Glycosaminoglycan profile in macrophages exposed to *Candida albicans* and interleukins

Maria Bodo,* Elisabetta Blasi, Ennio Becchetti, Monica Giammarioli, Carmela Conte,*
Silvia Bellocchio, Tiziano Baroni, Catia Bellucci, and Francesco Bistoni

*Dipartimento di Morfologia ed Embriologia, Università di Ferrara; and Dipartimento di Medicina Sperimentale e
Scienze Biochimiche, Università di Perugia, Italy*

Abstract: Glycosaminoglycans (GAG), are extra-cellular matrix macromolecules that affect the phagocytic properties of macrophages. In order to assess whether the interaction between macro-phages and *Candida albicans* (iCa) provokes changes in the phenotype, we analyzed the GAG profiles in two macrophage lines, ANA-1 (from murine bone-marrow) and BV-2 (from murine brain). We also investigated GAG modulation by interleukin-1α (IL-1α) and interleukin-6 (IL-6). During iCa treat-ment and even after the addition of ILs, ANA-1 accumulated less total GAG compared to controls. IL-1 treatment, combined with iCa exposure, in-duced a decrease in heparan sulfate and chondroi-tin sulfate chains, and an increase in the hyaluronic acid percentage. IL-6 treatment, with or without iCa, decreased the hyaluronic acid/sulfated GAG ratio. The GAG pattern in BV-2 appears to be different to ANA-1 and iCa exposure does not induce any difference in total GAG. The inhibitory effect induced by ILs on GAG synthesis is less than that observed in ANA-1 and the GAG elution profile is modulated to a lesser extent by treatment with ILs and/or iCa compared to the ANA-1. We suggest that the observed changes in the expression of the individual GAG classes may be responsible for the macrophage functional heterogeneity. *J. Leukoc. Biol.* **64: 650–656; 1998.**

INTRODUCTION

Macrophages belong to the mononuclear phagocyte system involved in many host defense functions such as the uptake and elimination of parasites, the processing and presentation of antigens to the immune system, and the destruction of trans-formed cells. Although deriving from a relatively homogenous population of precursor cells, their functional heterogeneity is determined on the basis of their capacity to secrete growth factors, depending on their developmental stage, and in particu-lar due to a specific stimulus dictated by the microenvironment to which the macrophages are exposed. These stimuli induce transmembrane and intracellular signaling events leading to diversity in gene expression and in macrophage phenotype [1, 2].

During inflammatory processes, the monocytes leave the circulation and migrate to the extravascular area where many cellular types (fibroblasts, neutrophils, lymphocytes, etc.) pro-duce extracellular matrix (ECM) proteins. ECM is principally composed of proteoglycans (PG), which include sulfated and non-sulfated GAG such as hyaluronic acid (HA). Sulfated GAG such as chondroitin sulfate (CS), dermatan sulfate (DS), and heparan sulfate (HS) are in turn covalently attached to a core protein. The ECM not only serves a mechanical role in supporting and maintaining tissue structures, but also modu-lates a multitude of cell functions such as development and proliferation [3], thus playing a critical role in the migration of macrophages to the target tissues or inflammatory foci. In fact, ECM components have been shown to interact with the cell surface molecules of leukocytes thus facilitating cell migration or proliferation [4–7]. Laminin, an ECM molecule present at sites of immune and inflammatory responses, enhances comple-ment and Fc-mediated phagocytosis in cultured human macro-phages [8–10]; fibronectin, another ECM glycoprotein, medi-ates microorganism uptake [11]; HA has been shown to be a potent inhibitor of phagocytosis *in vitro* [12]. Macrophages possess receptors for ECM proteins, which affect the biochemi-cal and functional profile of macrophages [13], and are able to release numerous proteases that degrade ECM macromolecules [14].

Quantitative or structural changes in ECM are often reflected in changes in the differentiation or functional state of cells because they have been shown to correlate to *in vitro* differentia-tion of monocytes into macrophages [15, 16]. Connective tissue proteins and phagocyte cell functions are thus strictly related.

Furthermore, ECM is a complex and dynamic reservoir of cytokines that regulate or modify cellular responses in inflam-matory and immune reactions inducing biological responses by

binding to specific cell surface receptors [17]. It has been shown that the *in vitro* exposure of human monocytes and monocyte-derived macrophages to microbial infections induces the release of proinflammatory cytokines such as interleukin-1 (IL-1) and interleukin-6 (IL-6) [18].

In previous studies we showed that macrophages, treated with HA or CS, underwent changes in shape and in the organization of the cytoskeleton and modified their adhesion to the substrate [19, 20]. The internalization of *Candida albicans*, evaluated as cell-associated radioactivity, diminished after HA or HS administration, whereas CS had no effects. Our results therefore show a relationship between the macrophage phenotype and phagocytic functions and a role of GAG as modulators of these functions.

To gain a better understanding of the relationship between ECM components and macrophage functional heterogeneity, we studied GAG profile of macrophages of different anatomical origin, bone marrow-(ANA-1) and brain-derived (BV-2) macrophages. Both cell lines were exposed or not to *C. albicans* and cultured in the presence of the two modulators of phagocytic processes, IL-1α and IL-6.

MATERIALS AND METHODS

Cell cultures

The bone marrow-derived macrophage cell line ANA-1 and brain-derived BV2 macrophages were obtained from C57/BL6 mice by immortalization with a recombinant retrovirus carrying the *v-raf/v-myc* oncogenes as previously described [21, 22]. Cells were seeded in 1.75-mm-diameter Petri dishes at a concentration of 10^6 cells/mL and cultured in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum (FCS), glutamine 2 mM, antibiotics in a humidified atmosphere of 5% CO_2 at 37°C.

