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TRANSCRIPTIONAL EXPRESSION OF MANNOSE RECEPTOR GENE DURING DIFFERENTIATION OF HUMAN MACROPHAGES

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SUMMARY : Mannose receptor is a differentiation marker of macrophages. Circulating monocytes isolated from plasma are devoid of this receptor; upon culture this receptor is rapidly expressed. Its expression is modulated by a variety of inflammatory and anti-inflammatory agents. In the present study, we investigated its transcription level during the differentiation process. Mannose receptor mRNA was monitored by quantitative RT-PCR on freshly harvested monocytes and on monocytes cultivated up to four days. No transcription was detected in freshly harvested cells, the transcription increased during the first 24 h upon adhesion and then decreased.

Macrophages carry out a number of functions that depend on highly specific interactions with their environnement. Many cell-molecule and cell-cell interactions are oligosaccharides mediated (1-4). Macrophages express a variety of cell surface lectins that support specific functionnal roles in inflammation, cancer cells trapping, pathogen neutralization and depending on their differentiation states (5). Among various binding proteins present on mononuclear phagocytes, the mannose receptor is a marker of differentiated macrophages (6). Expression of the mannose receptor is highly dependent on the macrophage phenotype. Freshly isolated monocytes do not express this cell surface receptor : conversely a functionnal protein is detected after 3 or more days in culture (7-8). Agents promoting macrophage activation decreased the mannose specific receptor levels while agents promoting an anti-inflammatory state increased the expression of the mannose specific receptor (6). Measurement of protein has allowed characterization of the cellular response to various stimuli. Little is known about the regulation of transcription during macrophage differentiation. We have previously identified a negative regulatory element within the promoter of the mannose specific receptor that apparently prevents its expression in nonmyeloid cells (9), suggesting that its transcription is activated during the process of

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differentiation. We monitored the modulation of mannose receptor gene expression upon adhesion of monocytes and their differentiation into macrophages in comparison with that of the galectin-3 gene. Galectin-3, also known as Mac-2, is another marker of macrophage differentiation; it is, for instance, absent on resident macrophages but it is expressed on mouse peritoneal macrophages elicited by thioglycollate (10). Its expression depends also on the differentiation state of different macrophages cells lines (11). Because northern analysis requires large amounts of total RNA or of purified poly A^+ RNA and because of the relatively small number of cells available, we have used an approach based on quantitative reverse transcription-PCR (RT-PCR).

MATERIALS AND METHODS

Cells : Monocytes were isolated from whole blood of heathly donors (Centre de transfusion sanguine, Orléans, France). They were either freshly harvested (designated J0) or cultivated during 1, 2, 3 and 4 days (designated J1, J2, J3, J4, respectively) in AIM V medium.

RNA isolation and cDNA synthesis : Total RNA were extracted by using the guanidinium isothiocyanate method (12). First strand cDNA was synthetized at 42°C for 2 h using 5 μg of total RNA in a total volume of 20 μl of 6 mM $MgCl_2$, 40 mM KCl, 1 mM DTT, 0.5 M Tris-HCL (pH 8.3) buffer containing 0.1 mg/ml BSA, 200 mM dNTP, 20 pmol of nonamer random primer and 37 units of AMV reverse transcriptase (Promega, Madison, WI).

Northern Blot : 10 μg of total RNA were glyoxylated, size fractionated through a 1.4 % agarose gel and transferred onto a N^+ nylon membrane in 2X SSC (sodium citrate, sodium chloride) (Appligene, Strasbourg, France). Hybridization was performed in 0.5 M sodium phosphate buffer (pH7.4) containing 10mg/ml bovine serum albumin, 0.25 M SDS, 2 mM EDTA and 37 KBq/ml of ^{32}P-labeled cDNA fragments prepared by a random priming method (Amersham, Buckinghamshire, UK).

