Recognition of Diverse Proteins by Members of the Immunoglobulin Superfamily: Delineation of the Receptor Binding Site in the Human CD6 Ligand ALCAM

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ABSTRACT: The CD6−ALCAM (activated leukocyte cell adhesion molecule) interaction, which mediates thymocyte−thymic epithelial cell adhesion, is a previously unobserved type of protein−protein interaction that involves members of the scavenger receptor cysteine rich protein superfamily (SRCRSF) and the immunoglobulin superfamily (IgSF). Targeted mutagenesis of ALCAM reveals that residues which constitute the CD6 binding site cluster on the predicted A′GFCC′C″ face of its N-terminal Ig domain. These results, in conjunction with recent analyses of interactions involving other IgSF members, suggest that this region in IgSF cell surface proteins is most suitable to mediate interactions with different ligands irrespective of their structure. The CD6 binding site in ALCAM is conserved across species, and nonconserved residues in ALCAM and its murine homolog map to the β-sheet face opposite to the CD6 binding site. This provides a molecular rationale for the inability to obtain murine monoclonal antibodies against the receptor binding domain which block the CD6−ALCAM interaction.

Cell surface receptors, ligands, and adhesion proteins belonging to the IgSF[1] (Williams & Barclay, 1988) interact not only with other IgSF members but also with structurally distinct molecules including integrins (Lee et al., 1995) and carbohydrates (Powell & Varki, 1995). CD6 (Aruffo et al., 1991) and ALCAM (Bowen et al., 1995) form a previously unobserved IgSF-mediated interaction. CD6 is a member of the SRCRSF (Resnick et al., 1994), and its extracellular region consists of three SRCR domains. ALCAM, which displays significant homology to the chicken neural adhesion molecule BEN (Pourquie et al., 1992), belongs to the IgSF (Bowen et al., 1995), and its extracellular region includes five Ig domains. ALCAM's N-terminal V-set (Williams & Barclay, 1988) domain specifically binds to the membrane-proximal domain of CD6 with 1:1 stoichiometry (Bowen et al., 1996).

CD6 is predominantly expressed by thymocytes and mature T cells (Reinherz et al., 1982; Morimoto et al., 1988), whereas ALCAM is expressed on activated T cells, B cells, and monocytes (Bowen et al., 1995) and on thymic epithelial (TE) cells (Wee et al., 1994; Patel et al., 1995). In addition, both proteins are expressed in the brain. In the immune system, CD6−ALCAM interactions have been implicated in thymocyte−TE cell adhesion and the regulation of T cell functions (Vollger et al., 1987; Bowen et al., 1995).

Using a systematic site-directed mutagenesis approach, we have characterized 31 ALCAM mutations and have identified six residues in ALCAM's receptor binding domain which are important for the interaction with CD6. These residues map to the predicted A′GFCC′C″ β-sheet face of the domain. The results are discussed in the context of recent studies of different interactions between cell surface proteins belonging to the IgSF and diverse molecules. The analysis of the novel IgSF−SRCRSF interaction between ALCAM and CD6 adds to our understanding of IgSF-mediated binding events on the cell surface. Although exceptions exist, these investigations suggest, in aggregate, that the A′GFCC′C″ face of IgSF V-set (Williams & Barclay, 1988) domains represents a preferred site for interactions of IgSF cell surface proteins with structurally distinct molecules.

MATERIALS AND METHODS

Construction and Expression of Mutant Proteins. The desired mutations and silent mutations for diagnostic restriction enzyme sites were introduced by overlap extension PCR as described (Bajorath et al., 1995b) into a cDNA encoding the three N-terminal ALCAM Ig domains (D1−D3). The PCR fragments were cloned into the expression vector CDM7B$^-$ which includes the hinge and constant regions of human IgG (Hollenbaugh et al., 1995). Each mutation was verified by restriction enzyme analysis and cDNA sequencing. The complete N-terminal ALCAM domain was sequenced for each mutant whose binding to CD6 and/or anti-D1 mAb was affected. Transient expression in COS cells as described (Bajorath et al., 1995b) yielded supernatants including the mutant Ig fusion proteins. Concentrations of mutant proteins were normalized using an ALCAM anti-D3 mAb ELISA assay following the protocol described below.

