0022-1767/87/1393-0842$02.00/0
THE JOURNAL OF IMMUNOLOGY

Vol. 139, 842–847, No. 3, August 1, 1987
Printed in U.S.A.

BIOSYNTHESIS AND GLYCOSYLATION OF p150,95 AND RELATED LEUKOCYTE ADHESION PROTEINS[1]

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The p150,95 cell surface protein is a member of a family of heterodimeric leukocyte adhesion proteins that have homologous α subunits, each noncovalently associated with a common β subunit. In this report we have metabolically labeled the U937 cell line at various timepoints during its phorbol myristic acetate-induced maturation to examine the kinetics of synthesis of these proteins during monocytic differentiation, and their maturation and glycosylation. The p150,95 α subunit was immunoprecipitated with p150,95-specific monoclonal antibody (MAb), or an antiserum to the denatured, purified αX subunit. The glycosylation and polypeptide chain length of the p150,95, Mac-1, and lymphocyte function associated antigen (LFA-1) α and β subunits were compared by immunoprecipitation with subunit specific MAb and antisera, and by digestion with Endo H and N-glycanase. The p150,95 α subunit is synthesized as a precursor of 146,000 M_r, has five to six N-linked oligosaccharides, and has a polypeptide chain backbone of 132,000 M_r. Over 50% of the carbohydrate on the mature α subunit of 150,000 M_r was sensitive to Endo H digestion. The p150,95 α and β precursors can associate before maturation into the mature form. Conversion to the mature form was accompanied by loss of reactivity with the antiserum to the denatured αX subunit, suggesting a change in conformation. Mac-1 and LFA-1 α subunits have precursors of 160,000 M_r and 165,000 M_r, respectively, and contain N-linked carbohydrates. The polypeptide chain length for the Mac-1 α subunit is 137,000 M_r, and for LFA-1 is 149,000 M_r. Only 14% of the oligosaccharide on the mature LFA-1 α subunit was sensitive to Endo H, suggesting that unlike p150,95, most is converted to the complex type. The differences noted in the M_r of the three homologous α subunits are therefore due to differences in both polypeptide chain length and carbohydrate processing during biosynthesis.

p150,95 is a cell surface protein expressed in greatest quantity on tissue macrophages and hairy cell leukemia cells, and in smaller amounts on circulating granulocytes and monocytes, some CTL clones, and selected B cell lines (1–3). It is a heterodimer consisting of an αX subunit of M_r ~150,000 noncovalently associated with a β subunit of M_r ~95,000. p150,95 was originally identified immunochemically with a β subunit-specific monoclonal antibody (MAb)[2] cross-reactive with both the lymphocyte-function-associated antigen-1 (LFA-1) and Mac-1 (4). Subsequently, MAb specific for the p150,95 protein have been obtained (5, 6). p150,95, Mac-1, and LFA-1 are a family of leukocyte adhesion proteins that have unique but homologous α subunits (αX, M_r ~150,000; αM, M_r ~165,000; and αL, M_r ~177,000, respectively) noncovalently associated with the common β subunit (4, 7–10).

In granulocytes and monocytes, p150,95 and Mac-1 are stored in intracellular compartments (11, 12), which are induced by inflammatory mediators to fuse with the plasma membrane. This mobilization results in greater than fourfold increases in cell surface expression of p150,95 and Mac-1 (5, 11–14), and appears to regulate the increased adhesiveness of monocytes and granulocytes to endothelial cells and at sites of inflammation (13, 15–19).

The differentiation of blood monocyte to tissue macrophage results in greatly increased cell surface expression of p150,95 (1, 3, 12). Differentiation of immature myelomonocytic cell lines has been used as a model of myelopoiesis. Maturation of the LFA-1$^+$, Mac-1$^-$, p150,95$^-$ cell line U937 along the monocytic pathway after stimulation by phorbol myristic acetate (PMA) or granulocyte/macrophage colony-stimulating factor is accompanied by greatly increased p150,95 and Mac-1 cell surface expression (1, 2, 4, 20).

