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# The role of the cysteine-rich region of the β2 integrin subunit in the leukocyte function-associated antigen-1 (LFA-1, αLβ2, CD11a/CD18) heterodimer formation and ligand binding

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**Abstract The cysteine-rich region (CRR) of the β2 integrin subunit was replaced by that of β1 to give the chimera β2NV1. β2NV1 can combine with αL to form a variant leukocyte-function-associated antigen (LFA)-1 on COS cell surface, suggesting that the specificity of the β2 interaction with αL does not lie in the CRR. Unlike those expressing wild-type LFA-1, COS cells expressing αLβ2NV1 are constitutively active in intercellular adhesion molecule (ICAM)-1 adhesion. These results suggest that activation of LFA-1 involves the release of an intramolecular constraint, which is maintained, in part, by the authentic β2 CRR.**



*Key words:* Integrin; Leukocyte-function-associated antigen-1; Heterodimer formation; Adhesion; Activation

## 1. Introduction

Integrins are a family of membrane glycoproteins which mediate cell adhesion to extracellular matrix components and to other cells [1,2]. Each integrin is a non-covalent heterodimer of an α and a β subunit. To date, 17 human α subunits and 8 human β subunits have been characterised and over 20 αβ heterodimers have been described. Both the α and the β subunits of the integrins are type I membrane glycoproteins. Relatively little is known about their higher order structures. The α subunits are predicted to contain a β-propeller structure formed by seven repeating elements [3]. Eight of the 17 characterized α subunits, including those of the four leukocyte integrins, have an I-domain inserted between the second and third repeating units of the predicted β-propeller. The recombinant I-domains of the αL [4], αM [5,6] and α2 [7] subunits have been crystallised and shown to assume a Rossmann fold. In addition, these recombinant I-domains have been shown to bind the ligands of their respective parent integrins [8–12].

The integrin β subunits are linearly organised into an N-terminal cysteine-rich region, a highly conserved region (HCR), a middle connecting region, an extensive cysteine-rich region (CRR), a transmembrane segment, and a cytoplasmic region of about 50 residues (Fig. 1). The exception is the β4 subunit, which has an extensive cytoplasmic domain of about 1000 residues, see CD104 entry in [13]. The HCR is thought to contribute both to heterodimer formation and ligand binding. Most of the single residue mutations that cause leukocyte adhesion deficiency (LAD), in which the patients fail to express all members of the β2 integrin subfamily, have been located in the HCR [14]. In addition, single residue substitutions in the HCR constructed in the laboratory were found either incapable of forming heterodimers or formed heterodimers which were defective in ligand binding activities [15,16]. The CRR is speculated to have regulatory functions since epitopes of many antibodies which activate ligand binding, including KIM185 used in this study [17], have been mapped to this region.

If the specificity of ligand binding lies in the α subunits, what is the role of the β subunits? If the role of the β subunit is mainly regulatory, how does it confer the regulation to the α subunits? How the two subunits interact with each other is therefore a relevant question in terms of understanding integrin activation. A starting point to address this problem is to ask how the integrin α and β subunits form heterodimers only with certain counter-subunits, but not others. We focus on the β2 subunit, which combines with the αL, αM, αX, and αD subunits to form leukocyte-function-associated antigen (LFA)-1 (αLβ2, CD11a/CD18), Mac-1 (αMβ2, CD11b/CD18), p150,95 (αXβ2, CD11c/CD18), and the αDβ2 antigen [18,19]. The four α subunits are not known to combine with any other β subunit and, conversely, the β2 subunit is not known to interact with any α subunit outside this set of four. In particular, leukocytes also express the β1 integrin subunit (see CD29 entry in [13]), but the expression of the

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*Abbreviations:* mAb, monoclonal antibodies; ICAM, intercellular adhesion molecule; LFA, leukocyte-function-associated antigen; HCR, highly conserved region; CRR, cysteine-rich region; Mg/EGTA, 5 mM $MgCl_2$ and 1 mM EGTA


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αL, αM, and αX subunits with β1 has not been detected. Peripheral blood leukocytes express only low levels of αDβ2 [19] and little is known about the capacity of αD to form heterodimers with other β integrin subunits.

