Cloning and expression of the chicken CD18 cDNA

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Abstract: The leukocyte integrins play a critical role in a number of cellular adhesive interactions during the immune response. We describe here the isolation and characterization of the chicken β2 (CD18) subunit, common to the leukocyte integrin family. The deduced 748-amino-acid sequence reveals a transmembrane protein with 65% and 64% identity with its human and murine homologues, respectively. The chicken β2 can associate on the cell surface with the human α subunit of LFA-1 and yields a hybrid molecule capable of binding to purified ICAM-1 and ICAM-3. *J. Leukoc. Biol.* 55: 501–506; 1994.

Key Words: *LFA-1 · chicken CD18 cDNA · leukocyte integrins*

INTRODUCTION

A variety of important cell adhesion interactions within the immune system are mediated by the leukocyte integrins [1, 2]. These structurally related adhesion proteins, LFA-1, Mac-1 and p150,95, are composed of unique α subunits noncovalently associated with a common β subunit (CD18) [3–6]. LFA-1 (CD11a/CD18) is expressed on all leukocytes and is involved in lymphocyte adherence to vascular endothelium, cytotoxic T lymphocyte and natural killing, antibody-dependent cytotoxicity mediated by granulocytes and monocytes, and helper T and B lymphocyte responses [7–9]. This range of functions demonstrated by LFA-1 is determined by the binding of its three counterreceptors, ICAM-1, -2, and -3. ICAM-1 (CD54) is expressed at low levels on resting lymphocytes, macrophages, endothelial and epithelial cells, and keratinocytes, but shows highly inducible expression in response to cytokines and other inflammatory mediators [10, 11]. ICAM-2 is constitutively expressed at a low level on lymphocytes and monocytes and at a high level on vascular endothelium [12]. Unlike ICAM-1 and -2, ICAM-3 is expressed only on leukocytes [13].

The β2 subunit appears to play an important role in the function of all three leukocyte integrins, because monoclonal antibodies directed against this subunit significantly inhibit leukocyte integrin-mediated functions [5, 6, 9, 14]. This is amply demonstrated in the leukocyte adhesion deficiency (LAD) syndrome, in which there is loss of expression of the β2 subunit arising from heterogeneous mutations [15, 16]. Thus far, β2 integrins have been characterized only in mammals. Therefore, we have examined whether the structure and function of the β2 integrin subunit are conserved in an evolutionarily distant species. We have cloned an avian β2 integrin cDNA and determined its nucleotide and deduced amino acid sequence. The chicken β2 can associate at the cell surface with the human LFA-1 α subunit and maintain binding to the human ICAM molecules.

MATERIALS AND METHODS

Isolation and characterization of an avian CD18 cDNA

A chicken spleen cDNA library in λgt11 (Clontech, Palo Alto, CA) was screened by cross-hybridization with both human [17] and murine [18] CD18 cDNAs using standard methods [19]. Two *Eco*RI-*Xba*I fragments (964, 1812 bp) encompassing the human CD18 cDNA and three similar fragments (668, 986, and 1174 bp) encoding the murine cDNA were labeled with [α-^{32}P]dCTP using a random primer DNA labeling system (Gibco BRL, Gaithersburg, MD) and hybridized with the library filters in 50% deionized formamide, 5× SSC, 5× Denhardt's solution, 0.1% SDS, 10 mM sodium phosphate buffer, pH 7.0, 1 mM EDTA, 50 μg/ml sheared herring sperm DNA at 37°C. Filters were washed at low stringency in 1× SSC, 0.1% SDS, 10 mM sodium phosphate buffer, pH 7.0, 1 mM EDTA, first at room temperature and then at 37°C. Hybridizing phage were plaque-purified twice and the phage inserts then subcloned into the *Eco*RI site of the plasmid pBluescript (Stratagene, La Jolla, CA). Three clones, A4, E4, and S1, were analyzed by restriction enzyme mapping and partial sequencing.

The complete nucleotide sequence of clones A4 and E4 was determined by the dideoxy chain termination method with Sequenase, a modified T7 DNA polymerase (United States Biochemical, Cleveland, OH). Both DNA strands were sequenced using pBluescript T3 and T7 primers and synthetic oligonucleotide primers complementary to the chicken β2 sequence. Alignment of nucleotide sequences was performed using the Best fit and Pileup programs from GCG [20].

Cell lines and monoclonal antibodies

The SV40-transformed African green monkey kidney fibroblastoid cell line COS [21] was used for transfection.

