Mapping the Collagen-binding Site in the I Domain of the Glycoprotein Ia/IIa (Integrin α₂β₁)*

Craig Smith, Dogaris Estavillo, Jonas Emsley‡, Laurie A. Bankston‡§, Robert C. Liddington‡§, and Miguel A. Cruz†

From the Hematology Division, Department of Medicine, Brigham and Women’s Hospital and Harvard Medical School, Boston, Massachusetts 02115 and the Department of Biochemistry, University of Leicester, Leicester LE1 9RH, United Kingdom

The I domain present within the α₂ chain of the integrin α₂β₁ (GPIa/IIa) contains the principal collagen-binding site. Based on the crystal structure of the α₂-I domain, a hypothetical model was proposed in which collagen binds to a groove on the upper surface of the I domain (Emsley, J., King, S. L., Bergelson, J. M., and Liddington, R. C. (1997) J. Biol. Chem. 272, 28512–28517). We have introduced point mutations into 13 residues on the upper surface of the domain. Recombinant mutant proteins were assayed for binding to monoclonal antibodies 6F1 and 12F1, to collagen under static conditions, and for the ability to retain adhesive activity under flow conditions. The mutations to residues surrounding the metal ion-dependent adhesion site that caused the greatest loss of collagen binding under both static and flow conditions are N154S in the β2α1 turn, N190D in the β2β4 turn, D219R in the α2α2 turn, and E256V and H285V in the β2α5 turn. Mutation in one of the residues that coordinate the metal binding, S155A, completely lost the adhesive activity under flow but bound normally under static conditions, whereas the mutation Y285F had the converse effect. We conclude that the upper surface of the domain, including the metal ion-dependent adhesion site motif, defines the collagen recognition site.

The glycoprotein (GP) Iα/IIa (integrin α₂β₁) is a major platelet-attachment receptor for collagen. Integrins are a family of heterodimeric cell adhesion molecules that mediate cell-cell and cell-matrix adhesion (1). The integrin α₂β₁ is expressed on platelet and fibroblastic cells, it functions both as a collagen and laminin receptor on endothelial and epithelial cells (2, 3). It also acts as the receptor for the human pathogen echovirus-1 (4).

α₂β₁ is composed of a 150-kDa α₂ and a 130-kDa β₁ subunit (5). Within the α₂ subunit, the 200-amino acid I domain shares homology with the A domains of von Willebrand factor, complement proteins, cartilage matrix protein, and certain other integrins. There is increasing evidence that I (A) domains play an important role in cell-adhesion protein and cell-matrix interaction.

Within the α₂β₁ integrin, the I domain (amino acids 140–349) provides the principal binding site for collagen. Thus, antibodies that block α₂β₁ interaction with collagen recognize epitopes within the I domain (6–8). Three groups have shown that recombinant α₂-I domain fusion proteins bind specifically to collagen in a divalent cation-dependent manner (9–11), and a fourth group has presented conflicting data suggesting that collagen binding is independent of divalent cation (12).

Like other I domains, the α₂-I domain contains a cation-binding site called the metal ion-dependent adhesion site (MIDAS) motif (13). In the crystal structure (14), the side chains of residues Ser-153, Ser-155, and Asp-254 directly coordinate a Mg²⁺ ion, and Asp-151 and Thr-221 provide water-mediated bonds. One study reported that mutation of residues Asp-151, Thr-221, or Asp-254 abolishes adhesion of the intact integrin α₂β₁ to collagen (12), whereas of these three residues only Thr-221 is absolutely critical for the binding of the recombinant glutathione S-transferase-α₂-I fusion protein to collagen.

Very recently we reported (15) the expression and characterization of a recombinant α₂-I protein that inhibits the interaction of platelets with collagen under flow conditions. Furthermore, this recombinant α₂-I binds specifically to collagen in a divalent cation-dependent manner.

Based on the crystal structure, a hypothetical model of an I domain-collagen complex has been proposed, and specific residues were identified that might be critical for collagen binding (14). In this study, we have tested this model by introducing point mutations into 13 residues surrounding the MIDAS motif. We then expressed recombinant α₂-I mutants and analyzed both the collagen binding activity and the ability to bind collagen under flow conditions. We coated beads with the proteins and tested in a flow chamber to resemble the function of a platelet membrane receptor exposed to collagen fibrils. We have identified several amino acids, distinct from the MIDAS residues, which are critical for α₂-I domain binding to collagen. The results provide direct experimental support for the hypothetical model.

