CHEMOTACTIC FUNCTION OF SKIN FIBROBLASTS IN PATIENTS WITH AMYLOIDOSIS

B. Ch. Karryeva, L. V. Kozlovskaya, B. V. Mchedlishvili, and V. N. Lyashko

UDC 616-003.821-07:616.5-076

KEY WORDS: amyloidosis; chronic glomerulonephritis; fibroblasts; chemotaxis

Weakening of activity of cellular immunity [2] and qualitative disturbances of the phagocytic function of leucocytes [3] and of platelet functions [1] have now been identified in amyloidosis. The inflammatory reaction of patisnts with amyloidosis, as has been demonstrated by studies, including our own, of acute inflammation by the skin window method [4], is distinguished by unique features, but it can evidently lead to the same disturbances in the kidney as immune inflammation in chronic glomerulonephritis (CGN). It is therefore interesting to compare cellular protective reactions of the body, participating in inflammation, and arising during CGN and amyloidosis.

In the modern view, along with other cells, tissue fibroblasts play an active role in inflammatory and repair processes. There is evidence that fibroblasts may be involved in the synthesis of the amyloid fibril [7], and also, probably, in the resorption of amyloid, which explains the importance of an evaluation of the role of fibroblasts in the general system of intercellular interrelations in this pathology.

The aim of this investigation was to study the functional state of skin fibroblasts in culture, taken from different groups of individuals: patients with amyloidosis, patients with CGN, who develop amyloidosis extremely rarely, and a control group. The functional properties of the cells were assessed on the basis of their ability to migrate (chemotaxis, CT). By studying functions of fibroblasts in a series of cell generations in vitro under standard conditions it is possible, first, to largely exclude the effect of the amyloid fibril on the CT function, and second, if native sera are used as chemoattractants, to approximate their function as closely as possible to the natural situation.

EXPERIMENTAL METHOD

Altogether five patients with amyloidosis (four men and one woman aged 23-28 years), two men with CGN (aged 16 and 32 years), and two healthy individuals (a boy aged 4 and a woman aged 29 years) were studied. Fibroblasts of strain No. 795 were obtained from the skin of a 9-week fetus obtained at medical abortion.

The diagnoses of CGN and amyloidosis were verified morphologically by renal biopsy.

Among the patients with amyloidosis, the disease was primary in one and secondary in four cases; the causes of secondary amyloidosis were rheumatoid arthritis (two patients), chronic osteomyelitis (one patient), and actinomycosis of the skin (one patient). Four patients had a nephrotic syndrome and one patient the proteinuric stage of amyloid nephropathy.

Both patients with CGN had a nephrotic syndrome, and pathological examination revealed membranous glomerulonephritis with a tubulointerstitial component in one case and mesangioproliferative glomerulonephritis, turning into glomerulosclerosis, in the other.

Department of Internal Medicine and Occupational Diseases, and Problem Laboratory for Nephrology, Central Research Department, I. M. Sechenov First Moscow Medical Institute. Institute of Medical Genetics, Academy of Medical Sciences of the USSR. Institute of Crystallography, Academy of Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. P. Bochkov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 109, No. 6, pp. 578-580, June, 1990. Original article submitted May 10, 1989.

 0007-4888/90/0006-0774$12.50

TABLE 1. Effect of Seeding Density of Cells on Membrane on Value of CTI

Strain	Concentration of fibroblasts in suspension (seeding density × 10^3)	Number of cells migrating into filter in chamber with			CTI	
		normal serum	amyloid serum	hanks's solution	on normal serum	on amyloid serum
156	9,3	39	28	17	2,29	1,64
	8,5	33	26	15	2,20	1,73
	3,1	19	15	9	2,11	1,67
161	9,2	44	33	21	2,09	1,57
	7,1	38	27	18	2,11	1,50
	4,8	24	19	12	2,0	1,58
795	8,2	52	32	17	3,05	1,88
	5,2	41	27	14	2,92	1,92
	3,8	32	23	11	2,91	2,09
1029	6,9	52	34	18	2,89	1,88
	2,5	38	23	12	3,16	1,91

A culture of fibroblasts was obtained from a biopsy specimen of skin from the anterior surface of the forearm. Serial subculture of the cells was carried out in Carrel flasks on Eagle's medium with the addition of 5% human umbilical serum and 5% bovine serum.

CT of the fibroblasts was studied in modified Boyden's chambers consisting of two divisions. A solution of chemoattractant (normal human serum, amyloid serum, or Hanks's solution — a zero chemoattractant) was poured into the bottom division, and a cell suspension in Eagle's medium with the addition of 0.02% bovine serum albumin was poured into the top division. The chambers were separated by a porous membrane nuclear filter, with pore diameter of 8 μ (made by the Nuclear Research Laboratory, Dubna), which were covered beforehand with gelatin. Preparation of the cell suspension and subsequent processing of the membranes were carried out by the method described previously [6]. After the cell suspensions had been seeded in the top division of the Boyden's chamber, they were incubated for 24 h in an atmosphere containing 5% CO_2 at 37°C, after which the membranes were taken out, and the top layer of cells which had not migrated into the upper layer of the membrane was removed, fixed in ethanol, and stained with hematoxylin. The total number of fibroblasts penetrating into the membrane was counted and the chemotactic index (CTI) calculated as the ratio of the number of cells migrating into the membrane along the autologous serum gradient to the number of cells migrating into the membrane in the presence of the zero chemoattractant (as a result of cytokinesis).

