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Biosynthesis and Assembly of the α and β Subunits of Mac-1, a Macrophage Glycoprotein Associated with Complement Receptor Function*

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Mac-1 is a macrophage surface antigen containing noncovalently associated α and β subunits of M_r = 170,000 and 95,000, respectively (Kürzinger, K., and Springer, T. A. (1982) *J. Biol. Chem.* 257, 12412-12418). To determine whether the subunits are derived from a common or separate precursor, the biosynthesis of Mac-1 was studied. [^{35}S]Methionine pulse-chase-labeled material was immunoprecipitated with either a monoclonal antibody recognizing an α chain determinant present in the associated $\alpha_1\beta_1$ complex or a polyclonal antiserum recognizing the $\alpha_1\beta_1$ complex as well as the free β subunit. In peritoneal exudate macrophages, the α subunit was derived from a precursor of M_r = 161,000 which was converted to the mature M_r = 170,000 chain with a $t_{1/2}$ of 30 to 45 min. The β subunit was derived from a M_r = 87,000 precursor which became associated with the α subunit and was converted to M_r = 95,000 with a $t_{1/2}$ of 2 h. Labeled β chain took longer than α to become associated with the $\alpha_1\beta_1$ complex in a number of different types of peritoneal macrophage populations, correlating with synthesis of an excess of β. In the P388D$_1$ macrophage-like tumor line, α and β were processed with $t_{1/2}$s of about 2 and 1 h. Both α and β precursors were present in the complex, suggesting that complex formation preceded processing.

Mac-1 is a monoclonal antibody-defined mouse surface antigen expressed in 1.7×10^5 surface sites/cell in thioglycolate-elicited macrophages, in similar quantities on other macrophages, and in lower amounts on blood monocytes, granulocytes, and natural killer cells, but absent from lymphocytes and nonhematopoietic tissues (2-4). Mac-1 has the same distribution on human cells, as shown with the cross-reactive M1/70 MAb[1] (5). Human Mac-1 is probably identical with the OKM1 (6) or Mo1 antigen (7). The anti-Mac-1 MAb and its F(ab')$_2$ fragment inhibit the mouse and human complement receptor type three (CR_3), but have no effect on several other types of macrophage/granulocyte receptors (8). Mac-1 thus appears to be associated or identical with the CR_3. The CR_3 is specific for inactivated C3b (C3bi) or its further degradation fragment C3d,g, and is physiologically important in the phagocytosis of immune complexes (9, 10).

Mac-1 contains α and β subunits of M_r = 170,000 and 95,000, respectively, which are noncovalently associated in an $\alpha_1\beta_1$ structure as shown by cross-linking (1, 11). Both subunits are glycosylated and appear to have surface exposure (1). Mac-1 is structurally related to the lymphocyte function-associated (LFA-1) antigen (reviewed in Ref. 12). MAb to LFA-1 block antigen-specific T lymphocyte-mediated killing and proliferative responses (12, 13). LFA-1 is expressed on lymphocytes and some myeloid cells, but not on thioglycolate-elicited macrophages (14). LFA-1 contains α and β subunits of M_r = 180,000 and 95,000 which, like those of Mac-1, are associated in an $\alpha_1\beta_1$ structure (1, 11). Tryptic peptide mapping has shown that the Mac-1 and LFA-1 α subunits have <10% shared tyrosyl peptides, while the β subunits are highly homologous or identical (11, 15). There are no tyrosyl peptides shared between the α and β subunits, showing that the β subunits are not α degradation products. The MAb defining Mac-1 and LFA-1 do not cross-react, suggesting that they recognize unique determinants on the α subunits. However, polyclonal antisera prepared against purified or partially purified Mac-1, and certain MAb, have been found to cross-react between Mac-1 and LFA-1 (11, 12, 15). The cross-reaction is between the β and not the α subunits.[2]