**EXPERIMENTAL PROCEDURES**

Construction of the α₂-I Mutants—As described previously (15), complementary DNA encoding the human α₂ integrin subunit I domain (amino acids 120–337) was generated by PCR using full-length human α₂ cDNA as template. The PCR end primers were designed to introduce a BamHI restriction site at the 5’ end and HindIII restriction site at the 3’ end. The PCR product was digested with BamHI and HindIII restriction enzymes and inserted into pQE9 (Qiagen, Chatsworth, CA) for

*This work was supported by Grant RR-9630227N from the American Heart Association and Grant R01 HL48576 from the National Institutes of Health. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed: Hematology Research, Brigham and Women’s Hospital, 221 Longwood Ave., LMRC-605, Boston, MA 02115; Tel.: 617-732-5812; Fax: 617-739-3324; E-mail: CRUZ@CALVIN.BWH.HARVARD.EDU.

‡ The abbreviations used are: GP, glycoprotein; MIDAS, metal ion-dependent adhesion site; PCR, polymerase chain reaction; IPTG, isopropyl-β-D-thiogalactopyranoside; BSA, bovine serum albumin; TBS, Tris-buffered saline; ELISA, enzyme-linked immunosorbent assay.

§ Current address: The Burnham Institute, 10901 N. Torrey Pines Rd., La Jolla, CA 92037.
expression in Escherichia coli. The recombinant α2-I domain was expressed as a His tag fusion protein containing 12 residues at the N terminus from the expression vector (MRGSHHHHHHHS).

We mutated residues that were predicted to be collagen contact sites and other residues on the surface of the domain surrounding the MIDAS residue, Ser-155 to Ala. Mutations were introduced into α2-I domain cDNA using two rounds of PCR amplification. First, each reverse primer was combined with the reverse orientation of the EcoRI primer and amplified. Second, the resultant DNA fragment was then combined with the opposite end primer to produce the cDNA fragment. Forward or reverse primers containing the changed nucleotides were prepared for the following: N154S, S155A, N190D D219R, L220R, E256V, H258V, Y285F, N287D, N289D, L291D, N295E, and R298E. The mutant cDNA fragments were isolated by digestion with BamHI and HindIII restriction enzymes and used to transform the expression vector, pQE9, as described above. The nucleotide sequence of the PCR products was confirmed by sequencing the insert of the subcloned vector.

**Expression and Purification of the α2-I Proteins**—Recombinant α2-I proteins were expressed in bacteria as described previously with some modifications. E. coli M15 (pREP4) (QIAGEN) containing each of the pQE9-α2-I variants was cultured overnight at 37 °C in 30 ml of 25 g/ltr tryptone, 15 g/liter yeast extract, 5 g/liter NaCl, pH 7.3, containing 100 μg/ml ampicillin and 25 μg/ml kanamycin. The culture was diluted 1:30 and grown to an A_600 nm of 0.8. The culture was adjusted to 1.5 mM IPTG and incubated for 3.5 h at 37 °C. The cells were then harvested and resuspended in 25 ml of lysis buffer (50 mM Tris-Cl, 0.1 mM EDTA, pH 8.0) containing a final concentration of 250 μg/ml lysozyme and allowed to stand for 15 min at 4 °C. The bacterial cells were lysed in the presence of 1.25 mg/ml deoxycholic acid and 7 μg/ml RNase/DNase. The lysate was centrifuged at 45,000 × g for 25 min. The supernatant was collected and passed over a C-4′-chelated Sepharose (TALON Superflow, CLONTech, Palo Alto, CA) column equilibrated with 50 mM Tris-Cl, 500 mM NaCl, pH 8.0, buffer. The α2-I proteins eluted from the column with 50 mM imidazole. The highly purified proteins were dialyzed against 35 mM Tris-HCl, 150 mM NaCl, pH 7.4. Protein concentration was determined by the BCA method (Pierce). Purity of all the α2-I proteins was assessed by Coomassie Blue staining of SDS-polyacrylamide gel electrophoresis gels (16).

**Radiolabeling of α2-I Protein—**E. coli were grown overnight and diluted to the medium described above. When grown to an A_600 nm of 0.8, the cells (800 ml) were pelleted by centrifugation, washed with M9 minimal medium supplemented with 150 mg/liter of each of the following amino acids: threonine, phenylalanine, leucine, isoleucine, valine, and proline. Bacteria were grown for 60 min; IPTG (0.1 mg/liter) and thiamine (0.5 μg/liter) were added. After 30 min of substrate conversion, reactions were stopped with 0.025 ml of 2N H2SO4. The beads were centrifuged, and the supernatant was transferred to microtiter plates, which were read at 490 nm. Net specific binding was determined by subtracting OD values from wells coated with BSA alone.

