PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 155, 51-54 (1977)

Defective Chemotactic Migration of Polymorphonuclear Leukocytes in Pelger-Huet Anomaly (39743)

BYUNG H. PARK,[1] JALE DOLEN, AND BARBARA SNYDER

Department of Pediatrics, State University of New York at Buffalo, Buffalo, New York 14222 and Children's Hospital, Buffalo, New York 14222

Pelger-Huet (P-H) anomaly is an autosomal dominant form of a hereditary disorder, characterized by incomplete segmentation of the nucleus of the polymorphonuclear leukocytes (PMN) (1-3). Thus, the majority of PMN with P-H anomaly contains single, unsegmented nucleus resembling immature forms of normal PMN. The homozygous form in man is extremely rare and associated with a high mortality rate (4). However, the incidence of the heterozygous form varies from 1:320 in Adivasi, India (5) to 1:43,000 in Spokane, Washington (6). Although the heterozygous form of this anomaly in man and rabbit does not appear to be associated with undue susceptibility to infection, the homozygous form in rabbits was reported to be uniformly lethal (3).

In 1963, Rebuck *et al.* reported in their skin-window study that the PMN of P-H anomaly migrated less in response to a single antigenic stimulation than did controls (7). These findings support the earlier postulate of Metchnikoff (8), i.e., the segmentation of nuclei in the PMN might be "more adequately explained as a special adaptation for passing through vessel walls." It is, therefore, conceivable that, in P-H anomaly, the failure of nuclear segmentation might lead to a mechanical hindrance to the migration of these cells through smaller openings.

We have investigated chemotaxis function *in vitro* and *in vivo* in a family with P-H anomaly.

Subjects, materials, and methods. Five individuals with P-H anomaly in three generations of a family (Fig. 1) were studied. Each affected member had minor furunculosis at some time. Unaffected members of the family and normal volunteers served as controls. A proper informed consent was obtained from all individuals before the test.

In vivo chemotaxis function was assessed using a skin-window technique (9) with some modification as outlined below. The volar surface of the forearm was covered with wet gauze for 1 hr or longer and cleaned with antiseptic solution and sterile water. An adhesive tape (Scotch, clear transparent, 3M Company, Minneapolis, Minn.) with a template hole of 6 mm in diameter (0.28 cm^2) was placed on the cleaned surface of the forearm. The template was created on the adhesive tape by use of a paper puncher. By repeated application (usually 150 times or more) of another adhesive tape (Magic Tape, white opaque, 3M Company, Minneapolis, Minn.) on the template hole, one could strip off the stratum corneum of the skin through the template hole and create a round skin window with a glistening dermal layer exposed. Then the adhesive tape with the template hole was detached from the forearm. A chemotaxis chamber (0.5-ml capacity) was constructed by cutting a plastic tube (Eppendorf micro-test tube, 1.5-ml polypropylene tube, Brinkmann Instruments, Westbury, N. Y.) and closing one end with the attached cap. The chemotaxis chamber was filled with 0.4 ml of 50% (v/v) zymosan-activated autologous serum in tissue culture medium (RPMI-1640) containing penicillin, streptomycin, and Hepes-bicarbonate buffer (Associated Biomedics Inc., Buffalo, N. Y.). The zymosan-activated serum was tested for its chemotactic activity *in vitro* before use in this procedure. The chamber was placed on the skin window and fastened to the forearm, which was allowed to move freely for normal activity. After an appropriate time (3-6 hr) the chamber was carefully removed, and the

[1] Send reprint requests to Byung H. Park, M.D., Department of Pediatrics, Children's Hospital, 219 Bryant Street, Buffalo, N. Y. 14222.

ISSN 0037-9727

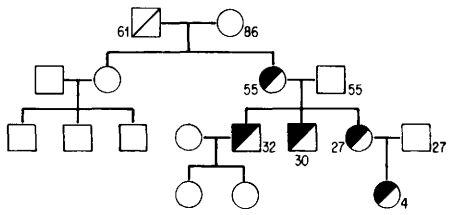

FIG. 1. Pedigree of B.S. family with Pelger-Huet anomaly. Peripheral blood smears from all members were examined. Diagnosis of Pelger-Huet anomaly was made on the basis of the characteristic, segmental arrest of nucleus in 95% or more of PMN. The numbers indicate the age of each member at the time of investigation. The five affected members were identified as heterozygous state. No evidence of homozygous offspring was found in the family.

fluid in the chamber was recovered and examined for the number and morphology of cells in the fluid. The result was expressed as the number of migrated cells per square centimeter of exposed dermal area. A portion of migrated cells in the fluid was deposited on a glass slide by use of a cytocentrifuge (Shandon Southern Instruments Inc., Sewickley, Pa.) and stained with Wright's stain for morphologic examination.

