The C3b/C4b Receptor Is Recognized by the Knops, McCoy, Swain-Langley, and York Blood Group Antisera

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Summary

Erythrocytes (E) lacking high incidence blood group antigens were screened by an antiglobulin test with a monoclonal antibody to human complement receptor type 1 (CR1; C3b/C4b receptor; CD35). Some examples of E lacking Knops, McCoy, Swain-Langley, and York antigens, a serologically related group, were not agglutinated. Moreover, E of the null phenotype for these same antigens were nonreactive. To further explore this relationship, E expressing these antigens were surface labeled, solubilized, and incubated with the corresponding blood group–specific antisera. CR1 was immunoprecipitated, indicating that the epitopes recognized by each of these antisera are expressed on CR1.

E of two individuals, putative null phenotypes for the Knops, McCoy, and Swain-Langley blood group antigens, expressed a very low number of CR1 (<30/E; ~10% of the normal mean). This observation accounts for their lack of reactivity in the antiglobulin test and their prior designation as null phenotypes. Also, the previously reported low as well as variable expression of CR1 on E explains prior difficulties in the serologic analyses of these blood group antigens.

Complement receptor type 1 (CR1; C3b/C4b receptor; CD35) is expressed on human erythrocytes (E)[1] and leukocytes (1, 2). Immune adherence, the attachment of C3b/C4b–bearing immune complexes to primate E or nonprimate platelets, is mediated by this receptor as part of its role in the metabolism of immune complexes (3). In 1980, Fearon (4) purified CR1, and subsequent studies by Wong et al. (5) and Dykman et al. (6–8) led to the discovery of an unusual size polymorphism of this receptor. Four codominantly inherited allelic size variants with M_r of 190,000, 220,000, 250,000 and 280,000 were identified (9). Subsequent biochemical and molecular characterization of the variants suggested that a highly homologous repeating unit was duplicated or deleted (reviewed in references 1 and 2). Further, these investigations pointed out that the structural gene for CR1 was not linked to HLA (10), as had been initially proposed (11, 12), and also led to the discovery of a new linkage group at 1q32 of complement regulatory and receptor proteins (13, 14).

In 1975, Rothman et al. (3) analyzed E of individuals lacking common blood group antigens by immune adherence. One individual was immune adherence negative and had a rare blood group phenotype, Rh null; however, other individuals of this phenotype were immune adherence positive. In the early 1980s, two of us (J. P. Atkinson, J. J. Moulds) unsuccessfully attempted to find a blood group system with allelic frequencies that paralleled those of the CR1 polymorphic size variants. In the present studies, screening of E negative for high frequency blood group antigens with a mAb to CR1 led to the identification of CR1 as a blood group antigen and multiple new alleles that encode protein polymorphisms of CR1.

Materials and Methods

Antisera. The following antibodies to CR1 were used: a rabbit polyclonal antiserum, provided by E. Medof (Case Western Reserve University, Cleveland, OH), and mouse mAbs E11, provided by N. Hogg (ICRF Laboratories, London, England), and 3D9, provided by E. Brown (Washington University School of Medicine, St. Louis, MO). Blood group antisera, stimulated by transfusion or pregnancy, were obtained from patient samples referred to the consultation laboratory of Gamma Biologicals Inc., Houston, TX. The antisera designated "Kn/McC" belong to a heterogeneous group related to Knops and McCoy due to their lack of reactivity with the null phenotype MH.

Serological Testing. The anti-CR1 mAbs were tested against E lacking multiple antigens in a known blood group system or those

[1] *Abbreviation used in this paper:* E, erythrocytes.

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that lacked a single antigen of high frequency. Rare E phenotypes obtained through the "Serum, Cells, and Rare Fluid (SCARF) Exchange" were stored in liquid nitrogen until tested. The mAbs were diluted 1:500 in PBS containing 8% BSA for use in a mouse antiglobulin technique (15).

Fresh or frozen E from selected individuals were serologically phenotyped for the "Knops-McCoy" ("Kn/McC"), Knops (Kn), McCoy (McC), Swain-Langley (Sl), York (Yk), and Cost (Cs) antigens. Saline-suspended E were incubated with the appropriate antisera for 1 h at 37°C followed by a standard antiglobulin procedure (15).

Immunoprecipitation and Affinity Chromatography. Immunoprecipitation, affinity chromatography, and SDS-PAGE followed by autoradiography were performed as described (6). E were surface labeled with ^{125}I by a modified lactoperoxidase-glucose oxidase method (6).