The following monoclonal antibodies (mAbs) were used in this study: TS1/22 (anti-CD11a, immunoglobulin G1) [22], RR1/1 (anti-ICAM-1, IgG1) [10], CBR-IC3/1 (anti-ICAM-3, IgG1) [13], CBR-IC3/2 (anti-ICAM-3, IgG2a) [23], X63 (nonbinding antibody from the myeloma P3X63Ag8, IgG1), CBRM1/23 (anti-Mac-1, IgG2a) [24], and CBRp150/2E1 (anti-p150,95) [25].

Abbreviations: FCS, fetal calf serum; IgG1, immunoglobulin G1; LAD, leukocyte adhesion deficiency; mAb, monoclonal antibody; PBS, phosphate-buffered saline; SDS, sodium dodecyl sulfate; SSC, standard saline citrate.

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COS cell transfection and adhesion assays

A cDNA clone containing the entire coding region of the chicken β2 was created by splicing the 1.42-kb *Sma*I-*Apa*LI fragment from clone A4 and the 1.93-kb *Sma*I-*Apa*LI fragment, obtained by partial *Apa*LI digestion, of clone S1. The *Sma*I sites of both clones are derived from the vector pBluescript polylinker. Addition of BstXI linkers allowed the spliced product to be subcloned into the transient expression vector Ap^{r}M8, an ampicillin-resistant derivative of CDM8, provided by Lloyd Klickstein (The Center for Blood Research, Boston, MA). COS cells were transfected with Ap^{r}M8 constructs expressing the leukocyte integrin β2 and α subunits using the DEAE-dextran method [26]. COS transfectants were suspended using trypsin-EDTA and reseeded 1 day prior to assay [27].

Purified ICAM-1 and ICAM-3 used in the adhesion assays were isolated from human spleen cell lysates by immunoaffinity chromatography [23, 28]. Immunoaffinity-purified proteins, appropriately diluted in 10 mM Tris, pH 8.0, 150 mM NaCl, 2 mM $MgCl_2$ (TSM) were adsorbed onto a 96-well plate (Linbro-Titertek, Flow Laboratories, McLean, VA) for 1.5 h at room temperature (or overnight at 4°C). Nonspecific binding sites were blocked for 60 min at room temperature with TSM, 1% BSA and three washes with 5% fetal calf serum (FCS), L15 medium (assay buffer). The specificity of receptor-ligand interactions was determined by pretreatment of wells with blocking and nonblocking mAb for 30 min at 4°C. The mAbs RR1/1 [10] and CBR-IC3/1 [13] plus CBR-IC3/2 [23] were used to block specifically ICAM-1 and ICAM-3 binding, respectively. Supernatant from the myeloma X63 was used as a nonblocking control.

Adhesion assays in 96-well plates were performed as described previously [29]. Briefly, COS cell transfectants were harvested in 10 mM EDTA, phosphate-buffered saline (PBS); washed with 5% FCS, L15 medium; and then labeled with 6.25 μg/ml 2',7'-bis-(2-carboxyethyl)-5(and -6) carboxyfluorescein (BCECF; Molecular Probes, Eugene, OR) for 30 min at 37°C with gentle agitation. Cells were washed twice with 5% FCS, L15 medium and finally resuspended to 4×10^5 cells/ml. Labeled cells (2×10^4) were added to each well and incubated at 37°C for 60 min. Unbound cells were then removed by several washes with assay buffer and aspiration using a 21-gauge needle. Fluorescence of the total input cells and of the cells bound after the assay was measured by a Pandex fluorimeter (IDEXX Corp., Westbrook, ME).

Flow cytometry

COS cell transfectants were harvested in 10 mM EDTA, PBS and washed with 5% FCS, L15 medium. Indirect immunofluorescence flow cytometry was performed as described previously [28].

RESULTS AND DISCUSSION

Identification and characterization of chicken β2 cDNA clones

A chicken spleen library constructed in λgt11 was screened by cross-hybridization with human and murine β2 cDNA probes. Three of the positive clones, A4, E4, and S1, were analyzed in detail (**Fig. 1A**). Clones A4 and E4 were sequenced (Fig. 1B). The ATG codon at position 100 is the first initiation codon after an in-frame stop codon and was chosen as the beginning of the open reading frame. The composite

