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Immunology

cDNA sequence for the αM subunit of the human neutrophil adherence receptor indicates homology to integrin α subunits

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ABSTRACT **The receptor on human neutrophils (polymorphonuclear leukocytes) that mediates cellular adherence consists of two noncovalently associated subunits, designated αM (Mac-1α, Molα, or CD11b; M_r, 170,000) and β (Mac-1β, Molβ, or CD18; M_r, 100,000). We isolated a cDNA clone for the human neutrophil αM subunit by screening a λgt11 cDNA library made from chronic myelogenous leukemia neutrophils by using an affinity-purified rabbit polyclonal antibody directed against the αM subunit. We used this cDNA clone to obtain additional clones from cDNA libraries made from differentiated HL-60 promyelocytic leukemia cells. Together these cDNAs constitute the complete 1137-amino acid sequence for the mature human αM subunit protein. The deduced amino acid sequence indicates the presence of an extensive extracellular domain with three putative metal-binding regions, (*i*) an amino acid region that is homologous to the A domain of von Willebrand factor, (*ii*) a 26-amino acid hydrophobic sequence that is a potential transmembrane domain, and (*iii*) a 19-amino acid cytoplasmic region. The amino acid sequence for the human neutrophil αM subunit contains regions that are closely related to amino acid sequences of adhesion receptors belonging to the integrin family.**

The human neutrophil (polymorphonuclear leukocyte or PMN) must adhere to the vascular endothelium, migrate through extravascular tissue to regions of inflammation, and ingest appropriately opsonized particles at the site of infection, to carry out its role in host defense. The receptor on the PMN surface that mediates these diverse adherence-related activities has been characterized. This receptor consists of two noncovalently associated subunits, designated αM (Mac-1α, Molα, or CD11b; M_r, 170,000) and β (Mac-1β, Molβ, or CD18; M_r, 100,000) (1–5). The evidence that this receptor plays a critical role in PMN adherence stems from two observations. First, monoclonal antibodies directed against the subunits of this receptor complex block PMN adherence *in vitro* (1–5). Second, children whose PMNs lack this receptor complex suffer severe, recurrent bacterial infections that frequently culminate in death (6).

The αM/β complex on the PMN is a member of a family of related adherence glycoproteins present on the surface of leukocytes in both mice and humans (1, 2). On T lymphocytes the adherence complex consists of a larger molecular weight α subunit, designated αL (LFA-1α or CD11a; M_r, 180,000) noncovalently linked to the common β subunit in an α1β1 heterodimer (1). The αL/β complex mediates cytotoxic T-lymphocyte functions (1). The third member of this family, αX (p150,95, or CD11c; M_r, 150,000), is coupled to the β subunit on the cell surface of T lymphocytes, PMNs, monocytes, and macrophages (1).

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These leukocyte-adherence glycoproteins are a subfamily within a larger family of receptors termed "integrins" that mediate attachment of cells to matrix components (for review, see refs. 7 and 8). The integrins include platelet glycoproteins IIb/IIIa, the fibronectin receptor (FNR), the vitronectin receptor (VNR), the very late activation antigens (VLA) on T lymphocytes, and the position-specific antigens in *Drosophila* (7–12). Amino acid sequences for the β subunits of the three major integrin subfamilies have been deduced from the corresponding cDNAs and indicate extensive homologies among the FNR β (13), the leukocyte β (14, 15), the VNR β, and the IIb/IIIa β subunits (16, 17). The integrin α subunits of the FNR (13, 17), VNR (17, 18), and IIb (19) and the leukocyte αX subunit (20) also appear to be related.

To further understand the structure–function activity of the human PMN αM/β receptor complex and to investigate the relationship of the α subunits of this PMN complex to the integrin α subunits, we isolated the cDNA¶ for the αM subunit and derived the amino acid sequence.

MATERIALS AND METHODS

Production of Affinity-Purified Rabbit Polyclonal Antibody Versus αM. We produced a rabbit polyclonal antibody directed against the αM subunit of the PMN adherence receptor by immunizing rabbits with αM antigen purified from chronic myelogenous leukemia (CML) PMNs by using monoclonal antibody 60.1-Sepharose (anti-αM) affinity chromatography and HPLC as described (21). Antibodies from immune rabbit sera were affinity-purified on αM-Sepharose and shown to be monospecific by immunoblotting of a PMN lysate.

