DISCOVERY OF TWO CELL SUBPOPULATIONS, INCORPORATING LOW-DENSITY LIPOPROTEINS DIFFERENTLY, IN A HUMAN ATHEROSCLEROTIC AORTIC PLAQUE

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KEY WORDS: atherosclerosis; human aortic cells; low-density lipoproteins; continuous flow cytofluorometry.

In the region of an atherosclerotic lesion may be seen what are called foam cells, whose cytoplasm is filled with inclusions containing cholesterol esters [3, 6, 13]. In the opinion of some workers the source of cholesterol and its esters which accumulate in these cells may be low-density lipoproteins (LDLP) [2, 13].

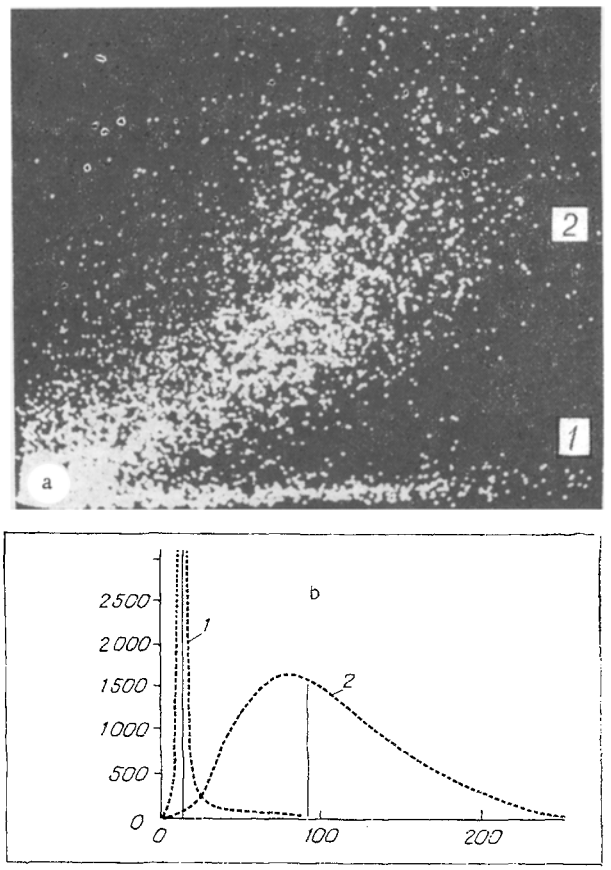

Fig. 1. Distribution of human aortic intimal cells. a) Abscissa, intensity of scattering of light; ordinate, intensity of fluorescence; b) abscissa, intensity of fluorescence (channel No.); ordinate, relative number of cells in channel. 1) After incubation of cells with 50 μg/ml of R-LDLP; 2) without addition of R-LDLP (autofluorescence).

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TABLE 1. Incorporation of R-LDLP into A and N Cells of Human Aortic Intima

Experiment No.	$\frac{\text{A cells}}{\text{N cells}} \cdot 10^*$
1	165
2	199
3	104
4	132
5	180
6	135
7	131
8	156
Mean . . .	150†

*Incorporation of R-LDLP estimated from intensity of fluorescence of cells; mean values of intensity of fluorescence obtained after subtraction of mean values of autofluorescence of cells.
†$P < 0.01$ (significance of differences determined by nonparametric sign test).