Mac-1 might be synthesized as a single polypeptide chain of about M_r = 265,000 which is then cleaved into the α and β subunits. Alternatively, the Mac-1 α and β subunits might be synthesized separately from different mRNAs, and then become associated after translocation of the polypeptide chains into the endoplasmic reticulum. There are good precedents for both mechanisms. The complement components C3, C4, and C5 (16, 17) and the influenza hemagglutinin (18) are synthesized as single polypeptide chains which are processed by proteolytic cleavage into two to three subunits. On the other hand, the immunoglobulins (19), Class I histocompatibility antigens (20), and hemoglobins (21) have subunits which are coded by separate genes and assembled after synthesis. The mode of biosynthesis of Mac-1 has important implications for the organization of the genes for the α and β subunits and the relationship to LFA-1. Therefore, the biosynthesis of Mac-1 has been studied in peritoneal macrophages and a macrophage-like tumor line.

EXPERIMENTAL PROCEDURES

Antibodies—MAb were obtained by growing the rat spleen cell × mouse myeloma hybridoma M1/70 (2) (IgG2b anti-Mac-1) to maximal density in Dulbecco's modified Eagle's medium, 5% fetal calf serum. Purification by $(NH_4)_2SO_4$ precipitation and DEAE and G-200 Seph-

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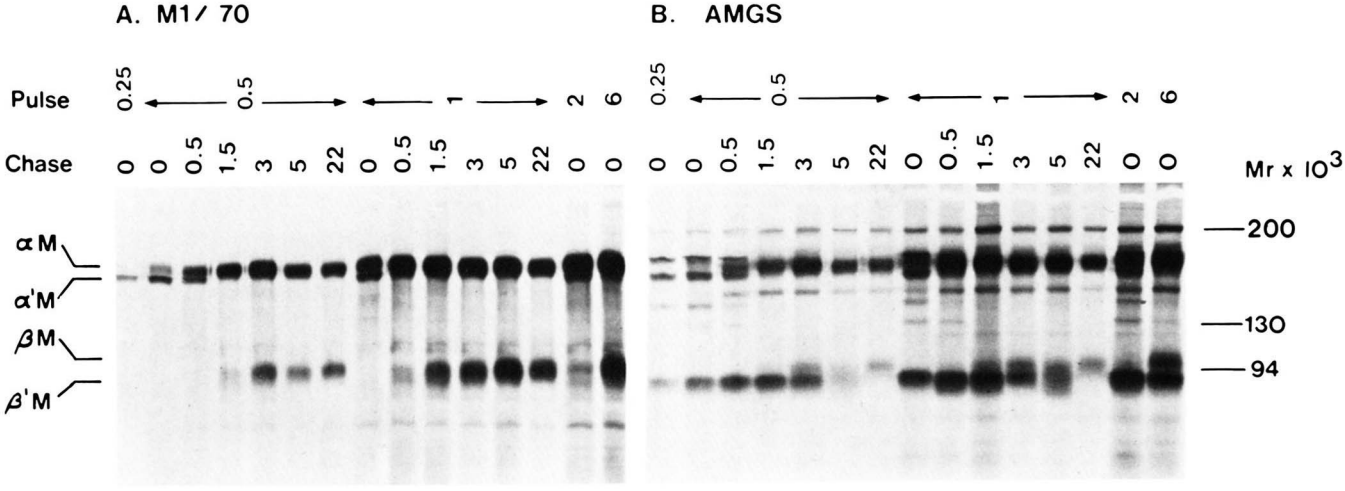
[1] The abbreviations used are: MAb, monoclonal antibody(ies); SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; LFA, lymphocyte function-associated.

[2] F. Sanchez-Madrid and T. A. Springer, manuscript in preparation.



adex chromatography (5) and coupling to Sepharose CL-4B at 1 to 2 mg/ml (22) were as described. The M1/70 hybridoma has been deposited with the American Type Culture Collection, Kensington, MD, and purified M1/70 MAb can be obtained from Hybritech, San Diego, CA. The antiserum against Mac-1 partially purified by *Lens culinaris* lectin chromatography and immunoadsorbent depletion of other immunodominant antigens has been described (11, 23).