**Mapping the Collagen-binding Site in the I Domain**

The purified 35S-α2-I protein was incubated with beads as described above. The coated beads were washed three times with TBS by centrifugation. Then, the 35S-α2-I protein-coated beads were counted using a scintillation counter. The number of α2-I molecules per bead was calculated by using the specific activity.

The adsorbed proteins on beads were measured by ELISA. Briefly, 0.025 ml of beads coated with α2-I proteins or control beads coated with BSA were centrifuged. The supernatant was removed, and the beads were incubated with 0.025 ml of TBS-T containing 1:10,000 dilution of peroxidase-conjugated monoclonal anti-polyhistidine antibody (Sigma) for 60 min at 37 °C. The beads were washed three times by centrifugation. The beads were resuspended in 0.1% Tween 20 for 60 min at 37 °C. After washing two times with phosphate-buffered saline, the beads were incubated with a 1:20,000 dilution of peroxidase-conjugated monoclonal antibody (12F1) or 8F1 (data not shown). After 30 min of substrate conversion, reactions were stopped with 0.01 ml of 2 N H2SO4. The beads were centrifuged, and the supernatant was transferred to microtiter plates, which were read at 490 nm. Net specific binding was determined by subtracting OD values from beads coated only with BSA from the total binding values obtained.

**Preparation of Collagen-coated Dishes—**Equine tendon collagen (100 μg/ml) (Helena Laboratories, Beaumont, TX) was added to 35-mm culture dishes. After 90 min incubation at 37 °C, coated dishes were subsequently rinsed with phosphate-buffered saline and incubated with TBS containing 3% BSA for 30 min at room temperature to block nonspecific interactions.

**Analysis of Recombinant α2-I Proteins under Flow Conditions—**The α2-I proteins were purified as described above by using the method for studying platelet adhesion in flowing blood that has been described (15) with some modifications. A parallel plate flow chamber was assembled as described by the manufacturer (GlycoTech, Rockville, MD). Briefly, the collagen-coated dish formed the lower surface of the chamber and a silicone rubber gasket determined the flow path height of 254 μm. The flow chamber was assembled and filled with TBS containing 2 mM MgCl2. A syringe pump (Harvard Apparatus Inc., Holliston, MA) was used to aspirate the bead suspension through the flow chamber. Using a gasket thickness of 0.025 cm, a flow rate of 0.48 ml/min produced a wall shear rate of 300 s⁻¹. Bead suspension was then perfused for 3 min and the coated dish was washed with TBS, 2 mM MgCl2. Attached beads were observed with phase contrast objectives and recorded by videomicroscopy. For the inhibition experiments, EDTA (5 mM) or monoclonal antibody (150 μg/ml of 6F1 or 12F1) was added to the wild type-coated bead suspension and incubated for 5 min before perfusion. The number of adherent beads was determined by overlaying a 36-square grid on at least 6–8 frames and counting and averaging the number of beads in 12 randomly selected squares. For some experiments, the whole frame was counted. All experiments were performed in duplicate on different days.

**RESULTS**

After induction with IPTG, bacteria transformed with pQE9-α2-I plasmids and expressing α2-I mutants were lysed. As described for wild type α2-I protein (15), the resultant bacterial lysate was then passed over a C4′ column, and the bound protein was eluted with a step imidazole gradient. The highly purified proteins were dialyzed against TBS. The final yields of the purified mutant proteins were between 1 and 10 mg/liter bacterial culture. All the proteins migrated identically to wild type α2-I protein, when analyzed by SDS-polyacrylamide gel electrophoresis under reduced and non-reduced conditions (data not shown).

