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Nuclear and Cytoplasmic Expressions of the Carbohydrate-Binding Protein CBP70 in Tumoral or Healthy Cells of the Macrophagic Lineage

Yasmina Hadj Sahraoui, Annie-Pierre Sève, Marie-Agnès Doyennette-Moyne, Line Saffar, Murielle Felin, Michèle Aubery, Liliane Gattegno, and Jean Hubert

INSERM U180, Centre Universitaire des Saints-Pères, Université René-Descartes, Paris 75006 (Y.H.S., A.-P.S., M.-A.D.-M., M.F., M.A., J.H.) and Laboratoire de Biologie Cellulaire, Faculté de Médecine Léonard-de-Vinci, Paris XIII, Bobigny (L.S., L.G.) France.

Abstract The expression of the carbohydrate-binding protein CBP70 was analyzed in undifferentiated HL60 cells, HL60 cells differentiated into monocytes/macrophages or granulocytes, healthy monocytes and in vitro monocyte-derived macrophages (MDM) using an anti-CBP70 serum. This study was performed by immunoblotting analysis of nuclear and cytoplasmic extracts before and after N-acetylglucosamine affinity chromatography and by indirect immunofluorescence microscopy. The results of this study show, for the first time, that CBP70 is expressed in the nucleus and the cytoplasm of healthy or leukemic cells of the macrophagic lineage. However, striking differences were observed depending upon the leukemic or normal state of cells and cell differentiation. Indeed, the level of expression and the intracellular distribution of CBP70 were found to be different in undifferentiated HL60 cells and monocytes/macrophages differentiated from these cells. Major differences were also observed according to whether macrophages differentiated from leukemic HL60 cells or healthy monocytes. Thus, the total cellular expression of CBP70 was markedly lower in MDM than in HL60-derived macrophages and the intracellular distribution of the protein was different. Nevertheless, in both cases, the total cellular expression of CBP70 was enhanced during cell differentiation. Another important result is the finding that CBP70 behaviour was totally different when HL60 cells were induced to differentiate into macrophages or granulocytes. These data could therefore suggest that CBP70 is involved in phagocytic cell differentiation. Moreover, we show that an additional 60 kDa polypeptide (p60), recognized by the anti-CBP70 serum, is expressed in HL60 cells differentiated into macrophages or granulocytes as well as in healthy monocytes or MDM but not expressed in undifferentiated HL60 cells. Although CBP70 and p60 appeared to be closely related polypeptides, their relationship remains to be precised. These findings are discussed with regard to data available on galectin-3.

Key words: carbohydrate-binding protein70, HL60, nucleus, macrophages, lectins

It is now well established that lectins, also called carbohydrate-binding proteins (CBPs), exist not only at the cell surface and in the cytosol but also in the cell nucleus [reviewed in Harrison, 1991; Wang et al., 1991; Hubert and Sève, 1994]. Since lectins were detected cytochemically in the animal cell nucleus [Hubert et al., 1985; Sève et al., 1985, 1986], six lectins (CBP14, CBP22, CBP33, CBP35, CBP67, and CBP70) have been purified from isolated nuclei or subnuclear fractions.

CBP67 and CBP33 recognize glucose [Schröder et al., 1992; Lauc et al., 1994], CBP22 is an N-acetylglucosamine (GlcNAc)-specific lectin [Felin et al., 1994] and CBP70 binds to glucose and GlcNAc residues with a better affinity for the latter [Felin et al., 1994]. CBP35, initially identified in whole cellular extracts [Roff and Wang, 1983], and CBP14 [Cuperlovic et al., 1995] specifically bind to galactose and lactose residues. On the basis of sequence homologies [re-

Abbreviations used: ATRA: all-*trans*-retinoic acid; AU: arbitrary unit; BCIP: 5-bromo-4-chloro-3-indolyl phosphate; BSA: bovine serum albumin; CBP: carbohydrate-binding protein; EDTA: ethylenediaminetetraacetic acid; FCS: fetal calf serum; FITC: fluorescein-isothiocyanate; GlcNAc: N-acetylglucosamine; IgG: immunoglobulin G; MDM: monocyte-derived macrophages; NBT: nitroblue tetrazolium; PBS: phosphate-buffered saline; p60: 60 kDa polypeptide; PMA: phorbol 12-myristate 13-acetate; SA: sodium azide; SDS-PAGE: sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TBS: Tris buffer saline.

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Address reprint requests to Jean Hubert, INSERM U180, Centre Universitaire des Saints-Pères, Université René-Descartes, 45, rue des Saints-Pères, 75270 Paris Cedex 06, France.

viewed in Hughes, 1994], CBP35 has been found to be homologous to several lectins discovered independently at the cell surface; all these lectins are now referred to as galectin-3 [Barondes et al., 1994]. Galectin-3 was found to be present in a number of normal and tumoral animal cells and it is the most documented so far [reviewed in Anderson and Wang, 1992]. For instance, it is known that galectin-3 expression, both at the mRNA and protein levels, varies as a function of cell aging [Hamman et al., 1991; Lauc et al., 1993], cell proliferation [Agrwal et al., 1989; Raz et al., 1990], and phenotype transformation following oncogene transfection [Hébert and Monsigny, 1994]. It was also found to be associated with nuclear hnRNP [Laing and Wang, 1988] and snRNP [Wang et al., 1992] complexes. In agreement with these data, in vitro experiment results indicated that galectin-3 is a splicing factor [Wang et al., 1992; Dagher et al., 1995] and a recent immunoelectron microscopy study suggested that it could also be involved in mRNA transport [Hubert et al., 1995]. Moreover, this lectin appeared to be predominantly nuclear or cytosolic depending upon the mitotic activity of 3T3 fibroblasts [Moutsatsos et al., 1987; Hamman et al., 1991] or the physiological state of colonic cells [Lotz et al., 1993]. CBP67 was also identified as a component of nuclear RNP complexes of rat liver nuclei [Schröder et al., 1992] and some data strongly suggested that it could act in mRNA transport [Schröder et al., 1992; Lauc et al., 1993]. In comparison, very little is known about other lectins identified to date in the nucleus. In this context, previous identification of CBP70 in undifferentiated HL60 cell nuclei [Sève et al., 1993] prompted us to initiate further investigations on this lectin.

Although CBP70 and galectin-3 can be isolated as a complex from undifferentiated HL60 cell nuclear extracts subjected to glucose affinity chromatography [Sève et al., 1993], the present study has been limited to CBP70 analysis. Indeed, we decided to isolate CBP70 by GlcNAc affinity chromatography, since CBP70 affinity for GlcNAc was recently reported to be better than for glucose [Felin et al., 1994]. Under such affinity chromatography conditions, CBP70 and galectin-3 cannot be coisolate because GlcNAc-binding to CBP70 disrupts CBP70-galectin-3 interaction [Felin et al., 1994] as does lactose-binding to galectin-3 [Sève et al., 1993, 1994].

The aims of this study were, therefore, to determine 1) whether CBP70 was expressed in the nucleus of differentiated leukemic HL60 cells and healthy cells of the macrophagic lineage; 2) whether CBP70 was confined to the nucleus or also expressed in the cytosol; and 3) whether its intracellular expression varied according to cell differentiation and/or malignant process.

The results presented here demonstrated that CBP70 can be expressed in the nucleus and the cytosol of leukemic or healthy cells but with striking differences depending on both normal or pathological state of cells and cell differentiation. Furthermore, a 60 kDa polypeptide, recognized by the anti-CBP70 serum, was visualized only in nuclear and cytoplasmic extracts of differentiated leukemic cells, healthy monocytes, and in vitro monocyte-derived macrophages, suggesting an involvement of p60 in some physiological process of phagocytic cells.

MATERIALS AND METHODS

Cell Culture and Differentiation

Myeloblastic leukemia HL60 cells (from Dr. C. Chomienne, Hôpital Saint-Louis, Paris, France) were grown in suspension in RPMI 1640 medium (Gibco, Paisley, Scotland) supplemented with 10% heat-inactivated fetal calf serum (FCS; Gibco), 2 mM L-glutamine (Gibco), 50 IU/ml of penicillin and 50 μg/ml of streptomycin (Gibco). Cells were incubated at 37°C in a humidified 5% CO_2 atmosphere and maintained at 2×10^5 to 1×10^6 cells/ml by resuspending the cells in fresh culture medium every 3 days. Cells were used between passages 15 and 40.

HL60 cells can be differentiated either into monocytes/macrophages [Huberman and Callaham, 1979; Rovera et al., 1979] or granulocytes [Collins et al., 1979; Breitman et al., 1980] upon treatment with various drugs. Thus, monocytic/macrophagic differentiation was induced by treating HL60 cells at a density of 5×10^5 cells/ml with 50 ng/ml of phorbol 12-myristate 13-acetate, (PMA; Sigma, St. Louis, MO) for 48 or 72 h. After removing the culture medium, adherent differentiated cells were detached with 0.05% trypsin and 0.02% ethylenediaminetetraacetic acid solution (trypsin-EDTA; Gibco). The reaction was stopped by adding 1 mg/ml of soybean trypsin inhibitor. In some experiments, cells were detached by scraping and harvested. Granulocytic differentiation was induced by treating cells at a density of 5×10^5 cells/ml with 10^{-6} M all-*trans*-retinoic acid (ATRA; Sigma) for 48 or 72 h. The cells, grown in

suspension, were collected by low-speed centrifugation.