Little is known about the biosynthesis, maturation, and glycosylation of p150,95. Previous studies on murine Mac-1 have shown that the α and β subunits are synthesized from independent precursors (21). After association of the α and β subunits, they undergo an increase in M_r that is accompanied by the conversion of most N-linked carbohydrates to an Endo H-resistant complex form (22). In an inherited disease in which the common β subunit appears defective, cell surface expression of the p150,95, Mac-1 and LFA-1 α subunits is lacking (13, 23–26). The LFA-1 α precursor is present in normal amounts in lymphocytic cell lines derived from these patients, but in the absence of association with β did not undergo the increase in M_r that is normally associated with maturation (14). A previous biosynthesis study utilizing the differentiated U937 cell line also suggested that association of the human LFA-1 and Mac-1 α subunits with the β subunit precedes conversion to higher M_r forms (4). However, by using an anti-β subunit MAb, little increase in M_r of

Received for publication February 20, 1987.
Accepted for publication May 4, 1987.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] This work was supported by National Institutes of Health Grants CA 31798 and 31799.

[2] Abbreviation used in this paper. MAb, monoclonal antibody.

the αX subunit of p150,95 was evident. This raised the question of whether the glycosylation of the p150,95 α subunit differed from that of Mac-1 and LFA-1.

In this report we first examine the time course of synthesis of p150,95, Mac-1, and LFA-1 in PMA-induced U937 cells. We then utilize p150,95-specific MAb, polyclonal antiserum to the native p150,95 αβ subunit complex, and antiserum raised against the isolated, SDS-denatured αX subunit, and digestion with Endo H and N-glycanase, to study p150,95 biosynthesis, glycosylation, and maturation. We also investigate the glycosylation of Mac-1 and LFA-1, and determine whether differences in M_r between the three homologous α subunits are due to glycosylation and/or to differences in protein backbone size.

MATERIALS AND METHODS

Cell lines and metabolic labeling. U937 cells were cultured in complete medium (RPMI 1640 with 5×10^{-5} M β-mercaptoethanol, 2 mM glutamine, and 5 μg/ml gentamicin) and 10% fetal calf serum (FCS) (Hyclone, Logan, UT). U937 cells (5×10^6/ml), in 20 ml of complete medium with 10% FCS and 2 ng PMA/ml, were plated into 100 mm Petri dishes. After incubation for 0, 1, 2, or 4 days, adherent cells were washed three times with Hanks' balanced salt solution (HBSS)/10 mM HEPES. The nonadherent cells were collected from the washes, were also washed three times, and were returned to the dishes. [^{35}S]Methionine (0.625 mCi) in 5 ml of methionine-free complete medium with 15% dialyzed FCS was then added to each plate. After a 30 or 60 min pulse at 37°C, 50% of each sample was washed three times in HBSS/10 mM HEPES and was lysed in 1 ml of lysis buffer (1.0% Triton X-100, 10 mM Tris-HCl, pH 8.0, 0.15 M NaCl, 1.0% bovine hemoglobin, 1.0 mM PMSF, and 0.025% NaN_3). Complete medium with 10% FCS and 100 μg/ml methionine (5 ml) was added to the remaining cells, and incubation at 37°C continued for either 2.5, 5, or 6 hr. The samples were lysed in 1 ml of lysis buffer, and all samples were dialyzed overnight against 10 mM Tris-HCl (pH 8.0), 0.15 M NaCl, and 0.025% NaN_3.

MAb and rabbit antisera. Anti-LFA-1 αL subunit TS1/22 MAb (27), anti-β subunit TS1/18 MAb (27), anti-p150,95 SHCL3 MAb (1), and anti-Mac-1 αM subunit LM2/1 MAb (2) have been described. SHCL3 is available from Becton-Dickinson as Leu-M5. MAb covalently coupled to Sepharose CL-4B was prepared as described (28). The native p150,95 αβ complex was affinity purified on an SHCL3 MAb-Sepharose column (7). The p150,95 αX subunit was separated from the β subunit by preparative SDS-PAGE, was excised from the gel, and was electrophoretically eluted (7, 29). Rabbit antisera were prepared by intramuscular injection of 600 pmol of either affinity purified p150,95, or electrophoretically isolated, SDS-denatured αX subunit in complete Freund's adjuvant. Each rabbit was subsequently boosted three times at 1½ mo intervals with 150 to 660 pmol of material in incomplete Freund's adjuvant.

