Characterization of C1 Inhibitor-Ta

A DYSFUNCTIONAL C1INH WITH DELETION OF LYSINE 251*

(Received for publication, March 28, 1996, and in revised form, July 12, 1996)

Rana Zahedi‡, Kulwant S. Aulak‡, Eric Eldering§, and Alvin E. Davis III‡¶

From the ‡Division of Nephrology, Children’s Hospital Research Foundation, Cincinnati, Ohio 45229 and the ¶Department of Autoimmune Diseases, Central Laboratory of Red Cross Blood Transfusion Service, 1006 AD Amsterdam, The Netherlands

Dysfunctional C1 inhibitor (C1INH)-Ta is a naturally occurring mutant from a patient with type II hereditary angioedema. This mutant has a deletion of the codon for Lys-251, which is located in the connecting strand between helix F and strand 3A, overlying β sheet A. Deletion of this Lys modifies the amino acid sequence at this position from Asn-Lys-Ile-Ser to Asn-Ile-Ser and creates a new glycosylation site. To further characterize the mechanism of dysfunction, we have analyzed the recombinant normal and Ta proteins expressed by COS cells in addition to the proteins in serum and isolated from serum. Recombinant C1INH-Ta revealed an intermediate thermal stability in comparison with the intact and reactive center cleaved normal proteins. Analysis of the reactivity of this recombinant protein with target proteases demonstrated no complex formation with C1s, C1r, or kallikrein. Inefficient complex formation was, however, clearly detectable with β-factor XIIa. Each protease produced partial cleavage of the recombinant mutant inhibitor. Recombinant C1INH-Ta, on 7.5% SDS-polyacrylamide gel electrophoresis and by size fractionation on Superose 12, showed a higher molecular weight fraction that was compatible in size with dimer formation. However, no multimerization of C1INH-Ta isolated from serum or of C1INH-Ta in serum, was observed. The C1INH-Ta dimer expressed the epitopes that normally are expressed only on the protease complexed or the cleaved inhibitor. These epitopes were not expressed on the monomeric inhibitor. The data suggest that the mutation in C1INH-Ta results in a folding abnormality that behaves as if it consists of two populations of molecules, one of which is susceptible to multimerization and one of which is converted to a substrate, but which retains residual inhibitory activity.

C1 inhibitor (C1INH)1 belongs to the superfamily of serine protease inhibitors or serpins. It is the sole inhibitor of the complement proteases C1s and C1r and is the major inhibitor of plasma kallikrein and coagulation factor XIIa (1, 2). Deficiency of C1INH or inheritance of a dysfunctional mutant molecule results in hereditary angioedema. Inhibition by serpins involves a region of the molecule termed the reactive center loop. Amino acids within this region mimic the substrate of the protease. However, following recognition of the reactive center residue, rather than cleavage, a stable, equimolar inhibitor-protease complex results. Amino acid substitutions within this region either cause loss of inhibitory activity or alter target protease specificity. Approximately 70% of dysfunctional C1INH proteins result from reactive center P1 (Arg-Glu-Asp-Glu) mutations (3, 4). The role of this region in specificity determination is well known. Several non-reactive center mutations have been described. As expected from analyses of other mutant serpins, replacement of the P2 residue modifies target protease specificity (5–7). Mutations within the hinge region (P10–P16) result in conversion of the inhibitor to a substrate (P12, P14, and one P10 mutant) (8–11); others lead to multimerization of the inhibitor (P10) (12, 13). Dysfunctional C1INH-Ta, a naturally occurring mutant from a patient with hereditary angioedema, does not inhibit C1s and C1r (14). It has deletion of Lys-251, which is widely separated, in linear sequence, from the reactive center loop. This residue is located in the connecting strand between helix F and strand 3A, and overlies β sheet A. Deletion of Lys-251 modifies the amino acid sequence at this position from Asn-Lys-Ile-Ser to Asn-Ile-Ser, which creates a new glycosylation site. The additional carbohydrate together with the deletion resulted in a slightly higher molecular weight band on SDS-PAGE and an altered electrophoretic mobility on agarose gel electrophoresis in comparison with the normal inhibitor. Enzymatic deglycosylation reduced the normal and Ta mutant inhibitors to the same size but did not restore activity of the mutant Ta inhibitor.