Newly synthesized GAG isolation and identification

For the biosynthetic labeling of GAG, ANA-1 and BV2 macrophage cells were incubated for 6 and 48 h in RPMI 1640 medium + 10% FCS and exposed to [³H]glucosamine (25 µCi/mL; 28 Ci/mmol, Amersham) in the last 6 h of culture.

In a parallel set of experiments, macrophages were maintained for 30 h in RPMI 1640 + 10% FCS with and without IL-1α or IL-6 (human recombinant, Boehringer Mannheim) both at doses of 5 U/mL. *Candida albicans*, heat inactivated for 30 min (iCa), ratio 1:5 compared to macrophages and [³H]glucosamine (25 µCi/mL), was added in the last 6 h of culture. At the end of the incubation times, cells and media were recovered separately. Cells were scraped in 1 mL ice-cold 0.01 M Tris-HCl and 1.5 mM $CaCl_2$ (pH 7.5) and lysed by sonicating (amplitude 18) with six 10-s bursts using a B15P model Branson sonicator. Media were dialyzed, lyophilized, and dissolved in the above buffer. Lysates and media were boiled for 5 min and then digested for 3 days at 37°C with 1 mg/mL predigested (for 30 min at 37°C) protease type XIV (Sigma Chemical Co., St. Louis, MO) in the presence of 1% toluene. Fresh protease was added daily. Proteins in the digested samples were precipitated with 10% trichloroacetic acid (TCA), pelleted by centrifugation (12,000 rpm), and discarded. GAG were precipitated from recovered supernatants with 3 volumes of 5% potassium acetate in absolute ethanol in the presence of 100 µg of GAG carrier and pelleted by centrifugation for 20 min at 12,000 rpm. GAG were dissolved in 0.1 M Tris-HCl pH 7.2 and aliquots of [³H]GAG were mixed with 3 mL of Ultima Gold (Canberra Packard Int. S.A., Zurich, Switzerland) and counted in a Packard Tri-Carb 2425 liquid scintillation counter.

Aliquots of media containing [³H]GAG were applied to a DE-52 cellulose anion exchange column (0.5 × 6 cm), equilibrated with 10 mM Tris-HCl (pH 7.2) and eluted at room temperature with increasing stepwise concentrations of NaCl (0.1, 0.3, 0.4, 0.5, 0.6 M). One-milliliter fractions were collected; 0.2-mL aliquots were mixed with 3 mL of Ultima Gold and counted in a liquid scintillation counter. Column fractions corresponding to specific types of GAG were pooled, dialyzed, and lyophilized. Standard GAG (Sigma) were then added and precipitated with 3 volumes of 5% potassium acetate in ethanol. Radioactivity was measured in both supernatants (digested GAG) and pellet (undigested GAG).

Individual GAG were identified by their susceptibility to specific enzymes. Testicular hyaluronate lyase (beef, Miles Italiana, S.P.A., Cavenago Brianza, Milano, Italy) and streptomyces hyaluronate lyase (*Streptomyces hyaluroniticus*, Seikagaku Kogyo Co.) digestions were performed by dissolving lyophilized samples in 0.02 M sodium acetate buffer, pH 5, containing 0.1 M NaCl, at 37°C for 24 h with 30 and 5 enzyme units, respectively. Chondroitin AC-II lyase (*Arthrobacter aurescens*, Seikagaku Kogyo Co.) digestions were performed in 0.01 M Tris buffer, pH 8, at 25°C for 24 h with 0.5 and 0.01 enzyme units. Nitrous acid was used to degrade heparan sulfate. Recovery of the fractionating procedure was about 85–95%. Results are expressed as cpm/mg of protein [23].

Statistical analysis

Statistical analysis was performed by Students' *t* test. Results reported in tables and figures are the mean ± SEM for quadruplicate cultures from a typical experiment. Similar results were obtained in three independent experiments.

RESULTS

Incorporation of [³H]glucosamine into GAG in ANA-1 and BV-2 macrophages as a function of time

ANA-1 and BV-2 macrophages were incubated for various times in complete medium containing [³H]glucosamine to analyze the kinetics of cellular and secreted GAG (**Fig. 1**). It is noteworthy that neosynthesized GAG were retained more in the cellular compartment. The radioactivity assayed in the medium was about half of that present in the cell pellet. The radioactivity of both cellular and extracellular GAG increased approximately linearly across the incubation times. After 6 h of culture the amount of cellular GAG from BV-2 cell line was higher compared to the ANA-1 line (Fig. 1A). On the other hand, after 48 h of culture the amount of endocellular GAG in the ANA-1 cells was significantly higher than that in the BV-2 cells. As far as the GAG secreted in the medium is concerned (Fig. 1B), the results demonstrate that after 36/48 h, the macrophages of the ANA-1 line secreted significantly more GAG compared to the macrophages of the BV-2 line. Therefore on the whole the macrophages of the ANA-1 line in culture produce a greater quantity of total GAG compared to that produced by the BV-2 line (Fig. 1C).