Binding of Mutant Proteins to ALCAM Anti-D1 mAb and Anti-D3 mAb by ELISA. A 96-well plate (Immulon 2, Dynatech, Chantilly, VA) was coated overnight at room temperature with either anti-D1 mAb (Bowen et al., 1996)

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[1] Abbreviations: ALCAM, activated leukocyte cell adhesion molecule; CDR, complementarity determining region; D1, N-terminal extracellular domain of ALCAM; D3, third extracellular domain of ALCAM; ELISA, enzyme-linked immunosorbent assay; Fv, variable fragment; Ig, immunoglobulin; IgSF, immunoglobulin superfamily; mAb, monoclonal antibody; PCR, polymerase chain reaction; SRCR domain, scavenger receptor cysteine-rich domain; SRCRSF, scavenger receptor cysteine-rich protein superfamily; TE, thymic epithelium; V-domain, immunoglobulin variable domain; WT, wild type.

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or anti-D3 mAb (Bowen et al., unpublished) at 2.5 μg/mL, blocked with 1× specimen diluent (Genetic Systems, Redmond, WA), washed, incubated with serial dilutions of COS cell supernatants, and washed again. Wells were incubated with HRP-conjugated donkey anti-human IgG (Jackson ImmunoResearch, West Grove, PA) and developed with chromogenic substrate TMB (Genetic Systems, Redmond, WA). Absorbance was measured at dual wavelength (450 nm, 630 nm).

Binding of Mutant Proteins to CD6 by ELISA. A 96-well plate (Immulon 2, Dynatech, Chantilly, VA) was coated overnight at room temperature with human CD6 immunoglobulin fusion protein (CD6-Ig) at 1 μg/mL, blocked with 1× specimen diluent (Genetic Systems, Redmond, WA), washed, and incubated with serial dilutions of COS cell supernatants (2−0.00012 μg/mL) of ALCAM mutant proteins. Wells were incubated, developed, and measured as described for the anti-D1/D3 mAb ELISA. In this assay, the interaction between defined oligomeric states (Bowen et al., 1996) of the fusion proteins is measured at equilibrium, which represents a high-avidity assay format.

BIAcore Analysis. The binding of mutant proteins to CD6 was studied by surface plasmon resonance analysis (Jönsson et al., 1991) using the BIAcore instrument (Pharmacia, Uppsala, Sweden). Amine coupling chemistry (Karlsson et al., 1991) was used to immobilize CD6−Ig on the CM5 sensor chip. ALCAM−Ig wild-type and mutant proteins in filtered cell culture supernatants were injected, and the dissociation of bound protein was followed. Abrupt changes in response at the beginning and end of the injections are due to refractive index differences between the cell culture supernatant and the Hepes-buffered saline running buffer. Binding was measured following these changes. The data were fit to an exponential decay to obtain dissociation rates (off-rates) which are concentration-independent.

RESULTS AND DISCUSSION

Selection of Mutagenesis Sites. ALCAM residues outside IgSF consensus positions (Williams & Barclay, 1988) were selected on the basis of IgSF structure-oriented sequence comparison (Bajorath et al., 1995) and a three-dimensional model of ALCAM's N-terminal domain (Bajorath et al., 1995) which was generated on the basis of the CD8 X-ray structure (Leahy et al., 1992). Thirty-four residues were selected for mutagenesis (Table 1) which systematically screen the predicted loop regions and β-sheet faces of ALCAM's N-terminal domain.