**Effect of Mutations in Binding Monoclonal Antibodies 6F1 and 12F1—**We previously reported that monoclonal antibodies 6F1 and 12F1 recognize a conformation-dependent epitope within the α2-I domain and block the interaction of α2-I protein with collagen (15). Therefore, reactivity with these antibodies

---

*Mapping the Collagen-binding Site in the I Domain*
was determined to provide an index of the structural integrity of the recombinant α2-I mutant proteins, and decreases in reactivity were used to localize the target epitope(s) of each antibody. The binding data for the α2-I mutants are summarized in Fig. 1. Compared with wild type, 10 mutants bound normally to both antibodies (>80% wild type binding) indicating that they are correctly folded and that the mutations are not a critical component of the bound epitope. Mutation N154S had a binding activity for the two antibodies between 65 and 75% of wild type binding. The mutant, K298E, had the lowest binding activity for antibodies 6F1 and 12F1 (<40%) but had normal collagen interaction (see below), suggesting that this residue, which lies on the edge of the upper surface of the domain, forms part of the target epitope for both antibodies. Mutation S155A had normal binding to 12F1 but showed 50% reduced binding to antibody 6F1, suggesting either that Ser-155 also forms part of the antibody epitope or that there is a partial misfolding of the upper surface of α2-I domain.

**Effect of Mutations in Binding to Collagen**—The collagen binding activity of the α2-I mutants relative to that of the wild type was examined over a range of concentrations (Fig. 2). As previously reported, the recombinant α2-I domain bound to immobilized collagen in a saturable manner with a half-maximal collagen binding of 0.40 and 1.4 μM (9–12, 15). By comparison, introduction of mutations N154S, N190D, D219R, E256V, and Y285F greatly increased the concentrations required for half-maximal collagen binding (>6.0 μM). Mutation H258V completely impaired the collagen binding activity (Fig. 2). Unexpectedly, mutation on the metal coordination residue, S155A, had normal collagen binding and a half-maximal binding of 0.75 μM (Fig. 2). The half-maximal binding values for the mutations L220R, N287D, N289D, L291D, N295F, and K298E were observed between 1.0 and 3.0 μM (data not shown).

**Analysis of Polystyrene Beads Coated with α2-I Proteins under Flow Conditions**—To evaluate better the effect of these mutations in a more physiologically relevant setting, we next studied the ability of 2-μm polystyrene beads coated with wild type and mutant α2-I proteins to mimic the function of platelet exposed to collagen fibrils in a flow chamber. Fig. 3A shows a photomicrograph of attached wild type α2-I protein-coated beads to collagen fibrils after 3 min perfusion at a shear rate of 300 s⁻¹. As expected, this interaction was blocked by the addition of either 5 mM EDTA (Fig. 3B) or monoclonal antibody 6F1 (Fig. 3C). The number of beads attached in the presence of EDTA or 6F1 was similar to beads coated with BSA (Fig. 3D).

These results confirmed the importance of the MIDAS motif and the structural integrity of this motif in the coupled α2-I protein.

Eleven of the 13 mutants had a parallel correlation between this assay and the collagen binding assay (static conditions). The mutants, D219R and H258V, that yielded half-maximal collagen binding values of 6.0 μM or more (Fig. 2) completely
lost their adhesive activity (Fig. 4). In addition, mutations N154S, N190D, and E256V that also had low collagen binding activity retained less than 38% of wild type adhesive activity. Discrepancies between the results of both assays were also observed. Mutant S155A, which had normal collagen binding activity, completely lost adhesive activity. In contrast, mutation Y285F, previously shown to possess a low collagen binding activity, retained 70% of wild type adhesive activity. N289D retained 50% activity, whereas the remaining mutants retained more than 75% of wild type activity. Fig. 4 also shows the inhibitory effect of EDTA and the monoclonal antibodies 6F1 and 12F1.

Polystyrene beads were coated with 35S-labeled α2-I protein to determine the number of α2-I protein molecules coupled per bead. The calculated number was approximately 5 × 10^5 molecules. Furthermore, protein-coated beads analyzed by ELISA with the anti-polyhistidine antibody demonstrated that the adsorption of protein on beads was similar for all the recombinant molecules. Furthermore, protein-coated beads analyzed by ELISA to determine the number of α2-I protein molecules coupled per bead. The calculated number was approximately 5 × 10^5 molecules. Furthermore, protein-coated beads analyzed by ELISA with the anti-polyhistidine antibody demonstrated that the adsorption of protein on beads was similar for all the recombinant proteins (data not shown).

**DISCUSSION**

Several studies have described the essential role of the integrin α2β1 as an adhesive receptor under flow conditions (18–20). The I domain present in the α2 chain (GPIa) contains the collagen-bonding site (7, 9–12). In previous studies, we reported the expression of a recombinant α2-I protein that binds to collagen in a metal-dependent manner and inhibits platelet interaction with collagen under flow conditions (15).