Cells—Peritoneal exudate macrophages were elicited from C57/BL6 mice (Jackson Labs, Bar Harbor, ME) by intraperitoneal injection of one of the following: 1.5 ml of Brewer's thioglycolate medium, 1.5 ml of 10% protease peptone, 40 μg of *Salmonella typhosa* lipopolysaccharide (Westphal) (all three from Difco), or 15 μg of concanavalin A (Sigma). Cells were harvested after 3 to 4 days by lavage with 0.01 M $NaPO_4$, 0.14 M NaCl, pH 7.3 containing 20 units/ml of heparin. The $P388D_1$ macrophage-like tumor line was maintained in Dulbecco's modified Eagle's medium supplemented with 10% horse serum.

Radiolabeling and Immunoprecipitation—Peritoneal cells were plated on T25 flasks (10^7 cells/flask) and incubated for 2 or 18 h at 37 °C. Nonadherent cells were washed off and the adherent cells preincubated with labeling medium (methionine-free Dulbecco's modified Eagle's medium supplemented with 10% dialyzed fetal calf serum) at 37 °C. After 45 min, 1 ml of fresh labeling medium containing 200 μCi of L-[^{35}S]methionine (New England Nuclear) was added for the indicated time. Cells were chased for the indicated time with 10 ml of warm Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum at 37 °C either immediately or after two rapid washes with warm 0.01 M $NaPO_4$, 0.14 M NaCl, pH 7.3. The adherent monolayer was then washed three times in 0.01 M $NaPO_4$, 0.14 M NaCl, pH 7.3, containing 0.1 mM phenylmethylsulfonyl fluoride, and detergent solubilized in 0.01 M Tris-HCl, pH 8.0, 0.14 M NaCl, 1% Triton X-100, 1% hemoglobin, 1 mM phenylmethylsulfonyl fluoride, 1 mM iodoacetamide. Lysates were centrifuged at 100,000 × *g* for 1 h and the supernatants recovered. $P388D_1$ cells were grown in monolayers almost to confluency and then processed as for adherent macrophages. Antigen isolation with MAb Sepharose (1) or immunoprecipitation (2), SDS-PAGE (24), and fluorography (25) were as described.

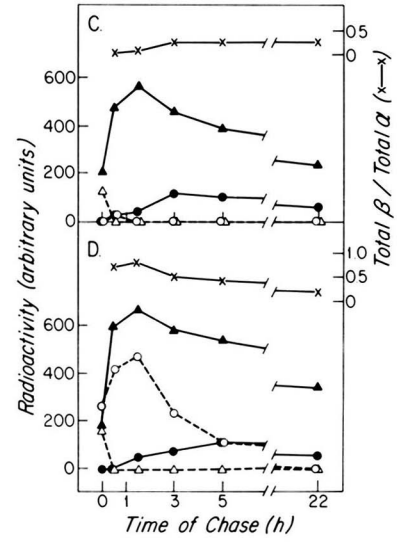


FIG. 1. **Biosynthesis of Mac-1 in thioglycolate-elicited macrophages.** Adherent macrophages were pulsed with [^{35}S]methionine and chased for the times indicated (expressed in hours). Cell lysates (2.7×10^5 cell equivalents) were immunoprecipitated directly with 15 μg of M1/70 coupled to Sepharose (*A*) or indirectly with 12 μl of 1:20 diluted anti-partially purified Mac-1 glycoprotein serum (*AMGS*) followed by 30 μl of rabbit anti-rat IgG (*B*). Precipitates were subjected to 9% SDS-PAGE and fluorography. The upper one-half of the gel is shown. Control immunoprecipitates with normal rat serum showed that only the bands marked *αM*, *α'M*, *βM*, and *β'M* were specifically precipitated. Molecular weight standards were myosin, β-galactosidase, and phosphorylase *a*. Radioactivity in chains was quantified as peak area after scanning hypersensitized fluorograms (25) of M1/70 (*C*) and AMGS (*D*) precipitates from cells pulsed for 1 h. ▲, αM; △, α'M; ●, βM; ○, β'M; ×, total β/total α.