We have therefore constructed β2/β1 chimeric subunits and studied their capacity to combine with the αL subunit and the interaction of the resultant heterodimers with intercellular adhesion molecule (ICAM)-1. We report here the study on the chimera β2NV1, in which the CRR of the β2 subunit was replaced by the corresponding region of β1 (Fig. 1). This chimeric subunit can combine with the αL subunit to form a variant LFA-1, which is constitutively active in binding ICAM-1.

## 2. Materials and methods

The monoclonal antibodies (mAbs) MHM24 (anti-αL), H52 (anti-β2), KIM185 (anti-β2), and OX33 (control) were previously described [20,21]. MEM83 (anti-αL) was a gift from V. Horejsi (Academy of Sciences of the Czech Republic, Prague, Czech Republic), YFC118 (anti-β2) and YTS191 (control) were gifts from S. Cobbold and H. Waldmann (Sir William Dunn School of Pathology, Oxford, UK), and mAb 24 (anti-CD11/CD18 heterodimers) was a gift from N. Hogg (ICRF, London, UK). ICAM-1/Fc was prepared as described [22].

The αL cDNA clone was reported previously [23]. The β1 (partial) cDNA clones were screened from a HPBALL library [24] using probes constructed by PCR based on the published sequences of β1 [25].

The original cDNA clone coding for the β2 integrin subunit, J8, was in the PATX vector [26,27]. Three *Nco*I sites, one in the coding region, and two in the 3′ untranslated region, are found in the cDNA of β2. The fragment from the internal *Sac*II site to the 3′ *Xho*I cloning site was removed from J8 and replaced by a synthetic polylinker containing the restriction enzyme sites for *Sac*II, *Stu*I, *Spe*I, *Sst*I, *Xba*I and *Xho*I to give J8.1SXp. The fragment from the same internal *Sac*II site to the first *Nco*I site in the 3′ untranslated region of J8 was blunt-ended at the *Nco*I site and inserted into the *Sac*II and *Stu*I site of J8.1SXp to give J8.1N. In this manipulation the two *Nco*I sites in the 3′ untranslated region were removed. The entire insert containing the entire coding region of β2 of J8.1N, a *Cla*I/*Sst*I fragment, was cloned into the same sites in the polylinker of pBluescript. The resultant clone, J8.1E, has unique sites for *Xba*I, *Bam*HI, *Eco*RI, *Nco*I and *Sac*II in the cDNA insert. An *Eco*RV site was introduced at the extracellular/transmembrane junction of J8.1E by PCR using the strategy of Horton et al. [28] to yield the variant clone J8.1EV. Fragments encoding the CRR of β1 was obtained by PCR using 5′ oligonucleotides with an *Nco*I site and blunt-end 3′ oligonucleotides. The fragment was digested with *Nco*I and used to replace the *Nco*I/*Eco*RV fragments of J8.1EV to yield the chimeric clone β2NV1. The chimeric clones were verified by cDNA sequencing. The inserts of the β2 and β2NV1 clones were removed with *Asp*718I and *Spe*I and cloned into the *Asp*718I and *Xba*I sites of the expression vector pcDNA3 (Invitrogen).

cDNAs of the αL, β2 and β2NV1, in pcDNA3, were transfected singly or in αβ pairs into COS-7 cells by the DEAE-dextran method [24]. Protocols for the harvesting of transfectants, the analysis of LFA-1 expression by flow cytometry and immunoprecipitation, and the adhesion of transfectants to ICAM-1 coated microtiter plates were described in Al-Shamkhani and Law [21].

## 3. Results

### 3.1. Expression of the αL, β2 and β2NV1 integrin subunits on COS-7 cells

COS-7 cells were transfected with plasmids containing the cDNA for integrins subunits either singly or in αβ combinations. Expression of the αL subunit was monitored by the mAb MHM24 and the β2 and β2NV1 subunits by the