DNA synthesis was assessed by (methyl-^{3}H)-thymidine (specific activity: 636 GBq/mmol; Amersham; Les Ulysses; France) incorporation. Briefly, cells were cultured at a density of 5×10^5 cells/ml with or without differentiation promotors, transferred into 96-well polystyrene microtiter plates (100 µl/well) and pulsed with 1 µCi of (^{3}H)-thymidine/well during the last 6 h of every culture period. Undifferentiated HL60 cells and cells differentiated into granulocytes, both growing in suspension, were transferred onto paper filters by using an automated Skatron harvester 3401 (Lierbyen; Norway); the filters were extensively washed and then dried. Adherent PMA-differentiated cells were washed twice with Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline (PBS^-), pH 7.4, and lysed by overnight incubation at 4°C with 100 µl of 0.2 N NaOH/well. The amount of radioactivity was determined by counting filters or cell lysates in an emulsifier-safe liquid (Packard Instruments, Rungis, France) with a Minaxi β Tri-Carb 4000 series liquid scintillation counter (United Technologies; Packard).

Monocyte and Monocyte-Derived Macrophages (MDM)

Blood was obtained from healthy volunteers at the Seine-Saint-Denis or the Pitié-Salpêtrière Blood Transfusion Center. Mononuclear cells, isolated from the buffy coat by Ficoll-Hypaque centrifugation [Boyüm, 1968] and adjusted to 2–3 $\times$ 10^6 cells/ml in 2.5–6 ml of RPMI 1640 containing 10% heat-inactivated human AB serum (Gibco) and 20% FCS, were deposited into culture flasks (Costar, Cambridge, MA) for 12 h. In some experiments, nonadherent cells were removed by several washes in PBS^- and adherent monocytes were detached by scraping and harvested. In other experiments, mononuclear cells were maintained in the culture flasks for 5 days. Under such culture conditions, the differentiation of monocytes into macrophages is induced by lymphokines secreted by autologous lymphocytes [Valentin et al., 1991, 1994]. After removing nonadherent cells by vigorous washing in PBS^-, adherent MDM were cultured in the presence of 20% FCS for 48 h and detached with trypsin-EDTA or by scraping and collected.

For all cell cultures, cell viability was assessed by trypan blue dye exclusion. Cell differentiation was evaluated as follows: 1) cells were subjected to nitroblue tetrazolium (NBT) reduction [Yen and Albright, 1984] which is generally used to assess differentiation of HL60 cells into monocytes/macrophages or granulocytes; 2) the binding of various monoclonal antibodies raised against surface antigens of phagocytic cells was analyzed by flow cytofluorometry as described below; 3) differentiation into mature cells was monitored by electron microscopy and by fluorescence microscopy after acridine-orange staining. (For details, see below).

Electron Microscopy

Pellets of cells or membrane-depleted nuclei were fixed with 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2, postfixed with 1% osmium tetroxide in the same buffer, dehydrated in acetone, and embedded in Epon 812. Ultrathin sections were contrasted with uranyl acetate and lead citrate.

Fluorescence Microscopy

Cells were washed in phosphate buffer containing 1 mM $CaCl_2$ and 1 mM $MgCl_2$ (PBS), fixed with 3.7% formaldehyde in PBS for 20 min at 4°C, permeabilized for 10 min at −20°C with acetone kept at −20°C, washed in PBS, then in PBS containing 1% bovine serum albumin (Sigma) (PBS-BSA) and incubated for 1 h at 4°C in the presence of an anti-CBP70 serum, previously characterized [Sève et al., 1994], diluted 1/200 in PBS-BSA. The cells were pelleted by low-speed centrifugation, washed in PBS-BSA and incubated with FITC-conjugated goat anti-rabbit IgG, diluted 1/100 in PBS-BSA for 1 h at 4°C. The cells were washed twice in PBS-BSA, and once in PBS, suspended in a drop of glycerol-PBS solution and spread between slide and coverslip prior to observation under the fluorescence microscope. Two controls were performed: 1) omission of the primary antibody; and 2) use of the preimmune serum instead of the specific antiserum. In some experiments, living cells were observed after acridine orange staining.

Flow Cytofluorometric Analysis

Adherent cells were detached by scraping, harvested, then pelleted by low-speed centrifugation. Cells grown in suspension or as monolayers were incubated with monoclonal antibodies directed against surface antigens either of differentiated phagocytic cells (CD14 and CD11b) or

lymphocytes (CD19 and CD3) as follows: for indirect immunofluorescence assays, cells were washed PBS, then in PBS containing 1% BSA and 1% sodium azide (Merck) (PBS-BSA-SA) prior to incubation for 1 h at 4°C in the presence of monoclonal antibodies CD11b (CR3, clone Bear-1; IgG_1; Tebu, Le Perray-en-Yvelines, France) or CD14 (anti-Leu M3; IgG_{2b}; Becton-Dickinson, Mountain View, CA), both diluted 1/5. After washing in PBS-BSA-SA, the cells were incubated with fluorescein isothiocyanate (FITC)-conjugated anti-mouse $(Fab)'_2$ IgG diluted 1/400 in PBS-BSA-SA. Control experiments: cells were incubated with mouse isotype IgG_1 (Immunotech, Marseille, France) or mouse isotype IgG_{2b} (MOPC 41; Sigma) both diluted 1/5, instead of CD11b or CD14, respectively, or with the buffer alone. For direct immunofluorescence assays, cells were incubated for 1 h at 4°C in PBS-BSA-SA with FITC-coupled anti-Leu-4 (CD3-FITC; IgG_1; Becton-Dickinson) in PBS-BSA-SA or FITC-IOB4a (CD19; IgG_1; Immunotech), both diluted 1/5 in PBS-BSA-SA. The isotype control was FITC-conjugated IgG_1 (Sigma). The fluorescence intensity of 5,000 cells from each experiment was recorded using a FACScan analyzer (Becton-Dickinson).

Cell Fractionation and Protein Extraction

Membrane-depleted nuclei were isolated according to the method described in [Facy et al., 1990]. Briefly, cells were homogenized in 10 mM Tris-HCl, pH 7.5, containing 20 mM KCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, and 0.2 mM spermidine (TKCM buffer) in the presence of the following protease inhibitors: 10 μg/ml each of leupeptin, pepstatin A, chymotrypsin, and 0.17 IU/ml of aprotinin. Triton X-100 was added to a final concentration of 0.5%. Membrane-depleted nuclei were pelleted by centrifugation at 1,000*g* for 10 min. The supernatant was centrifuged at 100,000*g* for 1 h at 4°C. The supernatant containing cytoplasmic proteins was then dialyzed against PBS^- at 4°C overnight. This dialyzed supernatant will be named cytoplasmic extract throughout the text. Nuclear proteins were extracted from membrane-depleted nuclei with NaCl adjusted to 2 M (final concentration) in TKCM buffer for 1 h at room temperature. Excess NaCl was removed by overnight dialysis against PBS^- and the proteins were concentrated on a Centricon 10 filter; (Amicon, Beverly, MA).

Affinity Chromatography

CBP70 was isolated by affinity chromatography using immobilized GlcNAc as previously described [Felin et al., 1994]. Briefly, N-acetyl-β-D-glucosamine phenylisothiocyanate (Sigma) was immobilized on acetone-dehydrated Trisacryl GF 2,000 M (IBF-Biotechnics, Villeneuve-La-Garenne, France). Nuclear or cytoplasmic extracts were adjusted to 0.7 mM $CaCl_2$ and 0.5 mM $MgCl_2$ prior to affinity chromatography. CBP70, bound to the immobilized sugar, was eluted with 0.2 M free GlcNAc.

Protein Electrophoresis

The polypeptides of total protein extracts or GlcNAc-eluted fractions from affinity chromatography columns were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on 12% acrylamide mini-slab running gels under denaturing conditions, according to Laemmli's procedure [Laemmli, 1970].

Immunoblotting Analysis

Polypeptides, resolved by SDS-PAGE, were transferred electrophoretically (60 V at room temperature for 1 h) onto Immobilon-P membranes (Millipore, Bedford, MA) in 10 mM (cyclohexylamino)-1-propane sulfonic acid, pH 10.7 (Caps buffer). The Immobilon membrane was incubated overnight in saturating buffer, 20 mM Tris-HCl, pH 7.4, 0.5 M NaCl (TBS) containing 5% milk powder. The membrane was then washed three times in TBS containing 0.5% Tween 20 (TBS-Tween) and incubated for 3 h with anti-CBP70 diluted 1/1,000 in TBS-Tween. The membrane was washed three times in TBS-Tween, incubated for 1 h at 4°C with alkaline phosphatase-conjugated goat anti-rabbit IgG (Sigma) at a dilution of 1/8,000, washed three times in TBS-Tween and finally in 0.1 M Tris-HCl, pH 9.5, containing 100 mM NaCl and 5 mM $MgCl_2$ (Tris buffer) before staining with 5-bromo-4-chloro-3-indolyl phosphate (BCIP) and NBT (Sigma). Controls were incubated with either preimmune serum or alkaline phosphatase-conjugated goat anti-rabbit IgG or only BCIP and NBT.