Mutational Analysis. With a few exceptions, selected residues were subjected to alanine scanning mutagenesis (Wells, 1991) as described in the Materials and Methods section. Correctly folded mutant proteins were identified by binding to a monoclonal antibody against ALCAM's N-terminal domain (anti-D1 mAb) and/or by binding to CD6. Anti-D1 mAb recognizes a conformational epitope which requires the presence of overall correctly folded D1 (Bowen et al., 1996). The mutagenesis experiments are summarized in Table 1. Thirty-two of 34 mutant proteins were expressed in sufficient quantities and further characterized. Only 1 of these 32 mutant proteins, Tyr53/Ala (Table 1), did not bind to either anti-D1 mAb or CD6, indicating a substantial structural perturbation. Thus, 31 of 34 attempted mutants were analyzed. These results suggest that it was possible to select ALCAM residues with some confidence whose mutation did not significantly compromise the global D1 structure.

Table 1: Summary of Mutagenesis and Binding Experiments[a]

ALCAM mutant	anti-D1	CD6 ELISA	CD6 BIAcore
Tyr2/Ala	+	+	+
Asn5/Ala	+	+	+
Ile13/Ala	+/−	+	+
Asp19/Ala	+	+	+
Pro21/Ala	+	+	+
Asn23/Ala	+	+	+
Leu24/Ala + Met25/Ala	+	+	+
Phe26/Ala	+	−	−
Lys28/Ala		not expressed	
Lys30/Ala	+	+	+
Glu32/Ala + Lys33/Ala	+	+	+
Phe40/Ala	+	−	−
Phe43/Ala	+	−	−
Ser46/Ala + Thr47/Ala	+	+	+
Glu52/Ala	+	+	+
Tyr53/Ala	−	−	−
Asp55/Ala	+	+	+
Glu58/Ala		not expressed	
Lys60/Ala	+	+	+
Arg62/Ala	+	+	+
Leu65/Ala	+/−	+	+
Glu67/Ala	+	+	+
Ser72/Tyr	+/−	+	+
Arg77/Ala	+	+	+
Arg83/Ala	+	+	+
Met87/Ala	+	+	+/−
Val89/Tyr	+	+	+
Thr90/Ala	+	+	+/−
Glu91/Ala	+	+/−	+/−
Asp92/Ala	+	+	+
Asn93/Ala	+	+	+
Phe95/Ala	+	+	+
Glu96/Ala	+	+	+
Thr99/Tyr	+	+	+

[a] Anti-D1 reports the binding of ALCAM anti-D1 mAb to mutant protein as comparable to wild-type ALCAM (+), up to 10-fold reduced (+/−), or not detectable (−). CD6 ELISA reports the binding of mutant proteins to immobilized CD6 (see Figure 1) as comparable to wild type (+), up to 5-fold reduced (+/−), or at least 100-fold reduced (−) (see Figure 1). CD6-BIAcore reports the kinetic off-rates of mutant protein binding to CD6 (see Figure 2) as comparable to wild type (+), ∼10-fold faster (+/−), or too fast to measure (−). Mutants with reduced binding to anti-D1 and/or CD6 are shown in bold face.

Residues Important for Binding to CD6 and Anti-D1 mAb. ALCAM mutant proteins which consistently bound to anti-D1 mAb in ELISA were tested for binding to CD6−Ig fusion protein in ELISA and by surface plasmon resonance (BIAcore). Figures 1 and 2 show representative binding experiments. Twenty six of 32 ALCAM mutations bound to CD6 at wild-type levels in both ELISA and BIAcore. Mutations of six residues in ALCAM (Phe26/Ala, Phe40/Ala, Phe43/Ala, Met87/Ala, Thr90/Tyr, Glu91/Ala), which displayed wild-type binding to anti-D1 mAb, were affected in their binding to CD6 in ELISA and/or BIAcore assays (Table 1). The binding of three ALCAM mutants, Thr90/Ala, Glu91/Ala, and Met87/Ala, to CD6 was partially reduced, ranging from wild-type to ∼5-fold reduced binding in the high-avidity ELISA assay (see Materials and Methods) (Figure 1) and having ∼10-fold faster off-rates by BIAcore (Figure 2). In contrast, the binding of three other Phe/Ala mutants at positions 26, 40, and 43 was significantly reduced or undetectable in both ELISA and BIAcore assays, although these mutant proteins bound to anti-D1 mAb at wild-type levels. Three mutant proteins (Ile13/Ala, Leu65/Ala, Ser72/Tyr) showed CD6 wild-type binding but reduced binding to