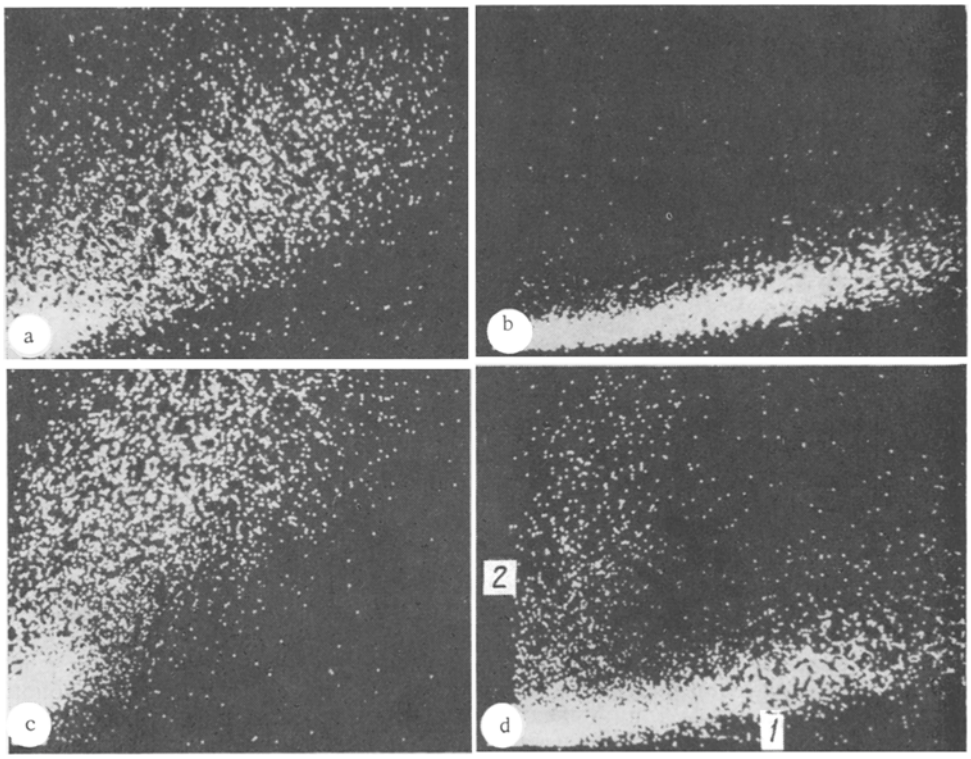

Fig. 2. Cytofluorograms of human aortic intimal cells. Abscissa, intensity of scattering of light; ordinate, intensity of fluorescence (channel No.). a) After incubation of N cells with 50 μg/ml of R-LDLP; b) the same with 50 μg/ml R-LDLP + 1 mg/ml LDLP; c) after incubation of A cells with 50 μg/ml R-LDLP; d) the same, with 50 μg/ml R-LDLP + 1 mg/ml LDLP.

Experiments on cultures of different types of cells have shown that there are at least two ways whereby the cell can utilize LDLP: through specific receptors for LDLP and through nonspecific uptake [2, 4]. The question of how cells of the blood vessel wall in the region of an atherosclerotic lesion utilize LDLP and what causes excessive accumulation of lipids in these cells remains unanswered.

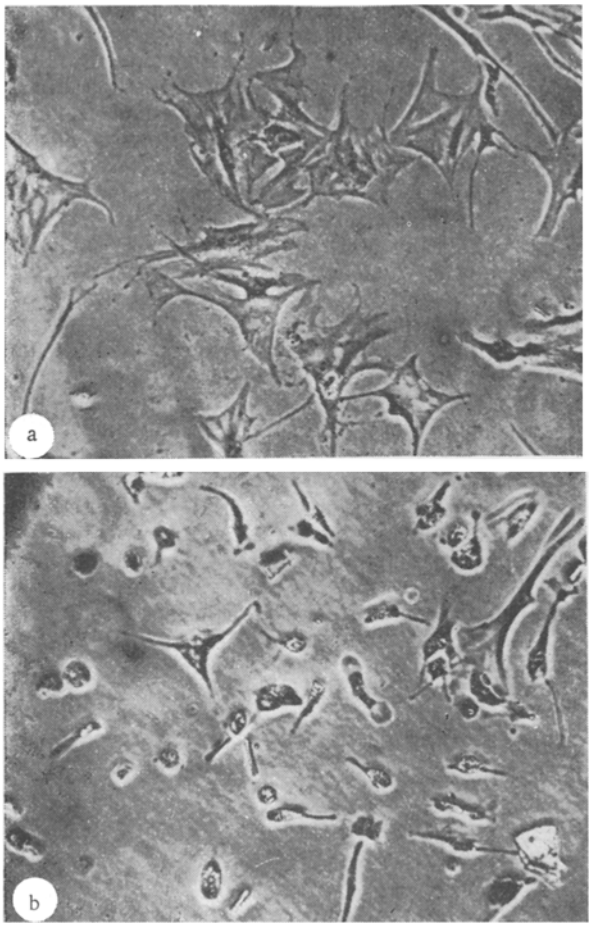

Fig. 3. General appearance of cells of subpopulation 1 (a) and subpopulation 2 (b) of A cells. Phase contrast, 140 ×.

In the present investigation the method of continuous-flow cytophotometry was used to compare the ability of cells from an atherosclerotic plaque and cells from the intima of a region of the human aorta unaffected by atherosclerosis to incorporate LDLP in primary culture. An approach whereby cell subpopulations with different ways of assimilating LDLP could be distinguished among the cell population from an atherosclerotic plaque also is described.