In vitro chemotaxis. Freshly drawn venous blood was transferred to a plastic tube containing sodium heparin (10 units/ml of blood, preservative free), and centrifuged at 900*g* for 10 min at room temperature. The plasma, buffy coat, and upper layer of red cells were carefully transferred to a new plastic tube and mixed thoroughly by use of a transfer pipet. This "leukocyte-rich plasma" was layered on the Ficoll-Hypaque mixture (Pharmacia Fine Chemicals Inc., Piscataway, N. J.) in a plastic tube and centrifuged at 400*g* for 20 min at room temperature. The bottom layer containing PMN was carefully transferred to a new plastic tube containing medium 199. The contaminating red blood cells were lysed by hypotonic shock. The isolated leukocytes contained more than 95% PMN with 98% viability as determined by trypan blue exclusion. These isolated PMN were resuspended in medium 199 at a concentration of 3×10^6/ml.

The PMN suspension in medium 199 (0.1 ml) was deposited on the surface of a Millipore filter in a circular area of 0.28 cm^2, while the suspending fluid was simultaneously absorbed by absorbant paper placed on the opposite side of the Millipore filter paper. The PMN on the Millipore filter paper was sandwiched quickly by placing another wet Millipore filter on the deposit. The two Millipore filters with "sandwiched" PMN were placed on the blind well chamber (Neuro Probe, Bethesda, Md.) with tissue culture medium in the lower compartment. Chemotactic attractant (activated serum or culture filtrate of *E. coli*) was introduced into the upper compartment of the chamber. Thus, the direction of chemotactic movement of neutrophils was made upward (10). These chambers were incubated in a water bath to ensure a constant, accurate temperature of 37°. After an appropriate time of incubation, the filter paper was removed, dipped quickly in 100% methyl alcohol, and then fixed in buffered formalin (3 vol of 33% formaldehyde and 7 vol of isotonic phosphate buffer, pH, 7.2) for 1 hr or longer, stained with hen•atoxylin, and examined under the microscope. The result was expressed as an average number of migrated cells per high power field (HPF).

Results. Figure 2 shows results of *in vivo* chemotaxis function tests using the skin-window technique. In healthy control subjects, the cells which migrated into the chemotactic chamber through the skin window were $120 \times 10^3/cm^2$ of skin-window area at 3 hr. In contrast, the number of migrated cells from patients with P-H anomaly was only one-tenth of the control.

Figure 3 shows the results of *in vitro*

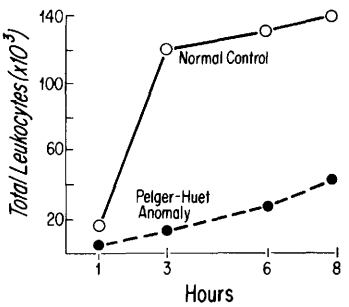

FIG. 2. *In vivo* chemotaxis function test using modified skin-window technique. The number of migrated cells in P-H anomaly was decreased as compared to normal controls and unaffected members of the family.

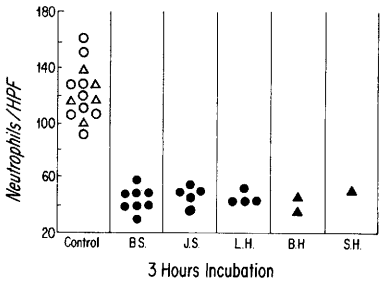

FIG. 3. Chemotaxis *in vitro* using modified Boyden chamber method: Millipore filter paper with 5-μm pore size was used. Circles indicate males and triangles indicate females. Affected individuals are shown by black circles or triangles. Each circle or triangle represents a separate test.

chemotactic function tests using the standard 5-μm Millipore filter paper. Controls showed an average of 120 migrated cells/HPF. In contrast, PMN of P-H anomaly showed a markedly decreased chemotactic activity (45 cells/HPF). Most of these migrated cells had two-lobe nuclei. The decreased chemotactic activity was observed in repeated tests over a period of more than 8 months in the affected members of this family.

Figure 4 shows the effect of the different pore sizes of Millipore filters on the chemotactic activity of P-H anomaly. When the pore size was small (3 μm), significantly fewer cells migrated in P-H anomaly. When the pore size was large (8 μm), the numbers of migrated cells in P-H anomaly approached those of the controls. In contrast, no significant difference was noted in the chemotaxis of PMN from normal controls when Millipore filters of different pore sizes were employed.

Discussion. Our study confirms the report of Rebuck *et al.* (7) that PMN of P-H anomaly have a decreased *in vivo* chemotactic activity. In addition, we were able to demonstrate the difference in a more quantitative manner by use of a modified method.

