The In Vivo Differentiation of Human Leukocytes into Histiocytes, Fibroblasts and Fat Cells in Subcutaneous Diffusion Chambers

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One of the important controversies of morphologic hematology concerns the concept that some of the nongranular mononuclear leukocytes of the circulating blood have the potential for further differentiation toward connective tissue and hematopoietic cells. A relationship between leukocytes of the blood and connective tissue was suggested by Cohnheim in 1876, who observed leukocyte diapedesis and suggested that the cells of the blood give rise to inflammatory exudates and, possibly, to connective tissue. Morphologic studies of leukocytes participating in inflammatory reactions and of leukocytes growing in tissue culture suggested to Maximow, Bloom and others that the nongranulated leukocytes of adult blood have latent mesenchymal potentialities, which under the proper circumstances, can differentiate toward macrophages, fibroblasts, and other components of connective tissue. However, other investigations of inflammatory exudates and of healing wounds, particularly those of Marchand, and of Herzog, did not support this view, but demonstrated that the leukocytes were phagocytes acting mainly as scavengers, which subsequently disintegrated. This latter view is essentially that held by most investigators today. In a recent review of wound healing, Edwards and Dimpy conclude, "As yet there is no conclusive evidence for a specific (i.e. blood borne) cell progenitor of the fibroblast." The historical aspects of this subject have been extensively reviewed by Bloom, Allgöwer, Reubke and Crowley, Florey, and Trowell.

An important factor which has obscured the possible role of the mononuclear leukocytes in the formation of connective tissue cells has been the great difficulty in following the fate of the individual leukocytes among the fibroblasts which rapidly proliferate from edges of the healing wound.

The present studies were undertaken to reinvestigate the developmental potentialities of the nongranular mononuclear leukocytes of the blood which had been cultivated subcutaneously in Algire diffusion chambers. The chambers were constructed with Millipore membranes which permit the diffusion of nutrients into the chamber, but do not allow the entry or exit of leukocytes. In this fashion, the chamber acts as a type of in vivo tissue culture.
receptacle which permits the enclosed leukocytes to be exposed to the environment of the wounded extravascular tissue and to reveal any inherent potential they might possess for further differentiation.

**Materials and Methods**

The diffusion chambers employed were a modification of that of Algire, et al. in their studies of mouse bone marrow. These consisted of plexiglass washers which were covered with Millipore filter membranes (type HA, 18 mm. diameter, 150 microns thick with an average pore size of 0.45 microns plus or minus 0.02 microns.) The membranes were sealed to the plexiglass washers along their outer edges with a 1 per cent leucite in acetone suspension applied with a cotton-tipped applicator. The plexiglass rings and Millipore membranes were sterilized by autoclaving at 15 pounds pressure for 15 minutes at 250 degrees.

Leukocytes were obtained from the blood by venepuncture, employing heparin as an anticoagulant. The blood was gently centrifuged at 500 to 1000 rpm for 10 minutes to obtain the buffy coat; and in order to manipulate the leukocytes as gently as possible, complete separation of the buffy coat from erythrocytes was not attempted. A suspension of leukocytes in plasma containing approximately $5 \times 10^5$ to $1.5 \times 10^5$ cells was sealed within the chambers. In several instances suspensions of lymph node lymphocytes obtained at laparotomy were placed within the chambers. The sealed chambers containing the leukocytes were kept in Petri dishes containing Hank's solution until they were implanted, usually within 15 minutes after sealing.

The chambers were implanted subcutaneously into volunteer subjects, primarily patients with advanced neoplastic disease from the wards of the Cancer Research Institute. Studies of autologous and homologous leukocytes were made. The technic of implantation and removal of the chambers was a minor surgical one which was often carried out as an outpatient procedure. The chambers were placed subcutaneously in small incisions along the axillary line of the chest. One per cent Xylocain [g] was used as a local anesthetic and the incisions were closed with surgical clips. The chambers were removed at weekly intervals up to six weeks and following their removal the diffusion chambers were fixed in Bouin's solution for one to two hours. The membranes were stained in toto, in hematoxylin and eosin in the usual manner employed for staining histologic sections and mounted on micro slides. The clot which formed within the chamber was sectioned and stained with Van Giesen and Mallory connective tissue stains and for reticulin.