Immunoprecipitation and oligosaccharide digestion. Fifteen microliters of a 1:1 slurry of MAb-Sepharose, or 10 μl of a 1/10 dilution of antiserum was rotated with 10^6 cpm [^{35}S]methionine labeled lysate for 2 hr at 4°C. Protein A coupled to cross-linked-agarose (Genzyme, Boston, MA) (15 μl) was added to the samples containing antiserum, incubation continued for 30 more min, and the immunoprecipitates were washed as described (28).

The sample and control immunoprecipitates for Endo H digestion studies were boiled for 5 min in 50 μl of 100 mM Tris (pH 8) and 1.0% SDS, to which 450 μl of 10 mM MES pH 5.5 (Calbiochem) and 0.14 M NaCl was then added, and the supernatants were removed to new tubes. The samples were incubated for 24 hr at 37°C with or without 0.5 mU Endo H (Genzyme, Boston, MA) in the presence of 1 mM PMSF.

Immunoprecipitates prepared for N-glycanase digestion were boiled for 5 min in 50 μl 0.5% SDS and 0.1 M β-mercaptoethanol. Distilled H_2O (60 μl) and 15 μl 100 mM 1,10-phenanthroline hydrate (in methanol) (Sigma, St. Louis, MO) were added, and the supernatants were removed to new tubes. After addition of 0.1 M Tris pH 8.6, 12.5% Triton X-100 (15 μl), 90 mU (10 μl) of N-glycanase (peptide: N-glycosidase F, Genzyme), and PMSF to a final concentration of 1 mM were added. The samples were incubated at 37°C overnight. The protein in all samples was then precipitated overnight at −20°C with 10 μg tRNA and 4 vol acetone, boiled in 25 μl of reducing Laemmli sample buffer, and subjected to SDS-PAGE (28).

RESULTS

Synthesis of p150,95, Mac-1 and LFA-1 during PMA-induced differentiation of U937 cells. We first examined the biosynthesis of p150,95, Mac-1 and LFA-1 α and β subunits at different times during the PMA-stimulated maturation of U937 cells along the monocytic pathway. U937 cells were induced with PMA for 0, 1, 2, or 4 days. By day 2 the majority of the cells were adherent and assumed a spread macrophage-like morphology. These cells were metabolically labeled with [^{35}S]methionine, and some cultures were "chased" with unlabeled methionine. Triton X-100 lysates were subjected to immunoprecipitation with MAb specific for each of the three α subunits or the common β subunit, and with rabbit antisera to the purified p150,95 αβ complex (anti-αXβ) or to the denatured, SDS-PAGE purified αX subunit (anti-αX). An equal number of cpm were used from each lysate; thus the amount of protein immunoprecipitated reflected the percentage of total protein synthesis devoted to the immunoprecipitated protein at a given time point during differentiation.

Synthesis of αX was not detected with the p150,95 MAb on day 0, but was induced by day 1, and appeared to be maximal on days 2 and 4 of PMA treatment (Fig. 1-*A*, lanes *3* through *10*). The same timecourse of synthesis was detected with rabbit anti-αX serum (Fig. 1-*A*, lanes *11* through *18*). Thus our results on the timecourse of αX induction appear to be independent of the conformation of αX or its association with β. The kinetics of Mac-1 αM subunit induction during U937 cell maturation were very similar to that of the p150,95 αX subunit: absent on day 0, present on day 1, and maximal by day 2 (Fig. 1-*B*, lanes *11* through *18*). However, synthesis of the LFA-1 αL subunit was detected on day 0, with maximal biosynthesis on days 1 and 2 (Fig. 1-*B*, lanes *19* through *26*). The β subunit MAb precipitated β and all three types of α subunits, demonstrating their association with β. Precipitation of β was maximal on day 2 (Fig. 1-*B*, lanes *3* through *10*). Immunoprecipitation with anti-αXβ serum yielded a similar pattern, suggesting the presence of anti-β antibodies (Fig. 1-*A*, lanes *19* through *26*). The anti-αXβ serum precipitated much more β precursor than did the β subunit MAb, but precipitated a similar amount of associated αX, αM, and αL subunits. This demonstrates that the rabbit anti-αXβ serum is much more efficient than the anti-β MAb in immunoprecipitating the unassociated β subunit precursor.

