

# Changes in the expression of cell surface sialoglycoproteins during transition of human monocytes into macrophages

Jyrki Heino, Hannu Larjava<sup>+</sup> and Risto Penttinen

*Departments of Medical Chemistry and <sup>+</sup>Periodontology, University of Turku, Kiinamylynkatu 10, SF-20520 Turku, Finland*

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Cell surface sialoglycoproteins of human mononuclear phagocytes in different maturation stages were labelled by the periodate/borohydride method and separated by SDS-polyacrylamide gel electrophoresis. The main surface glycoproteins of peripheral blood monocytes had molecular masses of 115 and 95 kDa. During in vitro transition into adherent macrophages, the monocyte-characteristic surface glycoproteins disappeared. Most of the changes in the surface glycoprotein pattern occurred during the first 24 h and after 96 h the changes were completed. The major sialoglycoproteins of the macrophage cell surface had molecular masses of 130 and 55 kDa. The macrophage cell surface showed further changes when cultured in the presence of synovial fluid (10%). These results may reflect the in vivo maturation of monocytes into tissue macrophages. In synovium, tissue-derived factors may also take part in differentiation.

*Glycoprotein    Plasma membrane    Monocyte    Macrophage    (Synovium)*

## 1. INTRODUCTION

Cell surface proteins are important in cell recognition, cell adherence and spreading and regulation of cellular metabolism [1]. Selective radiolabelling by periodate and tritiated borohydride is a widely used method for studying cell surface sialoglycoproteins [2]. This method has been used to characterize several normal cell lines [3] and transformed cells [4].

It is well documented that peripheral blood monocytes are able to change into macrophage-like cells in vitro. During this transition they elongate [5], remodel their cytoskeleton [6] and change their surface Fc and C3 receptor function [7]. We found previously that the electrophoretic patterns of sialic acid-containing surface glycoproteins of blood monocytes and synovial membrane macrophage-like cells were different [8]. Furthermore, on the basis of their surface glycoproteins synovial macrophages resembled fibroblasts more than monocytes [8]. Obviously, the surface glycoproteins of mononuclear phagocytes must

change during in vivo maturation of blood monocytes into tissue macrophages. Here, we studied changes in the expression of sialic acid-containing surface glycoproteins during in vitro transition of human peripheral blood monocytes into macrophages.

## 2. MATERIALS AND METHODS

Monocytes were separated from heparinized blood samples first by Ficoll-Paque (Pharmacia, Uppsala) centrifugation at  $800 \times g$  for 30 min [9]. The cells at the interface were washed and enriched further by centrifugation against a 60% Percoll cushion at  $800 \times g$  for 30 min [10]. After the second centrifugation the cells were suspended in Dulbecco's modification of Eagle's minimum essential medium (DMEM) supplemented with 10% fetal bovine serum (FBS). After a 2 h or an overnight incubation in petri dishes the nonadherent cells were washed off. Synovial fibroblasts (from synovial explants taken in or-

thopaedic operations) were cultured in DMEM with 10% FBS [11].

Synovial fluid was from a rheumatoid patient with hydrops. It was centrifuged and the supernatant was pressed through a Millipore MF filter (pore size  $0.45\ \mu\text{m}$ ) and stored at  $-70^\circ\text{C}$  until use. In the lipopolysaccharide (LPS) stimulation assays, the cells were cultured for 72 h in the presence of  $20\ \mu\text{g/ml}$  LPS (LPS W *E. coli* 055:B5, Difco, Detroit, MI).

In the indirect immunofluorescence assays, HLA-DR antigens were detected with  $\text{I}_2$  monoclonal antibodies (Coulter Immunology, Hialeah, FL) and mononuclear phagocytes with OKM-1 antibodies (Ortho Diagnostic System, Raritan, NJ). FITC-conjugated anti-mouse IgG (Miles Laboratories, Elkhart, IN) was used as a second antibody.

The cells were surface-labelled with the periodate/borohydride method of Gahmberg and Andersson [2]. In electrophoresis, SDS-polyacrylamide gels with a gradient from 5 to 22% were used (SDS-PAGE) [12]. After the run the gels were processed for fluorography [13] and exposed against Kodak X-Omat film.