In order to further characterize the potential role of the additional carbohydrate and to analyze the mechanism of dysfunction, we transfected COS-1 cells with expression vectors containing the cDNA for C1INH-Ta and the wild type C1INH and expressed the recombinant proteins in the presence or absence of tunicamycin. We also expressed a new mutant in which Lys-251 was replaced with a Thr; this results in glycosylation at Asn-249. Functional and structural analysis of the recombinant inhibitors indicate that the dysfunction of C1INH-Ta results from the deletion of Lys-251 and not from the additional carbohydrate. Furthermore, the data suggest that this mutant inhibitor behaves as if it consists of two conformers, one of which is converted primarily to a substrate, and one of which is susceptible to multimerization.

MATERIALS AND METHODS

Site-directed Mutagenesis—A polymerase chain reaction-based site-directed mutagenesis as described previously was performed (6). The mutagenic oligonucleotide primer used has the following sequence, K251T (GAA ACC CAA CAA CAC GAT CAG CC GG CTG CTAGA), which corresponds to nucleotides 8443–8475, with A → C transversion at position 8457. This point mutation results in replacement of Lys-251 by a Thr. The presence of the mutation was confirmed by DNA sequence analysis.

---

* These studies were supported by United States Public Health Service Grant HD22082. 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: Division of Nephrology, Children’s Hospital Research Foundation, 3333 Burnet Ave., Cincinnati, OH 45229-3039.

¶ The abbreviations used are: C1INH, C1 inhibitor; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; ELISA, enzyme-linked immunosorbent assay.

1 The abbreviations used are: C1INH, C1 inhibitor; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; ELISA, enzyme-linked immunosorbent assay.

© 1996 by The American Society for Biochemistry and Molecular Biology, Inc.

Printed in U.S.A.
Characterization of C1 Inhibitor-Ta

Expression of Recombinant Proteins—COS cells were transfected with expression vectors (pSVL) containing cDNA of normal C1INH, mutant C1INH-Ta, and mutant K251T C1INH. Transfection was performed using a modified DEAE-dextran technique (15). Newly synthesized protein is metabolically radiolabeled with [35S]Met (Amersham Life Sciences). Medium was harvested 72 h after transfection and dialyzed in phosphate-buffered saline (9.6 mM K/NaPO4, 140 mM NaCl, pH 7.2) (PBS).

Complex Formation of the Recombinant Proteins with Target Proteases—Radiolabeled recombinant C1INH wild type and Ta were incubated alone or with C1s, C1r, β-factor XIIa, and kallikrein for 1 h at 37 °C. C1r and C1s were generous gifts from Dr. David Bing, Center for Blood Research, Boston, MA. Factor XII and kallikrein were purchased from Enzyme Research Laboratories, Elkhart, IN. The reactions were then stopped by 1 μl of 0.1 M phenylmethylsulfonyl fluoride, Triton X-100 (0.5%), deoxycholic acid (0.25%), SDS (0.5%), and EDTA (5 mM) were added to each sample. Rabbit anti-human C1INH antibody (3 μl) was then added, and samples were incubated at 4 °C overnight. A suspension of 6 μl of fixed Staphylococcus aureus (IgG sorb, Enzyme Center, Boston, MA), sonicated and washed three times (with 1% Triton X-100, 1% SDS, 0.5% deoxycholate, and 5 mg/ml bovine serum albumin in 0.02 M sodium phosphate, 0.15 M NaCl, pH 7.4), was added to each sample and incubated at 4 °C for 1 h. The pellets (14,000 g for 3 min) were washed once with the above washing solution and three times with a washing solution lacking bovine serum albumin. Each precipitate was then dissolved in SDS non-reducing buffer (15 μl) (4.2% SDS, 20% glycerol, 0.1 M Tris/HCl, pH 6.5, and 0.01% bromophenol blue), vortexed, boiled for 3 min, centrifuged briefly, and subjected to electrophoresis on SDS-7.5% polyacrylamide gels. Gels were fixed, dried, and exposed to x-ray film (Kodak XAR-5, Eastman Kodak Co.) at −70 °C.

Thermal Denaturation—The heat stability of the recombinant C1INH was determined as described by Stein et al. (16). COS-1 cell supernatants (100 μl) containing normal or C1INH-Ta were incubated at 40, 50, 60, 70, and 80 °C for 120 min and then were centrifuged at 14,000 g for 30 min. The residual antigen in the supernatant was quantified by an ELISA technique as described by Aulak et al. (12).