Maturation and glycosylation of p150,95, Mac-1, and LFA-1. In the same experiment the maturation of the subunits was examined. Immunoprecipitation with the p150,95 MAb detected an αX precursor of M_r ~146,000 that was converted after 2.5 h of chase to a M_r ~150,000 (Fig. 1-*A*, lanes 5 through *10*). The antiserum produced by immunization with the purified, SDS-denatured αX subunit preferentially precipitated the αX subunit precursor (Fig. 1-*A*, lanes *11* through *18*); far less αX was immunoprecipitated from the chased cultures with this antiserum than with the MAb (Fig. 1-*A*, compare lanes *14*, *16*, and *18* to lanes *6*, *8*, and *10*, respectively). The αX and β precursors can associate before processing, as demonstrated by immunoprecipitation with β subunit MAb from lysates of cultures that were not chased (Fig. 1-*B*, lanes *5*, *7*, and *9*). As reported (4), Mac-1 and LFA-1

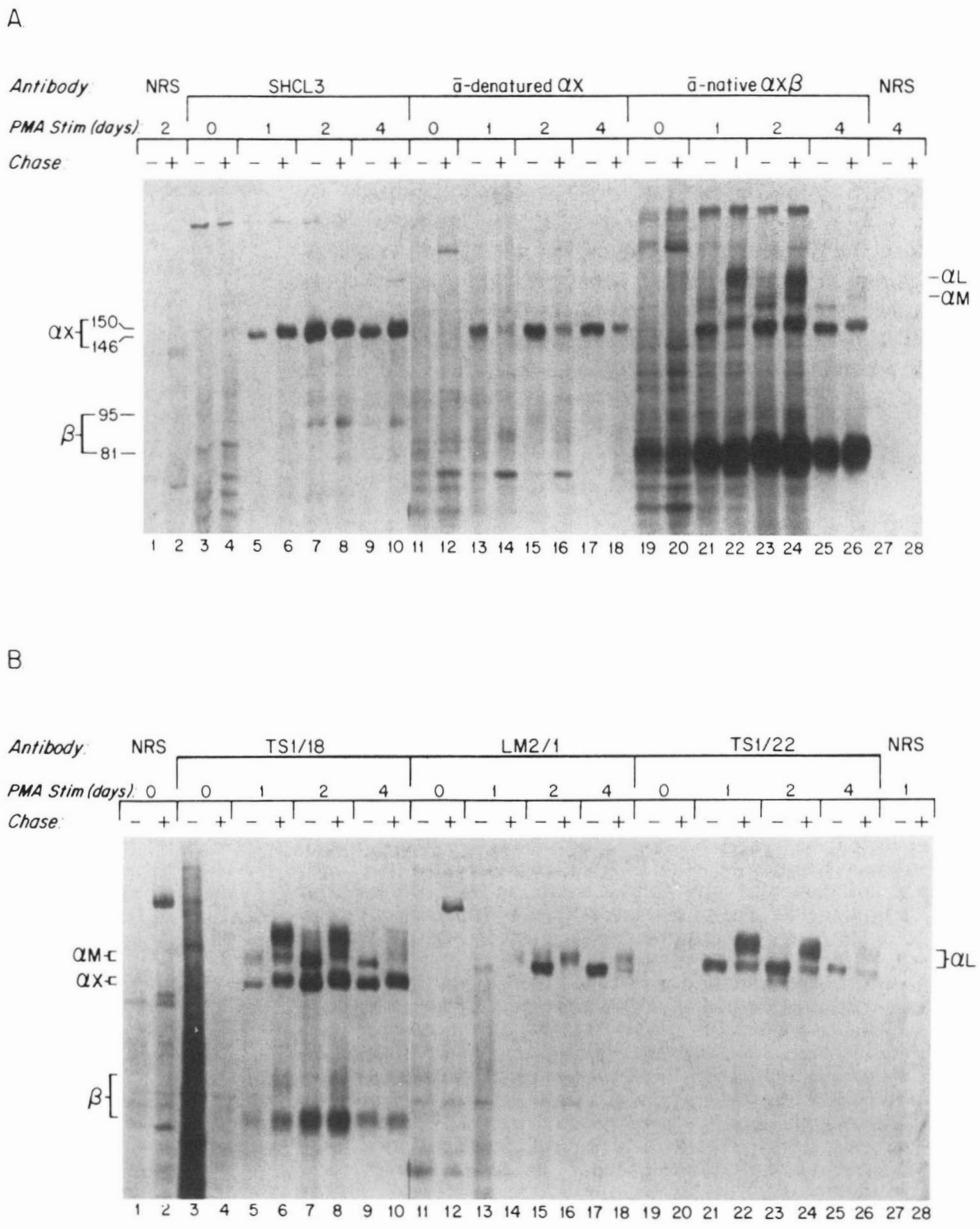

Figure 1. Biosynthesis of p150,95, Mac-1, and LFA-1. U937 cells were stimulated with PMA for the indicated number of days. [^{35}S]Methionine was biosynthetically incorporated for 30 min, then the label in 50% of the cells was chased for 2.5 hr with unlabeled methionine. The cells were lysed, and immunoprecipitates from 6×10^5 cpm of each lysate were formed with normal rabbit serum (NRS), SHCL3 anti-p150,95 MAb, rabbit anti-denatured αX serum, rabbit anti-native αXβ complex serum, TS1/18 anti-β subunit MAb, LM2/1 anti-αM MAb, or TS1/22 anti-αL MAb as indicated. The samples were reduced and were subjected to SDS 7%-PAGE.

α precursors underwent much greater increases in M_r than did the p150,95 α precursor when chased (Fig. 1-*B*, lanes *13* through *26*). The Mac-1 αM precursor of M_r ~160,000 matured to ~167,000, and the LFA-1 αL precursor of M_r ~165,000 increased to ~173,000 with chase.