**Results**

Following subcutaneous implantation of autologous leukocytes in millipore diffusion chambers, a characteristic sequence of morphologic transformation of the mononuclear leukocytes to fibroblasts was observed to occur. Similar results were obtained with both homologous and autologous leukocytes. A gradual disintegration of neutrophilic granulocytes and eosinophils took place over a two to three week period, and in most studies, no granulocytes were found beyond three weeks. It was not possible in all instances to distinguish positively lymphocytes from monocytes on the stained membranes. During the first two weeks, the mononuclear leukocytes (lymphocytes and/or monocytes) appeared to hypertrophy into cells having the morphology of macrophages and histiocytes (fig. 1A). These cells appeared to have a variety of morphologic forms and were designated as polyblast macrophages (after Maximow). At three weeks, the cells had assumed a stellate appearance or the spindle-shaped form of fibroblasts (fig. 1B). By three to four weeks, the surfaces of the membranes were covered with dense sheets of fibroblasts arranged in parallel rows or in
Fig. 1.—(A). Conversion of mononuclear leukocytes to “polyblast” macrophages at 10 days (1000X). (B). Fibroblast-like cells at 3 weeks (1000X). (C). Dense fibroblastic growth at 4 weeks (1000X).
interlacing patterns (fig. 1C). Numerous mitotic figures could be found in these cells. This sequence of transformation was observed on all occasions, the variation occurring in the time required and the degree of fibroplasia. In general, dense fibroblastic proliferation was not prominent until the third week, although it was occasionally seen as early as 10 to 14 days.

When lymphocytic suspensions were prepared from lymph nodes, recognizable small lymphocytes could be observed up to four weeks time, although the majority underwent dissolution during the first week. Large fibroblasts were found in the chamber as early as one week which were presumed to have arisen from either large lymphocytes or reticulum cells from the lymph node stroma, but direct evidence for this possibility was absent.

Experiments were performed to confirm the transformational sequence from polyblast macrophage to fibroblast by adding a small amount of India ink to the cell suspensions at the time the leukocytes were sealed into the chamber. At two weeks the India ink was found to be present in the macrophages (fig. 2A), and by four weeks, the ink particles could be identified in the cytoplasm of the fibroblasts growing in the less dense areas of the membranes (fig. 2B), indicating the conversion of macrophages to fibroblasts.

Histologic studies were made of the plasma clots within the chambers to determine the capacity of the fibroblasts for the formation of collagen and reticulin. The sections were stained with both Mallory's and Van Giesen stains and indicated that active collagen and reticulin production occurred in association with the fibroblasts (figs. 3A & B). The amount of collagen produced was observed to vary considerably. In some instances dense collagen bundles were produced, whereas in others, very scanty amounts were formed. These variations were attributed to possible nutritional differences among the subjects in whom the chambers were implanted and those from whom the leukocytes were obtained.

Other elements of loose connective tissue were found in the diffusion chambers and were attributed to a mononuclear leukocyte origin similar to that of the fibroblasts. Fat-containing cells were found in several instances after three to four weeks of implantation (fig. 4A). In addition, capillary-like structures were found in two studies (figs. 4B & C). In one, the cell nuclei were found to have aligned themselves along the length of a cotton fibril which inadvertently fell into the chamber at the time of sealing. The macrophage-like character of the cells was evident. However, in another study, a capillary-like tubule lined by endothelial-like cells was observed within the chamber. The absence of erythrocytes within the lumen of the tubule indicated that the cells had originated from the buffy coat which had been placed in the chamber, rather than from capillaries from the subcutaneous tissue of the host growing through a possible perforation of the millipore membrane.

DISCUSSION

The present investigations of human leukocytes from adult blood cultivated in subcutaneous diffusion chambers offer an experimental demonstration of the mesenchymal potentialities of the circulating nongranular leukocytes of
Fig. 2.—(A). Phagocytosis of India ink particles by macrophages at 2 weeks (250X). (B, C and D). Fibroblasts containing India ink at 4 weeks (1000X).
adult blood postulated by Maximow. They demonstrate that under the ecologic conditions encountered in the chamber, mononuclear leukocytes have the capacity for differentiation toward polyblast macrophages and eventually to fibroblasts capable of collagen and reticulin production. In several instances, they were found to form fat cells, and in one chamber, an endothelial-like tubule. The observations of fat cell formation correspond to those of Chang who reported the transformation of histiocytic macrophages of the peritoneal cavity to fat cells.