Gel Filtration—Size fractionation of normal serum and serum from Ta, of C1INH protein purified from each serum, and of each recombinant protein was performed on Superose 12 using an FPLC system (Pharmacia Biotech Inc.). The column was equilibrated in PBS. Fractions of 300 μl were collected at a flow rate of 0.5 ml/min.

Immunological Assays—C1INH antigen was detected using an ELISA technique. Ninety-six-well plates were coated with goat polyclonal anti-human C1INH diluted 1/1000 in PBS (from Atlantic Antibodies) and incubated overnight at 4 °C. Non-specific binding was inhibited by incubation in 2% bovine serum albumin in PBS for 1 h at 37 °C. The bound antigen was then detected with a rabbit polyclonal antiserum to human C1INH and subjected to SDS-PAGE; autoradiography was performed as described under “Materials and Methods.”

RESULTS

Interaction of Recombinant Wild Type C1INH and C1INH-Ta with Target Proteases—Previous experiments using enzymatic deglycosylation indicated that the additional carbohydrate was not simply blocking access to the reactive center (14). However, glycosidases do not restore the asparagine residue, which could be responsible for the inability to regenerate function. With peptide-N-glycosidase F, an aspartic acid is produced from deglycosylation, while with endoglycosidase H, one N-acetylglucosamine residue remains linked to the asparagine residue. Normal and dysfunctional C1INH were synthesized in the presence of tunicamycin to prevent glycosylation. This had no effect on the function of either protein; normal C1INH remained fully functional, and C1INH-Ta did not complex with C1r or C1s (data not shown).

Lys-251 was replaced with a Thr, using polymerase chain reaction-mediated site-directed mutagenesis (6). Replacement of Lys by Thr creates a new glycosylation site on Asn-249 (17, 18). Mouse monoclonal antibody 4C3 was a generous gift from Dr. Marc Shapira. Peroxidase-conjugated goat anti-rabbit or anti-mouse IgG antibody (Life Technologies, Inc.) was used to detect binding of the anti-C1INH antibodies, the color was developed using O-phenylenediamine dehydrochloride (Sigma) and H2O2, and the optical density was read at 492 nm. ELISA using the monoclonal antibody K11, which reacts only with reactive center cleaved C1INH, as the catching antibody was done as described previously (19, 20). Microtiter plates were coated with 1 μg/ml K11 in PBS overnight at 4 °C. Blocking was with 3% bovine serum albumin in PBS for 30–45 min at room temperature. Culture supernatants (100 μl) were incubated for 90 min at room temperature, and plates were washed four times with PBS containing 0.1% Tween 20. Adsorbed C1INH was detected with biotinylated rabbit polyclonal anti-C1INH antibody, followed by 30 min with streptavidin-coupled hors eradish peroxidase, both diluted 1:1000 in PBS, 0.1% (w/v) Tween 20, 0.2% (w/v) gelatin. Development was with 3,3′,5,5′-tetramethylbenzidine, and reactions were stopped after 5–10 min with 2 μl H2O2, or 200 kDa. The high molecular mass band was present and unchanged after incubation with C1r, C1s, β-factor XIIa, and kallikrein. As opposed to the reactions with the other target proteases, with
b-factor XIIa, a minor but reproducible band was observed that corresponded in size to that of the inhibitor-protease complex (Fig. 1B). Time-course analysis of the interaction of the Ta protein with b-factor XIIa revealed complex formation, which was visible by 15 min and appeared to reach a maximum after approximately 1 h (Fig. 2, A and B). In comparison with the b-factor XIIa reaction with wild type C1INH, therefore, C1INH-Ta reacts much more slowly. In addition, only a portion of the inhibitor appears capable of participating in complex formation. Time-course analysis of C1INH-Ta with C1s showed maximal cleavage within 30 s (Fig. 2C). This amount did not appear to change over an incubation period of 1 h at 37 °C, and no complex formation was observed.

Reactive Center Loop Conformation—Thermal stability of serpins is a measure of their conformation. Natural intact serpins multimerize at 50–60 °C. This multimerization is accompanied by decreased antigenicity. Serpins cleaved near the reactive center are stable (remain monomeric and retain antigenicity) at elevated temperatures (80–90 °C) (21, 22). The recombinant Ta molecule with an intact reactive center loop is more stable at elevated temperatures than the wild type protein, but it is not as stable as the reactive center cleaved molecule (Fig. 3). Cleavage of the reactive center loop with trypsin increases the thermal stability of both the wild type and Ta proteins, indicating that the cleaved mutant molecule undergoes the normal conformational rearrangement specific to serpins. The observation that reactive center loop cleavage of C1INH-Ta enhanced reactivity with the monoclonal antibody KII, which reacts with cleaved but not with native or with complexed C1INH, was consistent with this interpretation (Fig. 4). Reactive center loop cleavage was confirmed in both experiments by SDS-PAGE (data not shown).