RESULTS

To examine the biosynthesis of Mac-1, thioglycolate-elicited macrophages were pulsed with [^{35}S]methionine and chased for varying lengths of time, and lysates were immunoprecipitated and subjected to SDS-PAGE and fluorography. Two different types of antibodies were used for Mac-1 isolation. The M1/70 MAb appears to react with an α chain conformational determinant which is dependent on β chain association, as shown by 1) reaction with Mac-1 but not LFA-1, which differ in their α subunits (1, 11), 2) the lack of reactivity with the separated Mac-1 α or β subunits in immunoblotting, and 3) the lack of reactivity with the subunits dissociated at pH 11.5.[2] A conventional antiserum prepared to partially purified Mac-1 reacts with the native $\alpha_1\beta_1$ complex and determinants present on the associated or free β chain as shown by 1) reaction with native ^{125}I surface-labeled Mac-1 and LFA-1, which have common β subunits, and 2) reaction with the separated Mac-1 and LFA-1 β subunits after separation from α by SDS-PAGE or after dissociation at pH 11.5.[2]

Immunoprecipitation of Mac-1 with M1/70 MAb showed a precursor of M_r = 161,000 (α'M) after pulsing for 0.25 h (Fig. 1*A*). The M_r = 161,000 material decreased and then disappeared after 0.5 and 1.5 h of chase, concomitant with an increase in the amount of the mature M_r = 170,000 α chain, showing it is the α chain precursor. The mature α chain (αM) persisted for at least 22 h, and was identical in molecular weight to the α chain isolated after ^{125}I surface labeling (not shown). No labeled β chain (βM) was precipitated by M1/70 for the first 0.5 h of chase after a 0.5-h pulse. Small amounts of a β chain precursor (β'M) of M_r = 87,000 and mature β of

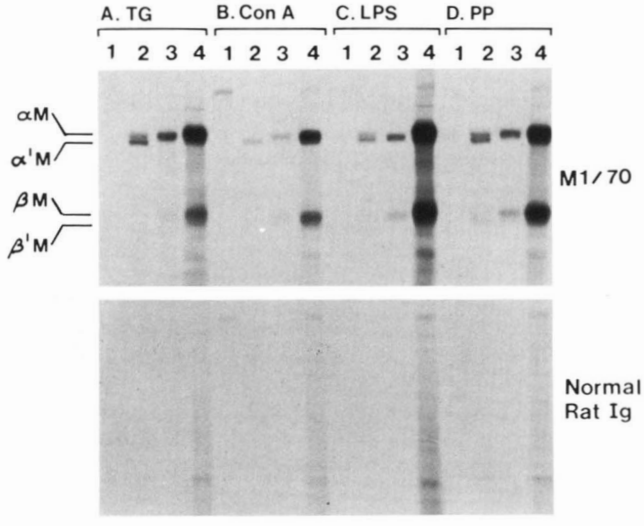


FIG. 2. **Biosynthesis of Mac-1 in macrophages elicited by different agents.** Macrophages elicited by thioglycolate (*TG*) (*A*), concanavalin A (*ConA*) (*B*), lipopolysaccharide (*LPS*) (*C*), or peptone (*PP*) (*D*) were plated on tissue-culture flasks. Labeling with [^{35}S]methionine was as follows: 15-min pulse (*lane 1*), 15-min pulse followed by 30-min chase (*lane 2*), 15-min pulse followed by 90-min chase (*lane 3*), and 4-h continuous labeling (*lane 4*). Cell lysates (5 × 10^5 cell equivalents) were immunoprecipitated with 11 μg of M1/70 (*top*) or 11 μg of normal rat Ig (*bottom*) coupled to Sepharose and the eluates subjected to 9% SDS-PAGE and fluorography.