Construction and Screening of cDNA Libraries. Five micrograms of poly(A)$^+$ RNA was obtained from CML PMNs and used to construct a λgt11 cDNA library as described (22). This library was screened by using the affinity-purified anti-αM rabbit polyclonal antibody (23). One positive clone from the CML PMN library was plaque-purified, and the cDNA insert was labeled to high specific activity by nick-translation. This cDNA was used to screen a λgt10 cDNA library made from retinoic acid-differentiated HL-60 cells and a λgt11 cDNA library made from dimethylformamide-induced HL-60 cells (24). Positive clones were isolated, and the cDNA inserts were sequenced.

cDNA Sequence Analysis. The DNA sequence of the cDNA inserts was determined by the dideoxy chain-termination

Abbreviations: CML, chronic myelogenous leukemia; PMN, polymorphonuclear leukocyte; FNR, fibronectin receptor; VNR, vitronectin receptor; vWF, von Willebrand factor.

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¶The sequence reported in this paper is being deposited in the EMBL/GenBank data base (accession no. J04145).

method of Sanger *et al.* (25) by using deoxyadenosine 5′-[α-[^{35}S]thio]triphosphate and the phage cloning vectors M13mp18 and M13mp19. All clones were sequenced in both directions.

Protein Purification, Tryptic Peptide Generation, and Amino Acid Sequencing. Approximately 1 mg of αM was isolated from CML PMNs with monoclonal antibody 60.1-Sepharose (anti-αM) affinity chromatography and HPLC as described (21). Affinity-purified αM was succinylated and digested with trypsin. Tryptic peptides were size-fractionated on an LH-60 column in the presence of formic acid/ethanol and separated by reverse-phase HPLC. NH_2-terminal amino acid sequence of the individual tryptic peptides was determined by automated Edman degradation with an Applied Biosystems (Foster City, CA) model 470A gas-phase sequencer (26).

Northern Blotting. Total RNA was extracted from undifferentiated HL-60 cells (27), HL-60 cells induced to differentiate by 1 μM retinoic acid (Sigma) (28) or 90 mM dimethylformamide (Sigma) (29), normal human PMNs, and CML PMNs (30) by a modification of the guanidine hydrochloride method (31). Poly(A)$^+$ RNA was isolated on oligo(dT)-cellulose (32) and 5 μg was electrophoresed on a formaldehyde/agarose gel and blotted to nitrocellulose (31). Northern blots were hybridized to a nick-translated, ^{32}P-labeled cDNA coding for the 378-base-pair (bp) *Eco*RI–*Eco*RI fragment of the αM cDNA. Conditions of the hybridization, washing, and autoradiography have been described (31).

Data Analysis. Sequence homology searches were performed by using the GENEPRO computer program (Riverside Scientific, Seattle).

RESULTS AND DISCUSSION

Isolation and Characterization of cDNA Clones. We screened 5 × 10^5 recombinants from a λgt11 cDNA library made from CML PMNs with our affinity-purified rabbit polyclonal antibody directed against the αM subunit. Two positive clones were detected; one clone was plaque-purified and its cDNA insert was sequenced. This clone contained a 378-bp insert with a continuous open reading frame. The predicted amino acid sequence from a segment of this cDNA exactly matched the amino acid sequence (Arg-Val-Met-Gln-His-Gln-Tyr-Gln-Val-Ser) obtained from a tryptic peptide of the αM subunit (Fig. 1). This 378-bp cDNA is identical to the 378-bp partial cDNA for human αM (33).

We used this cDNA insert to screen a λgt11 cDNA library constructed from dimethylformamide-induced HL-60 cells and a λgt10 cDNA library made from retinoic acid-differentiated HL-60 cells. Additional clones were obtained that spanned 3411 nucleotides of open reading frame ending with a stop codon and followed by 722 bp of 3′ noncoding sequence (Fig. 1). The 3′ untranslated region terminated in a poly(A) tail and possessed a polyadenylylation signal (AA-TAAA) 27 nucleotides upstream from the poly(A) tail (Fig. 1).