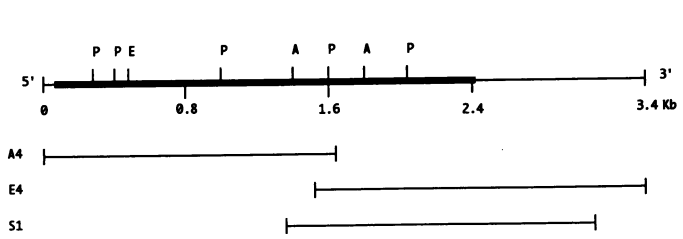

Fig. 1. Restriction map and sequence of chicken CD18 clones. (A) Restriction map of the CD18 clones (P, *Pou*II; E, *Eco*RV; A, *Apa*LI). The open reading frame is indicated by a thick line and the untranslated regions by a thin line. (B) (See next page.) The nucleotide sequence and deduced amino acid sequence of the chicken β2 integrin cDNA. The signal sequence, transmembrane region, and a potential polyadenylation site are underlined. Potential N-linked glycosylation sites are boxed. Sequence data are available from EMBL/GenBank/DDBJ under accession number X71786.

sequence of clones A4 and E4 contains an open reading frame of 2319 bp, a 5'-untranslated region of 99 bp, and a 3'-untranslated region of 923 bp.

The amino acid sequence deduced from the cDNA (Fig. 1B) reveals a transmembrane protein that consists of a 24-amino-acid hydrophobic signal sequence, a 678-amino-acid extracellular domain, a 23-amino-acid transmembrane domain, and a 47-amino-acid cytoplasmic domain. Six consensus sites for N-linked glycosylation (Asn-X-Ser/Thr) are present in the extracellular domain.

Amino acid sequence comparison between the chicken CD18 and its human and murine homologues shows 65% and 64% identity, respectively (**Fig. 2**), whereas the human and murine sequences show 82% amino acid identity. The area of highest sequence identity is found in the region previously found to be conserved between β1, β2, and β3 integrins, between amino acids 104 and 349 (numbering according to chicken β2 sequence, Fig. 2). Within this region, blocks of 24 of 27, 28 of 30, and 53 of 58 amino acid identity exist. The high degree of conservation of this region between chicken and human β2, 80% amino acid identity, suggests an important role for this segment of the protein that has been highly conserved.

The cytoplasmic domain of integrin β subunits has been shown to be important for transducing signals that regulate the adhesiveness of the extracellular domain [1, 2, 7, 30–32]. Two regions of striking sequence identity, 712–723 and 731–748, are found in the cytoplasmic domain. Within these two regions, six amino acids are also found to be highly conserved in the β1, β3, β5, β6, and β7 subunits and have been implicated in the localization of integrins to focal contacts [30]. The second region, from amino acid 731 to 748, contains two sets of residues that are required for human LFA-1 binding to ICAM-1. Deletional and mutational analyses of the human β2 subunit have revealed the importance of a cluster of three contiguous threonines and a phenylalanine residue that are necessary for ICAM-1 binding [31, 32]. The three threonines (740–742) and surrounding residues and the single phenylalanine (748) are completely conserved in the chicken β2 sequence (Fig. 2). Throughout the mature protein, all the cysteine residues, within the cysteine-rich domain and outside, are completely conserved with respect to the human and murine sequences.

B.

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1    AGCTCTGGTGCTGGTCCCAGCCAGGCAGTGCAGCCAGGGGGCTACCAGCCTCACTGCAGTCCCAGAAGAGGAGCTGAAGGGAAGGCTTGATGTGAGGAAATGCCCCGTGAC  111
                                                                                                  M  P  R  D          -21

112  TGCTGCCTCTGGCTGCCAGCAATGACCTGGGTGCTGTTGCTACTGACAACAGCCTTTGCTGCAGAGTGTCCCAAGATCAAGGTGGGGACATGCAAGAACTGCATCCAGTCA  222
-20  C  C  L  W  L  P  A  M  T  W  V  L  L  L  L  T  T  A  F  A  A  E  C  P  K  I  K  V  G  T  C  K  N  C  I  Q  S   17

223  GGCCCTGGCTGCGCCTGGTGCAAGAAGCTGAGTTTCACCAAAGCTGGTGAGCCAGACTCCAACCGCTGTGACACCATAGAACAGCTGCAGCAGAGGGGATGCCCAGAGAAT  333
 18  G  P  G  C  A  W  C  K  K  L  S  F  T  K  A  G  E  P  D  S  N  R  C  D  T  I  E  Q  L  Q  Q  R  G  C  P  E  N   54

334  GAGATTGAGTTTCCAGTAAATGAAATCAAAAGGACGCAGGACAGTGCGTTCAGCAATAAGATACAGCTGACTCCCCAGGAGGTGCATCTGAAGCTGAGGATAAGGGAGCCT  444
 55  E  I  E  F  P  V  N  E  I  K  R  T  Q  D  S  A  F  S  N  K  I  Q  L  T  P  Q  E  V  H  L  K  L  R  I  R  E  P   91