In some experiments, a Western blot chemiluminescence reagent for nonradioactive detection of proteins was used. (Du Pont NEN Research products, Boston, MA). The staining intensity of immunoblot bands, reported in arbi-

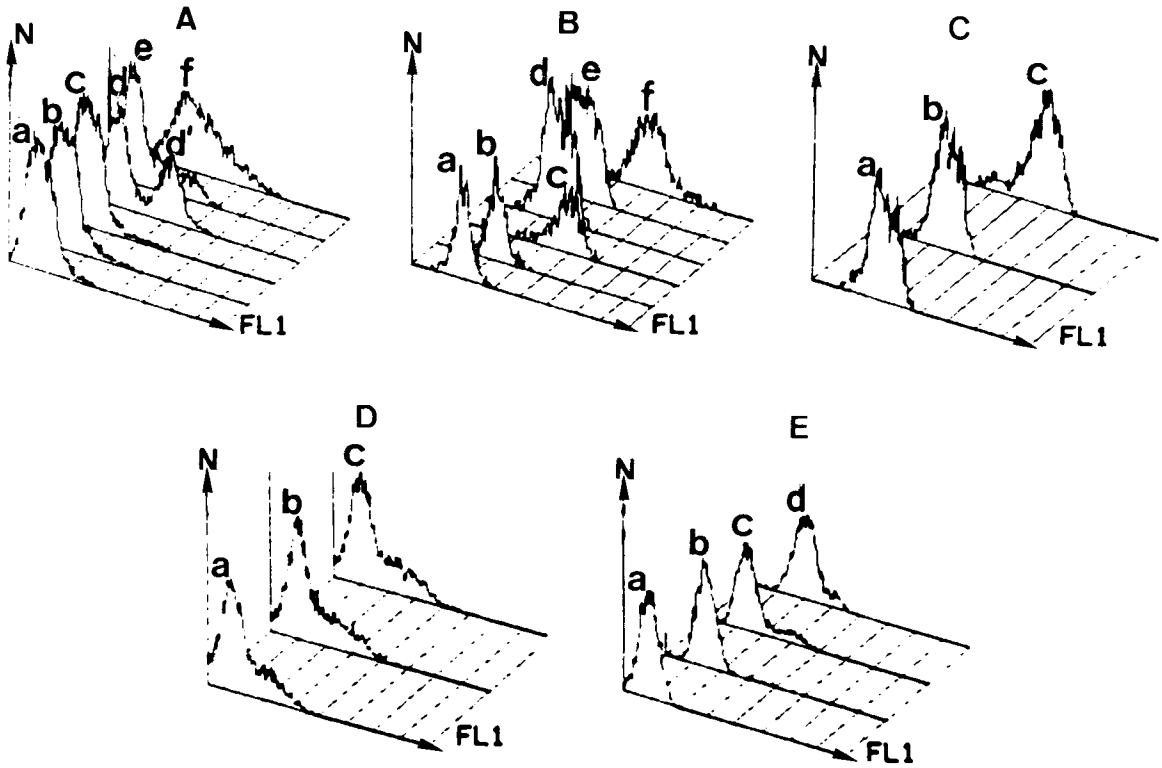

Fig. 1. Quantitative flow cytofluorometric analysis of cell-surface markers CD11b (**A, B**), CD14 (**C, D**), CD3 and CD19 (**E**) using monoclonal antibodies. *N:* Cell number; *FL1:* fluorescence intensity expressed in arbitrary units (log_{10} scale). Peaks represent the mean values of the fluorescence intensity of 5,000 cells. A: Undifferentiated cells incubated with buffer alone (**a**), FITC-conjugated antibody alone (**b**), mouse isotype IgG_1 (**c**), or anti-CD11b (**d, d'**). PMA-differentiated cells incubated with mouse isotype IgG_1 (**e**), or with CD11b (**f**). B: Healthy monocytes and monocyte-derived macrophages (MDM) incubated, respectively, with buffer alone (**a, d**), mouse isotype IgG_1 (**b, e**), or CD11b (**c, f**). C: MDM incubated with buffer alone (**a**), mouse isotype IgG_{2b} (**b**), or CD14 (**c**). D: PMA-differentiated cells incubated with buffer alone (**a**), mouse isotype IgG_{2b} (**b**) or CD14 (**c**). E: MDM incubated with buffer alone (**a**), FITC-IgG_1 (**b**), CD19 (**c**), or CD3 (**d**).

trary units, was quantified by immunoblot densitometric scanning.

RESULTS

Cell Viability and Assessment of Cell Differentiation

As determined by trypan blue dye exclusion, cell viability of untreated HL60 cells was 98%, those of HL60 cells treated with PMA for 48 or 72 h or treated with ATRA for 48 or 72 h were 85% or 82% and 80% or 81%, respectively; MDM viability was 87%.

In the context of the present study, it was very important to precisely determine the proportion of cells either spontaneously differentiated [Breitman et al., 1980; Dalton et al., 1988] or differentiated under experimental conditions. Results of quantitative analyses by flow cytofluorometry showed that up to 25% of cells could be CD11b positive in undifferentiated HL60 cell cultures (Fig. 1A, d') but, only culture batches containing from 1 to 10% of CD11b positive cells were used. In contrast, the percentage of cells differentiated upon various treatments was most often similar from one experiment to another. Thus, about 80% of HL60 cells treated with PMA for 72 h (Fig. 1A, f), 90% of healthy monocytes (Fig. 1B, c) and 92% of MDM (Fig. 1B, f) were CD11b positive. Most MDM were CD14 positive (Fig. 1C, c), whereas PMA-differentiated HL60 cells were CD14 negative (Fig. 1D, c). Very few MDM were CD19 or CD3 positive (Fig. 1E, c, d), indicating that cultured MDM were not contaminated with B or T lymphocytes. The binding of IgG_1-, IgG_{2b}-, or IgG_1-FITC was negligible as can be seen in the corresponding peaks in Figure 1.

In agreement with results of quantitative analyses of CD11b binding, 1 to 20% of cells, according to undifferentiated HL60 cell culture batches, were NBT positive. These NBT positive cells were not adherent, suggesting that they

were differentiated into granulocytes. That was confirmed by observations in fluorescence microscopy of aliquots from these culture batches, after acridine orange staining, since the number of NBT positive cells approximatively corresponded to this of cells exhibiting a polylobated nucleus which characterizes granulocytes. After 48 or 72 h of PMA treatment, 85% of the cells had become NBT positive and adherent; 70% of the cells treated with ATRA for 48 or 72 h were NBT positive but not adherent.

Phenotypic differentiation of cells was also monitored at the ultrastructural level. Healthy monocytes (Fig. 2a) exhibited a nucleus most often reniform, a well-developed Golgi apparatus generally localized in the concave invagination of the nucleus and only one nucleolus per nucleus. MDM appeared to be larger than monocytes and they exhibited an irregular-shaped nucleus which usually contained a single nucleolus (Fig. 2b). The rough endoplasmic reticulum was more extensively developed than in monocytes. In addition, the cytoplasm of some MDM contained numerous dense granules (Fig. 2c). Very few contaminating lymphocytes were observed.

Undifferentiated HL60 cells possessed a large, spherical nucleus containing a slightly condensed chromatin network and 1 to 3 nucleoli (Fig. 3a). After 48 h of incubation, many HL60 cells treated with PMA looked like healthy monocytes (compare Fig. 3b and Fig. 2a). After 72 h of treatment, some cells exhibited a monocyte phenotype, others an MDM phenotype (compare Fig. 3c and Fig. 2b) but dense cytoplasmic granules visible in MDM (Fig. 2c) were rarely seen in PMA-differentiated HL60 cells. When HL60 cells were treated for 72 h with ATRA, most of them exhibited typical nuclear aspects of granulocytes characterized by a polylobated nucleus (Fig. 3d). ATRA-differentiated cells contained either a small poorly developed nucleolus or a nucleolus looking like that of undifferentiated cells (Fig. 3d).

DNA synthesis, evaluated by (^{3}H)-thymidine incorporation, had almost completely stopped, after 48 h of culture, in the presence of PMA, being only about 14% of that of untreated HL60 cells, and remained stable thereafter (Table I). ATRA treatment led to a 36% reduction in mitotic activity at 48 h and untreated undifferentiated cells reached this same level of radiolabel incorporation 24 h later, when the (^{3}H)-thymidine uptake of ATRA-differentiated cells had declined an additional 32% (Table I). This difference between PMA- and ATRA-differentiated cells is not surprising if we consider that in the latter case the cells undergo several rounds of DNA synthesis during the differentiation [Gezer et al., 1988]. Taking into account that most ATRA-treated cells showed typical aspects of apoptosis at 92 h, and that cells exhibiting a macrophagic phenotype were observed after 72 h of PMA treatment, we have used HL60 cells treated for 72 h with PMA or ATRA in all experiments.