Human αM Subunit Amino Acid Sequence Deduced from cDNA Clones. The deduced amino acid sequence from the composite cDNA encoded 1137 amino acids. Edman degradation of peptides produced by tryptic cleavage of the αM subunit yielded unique amino acid sequences for four peptides that were identical to those predicted from the open reading frame of the cDNA (Fig. 1). In addition, the NH_2-terminal amino acid sequence from the intact αM subunit matched the deduced sequence (21).

The amino acid sequence for the αM subunit indicated the presence of a 19-amino acid COOH-terminal cytoplasmic domain and a 26-amino acid hydrophobic sequence that was a probable membrane-spanning domain. The presence of a transmembrane domain is consistent with the observation that the αM subunit is an integral membrane protein. Paired basic residues, located on the COOH-terminal cytoplasmic side of the membrane-spanning domain, may serve to anchor the protein into the membrane.

Within the extracellular portion of the human αM subunit are three short amino acid segments that are homologous to the calcium-binding regions of other proteins (Fig. 1) (34–39). These putative metal-binding domains contain the consensus sequence Asp-Xaa-Asp-Xaa-Asp-Gly-Xaa-Xaa-Asp, which is characteristic of the EF-hand type of calcium-binding site present in calmodulin, troponin C, myosin light chain, and thrombospondin, or the "lock-washer" type of calcium-binding site (34–39).

The extracellular portion of the αM subunit also contains a 187-amino acid domain inserted on the NH_2-terminal side of the metal-binding regions. This inserted domain is homologous to the type A repeats present in von Willebrand factor (vWF) (40–43). In vWF the region lies between residues 496 and 1111 in the mature protein and consists of three tandem repeats, designated A1, A2, and A3, with an identity among the three repeats ranging from 29% to 43% (40, 42). In human αM the homologous region begins with cysteine-128 in the mature protein. Although there exists an overall identity of 17% between the A domain of vWF and the inserted region in human αM, alignment of sequences indicates that residues 134–160, 233–243, and 264–272 in the αM subunit are the regions of greatest identity to the vWF repeats.

There are 19 potential sites of N-linked glycosylation in the deduced amino acid sequence for αM (Fig. 1). All of these sites are present in a region of the protein that is likely to be extracellular. The complete cDNA for the murine αM, which encodes 1144 amino acids, was shown to possess 17 potential N-glycosylation sites (43). Our results suggest that the majority of the sites for glycosylation are located in the proximal half of the extracellular region.

Expression of Human αM Subunit mRNA. The 378-bp *Eco*RI–*Eco*RI fragment of cDNA for the human αM subunit detected a transcript of ≈4.7 kilobases in dimethyl formamide- and retinoic acid-differentiated HL-60 promyelocytic leukemia cells, normal human PMNs, and CML PMNs (Fig. 2). No hybridization was observed on Northern blots with RNA from undifferentiated HL-60 cells (Fig. 2). These findings are consistent with previous studies (44) in which a marked increase in surface expression of the αM subunit in HL-60 cells treated with different chemical-inducing agents was noted. The αM subunit is known to be expressed in high levels on normal human PMNs and CML PMNs (21, 44).

Comparison of the Amino Acid Sequences for Human αM Subunit and Integrin α Subunits. Amino acid sequence for the human αM subunit predicted from the cDNA indicated that the human αM subunit is closely related to the murine αM subunit (43). There is 74% overall amino acid sequence identity between the two proteins (43). The most conserved regions are the three metal-binding domains and the transmembrane domain. The human αM subunit is also closely related to the human leukocyte αX (p150,95) subunit with 63% overall amino acid sequence homology (20).

Murine αM and human αX are homologous to the FNR α subunit, VNR α subunit, and platelet IIb (13, 18–20, 43). All of these integrin α subunits have a short COOH-terminal cytoplasmic portion, a transmembrane region located near the COOH terminus, and extracellular metal-binding domains (13, 18–20). Regions of strongest homology among all of these subunits are the membrane-spanning domains and the metal-binding sites.