EXPERIMENTAL METHOD

Aortas were taken under aseptic conditions from men aged 40-60 years 1.5-3 h after confirmation of death. Cells were isolated from atherosclerotic plaques (A cells) and from regions of unaffected intima (N cells) of the human aorta by the method described previously [1]. The cells were seeded in Petri dishes (60 mm, from Falcon Plastics, USA) at the rate of 4×10^5 cells per dish in medium 199 containing 10% embryonic calf serum, 2 mM glutamine, 2.5 µg/ml fungizone, and 100 µg/ml kanamycin (all reagents were from Gibco, USA). The primary cell cultures were kept in a CO_2 incubator in an atmosphere consisting of 5% CO_2 and 95% air at 37°C; the medium was changed every 3 days. To detect lipid inclusions the cells were stained with Oil red by Lillie's method [7]. LDLP (density from 1.019 to 1.063 g/cm^3) and delipidized human serum (DLHS) with a density of 1.215 g/cm^3 were obtained by consecutive ultracentrifugation [8]. The LDLP concentration was estimated from the protein content [10]. Fluorescence of LDLP labeled with rhodamine isothiocyanate (R-LDLP) was obtained by the method described in [11].

Experiments were carried out on 7-day primary cultures. Three dishes with N and three with A cells were used in each experiment. On the 6th day 2 ml of medium 199 containing 10% DLHS was added to each dish. The medium was changed for fresh on the 7th day and 50 µg/ml of R-LDLP was added to the first dish, 50 µg/ml R-LDLP and 1 mg/ml LDLP were added to the second dish, and no lipoproteins were added to the third dish. The cells were incubated for 5 h at

37°C in a CO_2 incubator, after which they were washed to remove unbound LDLP by the standard method [5]. The cells were removed from the substrate by trypsinization and the cell suspensions thus obtained were kept at 4°C until the beginning of analysis. The content of R-LDLP in the cells was estimated from their fluorescence in the SACS II fluorescent-activated cell sorter (Becton and Dickinson, USA); the wavelength of exciting light of the argon laser was 514 nm. The intensity of fluorescence of the cells was recorded in the region of the spectrum with a wavelenght of over 580 nm. The method of analysis of cell fluorescence with the SACS II instrument was described previously [10].

EXPERIMENTAL RESULTS

By flow cytophotometry it is possible to analyze the level of fluorescence of single cells [10]. If R-LDLP are used the method enables incorporation of LDLP into the cell to be estimated. As a result of incubation of human aortic intimal cells for 5 h with R-LDLP at 37°C the intensity of fluorescence of most cells greatly exceeded the intensity of autofluorescence of the cells (Fig. 1). As a result of incubation of the cells with R-LDLP at 4°C, when binding of LDLP with the cell surface is all that happens without any further incorporation within the cell, the intensity of fluorescence of the cells was unchanged compared with their autofluorescence. This is evidence that this method records fluorescence of R-LDLP located intracellularly.

According to the results of light microscopy the A cell population differed from the N cell population by possessing a large number of cells with lipid inclusions in their cytoplasm. Among cells with lipid inclusions polymorphism was observed for the number of lipid inclusions: from cells containing single small lipid inclusions in their cytoplasm to typical foam cells, whose cytoplasm was overloaded with large and small inclusions. According to the results of light microscopy incubation of both A and N cells with R-LDLP for 5 h did not affect the morphology and viability of the cells estimated by the incorporation of trypan blue (about 90%).

Comparison of the A and N cell populations by ability to incorporate R-LDLP showed that the content of R-LDLP in the A cells was on average 1.5 times higher per cell than that of N cells (Table 1). The relatively high R-LDLP content in the A cells compared with the N cells may be due to two causes: the higher endocytic activity and (or) the lower efficiency of lysosomal hydrolysis of R-LDLP. Typical cytofluorograms of cells obtained by analysis of H and N cell populations are shown in Fig. 2. As Fig. 2: A, B shows the intensity of fluorescence of all N cells fell sharply when a 20-fold excess of unlabeled LDLP was added to the incubation medium.