This is the first study to demonstrate that polystyrene beads, similar in size to platelets, coated with the recombinant α2-I protein adhere to collagen with the adhesive properties found in the intact platelet α2β1 complex. Similar to the studies that used platelets at shear rates of 300 s⁻¹, our α2-I protein-coated beads adhered to collagen fibrils in a metal-dependent manner (21, 22), and this adhesion was blocked by an anti-functional monoclonal antibody (20). The inhibitory effect of both EDTA and antibodies 6F1 or 12F1 on the adhesion of coated beads provides evidence that the folding and conformation of the isolated I domain is similar to that found in the native GPIa-IIa complex. Our findings, therefore, confirm the important role of GPIa-IIa in mediating platelet adhesion to collagen and show that the I domain is the smallest fragment of the GPIa-IIa complex to mediate this interaction under flow conditions.

Based on the crystal structure of the α2-I domain, a hypothetical model was proposed for the collagen-binding site (14).

---

**FIG. 4. Effect of the mutations on the adhesion of α2-I protein-coated beads to collagen fibrils.** Adhesive properties of the α2-I mutant proteins were analyzed as described in the legend to Fig. 3 and under “Experimental Procedures.” The values were normalized to the value obtained for wild type α2-I protein. Each column represents the mean ± S.D. of values obtained in 2–4 independent duplicate experiments.

---

**FIG. 5. Mapping mutations to the structure of the α2-I domain-collagen complex.** All atom representation of the α2-I domain, looking down onto the MIDAS face, showing sites of mutation, color-coded according to their effect on adhesion under flow. The collagen triple helix is shown as three coils, and the metal ion in the MIDAS motif as M. A, the high affinity conformation from the crystal structure of the I domain-collagen complex. Red balls indicate mutations that reduce adhesive activity by 60% or more. Yellow ball indicates N289D, a mutation that retained 50% of adhesive activity. Blue balls indicate mutations that show 70% or greater adhesive activity to collagen. The green ball indicates K289E, a mutation that reduces 6F1 and 12F1 antibody binding but shows >80% adhesive activity. B, a hypothetical low affinity complex, in which the α2-I domain structure, is that of the unliganded conformation (14). Note the location of Tyr-285 in the two states; the mutation Tyr-285 reduces binding under static conditions but has little effect under flow, consistent with two different conformations in the two assays.

We mutated residues that were predicted to be collagen contact sites and other residues on the surface of the domain surrounding the MIDAS motif. In general, the mutated residues were completely exposed, did not form salt bridges, hydrogen bonds, or have other contacts with the rest of the domain. Mutations were either uncharged to charged or charge reversal. We also mutated the metal-coordinating MIDAS residue, Ser-155 to Ala.

We used the previously described flow system since it provided an efficient and physiologically relevant method to study the function of small quantities of recombinant α2-I mutant proteins. The analysis is also simplified as it utilizes purified recombinant α2-I protein rather than the heterodimeric platelet α2β1 complex. Using flow studies as one of the functional end points also eliminates the need to express the α2 chain in the surface membrane of other cell types. The close correlation between the reduced ability of the α2-I mutant proteins to adhere to collagen under shear conditions and results obtained in binding assays (static conditions) helps to validate the flow chamber method and supports the hypothesis that the residues that we have identified by structural studies play a role in platelet adhesion.
Very recently, the crystal structure of a collagen-I domain complex has been determined.\(^2\) The structure confirms the location of the collagen-binding site (Fig. 5A), with a glutamic acid from the collagen directly coordinating the metal ion in the MIDAS motif. Four mutations that resulted in the greatest disruption of collagen interaction, N154S in the βα-α1 turn, D219R in the α3-α4 turn, and E256V and H258V in the βD-α5 turn, form part of the surface surrounding the MIDAS motif that make direct contact with collagen. Other mutations that do not affect collagen binding under flow conditions (Y285F, N287D, N289D, L291D, N295E, and K298E in the C-helix, α6 and C-α6 turn) do not contact collagen.