The leukocyte subfamily of integrins possesses several distinctive features when compared to the members of the other two subfamilies. The FNR α subunit, VNR α subunit, and platelet IIb are composed of two-chain disulfide-linked polypeptides in which both chains are encoded by a single gene (13, 18, 19). The protein product is cleaved at a Lys-Arg

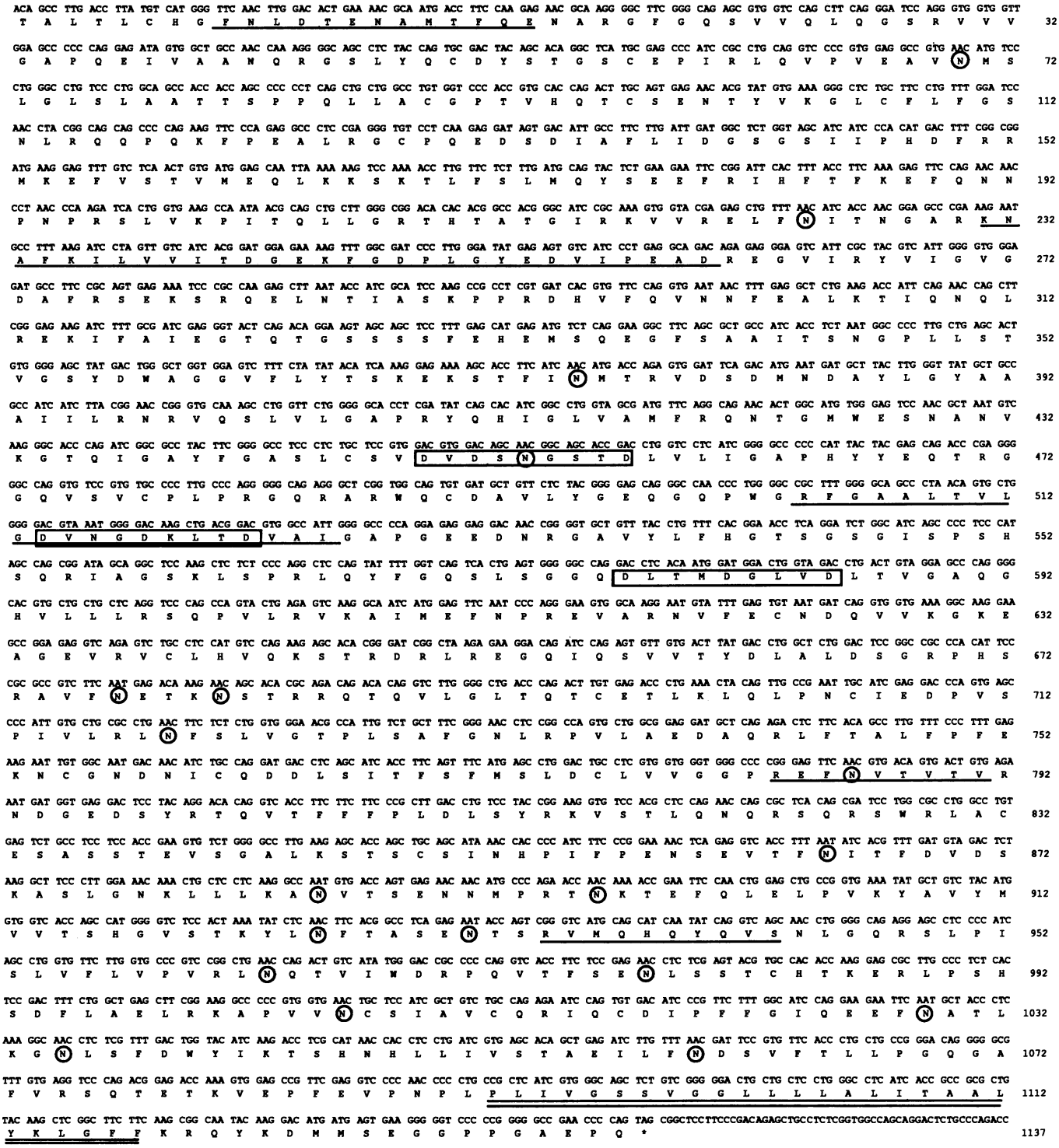

FIG. 1. DNA sequence and predicted amino acid sequence of human αM subunit. Both strands of all clones were sequenced by the dideoxynucleotide chain-termination method. Amino acids are indicated by the single-letter code under the nucleotide sequence and are numbered starting with the NH_2 terminus determined by NH_2-terminal amino acid sequencing. Amino acid sequences from the NH_2-terminal end of the protein, and from tryptic peptides, are underlined once. The potential membrane-spanning domain is underlined twice. The three metal-binding sequences are boxed. Potential N-linked glycosylation sites are circled.