445  GCTGAATTTGATGTGAAGTTTCGTCGTGCTACGGGGTACCCAATTGATATCTACTACCTCATGGACCTCTCCTATTCCATGCTGGATGACCTAGAGAACGTGAAGAAGCTG  555
 92  A  E  F  D  V  K  F  R  R  A  T  G  Y  P  I  D  I  Y  Y  L  M  D  L  S  Y  S  M  L  D  D  L  E  N  V  K  K  L  128

556  GGAGGACAACTGCTTAGAGCCCTGGAGAGCACCACACCCTCTCGCCGCATAGGTTTTGGTTCCTTTGTGGACAAGACTGTACTGCCTTTTGTCAACACACATCCTGAGAAG  666
129  G  G  Q  L  L  R  A  L  E  S  T  T  P  S  R  R  I  G  F  G  S  F  V  D  K  T  V  L  P  F  V  N  T  H  P  E  K  165

667  CTGAAGAACCCTTGCCCCAACAAGGACAGTAATTGCCAGCCTCCCTTCGCCTTCAAGCACATCCTCTCACTGACTGACAATGCCGAGAAGTTTGAGAGTGAAGTGGGGAAG  777
166  L  K  N  P  C  P  N  K  D  S  N  C  Q  P  P  F  A  F  K  H  I  L  S  L  T  D  N  A  E  K  F  E  S  E  V  G  K  202

778  CAGTTCATCTCAGGGAACCTGGATGCCCCAGAAGGGCTGGATGCCATGATGCAGGCGGCAGTGTGTGGAGACTTGATTGGCTGGCGCAATGTGACCCGATTGCTGGTGTAT  888
203  Q  F  I  S  G  N  L  D  A  P  E  G  L  D  A  M  M  Q  A  A  V  C  G  D  L  I  G  W  R  N  V  T  R  L  L  V  Y  239

889  GCAACTGATGATGGCTTCCACTTCGCTGGTGATGGCAAGCTCGGGGGCATTTTGACCCCCAATGATGGCCAGTGCCACTTGGAGGACAACATGTACAAAAAGAGCAATGAG  999
240  A  T  D  D  G  F  H  F  A  G  D  G  K  L  G  G  I  L  T  P  N  D  G  Q  C  H  L  E  D  N  M  Y  K  K  S  N  E  276

1000 TTTGACTATCCTTCTGTTGGCCAGCTGGTCCAGAAACTTGCTGAAAACAACATTCAGCCCATTTTTGCTGTCACCAGCAAGATGGTGGATGTTTACAAAAAACTCAGTGAT 1110
277  F  D  Y  P  S  V  G  Q  L  V  Q  K  L  A  E  N  N  I  Q  P  I  F  A  V  T  S  K  M  V  D  V  Y  K  K  L  S  D  313

1111 ATGATCCCAAAGTCAGCAGTAGGGGAGTTGAACGAGGACTCCAGCAACATCATTGAACTCATCCAGGTGGCCTACAATAACCTCTCTTCACGGATCATCCTGGACCACTCC 1221
314  M  I  P  K  S  A  V  G  E  L  N  E  D  S  S  N  I  I  E  L  I  Q  V  A  Y  N  N  L  S  S  R  I  I  L  D  H  S  350

1222 ACCCTGCCAGATGTCCTGGATGTCAAATATGACTCCATCTGCAATAACAACACAGGAGCCAAGAATGAAGCAAGAGGGCAATGCGACAATGTTAAGATCAATGATGAGGTC 1332
351  T  L  P  D  V  L  D  V  K  Y  D  S  I  C  N  N  N  T  G  A  K  N  E  A  R  G  Q  C  D  N  V  K  I  N  D  E  V  387

1333 ACCTTCAAAGTGAAGGTCACAGCAAATGAGTGCATCAAAAGCCAGTCCTTCACCATCCGGCCCCTGGGCTTCACAGACACGCTCACTGTGCACCTGGACAGCATCTGTGAC 1443
388  T  F  K  V  K  V  T  A  N  E  C  I  K  S  Q  S  F  T  I  R  P  L  G  F  T  D  T  L  T  V  H  L  D  S  I  C  D  424

1444 TGTGACTGCAGAGAGCAGCCTGATCCAACTGCCTGCAGTGGAAATGGCAAGGTCGTCTGTGGGATCTGCAGTTGCAATTTGAGCTACACGGGGAAGAACTGTGAGTGTGAC 1554
425  C  D  C  R  E  Q  P  D  P  T  A  C  S  G  N  G  K  V  V  C  G  I  C  S  C  N  L  S  Y  T  G  K  N  C  E  C  D  461