ELISA. mAbs were purified by ammonium sulfate precipitation. Microtiter wells (MaxiSorp; Nunc, Roskilde, Denmark) were coated with 2.5 μg/ml of 3D9, the capture mAb. Horseradish peroxidase (Boehringer Mannheim Biochemicals, Indianapolis, IN)–conjugated E11, the detection mAb, was prepared by a modification of the procedure of Nakane and Kawaoi (16). After a 2-h incubation at 37°C with the conjugated E11 (10 ng/ml), color was developed with *o*-phenylenediamine and the absorbance was read at 490 nm in a microplate reader (Dynatech, Chantilly, VA). Samples were referenced relative to a standardized preparation of E ghosts of known CR1 number (9) and to purified CR1 (17).

Results

Identification by Hemagglutination of Blood Group Antigens Related to CR1. E negative for high frequency blood group antigens were tested by agglutination with a mAb to CR1 (Table 1). Initially, one example each of Rh null, Jk-3, i adult, Lan-, "Kn/Mc"-, Sl^a-, Yk^a-, and Yk^a-/Cs^a- was nonreactive. A second example of Rh null, Jk-3, i adult, and Lan- was reactive. These blood groups were not investigated further since they had no apparent relationship to one another and because biochemical data indicate that they could not be CR1 (18). However, additional examples of E negative for the related antigens "Kn/McC", Sl^a, and Yk^a gave variable reactions, including negative ones. Moreover, E from MH, the null phenotype for "Kn/McC", Kn^a, McC^a, and Sl^a, were not agglutinated. Because of these results and the known serologic relationship between Kn^a, McC^a, and Yk^a (19), E expressing these antigens were investigated further by immunoprecipitation.

Knops, McCoy, Swain-Langley, and York Blood Group–specific Antisera Immunoprecipitate CR1. Antisera that reacted with the "Kn/McC", Kn^a, McC^a, Sl^a, or Yk^a blood group antigens were incubated with ^{125}I-surface-labeled, solubilized E. Multiple examples of each of these antisera precipitated a 220-kD protein that aligned with the common polymorphic size variant of CR1 (Fig. 1 *A*). In most experiments CR1 was the only labeled band identified and, in those cases where another band was noted, it was also present in control (normal serum) lanes.

Further evidence that these blood group antigens are present on CR1 included the following: (*a*) the protein precipitated by these antisera had an M_r of 190,000 under nonreducing conditions (Fig. 1 *B*) and again aligned with CR1; (*b*) the antisera recognized the two common polymorphic size variants of CR1 (Fig. 1 *A*, lanes *9–11*); (*c*) a protein corresponding to CR1 was not precipitated by these antisera if solubilized E preparations were absorbed with mAbs to CR1 (Fig. 2); and (*d*) sera of normal individuals (Fig. 1 *A*) and antisera to other blood group antigens (data not shown) did not precipitate CR1.