### 3. RESULTS AND DISCUSSION

After 24 h in culture the cells were round-shaped, morphologically typical monocytes (figs 1a,2a), 95% containing OKM-1 and 75% HLA-DR antigens (not shown). These plastic-adherent cells are known to differ from other blood monocytes because of their HLA-DR antigens and larger cell size [14]. Between 24 and 96 h several cells became elongated (after 96 h about 20–44%)

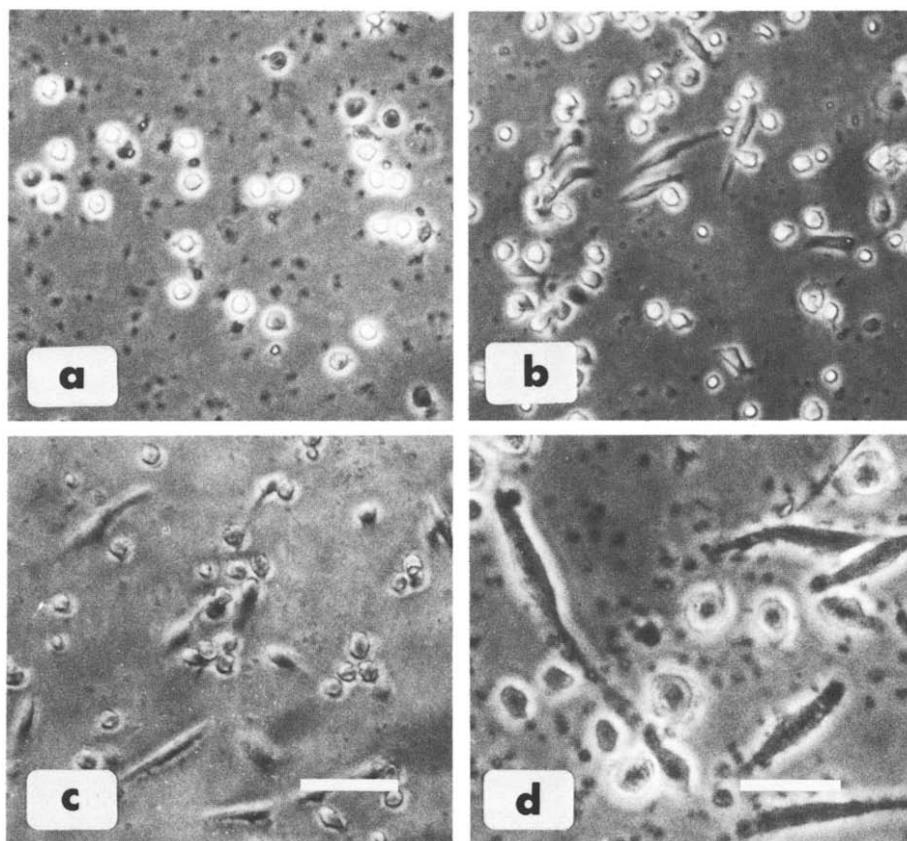


Fig.1. Transition of monocytes into macrophages as seen using a phase-contrast microscope. (a) 24 h, (b) 48 h, (c,d) 96 h. Bar in a–c is  $60\ \mu\text{m}$  and in d  $30\ \mu\text{m}$ .



Fig.2. (a) Monocytes after 24 h in culture. (b) Cells after 96 h in culture. May-Grünwald-Giemsa staining. Bar = 10  $\mu$ m.

and the round-shaped cells spread and flattened (figs 1b–d,2b).

The main sialoglycoproteins of adherent monocytes (after 1 h in culture) were 115 and 90 kDa proteins (figs 3,4), as described for total blood monocyte populations by Gahmberg and Andersson [3]. They changed dramatically in 24 h. After 96 h the cell surface seemed to be unchanged, where all monocyte-derived proteins disappeared while the prominent 130 and 55 kDa protein bands had appeared (figs 3,4). These

changes were relatively rapid, as the estimated half-life of macrophage membrane proteins is more than 80 h [15].

The macrophage 55 kDa protein was trypsin-sensitive like a fibroblast surface sialoglycoprotein of the same molecular mass [16]. It may have a function in cell spreading, while the attachment of macrophages is known to be mediated by a protease-resistant 46 kDa protein [17].

The maturation of monocytes into macrophages in vivo probably requires tissue-related factors. In

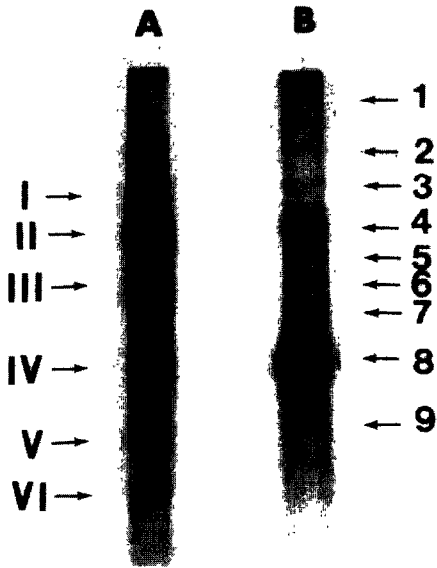


Fig.3. The main surface sialoglycoproteins separated with SDS-PAGE. (A) Monocytes (1 h in culture). (B) Macrophage-like cells (96 h in culture). Approximated molecular masses (shown in kDa, means and ranges are from 3–5 determinations):