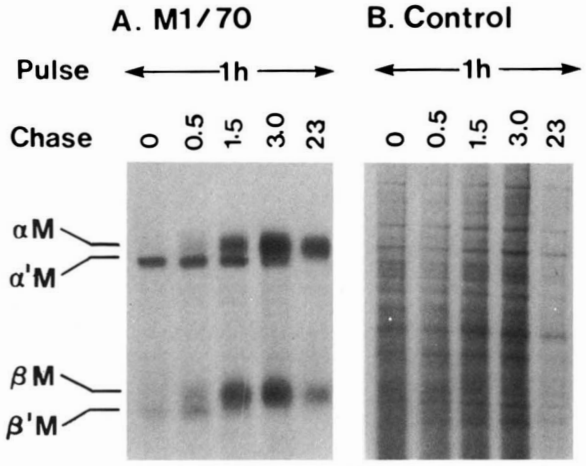


FIG. 3. **Biosynthesis of Mac-1 in P388D$_1$.** P388D$_1$ cells were pulsed with [^{35}S]methionine for 1 h followed by the indicated chase periods (expressed in hours). Lysates from 10^5 cells were immunoprecipitated with 11 μg of M1/70 (*A*) or 11 μg of normal rat Ig (*B*) coupled to Sepharose, and the eluates subjected to 8% SDS-PAGE and fluorography.

M_r = 95,000 were first seen after a 1.5-h chase of the 0.5-h pulse. βM and β′M were also seen in the M1/70 precipitates after a 1.5-h chase of the 1-h pulse and after a 2-h pulse. At 3 h of chase and later, large amounts of βM but no β′M were precipitated.

Similar results were obtained when material was precipitated with the conventional antiserum to Mac-1, except large amounts of the β′M precursor of M_r = 87,000 were found from the earliest times onward (Fig. 1*B*). A band appearing just above the position of the mature α chain at 0 to 0.5 h of chase and some other weak bands were nonspecifically precipitated by normal rat serum (not shown). β′M was present after labeling for 0.25 h and remained for longer than 5 h. The precursor decreased in intensity beginning after a chase of 3 h, concomitantly with the appearance of the mature M_r = 95,000 β subunit with the same kinetics as in the M1/70 immunoprecipitated material. β′M had disappeared by 22 h. Immunoprecipitated chains were quantitated by film scanning (Fig. 1, *C* and *D*). The ratio of total β to total α was constant in the M1/70 precipitate after 1.5 h. In the conventional antiserum precipitate, the total β/total α ratio was 3-fold higher than for M1/70 at 1.5 h and declined to a ratio close to that for M1/70 at 22 h, showing that β′ is synthesized in excess over α and then degraded.

Mac-1 biosynthesis was also examined in peritoneal macrophages elicited by three other agents and in resident macrophages. These macrophages have been shown to differ in function, morphology, and enzyme profiles, and to process the Mac-3 antigen to mature forms differing in molecular weight (26, 27). Biosynthesis was similar in all cases, with the immature α chain and smaller amounts of the mature α chain found after pulsing for 15 min and chasing for 30 min (Fig. 2, *lanes 2*), and the mature α chain found after a 90-min chase (Fig. 2, *lanes 3*) or 4-h continuous labeling (Fig. 2, *lanes 4*). The mature β subunit was seen after 90 min of chase (Fig. 2, *lanes 3*).

The same precursors were found in the P388D$_1$ macrophage-like cell line, but the kinetics of biosynthesis differed (Fig. 3). The M_r = 87,000 β′M precursor was precipitated by M1/70 MAb after a 1-h pulse and was partially and fully converted to the mature β subunit at 0.5 and 1.5 h of chase, respectively. Conversion of the α subunit precursor to the mature form occurred more slowly and was largely complete after 3 h of chase.