Immunodetection of CBP70 in Nuclear and Cytoplasmic Extracts Using Anti-CBP70 Serum

The cleanness of isolated nuclei was carefully controled by electron microscopy prior protein extraction. Examination of multiple cell preparations showed that nuclei were generally devoid of their nuclear envelope and cytoplasmic remnants, and the ultrastructures of the nucleoplasm and the nucleoli were relatively well preserved (Fig. 4a, b).

Two polypeptides of 70 kDa and 80 kDa were visualized in immunoblots of total cytoplasmic extracts of undifferentiated HL60 cells (Fig. 5a), while only CBP70 was visualized in nuclear extracts of these cells (Fig. 5b). In contrast, a major 70 kDa polypeptide and an additional minor 60 kDa polypeptide (p60) were detected in the nuclear extract of PMA-differentiated cells (Fig. 5d). The immunoblotting pattern of the corresponding cytoplasmic extracts was identical to that of undifferentiated cells (compare Fig. 5a and Fig. 5c).

To ascertain whether the 70 kDa polypeptide immunodetected in total protein extracts of PMA-differentiated cells possessed the same sugar-binding ability as that of CBP70, nuclear and cytoplasmic extracts of undifferentiated and PMA-differentiated cells were subjected to affinity chromatography using immobilized GlcNAc. Then, GlcNAc-eluted fractions were immunoblotted with anti-CBP70. No polypeptide was recognized by the antiserum in the GlcNAc- eluted fraction of a nuclear extract prepared from 3×10^7 undifferentiated cells (Fig. 6a) whereas two major polypeptides corresponding to CBP70 and p60 respectively were identified in the GlcNAc-eluted fraction of a nuclear extract of 3×10^7 PMA-differentiated cells (Fig. 6b). Results of several similar analyses showed that the antiserum generally recognized both CBP70 and p60 in this fraction, while only CBP70 was visual-

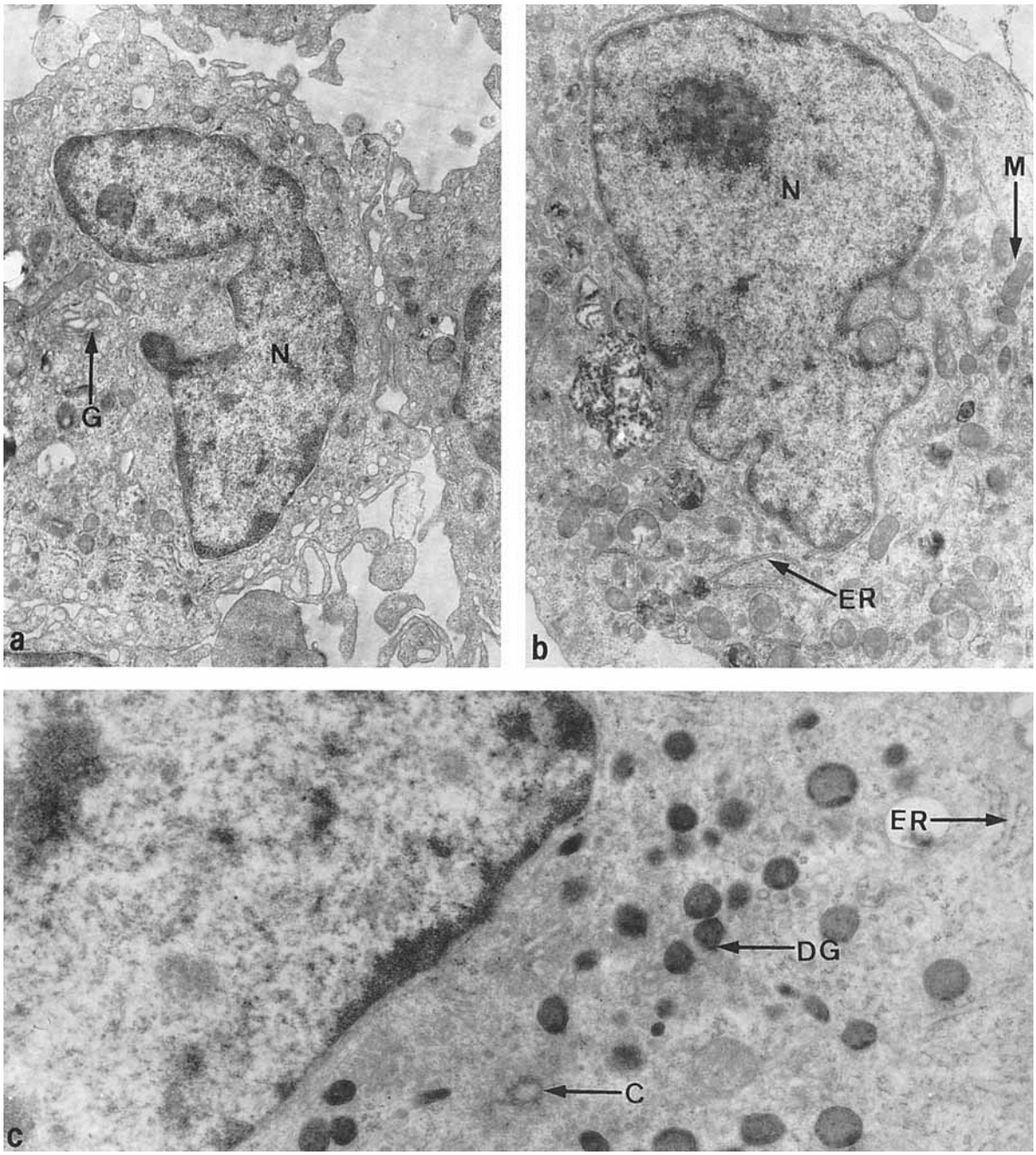

Fig. 2. Electron micrographs showing the ultrastructure of healthy human monocytes and MDM. **a:** Monocyte exhibiting a reniform nucleus (*N*) and a well-developed Golgi apparatus (*G*) located in the concave invagination of the nucleus. **b:** This section of a MDM shows the irregularly shaped nucleus, the extensive development of the rough endoplasmic reticulum (*ER*) and numerous mitochondria (*M*). **c:** Cytoplasmic dense granules (*DG*) observed in MDM. C: Centrosome. (a × 9,000; b × 10,500; c × 20,000.)

ized in immunoblots of GlcNAc-eluted fractions obtained from a nuclear extract of 1×10^9 undifferentiated cells (Fig. 6c). Thus, the results of these experiments indicated that the level of CBP70 expression was higher in the nucleus of PMA-differentiated cells than in those of undifferentiated cells. The densitometric scanning of immunoblots (Table II) showed that the immunolabeling intensity of CBP70 was even stronger in the fraction eluted from the extract prepared with 3×10^7 PMA-differentiated cells than in that using 1×10^9 undifferentiated cells.

