Gene, 171 (1996) 291–294

GENE 09571

Cloning and chromosomal localization of a novel gene-encoding a human β_2-integrin α subunit

(Recombinant DNA; polymerase chain reaction; *ITGAD*; sequence homology)

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Received by J.K.C. Knowles: 13 March 1995; Revised/Accepted: 16 August/22 August 1995; Received at publishers: 11 December 1995

SUMMARY

We isolated a partial genomic clone encoding ITGAD, a novel β_2-integrin α subunit. The *ITGAD* gene is highly homologous to the three previously known α subunit-encoding genes, that compose the β_2 integrin family, in deduced amino acid sequence, intron/exon structure and mapping location (chromosome 16p11).

INTRODUCTION

Integrins (Itg) are a large family of cell surface $\alpha\beta$ heterodimers involved in cell-cell and cell-extracellular matrix interactions (Hynes, 1992). Three Itg that share the β_2 (CD18) subunit are restricted in expression to leukocytes and have homologous α subunits: α^L (LFA-1, CD11a), α^M (Mac-1, CD11b), and α^X (p150/95, CD11c) (Larson and Springer, 1990). These Itg are important in the emigration of leukocytes from the vasculature, interaction with target cells and antigen-presenting cells, and binding to iC3b and fibrinogen (Springer, 1995). Inherited defects in the β_2 subunit, which lead to the absence of these three heterodimers in leukocyte adhesion deficiency, result in life-threatening bacterial infections (Anderson and Springer, 1987).

To study the possibility of an alternative Itg α subunit which could associate with β_2, a genomic molecular cloning approach was used and a novel gene was found.

EXPERIMENTAL AND DISCUSSION

(a) The cloning of a novel *ITG* gene

A genomic approach was used, because the genome would contain all α subunit-encoding genes, regardless of cellular distribution, in equal numbers. The most conserved region of α^L, α^M and α^X is in the EF hand-like putative divalent cation binding repeats. To identify novel leukocyte integrin α subunits, a number of degenerate PCR primers complementary to this region were designed based on the known intron/exon structure of the human α^X-encoding gene, *ITGAX* (Corbi et al., 1990). primers A, 5′-GGGRGCMCCYCGMTAYCAGCACA; B, 5′-ATYGGCKCYTAYTTCGGKG; W, 5′-ASAYRRACACCYGGCCYCCTC; and X, 5′-CTCCTCYMSWGGGGCCCCIAYRRCCACGTC (where K = G or T; M = A or C; R = A or G; S = C or G; Y = C or T) were able to generate PCR products of the appropriate length. Using the exon numbering system for *ITGAX*, primers

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Abbreviations: aa, amino acids(s); bp, base pair(s); cDNA, DNA complementary to RNA; iC3b, inactivated third component of complement b fragment; Itg, integrin(s); *ITG*, gene encoding Itg; *ITGAD*, gene encoding a novel Itg α^D subunit; *ITGAX*, gene encoding the Itg α^X subunit; kb, kilobase(s) or 1000 bp; nt, nucleotide(s); ORF, open reading frame; PCR, polymerase chain reaction; RT, reverse transcription(tase).

SSDI 0378-1119(95)00869-1

B and W were located within exon 14. PCR products from genomic DNA using primers B and W were cloned and sequenced and included known leukocyte integrin α subunit gene sequences as well as products that were most similar to *ITGAX*, but differed by 5 nt. The products of primers A (from exon 13) and X (from exon 15) included *ITGAX* and a product that corresponded to the novel BW product in exon 14 and was homologous to known α subunit genes in the exons, but differed substantially in the two introns.

The intron between exons 14 and 15 of this novel product was used to probe 5×10^5 clones of a human pWE15 genomic cosmid library (kindly provided by Glen Evans, Salk Institute). A clone with a 37-kb insert designated pWE15αW.1 was isolated.

(b) Sequencing a novel *ITG* gene and aa comparison

Sequences that hybridized with the full-length α^X and α^M cDNAs were localized to a 21-kb *Not*I-*Eco*RI fragment on the 5′ end of the cosmid insert. *Sau*3A, *Taq*I, *Hae*I and *Bgl*II fragments that hybridized with the α^X cDNA were selected from this region for sequencing. Over 5000 nt were sequenced, including a 4153-nt fragment containing exons 25–30. Eleven putative exons were identified by sequence homology (Fig. 1A). A twelfth exon, exon 14, was sequenced in the PCR fragment, but did not appear in the cosmid clone, in which the intron between exons 14 and 15 is at the 5′ end. Seven exons were completely sequenced (21 and 25–30) and five were sequenced through one intron boundary. Exons 18–20, 22, and 23 have not been localized and are predicted to be present in regions that remain to be sequenced. All exons thus far identified contained ORFs, consensus splice sites, and the same intron phase as in the α^X (Corbi et al., 1990) and α^M (Fleming et al., 1993) genes.