To examine whether N-linked oligosaccharides of the p150,95 αX subunit undergo maturation from the high mannose to the complex type, immunoprecipitates were subjected to digestion by Endo H (Fig. 2). Endo H cleaves high mannose and some hybrid, but not complex type N-linked oligosaccharides (30). The p150,95 αX precursor of M_r ~146,000 was cleaved by Endo H to a M_r ~132,000 (Fig. 2, lanes *1* through 4). The p150,95 MAb immunoprecipitated two forms of the Endo H digested αX subunit from cells pulsed for 1 hr (Fig. 2, lane 2), indicating that some of the αX had been converted to a partially resistant (mature) form during the pulse. The anti-αX serum precipitated only the form fully sensitive to Endo H (Fig. 2, lane 4), confirming its specificity for the αX precursor. After a 5 hr chase the mature p150,95 αX subunit was immunoprecipitated with the p150,95 MAb, but not with the anti-αX serum, also confirming the specificity of the serum for the αX precursor (Fig. 2, lanes *9* through *12*). The mature αX subunit of M_r ~150,000 was digested by Endo H to M_r ~140,000, showing that a portion of the N-linked carbohydrate remains Endo H sensitive.

The longer chase period in this experiment allowed better visualization of the β subunit associated with αX (Fig. 2, lanes *9* and *10*). The β precursor of M_r ~81,000 was cleaved by Endo H to 74,000 (Fig. 2, lanes *7* and *8*), and the 95,000 M_r mature β subunit associated with the αX subunit was digested to M_r ~90,000 (Fig. 2, lanes *9* and *10*). During the chase period used, only a small proportion of the β subunit was converted to the mature form. Previous studies have suggested that this is due to the longer maturation period required for β than for the α subunits (4, 21). Although in the mature αXβ complex precipitated by the p150,95 MAb there is considerably more labeled α than β subunit, this is misleading. The time required for β subunit maturation (>22 hr) (4) suggests that much of the immunoprecipitated labeled α subunit is associated with unlabeled β subunit synthe-