1555 ACCAAAGGCAAGACCAGCAAAGAGCTGGAGGGCAGCTGCCGGAAGGACAACAGCTCAGTCATCTGCTCAGGGCTGGGGGACTGCGTGTGTGGGCAGTGCGTCTGCCACACC 1665
462  T  K  G  K  T  S  K  E  L  E  G  S  C  R  K  D  N  S  S  V  I  C  S  G  L  G  D  C  V  C  G  Q  C  V  C  H  T  498

1666 AGTGACGTACCTGGCAAGGAGATCTATGGCACCTTCTGCGACTGTGACAACATGAACTGCGAGTTTCACAACGGCTCACTGTGTGGTGGCGAGGAGCGTGGACGATGCGAC 1776
499  S  D  V  P  G  K  E  I  Y  G  T  F  C  D  C  D  N  M  N  C  E  F  H  N  G  S  L  C  G  G  E  E  R  G  R  C  D  535

1777 TGTGGTGAGTGCAAGTGCACACCCAAGTACGAGGGCAGTGCCTGCCAATGCAAGAAGTCGACTGATGGCTGTAGGAACAGCCGGCAAAATGAATGCAGCCTGCGTGGCTCC 1887
536  C  G  E  C  K  C  T  P  K  Y  E  G  S  A  C  Q  C  K  K  S  T  D  G  C  R  N  S  R  Q  N  E  C  S  L  R  G  S  572

1888 TGCCCATGCAACCGCTGCCAGTGCCGAGGGGGCTACCAGCCCCCCTTCTGCGAGGAGTGTCCTGGCTGCCCCTCACCCTGTGGCAGGCACATTTCCTGCGTGGAGTGCAAG 1998
573  C  P  C  N  R  C  Q  C  R  G  G  Y  Q  P  P  F  C  E  E  C  P  G  C  P  S  P  C  G  R  H  I  S  C  V  E  C  K  609

1999 TCATTCAATAGTGGGCCACTGGCAAAGAACTGCTCTGTGGCCTGCACCAGCATCCAGCTGGCTGATGAGCCACGGGCAGGGAGTCGGCAGTGCAAGGAGAAGGACTCTGAG 2109
610  S  F  N  S  G  P  L  A  K  N  C  S  V  A  C  T  S  I  Q  L  A  D  E  P  R  A  G  S  R  Q  C  K  E  K  D  S  E  646

2110 AACTGCTGGATCTCTTTCTATATGGCCCAGGATGATGGAGAGGAGATGTACACCGTCACTGTTGACCCTAAGAAAGAGTGCCCAGAGCCTCCCAACATCGCGCTGATCGTA 2220
647  N  C  W  I  S  F  Y  M  A  Q  D  D  G  E  E  M  Y  T  V  T  V  D  P  K  K  E  C  P  E  P  P  N  I  A  L  I  V  683

2221 GGCAGCACCATTGCCGGTGTGGCTCTCATTGGCCTGCTGCTCCTGCTGACCTGGCGGCTCTTGACAGAGATCTTTGACCGCCGAGAATACCGCAGGTTTGAGAAGGAGAAA 2331
684  G  S  T  I  A  G  V  A  L  I  G  L  L  L  L  L  T  W  R  L  L  T  E  I  F  D  R  R  E  Y  R  R  F  E  K  E  K  720

2332 TCCAAGGCCAAGTGGAACGAAGCTGATAATCCTCTGTTCAAGAGTGCCACCACCACCGTCATGAATCCCAGATTTGATGGGCAATGAACTGGAGTGATGCCTGAAGCACTA 2442
721  S  K  A  K  W  N  E  A  D  N  P  L  F  K  S  A  T  T  T  V  M  N  P  R  F  D  G  Q  *                          757