The exons correspond to the C-terminal two-thirds of the putative a subunit, from the three EF hand-like putative divalent cation binding repeats to the transmembrane domain. The translated aa sequence is most homologous to the α^X subunit (70% identity) compared with α^M (59%) and α^L (32%) (Fig. 1B). We designate this novel gene *ITGAD* and predict it is expressed as a novel β_2 integrin α^D subunit. Nested RT-PCR on tonsillar RNA using primer BW, 5′-GACAGCGATGGCAGCACCGACCTGA, derived from the *ITGAD* exon 14 sequence and primer X after an initial round of PCR with primers A and X, showed the novel gene to be processed at least to the point of having the intron between exons 14 and 15 spliced out. This 220-nt product when subcloned and sequenced was 86% identical to the α^X sequence, as compared to the 92% identity of α^X and α^M over the same region. Using this product as a probe and by combining RT-PCR and enrichment using specific restriction enzymes, the predicted transcription product of this gene could be shown to be present in tonsilar mRNA (Fig. 2). Furthermore another group of investigators have independently, using an alternative strategy, cloned the cDNA of an α subunit with essentially identical sequence to the predicted sequence from our gene (W.M. Gallatin, unpublished personal communication). Our mutual use of the letter D for this integrin is based on the likelihood that the protein will be designated CD11d in the future.

B

..exon 14 |exon 15 ...|exon 16 ...|...exon 17 |exon21
DSDGSTDLILIGAPHYYEQTRGGQVSVCPLPRGRVQWCCDAVLPGEQATPGRFGAALTVLGDVNEDKLIRIASSQLSPLQYFGQALSGGQDLTQDGLMDLAKAVYRCWEEKPSALEAGDATVCLTIQKSSLDQLLQTLTVGSSLELNVI
|...exon 24 |exon25 |exon26 |exon27 |exon28
VTVWNAGEDSYGTVVSLYYPAGVSHRRVSGAQATFQLELPVKYAVYTMISRQEFSTKYFNFATSDEKKMKEAEHRYRVNNLSQRDLAISINFWVPVLLNGVAVWDVAMEAPSQSLPCVSERKPPQHSDFLTQISRSPMLDCSIADCLQFR
|exon29 |exon30
CDVPSFSVQEELDFTLKGNLSFGWVRETLQKKVLVVSVAEITFDTSVYSQLPGQEAFMRAQMEMVLEEDEVYNAIPIIMSSGVGALLLLALITATLYK

Fig. 1. Organization and partial sequence of *ITGAD*. **A**: Genomic organization. Restriction map of the human genomic 37-kb pWE15αW.1 cosmid insert to an accuracy of 250 bp. *Bam*HI (B), *Bgl*II (Bg), *Eco*RI (E), *Hin*dIII (H), and *Not*I (N). Filled boxes represent possible exons that are numbered based on homology to the exons of *ITGAX* (Corbi et al., 1990). Thicker lines indicate flanking vector sequence containing *Not*I sites. Mapping was done by Southern hybridization of digested fragments with ^{35}S-labeled exons, as well as PCR between exons and between the T3 promoter site (at the left or 5′ end) and certain exons. **B**: Homology of the translated putative exons of *ITGAD* with the α^L (CD11a), α^M (CD11b), and α^X (CD11c) subunits are underlined when shared by two of the other subunits (GenBank accession Nos.: U40274, U40275, U40276, U40277, U40278, U40279). Translated exons from α^D were combined for alignment with the corresponding segments from other leukocyte integrin subunits. Exon boundaries are indicated by vertical lines and three periods indicate segments that do not extend fully to the predicted boundary. Segments used for alignment and Swiss-prot database accession Nos. were: α^L, aa 470–537, 567–600, 631–662, 789–836, and 910–1113 (P20701); α^M, aa 467–537, 571–602, 633–666, 790–836, and 914–1130 (P11215); α^X, aa 468–537, 571–602, 633–666, 790–836, and 912–1129 (P20702).