Gel Filtration of Wild Type and the Mutant C1INH-Ta—In order to further analyze the apparent dimer formation observed on SDS-PAGE, the recombinant WT and Ta proteins were size-fractionated on Superose 12 (Fig. 5). As pointed out previously, because of its highly glycosylated nature, normal monomeric C1INH elutes earlier than expected on gel filtration, at a position corresponding to a molecular mass of nearly 400 kDa (Figs. 5 and 6) (23, 24). When fractions were analyzed with the polyclonal anti-C1INH antibody, recombinant C1INH-Ta eluted in two peaks, one of which corresponded to the elution position of normal C1INH (Fig. 5). The earlier, smaller peak eluted at a position corresponding to a molecular mass of approximately 800 kDa. It is, therefore, possible that this peak represents a dimer of the Ta protein. The thermal stability of the protein within each peak was analyzed and each gave a profile the same as that shown in Fig. 3 for C1INH-Ta (data not shown). Antithrombin Rouen VI, with a mutation within helix F, and thus at a position near that of C1INH-Ta, undergoes spontaneous multimerization and also forms a stable inactive monomeric form after prolonged storage at 4 °C or incubation at 41 °C (25). This monomeric form probably is equivalent to the L-form of antithrombin or the latent form of plasminogen activator inhibitor-1. Therefore, we attempted to enhance these conformational changes in the Ta protein by incubation for 24 h at 37 and 41 °C. No change in the interac-
tion with any of the proteases was observed (data not shown), nor was there any change in the quantity of high molecular weight C1INH-Ta (on either gel filtration or SDS-PAGE analysis). Gel filtration of the mutant Ta protein and the wild type protein in serum or purified from serum showed identical elution profiles, using the polyclonal antiserum as a probe (Fig. 6). Only one peak was observed with C1INH-Ta, and this peak corresponded to the size of the monomer, which was identical in its elution position to the native normal C1INH.

In order to clarify the conformation of recombinant C1INH-Ta and wild type C1INH, reactivity with the monoclonal antibodies 4C3 and KOK12 was analyzed. Only the experiments with KOK12 are illustrated; the reactivities with 4C3 differed in intensity, but otherwise the results were identical to those with KOK12. These monoclonal antibodies detect C1INH that has undergone structural rearrangement due either to cleavage, complex formation, or loop-sheet polymerization (12, 17). Both monoclonal antibodies reacted with the higher molecular weight form of the recombinant C1INH-Ta (Fig. 7B). The reaction of each with the monomeric C1INH-Ta, like the reaction with the normal inhibitor, was minimal. This was true even though the amount of monomer present greatly exceeded the amount of the higher molecular weight form, as shown by the reactivity with the polyclonal antiserum. These data indicated that the dimer expresses the epitope that is expressed on complexed normal C1INH. The reactivity of the C1INH-Ta monomer with the monoclonal antibodies, like that of the wild type C1INH, was strikingly increased following cleavage at the reactive center with trypsin (Fig. 7, A and B).

**DISCUSSION**

The naturally occurring dysfunctional mutant serpins provide a starting point to help understand the structural requirements and the inhibitory mechanism used by these inhibitors. In this paper, we have characterized several aspects of the mechanism of dysfunction in a variant of C1INH that has deletion of Lys-251, which is situated outside the reactive center region. While the recombinant mutant protein did not form an SDS-stable complex with the target proteases Cls, Clr, and kallikrein, it demonstrated partial susceptibility to cleavage by target proteases. The intermediate thermal stability of the Ta protein together with the presence of a high molecular mass band on SDS-PAGE led to an analysis of potential multimer formation. Multimers were, in fact, detected, and both the monomer and multimer revealed intermediate thermal stability. Multimerization has been observed with several different dysfunctional serpins with mutations in several distinct regions.