Composition of labeled secreted GAG

Ionic exchange chromatography demonstrated the presence of five classes of secreted GAG, namely hyaluronic acid (HA), chondroitin (Ch), heparan sulfate (HS), chondroitin sulfate (CS), and dermatan sulfate (DS; **Fig. 2**). In both macrophage populations, after 6 h HA was the dominant GAG and HS and CS represented about 50% of the HA. After 48 h of maintenance *in vitro*, the ANA-1 macrophages produce a lower quantity of HA compared to that produced by the BV-2 macrophages. In both cell lines it was seen that increasing the *in vitro* maintenance time from 6 to 48 h, the HA/sulfated GAG ratio shifted in favor of the latter.
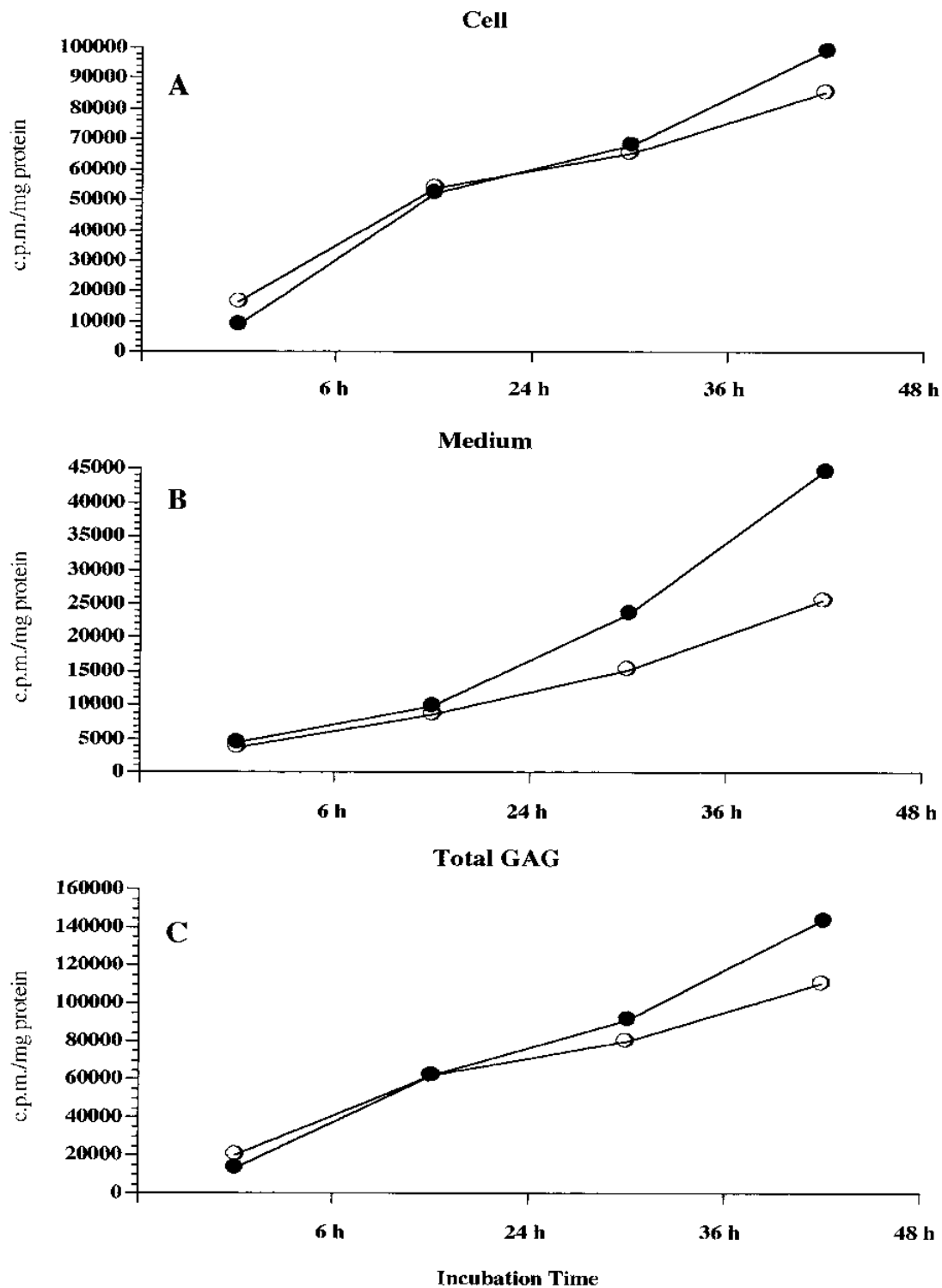
Cell

Medium

Total GAG

Fig. 1. Kinetics of GAG synthesis in bone marrow- and brain-derived macrophage cell lines (ANA-1, filled circles; BV-2, open circles). The periods of incubation time are indicated. [^{3}H]glucosamine (25 μCi/mL) was added in the last 6 h of culture. Values are expressed as cpm/mg protein and represent the mean for four determinations from a typical experiment. For all determinations, standard deviation was less than 15%. Similar results were seen in three independent experiments.

GAG synthesis and profile in IL-1- or IL-6-treated ANA-1 and BV-2 macrophages during iCa exposition