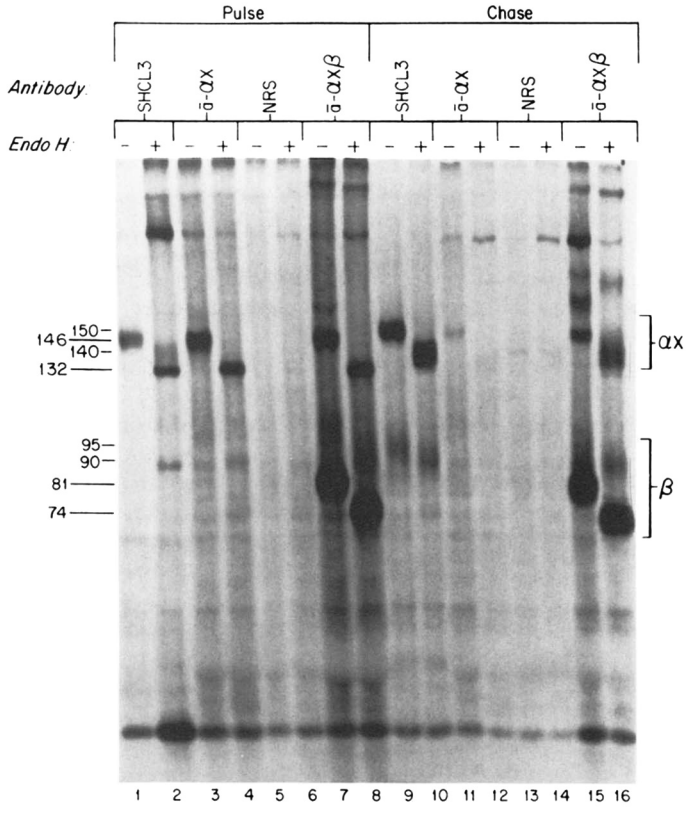

Figure 2. Endoglycosidase H treatment of p150,95. U937 cells were PMA-stimulated for 2 days, then were biosynthetically labeled with [^{35}S] methionine for 1 hr. Fifty percent of the cells were chased with unlabeled methionine for an additional 5 hr. Lysates (10^6 cpm) were immunoprecipitated with anti-p150,95 MAb SHCL3 (*lanes 1, 2, 9,* and *10*), rabbit anti-denatured αX serum (*lanes 3, 4, 11,* and *12*). NRS (*lanes 5, 6, 13,* and *14*) and rabbit anti-native αXβ complex serum (*lanes 7, 8, 15,* and *16*). These samples were then treated with or without 0.5 mU Endo H as indicated, were reduced, and were subjected to SDS 8%-PAGE.

sized before the pulse. The SHCL3 MAb used for immunoprecipitation and purification of p150,95 does not appear to induce dissociation of the αXβ complex, as judged by silver staining (7), and the α and β subunits contain 19 and nine methionines, respectively (31) (Corbi, Miller, and Springer, manuscript in preparation). In general, subunits with long maturation times will appear to be under-represented in complexes when the chase period is short relative to the time required for maturation.

To confirm whether the differences of 8000 M_r between the Endo H treated precursor and mature αX subunits was due to the presence of complex N-linked oligosaccharides, or could be accounted for by other covalent modifications such as O-linked glycosylation, p150,95 was digested with N-glycanase (Fig. 3-*A*). This enzyme cleaves nearly all N-linked oligosaccharides between the asparagine and the terminal carbohydrate residue, generating an aspartic acid residue and a free oligosaccharide. p150,95 immunoprecipitated with MAb SHCL3 was subjected to either Endo H or N-glycanase digestion. Both Endo H and N-glycanase reduced the apparent m.w. of the p150,95 αX precursor by 14,000, from ~146,000 to ~132,000 M_r (Fig. 3-*A*, lanes *2* and *3*). However, N-glycanase, but not Endo H reduced the mature αX subunit from M_r ~150,000 to a M_r ~132,000, which was equivalent to the size of the polypeptide backbone (Fig. 3-*A*, compare lanes *3*, *4*, and *5*, and Fig. 2, compare lanes *8* and *10*).

Mac-1 and LFA-1 were subjected to Endo H and N-glycanase digestion to compare the oligosaccharide structure and polypeptide backbone M_r of their α subunits with those of the p150,95 αX subunit (Fig. 3*B*). Both N-glycanase and Endo H cleaved the Mac-1 αM precursor of M_r ~160,000 to a polypeptide chain backbone of M_r ~137,000 (Fig. 3*B*, lanes *2* and *4*). The mature αM subunit of M_r ~167,000 was partially resistant to Endo H (digested to M_r ~153,000), but was wholly susceptible to N-glycanase (digested to M_r ~137,000), as shown in Figure 3*B*, lanes *6* and *8*.