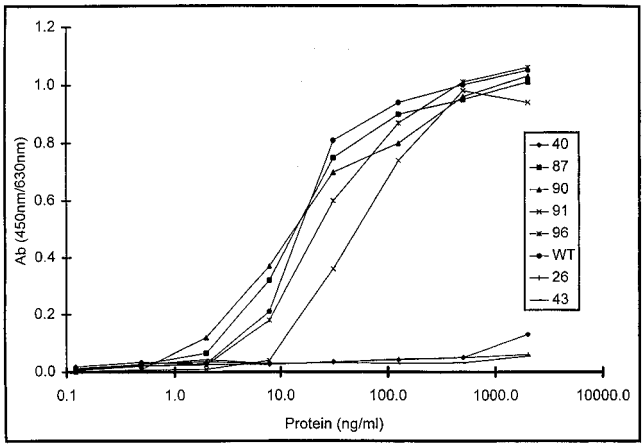

FIGURE 1: Binding of ALCAM mutant proteins to CD6 assayed by ELISA. Representative binding experiments are shown for mutants with wild-type, intermediate, or significantly reduced binding.

anti-D1 mAb by ELISA (Table 1). The mutated residues may therefore constitute part of the anti-D1 mAb epitope.

Mapping of Mutants. The three-dimensional model of ALCAM's N-terminal V-domain (Bajorath et al., 1995) was used to study the predicted location of mutants which affect CD6 or anti-D1 mAb binding. The results are shown in Figure 3. Two of the three ALCAM mutants whose binding to CD6 was partially reduced, Thr90/Ala and Glu91/Ala, map to the F−G loop (CDR3-analogous region). The third, Met87/Ala, is located in the F-strand. Two of the three mutants which significantly affect the binding to CD6, Phe40/Ala and Phe43/Ala, map to the C′-strand, whereas Phe26/Ala maps to the adjacent C-strand. The six mutations which affect CD6 binding therefore cluster in one region of the A′GFCC′C″ face of the N-terminal domain, whereas mutant proteins with wild-type binding to CD6 are scattered throughout the domain (data not shown). In contrast, the mutations which only affect anti-D1 mAb but not CD6 binding map to the B-strand, the D−E loop, and the E-strands, respectively.

Binding Site Conservation. The residues implicated in CD6 binding are conserved in the chicken (Pourquie et al., 1992) and murine (Bowen et al., unpublished) ALCAM homologs and are predicted to map to the A′GFCC′C″ face of the domain (Figure 4). The mapping of ALCAM residues important for anti-D1 mAb and CD6 binding to opposite faces of D1 is consistent with the finding that the anti-D1 mAb is not capable of blocking the CD6−ALCAM interaction (Bowen et al., 1996). In fact, despite repeated attempts, it has not been possible to obtain any blocking murine anti-D1 mAbs. Nonconservation of residues at positions outside IgSF consensus residues in ALCAM and its murine homolog is limited to the BED face of the D1 V-domain (Figure 4) which is opposite the CD6 binding site. Thus, due to its conservation, the CD6 binding site of ALCAM is not targeted during the murine humoral immune response.