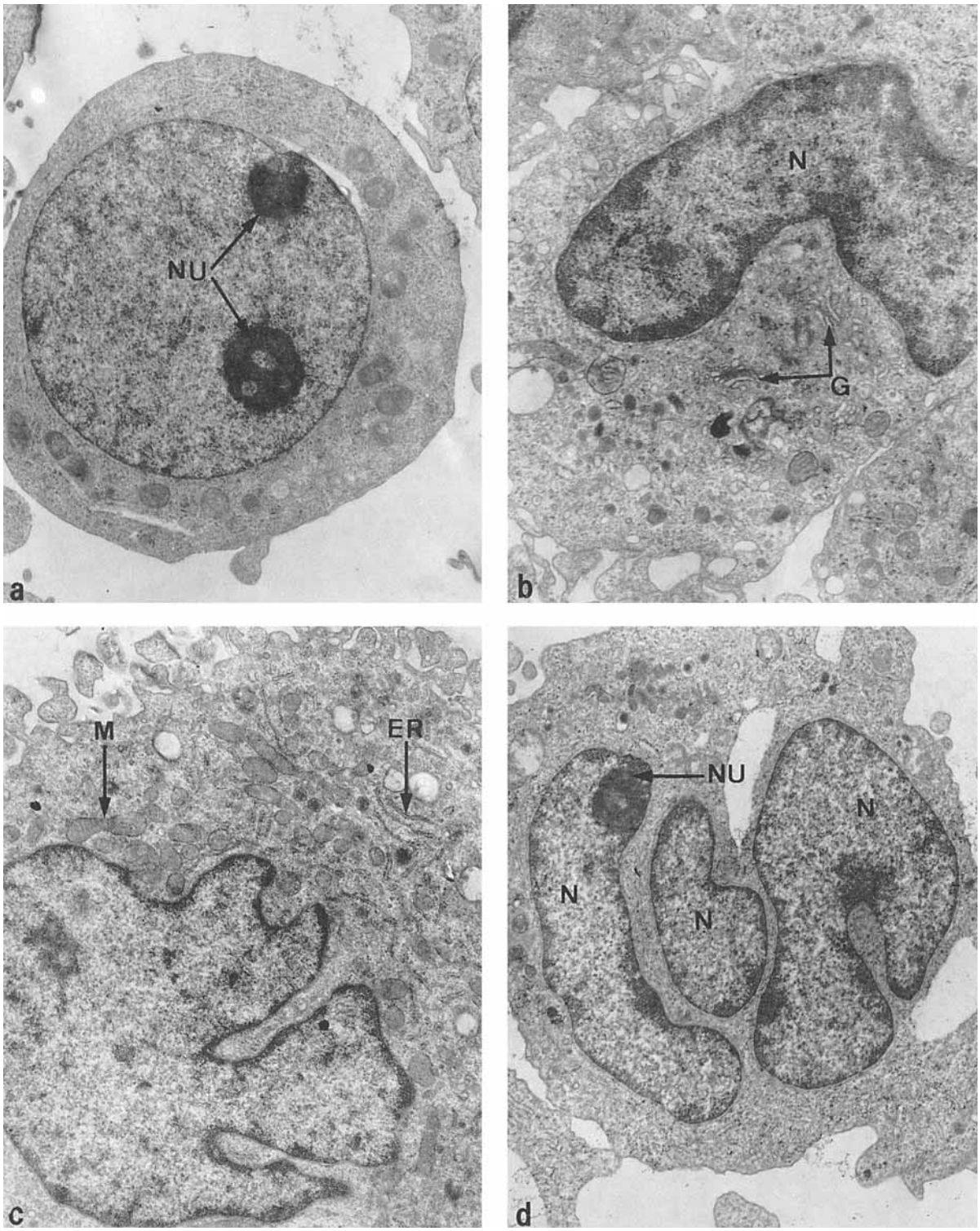

Fig. 3. Ultrastructure of undifferentiated and differentiated HL60 cells. **a:** Undifferentiated cell exhibiting a large spherical nucleus containing 2 nucleoli (*NU*). **b:** Cell differentiated into a monocyte-like cell after 48 h of PMA treatment. Note the reniform nucleus (*N*) and the Golgi apparatus (*G*) in the concave invagination of the nucleus. **c:** Cell differentiated into a macrophage-like cell after 72 h of PMA treatment exhibiting a well-developed rough endoplasmic reticulum (*ER*) and numerous mitochondria (*M*). **d:** Cell differentiated into a granulocyte. Note the typical polylobed nucleus (N) containing 1 nucleolus (NU). (a, b, c × 12,000; d × 8,000.)

TABLE I. Representative Experiment of the Mitotic Activity of Undifferentiated, PMA-Treated, and ATRA-Treated HL60 Cells

	^{3}(H)-thymidine uptake after initiation of treatment (cpm/10^5 cells)	
Treatment	48 h	72 h
None	7,144	4,529
PMA	1,017	983
ATRA	4,578	3,098

To know whether these variations, including p60 expression, were specifically related to macrophagic differentiation pathway, the nuclear expressions of CBP70 and p60 were compared in PMA- and ATRA-differentiated HL60 cells. Neither CBP70 nor p60 were detected in GlcNAc eluted fractions from nuclear extracts of 3×10^7 ATRA-differentiated cells but they were visualized when nuclear extracts were prepared from 1×10^9 cells (Fig. 6d). However, even in this case, the levels of expression of both proteins appeared to be lower than those observed in PMA-differentiated cells (Table II). It is to note that CBP70 and p60 labeling intensities were almost similar either in ATRA- or PMA-treated cell nuclear extracts (Fig. 6b, d).

As far as the cytoplasmic 70 kDa polypeptide is concerned, analysis of GlcNAc-eluted fractions of cytoplasmic extracts of 3×10^7 undifferentiated or PMA-differentiated cells showed that this protein corresponds to CBP70 (Fig. 7a, b). The 80 kDa polypeptide recognized by the antiserum in total cytoplasmic extracts was not retained on the affinity column, whereas, in either case, CBP70 and p60 were visualized, but the staining intensity of p60 was very low compared to that of CBP70. Densitometric scanning of immunoblots (Table II) revealed that the labeling intensity of CBP70 band was about the same in immunoblots of GlcNAc-eluted fractions from the cytoplasmic extracts of 3×10^7 undifferentiated or 3×10^7 PMA-differentiated HL60 cells. We failed to detect CBP70 and p60 in immunoblots (not shown) of fractions eluted from cytoplasmic extract prepared either from of 3×10^7 or 1×10^9 ATRA-differentiated cells (Table II).

Even if PMA-differentiated HL60 cells became mitotically inactive and exhibited monocyte/macrophage phenotype, they could not be considered as true healthy cells. Nuclear and cytoplasmic extracts of 3×10^7 healthy monocytes and MDM were therefore subjected to GlcNAc affinity chromatography, then analyzed by immunoblotting. No polypeptide was visualized either in nuclear or cytoplasmic fractions of monocytes. CBP70 was faintly distinguishable in the MDM cytoplasmic fractions of cells, but not visible in the corresponding nuclear fraction (not shown). All immunoblotting experiments described above being performed using a colorimetric enzymatic method, CBP70 and p60 could not be detected because they were weakly expressed in healthy monocytes and MDM. Similar immunoblotting analyses were therefore carried out using a more sensible system of detection, based on chemiluminescence (see Material and Methods). Under such conditions, both CBP70 and p60 were visualized in GlcNAc-eluted fractions of nuclear and cytoplasmic extracts of monocytes (Fig. 8a, b) or MDM (Fig. 8c, d). As judged by densitometric scanning of immunoblots (Table II), in both cases, the labeling intensities of CBP70 and p60 bands were higher in cytoplasmic fractions than in nuclear ones (Table II). Furthermore, cytoplasmic and nuclear expressions of CBP70 and p60 appeared to be increased in MDM with regard to monocytes (Table II).

Fluorescence Microscopy Analysis of Anti-CBP70 Binding in Whole Cells

When cells were permeabilized and subsequently incubated with anti-CBP70 and the second FITC-coupled antibody, the nucleus and the cytoplasm, easily distinguishable by phase-contrast microscopy (Fig. 9a, b, c), were labeled in undifferentiated HL60 cells (Fig. 9d), PMA-differentiated cells (Fig. 9e), healthy monocytes (not shown), and MDM (Fig. 9f). However, in most undifferentiated HL60 cells, nuclear labeling was weaker than that noted in the cytoplasm (Fig. 9d). In PMA-differentiated cells the labeling intensity of the nucleus was slightly higher than that observed in the cytoplasm (Fig. 9e) whereas the cytoplasm appeared to be a little more labeled than the nucleus in MDM (Fig. 9f). Moreover, labeling intensity of nuclei was obviously stronger in PMA-treated cells than in undifferentiated HL60 cells (compare Figs. 9d and e). The nucleoli, visible by phase-contrast microscopy (Fig. 9a, b), were not labeled (Fig. 9d, e). In ATRA-differentiated cells the nucleus was clearly labeled while the cytoplasm exhibited only a faint fluorescence (not shown).

