice remains approximately constant. This means that in experiments in which the level of bone-marrow eosinophils is to be influenced, it is not necessary to determine the ‰ value in untreated mice as control throughout the experiment but only at the start. Nevertheless, it is advisable to make occasional determinations in an untreated animal in order to be certain that no process that would elevate the level has developed.

3. Predictive statistical analysis yielded the results shown in Tables 3, 4 and 6. Such Tables can be used for quantitative prediction of the outcome of new ‰ determinations, and therefore also serve as a check on the latter. If the fulfilment of the prediction is not reasonably good, the divergence is a strong indication that a change has occurred in the experimental conditions. This kind of check is preferable to statistical testing and estimation techniques whose results are not readily verifiable.

Thus, on the basis of these Tables we can not only distinguish significant statistical differences between the four groups of mice evaluated at given intervals but also convert these differences into predictions about new observations. In the present case we can say the four groups sampled at intervals differ significantly, and so much that for observations in a new group we can only say, with a 99 per cent probability of being correct, that the value will lie between 5 and 139, which is of course a very weak prediction. This means, for instance, that the number of eos per thousand cells from a number of untreated mice (controls) must be determined in all new experiments.

SUMMARY

The number of eosinophil leukocytes in the bone marrow of untreated mice (Cpb:SE) has been shown to vary widely. A systematic investigation of this variability is reported here.

The study was done a) within a group of mice, b) within groups of mice at roughly 3-month intervals, and c) within a group over a time course.

The following are the most important results of this study:

1. Analysis of variance of eosinophils/1000 cells in 165 mice over a 35-day period showed that the results were consistent with the assumption that there are no changes during this interval ($P > 0.05$).
2. The number of eosinophils/1000 cells found in the various groups are not directly comparable; the mean number for the four groups investigated at intervals of about 3 months differed by a factor of up to 3.
3. On the basis of the variability found, the number of eosinophils/1000 cells in the bone marrow of a given untreated mouse in a group to be investigated cannot be reliably predicted. At a 99 per cent confidence level, the value would lie between 5 and 139.

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