Both IL-1 and IL-6 treatment of ANA-1 macrophages reduced cellular and extracellular GAG; the inhibition effect on secreted GAG was seen to a greater extent with IL-6 (decrease of 78% for IL-6, decrease of 61% for IL-1; **Table 1**). ANA-1 macrophages exposed to iCa had a lower GAG content compared to the controls both in the cellular (decrease of 25%) and extracellular compartment (decrease of 36%). Therefore, the inhibitory effect induced by iCa on the intra- and extracellular GAG quota was less than that induced by the two cytokines.
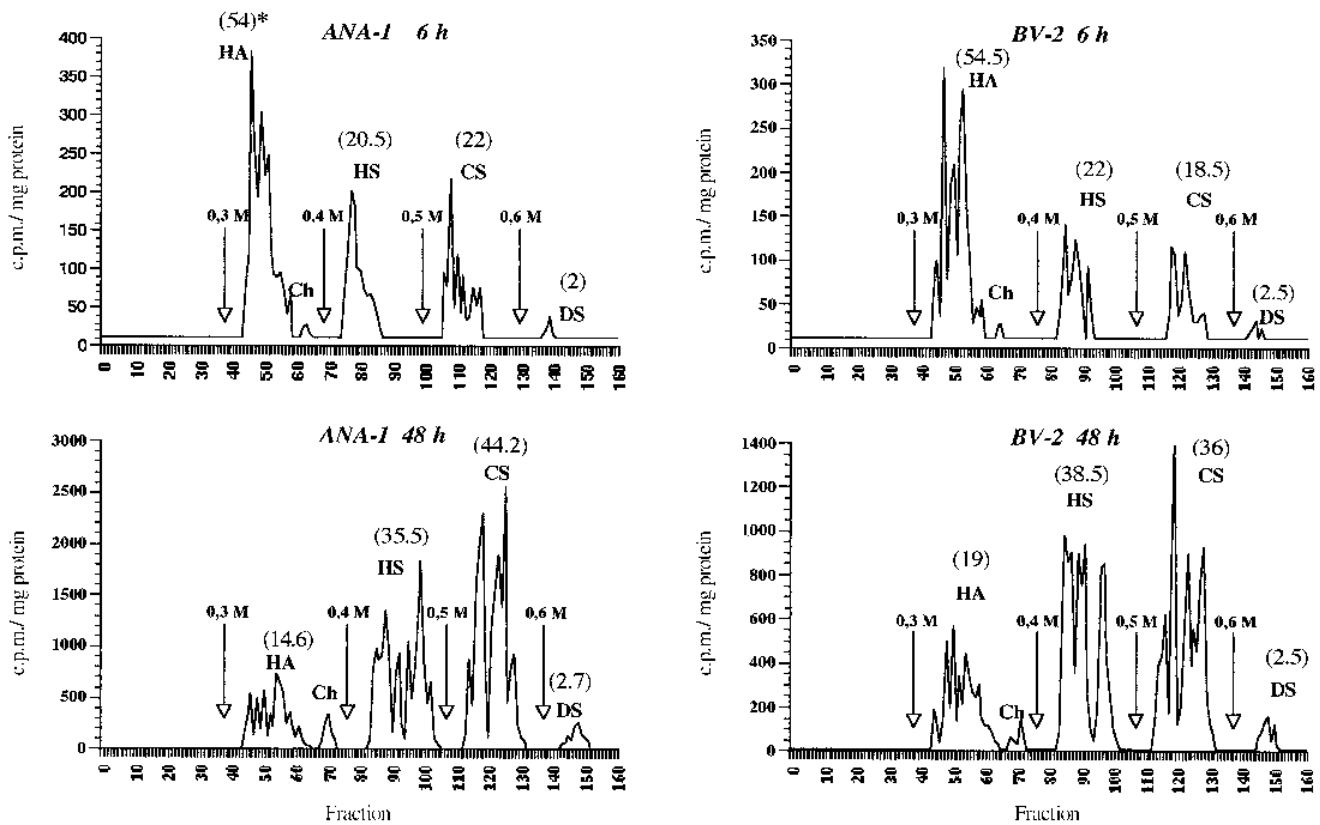
Fig. 2. Anion exhange elution profile of GAG synthesized by ANA-1 and BV-2 macrophage cell lines maintained in RPMI 1640 + 10% FCS for 6 and 48 h. [³H]glucosamine (25 µCi/mL) was added in the last 6 h of culture. Values are representative of a single chromatography. Similar results were obtained in three independent experiments. *Percent secretion in the medium.

Treatment of BV-2 macrophages with IL-1 also induces a reduction in GAG, but the effect is limited to the intracellular compartment (decrease of 35%, data not shown). Exposure of BV-2 to iCa alone or in combination with IL-1 does not show any modifications in the total GAG. In the presence or absence of iCa, IL-6 induces a significant reduction both of the GAG

TABLE 1. Effects of IL-1 or IL-6 on [³H]glucosamine Incorporation in ANA-1 Macrophage Cell Line Exposed or not to *Candida albicans* (iCa)

	Cellular GAG	Extracellular GAG	Total GAG	Cellular Proteins mg/well
Control	23,736 ± 1,945	13,895 ± 975	37,631	1.37 ± 0.01
IL-1	18,207 ± 3,456[b]	5,370 ± 517[a]	21,664	1.32 ± 0.04[d]
iCA	17,675 ± 460[a]	8,951 ± 1,178[a]	26,626	1.35 ± 0.02[d]
IL-1 + iCa	16,363 ± 1,308[a]	6,225 ± 330[a]	22,588	1.45 ± 0.01[d]
IL-6	20,690 ± 1,892[c]	3,018 ± 70[a]	23,708	1.36 ± 0.03[d]
IL-6 + iCa	17,137 ± 1,872[a]	7,342 ± 841[a]	24,479	1.36 ± 0.01[d]

ANA-1 macrophages were maintained in RPMI 1640 + 10% FCS for 30 h with or without IL-1α or IL-6 (both at the dose of 5 U/mL). [³H]glucosamine (25 µCi/mL) and iCa (heat inactivated for 30 min, ratio 1:5) were added in the last 6 h and processed as described in Materials and Methods. [³H]glucosamine incorporation was evaluated into cellular and extracellular GAG. Values are expressed as cpm/mg protein and represent the mean ± SD for four determinations from a typical experiment. Similar results were seen in three independent experiments. Differences versus each control are significant for [a] $P < 0.001$, [b] $P < 0.01$, [c] $P < 0.05$, [d] not significant.

accumulated in the cells and the secreted GAG (decrease of 27 and 21%, respectively, data not shown).

