that case the toxin, by blocking transmission of the signal from receptors to N-protein, may raise the cAMP level and thereby inhibit the Ca-response to the mitogen. Another possibility is that the target for CT in thymocytes is the N-protein coupling the receptor with phospholipase C, and for that reason the toxin suppresses the rise of $[Ca^{++}]_i$ by inhibiting hydrolysis of polyphosphoinositides. Irrespective of how this is brought about, our results are evidence that N-proteins are involved in transmission of the signal from the receptor inside the cell and in generation of the Ca-response during mitogenic activation of thymocytes.

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HEMATOPOIETIC PRECURSOR CELLS IN THE INTIMA OF THE ATHEROMATOUS HUMAN AORTA

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KEY WORDS: colony-forming units; precursors of granulocytes and macrophages; atherosclerosis; intima of the aorta

The presence of blood cells in the intima of human arterial walls affected with atherosclerosis has been noted in several investigations [2, 7, 8, 10, 15]. The appearance of leukocytes in the intima of atheromatous vessels is associated with their penetration from the circulating blood through the endothelium, after preliminary adhesion to its surface, but the possibility has never been envisaged that colony-forming units (CFU), cells capable of forming colonies in culture, can proliferate and differentiate in the intima. It is with this possibility that our data, some of which were published previously in abstract form [14], are concerned. In this paper we present data indicating that precursor cells of granulocytes and macrophages (CFU-GM) may be present in the intima of the atheromatous human aorta.

EXPERIMENTAL METHOD

The aortas from 22 persons dying accidentally or suddenly between the ages of 40 and 80 years were investigated. The thoracic part of the aorta was taken not later than 6 h after death. After mechanical separation of the adventitia the luminal surface of the vessel was carefully washed with phosphate-buffered saline to remove blood. The endothelium was taken from the surface of the vessel with 0.1% collagenase solution in medium 199 for 15 min at 37°C.

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After removal of the endothelium, the intima of the vessel was separated from the media. Intimal cells were isolated from intact parts of the aorta, and from zones of lipid and fibrous lesions. Pieces of intima were treated with type II collagenase solution (Worthington), made up in medium No. 199 containing 2 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. The duration of incubation at 37°C was 3.5-4 h. The viability of the isolated cells was 80-98%. Blood which was found in the aorta after ligation of its ends also was tested for the presence of CFU-GM. Blood mononuclears were isolated by centrifugation at 400g for 30 min on Ficoll-Paque, with a density of 1.077. Intimal cells, like blood mononuclears, were suspended in Dulbecco's medium (Flow Laboratories) containing 0.9% methylcellulose or 0.3% agar (Bacto-Agar, "Difco"), 30% human group AB(IV) serum, 5×10^{-5} M 2-mercaptoethanol, and 5% of medium conditioned by leukocytes in the presence of phytohemagglutinin (PHA-LCS), which served as the source of colony-stimulating factor [4]. Intimal cells, numbering not less than 2×10^5 nucleated cells, and blood mononuclears numbering not less than 5×10^5 in 1 ml of medium were transferred into three or four plastic Petri dishes (35 mm, "Lux") and incubated at 37°C in an atmosphere with 5% CO_2 and 100% humidity for 14 days, after which the number of colonies in the dishes was counted in an inverted microscope. For morphological analysis of cells in the colonies from the agar cultures, preparations were made by the method in [13] and stained with May–Gruenwald–Giemsa mixture. Separate colonies were taken from the agar with a micropipet, placed on a glass slide, and treated with the histochemical reaction for nonspecific esterase [9]. Pieces of intima were excised from the intact region of the aorta and from zones of lipid and fibrous lesions. To obtain histological preparations the pieces were fixed in Carnoy's mixture, dehydrated, and embedded in paraffin wax; sections of the intima were stained with Giemsa's mixture. To obtain semithin and ultrathin sections pieces of intima were fixed in 2.5% glutaraldehyde solution in phosphate buffer. After postfixation with osmium tetroxide the pieces were dehydrated and embedded in an Epon mixture. Sections of the intima, oriented parallel and perpendicular to the plane of the aorta, were cut from the blocks on an LKB ultratome. Sections 1 μ thick were stained with methylene blue [3] and examined in the light microscope. Transmission electron microscopy (TEM) was carried out on a JEM-100CX electron microscope.