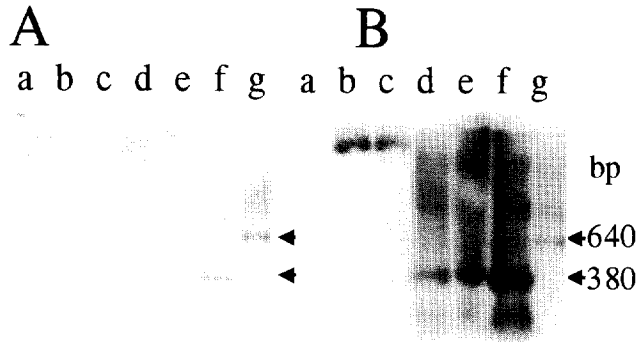

Fig. 2. *ITGAD* transcription product present in tonsilar RNA after enrichment. **A**: To detect transcription of the novel gene, tonsilar mRNA was submitted to RT-PCR with degenerate primers A and X. The RNA was then enriched for the novel sequence by digesting with three specific restriction enzymes *Fok*I, *Sal*I, and *Nar*I which cut the known α subunit cDNAs in this region and then resubmitted for PCR with A and X. Only after two rounds of this type of enrichment could the appropriate 380-bp product be detected with the ^{32}P-labeled 220-bp probe from the new sequence. In genomic DNA where the novel gene sequence would be expected to be expressed in equal amounts to the other α subunits the appropriate 640-bp product with the two introns still present is readily detected. **B**: In contrast, α ^{32}P-labeled probe from the same region of α^M is able to detect α^M sequence without enrichment on the same blot. The 92% homology between α^M and α^X over this region leads to some cross-reactivity between the α^M probe and α^X. An equal number of copies of sequence was loaded on a 1.2% agarose gel in each lane by weighting for the expected length of an individual copy. Lanes: a, cDNA α^L; b, cDNA α^M; c, cDNA α^X; d, RT-PCR product of degenerate primers A and X; e, PCR product of primer A and X after triple digestion; f, PCR product of primer A and X after a second round of triple digestion; g, PCR product of primers A and X using genomic DNA as a template.

(c) The chromosomal localization of *ITGAD*

To localize the *ITGAD* gene, we performed fluorescence in situ hybridization with a biotin-labeled probe of the cosmid insert on normal human metaphase chromosomes (Rowley et al., 1990). Specific labeling of chromosome 16, band p11 (Fig. 3) was observed on four chromatids (16 cells), three chromatids (7 cells) or two chromatids (2 cells) in 25 cells examined. A single background signal was observed on 3p12. Localization to 16p11 was confirmed in a second hybridization experiment. *ITGAD* thus clusters with the α^L, α^M, and α^X genes, which also localize to chromosome 16, band p11 (Corbi et al., 1988).

(d) Conclusions

The similarity in gene organization, sequence, and chromosomal location suggest that *ITGAD* encodes a fourth leukocyte integrin α subunit. This subunit is much more homologous to the three β_2 integrin α subunits than to other integrin α subunits, and may associate with the β_2 subunit and be expressed on leukocytes. Previous studies have shown a subunit lower in M_r than α^L, α^M, or α^X that associates with β_2 on human monocytes (Sanchez-Madrid et al., 1983), and an α subunit that associates with β_2 on canine macrophages and lymphocytes that appears distinct from α^L, α^M, and α^X (Danilenko et al., 1990). The relationship of these subunits to α^D remains to be characterized.

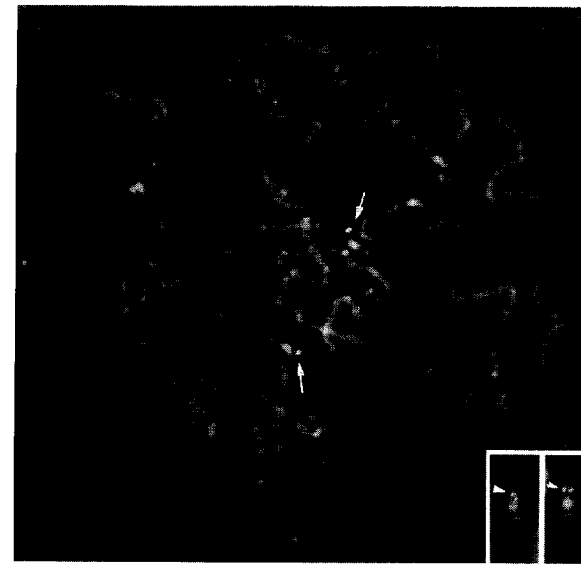

Fig. 3. Chromosome localization. The chromosome 16 homologues are identified with arrowheads. The inset shows partial karyotypes of two chromosome 16 homologues illustrating specific labeling at 16p11 (arrow). A biotin-labeled human *ITGAD* cosmid probe was prepared by nick translation using Bio-11-dUTP (Enzo Diagnostics) and used in fluorescence in situ chromosomal hybridization with metaphase phytohemogglutin-stimulated peripheral blood lymphocytes as described (Rowley et al., 1990). Hybridization was detected with flourescein-conjugated avidin (Vector Laboratories), and chromosomes were identified by staining with 4,6-diamidino-2-phenylindole-dihydrochloride.

ACKNOWLEDGMENTS

Supported by NIH grants CA31799 and CA40046. D.A.W. was supported by an MRC of Canada Clinician Scientist Award. We thank Lloyd Klickstein for advice and Steven Minaglia for assistance with chromosomal localization.

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