The numerical data was subjected to statistical analysis by Wilcoxon—Mann—Whitney nonparametric tests.

EXPERIMENTAL RESULTS

To rule out any effect of seeding density of the fibroblasts on the value of CTI, a series of experiments was carried out to estimate the value of CTI in several suspensions containing different concentrations of fibroblasts. The results obtained on four sample serains of fibroblasts (normal blood donors, patients with CGN and with amyloidosis) showed that the value of CTI was virtually independent of the seeding density of the cells on the membrane (Table 1). During the subsequent course of the work it was therefore unnecessary to ensure a strictly equal concentration of fibroblasts in the cell suspension.

Investigation of CTI with standard serum showed that this parameter in fibroblasts obtained from patients with amyloidosis was significantly lower than in the control group: not only in normal blood donors and the fetus, but also in patients with CGN (Fig. 1). CT was studied after different periods of culture of the cells, and the migration index of the fibroblasts from patients with amyloidosis remained low even after several subcultures (at least eight). Migrating activity of the fibroblasts depended neither on the clinical form of amyloidosis nor on the stage of the disease: CTI of fibroblasts from patients in the nephrotic stage averaged 2.08, and from the patient with the proteinuric stage 2.24.

When amyloid serum was used as the chemoattractant, CTI of all strains of fibroblasts tested was lower than CTI when normal serum was used as the chemoattractant.

The causes of the decrease in CT of the fibroblasts from patients with amyloidosis which we found in response to standard normal serum are not clear, but a number of suggestions may be put forward. Fibroblasts of patients with amyloidosis may be more sensitive to different kinds of inhibitory factors present in the serum (protein degradation products and other natural metabolites). However, the second part of our experiments in which amyloid serum was used largely contradicted this hypothesis. We showed that amyloid serum possesses a marked inhibitory effect on CT in relation to fibroblasts of all the strains studied; this property of amyloid serum, moreover, was relatively weaker against fibroblasts from patients with amyloidosis.

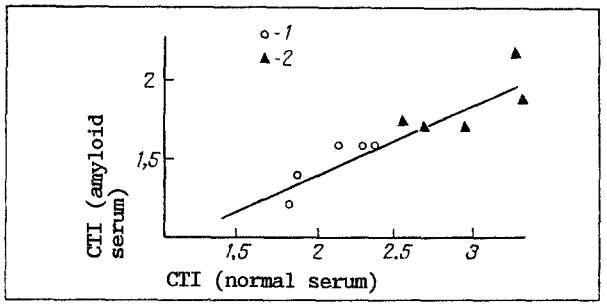

Fig. 1. Comparison of CTI using normal serum and CTI using amyloid serum. Differences between fibroblasts of patients with amyloidosis and fibroblasts of control group statistically significant ($p < 0.05$).

Meanwhile, other tentative conclusions can be drawn from the fact that CT of the fibroblasts was inhibited by amyloid serum. The possibility cannot be ruled out that the preceding (in vivo) action of the amyloid fibril and of humoral precursors of amyloid on the cells may lead to long-term modifications of the function under investigation, and these may persist also in culture. Meanwhile lowering of CT of the fibroblasts of patients with amyloidosis was observed in relation not only to the amyloid, but also to the control serum. This decrease, moreover, was reproduced consistently in a series of cellular generations and was similar in type in fibroblasts from patients with different clinical forms of amyloidosis and at different stages of amyloid naphropathy. The evidence thus strongly supports the view that there is an initial inherited variant (or variants) of this cellular function which, under certain conditions, leads to the development of pathology.

The essence of the disturbance of CT of the fibroblasts of patients with amyloidosis remains unclear, but if the results of our previous studies of the phagocytic function of leucocytes in amyloidosis, demonstrating a disturbance mainly of the first phase of phagocytosis, namely adhesion [3], and also lengthening of the latent period of collagen aggregation and aggregation in response to stimulation by arachidonic acid [1, 5], are taken into account, it is possible to suggest by analogy that the disturbance of CT of the fibroblasts is also based on a change in the cell membranes and/or cytoskeleton.

Depression of the chemotactic function of fibroblasts was thus discovered in patients with amyloidosis, and it persisted in a series of successive cell generations in culture and was observed in patients with both initial and fully developed stages of the disease. The results are interesting from the standpoint of development of approaches to the study of the pathogenesis of amyloidosis as an inherited pathology, realized at the cellular level, and in particular, in the form of disturbances of chemotactic function.

LITERATURE CITED

1. N. L. Kozlovskaya, Author's Abstract of Candidate's Dissertation, Medical Sciences, Moscow (1985).
2. O. P. Kuznetsova, V. V. Sura, N. A. Mukhin, et al., Sov. Med., No. 8, 99 (1975).
3. N. A. Mukhin, L. V. Kozlovskaya, and O. V. Roganova, Ter. Arkh., No. 8, 116 (1980).
4. O. V. Roganova, L. V. Kozlovskaya, N. A. Mukhin, et al., Ter. Arkh., No. 7, 37 (1984).
5. B. M. Charyev, M. L. Kozlovskaya, L. V. Kozlovskaya, et al., Ter. Arkh., No. 7, 85 (1988).
6. J. H. Greenberg, S. Seppa, and A. T. Hewitt, Develop. Biol., **87**, 259 (1981).
7. K. Hashimoto and M. H. Brownstein, Am. J. Path., **68**, 371 (1972).