Analysis of individual secreted GAG classes secreted by ANA-1 macrophages (**Fig. 3** and **Table 2**) showed that IL-1 addition induced an increase of the percentage of HA (more than double) and a decrease of HS and CS with an increase of the HA/sulfated GAG. In iCa-treated macrophages, the HA percentage was moderately enhanced compared to the controls; the radioactivity incorporated into HS and CS was significantly reduced, whereas the percentage of DS was about fourfold above the controls. After combined treatment with iCa and IL-1, the percentage of HA increased, as also seen following treatment with iCa alone. A further decrease in HS and a more significant increase in DS percentage were seen. IL-6 treatment induced a slight increase in the percentage of HA and HS, a considerable increase in DS, and a sharp decrease in CS. The HA/sulfated GAG ratio was in favor of sulfated GAG. Combined treatment with IL-6 and iCa produced an increase in the HS percentage.

In BV-2, treatment with IL-1 or IL-6 induced fewer modifications in redistribution of the percentage of individual GAG classes (Table 2). In fact both the ILs determine only a slight decline in the HA and a modest increase in the CS. The most significant event during exposure to iCa is the increase in the HS percentage.

DISCUSSION

Relatively little is known concerning the relationship among ECM components and cells of the immune system. Neverthe-
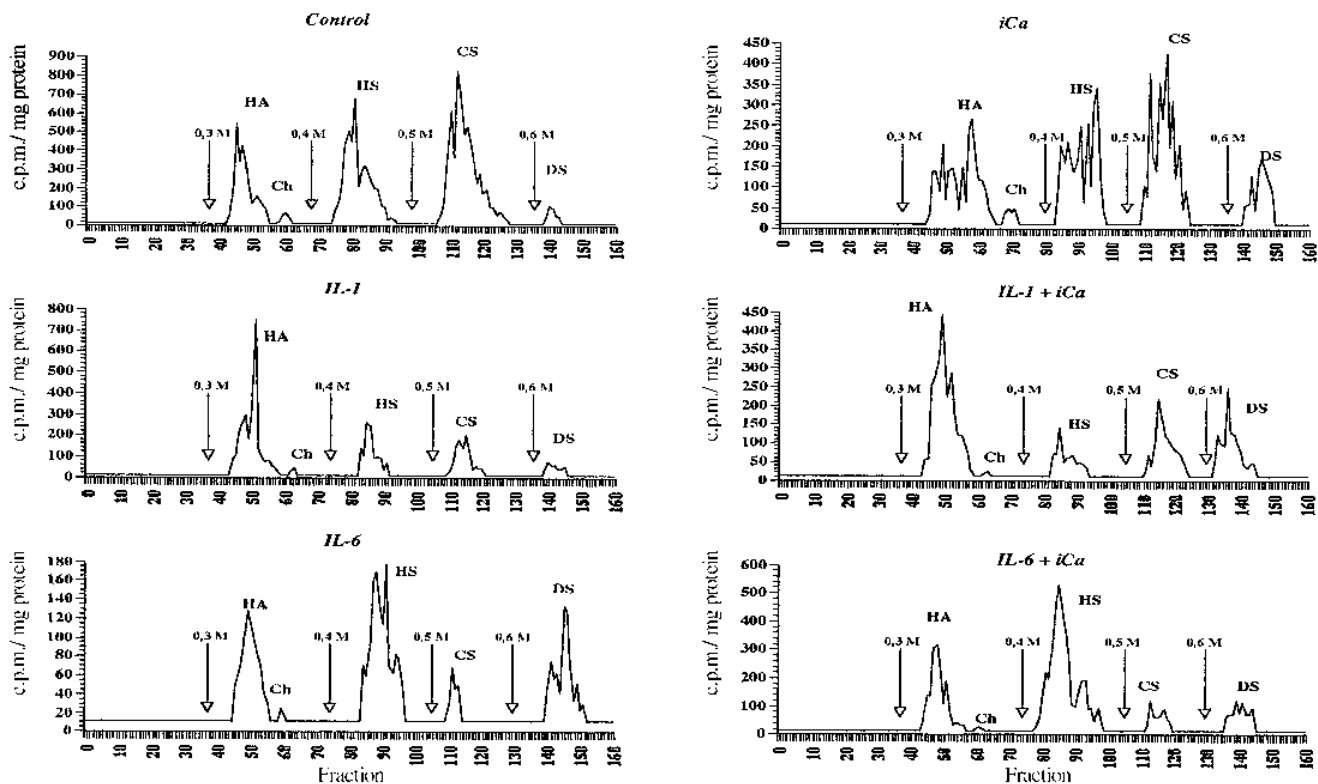
Fig. 3. Anion exchange profile of GAG synthesized by ANA-1 macrophages maintained in RPMI 1640 + 10% FCS for 30 h with or without IL-1α or IL-6 (both 5 U/mL) in presence or absence of *Candida* (iCa, heat inactivated for 30 min, ratio 1:5). Values are representative of a single chromatography. Similar results were obtained in three independent experiments.

less, it is well-known that the interactions between monocytes and ECM proteins represent an important stimulus for differentiation of macrophages [24–26]. ECM macromolecules, generated at inflammatory sites, affect macrophage adherence,

TABLE 2. Percentage of [³H]Glucosamine Incorporation into Individual GAG Classes Secreted by ANA-1 and BV-2 Macrophage Cell Lines

	HA	Ch	HS	CS	DS
			ANA-1		
Control	20	1.5	32.8	43.2	2.5
IL-1	50	1.5	21.5	20	7
iCa	27	2	29	31	11
IL-1 + iCa	48	1	10.2	19.8	21
IL-6	28	1.5	37	6.5	27
IL-6 + iCa	23.3	1	59	7.2	9.5
			BV-2		
Control	66	2	25	5	2
IL-1	61.3	3.5	25	8	2.2
iCa	57.2	3	32	6	1.8
IL-1 + iCa	59	2	25	12	2.1
IL-6	64.5	1.5	26	6	2
IL-6 + iCa	62	1	30	5	2