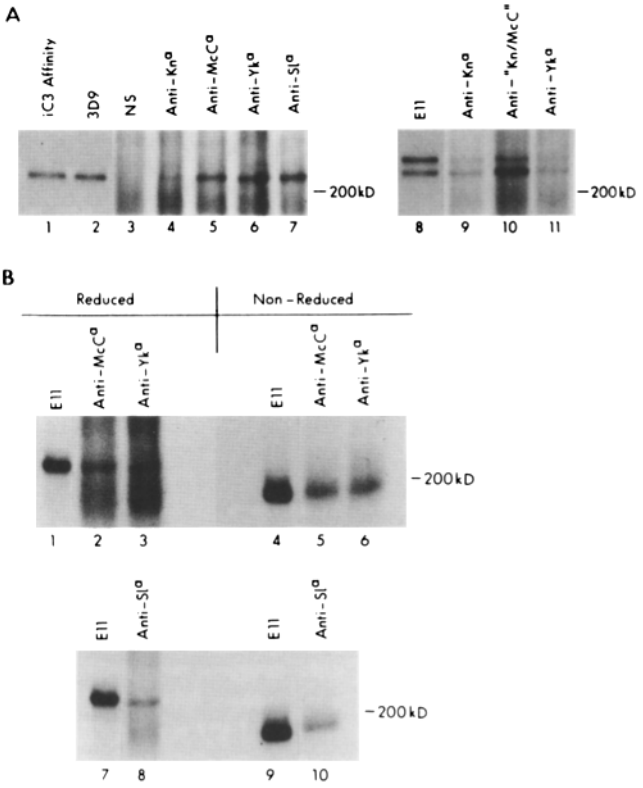

Figure 1. Blood group–specific antisera (Kn^a, McC^a, Yk^a, Sl^a, and "Kn/McC") immunoprecipitate CR1. (*A*) In this and subsequent Figures, ^{125}I-surfaced-labeled, detergent-solubilized E preparations are the starting material. E of a homozygous donor expressing the common CR1 phenotype were assessed by affinity chromatography with iC3-Sepharose (lane *1*) or by immunoprecipitation (lanes *2–7*). E of a heterozygous donor expressing the two most common polymorphic size variants of CR1 (lanes *8–11*) were similarly analyzed. For immunoprecipitation, a mAb to CR1, *3D9* (lane *2*) or E11 (lane *8*), and the blood group–specific antisera (lanes *4–7* and *9–11*) were used. A normal human serum was used as a control (lane *3*). The relatively weak reaction with the anti-Kn^a antisera (lane *4*) is likely due to its low titer (1:2 vs. 1:64 or greater for the others). These and subsequent autoradiographs are of 5% gels analyzed by SDS-PAGE (reducing conditions). (*B*) Comparison of the immunoprecipitated proteins under reducing and nonreducing conditions. Experimental conditions are as in *A*. Lanes *1–6* and *7–10* were analyzed on separate gels.

CR1 Is Present on E of Antibody Producers. To assess if the antibody producers represent a null phenotype or expressed an anomalously migrating species of CR1, labeled E of four antibody producers (Kn^a, McC^a, "Kn/Mc", and Sl^a) were analyzed by immunoprecipitation followed by SDS-PAGE and autoradiography. In each case, their CR1 had an identical M_r to the common polymorphic size variant (data not shown).

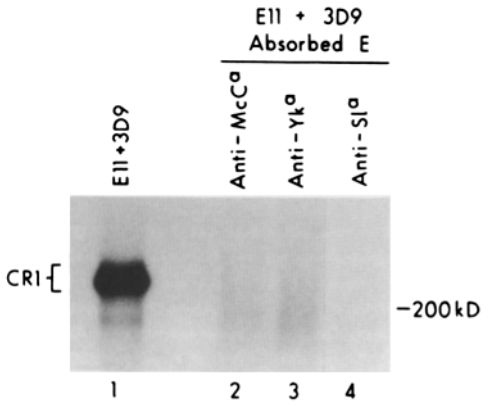

Figure 2. mAbs to CR1 absorb the antigen recognized by the blood group–specific antisera. CR1 was immunoprecipitated from an E preparation by a mixture of mAbs (E11 and 3D9) to CR1 (lane *1*). This E preparation was then incubated with the blood group–specific antisera (lanes *2–4*). A threefold longer exposure of this gel (10 d) did not demonstrate CR1 in lanes *2–4*. The same result was obtained with an antiserum to Kn^a (data not shown). These same antisera precipitated CR1 in this and other experiments (Fig. 1) from E preparations that had not been precleared of CR1.

The Null Phenotypes for the CR1-related Blood Group Antigens Express Low Copy Number. The E of MH are the null phenotype for the blood group antisera recognizing CR1. A second individual's E, LM, are the Rh null sample that was nonreactive with E11 (Table 1) and those previously described as immune adherence negative (11). LM's E typed identically to the rare MH phenotype.

MH's and LM's E were radiolabeled, solubilized, and then incubated with antibodies to CR1. CR1 was immunoprecipitated in both cases and aligned with the common polymorphic CR1 size variant. Their CR1 also bound iC3 (Fig. 3). Densitometric analysis of these autoradiographs suggested that E of MH and LM expressed a low number of CR1. An ELISA was used to more quantitatively assess this observation. The range of CR1/E for 25 unrelated healthy donors was 60–483 (mean, 300) while MH expressed 28 and LM 20 CR1/E. These results confirm the presence of CR1 on E of MH and LM and provide an explanation, i.e., low copy number, for the discrepancy between the negative results by agglutination (Table 1) but positive results by immunoprecipitation (Fig. 3).