To investigate whether the decreased chemotactic activity is due to the large unsegmented nucleus in the PMN of P-H anomaly, we examined the chemotactic activity *in vitro* by use of the newly modified Boyden chamber technique, in which filter papers of different pore sizes were employed. A markedly decreased chemotaxis was noted in PMN of P-H anomaly when the filter paper of a small pore size was employed. The decreased chemotactic activity was constantly observed in the affected members of the family for more than 8 months of the study period. Our results indicate that the large, unsegmented nucleus in P-H anomaly may indeed hinder the migration of PMN through a smaller opening. This finding lends support to the earlier prediction by Metchnikoff (8), namely, that the segmentation of the nucleus in PMN may be advantageous to the migration of these cells and that a large, unsegmented nucleus may result in reduced deformability of cells and thus cause a mechanical hindrance to the migration of cells through a smaller opening. Reduced deformability of cells might also be caused by alteration of outer membrane (12), or by cytoskeletal changes such as dysfunction of actin (13). However, our preliminary study indicated that the outer membrane and actin in the PMN of P-H anomaly appeared to be normal.

Although the P-H anomaly in man appears to be a benign condition in heterozygous form, its recognition is important since these abnormal leukocytes can be confused with immature forms of normal PMN and, thus, may lead to clinical misinterpretation. Since homozygous offsprings of P-H anomaly seem to have a high mortality rate (4), identification of this anomaly may become important in genetic counseling in areas where the frequency of heterozygous form is

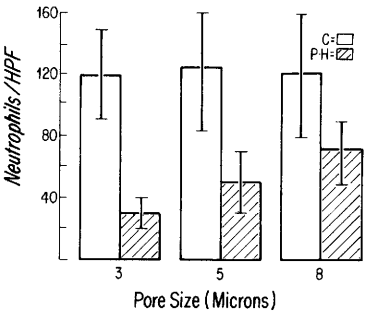

FIG. 4. The effect of different pore sizes on the chemotaxis. Chemotactic migration in PMN of Pelger-Huet anomaly was significantly impaired when filter papers with small pore size (3 or 5 μm) were used. Open bars represent controls; hatched bars represent P-H anomaly. (Mean ± 2 SD.)

reported to be as high as 1:320 (5).

Leitner and Gugelot (11) reported that although more mature PMN with P-H anomaly had near normal phagocytic function, the immature unsegmented PMN were less effective than the immature unsegmented PMN from normal individuals. They suggested that the individual with P-H anomaly might more easily succumb to severe infection. Our study (unpublished) showed that the ingestion and killing of *Staphylococcus aureus* (502A), as well as the capacity to reduce nitroblue tetrazolium dye, by PMN of P-H anomaly did not differ significantly from normal PMN. We also found that individuals with P-H anomaly had normal immune function as indicated by normal numbers of T- and B cells in the peripheral blood, and normal serum immunoglobulin levels.

Summary. A decreased chemotactic migration of PMN *in vivo* and *in vitro* was observed in PMN from five individuals with P-H anomaly in a family. The decreased chemotactic migration was more pronounced when the PMN were made to migrate through a filter paper with small pore size. The decreased migration may be in part due to the mechanical hindrance of the large unsegmented nucleus to the migration of the cells.

We thank Dr. Elliot F. Ellis, Professor and Chairman, Department of Pediatrics, for his support and guidance throughout this investigation.

A portion of this report was presented in the annual meeting of the Federation of the American Society for Experimental Biology on April 13, 1976.

This work was supported in part by Grants HL-19628-01 and BRS-342.23.148 from the NIH.

1. Pelger, K., Ndld. Tschr. Geneesk. **72,** 1178 (1928).
2. Huet, G. J., Klin. Wschr. **11,** 1264 (1932).
3. Nachtsheim, H. J., Heredity **41,** 131 (1950).
4. Haverkamp Begemann, N., and Van Lookern Campagne, A., Acta Hematol. **7,** 295 (1952).
5. Gehlot, G. S., and Monga, N. N., Indian J. Med. Res. **61,** 653 (1973).
6. Ludden, T. E., and Harvey, M., Amer. J. Clin. Pathol. **37,** 302 (1962).
7. Rebuck, J., Barth, C. L., and Petz, A. J., Fed. Proc. **22,** 427 (1963).
8. Metchnikoff, E., "Lecture on the Comparative Pathology of Inflammation" (Delivered at the Pasteur Institute in 1891). pp. 185–186. Dover, New York (1968).
9. Mass, M. F., Dean, P. B., Weston, W. L., and Humbert, J. R., J. Lab. Clin. Med. **86,** 1040 (1975).
10. Park, B. H., Pediat. Res. **9,** 333 (1975).
11. Leitner, S. J., and Gugelot, P. C., Nederl. T. Geneesk **82,** 3959 (1938).
12. Miller, M. E., *in* "The Phagocytic Cell in Host Resistance" (J. A. Bellanti and D. Dayton, eds.), p. 295. Raven, New York (1975).
13. Boxer, L. A., Hedley-Whyte, E. T., and Stossel, T. P., N. Engl. J. Med. **291,** 1093 (1974).

Received December 28, 1976. P.S.E.B.M. 1977, Vol. 155.