IgSF-Mediated Interactions on the Cell Surface. The region corresponding to the A′GFCC′C″ face of the ALCAM D1 V-domain has recently been implicated in several IgSF−IgSF interactions including CD2−CD58 (Arulanandam et al., 1993) and CD2−CD48 (van der Merwe et al., 1995) and CD80−CD28/CTLA-4 (Peach et al., 1995). Equivalent results have been obtained in studies of IgSF interactions with structurally distinct molecules which include the

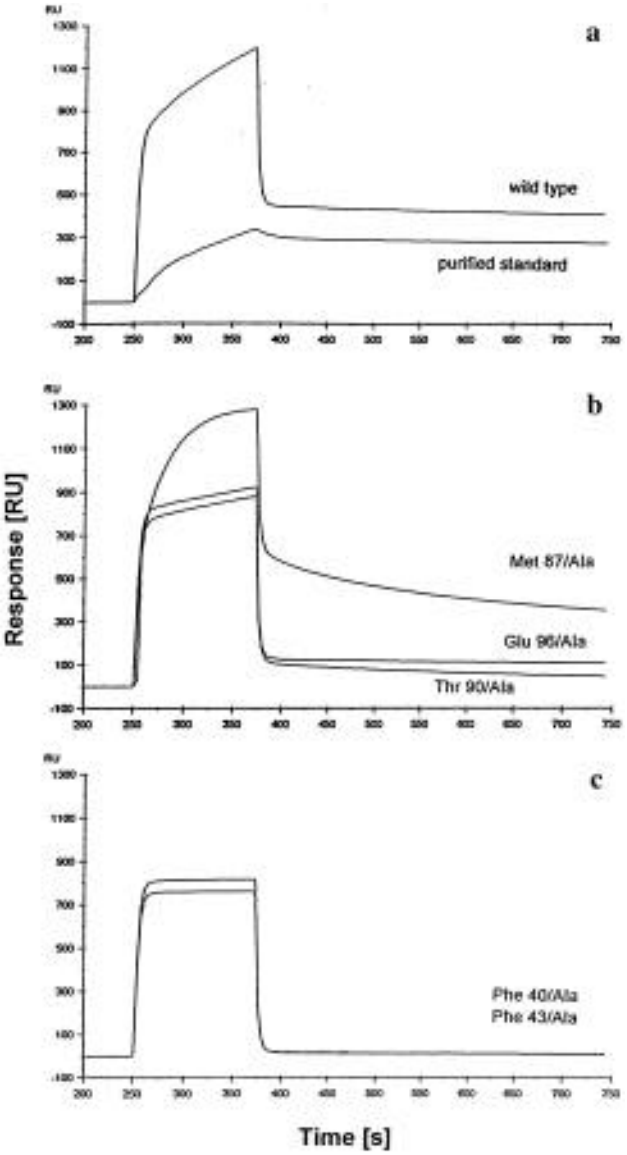

FIGURE 2: Dissociation rates of ALCAM mutant proteins determined by BIAcore analysis. Binding levels are given in response units and dissociation rates in s^{-1}. Data format: mutant (binding level, off-rate). Kinetic off-rates of binding are concentration-independent. Panel a shows purified ALCAM-Ig (P) and culture supernatant (WT) wild-type proteins. The dissociation rates are similar, but the binding levels differ as a consequence of concentration differences: P (299, 2×10^{-4}), WT (442, 2×10^{-4}). In panel b, representative sensorgrams of mutants with wild-type binding to CD6 or faster off-rates are shown: Met87/Ala (577, 1×10^{-3}), Thr90/Ala (104, 2×10^{-3}), Glu96/Ala (127, 4×10^{-4}). In panel c, mutants with essentially nondetectable binding are shown.

VCAM-1−VLA4 (IgSF−integrin) (Jones et al., 1995), CD22−sialic acid (IgSF−carbohydrate) (van der Merwe et al., 1996), sialoadhesin−sialic acid (Vinson et al., 1996), and ALCAM−CD6 (IgSF−SRCRSF) (reported herein) interactions. Despite their diversity, all these interactions critically depend on residues on the A′GFCC′C″ face of an IgSF V-domain or, as in the case of VCAM-1, the corresponding region of an I-set (Harpaz & Chothia, 1994) domain.