To determine their correct phenotype, E of MH and LM were typed by immunoprecipitation with the same antisera used in the agglutination assay. CR1 of LM was precipitated by antisera to Kn^a, McC^a, Sl^a, and Yk^a. CR1 of MH was precipitated by antisera to McC^a and Yk^a, but this analysis was inconclusive using anti-Kn^a or anti-Sl^a.

Table 1. *Analysis of E Lacking High Incidence Antigens with an Agglutination Assay Using a Mouse mAb (E11) to CR1*

Null phenotype	Rx with E11*	High incidence Antigen	Rx with E11
Ko	+	i (adult) (2)	−/+
Oh	+	U-	+
Rh null (2)‡	−/+	En^a-	+
Tj^a-	+	Ge-1, -2, -3	+
Jk-^{3} (2)	−/+	k-	+
Leach	+	Kp^b-	+
Helgeson-frozen§	−	Js^b-	+
Helgeson-fresh§	−	Fy-^{3}	+
		Lan- (2)	−/+
		Yt^a-	+
		Vel-	+
		Co^a-	+
		Lu (a-b-)	+
		Au^b-	+
		Ok^a-	+
		Dr^a-	+
		At^a-	+
		Er^a-	+
		Rg- (C4AQO)	+
		Ch- (C4BQO)	+
		Hy^a-	+
		Gy^a-	+
		JMH- (acquired)	+
		JMH- (genetic)	+
		"Kn/McC"- (2)	−
		Sl^a- (5)	−/+
		Yk^a- (2)	−
		Cs^a- (2)	+
		Yk^a-/Cs^a-(3)	−/+

This table is divided into two parts. The "Null Phenotype" section refers to E that are negative for several antigens in a blood group system. For the remainder of the Table, E lacking a single high incidence antigen are shown.

* − = negative, + = positive. −/+ , indicates a group in which at least one example was negative.

‡ Numbers in parentheses represent the number of samples evaluated. The nonreactive Rh null sample is LM (see text).

§ Helgesen cells were stored in liquid nitrogen and thawed before testing. Fresh Helgesen cells were tested within 48 h of obtaining the blood sample.

Discussion

The goal of this study was to find a CR1-related blood group system. The availability of mAbs to CR1 provided a tool to screen, by an antiglobulin assay, E deficient in blood group antigens. Some examples of E, lacking a related group, "Kn/McC", Sl^a and Yk^a, were not agglutinated by a mAb to CR1. Further, E of the null phenotype (MH) for these antigens were also nonreactive. These serologic results prompted analysis of the alloantisera specific for these blood group antigens. These antisera specifically precipitated CR1; therefore, these blood group antigens are expressed on CR1.

Antisera to these CR1-related blood group antigens have

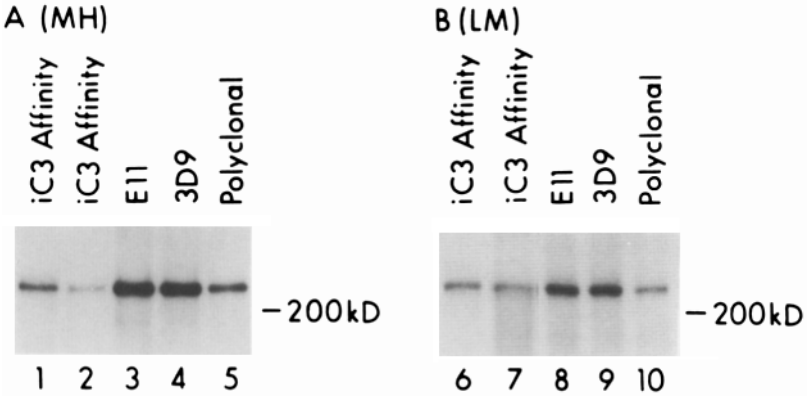

Figure 3. E of two individuals (MH and LM) with the putative null phenotype for the Kn, McC, and Sl blood group antigens express CR1. CR1 was purified from E preparations of these two individuals by iC3 affinity chromatography (lanes *2* and *7*) or immunoprecipitation (lanes *3–5* and *8–10*). In parallel, CR1 of E preparations of a control individual was purified (lanes *1* and *6*).

traditionally been placed in a collection referred to as high-titer, low-avidity (HTLA) antibodies (19). They are characterized by their weak reactivity and wide variation in strength of their agglutination reactions. The serological data indicate that "Kn/McC", Kn^a, McC^a, Sl^a, and Yk^a define distinct but related blood group antigens (18, 20–22). Thus, the prior serological data and the present investigation have led to the identification of many alleles that encode new protein polymorphisms of CR1.

