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in patients with various eosinophilia

Kiyoshi Hiraki*

Masakatsu Inoue†

*Okayama University,

†Okayama University,

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Abstract

Of eosinophilias that we often encounter clinically, we selected two of the most representative ones, namely, hookworm disease and bronchial asthma, for our present sternal bone-marrow tissue culture, and studied the movement patterns and wandering capacity of eosinophils. As the results, even in those eosinophils that show no significant change other than the increase in number in ordinary stained-smear specimens of peripheral blood or bone marrow, it has been clarified that, when observed under living condition, they reveal a picture specific to individualistic behaviors according to diseases. Therefore, it can be assumed that in the pathologic condition what is known as eosinophilia not only eosinophils increase in number but also qualitative changes of eosinophils specific to each disease are brought about, and consequently these specific changes are reflected on the movement patterns of the eosinophil.

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**STUDIES ON EOSINOPHILS IN BONE-MARROW TISSUE
CULTURE OF THE HUMAN STERNUM**

**PART 1. OBSERVATIONS ON THE BEHAVIORS OF
EOSINOPHILS IN BONE-MARROW TISSUE
CULTURE OF THE STERNUM IN PATIENTS
WITH VARIOUS EOSINOPHILIA**

Kiyoshi HIRAKI and Masakatsu INOUE

*Department of Internal Medicine, Okayama University Medical School
Okayama, Japan (Director: Prof. K. Hiraki)*

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Because of the marked clinical variability of the eosinophil count in various patients, there are numerous studies on the biological significance of these cells. However, the majority of these studies on eosinophils is based on the research works dealing with stained specimens or on histopathological studies, and the studies based on the patterns of movement, motility or on the change in number of living eosinophils in locomotion are extremely rare. Recently SHIMIZU and HATTA³ classified eosinophils into three types (Type I, anchylostomiasis; Type II, bronchial asthma type; Type III, normal type) by peroxidase reaction and silver impregnation; and they clarified that the differences in these reactions depended on the amount of reducing substance contained in eosinophils. Therefore, as a link in the studies of bone-marrow tissue culture^{1,2} which has been jealously carried out in our laboratory for the past several years, we have conducted a series of bone-marrow tissue culture of normal persons and of patients with eosinophilia (anchylostomiasis, bronchial asthma), and have studied the patterns of movement and motility of eosinophils.

In the present paper are described the results in which we found the existence of eosinophils possessing a fairly characteristic patterns of movement specific to each disease, and in addition, the description is made on the classification of movement patterns of eosinophils in human bone marrow, the classification newly devised by us.

MATERIALS AND METHODS

Tissue slices were prepared from the bone-marrow tissue aspirated by puncture of the sternum of normal persons, and patients with anchylostomiasis or bronchial asthma. These bone-marrow slices were cultured

by the simple culture method¹ of our own device, and keeping in a warm-box, observations and estimation of the wandering velocity of eosinophils were carried out for the period of 24 hours under the bright field microscope and the phase-contrast microscope.

RESULTS

The classification of movement patterns of eosinophils in the human bone marrow. Although there are many ways in classifying the movement pattern of leucocytes including eosinophils, we have classified them anew as shown in Fig. 1 in order to have as much intimate relationship between the movement pattern and the motility of eosinophils as possible. In making such a classification we have placed a special emphasis on the ratio between axes of the cell in the forward movement stage, the manner of pseudopodes branching, and the position of the nucleus within the cell.

Pattern	I				II	III	IV	V
	A	B	C	D				
average velocity	15 μ /m.	18 μ /m.	9 μ /m.	7 μ /m.	16 μ /m.	8 μ /m.	5 μ /m.	2 μ /m.
arrest stage								
tail-contraction stage								
forward movement stage								
Pseudopode formation stage								
prototype stage								

Fig. 1. The Classification of Movement Patterns of Eosinophils in the Human Bone Marrow (Inoué)

It seems that the position of the nucleus and the motility of the cell are closely associated with each other, and in general when the motility is high, granules occupy the anterior part of the cell and the nucleus is situated in the posterior half (for example, Types I-A, I-B, II); when the

motility is low, the nucleus occupies the central or anterior half of the cell (Types I-C, IV). In Type I-D the long axis of the cell is more than three times the short axis, and its motility is weakened. Type III is side-crawl type and its motility is likewise weakened. Type V is almost motionless. Type I-A (Plate 1) represents the basic type of the movement pattern of human eosinophils, and this type often changes to Type II or vice versa. Type II is the type when pseudopodes of Type I are distinctly branching out in two.

Normal human eosinophils. Normal human eosinophils consist mostly of Type I-A, followed by Type I-C and Type II. The wandering velocity of eosinophils is always a little lower than that of neutrophils (Fig. 2).