2443 GGACCCACCACTAAATAAGGAAACACCAAACAAGGAAATCTCAGGCTCCCACCTCTTCTTCTTTATTGTGTTGTTTTTAACCCTTCTTCCAAGAGACCACCAGTAGCCAGG 2553
2554 TGCTGGCTGAGGGGCTGCACAGTACCTATCCTCTCTCCCAGAGCCATCTGGTTGCACTGTAGGAGGAGGGAACAGCAGTGCCATGGACGGGGCTGCAGAGCAGATCTTACC 2664
2665 AACAGGCACTCACCCCAGCTCCAGTTTCCATCTGTCCTAGCTCCTGGTGCTAACTTTACTTTGTGGCAGGACCTTCAGACACCAAGTCCCATAGGGACTTAGCACAGAAAG 2775
2776 TGCTCTCAATGAGGAGTTCAAAACTGCTATTTCCCTTCTGCTCCAGCAGGCTCCACTCCTAGGCTGCTCCATGCCCTGCTGCACTGCTCTGCACAGTTACACTTGCAGCTC 2886
2887 ATTTCTAAACTACTCACTGCTGAGATGGATGGGGAAGGTCTCCTAGGTGGGCTGTCTGCTCCTGTTGCTGTACCTGGAAATGATGGACAGCCAGAAAACAAACAGTGAATC 2997
2998 TTATTTTTAAATCATCAGCTTCTCCCCAGTGTTACTTCCCTCTTAGACCTACCTCAGGCTCCTCCACGTTTCTACCTGGGCTTCCATGAGCTTGGCTTACCTCCACCATGC 3108
3109 AGCCCACTGCATGGCAGAGAAGCAAAGGAGCAGCGGATTGAATTGTGGAACATCAGTATCAGAGGCGCTGAATGTGTGAAACAGTGCTTGCAGCTTTGAGCTCAACCACAG 3219
3220 CTTCTTCTGCCACCTCTAGGACCAGATCTCTTCCCTACTCTGTGAGTCCATCAGACTTGTTGAGGATCAGCCATGCAAAAGACCTAGCATCACAATAAAATCTCCAGCTC 3330
3331 CGTGCTTTTTCAAAAAAAAAAAAA  3354
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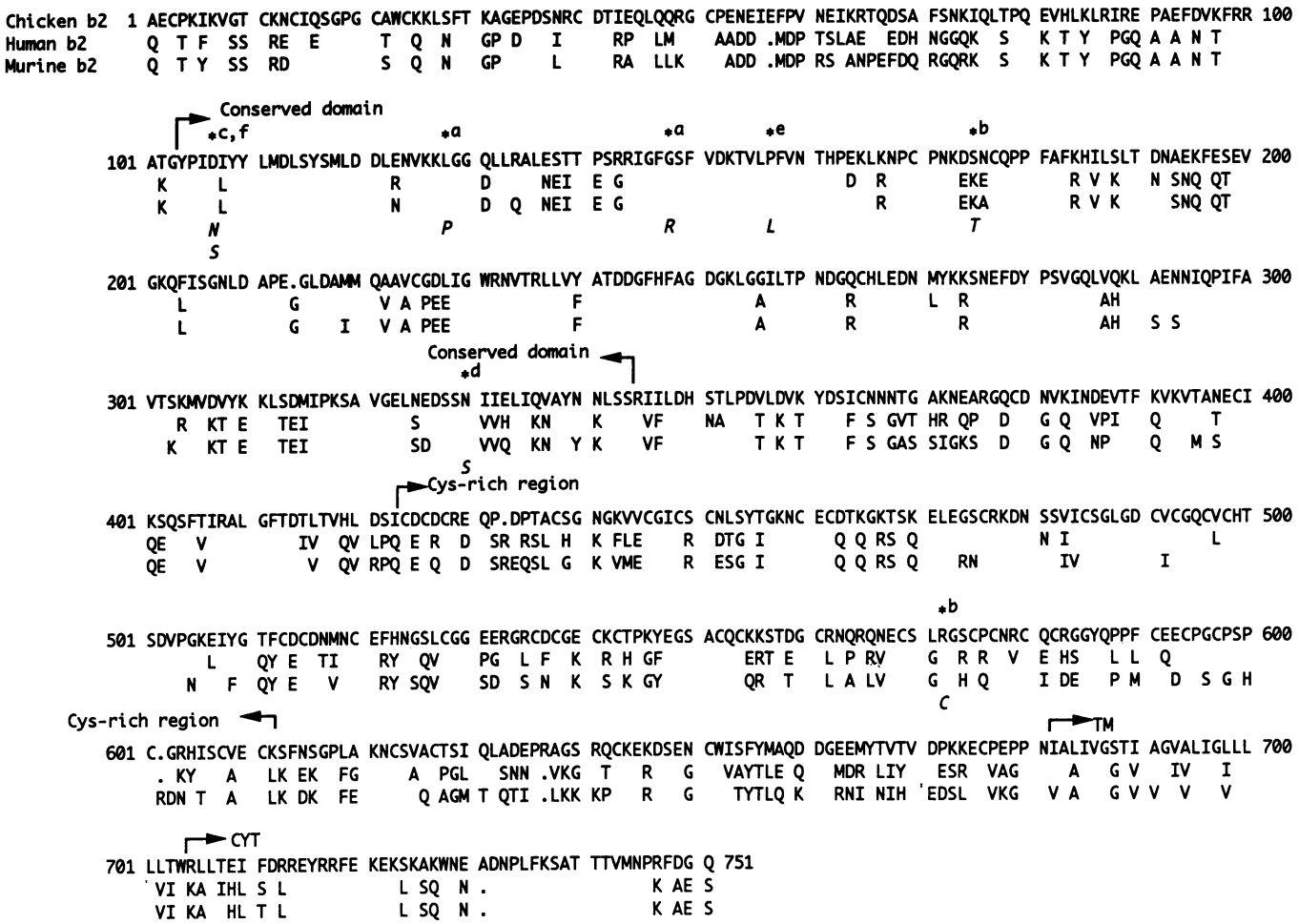