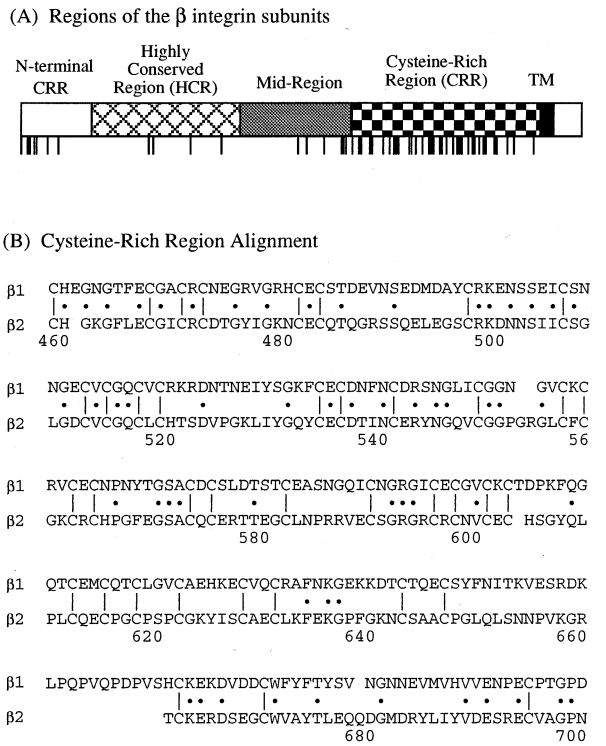


Fig. 1. A: The regional organization of the β integrin subunits. The locations of the 56 conserved cysteine residues in the extracellular domain are indicated by vertical lines under the main diagram. B: Alignment of the CRR of the β1 and β2 integrin subunits. Identical residues are indicated by (●) between the sequences; identical conserved cysteine residues are marked by (|)s. The numbering underneath is that of the human β2 sequence.

mAbs YFC118, H52 and KIM185 (Fig. 2). All three subunits are expressed on COS-7 cells. In addition, whereas the epitopes of the three anti-β2 antibodies are expressed on the β2 transfectants, only the epitopes of YFC118 and H52, but not that of KIM185, are expressed on the β2NV1 transfectants. This is consistent with the mapping of the epitopes of YFC118, H52, and KIM185 to the HCR [29], mid-region [21] and CRR [17], respectively. The expression profiles of the anti-β2 epitopes in the αLβ2 and αLβ2NV1 transfectants are correspondingly similar to those of the β2 and β2NV1 transfectants. However, the expression of the MHM24 epitope is significantly enhanced in the αβ transfectants when compared with the αL transfectants.

Monomeric and heterodimeric expression were analysed by immunoprecipitation of the integrin subunits from surface-labelled cell lysates of the transfectants (Fig. 3). Only the β2 and β2NV1 subunits were precipitated with H52 from the respective transfectants, confirming that they are expressed as monomers on the cell surface without combining with endogenous α subunits from the COS cells. Similarly, only the αL subunit is precipitated with MHM24 from the αL transfectants. However, in addition to the αL subunit, the β2 and β2NV1 subunits were also present in the immunoprecipitate from the lysates of the αLβ2 and αLβ2NV1 transfectants, respectively, with the αL-specific mAb MHM24. These results showed that the β2NV1 chimera can combine with the αL subunit to form a variant form of LFA-1.