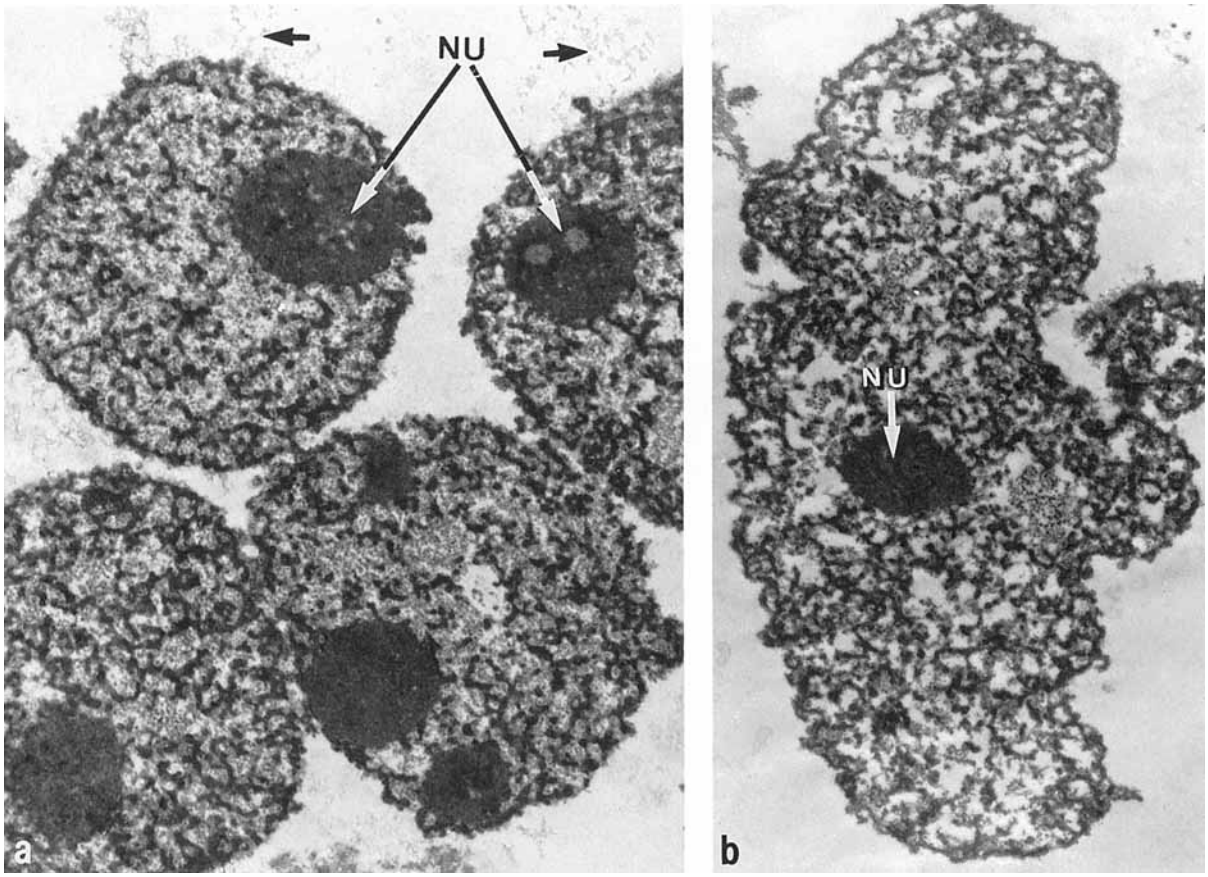

Fig. 4. Representative electron micrographs of membrane-depleted nuclei from undifferentiated HL60 cells (**a**) and MDM (**b**). In both cases, note the absence of cytoplasmic remnants and the nuclear envelope. The sparse material (*arrows*) visible around the nuclei probably corresponds to DNA fibers. *NU:* Nucleoli. ×8,000.

DISCUSSION

To date, the carbohydrate-binding protein CBP70 has been identified only in isolated nuclei or nuclear extracts of leukemic undifferentiated HL60 cells [Sève et al., 1993, 1994; Felin et al., 1994]. The results of the present study show that CBP70 is also present in the cytoplasm of these cells as well as in the nucleus and the cytoplasm of PMA-differentiated HL60 cells, healthy monocytes, and MDM. The presence of CBP70 in the cytoplasm raised the question to know whether CBP70 detected in nuclear extracts corresponded to a true nuclear component or a cytoplasmic contamination during nuclei isolation. The latter possibility can reasonably be excluded for the following reasons: 1) the nuclei isolation procedure used here has previously been shown to yield nuclei uncontaminated by soluble cytoplasmic proteins [Sève et al., 1993]; 2) the absence of cytoplasmic remnants was carefully controled by examining sec-

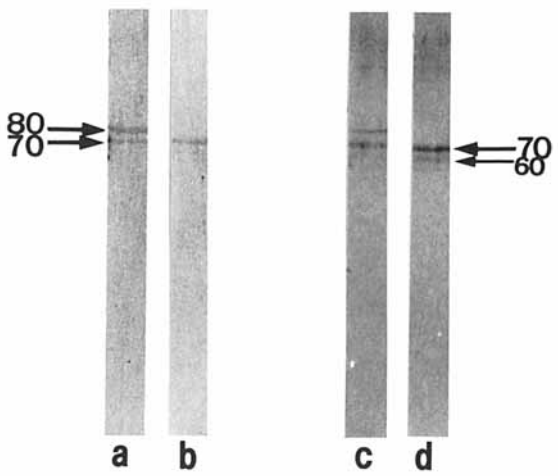

Fig. 5. Immunoblotting analysis of total nuclear and cytoplasmic protein extracts using anti-CBP70 serum. Approximately 20 µl of each extract were electrophoresed in a 12% acrylamide mini-slab gel prior to immunoblotting. The second antibody was revealed using a colorimetric enzymatic method. Cytoplasmic extracts of undifferentiated (**a**) and PMA-differentiated HL60 cells (**c**), nuclear extracts of undifferentiated (**b**), and PMA-differentiated HL60 cells (**d**).

TABLE II. Levels of Nuclear and Cytoplasmic Expressions of CBP70 and p60*

	Cell number	Cells	Nuclear extracts p60	Nuclear extracts CBP70	Cytoplasmic extracts p60	Cytoplasmic extracts CBP70
a	3×10^7	HL60	ND	ND	0.3	1.4
	1×10^9	HL60	ND	1	0.4	1.5
	3×10^7	HL60 + PMA	1.6	1.7	0.2	1.3
	1×10^9	HL60 + ATRA	1.3	1	ND	ND
b	3×10^7	Monocytes	0.2	0.4	0.9	1.2
	3×10^7	MDM	1	1.3	1.8	2

*The immunoblots of GlcNAc-eluted fractions from nuclear or cytoplasmic extract shown in Figures 6, 7, and 8 were subjected to densitometric scanning. Immunoblots were performed using anti-CBP70 serum and a second antibody revealed by a colorimetric enzymatic method (a) or by a chemiluminescent enzymatic method (b). The intensities of CBP70 and p60 bands are expressed as arbitrary units. ND: nondetected.

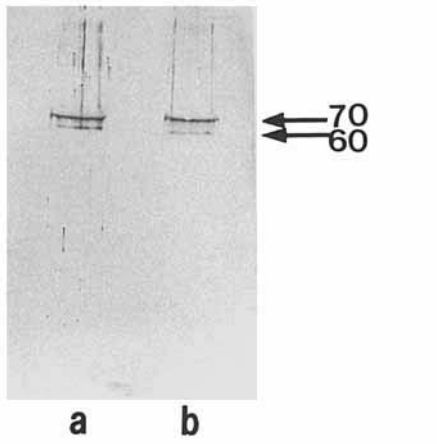

Fig. 7. Immunoblotting analysis of GlcNAc-eluted cytoplasmic polypeptides, with anti-CBP70 serum (colorimetric method). Undifferentiated (**a**) or PMA-differentiated HL60 cell extracts (**b**).

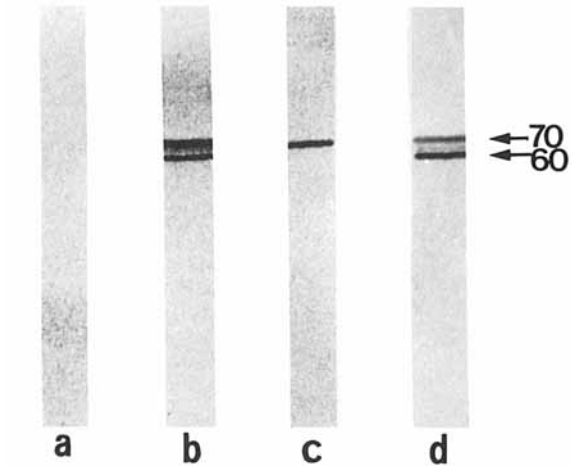

Fig. 6. Immunoblotting analysis of GlcNAc-eluted nuclear polypeptides, with anti-CBP70 serum (colorimetric method). Fraction eluted from a nuclear extract of 3×10^7 undifferentiated (**a**) or PMA-differentiated HL60 cells (**b**). Fraction eluted from a nuclear extract of 1×10^9 undifferentiated HL60 cells (**c**) or ATRA-differentiated cells (**d**). 20 µl of each concentrated fraction were loaded per lane.

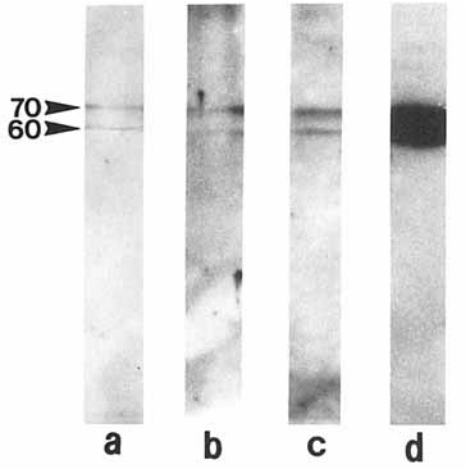

Fig. 8. Immunoblotting analysis of GlcNAc-eluted polypeptides from nuclear or cytoplasmic extracts of 3×10^7 healthy monocytes and MDM, with anti-CBP70 serum (chemiluminescent enzymatic method). Nuclear (**a**) and cytoplasmic (**b**) fractions of healthy monocytes. Nuclear (**c**) and cytoplasmic (**d**) fractions of MDM. 20 µl of each eluted fraction were loaded per lane.

tions through isolated nuclei at the ultrastructural level; and 3) results of immunoblotting analyses of nuclear and cytoplasmic extracts were concordant with intracellular immunostaining in whole cells.