EXPERIMENTAL RESULTS

Examination of histological and semithin sections from unaffected parts of the intima or parts with traces of lipid infiltration showed that the leukocytes infiltrating these areas often formed agglomerations. These were characterized by loose or dense packing of the cells, which differed in number from 3 to 50 or more, so that they resembled colonies and clusters (Fig. 1a, b). The cells in these agglomerations were analyzed using classical morphological criteria for mononuclears and granulocytes; the size, shape, and density of the nucleus, the presence of nucleoli in it, the nucleocytoplasmic ratio, and also the degree of basophilia of the cytoplasm and the presence of granulation. On the basis of these criteria the cells in agglomerations of this type could be classed as cells following the granulocytic-macrophagal direction of differentiation, from blast cells to mature forms (Figs. 1 and 2). Among cells forming agglomerations, unidentified blast cells with a pale, large nucleus, with one or more nucleoli, and with a narrow rim of cytoplasm, with a varied degree of basophilia, were frequently found (Fig. 1a, b; Fig. 2b). Promonocytes and monocytes, and also promyelocytes, the largest cells in the series of granular leukocytes, with abundant azurophilic granules, lipophages, round or polygonal cells with cytoplasm filled with lipid inclusions, could be distinguished. An increase in the number of mature forms and, in particular, of lipophages, was characteristic of agglomerations of mononuclear cells in areas with lipid infiltration, evidence of the predominantly macrophagal direction of their differentiation. Lipophages were predominant in lipid infiltrations of a fibrous-lipid plaque. An essential component of the hematogenous cellular agglomerations of the intima was the undifferentiated lymphocyte-like cells in whose narrow cytoplasm there were many free ribosomes; mitochondria, and dispersed nuclear chromatin (Fig. 2a). Among the lymphocyte-like cells there were also some in whose cytoplasm elements of organoids characteristic of later and morphologically identifiable stages of monocytopoiesis could be seen: solitary cisterns of the endoplasmic reticulum and small vesicles (Fig. 2c). These cells, in particular, have been called monocytoid [6] and they are classed as intermediate forms from CFU-GM to the early stages of monocyte maturation. Incidentally, the lymphocyte-cell or the pale transitional cell intermediate between it and the blast cell [12], are regarded as a hematopoietic cell capable of colony formation, whereas other workers [5] associate ability to form colonies with cells of the monocyte type.

Morphological analysis of the intima of human atherosclerotic vessels suggested that