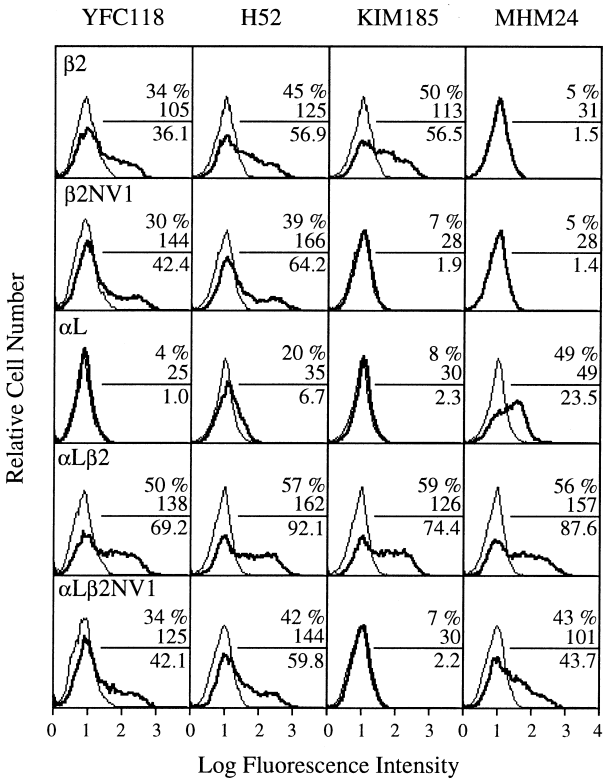


Fig. 2. Flow cytometry analysis of COS-7 transfected with LFA-1 integrin subunits. The expression profiles of the epitopes of YFC118, H52, KIM185 and MHM24 are shown in bold. The background profiles for the rat mAb YFC118 expression were obtained with the rat mAb YTS191, and those for mouse mAbs, i.e. H52, KIM185, and MHM24, were obtained with OX33. All first antibody staining was done in RPMI-1640 supplemented with 5% fetal calf serum and 10 mM HEPES, pH 7.5. The positive gates, shown as horizontal lines, were set to include the brightest 5% of the cells in the background profiles. % positive cells, obtained with the specific mAbs in the gated region, and their mean fluorescence intensity (MFI), are indicated above the bar, and an expression index, defined as the product of (% positive cells)×(MFI of the positive population), is shown below the bar.

### 3.2. Adhesion of COS-7 transfectants expressing wild-type and variant LFA-1 to ICAM-1

COS-7 transfectants were studied for their adhesion to ICAM-1 at different ligand densities (Fig. 4). The β2 and β2NV1 transfectants were not adherent to ICAM-1 including the β2 transfectants in the presence of the stimulatory mAb KIM185. The αL transfectants can be stimulated to adhere to ICAM-1 with the αL-specific mAb MEM83. Transfectants expressing wild-type LFA-1 also require activation for adhesion to ICAM-1. However, this is not simply due to the more effective expression of the αL subunit since activation can also be promoted with the β2-specific mAb KIM185. The αLβ2NV1 transfectants adhere to ICAM-1 readily in the absence of activating mAbs, and adhesion can be detected at $<1$ ng of ICAM-1 per microtitre well. The promotion of adhesion by MHM83 is minimal. Since the KIM185 epitope is not expressed, the mAb has no effect on adhesion (data not shown). In all cases, adhesion was specific: no adhesion was detected in wells not coated with ICAM-1. Furthermore, adhesion can be blocked with the mAb MHM24 and, for the αβ transfectants, also with the anti-β2 mAb YFC118 (data not shown).

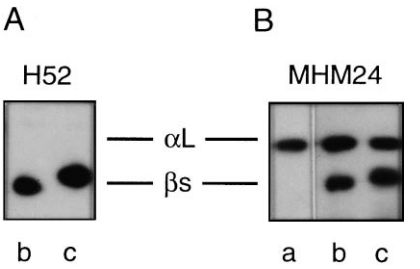


Fig. 3. Immunoprecipitation of surface-labeled LFA-1 subunits. A: Immunoprecipitates with H52 from β2 (lane b) and β2NV1 (lane c) transfectants. B: Immunoprecipitates with MHM24 from αL (lane a), αLβ2 (lane b), and αLβ2NV1 (lane c) transfectants.

### 3.3. Expression of the mAb 24 epitope on the wild-type and variant LFA-1

The expression of the epitope for mAb 24 on LFA-1 has been shown to correlate with a high affinity conformation and ICAM-1 binding capacity [30]. The epitope was detected on wild-type LFA-1 transfectants at 37°C in the presence of Mg/EGTA, at very low levels at 37°C, and not at all at 4°C (Fig. 5), in agreement with previous reports on the property of this epitope [30]. However, the epitope was detected on the αLβ2NV1 transfectants in the absence of Mg/EGTA, and not only at 37°C but also at 4°C (Fig. 5). Expression of mAb 24 epitope cannot be detected under the three conditions on the transfectants expressing the monomeric αL, β2 and β2NV1 subunits (data not shown).

## 4. Discussion

How the α and β subunits interact with each other to bind ligands in a highly regulated fashion is a key question of integrin-mediated adhesion. In this article, we report the construction of a chimeric β2/β1 integrin subunit, β2NV1, in which the CRR of β2 is replaced with that of β1, and the study of β2NV1 on its ability to combine with the αL subunit to form a variant LFA-1. Since COS-7 cells do not normally express any subunits of the β2 integrin subfamily, they provide a suitable cell background for these studies.

Both the αL and β2 subunits can be expressed on COS cells independently, in agreement with previous reports [31]. In addition, we showed that they are expressed as monomers.