Polymerase Chain Reaction : 5 μl of reverse transcription reaction were added to 45 μl of 50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris-HCl (pH 8,8) buffer containing 1mg/ml Triton X-100, 200 μM each dNTP, 100KBq of [α^{32}P]dCTP (110TBq/mmol, Amersham), 1 μM of primer pairs either specific for the mannose receptor or for the galectin-3 genes and 0.03 Units/μl Taq DNA polymerase (Promega Corporation, Madison, WI). Twenty five amplification cycles were performed on a Perkin Elmer thermocycler (1 min at 94°C, 2 min at 55°C, 2 min at 72°C). Seven μl of the PCR product mixture were then loaded on 2% agarose gels. Radioactive bands were quantitated by scanning the dried gel with a Phosphorimager (Molecular Dynamics, Sunnywale, CA). Each sample was assayed in triplicate. Quantitation was conducted in reference with a standard curve generated by amplification of a serial dilution of a plasmid containing mannose receptor or galectin-3 cDNA. The quantitative RT-PCR was validated by amplification of serial dilutions of mannose receptor and galectin-3 cDNA derived from J774E cells which express constitutively both genes.

Oligonucleotides and DNA templates : Oligonucleotides were purchased from Eurogentech (Seraing, Belgium). Mannose receptor mRNA were amplified with 5'-CCT AAC GTT TGC TGT TGA CC-3' and 5'-CAT AGC GCT GCT GTA GAC CTG-3' primers and probed with 5'-GGA CTC GTG CTG TTG ACC GCA T-3'. Galectin-3 mRNA were amplified with both

primers 5'-CGT CAC GAG CTG ACG ACT CAT A-3' and 5'-CGT ATC ATG CTG TGG ACA AGC-3' and probed with 5'-CGT ATC GTG CTG TTG GGC AT-3'. The cDNA used to construct the mannose receptor standard curve was a generous gift from Dr. K. Drickamer, Oxford, UK, and that of galectin-3 from Dr A.Raz, University of Illinois,MI.

RESULTS AND DISCUSSION

Northern blot analysis of mannose receptor and galectin-3 gene expression : In order to measure changes occuring in mannose receptor mRNA levels during differentiation, freshly isolated monocytes were cultivated up to three days. The expression of differentiation markers of macrophages, c-*fms* and *Fc* receptor, were followed by RT-PCR (data not shown). c-fms is closely related to the receptor for the macrophage colony stimulating factor CSF-1 (13) and its expression is the earliest and most accepted marker of monocytic differentiation (14). Receptors for the Fc domain of IgG (FcgRII) were found on both monocytes and leukemic cell lines (15). No expression of c-*fms* and *Fc* receptor genes was found at J0, but was detected at J3 indicating that the cells have differentiated along the experiments. In addition FACS analysis of the OKM1 marker indicates that 95% of the cell population is positive for this marker of mature monocytic phenotype (data not shown).

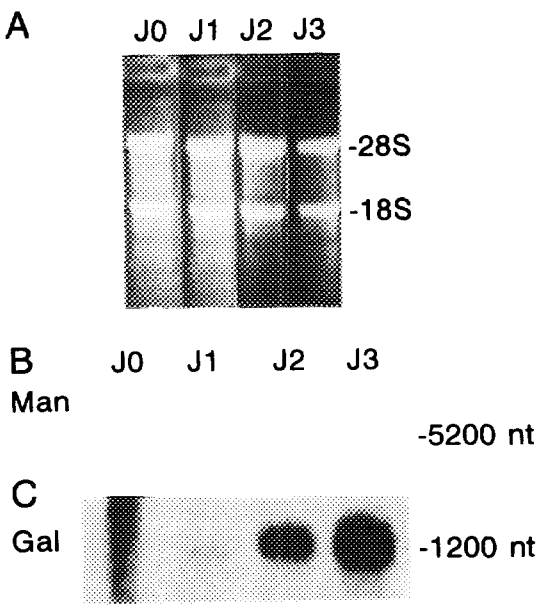

Figure 1. Analysis of mannose receptor gene and galectin-3 gene expression in human monocytes freshly harvested (J0) or differentiated upon 1 to 3 days culture (J1, J2 and J3). **A.** Ten micrograms of total RNA were loaded on a 1.2% agarose gel, blotted and hybridized either with a probe specific to the mannose receptor gene or to the galectin-3 gene. **B.** No transcripts specific for mannose receptor gene (Man) (5200 nt) were detected by Northern blot. **C.** No transcripts specific of the galectin-3 gene (Gal) (1200 nt) were visualized in extracts from J0 monocytes but the expression increased upon culture.