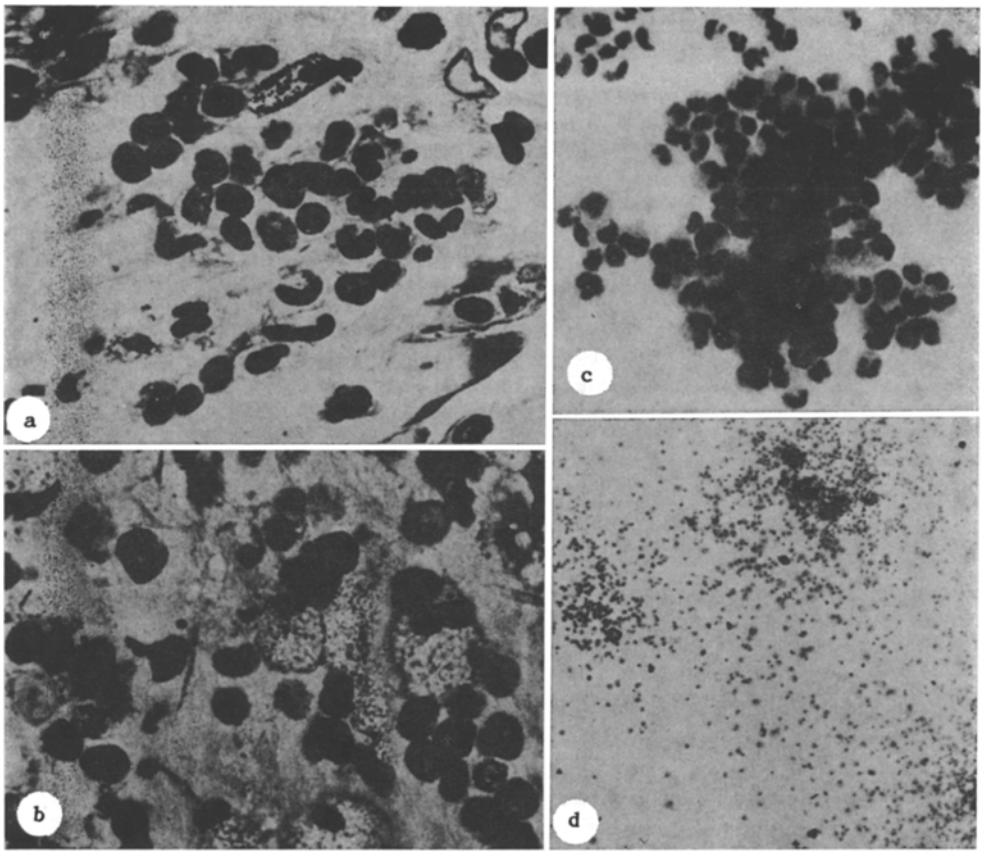

Fig. 1. Hematogenous cells of intima of atheromatous human aorta in vivo (a, b) and in vitro (c, d). a, b) Semithin sections stained with methylene blue. a) Agglomeration of cells with granulocytic-macrophagal direction of differentiation, in unaffected part of intima, from blast cells to mature forms. 400 ×; b) Zone of lipid streak on border with unaffected area. Blast cells with a large leptochromatic nucleus and narrow basophilic cytoplasm are concentrated around lipophages. 400 ×; c, d) Granulocytic-macrophagal colonies in agar cultures of intimal cells of human aorta on 14th day of culture. May–Gruenwald–Giemsa stain; c) general view. 60 ×; d) Nuclear polymorphism in cells of mixed granulocytic-macrophagal colony. 250 ×.

TABLE 1. Efficiency of Colony Formation in Suspensions of Intimal Cells and Blood Mononuclears from Atheromatous Human Aorta

Source of cells	CFU-GM/10^5 cells (M ± m)	Number of independent determinations (n)
Macroscopically unchanged areas of intima	1.3 ± 0.3	12
Areas of lipid infiltration (lipid spots, stains, streaks)	2.8 ± 1.6	9
Lipid-fibrous plaque	0.078 ± 0.068	12
Fibrous plaque	0	16
Blood from atheromatous vessels	0.022 ± 0.027	4