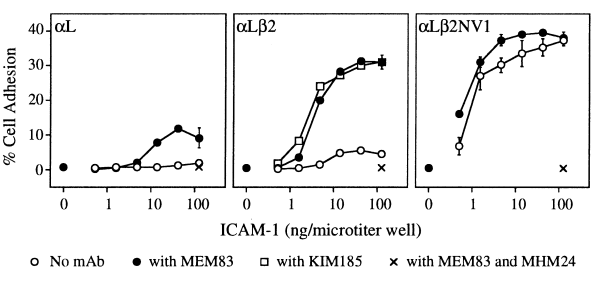


Fig. 4. Adhesion of COS-7 transfectants to ICAM-1. Adhesion was carried out in the absence of activating mAb (○), in the presence of MEM83 (●) and KIM185 (□). Only the results obtained in the presence of MEM83 are shown for adhesion with no ICAM-1 coating are shown. Adhesion was also done in the presence of the blocking mAb MHM24 at the highest ligand density. Only the results obtained in the presence of MEM83 (×) are shown.

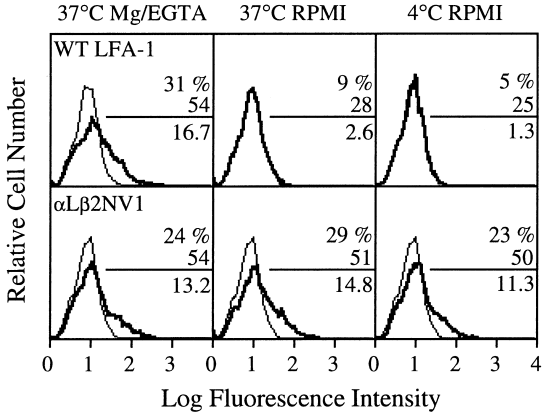


Fig. 5. Expression of the epitope of mAb 24. COS-7 transfectants of wild-type LFA-1 (αLβ2) and variant αLβ2NV1 were stained for mAb 24 at 4°C or 37°C in RPMI-1640 supplemented with 5% foetal calf serum and 10 mM HEPES, pH 7.5, or at 37°C in the same medium with 5 mM $MgCl_2$ and 1 mM EGTA (Mg/EGTA). Second antibody staining was done in the same medium without Mg/EGTA at 4°C. % positive cells, their MFI and expression indices are shown similarly as in Fig. 2.

The epitopes of the mAbs YFC118 [32], H52 [20], and KIM185 [17,33] are expressed on the monomeric β2 subunit (Fig. 2). It has been suggested that the HCR of the β2 subunit does not fold 'correctly' in the absence of a proper α subunit based on the observation that a panel of mAbs, whose epitopes map to the HCR, can only recognise the β2 subunit in the heterodimer state [33]. However, one of these mAbs was YFC118, which, in our hands, can detect the monomeric β2 subunit. Thus the structural differences between β2 in its monomeric and heterodimeric states remain to be resolved. The αL transfectants, in the presence of the stimulating mAb MEM83 [34], can adhere to ICAM-1 coated surfaces. This observation is consistent with previous reports that the I-domain can be expressed independently [4], that it can interact with ligands [8,35], and that it is properly folded as part of the αL subunit though proper folding of the β-propeller appears to require the β2 subunit [36].

On the other hand, there is ample evidence that monomeric αL and β2 subunits are not expressed on leukocytes. The αL, αM, nor αX subunits are not detected on leukocytes of LAD patients, who have defective β2 subunits [14]. Thus, these α subunits are not expressed as monomers, nor as heterodimers by combining with another β integrin subunit, such as β1 or β3, which is expressed on leukocytes [13]. By chemical mutagenesis, the genes for the αL and β2 subunits have been made defective in the T lymphoblastoid cell lines Jurkat and SKW3, respectively [37]. Surface expression of the non-defective subunit could only be detected if the defective counter-subunits were restored by transfection [37]. Since both the αL and β2 subunits are expressed as monomers on COS cells, there is no inherent reason why they cannot be expressed on other cells. We therefore argue that there are certain regulatory mechanisms, yet to be characterised, in leukocytes that prohibit surface expression of the monomers.