Fig. 2. Comparison of the chicken, human, and murine integrin $\beta 2$ subunit amino acid sequences. The mature amino acid sequence of the chicken $\beta 2$ subunit is shown and only residues that differ in the human and murine sequences are indicated. Gaps in the sequence are indicated with a period. Alignment of the sequences was performed using the Pileup program from GCG [20]. Boundaries of the conserved domain, cysteine-rich domain, transmembrane (TM) region, and cytoplasmic (CYT) domain are indicated. The amino acid residues that when substituted in LAD appear to impair α-β subunit association are marked above with an asterisk and below with the substitution in italics. References for mutations are a [33]; b [34]; c [35]; d [36]; e [37]; f [38].

Expression of chicken $\beta 2$ with leukocyte integrin α subunits

Transient expression in the fibroblastoid COS cell line was used to determine the efficiency of interspecies hybrid formation between the chicken $\beta 2$ subunit and the human αL subunit of LFA-1. Expression of the interspecies heterodimer was monitored using mAbs against the human αL subunit, which requires the $\beta 2$ subunit for cell surface expression [28]. The α subunit of human LFA-1 was expressed equivalently on COS cells transfected with the human or chicken $\beta 2$ subunit but was poorly expressed when transfected alone (**Fig. 3**). Thus the sequences required for association between $\beta 2$ and the LFA-1 α subunit have been conserved between avian and mammalian $\beta 2$.

Analysis of leukocyte adhesion deficiency (LAD) patients has revealed amino acid substitutions that result in impaired α-β subunit association (asterisks, Fig. 2), highlighting the importance of these residues in heterodimer formation [33–38]. Seven of these substitutions are located in the conserved domain. All but one are conserved in the chicken $\beta 2$ sequence. The single exception, a human K-to-T substitution that is S175 in the chicken sequence, is only weakly impaired in ability to associate with the LFA-1 α subunit, because the mutant allele is expressed 60% as well as the wild type [34].

Binding of hybrid chicken/human LFA-1 to ICAM-1 and -3

To study the role of the LFA-1 $\beta 2$ subunit in binding of the ICAM molecules, COS cells were cotransfected with the chicken $\beta 2$ cDNA and the human αL cDNA and then tested for binding to purified human ICAM-1 and ICAM-3 coated on 96-well plates. Both wild-type human LFA-1 and hybrid chicken-human LFA-1 were expressed equivalently on transfected COS cells, as determined by flow cytometry (Fig. 3). The hybrid LFA-1 molecules were as active as wild-type LFA-1 in binding to ICAM-1 and ICAM-3 (**Fig. 4**). Antibodies to ICAM-1 and ICAM-3, as appropriate, reduced binding to background levels, showing that the binding interactions were specific (Fig. 4). The human LFA-1 α chain transfected alone in COS cells showed no binding to ICAM-1 or -3.

Concluding remarks

The isolation and characterization of the chicken β2 subunit described here reveal that this molecule has been highly conserved in structure and function. The chicken β2 subunit efficiently associates with the human LFA-1 α subunit and thus demonstrates that sequences required for subunit association have been conserved. In addition, hybrid chicken β2-human αL heterodimers are functionally active when tested for binding to ICAM-1 and ICAM-3, showing that the chicken β2 subunit can support these ligand binding interactions. Previous studies have shown that human LFA-1 α complexed with murine β2, but not murine LFA-1 α complexed with human β2, binds to human ICAM-1 [39]. The present study shows that although the chicken and human β2 subunits are twice as divergent (35% amino acid difference) as the human and mouse β2 subunits (18% difference), residues required for subunit association and ligand binding are either identical or conservatively substituted. The regions on LFA-1 involved in binding the ICAM molecules have not yet been determined. Several of the highly conserved regions in the extracellular domain of β2 may contribute. Further work by us (C.A.G. Bilsland, M. Diamond, and T.A. Springer, manuscript in preparation) has shown that the chicken β2 subunit can also efficiently associate with the αM subunit of the leukocyte integrins Mac-1 and the αX subunit of p150,95.