However, IgSF−MHC interactions depart from this theme. Residues in CD4 (Wang et al., 1990) which are important for the interactions with MHC class II molecules are more widely distributed over its N-terminal domain (Moebius et al., 1991). CD8, on the other hand, forms an antibody Fv-like (Chothia et al., 1985) homodimer (Leahy et al., 1992),

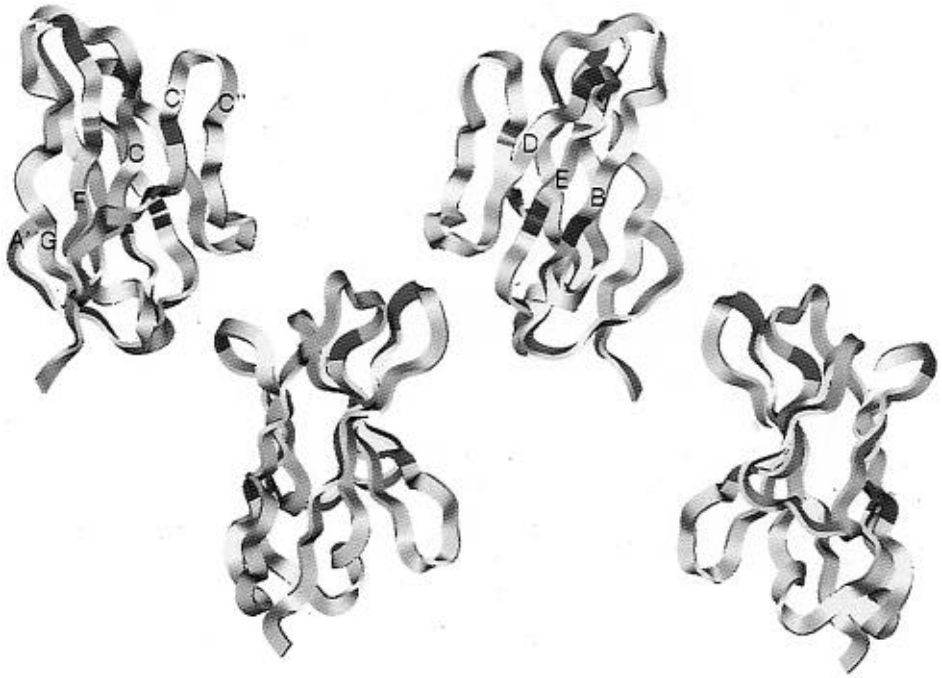

FIGURE 3: Mapping of ALCAM mutations. Positions of mutated residues were mapped on a three-dimensional model of the N-terminal Ig V-set domain depicted as a solid ribbon. In this orientation, the CDR analogous regions are at the top of the domain. The top left image focuses on the A′GFCC′C″ face. The β-strands are labeled. From the left to the right, the model was subsequently rotated by ~90° around the vertical axis to provide a complete view. Positions of mutations that only affect anti-D1 mAb but not CD6 binding are shown in blue. Positions of mutations that only affect CD6 binding are shown in red. Studies on different IgSF cell surface proteins have implicated residues on the A′GFCC′C″ face (top left) of Ig V- or V-like domains in IgSF−IgSF, IgSF−integrin, IgSF−carbohydrate, and IgSF−SRCRSF interactions (see text).