It has been reported that COS cell transfectants expressing LFA-1 are capable of adhesion to ICAM-1 coated surfaces without stimulation [15,16,31]. In our hands, the adhesion of LFA-1 transfectants to ICAM-1 requires the presence of activating mAbs. Since the adhesion properties of LFA-1 may depend on the cell type on which it is expressed [38], the discrepancy may therefore be due to the COS cells in different laboratories having diverged through passages and providing different cellular background for LFA-1 mediated adhesion activities.

Does the CRR play any part in interacting with the α subunit? The CRR of β3 is not required to combine with the αIIb subunit to form a truncated integrin heterodimer with ligand binding activities [39]. However, we should be cautious in extrapolating this conclusion to other integrins. In this paper, we have shown that by replacing the CRR of β2 with that of β1, the resultant chimera is fully capable of combining with the αL subunit to form a variant LFA-1. We did not address the question of whether a CRR is required, but we have demonstrated that the specificity of the β2 subunit to combine with αL does not lie in the CRR.

Why are the transfectants expressing αLβ2NV1 more adherent than those expressing wild-type LFA-1? It cannot be due to expression level, since αLβ2NV1 is expressed consistently at a lower level, as judged by the expression of the YFC118, H52, and MHM24 epitopes, than the wild-type LFA-1. Results of a representative experiment are shown in Fig. 2. One emerging view of integrin activation is that resting state integrins are constrained from binding ligands, and that activation involves the release of this constraint. The intramolecular constraint of the integrins would require inter-subunit and intra-subunit interactions between different regions of the heterodimer. The most direct evidence in support of this model has come from the study of the platelet integrin αIIbβ3 [40]. A putative salt bridge in the cytoplasmic domain of αIIbβ3 was identified between an Arg of the αIIb subunit and an Asp of the β3 subunit. It was proposed that this salt bridge may be a constraining device in maintaining αIIbβ3 in its resting state. This experiment also provided a possible means, by disrupting the salt bridge, of how a signal originating inside the cell might propagate through the integrin heterodimer to affect the structure of the ligand binding site and hence the ligand binding activities [41]. There are a number of mAbs, not only against the β2 integrins [17,33], but also against the β1 [42–48] and β3 [41,49–51] integrins, that have been described as activating mAbs by virtue of their ability to promote ligand binding. In addition, their epitopes often become more accessible upon binding to ligand or activation by another mAb. The locations of these eptiopes have been described for all regions of the extracellular domain: the mAbs N29 [45] and AP5 [51] have been mapped to the N-terminal CRR of β1 and β3, respectively, TS2/16 [42] and 12G10 [43] to the HCR of β1, the HUTS mAbs [44] to the β1 mid-region, and JB1B [45], 9EG7 [46], and AG89 [47,48] to the β1 CRR, KIM127 and KIM185 to the β2 CRR [17] and D3GP3 [50] and the anti-LIBS2 [41] to the β3 CRR. A plausible model, consistent with the constraint hypothesis, is that the binding of these mAbs perturbs the intramolecular interaction of the integrins, thereby releasing them into their activated states. The locations of their epitopes are not important, activation is achieved as long as the constraining intramolecular interactions are perturbed. Our results on the αLβ2NV1 variant, which is constitutively active with respect to ICAM-1 binding and expresses the activation epitope of mAb 24 in the absence of activating agents, show that the authentic CRR of the β2 subunit is required for the construction of the constraint.

Since the monomeric αL subunit can also be activated to

bind ICAM-1, one possible interpretation of these results is that the β2 subunit is purely regulatory in restricting the binding activity of wild-type LFA-1. This restriction is lost in αLβ2NV1 and the αL subunit is freed to bind ICAM-1. However, this description cannot be complete. The β2 subunit must also contribute to binding because several laboratory constructed β2 mutants have been shown to form LFA-1 heterodimers which cannot bind ligands [15,16].

The immediate extensions of this work are to study the adhesion properties of the αLβ2NV1 to other ligands for LFA-1, i.e. ICAM-2 and ICAM-3, and to determine if β2NV1 can form heterodimers with the αM and αX subunits, and if the resultant heterodimers can bind ligands. We also plan to construct chimeras of other regions, such as the mid-region, of the integrin β subunit, and chimeras involving the exchange of smaller regions. Ultimately, we aim to map the residues in the integrins which are involved in heterodimer formation, ligand binding, and regulation of their adhesion activities.