Transcription of the mannose receptor gene was followed by northern analysis on total RNA extracted from freshly harvested monocytes (J0) or from monocytes cultured from one (J1) to three days (J3) (Fig. 1). Filter was hybridized either with a mannose receptor probe or a galectin-3 probe. No transcripts for the mannose receptor gene were detected in all samples. For the galectin-3 gene, no transcripts were detected in J0 cells but the expression greatly increased with time of culture. These results indicate that northern blot analysis is not suitable for testing the mannose receptor gene expression because of the lack of sensitivity of the technique. As a consequence, the modulation of the transcription of the mannose

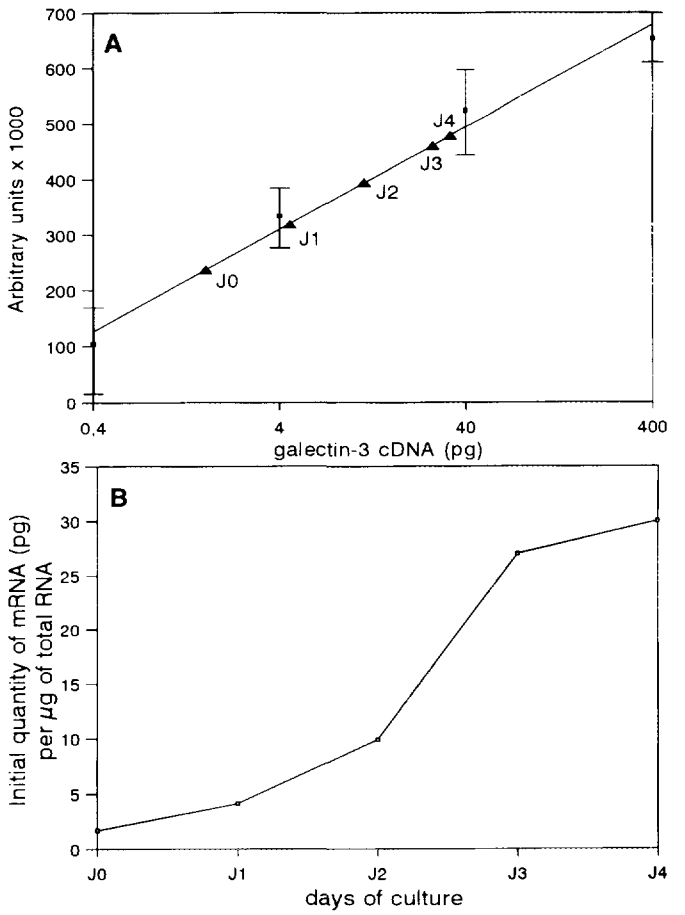

Figure 2. Expression of the galectin-3 gene during macrophage differentiation. **A.** A plasmid containing the human galectin-3 cDNA was amplified. A standard curve was generated by plotting the total amount of radioactivity incorporated into the PCR fragments (arbitrary units) versus the amount of plasmid cDNA template (log scale). mRNA from cultured monocytes were reverse transcribed and galectin-3 cDNA was subsequently amplified. Quantitations were performed by plotting the amount of radioactivity incorporated into the PCR fragments in the standard curve (triangles). **B.** Modulation of the galectin-3 gene expression. The amount of mRNA deduced from panel A is plotted versus days in culture.

receptor was evaluated by quantitative RT-PCR, a much more sensitive technique. The expression of the galectin-3 gene during monocyte differentiation was used as a positive control.