ANA-1 and BV-2 macrophage cell lines were maintained in RPMI 1640 + 10% FCS for 30 h with or without IL-1α (5 U/mL) or IL-6 (5 U/mL). *Candida albicans* (heat inactivated for 30 min, ratio 1:5) and [³H]glucosamine were added in the last 6 h of culture. Aliquots of media were applied to DE-52 cellulose anion exchange column and processed according to Materials and Methods. Values are representative of a single chromatography. Similar results were obtained in three independent experiments.

phagocytosis, and local cytokine production. Various hypotheses have been put forward regarding the mechanisms by which the ECM components influence phagocytosis. These hypotheses include the following: a direct effect on the number of ECM surface receptors, a change in the receptor typology, an effective link between receptors and cytoskeletal components affecting in turn the endocytosis vesicles. [19, 20, 27, 28].

This study was conducted in two macrophage cell lines obtained from different anatomical areas with different functional and secretory activities [29–31]. Previous studies showed that the two cell lines actively phagocytize *Candida*, although macrophages of microglial origin were the most effective [31, 32]. We have now shown that in ANA-1 and BV-2 macrophages, the biosynthetic program for GAG is different, since each cell line exhibited a peculiar GAG pattern, modulated by IL-1α and IL-6 treatment and further altered after exposition to a phagocytic stimulus. In ANA-1, more than in BV-2, total GAG synthesis is greatly inhibited by treatment with IL-1α and IL-6. In both macrophage populations, iCa exposure tends partially or completely to remove the strong inhibitory effect induced by ILs.

Chromatographic analysis of the media showed that ILs have a greater modulating effect on the ANA-1 line than on BV-2. In ANA-1, in fact, IL-1α treatment decreases the quota of sulfated GAG, in particular shifting the HA/sulfated GAG ratio in favor of HA. This ratio is modified during iCa exposure or after treatment with IL-6 and/or iCa. BV-2 reveals a different phenotype compared to ANA-1 with increased production of HA. Exposure to iCa lowers the HA/GAG sulfated ratio

inducing a relative increase in HS, whereas the ILs induce a modest modification in the expression of individual GAG classes. On the whole the results prove that since ANA-1 and BV-2 come from different anatomical sites, they each have particular GAG patterns and respond differently to treatment with the cytokines. These data may have functional implications. In fact macrophages bind HA and possess HA receptors, as well as being able to degrade HA [33–35]. HA, in its turn, reduces IL-1-stimulated proteoglycan degradation [36]. The altered balance between HA amount and sulfated GAG during iCa and IL treatment may be important during the process of migration of macrophages through the ECM and represents a critical aspect of phagocytic functions [37].

Cytokines show selective and differential binding to glycosaminoglycans or core protein of proteoglycans [38]. IL-1β partially binds to hyaluronic acid, whereas IL-1α shows essentially no retention. Selective, partial binding to acidic polysaccharides is also exhibited by IL-6, which binds heparin, dextran sulfate, and dermatan sulfate but is poorly retained on chondroitin sulfate [39, 40].

Furthermore, a cascade control of cytokine production has been indicated [41–43], as well as the role of cytokines in host defense [44–46]. Cytokines secreted by macrophages greatly influence, in an autocrine manner, the ability of macrophages to defend against microbial challenge [47]. The up-regulation of IL-1α and the down-regulation of IL-6 due to changes in IL-1 and/or HA amounts might broadly affect aspects of the delicate balance of the cytokine network during immunity response.

Furthermore we, like others [48–52], have shown that macrophage-derived growth factors are able to modulate the function of normal and pathological fibroblasts affecting collagen and GAG synthesis and driving the fibro-proliferative responses. Therefore, ECM components become the modulatory signals of a microenvironment, where they modulate the genetic program and control the cytokine release responsible for the fibrotic processes of many connective disorders.

In conclusion, our results provide the first evidence that GAG profile of macrophages is modulated by incubation with IL-1, IL-6, or iCa, suggesting a close relationship between GAG-sulfated and non-sulfated composition and release, IL production, and macrophage functions. Further studies are necessary to verify the mechanism through which the composition in the individual GAG classes constitutes an important means of metabolic control of the different macrophage functions.