Individuals lacking all Kn, McC, and Sl antigens by standard agglutination assays are said to express the "Helgeson phenotype". MH was the first such example with this pattern of reactivity (21). Our results indicate that this lack of reactivity in the agglutination assay for MH and another individual of the same phenotype (LM) is due to a low number of CR1/E (<30). This explains why such E are nonreactive in antiglobulin assays and their prior designation as a null phenotype.

A characteristic of HTLA antibodies is a wide variation in the strength of their agglutination reactions with E from different individuals. This serologic observation is accounted for by what is known about the expression of CR1 on E. Thus, the number of CR1 molecules/E vary from individual to individual by >10-fold (23). This variation of E/CR1 is regulated in part in a Mendelian fashion and is associated with a HindIII RFLP (23). The presence (L or low allele; gene frequency of 0.24) or absence (H or high allele; gene frequency of 0.76) of a HindIII site in an intron in the COOH-coding half of the gene (24) correlates with CR1/E. This means that ~6% of the population are homozygous for expression of a low CR1 copy number/E. The relative low expression of CR1/E, as compared with other proteins bearing blood group antigens, as well as the variability in expression between individuals, undoubtedly accounts for the difficulties experienced by serologists using agglutination assays. It also explains the variable reactions we observed in the analysis of E with a mAb to CR1 by agglutination (Table 1). For example, some Sl^a-, Yk^a-, or Cs^a- E samples were reactive and others were nonreactive with a mAb to CR1. When these E were tested with mAbs and the human blood group antisera specific for CR1, a pattern of reactivity was observed that was correlated with the number of CR1/E (our unpublished observations). Thus, E with <100–150 CR1/E were negative with all antibodies, and those with >300 CR/E were positive with all antisera.

Prior serological studies have indicated a relationship between Yk^a and Cs^a. However, while subsequent analyses indicated Yk^a was a CR1-related blood group antigen, two lines of evidence suggested that this was not the case for Cs^a. First, three Cs^a-specific antisera did not precipitate CR1. Second, Cs^a was readily detected on E of donors with a low number of CR1/E, including one of the null phenotypes (LM) (our unpublished data). Consequently, the serologic association of Yk^a and Cs^a is not accounted for by our data.

While this work was in progress, an abstract by Rao et al. (25) reported an association between CR1 and the Knops-McCoy group. In contrast to their conclusion that E of the null phenotype (Helgeson) expressed no or a highly abnormal form of CR1, we found that this phenotype is characterized by expression of a low number of CR1, which has normal molecular weight and binds iC3. In addition, our results indicate that Yk^a is also expressed on CR1.

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References

1. Ahearn, J.J., and D.T. Fearon. 1989. Structure and function of the complement receptors, CR1 (CD35) and CR2 (CD21). *Adv. Immunol.* 46:183.

2. Hourcade, D., V.M. Holers and J.P. Atkinson. 1989. The regulators of complement activation (RCA) gene cluster. *Adv. Immunol.* 45:381.