Monocytes			Macrophage-like cells		
Number of protein band	Molecular mass		Number of protein band	Molecular mass	
	Mean	Range		Mean	Range
I	165	160–170	1	340	280–400
II	115	110–120	2	240	200–300
III	90	85– 94	3	185	170–200
IV	58	56– 60	4	130	120–135
V	39	38– 40	5	110	105–120
VI	30	28– 32	6	90	85–100
			7	75	68– 84
			8	55	54– 60
			9	40	37– 46

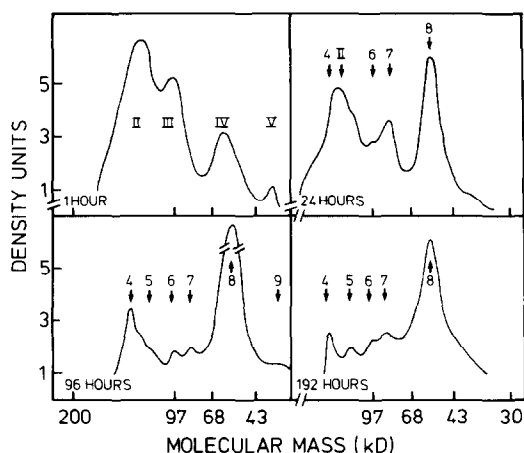


Fig.4. Cell surface sialoglycoproteins during in vitro transition of monocytes into macrophages. Densitograms of protein bands separated with SDS-PAGE.

monocytes cultured in the presence of 10% synovial fluid remarkable changes in the sialoglycoprotein pattern were detected (fig.5). The main changes were relative decreases in the amounts of 130 and 55 kDa proteins. In addition, two other proteins containing minor amounts of sialic acid (190 and 75 kDa) disappeared and two new proteins (140 and 80 kDa) appeared.

The synovial fluid-induced macrophages resembled fibroblasts in their surface glycoprotein pattern (main fibroblast sialoglycoproteins: 140, 80 and 55 kDa, not shown), but were not identical. A predominating fibroblast glycoprotein (a 140 kDa protein), was not reducible by mercaptoethanol, in contrast to the macrophage protein of the same molecular mass. The 140 kDa sialoglycoprotein of fibroblasts is suggested to be the cell surface receptor for fibronectin [18]. Thus, although the mononuclear phagocytes can synthesize fibronectin after in vitro maturation into macrophages [19], they probably did not express a fibroblast-like fibronectin receptor.

The synovial fluid-induced changes can partially explain the glycoprotein pattern found in synovial macrophages [8]. Several synovium-related factors may affect the membrane proteins of mononuclear phagocytes. Proteolysis of surface proteins is possible, supported by the decrease in the relative amount of the two trypsin-sensitive proteins of the

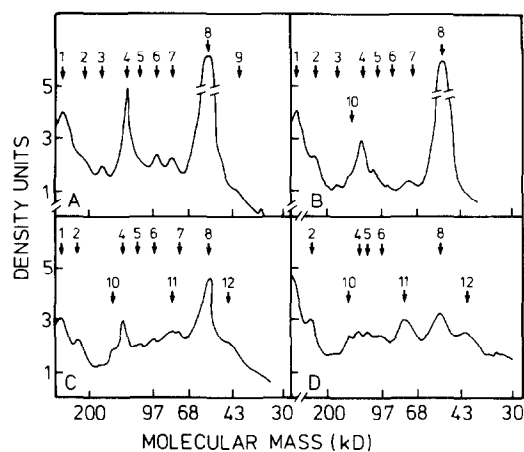


Fig.5. Changes in the surface sialoglycoprotein pattern induced by synovial fluid. (A) Cells cultured 96 h with medium containing 10% fetal bovine serum (FBS). (B) Cells cultured first 72 h with FBS and then 24 h with medium containing 10% synovial fluid (SF). (C) Cells cultured 48 h with FBS and then 48 h with SF. (D) Cells cultured 24 h with FBS and then 72 h with SF. Densitograms of protein bands separated by SDS-PAGE.

macrophage surface (the 130 and 55 kDa proteins were trypsin-sensitive; not shown). However, culture medium containing synovial fluid did not alter surface proteins of synovial fibroblasts (not shown). Factors stimulating or activating macrophages can also change their surface proteins [20]. However, in this study LPS stimulation had no effect on mononuclear phagocyte-related proteins (not shown). The factors regulating the maturation of monocytes into synovial macrophages are poorly understood, although they may play an important role in pathogenesis of inflammatory diseases like rheumatoid arthritis.

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