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      1 (A')    10   (B)    20        (C)     35   (C')   45
hALC: WYTVNSA YGD TIIIPCRLD VPQNLM FGKWKYEK PDGS PVFIAFR SSTK
cBEN: L    AV       TM    E  DG           M  N
mALC:               VM
       (C")  55     (D)   65     (E)  75        (F)    90
hALC: KSVQY DDVP   EYKDRLN LSEN YTLSIS NARISDE KRFVCMLV TEDN
cBEN:  N           D     S           K                    D
mALC:              S           A   K
          (G)  105
hALC:  VFEAPTIVKVFKQ
cBEN:   S E  V
mALC:        L
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FIGURE 4: Comparison of sequences corresponding to the N-terminal V-like domain in ALCAM (hALC) and its chicken (cBEN) and murine (mALC) homologs. Shown are only residues in cBEN and mALC which are not conserved in hALC. Predicted β-strands are labeled. Residue numbers are given for hALC.

and residues critical for the interaction with MHC class I map to CDR-analogous regions and parts of the ABED face (Giblin et al., 1994).

Implications for Molecular Recognition. Too few IgSF-mediated cell surface interactions have been analyzed in sufficient detail to firmly establish the A′GFCC′C″ face as the general site for IgSF-mediated cell surface interactions. This is illustrated by the analyses of IgSF−MHC interactions. In light of the results reported here, it is striking that the A′GFCC′C″ face in ALCAM is also responsible for the interaction with CD6. Although the positions of the A′GFCC′C″ residues which are critical for the interactions discussed above generally differ, the A′GFCC′C″ face of IgSF cell surface proteins emerges as a prominent binding site for structurally diverse molecules.

We conclude that this region, which includes the V-domain CDR2- and CDR3-analogous loops, represents the largest and most variable exposed surface in IgSF V-set or I-set domains. It thus provides a versatile binding surface for IgSF interactions with other proteins irrespective of their structure.

In the case of ALCAM, rigorous conservation of residues forming the A′GFCC′C″ binding site is sufficient to prevent the generation of murine anti-ALCAM mAbs which are capable of blocking the CD6−ALCAM interaction. In addition, this conservation is likely to provide the structural basis for previously observed CD6−ligand interactions across species (Whitney et al., 1995).

REFERENCES

Aruffo, A., Melnick, M. B., Linsley, P. S., & Seed, B. (1991) *J. Exp. Med. 174*, 949−952.

Arulanandam, A. R. N., Withka, J. M., Wyss, D. F., Wagner, G., Kister, A., Pallai, P., Recny, M. A., & Reinherz, E. L. (1993) *Proc. Natl. Acad. Sci. U.S.A. 90*, 11613−11617.

Bajorath, J., Bowen, M. A., & Aruffo, A. (1995a) *Protein Sci. 4*, 1644−1647.

Bajorath, J., Chalupny, N. J., Marken, J. S., Siadak, A. W., Skonier, J., Gordon, M., Hollenbaugh, D., Noelle, R. J., Ochs, H. D., & Aruffo, A. (1995b) *Biochemistry 34*, 1834−1844.

Bowen, M. A., Patel, D. D., Li, X., Modrell, B., Malacko, A. R., Wang, W.-C., Marquardt, H., Neubauer, M., Pesando, J. M., Francke, U., Haynes, B. F., & Aruffo, A. (1995) *J. Exp. Med. 181*, 2213−2220.

Bowen, M. A., Bajorath, J., Siadak, A. W., Modrell, B., Malacko, A. R., Marquardt, H., Nadler, S. G., & Aruffo, A. (1996) *J. Biol. Chem. 271*, 17390−17396.

Chothia, C., Novotny, J., Bruccoleri, R. E., & Karplus, M. (1985) *J. Mol. Biol. 186*, 651−663.

Giblin, P. A., Leahy, D. J., Mennone, J., & Kavathas, P. B. (1994) *Proc. Natl. Acad. Sci. U.S.A. 91*, 1716−1720.

Harpaz, Y., & Chothia, C. (1994) *J. Mol. Biol. 238*, 528−539.

Hollenbaugh, D., Doutwright, J., McDonald, V., & Aruffo, A. (1995) *J. Immunol. Methods 188*, 1−7.