The nuclear and cytoplasmic expressions of CBP70 were found to be different depending upon cell differentiation. Indeed, striking differences were observed between undifferentiated and differentiated HL60 cells, as well as between healthy monocytes and MDM. Furthermore, differences were also observed according to whether macrophages were derived from healthy monocyte or tumoral HL60 cells. The fact that CBP70 expression was not comparable in PMA-treated cells and MDM added to the absence of CD14 binding to the former, showed that PMA-differentiated HL60 cells do not exhibit all the features of healthy macrophages.

The difference in CBP70 expression between PMA- and ATRA-differentiated cells suggests that it could be involved in the phagocytic cell differentiation processes. However, CBP70 was mainly cytoplasmic in proliferative undifferentiated HL60 cells as well as in monocytes or MDM

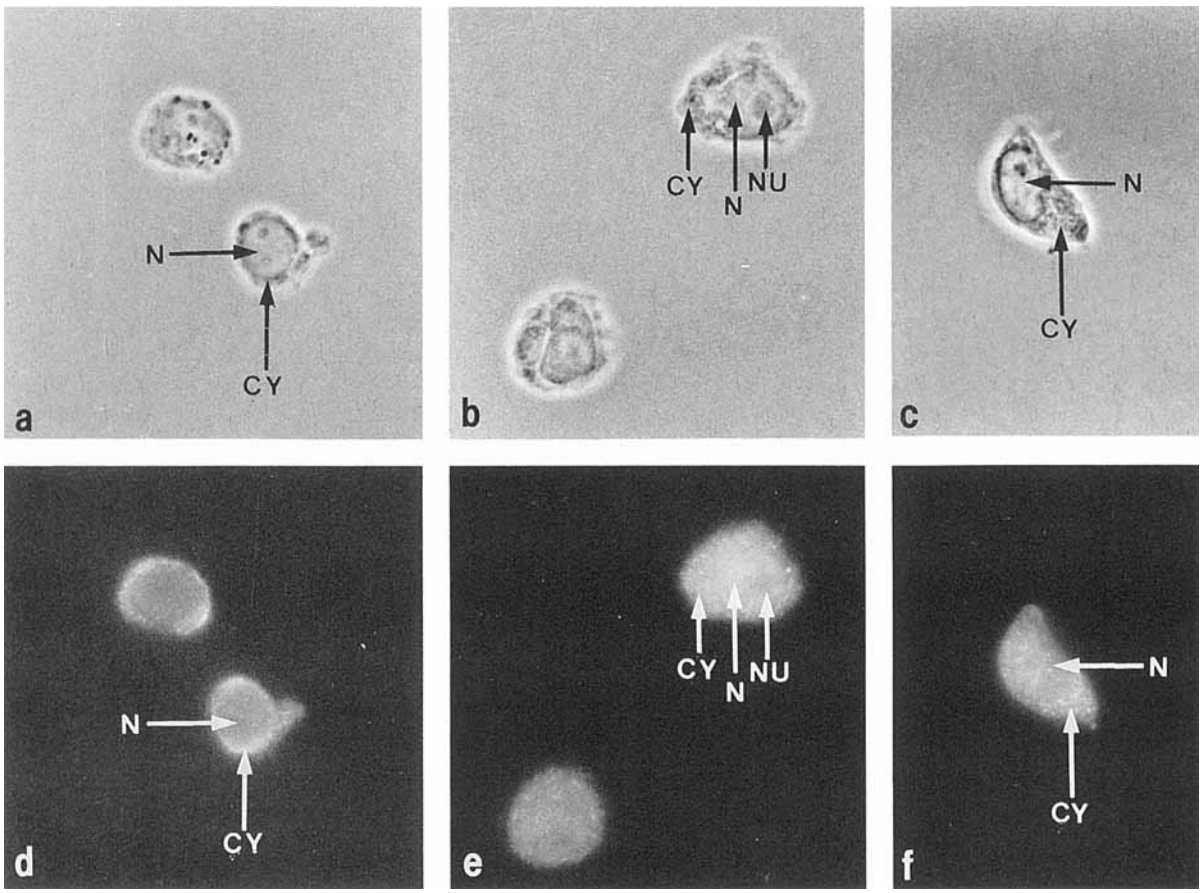

Fig. 9. Undifferentiated HL60 cells (**a, d**), PMA-differentiated HL60 cells (**b, e**) and MDM, (**c, f**) incubated with the anti-CBP70 serum followed by FITC-conjugated goat anti-rabbit IgG and observed under phase-contrast (a, b, c) or fluorescence microscopy (d, e, f), after permeabilization. *CY:* Cytoplasm; *N:* nucleus; *NU:* nucleolus. (×750.)

which are mitotically inactive cells and predominantly or solely nuclear in ATRA-differentiated cells which still exhibited a noticeable mitotic activity. Therefore, the intracellular distribution of CBP70 did not appear to correlate with the modulation of cell mitotic activity but rather with the cell type. This behaviour is quite reminiscent of this of galectin-3 [Hamman et al., 1991; Lotz et al., 1993].

The finding that the level of CBP70 expression was higher in the nucleus of PMA-differentiated cells than in the nucleus of undifferentiated ones and results of a previous study [Facy et al., 1990] could appear paradoxical. Indeed, that analysis of nuclear lectins, carried out using FITC-conjugated neoglycoproteins, including α-D-glucosyl-FITC-BSA but excluding α-D-N-acetylglucosamine FITC-BSA, in all cases, showed markedly lower neoglycoprotein binding to PMA-differentiated cell nuclei than to undifferentiated ones. However, it must be kept in mind that the affinity of CBP70 was recently found to be much stronger for GlcNAc than for glucose residues [Felin et al., 1994]. If we consider that several endogenous lectins can bind the same neoglycoprotein, the enhanced expression of CBP70, as revealed here with anti-CBP70, could have been masked in the earlier study by the decreased expression of other lectins able to recognize glucosyl-FITC-BSA.

Another unexpected result is the finding that the anti-CBP70 serum recognized an additional 60 kDa polypeptide (p60) in GlcNAc-eluted fractions of extracts from HL60 cells differentiated either into monocytes/macrophages or granulocytes as well as in healthy monocytes or MDM. In accordance with previous data [Sève et al., 1993; Felin et al., 1994] only CBP70 was visualized in the case of undifferentiated HL60 cells. This difference suggests that p60 expression might be related to phagocytic cell differentiation. However, increased expression of p60 in

MDM and PMA-treated HL60 cells with regard to monocytes and undifferentiated HL60 cells respectively inclines us to believe that p60 could be rather involved in a physiological process specific of mature phagocytic cells. The meaning of this cross-reaction is unclear. However, because p60 and CBP70 were specifically co-eluted from GlcNAc affinity chromatography columns, they could correspond to two different GlcNAc binding proteins with common epitopes. Another plausible explanation could be that they correspond to a different post-translational maturation of the same protein. Recent results obtained in our group, indicating that CBP70 is glycosylated [Rousseau et al., submitted], could support the latter hypothesis. Finally, preliminary results (unpublished data) are suggesting that CBP70 and p60 are associated with nuclear hnRNP/snRNP complexes. Thus, they might be two isoforms produced by alternative splicing like most hnRNPs [reviewed in Dreyfuss et al., 1993]. Experiments are currently in progress to characterize p60 with regard to CBP70.

Whatever p60 may be, the results of the present study demonstrate that CBP70, like galectin-3 [Moutsatsos et al., 1987; Lotz et al., 1993], is not a nuclear protein *stricto-sensu* and they evidence striking differences in level of expression as well as in the intracellular distribution of CBP70 depending upon the malignant or normal state of cells and phagocytic cell differentiation pathways. Finally, it is noteworthy that CBP70 expression was found to be increased during in vitro differentiation of healthy monocytes into macrophages as well as during differentiation of HL60 cells into monocytes/macrophages. Likewise, the total cellular level of galectin-3 increases as the monocytes differentiate into macrophages in vitro [Liu et al., 1995] or during differentiation of HL60 cells into monocytes/macrophages [Nangia-Makker et al., 1993]. If we consider that CBP70 and galectin-3 were found to be associated by protein-protein interaction in undifferentiated HL60 cell nuclei [Sève et al., 1993; Sève et al., 1994], it is tempting to speculate that CBP70 and galectin-3 could act as a complex in phagocytic cells. Another important question is to know whether these proteins have different functions in the cytoplasm and the nucleus or they merely shuttle between nuclear and cytoplasm as a regulatory system of their nuclear function. Experiments are under way to answer these questions.

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REFERENCES

Agrwal N, Wang JL, Voss PG (1989): Carbohydrate-binding protein 35. Levels of transcription and mRNA accumulation in quiescent and proliferating cells. J Biol Chem 264:17236–17242.

Anderson RL, Wang JL (1992): Carbohydrate-binding protein 35. Trends Glycosci Glycotechnol 4:43–52.