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## References

[1] Springer, T.A. (1990) Nature 346, 425–434.
[2] Hynes, R.O. (1992) Cell 69, 11–25.
[3] Springer, T.A. (1997) Proc. Natl. Acad. Sci. USA 94, 65–72.
[4] Qu, A. and Leahy, D.J. (1995) Proc. Natl. Acad. Sci. USA 92, 10277–10281.
[5] Lee, J.O., Rieu, P., Arnaout, M.A. and Liddington, R. (1995) Cell 80, 631–638.
[6] Lee, J.O., Bankston, L.A., Arnaout, M.A. and Liddington, R.C. (1995) Structure 3, 1333–1340.
[7] Emsley, J., King, S.L., Bergelson, J.M. and Liddington, R.C. (1997) J. Biol. Chem. 272, 28512–28517.
[8] Randi, A.M. and Hogg, N. (1994) J. Biol. Chem. 269, 12395–12398.
[9] Michishita, M., Videm, V. and Arnaout, M.A. (1993) Cell 72, 857–867.
[10] Zhou, L., Lee, D.H.S., Plescia, J., Lau, C.Y. and Altieri, D.C. (1994) J. Biol. Chem. 269, 17075–17079.
[11] Cai, T.Q., Law, S.K.A., Zhao, H.R. and Wright, S.D. (1995) Cell Adhes. Commun. 3, 399–406.
[12] Tuckwell, D.S., Reid, K.B.M., Barnes, M.J. and Humphries, M.J. (1996) Eur. J. Biochem. 241, 732–739.
[13] Barclay, A.N., Brown, M.H., Law, S.K.A., McKnight, A.J., Tomlinson, M.G. and van der Merwe, P.A. (1997) The Leucocyte Antigen Facts Book, 2nd Edn., Academic Press, London.
[14] Anderson, D.C., Kishimoto, T.K. and Smith, C.W. (1994) in: The Metabolic Basis of Inherited Diseases, 7th Ed., Chapter 132 (Shriver, C.R., Beaudet, A.L., Sly, W.S. and Valle, D., Eds.) pp. 3955–3994, McGraw-Hill, New York, NY.
[15] Bajt, M.L., Goodman, T. and McGuire, S.L. (1995) J. Biol. Chem. 270, 94–98.
[16] Goodman, T.G. and Bajt, M.L. (1996) J. Biol. Chem. 271, 23729–23736.
[17] Stephens, P., Romer, J.T., Spitali, M., Shock, A., Ortlepp, S., Figdor, C.G. and Robinson, M.K. (1995) Cell Adhes. Commun. 3, 375–384.
[18] Sanchez-Madrid, F., Nagy, J.A., Robbins, E., Simon, P. and Springer, T.A. (1983) J. Exp. Med. 158, 1785–1803.
[19] Van der Vieren, M., Trong, H.L., Wood, C.L., Moore, P.F., St John, T., Staunton, D.E. and Gallatin, W.M. (1995) Immunity 3, 683–690.
[20] Hildreth, J.E.K. and August, J.T. (1985) J. Immunol. 134, 3272–3280.
[21] Al-Shamkhani, A. and Law, S.K.A. (1998) Eur. J. Immunol. 28, 3291–3300.
[22] Fawcett, J., Holness, C.L.L., Needham, L.A., Turley, H., Gatter, K.C., Mason, D.Y. and Simmons, D.L. (1992) Nature 360, 481–484.
[23] Wright, A.H., Douglass, W.A., Taylor, G.M., Lau, Y.L., Higgins, D., Davies, K.A. and Law, S.K.A. (1995) Eur. J. Immunol. 25, 717–722.
[24] Simmons, D.L. (1993) in: Cellular Interactions in Development (Hartley, D., Ed.), pp. 93–128, Oxford University Press, Oxford.
[25] Argraves, W.S., Susuki, S., Arai, H., Thompson, K., Pierschbacher, M.D. and Ruoslahti, E. (1987) J. Cell Biol. 105, 1183–1190.
[26] Law, S.K.A., Gagnon, J., Hildreth, J.E.K., Wells, C.E., Willis, A.C. and Wong, A.J. (1987) EMBO J. 6, 915–919.
