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Colony Growth of Human Peripheral White Blood Cells In Vitro

By JOHN E. KURNICK AND WILLIAM A. ROBINSON

Peripheral blood from normal humans and patients with circulating atypical mononuclear cells have been studied for their ability to form granulocytic colonies in vitro in the agar-gel method of hematopoietic cell culture. WBC from patients with chronic lymphocytic leukemia failed to proliferate. Normal human white blood cells gave rise to 0.5–8 colonies per 1×10^6 cells plated, while peripheral blood containing large mononuclear cells gave rise to 15 to 120 colonies per 1×10^6 cells plated, depending upon the number of such cells plated. Colonies averaged 500 cells in size by day 20 of culture and appeared to be granulocytic.

ANIMAL STUDIES HAVE SUGGESTED that cells capable of repopulating the entire hematopoietic system can be found circulating in the blood stream.[1,2] It has been debated whether such cells circulate in man. The systems which have been utilized in animal models necessitate irradiated hosts, and have not been applicable to the study of dividing cells in human peripheral blood. Recently, the method of hematopoietic cell culture in agar-gel medium with which colonies of maturing granulocytes can be grown from single cells has been adapted for human bone marrow.[3,4] We have utilized this system to study the in vitro colony-forming potential of human peripheral blood cells in normal and abnormal states. These studies have shown that blood from normal humans gives rise to from one to eight colonies per 10^6 cells plated. Peripheral blood from patients with infectious mononucleosis and other common viral illnesses associated with large mononuclear cells (commonly referred to as large, or atypical lymphocytes) yields a much greater number of colonies. In all instances the colonies formed appear to be granulocytic in character. These studies have demonstrated that there are cells circulating in the peripheral blood of normal humans which are capable of giving rise to granulocytic colonies in vitro and that the number of such cells increases in certain infections in direct proportion to the numbers of atypical mononuclear cells in the circulation.

MATERIALS AND METHODS

Peripheral blood was obtained from 15 normal human subjects, 17 patients with atypical mononuclear cells due to infectious mononucleosis or other presumptive viral

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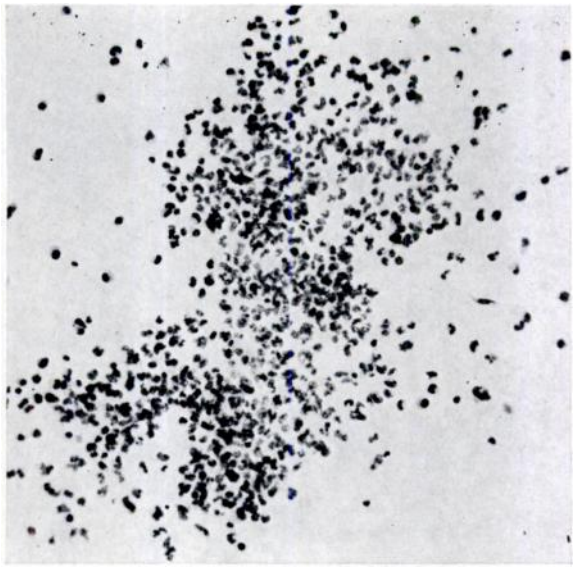

Fig. 1.—Low power microscopic appearance of a single colony grown from normal human peripheral blood at day 20 of incubation.

infections, and four patients with chronic lymphocytic leukemia. Blood was collected in 20 ml. vacuum tubes containing two drops of 1:1000 heparin (Liquaemin sodium 10, Organon, Inc., West Orange, N.J.). It was allowed to stand at room temperature for 2 hours during which time the red blood cells sedimented, leaving the white blood cells (WBC) and platelets in the plasma. The plasma containing WBC and platelets was then removed with a Pasteur pipette and the number of cells counted in a hemocytometer. The yield was usually 5–20 × 10^6 nucleated WBC per ml. of plasma with a 1:1 WBC to red cell ratio. The cells were then plated into 35 mm. Falcon plastic Petri dishes over an underlay of 1 × 10^6 normal WBC in 0.5 per cent in McCoy's 5A medium, as previously described for human bone marrow culture.[3] Each culture contained 1 × 10^6 nucleated WBC incorporated in a 1 ml. mixture of nine parts of McCoy's 5A medium and one part of 3 per cent boiled agar. The plates were then incubated at 37°C under humidified conditions with a constant flow of 7.5 per cent CO_2 in air. Aseptic technique was observed throughout.