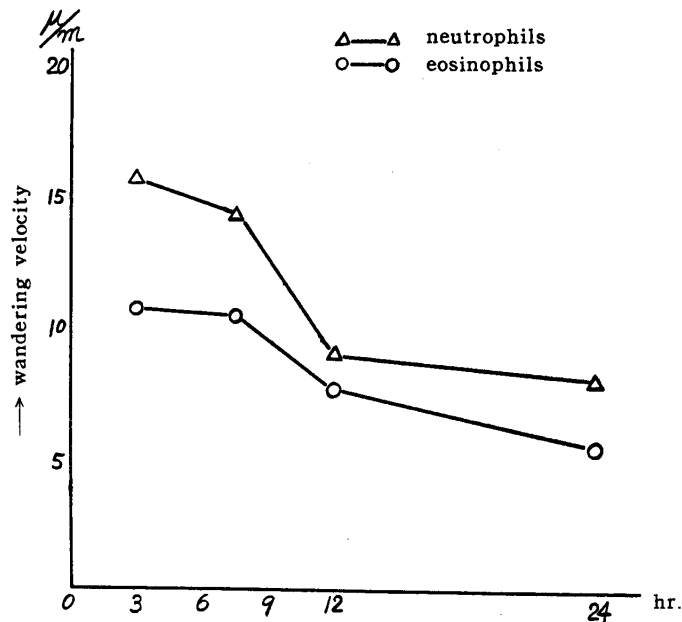


Fig. 2. Sternal Bone-Marrow Tissue Culture of Normal Person

Eosinophils in the patients with hook-worm eosinophilia. It is characteristic with hook-worm eosinophilia in that in this disease besides Types I-A, I-C and III, Type I-B occupies 20 per cent of eosinophils (Type I-B does not occur in other diseases). Type I-B, as shown in Fig. 3 and Plate 2, has a constriction approximately in the middle of the cell; and the posterior half of the cell body from this constriction is occupied practically all by nuclei, and the cell membrane of this part is tightly adhered



Fig. 3. Eosinophils in Hook Worm Disease (Type I-B)

to the nuclear membrane.

This finding indicates that the contractile power of cell at this part is extremely great. The anterior half from the constriction can freely change its shape and granules flow into pseudopodes. The wandering velocity of this type is greatest with average of $17.7 \mu/m$; and this type was observed in the bone-marrow tissue culture of hookworm disease and in the bone-marrow culture of normal person with addition of the serum of the patient with this disease. Furthermore, among these eosinophils of this disease there can often be observed ones with broken cell membrane, whose granules are scattered, and this finding suggests that in the eosino-

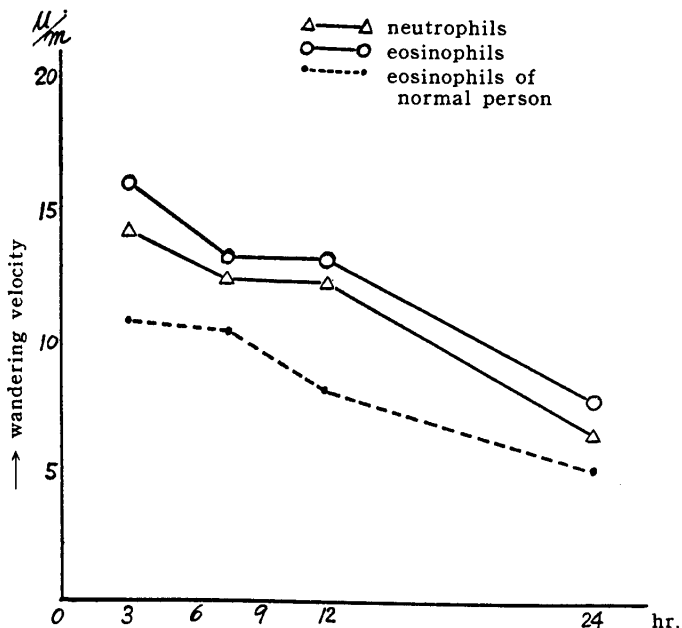


Fig. 4. Sternal Bone- Marrow Tissue Culture of the Patient with Hook Worm Disease

phils of eosinophilia there are cells with weak cell membrane (Plate 4).

The wandering velocity of eosinophils in this disease is higher than that of neutrophils, and consequently it is far more so than that of eosinophils in normal persons. This point is also one of the important characteristics of this eosinophilia.

Eosinophils in bronchial asthma patient. As for the movement pattern of eosinophils in this disease there are Types I-A, I-C and II almost the same as in the normal person, and the wandering velocity after 9-hour culture is somewhat increased (Fig. 5). However, a specific picture