The crystal structure also reveals that collagen binding induces dramatic changes in the upper surface of the I domain (compare Fig. 5, A and B), and it was proposed that the conformational shift switches the I domain from a low affinity to a high affinity state. Interestingly, mutation Y285F, which retained 70% of wild type activity in the flow assay, had a low collagen binding activity under static conditions. As seen in Fig. 5A, Tyr-285 lies atop the MIDAS motif in the “low affinity” conformation and makes contact with collagen. In assuming the high affinity conformation, this residue undergoes a positional change that removes it from the collagen-binding surface. It is therefore possible that in the static assay, binding to the low affinity conformation occurs, whereas in the flow assay, binding to the “high affinity” conformation is observed. Distinct binding modes, and perhaps distinct collagen sequence specificities, under flow versus static conditions are further suggested by both the different cation requirements and the effects of the S155A mutation (Ser-155 forms a direct bond to the metal in the MIDAS motif). The high affinity conformation has a more extended shape than the low affinity conformer, suggesting that the shear forces experienced by the I domain when the platelet engages collagen might provide a direct mechanism of activation by “molecular stretching.” “Catch bonds,” which strengthen when tensile force is applied, have been predicted theoretically.\(^23\).

Acknowledgments—We thank Dr. Barry Coller (Mt. Sinai Medical Center, New York) for providing the monoclonal antibody 6F1 and Dr. Virgil L. Woods (University of California, San Diego) for providing monoclonal antibody 12F1. We thank Andrew Ritchie for valuable suggestions on the manuscript. We also thank Dr. Robert I. Handin for continuing support.

REFERENCES

1. Hynes, R. O. (1992) Cell 69, 11–25
2. Elices, M. J., and Hemler, M. E. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 9906–9910
3. Langenau, L. R., Gehlsen, K. R., Wayner, C., Carter, W. G., Engvall, E., and Rusnac, L. E. (1989) J. Cell Biol. 109, 2455–2462
4. Bergelson, J. M., Shepley, M. P., Chan, B. M. C., Hemler, M. E., and Finberg, R. W. (1992) Science 255, 1718–1720
5. Santoro, S. A., and Zutter, M. M. (1995) Thromb. Haemostasis 74, 813–821
6. Kamata, T., Puzon, W., and Takada, Y. (1994) J. Biol. Chem. 269, 9659–9663
7. Bahou, W. F., Potter, C. L., and Mirza, H. (1994) Blood 84, 3734–3741
8. Coller, B. S., Beier, J. H., Scudder, L. E., and Steinberg, M. H. (1989) Blood 74, 182–192
9. Tuckwell, D., Calderwood, D. A., Green, L. J., and Humphries, M. J. (1995) J. Cell Sci. 109, 1629–1637
10. Dickeson, S. K., Walsh, J. J., and Santoro, S. A. (1997) J. Biol. Chem. 272, 7661–7668
11. Depraetere, H., Wille, C., Gansemans, Y., Stanssens, P., Laumers, M., Baruch, D., De Reys, S., and Deckmyn, H. (1997) Thromb. Haemostasis 77, 981–985
12. Kamata, T., and Takada, Y. (1994) J. Biol. Chem. 269, 26006–26010
13. Lee, J. G., Rieu, P., Arnason, M. A., and Liddington, R. (1995) Cell 80, 631–638
14. Emsley, J., King, S. L., Bergelson, J. M., and Liddington, R. C. (1997) J. Biol. Chem. 272, 28512–28517
15. Estavillo, D., Ritchie, A., Diacono, T. G., and Cruz, M. A. (1999) J. Biol. Chem. 274, 35921–35926
16. Laemmli, U. K. (1970) Nature 227, 680–685
17. Pischel, K. D., Hemler, M. E., Huang, C., Bluestein, H. G., and Woods, V. L. (1987) J. Immunol. 138, 226–233
18. Saelman, E. U. M., Horton, L. F., Barnes, M. J., Granick, H. R., Hese, K. M., Nieuwenhuis, H. K., de Groot, Ph. G., and Sixma, J. J. (1993) Blood 82, 2749–2753
19. Saelman, E. U. M., Nieuwenhuis, H. K., Hese, de Groot, Ph. G., Heijnen, H. F. G., Sage, E. H., Williams, S., McKeown, L., Granick, H. R., and Sixma, J. J. (1994) Blood 83, 1244–1250
20. Verkleij, M. W., Morton, L. F., Knight, C. G., de Groot, Ph. G., Barnes, M. J., and Sixma, J. J. (1998) Blood 91, 3808–3816
21. Santoro, S. A. (1986) Cell 46, 913–920
22. Zijenah, L. S., Morton, L. F., and Barnes, M. J. (1990) Biochem. J. 268, 481–486
23. Dembo, M., Torney, D. C., Saxman, K., and Hammer, D. (1988) Proc. R. Soc. Lond. Ser. B Biol. Sci. 234, 55–83

\(^2\) J. Emsley, C. G. Knight, M. J. Barnes, R. W. Farndale, and R. C. Liddington, submitted for publication.