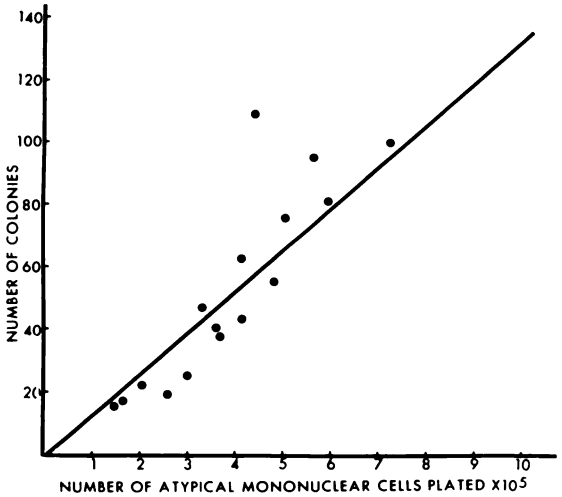

Fig. 2.—Correlation between the number of colonies formed from the peripheral blood of patients with atypical mononuclear cells and the absolute number of such cells plated. On the abscissa is plotted the number of atypical mononuclear cells on a single plate, calculated from the per cent present in the peripheral blood. Each point is a single patient and is the mean colony count of five plates.

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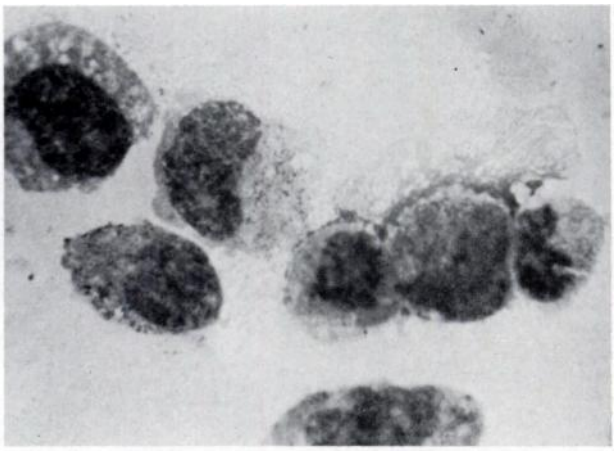

Fig. 3.—Portion of a colony from normal human peripheral blood at day 6 of incubation showing the large mononuclear cells comprising the colony. Giemsa stain. × 1200.

With the aid of a dissecting microscope, colony counts were done at day 15–25 of incubation. For the study of cell morphology, colonies were removed from the agar with a finely drawn Pasteur pipette, placed on glass slides, flattened with cover slips, and stained by floating aceto-orcein under the cover slips, as previously described.[3] Smears of colonies were also fixed in methanol, air dried, and immersed in a 1:50 solution of Giemsa stain in distilled water for 10 minutes.

Results

Colonies were visible on plates by days 5–6 of incubation. They appeared to reach maximum size at day 20, after which no further increase in size was noted (Fig. 1). Colony size was fairly uniform in any given culture, but bloods from different individuals, both normal and those with viral diseases, yielded colonies which varied noticeably in size. Colonies in both groups at day 20 consisted of 500–700 cells. The colonies from patients with viral illness tended to be at the higher end of the range.

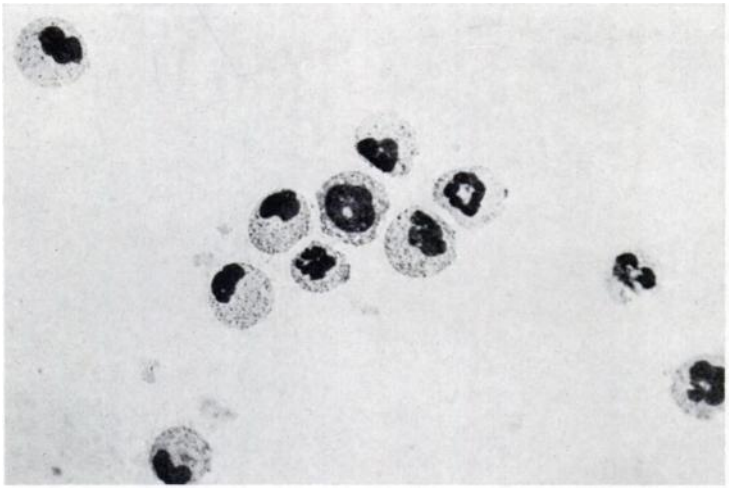

Fig. 4.—Portion of a colony from normal human peripheral blood at day 22 of incubation showing the predominance of cells which appear to be mature granulocytes.