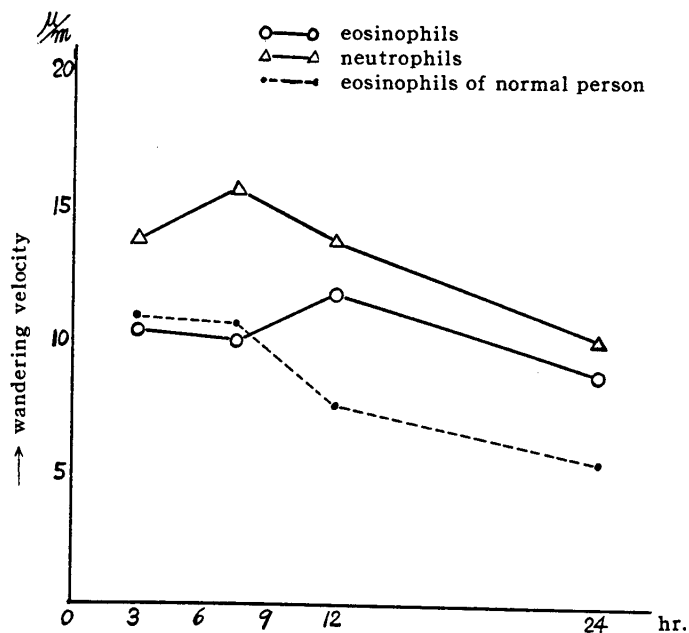


Fig. 5. Sternal Bone-Marrow Tissue Culture of the Patient with Bronchial Asthma

can be observed in those eosinophils whose wandering velocity is decreased (Fig. 6, Plate 5). Namely, there are some whose posterior part of the cell is stretched out forming a slender tail, or some projecting many thread-like branches and presenting a picture like branches of a tree. From these observations it is assumed that the adhesive capacity of eosinophils is abnormally increased. In addition, the granule movement of eosinophils is also somewhat more active and this seems to be a phenomenon indicative of an unbalance between the forward speed of the cell and the rate of the cytoplasm flow.

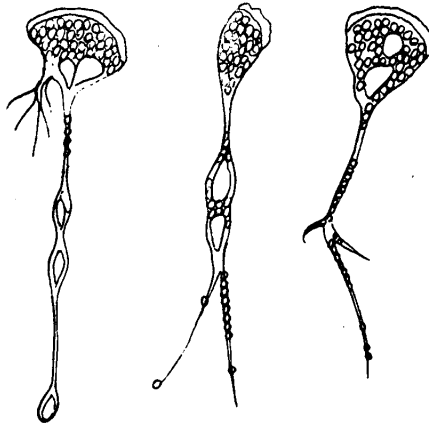


Fig. 6. Eosinophils of Patient with Bronchial Asthma

SUMMARY

Of eosinophilias that we often encounter clinically, we selected two of the most representative ones, namely, hookworm disease and bronchial asthma, for our present sternal bone-marrow tissue culture, and studied the movement patterns and wandering capacity of eosinophils. As the results, even in those eosinophils that show no significant change other than the increase in number in ordinary stained-smear specimens of peripheral blood or bone marrow, it has been clarified that, when observed under living condition, they reveal a picture specific to individualistic behaviors according to diseases. Therefore, it can be assumed that in the pathologic condition what is known as eosinophilia not only eosinophils increase in number but also qualitative changes of eosinophils specific to each disease are brought about, and consequently these specific changes are reflected on the movement patterns of the eosinophil.

CONCLUSIONS

By performing the sternal bone-marrow tissue culture of normal persons and patients with hookworm disease or with bronchial asthma, the authors studied the movement of eosinophils, and obtained the following results:

1. Movement patterns of eosinophils in human bone marrow were classified newly into Types I-A, I-B, I-C, I-D, II, III, IV, and V (Fig. 1).
2. In eosinophils of the bone marrow of patients with hookworm dis-

ease or bronchial asthma there are eosinophils that show characteristic movement pattern specific to each disease and in the case of eosinophilia, not only the increase of eosinophils in number but also characters in their movement patterns specific to each disease can be recognized.

EXPLANATION OF PLATES

- Plate 1. Eosinophil Type I-A
- Plate 2. Eosinophil Type I-B
- Plate 3. Eosinophil Type I-B
- Plate 4. A picture showing scattered granules with destruction of the nuclear membrane (2, 3, and 4 are all of the patient with hook worm disease)
- Plate 5. An eosinophil possessing a strikingly elongated tail (bronchial asthma)

REFERENCES

- 1) HIRAKI, K. and Ofuji, T.: Microcinematographic observations on the blood cells and their clinical applications, particularly by means of bone marrow culture, ACTA HAEM. JAP. 19, 406, 1956.
- 2) HIRAKI, K. : Fundamental and clinical studies on the bone marrow, OKAYAMA IGAKKAI ZASSHI, 67, 2, special volume, 1955.
- 3) SHIMIZU, M. and Hatta, Y. : Studies on eosinophils, NISSHIN IGAKU, 42, 309, 1955 a.



Plate 1.

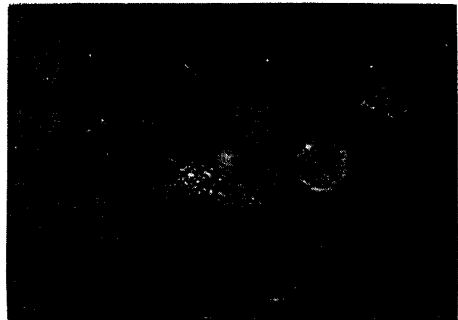


Plate 2.



Plate 3.



Plate 4.



Plate 5.