Quantitative analysis of mannose receptor and galectin-3 mRNA by RT-PCR : Transcription of galectin-3 occurred very early upon adhesion (Fig.2). A weak signal was visualized by RT-PCR after one hour of adhesion corresponding to the detection of 1.8 pg of mRNA per μg of total RNA at J0 and then increased fifteenfold by two days in culture,

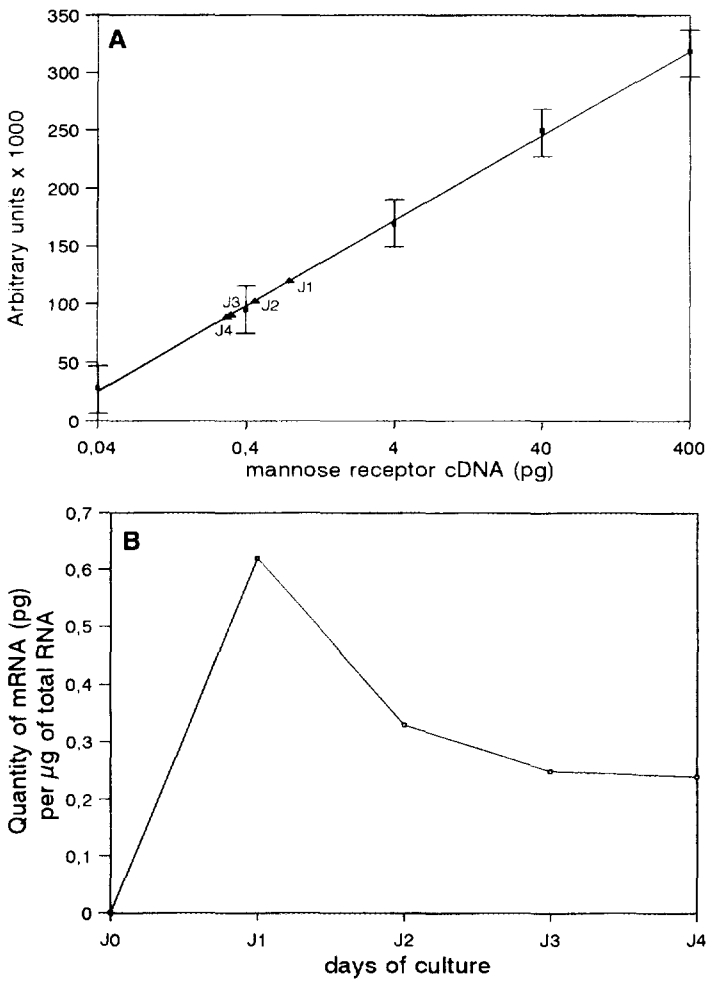

Figure 3. Expression of the mannose receptor gene during macrophage differentiation. **A.** A standard curve was generated under conditions described above, except that the human mannose receptor cDNA was used as a template for PCR. mRNA from cultured monocytes were reverse transcribed and mannose receptor cDNA was subsequently amplified and quantitated by plotting the amount of radioactivity incorporated into the PCR fragments in the standard curve (triangles). **B.** Modulation of the mannose receptor gene expression. The amount of mRNA deduced from panel A is plotted versus days in culture.

a result in agreement with the northern blot data (Fig.1). No transcripts of mannose receptor gene were detected one hour upon adhesion (J0 cells) but later transcription occured (Fig.3). Transcription reached a maximum 24 hours (J1) upon adhesion then decreased to one third over a 72 hours period (J2, J3). The expression remained constant from day 3 to day 4 (J4). It has been shown that the receptor is not present on freshly isolated peripheral blood monocytes or bone marrow monocytic precursor cells prior to cell culture and that the receptor mediated uptake of mannosylated ligands after 3 days in culture (7-8). An unglycosylated precursor of 154.000 is synthetized and matured to a 162.000 form by 90 minutes within 24 hours upon plating (16). It takes at least 3 days to find it at the cell surface of macrophage derived monocyte. The maximun amount of transcription detected at J1 could serve to synthetize the intracellular pool of the mannose receptor then the transcription decreases to assume the turnover of the protein.

Control of temporal and tissue-specific expression of several genes tighly regulated during macrophage differentiation such as the lysozyme gene (17), the *gp91-phox* gene (18) or the c-*fps/fes* gene (19), are mediated by silencer type negative regulatory elements. In a precedent paper, the promoter of the human mannose receptor gene has been characterized and a negative *cis* regulatory element has been described (9). These results together with the present data suggest that the tissue-specific expression of the mannose receptor gene is also dictated by a similar mechanism of regulation which involves blockage release of gene activity. Identification of the factor(s) involved in the control of the silencer activity will help to understand further the mechanism of regulation of expression of the mannose receptor gene during differentiation of macrophages.

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