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Table 1.—Colony Formation by Human Peripheral WBC

Subject	Percentage Large Mononuclears	Colonies/1 × 10^6 Cells
Normals		
M.E.		8.0*
E.B.		6.1
L.K.		5.0
J.G.		5.0
M.P.		3.5
J.S.		2.8
D.P.		1.9
R.V.		1.8
M.H.		1.6
W.R.		1.0
R.E.		1.0
N.B.		0.7
C.R.		0.6
P.S.		0.5
B.P.		0.5
	Mean	4.0
Chronic Lymphocytic Leukemia		
M.M.		0
R.P.		0
W.W.		0
L.J.		0
	Mean	0
Viral Infections		
J.J.†	46	120
E.H.	57	102
P.R.†	72	101
C.R.	52	87
G.S.†	60	86
A.T.	42	70
D.D.†	48	59
J.K.	33	49
E.K.†	42	42
H.W.	37	40
A.C.	37	39
S.M.†	28	35
S.S.	30	23
W.C.†	20	22
B.W.	24	18
S.K.	15	17
E.F.†	14	15
	Mean	54

* Mean number of colonies on five plates.

† Infectious mononucleosis as demonstrated by heterophil titer greater than 1:56 after guinea pig kidney absorption.

The number of colonies formed from the blood of normal humans and from patients with infectious mononucleosis and other viral illnesses is shown in Table 1. Normal human peripheral blood gave rise to 0.5–8.0 colonies per 10^6 cells plated, with a mean of 4.0. The number of colonies formed from the peripheral blood of patients with atypical mononuclear cells was considerably

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greater, 15–120 with a mean of 54. The number of colonies formed in cultures from these patients was directly related to the number of atypical mononuclear cells plated, Fig. 2. None of the cultures prepared with WBC from patients with chronic lymphocytic leukemia yielded any colonies, Table 1.

The morphology of colony cells from normal bloods and from those with atypical mononuclear cells was similar. All colonies appeared to begin as large mononuclear cells (5–10 days), Fig. 3, with a gradual progression to cells with the morphology of mature granulocytes (20–25 days), Fig. 4. Subsequently, many large phagocytes appeared in the colonies. The types of cells noted in the colonies and their maturation progression is similar to that previously noted with normal human bone marrow.[3,4]

Discussion

These studies have demonstrated that normal human peripheral blood contains cells capable of division and granulocytic maturation in vitro. The number of such cells is greatly increased in bloods containing large atypical mononuclear cells. The linear relationship between the number of these large atypical mononuclear cells plated and the number of colonies obtained suggests that the colony-forming cells in the peripheral blood of normal human subjects are of the same cell type. Such cells can be found in the blood of almost all normal subjects if a sufficiently detailed search is made. These cells may also have the potential for erythropoiesis and thrombopoiesis, or other circulating cells may be capable of these specific proliferative capacities. Rickard et al.[5] provided evidence that the cells which give rise to colonies in vitro are committed granulocytic precursors and not multipotential stem cells. Our experiments do not permit us to decide whether circulating cells with the capacity to produce the complete hematopoietic trilineage exist in man, because our culture method may select only cells committed to granulopoiesis or favor only granulopoietic differentiation.

These findings should be of interest to those using this culture system to grow human bone marrow in vitro. The method described utilizes a feeder layer of normal human WBC.[3] In determining the numbers of colonies formed in such a system, one should be aware of the fact that the feeder layer itself may be capable of colony formation. Peripheral blood from individuals with numerous atypical mononuclear cells should therefore be avoided as a source of feeder layers.

Others[6-13] have observed that peripheral blood cells morphologically resembling those plated in this study have the capacity for DNA synthesis and mitosis in vitro. MacKinney[9] and co-workers[7] have suggested that the spontaneous proliferation of such cells might be anticipated, while Tanaka et al.[14] made detailed observations on the transformation of small lymphocytes from normal human peripheral blood into larger cells capable of division. We are uncertain as to whether the cells cultured in our study actually represent large lymphocytes, as is generally assumed, or are phagocytic cells (monocytes) which, according to Leder,[15] derive from promyelocytes. This suggested common lineage of monocytes and granulocytes is supported by the observed appearance of large phagocytes in our older granulocytic colonies,

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which may result from differentiation. In this discussion we have therefore, attempted to avoid the ongoing controversy as to whether the stem cell is a lymphocyte by referring to the colony-forming cell in our system as an atypical mononuclear cell. That the unstimulated small lymphocyte is not the colony-forming unit in agar culture is suggested by the relative proliferative failure of normal WBC when compared to bloods with atypical mononuclear cells, and also by the failure of the lymphocyte of chronic lymphocytic leukemia to give rise to any in vitro colonies. We are investigating the possibility that stimulation of small lymphocytes may, however, impart to them this capacity.

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