REFERENCES

1. Bitterman, P. B., Wewers, M. D., Rennard, S. I., Adelberg, S., Crystal, R. G. (1986) Modulation of alveolar macrophage-driven fibroblast proliferation by alternative macrophage mediators. J. Clin. Invest. 77, 700–708.
2. Brown, E. J. (1986) The role of extracellular matrix proteins in the control of phagocytosis. J. Leukoc. Biol. 39, 579–591.
3. Adams, J. C., Fiona, M. W. (1993) Regulation of development and differentiation by the extracellular matrix. Development 117, 1183–1198.
4. Butcher, E. C. (1990) Cellular and molecular mechanisms that direct leukocyte traffic. Am. J. Pathol. 136, 3–11.
5. Newman, S. L., Tucci, M. A. (1990) Regulation of human monocyte/macrophage function by extracellular matrix. J. Clin. Invest. 86, 703–714.
6. Shimizu, Y., Shaw, S. (1991) Lymphocyte interactions with extracellular matrix. FASEB J. 5, 2292–2299.
7. McKay, D. B., Vazquez, M. A., Redline, R. W., Lu, C. Y., Schmalzried, S. R., Shea, C. M. (1992) Macrophage functions are regulated by murine decidual and tumor extracellular matrices. J. Clin. Invest. 89, 134–142.
8. Giavazzi, R., Hart, I. R. (1983) Mononuclear phagocyte adherence in the presence of laminin. Exp. Cell Res. 146, 391–399.
9. Bohnsack, J. F., Kleinman, H. F., Takahashi, T., O'Shea, J. J., Brown, E. J. (1985) Connective tissue proteins and phagocytic cell function. Laminin enhances complement and Fc-mediated phagocytosis by cultured human macrophages. J. Exp. Med. 161, 912–923.
10. Mercurio, A. M., Shaw, L. M. (1988) Macrophage interactions with laminin: PMA selectively induces the adherence and spreading of mouse macrophages on a lamin substratum. J. Cell Biol. 107, 1873–1880.
11. Proctor, R. A., Textor, J. A., Vann, J. M., Mosher, D. F. (1985) Role of fibronectin in human monocyte and macrophage bacterial activity. Infect. Immun. 47, 629–637.
12. Suzuki, Y., Yamaguchi, T. (1993) Effects of hyaluronic acid on macrophage phagocytosis and active oxygen release. Agents Action 38, 32–37.
13. Levitz, S. M., Farrell, T. P. (1990) Growth inhibition of Cryptococcus neoformans by cultured human monocytes: role of the capsule, opsonins, the culture surface, and cytokines. Infect. Immun. 58, 1201–1209.
14. Alexander, C. M., Werb, Z. (1989) Proteinases and extracellular remodeling. Curr. Opin. Cell Biol. 1, 974–982.
15. Uhlin-Hansen, L., Eskeland, T., Kolset, S. O. (1989) Modulation of the expression of chondroitin sulfate proteoglycan in stimulated human monocytes. J. Biol. Chem. 264, 14916–14922.
16. Michelacci, Y. M., Petricevich, V. L. (1991) Glycosaminoglycan profile of peritoneal and bone marrow-derived macrophages. Changes associated with macrophage activation. Comp. Biochem. Physiol. 100b, 617–625.
17. Gehr, G., Braun, T., Lessluer, W.(1992) Cytokines, receptors, and inhibitors. Clin. Invest. 70, 64–69.
18. Marcinkiewicz, J., Czajkowska, B., Grabowska, A., Kasprowicz, A., Kociszewska, B. (1994) Differential effects of chlorination of bacteria on their capacity to generate NO, TNF-α and IL-6 in macrophages. Immunol. 83, 611–616.
19. Bodo, M., Baroni, T., Mocci, S., Merletti, L., Giammarioli, M., Calvitti, M., Sbaraglia, G. (1995) Internalization of Candida albicans and cytoskeletal reorganization in macrophages and fibroblasts treated with Concanavalin A. Med. Sci. Res. 23, 89–94.
20. Bodo, M., Baroni, T., Mocci, S., Giammarioli, M., Bellucci, C., Sbaraglia, G. (1995) Extracellular matrix affects Candida albicans internalization and cytoskeleton pattern in mouse macrophages. Cell Mol. Biol. 41, 297–305.
21. Blasi, E., Mathieson, B. J, Varesio, L., Cleveland, L., Borchert, P. A., Rapp, U. (1985) Selective immortalization of murine macrophages from fresh bone marrow by a v-myc:/v-raf recombinant retrovirus. Nature [Lond] 318, 667–670.
22. Blasi, E., Barluzzi, R., Bocchini, V., Mazzolla, R., Bistoni, F. (1990) Immortalization of murine microglial cells by a v-myc/v-raf carryng retrovirus. J. Neuroimmunol. 27, 229–237.
23. Lowry, O. H., Rosebrough, N. J., Farr, A. J., Randal, R. J. (1951) Protein measurement with the folin phenol reagent. J. Biol. Chem. 193, 265–275.
24. Redline, R. W, McKay, D., Vazquez, M. A., Papaioannou, V. E., Lu, C. Y., Shea, C. M., Schmaizried, S. R. (1990) Macrophage functions are regulated by the substratum of murine decidual stromal cells. J. Clin. Invest. 85, 1951–1958.
25. Petricevich, V. L., Michelacci, Y. M. (1990) Proteoglycans synthesized in vitro by nude and normal mouse peritoneal macrophages. Biochim. Biophys. Acta 1053, 135–143.
26. Busiek, D. F., Baragi, V., Nehring, L. C., Parks, W. C., Welgus, H. G. (1995) Matrilysin expression by human mononuclear phagocytes and its regulation by cytokines and hormones. J. Immunol. 154, 6484–6491.
27. Writh, S. D., Licht, M. R., Craigmyle, L. S., Silverstein, S. C. (1984) Communication between receptors induces a reversible alteration in the function of complement receptors on cultured human monocytes. J. Cell. Biol. 99, 336–339.
28. Gresham, H. D., Goodwin, J. L., Allen, P. M., Anderson, D. C., Brown, E. J. (1989) A novel member of the integrin family mediates Arg-Gly-Asp-stimulated neutrophil phagocytosis. J. Cell Biol. 108, 1935–1943.
29. Blasi, E., Puliti, M., Pitzurra, L., Barluzzi, R., Mazzolla, R., Adami, C., Cox, G. W., Bistoni, F. (1994) Comparative studies on functional and secretory properties of macrophage cell lines derived from different anatomical sites. FEMS Immunol. Med. Microbiol. 9, 207–216.
30. Blasi, E., Puliti, M., Pitzurra, L., Bartoli, A., Bistoni, F. (1994) Heterogeneous secretory response of phagocytes from different anatomical districts to the dimorphic fungus Candida albicans. Cell. Immunol. 153, 239–247.
31. Blasi, E., Pitzurra, L., Puliti, M., Lanfrancone, L., Bistoni, F. (1992) Early differential molecular response of a macrophage cell line to yeast and hyphal form of Candida albicans. Infect. Immun. 60, 832–837.