In 1902, Maximow published his classical monograph on the relationship of the leukocytes of the blood to connective tissue. He noted the prompt appearance of leukocytes in fresh wounds and the subsequent transformation of lymphocytes and monocytes into macrophages. He named the macrophages
Fig. 4.—(A). Formation of fat containing cells at 4 weeks (1000X). (B). Capillary-like formation resulting from alignment of macrophages along cotton fibrile (1000X). (C). Endothelial tubule resembling capillary. Note absence of erythrocytes and leukocytes in lumen (1000X).
“polyblasts” because they exhibited a variety of morphologic forms, and because he believed them to be an intermediate cell phase which was capable of further transformation into fibroblasts and other elements of connective tissue such as cartilage, bone and hematopoietic tissue.

Studies of blood and thoracic duct leukocytes in tissue culture have been reported by many investigators, demonstrating the formation of macrophages, polyblasts, and fibroblast-like cells from lymphocytes and monocytes.5,16-25 Maximow26 reported the formation of argyrophilic fibrils in association with the fibroblast-like cells, indicating their connective tissue function, but these observations were not confirmed by others. However, it is doubtful that the in vitro tissue culture technics employed provided an environment comparable to injured extravascular connective tissues which could permit a significant degree of collagen formation.

These conclusions indicating a mesenchymal potential of mononuclear leukocytes, particularly for the lymphocyte, were not supported by the tissue culture experiments of Hall and Furth,27 Medawar,28 Ebert, Sanders and Florey29 and recently Gowans.30,31 These investigators did not observe the further transformation of lymphocytes, but reported that they disintegrated. They attributed the origin of the fibroblast-like cells to monocytes. While admitting the transformation of monocytes to macrophages and histiocytes, Florey28 emphasizes that their further transformation to fibroblasts is never seen in vivo.

The present studies indicate that once polyblast macrophages are formed, they are able to metamorphose into fibroblasts, but they do not clarify the question of whether or not both lymphocytes and monocytes possess the ability to become polyblast macrophages, in vivo, or whether transformation is a property of only one of these mononuclear forms. Most observers agree that monocytes possess the ability to form macrophages and giant cells, but strong doubt has been expressed that lymphocytes can undergo this transformation. Recently, Rebuck32 has demonstrated the morphologic transformation of lymphocytes to macrophages in vivo by means of a coverslip window technic. Bloom33 and Koszewski, et al.34 have demonstrated that lymphocytes are phagocytic for India ink which has been administered intravenously. The demonstrations by these investigators of phagocytic ability of lymphocytes under in vivo conditions indicate that negative results obtained under the artificial conditions of tissue culture and slide preparations must be applied with caution in the interpretation of possible in vivo function.

It has been considered by various authors35,36 that the nongranular mononuclear leukocytes of blood comprise a dynamic mesenchymal progenitor-cell pool which is readily available for functions such as phagocytosis and participation in the immune response. The present studies indicate that, in addition, under the proper conditions some mononuclear leukocytes of the circulating blood have the capacity to form various elements of the loose connective tissues, particularly collagen-producing fibroblasts.

**Summary**

Normal human leukocytes were cultivated in millipore diffusion chambers which had been implanted subcutaneously in autologous and homologous
IN VIVO DIFFERENTIATION OF HUMAN LEUKOCYTES

subjects. The observations were made over periods of a few days to six weeks. It was found that mature granulocytes underwent disintegration within one to two weeks. Mononuclear leukocytes underwent differentiation into macrophages and "polyblasts" within a few days and by three weeks had assumed the morphologic appearance characteristic of histiocytes and fibroblast-like cells. By four to six weeks extensive fibroblastic proliferation and marked collagen formation was found. In several chambers numerous fat cells were seen.

These in vivo studies demonstrate the mesenchymal potential for differentiation possessed by circulating mononuclear leukocytes of adult blood.

SUMMARIO IN INTERLINGUA

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REFERENCES


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