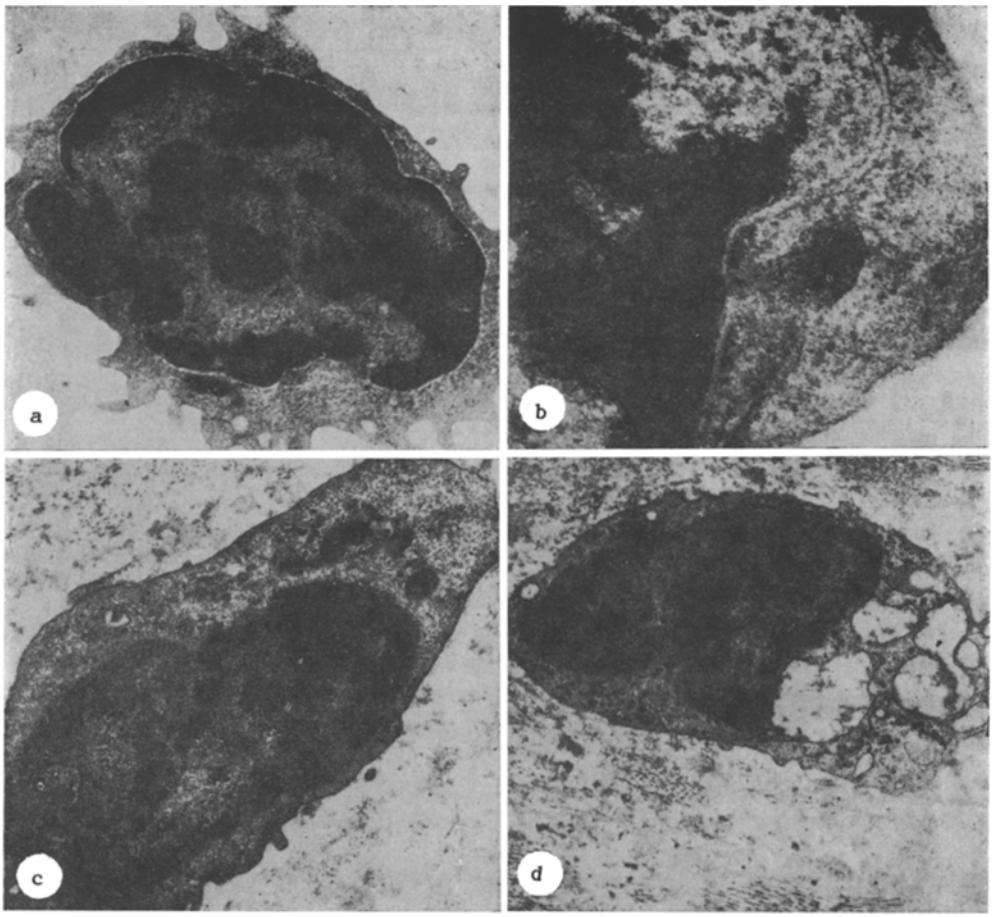

Fig. 2. TEM of hematopoietic cells differing in their degree of maturity, in intima of atheromatous human aorta. a) Lymphocyte-like cell with many free ribosomes and single mitochondria. Condensed chromatin in nucleus lies next to nuclear membrane. 8300 ×; b) Blast cell from agglomeration of mononuclears illustrated in Fig. 1b. Many ribosomes present in cytoplasm. 33,000 ×; c) Monocystoid cell from unaffected area. Occasional profiles of rough endoplasmic reticulum and many small vesicles — elements of later stages of differentiation — are present in the cytoplasm among the numerous ribosomes. 8300 ×; d) Mature monocytes with characteristically shaped nucleus, small vesicles, and large vacuoles, with electron-dense lysosomal granules and Golgi zone. 5000 ×.

leukocytes in the intima could develop from hematogenous precursor cells penetrating into it. To detect CFU-GM in the intimal cells we used clonal methods, which are usually used in hematology for culturing cells of the stem type, committed to and capable of forming colonies in culture. By the 14th day of culture of the intimal cells in the presence of PHA-LCS, cell clusters and colonies were found in the agar. On examination of the agar cultures in situ, granulocytic-macrophagal colonies were identified on the basis of morphological criteria, suggested in classical studies of CFU-GM in hematopoietic organs [1, 12]. The characteristic structure of the nuclei in preparations from agar cultures stained by the May—Gruenwald—Giemsa method was evidence that these cells belonged to the granulocytic-macrophagal series (Fig. 1c, d). High nonspecific esterase activity of the cells (diffuse red staining of the cytoplasm) in colonies extracted from the agar was an additional marker of monocyte-macrophages. During counting, agglomerations containing more than 40 cells, which corresponded to the above-mentioned morphological and histochemical criteria, were taken as a granulocytic-macrophagal colony. Groups of cells of this series, numbering under 40, were classed as clusters and were not taken specially into account. Data on the cloning efficiency of CFU-GM, calculated per 10^5 intimal cells transplanted into agar culture, are given in Table 1. The number of CFU-GM in a suspension of intimal cells from different parts of the aorta, indicated in Table 1, differed and exceeded their number in the population of nucleated blood cells from the same vessel. Differences in the number of CFU-GM in the intimal

regions depending on the degree of severity of the lesion suggest that proliferation and differentiation of CFU-GM in the subendothelium of the human aorta take place in early, mainly prefibrotic, stages of atherogenesis.

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CORRECTION OF METABOLIC DISTURBANCES IN EXPERIMENTAL CIRRHOSIS OF THE LIVER BY CRYOSURGICAL DESTRUCTION AND PLASMA FLOW RESECTION

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KEY WORDS: liver, cirrhosis; cryosurgical destruction; metabolic disturbances

In order to stimulate regeneration of the liver in cirrhosis, resection or cryosurgical destruction of part of the liver has been used successfully [5]. The possibility of using plasma resection has been demonstrated experimentally in liver surgery [4, 6, 7]. However, when large vascular trunks have to be divided, unless the liver is completely excluded from the circulation, bleeding is unavoidable. In addition, the incandescent gas flow of the plasma jet can cause embolism.

In the investigation described below, in order to eliminate complications during the operations we studied the possibility of liver resection in experimental animals by plasma flow after preliminary freezing of the resection line.

EXPERIMENTAL METHOD

Experiments were carried out on 36 Chinchilla rabbits weighing 3-3.5 kg. In six series of experiments the effects of cryosurgical destruction, plasma resection, and a combination of the two methods, used on the intact and cirrhotically changed liver were compared. Experimental cirrhosis of the liver was produced in rabbits by subcutaneous injection of 40% CCl_4 solution by the method in [8]. All operations on the liver were performed under

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