32. Blasi, E., Mathieson, B. J., Varesio, L., Cleveland, L., Borchert, P. A., Rapp, U. (1985) Selective immortalization of murine macrophages from fresh bone marrow by a *v-myc/v-raf* recombinant retrovirus. Nature [Lond.] 318, 667–670.

33. Shannon, B. T., Love, S. H., Myrvik, Q. N. (1980) Participation of hyaluronic acid in the macrophage disappearance reaction. Immunol. Commun. 9, 357–370.

34. Green, S. J., Tarone, G., Underhill, C. B. (1988) Distribution of hyaluronate and hyaluronate receptors in the adult lung. J. Cell Sci. 89, 145–156.

35. Culty, M., Nguyen, H. A., Underhill, C. B. (1992) The hyaluronan receptor (CD44) participates in the uptake and degradation of hyaluronan. J. Cell Biol. 116, 1055–1062.

36. Morris, E. A., Wilcon, S., Treadwell, B. V. (1992) Inhibition of interleukin 1-mediated proteoglycan degradation in bovine articular cartilage explants by addition of sodium hyaluronate. Am. J. Vet. Res. 53, 1977–1982.

37. Wessel, M. R., Moses, A. E., Goldberg, J. B., DiCesare, T. J. (1991) Hyaluronic acid capsule is a virulence factor for mucoid group A streptococci. Proc. Natl. Acad. Sci. USA 88, 8317–8321.

38. Andres, J. L., De Falcis, D., Noda, M., Massaguè, J. (1992) Binding of two growth factor families to separate domains of the proteoglycan betaglycan. J. Biol. Chem. 267, 5927–5930.

39. Ramsden, L., Rider, C. C. (1992) Selective and differential binding of interleukin (IL)-1 alpha, IL-1 beta, IL-2 and IL-6 to glycosaminoglycans. Eur. J. Immunol. 22, 3027–3031.

40. Ruoslathi, E., Yamaguchi, Y. (1991) Proteoglycans as modulators of growth factor activities. Cell 64, 867–869.

41. Hart, P. H., Jones, C. A., Finlay-Jones, J. J. (1995) Monocytes cultured in cytokine-defined environments differ from freshly isolated monocytes in their responses to IL-4 and IL-10. J. Leukoc. Biol. 57, 909–918.

42. Armbrust, T., Schmitt, E., Ramadori, G. (1995) Viable kupffer cells synthesize but do not secrete interleukin-1: indications for necrosis-induced maturation of interleukin-1α, but not of interleukin-1β. Biochem. Biophys. Res. Commun. 207, 637–645.

43. Zhou, D., Munster, A., Winchurch, R. A. (1991) Pathologic concentrations of interleukin 6 inhibit T cell responses via induction of activation of TGF-β. FASEB J. 5, 2582–2585.

44. Morrissey, P. J., Charrier, K., Vogel, S. N. (1995) Exogenous tumor necrosis factor alpha and interleukin-1α increase resistance to *Salmonella typhymurium*: efficacy is influenced by the Ity and Lps loci. Infect. Immun. 63, 3196–3198.

45. Beaman, M. H., Hunter, C. A., Remington, J. S. (1994) Enhancement of intracellular replication of *Toxoplasma gondii* by IL-6. Interactions with IFN-γ and TNF-α. J. Immunol. 153, 4583–4587.

46. Hsu, N., Young, L. S., Bermudez, L. E. (1995) Response to stimulation with recombinant cytokines and synthesis of cytokines by murine intestinal macrophages infected with the *Mycobacterium avium* complex. Infect. Immun. 63, 528–533.

47. Neta, R., Sayer, T. J., Oppenheim, J. J. (1992) Relationship of TNF to the interleukins. In *Tumor Necrosis Factors; Structure, Function and Mechanism of Action* (B. B. Aggarwal and J. Vilcek eds.), New York: Marcel Dekker, 499–566.

48. Bodo, M., Carinci, F., Baroni, T., Becchetti, E., Giammarioli, M., Bellucci, C., Pezzetti, F., Evangelisti, R., Carinci, P. (1996) Effect of interleukins on Crouzon fibroblast phenotype in vitro. Release of cytokines and IL-6 mRNA expression. Cytokine 8, 772–783.

49. Bodo, M., Carinci, F., Baroni, T., Giammarioli, M., Bellucci, C., Pezzetti, F., Becchetti, E., Evangelisti, R., Carinci, P. (1997) Apert's syndrome: differential in vitro production of matrix macromolecules and its regulation by interleukins. Eur. J. Clin. Invest. 27, 36–42.

50. Bodo, M., Carinci P., Venti, G., Giammarioli, M., Donti, E., Stabellini, G., Paludetti, G., Becchetti, E. (1998) Glycosaminoglycan metabolism and cytokine release in normal and otosclerotic human bone cells interleukin-1 treated. Connective Tissue Res. 36, 231–240.

51. Bodo, M., Carinci, F., Baroni, T., Becchetti, E., Bellucci, C., Giammarioli, M., Pezzetti, F., Tognon, M., Carinci, P. (1998) Interleukin pattern of Apert fibroblast in vitro. Eur. J. Cell Biol. 75, 383–388.

52. Bodo, M., Carinci, P., Baroni, T., Bellucci, C., Giammarioli, M., Pezzetti, F., Becchetti, E. (1998) Role of growth factors on extracellular matrix production by chick embryo fibroblasts in vitro. Antagonist effect of TGFβ through the control of IL-1 and IL-1ra secretion. Cytokine 10, 353–360.