[27] Weitzman, J.B., Wells, C.E., Wright, A.H., Clark, P.A. and Law, S.K.A. (1991) FEBS Lett. 294, 97–103.
[28] Horton, R.M., Hunt, H.D., Ho, S.N., Pullen, J.K. and Pease, L.R. (1989) Gene 77, 61–68.
[29] Huang, C., Lu, C. and Springer, T.A. (1997) Proc. Natl. Acad. Sci. USA 94, 3156–3161.
[30] Dransfield, I., Cabanas, C., Craig, A. and Hogg, N. (1992) J. Cell Biol. 116, 219–226.
[31] Larson, R.S., Hibbs, M.L. and Springer, T.A. (1990) Cell Regul. 1, 359–367.
[32] Pope, I., Hale, G. and Waldmann, H. (1989) in: Leukocyte Typing IV: White Cell Differentiation Antigens (Knapp, W., Dorken, B., Gilks, W.R., Rieber, E.P., Schmidt, R.E., Stein, H., and von dem Borne, A.E.G., Kr., Eds.), pp. 559–560, Oxford University Press, Oxford.
[33] Andrew, D., Shock, A., Bell, E., Ortlepp, S., Bell, J. and Robinson, M. (1993) Eur. J. Immunol. 23, 2217–2222.
[34] Landis, R.C., Bennett, R.I. and Hogg, N. (1993) J. Cell Biol. 120, 1519–1527.
[35] Knorr, R. and Dustin, M.L. (1997) J. Exp. Med. 186, 719–730.
[36] Huang, C. and Springer, T.A. (1997) Proc. Natl. Acad. Sci. USA 94, 3162–3167.
[37] Weber, K.S.C., York, M.E., Springer, T.A. and Klickstein, L.B. (1997) J. Immunol. 158, 273–279.
[38] Buckley, C.D., Ferguson, E.D., Littler, A.J., Bossy, D. and Simmons, D.L. (1997) Eur. J. Immunol. 27, 957–962.
[39] Wippler, J., Kouns, W.C., Schlaeger, E.J., Kuhn, H., Hadvary, P. and Steiner, B. (1994) J. Biol. Chem. 269, 8754–8761.
[40] Hughes, P.E., Diaz-Gonzalez, F., Leong, L., Wu, C., McDonald, J.A., Shattil, S.J. and Ginsberg, M.H. (1996) J. Biol. Chem. 271, 6571–6574.
[41] Du, X., Gu, M., Weisel, J.W., Nagaswami, C., Bennett, J.S., Bowditch, R. and Ginsberg, M.H. (1993) J. Biol. Chem. 268, 23087–23092.
[42] Takada, Y. and Puzon, W. (1993) J. Biol. Chem. 268, 17597–17601.
[43] Mould, A.P., Garratt, A.N., Puzon-McLaughlin, W., Takada, Y. and Humphries, M.J. (1998) Biochem. J. 331, 821–828.
[44] Luque, A., Gomez, M., Puzon, W., Takada, Y., Sanchez-Madrid, F. and Cabanas, C. (1996) J. Biol. Chem. 271, 11067–11075.
[45] Wilkins, J.A., Li, A., Ni, H., Stupack, D.G. and Shen, C. (1996) J. Biol. Chem. 271, 3046–3051.
[46] Bazzoni, G., Ma, L., Blue, M.L. and Hemler, M.E. (1998) J. Biol. Chem. 273, 6670–6678.
[47] Takagi, J., Isobe, T., Takada, Y. and Saito, Y. (1997) J. Biochem. 121, 914–921.
[48] Tsuchida, J., Ueki, S., Takada, Y., Saito, Y. and Takagi, J. (1998) J. Cell Sci. 111, 1759–1766.
[49] Frelinger III, A.L., Du, X., Plow, E.F. and Ginsberg, M.H. (1991) J. Biol. Chem. 266, 17106–17111.
[50] Kouns, W.C., Newman, P.J., Puckett, K.J., Miller, A.A., Wall, C.D., Fox, C.F., Seyer, J.M. and Jennings, L.K. (1991) Blood 78, 3215–3223.
[51] Honda, S., Tomiyama, Y., Pelletier, A.J., Annis, D., Honda, Y., Orchekowski, R., Ruggeri, Z. and Kunicki, T.J. (1995) J. Biol. Chem. 270, 11947–11954.