The overall sequence identity between the chicken cytoplasmic domain and the human (69%) and murine (71%) sequences is considerably lower than the 96% identity between the same human and murine sequences. Nevertheless, particular residues shown to be important by mutational analysis [32] are completely conserved, adding further support to the mutational studies.

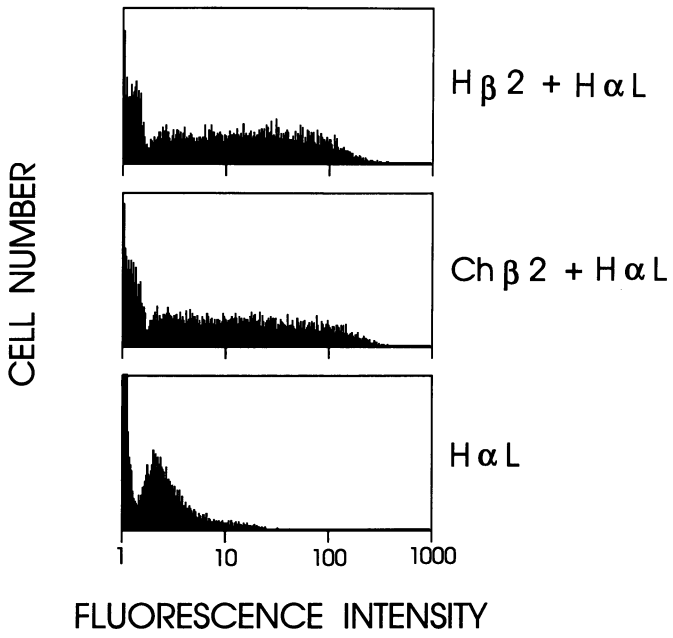

Fig. 3. Flow cytometry analysis of COS cells expressing human and human-chicken hybrid LFA-1. COS cells were cotransfected with the human α subunit of LFA-1 (HαL) plus either the human β2 subunit (Hβ2) or the chicken β2 subunit (Chβ2) or with the HαL subunit alone. Transfectants were immunostained with an anti-LFA-1 α subunit mAb (TS1/22) and subjected to immunofluorescence flow cytometry.

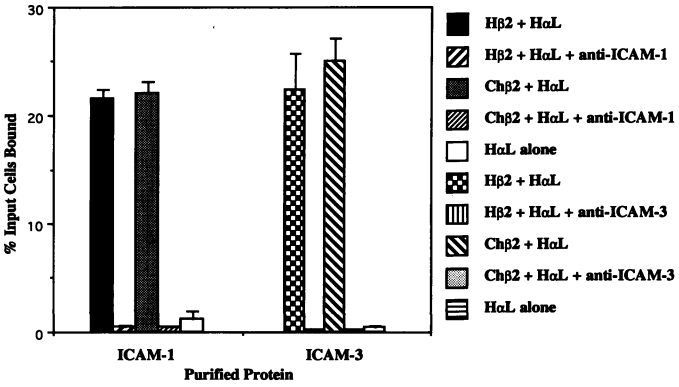

Fig. 4. Adhesion of COS cells expressing either human LFA-1 (Hβ2+HαL) or chicken-human hybrid LFA-1 (Chβ2+HαL) to ICAM-1 and -3. Transfected cells were allowed to bind to ICAM-1 or ICAM-3 coated on microtiter wells for 60 min at 37°C and were then washed several times by aspiration. Binding was blocked by the anti-ICAM-1 mAb RR1/1 or by the combination of the anti-ICAM-3 mAbs CBR-IC3/1 and CBR-IC3/2. COS cells transfected with the LFA-1 α chain alone (HαL) were used as a control. The data shown are for one representative experiment of three, with the mean of duplicate wells and standard deviation indicated.

Several other chicken integrin subunits have been cloned: β1 [40], α6 [41], α8 [42], and αv [43]. Comparison of these subunits with their human homologues reveals that they have been highly conserved in evolution: β1 (85% amino acid identity), α6 (73%), and αv (83%). The level of conservation of these subunits is thus higher than that seen between the chicken β2 subunit and its human (65%) and murine (64%) counterparts.

We have isolated and sequenced an avian leukocyte β2 integrin cDNA and shown that it is highly homologous to its human and murine counterparts. Regions of potential functional importance, particularly in the cytoplasmic domain, have been defined on the basis of sequence conservation. The chicken β2 molecule can associate on the cell surface with the α subunit of the leukocyte integrin LFA-1 and the hybrid molecule is functionally competent in ligand binding.

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