The mutant Z α1-antitrypsin, with replacement of Glu-342 by a Lys, undergoes spontaneous loop-sheet polymerization (26). This replacement disrupts a critical salt bridge between Glu-342 and Lys-290 at the junction between strand 5A and the proximal end of the reactive center loop. The result therefore
probably is to open the gap in β sheet A between strands 3 and 5. Initially, it was suggested that this gap provided a receptor site for the reactive center loop of a second antitrypsin molecule. Data from more recent mutants and from a crystalized antithrombin dimer suggest that overinsertion of the reactive center loop may result, and that this disrupts strand 1C (27). The reactive center loop of a second molecule then inserts into the gap in sheet C. Another example is mutant C1INH-Mo with replacement of Ala-436 by a Thr (12). This mutation is located within the hinge region amino-terminal to the reactive center and appears to interfere with the movement of the reactive center loop. The presence of Thr at this position apparently prevents formation of the multimer. It also is theoretically possible that the multimers in plasma are simply below the detection limits of the ELISA. However, this seems unlikely because the plasma used contained similar amounts of mutant and normal protein (14). It seems most likely that the multimers are rapidly cleared from the circulation in vivo. C1INH-protease complexes have a rapid in vivo clearance rate (28, 29). Since the C1INH-Ta multimer shares several characteristics with the inhibitor-protease complex, it may be rapidly cleared by a similar mechanism. It also is possible that multimers form intracellularly in vivo and, unlike with COS cells, are not secreted or that some other undefined mechanism in plasma prevents formation of the multimer. It also is theoretically possible that the multimers represent an artifact of expression of C1INH-Ta in COS cells. However, if this is true, it is specific for this mutant and therefore indicates that its conformation is different from that of the wild type protein.

Thermal denaturation analysis showed that the mutant protein was more stable at elevated temperature than the wild type. However, it was not as stable as the P10 Ala → Thr mutant or as reactive center cleaved wild type C1INH. Cleavage of the reactive center loop with trypsin increases the thermal stability of C1INH-Ta to a degree similar to that of the P10 Ala → Thr mutant or as reactive center cleaved wild type C1INH. Cleavage of the reactive center loop with trypsin increases the thermal stability of C1INH-Ta to a degree similar to that of the cleaved wild type protein. This further indicates that the cleavage of the mutant inhibitor results in complete insertion of the released reactive center loop. This susceptibility to cleavage by trypsin and by target proteases contrasts with the lack of
susceptibility of the P10 Ala → Thr mutant, of antithrombin Rouen and of latent plasminogen activator inhibitor (12, 25, 30). Therefore, the reactive center must be exposed in the Lys-251 deletion mutant as it is in the wild type inhibitor and in the P12 Ala → Glu and P14 Val → Glu mutant monomer. Finally, the lack of expression of the neoepitope on the monomer indicates the lack of complete insertion. Appearance of the neoepitope on the cleaved molecule confirms that the mutant is able to undergo the normal conformational rearrangement.

The fact that C1INH-Ta complexed with β-factor XIA indicates that the molecule has a reactive center that can be recognized by protease and is capable of forming a complex, albeit inefficiently. Both the rate and extent of complex formation were considerably diminished compared with the β-factor XIA-wild type C1INH reaction. C1INH-Ta, therefore, clearly is capable of undergoing the conformational rearrangements required for complex formation. It also can be concluded that the functional changes in C1INH-Ta are more subtle than in many of the other mutants that lead to loop-sheet polymerization and/or conversion of the inhibitor to a substrate. The only other mutant of this type that retains significant activity is antithrombin Rouen VI, which has a mutation within helix F, very near the site of the deletion in C1INH-Ta. However, the minimal activity retained by C1INH-Ta almost certainly is not physiologically relevant. Patients with hereditary angioedema express one normal C1INH gene. The small amount of activity contributed by this dysfunctional mutant would be insignificant in comparison with that contributed by the product of the normal allele.

In conclusion, the data presented here suggest that deletion of Lys-251 results in aberrant folding of the mutant molecule that results in two populations of molecules. One of these is probably characterized by loop overinsertion to the extent that it resembles a latent form. This form is susceptible to multimerization and is not recognized by target proteases. It also, at least in the dimerized form, expresses epitopes normally present on the complexed form of the protein. The other form remains in a conformation recognized by target proteases and by trypsin. This is probably the form capable of complexing with β-factor XIA. It does not express the epitopes present on the protease-complexed normal molecule. Although there may be some degree of loop insertion, appropriate insertion to allow efficient complex formation appears to be prevented, or the rate of loop insertion may be slowed. This combination of characteristics (substrate-like behavior, multimerization, and retention of residual ability to complex) is unusual and perhaps unique. The analysis of this inhibitor illustrates the fine line between function and dysfunction among the serpins.