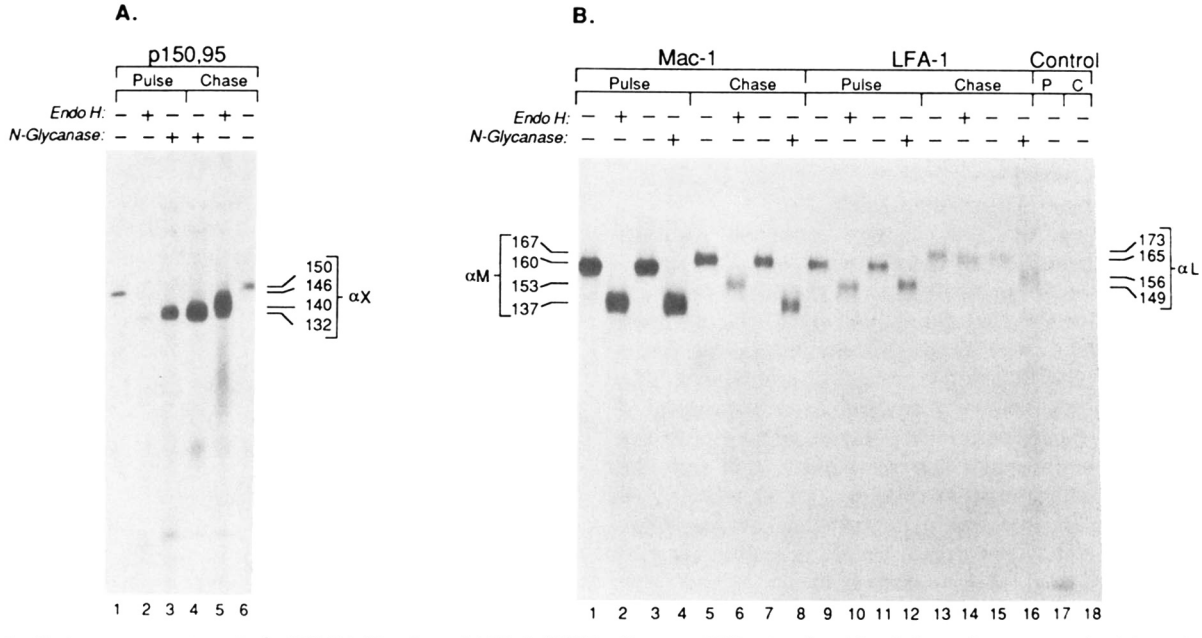

Figure 3. N-glycanase treatment of p150,95, Mac-1, and LFA-1. U937 cells were PMA-stimulated for 2 days, then were pulsed for 1 hr with [^{35}S] methionine. Additional cultures were chased for 6 hr with unlabeled methionine. Cells were lysed in Triton X-100, and immunoprecipitates formed with SHCL3 anti-p150,95 MAb-Sepharose (*Panel A*), LM2/1 anti-Mac-1 αM subunit MAb-Sepharose (*Panel B, lanes 1* through *8*), TS1/22 anti-LFA-1αL MAb-Sepharose (*Panel B, lanes 10* through *16*), or control CNBr activated, quenched-Sepharose (*Panel B, lanes 17* and *18*). The samples were then treated ± Endo H or ± N-glycanase as indicated, were reduced, and were subjected to SDS 8%-PAGE (*Panel A*) or SDS 7%-PAGE (*Panel B*).

TABLE I
Subunit M_r of the leukocyte adhesion proteins

	αX	αM	αL	β
Precursor form ($M_r \times 10^{-3}$)	146	160	165	81
Polypeptide chain backbone ($M_r \times 10^{-3}$)	132	137	149	74
N-linked high mannose carbohydrate ($M_r \times 10^{-3}$)	14	23	16	7
Mature form ($M_r \times 10^{-3}$)	150	167	173	95
Mature after Endo H digestions ($M_r \times 10^{-3}$)	140	153	170	90
Endo H sensitive carbohydrate (%)	56	47	14	24

The αL precursor of M_r ~165,000 was digested by both Endo H and N-glycanase to yield a protein backbone of M_r ~149,000 (Fig. 3*B*, lanes *10* and *12*). Endo H cleaved the mature αX from M_r ~173,000 to M_r ~170,000, suggesting the presence of only a single non-complex oligosaccharide (Fig. 3*B*, lane *14*). N-glycanase was more effective in cleaving the mature αL subunit, reducing it to M_r ~156,000 (Fig. 3*B*, lane *16*). The failure of N-glycanase to completely digest the LFA-1 αL subunit to its protein backbone of M_r ~149,000 suggests either the presence of unusual modifications of the N-linked carbohydrates, steric hindrance of the enzyme, or the presence of additional modifications such as O-linked oligosaccharides.