Barondes SH, Castronovo V, Cooper DNW, Cummings RD, Drickamer K, Feizi T, Gitt MA, Hirabayashi J, Hughes C, Kasai KI, Leffler H, Liu FT, Lotan R, Mercurio AM, Monsigny M, Pillai S, Poirier F, Raz A, Rigby PWJ, Rini JM, Wang JL (1994): Galectins: A family of animal β-galactoside-binding lectins. Cell 76:597–598.

Boyüm A (1968): Isolation of mononuclear cells and granulocytes from human blood. Scand J Clin Lab Invest Suppl 21:1–5.

Breitman TR, Selonick SE, Collins SJ (1980): Induction of differentiation of the human promyelocytic leukemia cell line (HL60) by retinoic acid. Proc Natl Acad Sci USA 77:2936–2940.

Collins SJ, Ruscetti FW, Gallanger RE, Gallo RC (1979): Terminal differentiation of human promyelocytic leukemia cells induced by dimethyl sulfoxide and polar compounds. Proc Natl Acad Sci USA 75:2458–2462.

Cuperlovic M, Jankovic M, Pfeifer K, Müller WEG (1995): Isolation and characterization of a β-galactoside-binding protein (14 kDa) from rat liver nuclei. Cell Physiol Biochem 5:34–44.

Dagher SF, Wang JL, Patterson RJ (1995): Identification of galectin-3 as a factor in pre-mRNA splicing. Proc Natl Acad Sci USA 92:1213–1217.

Dalton Jr WT, Ahearn MJ, McCredie KB, Freireich EJ, Stass SA, Trujillo JM (1988): HI60 cell line was derived from a patient with FAB-M2 and not FAB-M3. Blood 71:242–247.

Dreyfuss G, Matunis MJ, Pino-Roma S, Burs CG (1993): hnRNP proteins and the biogenesis of mRNA. Ann Rev Biochem 62:289–321.

Facy P, Sève AP, Hubert M, Monsigny M, Hubert J (1990): Analysis of nuclear sugar-binding components in undifferentiated and *in vitro* differentiated human promyelocytic leukemia cells (HL60). Exp Cell Res 190:151–160.

Felin M, Doyennette-Moyne M-A, Hadj Sahraoui Y, Aubery M, Hubert J, Sève A-P (1994): Identification of two nuclear N-acetylglucosamine-binding proteins. J Cell Biochem 56: 527–535.

Gezer S, Yasin Z, Imren S, Freeman J, Black A, Raza A (1988): Changes observed in the growth fraction, labeling index, duration of S phase, and total cell cycle times of HL60 cells as they undergo differentiation in response to retinoic acid. Cancer Res 48:5989–5994.

Hamman KK, Cowles EA, Wang JL, Anderson RL (1991): Expression of carbohydrate-binding protein 35 in human fibroblasts: variations in the levels of mRNA, protein, and isoelectric species as a function of replicative competence. Exp Cell Res 196:82–91.

Harrison FL (1991): Soluble vertebrate lectins: ubiquitous but inscrutable proteins. J Cell Sci 100:9–14.

Hébert E, Monsigny M (1994): Galectin-3 mRNA level depends on transformation phenotype in *ras*-transformed NIH 3T3 cells. Biol Cell 81:73–76.

Huberman E, Callaham MF (1979): Induction of terminal differentiation in human promyelocytic leukemia cells by tumor-promoting agents. Proc Natl Acad Sci USA 76: *1293–1297*.

Hubert J, Sève A-P, Bouvier D, Masson C, Bouteille M, Monsigny M (1985): *In situ* ultrastructural localization of sugar-binding sites in lizard granulosa cell nuclei. Biol Cell 55:15–20.

Hubert J, Sève A-P (1994): Nuclear lectins: In: Driessche EV, Fischer J, Beeckmans S, Bog Hansen TC (eds): "Lectins-Biology-Biochemistry Clinical Biochemistry." Denmark: Textop Hellrup, Vol 10, pp 220–226.

Hubert M, Wang SY, Wang JL, Sève A-P, Hubert J (1995): Intranuclear distribution of galectin-3 in mouse 3T3 fibroblasts: Comparative analyses by immunofluorescence and immunoelectron microscopy. Exp Cell Res 220:397–406.

Hughes RC (1994): Mac-2: A versatile galactose-binding protein of mammalian tissues. Glycobiology 4:5–12.

Laemmli UK (1970): Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685.

Laing JG, Wang JL (1988): Identification of carbohydrate binding protein 35 in heterogeneous nuclear ribonucleoprotein complex. Biochemistry 27:5329–5334.

Lauc G, Sève A-P, Hubert J, Flögel-Mrsic M, Müller WEG, Schröder HC (1993): HnRNP CBP35-CBP67 interaction during stress response and aging. Mech Ageing Dev 70: 227–237.

Lauc G, Flögel M, Diehl-Seifert B, Schröder HC and Müller WEG (1994): Identification and purification of a stress-associated nuclear carbohydrate binding protein (Mr 33000) from rat liver by application of a new photoreactive carbohydrate probe. Glycoconj J 11:541–549.

Liu FT, Hsu DK, Zuberi RI, Kuwabara I, Chi EY, Henderson Jr WR (1995): Expression and function of galectin-3, a b-galactoside-binding lectin, in human monocytes and macrophages. Am J Pathol 147:1016–1028.

Lotz MM, Andrews CW, Cynthia JR, Korzelius A, Lee EC, Steele GD, Astrid Clarke JR, Mercurio AM (1993): Decreased expression of Mac-2 (carbohydrate-binding protein 35) and loss of its nuclear localization are associated with the neoplastic progression of colon carcinoma. Proc Natl Acad Sci USA 90:3466–3470.

Moutsatsos IK, Wade M, Schindler M, Wang JL (1987): Endogenous lectins from cultured cells: nuclear localization of carbohydrate-binding protein 35 in proliferating 3T3 fibroblasts. Proc Natl Acad Sci USA 84:6452–6456.

Nangia-Makker P, Ochieng J, Christman JK, Raz A (1993): Regulation of the expression of galactoside-binding lectin during human monocytic differentiation. Cancer Res 53: 5033–5037.

Raz A, Zhu D, Hogan V, Shah N, Raz T, Karkash R, Pazerini G, Carmi P (1990): Evidence for the role of 34-kDa galactoside-binding lectin in transformation and metastasis. Int J Cancer 46:871–877.

Roff CF, Wang JL (1983): Endogenous lectins from cultured cells. Isolation and characterization of carbohydrate-binding proteins from 3T3 fibroblasts. J Biol Chem 258: 10657–10663.

Rovera G, Santolini D, Damsky C (1979): Human promyelocytic leukemia cells in culture differentiate into macrophage-like cells when treated with a phorbol diester. Proc Natl Sci Acad USA 76:2779–2783.

Schröder HC, Facy P, Monsigny M, Pfeifer K, Bek A, Müller WEG (1992): Purification of a glucose-binding protein from rat liver nuclei. Evidence for a role in targeting of nuclear mRNP to nuclear pore complex. Eur J Biochem 205:1017–1025.

Sève AP, Hubert J, Bouvier D, Bouteille M, Maintier C, Monsigny M (1985): Detection of sugar-binding proteins in membrane-depleted nuclei. Exp Cell Res 157:533–538.

Sève A-P, Hubert J, Bouvier D, Bouteille M, Maintier C, Monsigny M (1986): Analysis of sugar-binding sites in mammalian cell nuclei by flow microfluorometry. Proc Natl Acad Sci USA 83:5997–6001.

Sève A-P, Felin M, Doyennette-Moyne M-A, Sahraoui T, Aubery M, Hubert J (1993): Evidence for a lactose-mediated association between two nuclear carbohydrate-binding proteins. Glycobiology 3:23–30.

Sève A-P, Hadj Sahraoui Y, Felin M, Doyennette-Moyne M-A, Aubery M, Hubert J (1994): Evidence that lactose binding to CBP35 disrupts its interaction with CBP70 in isolated HL60 cell nuclei. Exp Cell Res 213:191–197.

Valentin A, Von Gegerfelt A, Matsuda S, Nilsson K, Asjö B (1991): *In vitro* maturation of mononuclear phagocytes and suceptibility to HIV_1 infection. J Acquir Immune Defic Syndr 4:751–759.

Valentin A, Albert J, Fenyö EM, Asjö B (1994): Dual tropism for macrophages and lymphocytes is a common feature of primary human immunodeficiency virus type 1 and 2 isolates. J Virol 68:6684–6689.

Wang JL, Laing JG, Anderson RL (1991): Lectins in the cell nucleus. Glycobiology 1:243–252.

Wang JL, Werner EA, Laing JG, Patterson RJ (1992): Nuclear and cytoplasmic localization of a lectin-ribonucleoprotein complex. Biochem Soc Trans 20:269–274.

Yen A, Albright KL (1984): Evidence for cell cycle phase-specific initiation program of HL60 cell myeloid differentiation mediated by inducer uptake. Cancer Res. 44:2511–2515.