REFERENCES
1. Schapira, M., de Agostini, A., Schifferti, J. A., and Colman, R. W. (1985) Curr. Issues Complement 2, 111–126
2. Davis, A. E., III (1988) Annu. Rev. Immunol. 6, 595–628
3. Aulak, K. S., Pemberton, P. A., Rosen, F. S., Carrell, R. W., Lachmann, P. J., and Harrison, R. A. (1988) Biochem. J. 253, 615–618
4. Aulak, K. S., Lachmann, P. J., Rosen, F. S., and Harrison, R. A. (1989) Complement Inflammation 6, 310 (Abst.)
5. Schapira, M., Ramus, M. A., Jallat, S., Carvallo, D., and Courtney, M. (1985) J. Clin. Invest. 76, 655–667
6. Zahedi, R., Bissler, J. J., Davis, A. E., III, Andreadis, C., and Wiesner, J. S. (1985) J. Clin. Invest. 95, 1299–1305
7. York, D. J., Li, P., and Cardell, S. J. (1991) J. Biol. Chem. 266, 8495–8500
8. Skriver, K., Wikoff, W. R., Patston, P. A., Tauxe, F., Schapira, M., Kaplan, A. P., and Bock, S. C. (1991) J. Biol. Chem. 266, 9216–9221
9. Siddique, Z. M., McPhaden, A. R., and Whaley, K. (1992) Hum. Hered. 2, 231–234
10. Devraj-Kizuk, R., Chui, D. H., Prochownik, E. V., Carter, C. J., Ofosu, F. A., and Blajchman, M. A. (1988) Blood 72, 1518–1523
11. Davis, A. E., III, Pemberton, R. E., Stecklein, H. P., Eldering, E., Hack, C. E., Kramer, J., Strunk, R. C., Bissler, J. J., and Rosen, F. S. (1992) Nat. Genet. 1, 354–358
12. Aulak, K. S., Eldering, E., Hack, C. E., Lachmann, P. J., and Hack, C. E. (1988) FEBS Lett. 254, 174–176
13. Davis, A. E., III, Pemberton, R. E., Stecklein, H. P., Eldering, E., Hack, C. E., Lachmann, P. J., and Hack, C. E. (1988) J. Biol. Chem. 263, 7013–7020
14. Stein, P. E., Tewkesbury, D. A., and Carrell, R. W. (1989) Biochem. J. 262, 103–107
15. de Agostini, A., Patston, P. A., Marotti, V., Carrell, S., Marpel, P. C., and Schapira, M. (1988) J. Clin. Invest. 82, 700–705
16. Aulak, K. S., Pemberton, P. A., Roman, M. A., Jallat, S., Carvallo, D., and Courtney, M. (1985) J. Clin. Invest. 76, 655–667
17. Eldering, E., Huijbregts, C. C. M., Lachmann, P. J., and Hack, C. E. (1988) Blood 72, 1841–1848
18. de Smet, B. J. G. L., de Boer, J. P., Agterberg, J., Rigter, G., Bleeker, W. K., and Hack, C. E. (1993) J. Clin. Invest. 81, 56–61
19. Nuijens, J. H., Huijbregts, C. C. M., Eerenberg-Belger, A. J. M., Abbinck, J. J., Strack van Schijndel, R. J. M., Felt-Bersma, R. J. F., Thijs, L. G., and Hack, C. E. (1988) J. Biol. Chem. 263, 193–198
20. Pemberton, P. A., Harrison, R. A., Lachmann, P. J., and Carrell, R. W. (1989) J. Biol. Chem. 264, 6145–6142
21. Pemberton, P. A., Harrison, R. A., Lachmann, P. J., and Carrell, R. W. (1995) J. Biol. Chem. 270, 2579–2587
22. Lewis, M. F., Harpel, P. C., and Kaplan, A. P. (1983) J. Biol. Chem. 258, 2265–2274
23. de Smet, B. J. G. L., de Boer, J. P., Agterberg, J., Rigter, G., Bleeker, W. K., and Hack, C. E. (1993) Blood 81, 56–61
24. Malek, R., Aulak, K. S., and Davis, A. E., III (1996) Clin. Exp. Immunol. 105, 191–197
25. Mattoon, J., Strand, A., Szymersky, J., Sweet, R. M., Danley, D. E., Georghegan, K. F., Gerard, R. D., and Goldsmith, E. J. (1999) Nature 355, 270–273