DISCUSSION

In this report we have studied the biosynthesis of p150,95 and compared its oligosaccharide structure and protein backbone with the homologous proteins Mac-1 and LFA-1. Additionally, we have studied at the level of protein synthesis the timecourse of the expression of these proteins during monocytic differentiation of the U937 cell line. Our metabolic labeling studies demonstrate that Mac-1 and p150,95 synthesis is induced after 1 day of stimulation of U937 cells with PMA. This is consistent with previous immunofluorescence flow cytometric observations that p150,95 and Mac-1 show little cell surface expression on days 0 and 1, but are greatly increased to half-maximal levels by day 2 (2). Our studies show that cell surface expression of these molecules in PMA-differentiated U937 cells is due to de novo protein synthesis. In contrast, short term stimulation of monocytes with PMA or inflammatory mediators induces rapid mobilization of a preformed intracellular pool of Mac-1 and p150,95 to the cell surface (5, 12, 32).

Previous studies on the biosynthesis of human p150,95, Mac-1, and LFA-1 have been limited to observations of changes in subunit M_r (4). We have used an antiserum specific for the denatured p150,95 αX subunit, a MAb specific for p150,95, and enzymatic digestion of the carbohydrates to study p150,95 biosynthesis. The p150,95 αX subunit was synthesized as a precursor of 146,000 M_r that matured to 150,000 M_r. The reactivity of an anti-αX serum with the precursor, but not the mature p150,95 αX subunit suggested that αX undergoes a conformational change during maturation, perhaps due to association with the β subunit. Both the precursor and mature forms of p150,95 subunit could be immunoprecipitated with a β subunit MAb, showing that formation of the αβ complex can precede maturation.

Cleavage of the p150,95 αX precursor with either Endo H and N-glycanase yielded a polypeptide backbone size of M_r ~132,000. Assuming each high mannose sidechain to be ~2500 M_r, the p150,95 αX subunit is predicted to have five to six N-linked oligosaccharides. The mature p150,95 αX subunit of 150,000 M_r was cleaved to 140,000 M_r by Endo H, showing that a portion of the N-linked carbohydrates are converted to the complex type during maturation. The mature αX subunit was completely digested to the polypeptide backbone of 132,000 M_r by N-glycanase, suggesting little or no O-linked glycosylation. Lack of cleavage with O-glycanase provided additional evidence suggesting no O-linked oligosaccharides on the αX subunit (Miller and Springer, data not shown).

The findings on the glycosylation and processing of the αX, αM, αL, and β subunits are summarized and compared in Table I. The results on human Mac-1 are consistent with our previous studies on in vivo and in vitro synthesis of murine Mac-1 (21, 22). The greatest difference among the three α subunits was in the more extensive processing of LFA-1 α subunit carbohydrates, as evidenced by its greater resistance to Endo H. The αM and αX subunits also differ from LFA-1 in isoelectric points (4), and cellular compartmentalization (11, 12). Furthermore, unlike the Mac-1 and p150,95 α subunits, the mature LFA-1 α subunit was partially resistant to N-glycanase. This unusual resistance of LFA-1 N-linked carbohydrates is not likely to be due to its sulfation on some cell types (33). Studies on murine Mac-1 and LFA-1 have recently shown that the α subunit influences β subunit glycosylation, and some β subunit N-linked oligosaccharides are resistant to N-glycanase (34).

This report extends our knowledge about the glycosylation and polypeptide backbone size of p150,95, Mac-1, and LFA-1, and the regulation of their biosynthesis during monocytic differentiation. These studies demonstrate that the three α subunits differ in polypeptide chain length, as well as in glycosylation. The differentiated U937 cells studied here have previously been shown to lack a rapidly mobilizable pool of p150,95 and Mac-1 (2). Circulating neutrophils and monocytes, however, have much higher quantities of p150,95 and Mac-1 in secretory granules than on the cell surface (5, 11, 12, 14). Mobilization of p150,95 and Mac-1 adhesive glycoproteins to the cell surface by inflammatory mediators regulates localization of neutrophils and monocytes in inflammatory sites (13, 15–19, 35). LFA-1 is present on the neutrophil cell surface, but not in the intracellular compartments. How Mac-1 and p150,95 are selectively targeted to intracellular compartments during their biosynthesis in myeloid precursor cells is an important area for further investigation.

Acknowledgments. We would like to thank A. Corbi, N. Hollander, T. Kishimoto, and S. Marlin for fruitful discussion and critical reading of the manuscript.

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