A different picture was observed in the population of A cells (Fig. 2: C, D). Addition of a 20-fold excess of unlabeled LDLP to the incubation medium led to a decrease in the intensity of fluorescence in only part of the A cell population (subpopulation 1), whereas the intensity of fluorescence of the rest of the cells was virtually unchanged (subpopulation 2). It will be evident that the population of A cells, unlike the population of N cells, consists of two cell subpopulations that differ in their ability to incorporate LDLP. By means of a cell sorter, these subpopulations of cells were separated on the continuous-flow cytofluorometer and were again seeded in culture. After 14 days the cells of both subpopulations were adherent and spread out in a layer. In Fig. 3, which shows the external appearance of these cells, it will be clear that subpopulation 2 consists of cells whose cytoplasm is overloaded with many lipid inclusions, whereas cells of subpopulation 1 are mainly without lipid inclusions.

It can be concluded from the data described above that cells isolated from an atherosclerotic plaque (A cells) differ from cells isolated from an area of the human aorta unaffected by atherosclerosis (N cells) in their ability to incorporate LDLP. The more marked ability to incorporate LDLP may lead to a further increase in the lipid pool in the A cells [12]. Cells overloaded with lipid inclusions were shown to incorporate R-LDLP without going through specific LDLP receptors, i.e., by a nonspecific route, for an excess of unlabeled LDLP does not inhibit uptake of R-LDLP by these cells. Conversely, uptake of R-LDLP by N cells and by cells of subpopulation 1 evidently takes place through receptors specific for LDLP.

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CIRCADIAN RHYTHM OF LIVER PHOSPHOLIPIDS IN NORMAL HAMSTERS AND HAMSTERS WITH OPISTHORCHIASIS

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Opisthorchiasis modifies circadian changes in activity of the enzymes responsible for plastic functions of the liver [5] and cell renewal in the digestive system [4, 9] in the acute phase of the disease. Changes may accordingly be expected in the circadian rhythm of phospholipids (PL), which play a major role in the activity of membrane-bound enzymes [6].

The object of this investigation was a biohistochemical analysis of PL in the liver of golden hamsters at different times of the 24-h period in the acute and chronic phases of opisthorchiases, and also after dehelminthization.

EXPERIMENTAL METHOD

Experiments were carried out on 204 sexually mature male golden hamsters, divided into five groups. Groups 1 and 2 consisted of intact animals aged 3 and 8 months respectively; groups 3 and 4 were animals infected 30 and 150 days respectively after infestation, treated with chloxyle (0.4 g/kg, 2-day course) on the 45th day after infection; they were investigated

TABLE 1. Phospholipid Concentration (in μg/g inorganic phoshporus) in Liver of Normal Golden Hamsters (aged 3 months, series I) and Hamsters in the Acute Phase of Opisthorchiasis (30th day of infection, series II) during the 24-h Period (M ± m)

PL	Series of experiments	Clock time				M
		9 a.m. ($n=20$)	3 p.m. ($n=20$)	9 p.m. ($n=20$)	3 a.m. ($n=22$)	
Total PL	I	1030±35,0	1210±45,0	1190±15	1130±30	
	II	820±25,0	640±25,0	880±70	840±40	
PA	I	99,0±4,1	89,8±4,4	101,6±5,6	84,0±4,3	93,2±4,0
	II	52,4±4,5	41,9±3,2	45,9±2,7	70,6±5,0	54,0±2,8
CL	I	78,7±3,6	104,0±5,8	107,8±5,3	88,3±5,0	94,4±5,6
	II	50,8±4,2	40,9±3,1	44,7±2,6	68,8±5,8	52,5±2,9
PEA	I	141,9±7,7	166,4±7,9	135,3±10,9	156,7±6,8	148,6±6,2
	II	164,6±8,8	132,3±11,4	199,1±20,4	120,2±10,2	151,7±9,5
PCh	I	338,5±13,5	430,4±15,7	413,1±16,0	372,3±13,2	387,8±24,3
	II	183,0±8,9	144,1±16,2	203,5±27,5	258,5±34,1	201,4±8,6
PS	I	136,9±5,1	133,4±12,9	151,1±8,9	157,5±11,3	145,5±3,1
	II	157,3±13,5	122,5±14,8	167,3±20,7	101,2±15,8	134,6±6,1
SM	I	103,5±2,2	151,6±10,6	143,8±13,4	139,4±10,6	134,8±5,7
	II	102,6±7,1	123,6±6,4	96,3±9,3	81,5±4,1	90,4±3,0
LL	I	116,6±3,2	129,4±9,0	140,0±10,8	140,5±9,3	132,1±2,3
	II	120,3±13,0	92,0±13,0	142,3±19,9	133,5±14,5	122,8±7,9

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