DISCUSSION

We examined 1) the kinetics of Mac-1 biosynthesis in different macrophage populations and a cell line, and 2) whether the subunit structure of Mac-1 arose from the proteolysis of a single precursor protein or, alternatively, from the assembly of independently synthesized precursors. In thioglycolate-elicited macrophages, an α chain precursor of M_r = 161,000 was found which was converted to the mature α chain of M_r = 170,000 with a $t_{1/2}$ of 30 to 45 min. The β subunit of M_r = 95,000 was found to be derived from a precursor of M_r = 87,000. Free β chain precursor remained present in the thioglycolate-elicited peritoneal macrophages for more than 5 hours, as shown by its precipitation with the polyclonal anti-Mac-1 serum but not with the M1/70 MAb. The mature form of the β chain was first seen in large amounts at 3.5 h, in both the MAb and polyclonal antiserum precipitates. Precipitation with M1/70 MAb showed that first the immature and then the mature β chain was found associated with the α chain. These findings suggest that association with the α chain triggers the processing of the β chain precursor to the M_r = 95,000 mature form. Biosynthesis studies in P388D$_1$ confirmed that association of the α and β precursors preceded processing to the mature chains. The longer time required for processing of the β than the α chain in thioglycolate-elicited macrophages correlated with the synthesis of excess β chains. Precipitation by M1/70 MAb of labeled α but not β chain at 0 to 0.5 h of chase most likely represents labeled α chain associated with unlabeled β chain from the previously synthesized pool, but precipitation of α unassociated with β cannot be ruled out. In P388D$_1$, α and β processing was slower and faster, respectively, than in thioglycolate-elicited macrophages. Whether this reflects a more balanced synthesis of α and β in P388D$_1$ remains to be determined.

Despite the use of monoclonal and polyclonal antisera of differing specificities in these studies, no trace could be found of a larger precursor which could give rise to both the α and β subunits by proteolytic cleavage. Synthesis of an excess of

β' chains in thioglycolate-elicited macrophages and the time required for assembly argue against an undetectable common precursor. The evidence obtained in different types of normal macrophages and a macrophage cell line shows that α and β are derived from separate precursors of M_r = 161,000 and 87,000, respectively, which become associated at a later time. In turn, this suggests that α and β are derived from separate mRNA transcripts and genes.

Mac-1 and LFA-1 comprise a family of antigens of $\alpha_1\beta_1$ structure sharing a highly homologous or identical β subunit (reviewed in Ref. 12). The antigens have different distributions on hematopoietic cells and different functions. LFA-1 participates in antigen-specific T lymphocyte-mediated killing and other T-cell responses, whereas Mac-1 is functionally associated or identical with the type three complement receptor on macrophages and granulocytes (8). The Mac-1 and LFA-1 α subunits are distinct in structure and antigenic determinants. Furthermore, the LFA-1 α subunit is synthesized from a M_r = 164,000 precursor which is distinct from that of Mac-1. As shown by preclearing experiments, anti-Mac-1 MAb do not precipitate the LFA-1 α precursor, and vice versa.[3] This suggests that differences between the Mac-1 and LFA-1 β chains are not due to processing, and lends further support to the idea that the α chains, which differ in 16 of 17 tyrosyl tryptic peptides, are products of different genes (11). The separate synthesis of the Mac-1 α and β subunits, followed by assembly into the mature $\alpha_1\beta_1$ structures, would allow the use of a single β subunit gene for both Mac-1 and LFA-1 antigens. This mode of biosynthesis and assembly parallels that of other multisubunit protein families which have alternative forms of one subunit which can be associated with a common or highly homologous second subunit, such as the Class I histocompatibility antigens (20), the immunoglobulins (19), and the hemoglobins (21).

[3] M.-K. Ho and T. A. Springer, unpublished observations.

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