Jones, E. Y., Harlos, K., Bottomley, M. J., Robinson, R. C., Driscoll, P. C., Edwards, R. M., Clements, J. M., Dudgeon, T. J., & Stuart, D. I. (1995) *Nature 373*, 539−544.

Jönsson, U., Fägerstam, L., Ivarsson, B., Johnsson, B., Karlsson, R., Lundh, K., Löfas, S., Persson, B., Roos, H., Rönnberg, I., Sjölander, S., Stenberg, E., Stahlberg, R., Urbaniczky, C., Östlin, H., & Malmquist, M. (1991) *BioTechniques 11*, 620−627.

Karlsson, R., Michaelsson, A., & Mattsson, L. (1991) *J. Immunol. Methods 145*, 229−240.

Leahy, D. J., Axel R., & Hendrickson, W. A. (1992) *Cell* 68, 1145−1162.

Lee, J.-O., Rieu, P., Arnaout, M. A., & Liddington, R. (1995) *Cell 80*, 631−638.

Moebius, U., Pallai, P., Harrison, S. C., & Reinherz, E. (1991) *Proc. Natl. Acad. Sci. U.S.A. 90*, 8259−8263.

Morimoto, C., Rudd, C. E., Letvin, N. L., Hagan, M., & Schlossman, S. F. (1988) *J. Immunol. 140*, 2165−2170.

Patel, D. D., Wee, S.-F., Whichard, L. P., Bowen, M. A., Pesando, J. M., Aruffo, A., & Haynes, B. F. (1994) *J. Exp. Med. 181*, 1563−1568.

Peach, R. J., Bajorath, J., Naemura, J., Leytze, G., Greene, J., Aruffo, A., & Linsley, P. S. (1995) *J. Biol. Chem. 270*, 21181−21187.

Pourquie, O., Corbel, C., Le Caer, J.-P., Rossier, J., & Le Douarin, N. M. (1992) *Proc. Natl. Acad. Sci. U.S.A. 89*, 5261−5265.

Powell, L. D., & Varki, A. (1995) *J. Biol. Chem. 270*, 14243−14246.

Reinherz, E. L., Meuer, S., Fitzgerald, K. A., Hussey, R. E., Levine, H., & Schlossman, S. F. (1982) *Cell 30*, 735−743.

Resnick, D., Pearson, A., & Krieger, M. (1994) *Trends Biochem. Sci. 19*, 5−8.

van der Merwe, P. A., McNamee, P. N., Davies, E. A., Barclay, A. N., & Davies, S. J. (1995) *Curr. Biol. 5*, 74−84.

van der Merwe, P. A., Crocker, P. R., Vinson, M., Barclay, A. N., Schauer, R., & Kelm, S. (1996) *J. Biol. Chem. 271*, 9273−9280.

Vinson, M., van der Merwe, P. A., Kelm, S., May, A., Jones, E. Y., & Crocker, P. R. (1996) *J. Biol. Chem. 271*, 9267−9272.

Vollger, L. W., Tuck, D. T., Springer, T. A., Haynes, B. F., & Singer, K. H. (1987) *J. Immunol. 138*, 358−363.

Wang, J., Yan, Y., Garrett, T. P. J., Liu, J., Rodgers, D. W., Garlick, R. L., Tarr, G. E., Husain, Y., Reinherz, E. L., & Harrison, S. C. (1990) *Nature 348*, 411−418.

Wee, S.-F., Wang, W.-C., Farr, A. G., Nelson, A. J., Patel, D. D., Haynes, B. F., Linsley, P. S., & Aruffo, A. (1994) *Cell. Immunol. 158*, 353−364.

Wells, J. A. (1991) *Methods Enzymol. 202*, 390−411.

Whitney, G. S., Starling, G. C., Bowen, M. A., Modrell, B., Siadak, A. W., & Aruffo, A. (1995) *J. Biol. Chem. 270*, 18187−18190.

Williams, A. F., & Barclay, A. N. (1988) *Annu. Rev. Immunol. 6*, 381−406.

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