3. Rothman, I.K., J.A. Gelfand, A.S. Fauci, and M.M. Frank. 1975. The immune adherence receptor: dissociation between the expression of erythrocyte and mononuclear cell C3b receptors. *J. Immunol.* 115:1312.
4. Fearon, D.T. 1980. Identification of the membrane glycoprotein that is the C3b receptor of the human erythrocyte, polymorphonuclear leukocyte, B lymphocyte, and monocyte. *J. Exp. Med.* 152:20.
5. Wong, W.W., J.C. Wilson, and D.T Fearon. 1983. Genetic regulation of a structural polymorphism of human C3b receptor. *J. Clin. Invest.* 72:685.
6. Dykman, T.R., J.L. Cole, K. Iida, and J.P. Atkinson. 1983. Polymorphism of the human erythrocyte C3b/C4b receptor. *Proc. Natl. Acad. Sci. USA.* 80:1698.
7. Dykman, T.R., J.A. Hatch, and J.P. Atkinson. 1984. Polymorphism of the human C3b/C4b receptor: Identification of a third allele and analysis of receptor phenotypes in families and patients with systemic lupus erythematosus. *J. Exp. Med.* 159:691.
8. Dykman, T.R., J.A. Hatch, M.S. Aqua, and J.P. Atkinson. 1985. Polymorphism of the C3b/C4b receptor: Identification of a rare variant. *J. Immunol.* 134:1787.
9. Van Dyne, S., V.M. Holers, D.M. Lublin, and J.P. Atkinson. 1987. The polymorphism of the C3b/C4b receptor in the normal population and in patients with systemic lupus erythematosus. *Clin. Exp. Immunol.* 68:570.
10. Hatch, J.A., J.P. Atkinson, B.K. Suarez, and T.R. Dykman. 1984. Evaluation of linkage of the human C3b/C4b receptor to HLA. *J. Immunol.* 132:2168.
11. Curry, R.A., M.P. Dierich, M.A. Pellegrino, and J.A. Hock. 1976. Evidence for linkage between HLA antigens and receptors for complement components C3b and C3d in human-mouse hybrids. *Immunogenetics.* 3:465.
12. Gelfand, M.C., D.H. Sachs, R. Lieberman, and W.E. Paul. 1974. Ontogeny of B lymphocytes. III. H-2 linkage of a gene controlling the rate of appearance of complement receptor lymphocytes. J. Exp. Med. 139:1142.
13. Rodriguez de Cordoba, S., T.R. Dykman, F. Ginsberg-Fellner, G. Ercilla, M. Aqua, J.P. Atkinson, and P. Rubinstein. 1984. Evidence for linkage between the loci coding for the binding protein for the fourth component of human complement (C4BP) and for the C3b/C4b receptor. *Proc. Natl. Acad. Sci. USA.* 81:7890.
14. Rodriguez de Cordoba, S., D.W. Lublin, P. Rubinstein, and J.P. Atkinson. 1985. The human genes for three complement components that regulate the activation of C3 are tightly linked. *J. Exp. Med.* 161:1189.
15. Walker, R.H., editor. 1990. Technical Manual. American Association of Blood Banks, Arlington, VA. 150–155.
16. Nakane, P.K., and A. Kawaoi. 1974. Peroxidase-labeled antibody, a new method of conjugation. *J. Histochem. Cytochem.* 22:1084.
17. Holers, V.M., T. Seya, E. Brown, J.J. O'Shea, and J.P. Atkinson. 1986. Structural and functional studies on the human C3b/C4b receptor (CR1) purified by affinity chromatography using a monoclonal antibody. *Complement.* 3:63.
18. Issitt, P.D. 1985. Applied Blood Group Serology, 3rd ed. Montgomery Scientific Publications, Miami, FL. 429–433.
19. Vengelen-Tyler, V. 1979. Nonneutralizable HTLA antibodies and the antigens they define. *In* Recognition and Resolution of High-titer, Low-avidity Antibodies. S.D. Rolih, editor. American Association of Blood Banks, Washington, D.C. 21–32.
20. Lacey, P., B. Laird-Fryer, U. Block, J. Lair, L. Guilbeau, and J.J. Moulds. 1980. A new high incidence blood group factor, Sl^a, and its hypothetical allele. *Transfusion (Arlingt.).* 20:632 (Abstr.)
21. Helgeson, M., J. Swanson, and H.F. Polesky. 1970. Knops-Helgeson (Kn^a), a high-frequency erythrocyte antigen. *Transfusion (Arlingt.).* 10:738.
22. Moulds, M.K. 1981. Serological investigation and clinical significance of high-titer, low-avidity (HTLA) antibodies. *Am. J. Med. Tech.* 47:789.
23. Wilson, J.G., E.E. Murphy, W.W. Wong, L.B. Klickstein, J.H. Weiss, and D.T. Fearon. 1986. Identification of a restriction fragment length polymorphism by a CR1 cDNA that correlates with the number of CR1 on erythrocytes. *J. Exp. Med.* 164:50.
24. Wong, W.W., J.M. Cahill, M.D. Rosen, C. Kennedy, E.T. Bonaccio, M.J. Morris, J.G. Wilson, L.B. Klickstein, and D.T. Fearon. 1989. Structure of the human CR1 gene: molecular basis of the structural and quantitative polymorphisms and identification of a new CR1-like allele. *J. Exp. Med.* 169:847.
25. Rao, N.C., D.J. Ferguson, S.F. Lee, and M.J. Telen. 1990. The relationship between the complement receptor type 1 and the Knops/McCoy antigen system. *Clin. Res.* 38:303*a* (Abstr).