recognition site, and the two subunits are then disulfide-linked and placed into the membrane with the light chain containing the hydrophobic membrane-spanning region. The human αM subunit lacks this cleavage site, as do the murine αM subunit (43) and the human αX subunit (20). These observations are consistent with previous studies demonstrating that the leukocyte α subunits are single-chain polypeptides (1, 2).

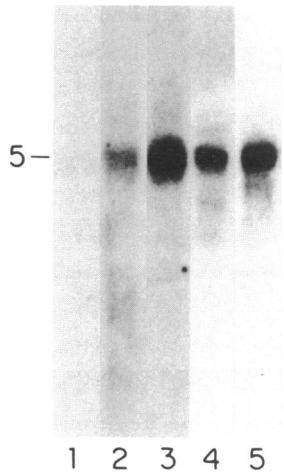

FIG. 2. Northern blot analysis. Five micrograms of poly(A)$^+$ RNA from each cell population was separated on formaldehyde/agarose gels, transferred to nitrocellulose, and hybridized to a ^{32}P-labeled 378-bp *Eco*RI–*Eco*RI cDNA fragment of the human αM subunit. Lanes: 1, undifferentiated HL-60 cells; 2, HL-60 cells differentiated with 90 mM dimethylformamide for 3 days; 3, HL-60 cells differentiated with 1 μM retinoic acid for 5 days; 4, normal human PMNs; and 5, CML PMNs. The band at 5 kilobases is indicated.

The putative metal-binding domains are present in all integrin α subunits described. However, in the leukocyte subfamily αM and αX possess only three metal-binding domains, whereas the individual α subunits of the other families possess four repeats of this divalent cation-binding site (13, 18–20). Since these metal-binding sites are highly conserved in all of the integrin α subunits and the binding of Arg-Gly-Asp (RGD) receptors to their ligands is divalent cation dependent (45, 46), these metal-binding sites are likely to be physiologically important. However, the precise function of these regions is not clear since, in most of the other proteins with calcium-binding elements, these elements are located intracellularly and bind calcium with high affinity (34–39). In calmodulin and calmodulin-like calcium-binding proteins, the calcium-binding β-turn is flanked by segments with an α-helical secondary structure producing the EF-hand (34–39). These α-helical segments are missing in the integrin α chains. In addition, the integrin metal-binding regions lack the conserved glutamic acid residue in the EF-hand consensus region. The side-chain oxygen on the glutamic acid residue coordinates with the metal in the calcium–calmodulin complex.

The 187-amino acid region that is inserted into the human αM subunit and contains significant homology with the sequence for the A domain of vWF is not found in the FNR, VNR, or platelet glycoprotein IIb/IIIa (13, 16–19). However, human leukocyte αX contains an inserted sequence in the same region of the molecule that is 57% identical to the inserted sequence in human αM (20). This insert may be important in cellular physiology since the A1 and A3 domains of vWF have been demonstrated to contain two binding sites for type III collagen (47). This homologous region within the αM subunit may serve to localize PMNs to inflammatory sites where subendothelial collagen is exposed.

In summary, the cDNA and derived amino acid sequence for the αM subunit of the receptor on human PMNs that mediates cellular adherence indicates that this subunit is a member of the integrin family of adherence receptors and that it is closely related to the murine αM subunit and the human leukocyte αX subunit.

Note Added in Proof. The cDNA sequence for the coding region of the αM subunit published here differs by 1 bp from that published by Corbi *et al.* (48). This difference does not result in an amino acid change. There are two differences in the amino acid sequence for the αM subunit published here and that published by Arnaout *et al.* (49). The glutamine at position 484 in our sequence and that of Corbi *et al.* (48) is not present in the sequence of Arnaout *et al.* (49). In addition, the leucine present at residue 965 in our sequence, and that of Cordi *et al.* (48), is replaced by a phenylalanine in the sequence of Arnaout *et al.* (49).

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