Kell Blood Group Antigens Are Part of a 93,000-Dalton Red Cell Membrane Protein*

(Received for publication, December 2, 1985)

Colvin M. Redman, Gabriella Avellino, Susan R. Pfeffer, Tarit K. Mukherjee, Margaret Nichols, Pablo Rubinstein, and W. Laurence Marsh

From The Lindsey F. Kimball Research Institute, The New York Blood Center, New York, New York 10021

Monospecific Kell blood group antibodies, of either human alloimmune or mouse monoclonal origin, react with a single surface-exposed protein of 93,000 daltons. Chymotryptic peptide maps of the 93,000-dalton protein isolated by antibodies of two different specificities (anti-K7 or anti-K14) indicate that Kell epitopes reside on the same protein. Kell protein is similar in size to band 3 protein but differs markedly in its tryptic and chymotryptic peptide maps, indicating that they are different proteins. In addition, sheep antibody to human band 3 does not react with Kell protein. Rabbit antibody to Kell protein reacts, by Western immunoblotting, with membrane proteins from Kell antigen positive red blood cells but not from those of a K0 (Kell null) cell. In intact red cells only a small portion of the Kell protein is available to lactoperoxidase-catalyzed iodination. Under nonreducing conditions Kell antigen is isolated not only as a 93,000-dalton protein but also as larger protein complexes ranging in size from above 200,000 to 115,000 daltons. Treatment of red cells with iodoacetamide, prior to isolation of Kell protein, reduces the amount of the very large complexes, but Kell protein occurs both as 115,000- and 93,000-dalton proteins.

The Kell blood group is one of the major antigenic systems in human red cells. It was first identified in 1946 (1) and 23 alloantigens have subsequently been determined to be part of, or related to, the system (2). It is clinically important, for incompatibility involving Kell blood group antigens can cause severe hemolytic reactions to blood transfusions and erythroblastosis in newborn infants. In addition, one of the variant Kell system phenotypes, named McLeod, which is characterized by a marked weakening of all Kell antigens and the absence of a universal antigen (Kx), has grossly abnormal morphology and reduced in vivo survival (3, 4). Kell antigens are present in only small amounts, about 5000 antigen sites per red cell (5, 6). Red cells of rare individuals expressing a null phenotype, called K0 (Kell null) cell. In intact red cells only a small portion of the Kell protein is available to lactoperoxidase-catalyzed iodination. Under nonreducing conditions Kell antigen is isolated not only as a 93,000-dalton protein but also as larger protein complexes ranging in size from above 200,000 to 115,000 daltons. Treatment of red cells with iodoacetamide, prior to isolation of Kell protein, reduces the amount of the very large complexes, but Kell protein occurs both as 115,000- and 93,000-dalton proteins.

* This work was partially supported by Grants HL 09011 and HL 33841 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 1754 solely to indicate this fact.

EXPERIMENTAL PROCEDURES

Iodination of Surface Proteins and Isolation of Kell-related Proteins—The separation procedures are adapted from those described by Moore et al. (12). Surface tyrosine residues on antigen-positive and antigen-negative erythrocytes were labeled with 125I using a modification of the procedure of Reichstein and Blostein (13). Thrice-washed packed cells (1 ml) were preincubated for 90 min at 37 °C with 20 volumes of 4 mM sodium phosphate, pH 7.4, 134 mM NaCl, 10 mM KCl, 5 mM α-D-glucose. Cells were then washed (3 × 10 volumes) at 4 °C with PBS (2 mM sodium phosphate, 0.15 M NaCl, pH 7.4). Packed cells (1 ml) were incubated for 15 min at 37 °C with the following iodination mixture: 0.7 ml of PBS, 13 μl of 2.5 mg/ml glucose oxidase, and 15 μl of 20 mg/ml 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid). The iodinated cells were washed (4 × 10 volumes) in PBS at 4 °C. Packed iodinated red cells of positive and negative Kell phenotype were incubated for 60 min at 37 °C and overnight at 4 °C with Kell system antibodies. A control test containing inert human serum, or PBS, and positive phenotype iodinated red cells was set up in parallel. Following incubation, the supernatant serum was removed and cells were again washed (4 × 10 volumes) in PBS.

Cell membranes were prepared from washed, antibody-sensitized cells by hypotonic lysis in 5 mM sodium phosphate, pH 7.4, buffer (50 mM and 1% fetal calf serum) and centrifuged at 20,000 × g for 20 min at 4 °C. The membranes were washed at 4 °C (2 × 5 volumes) in the same buffer and solubilized by mixing for 15 min at ambient temperature with 10 volumes of PBS containing 1% Triton X-100 and 0.5% sodium deoxycholate. Insoluble material was removed by centrifugation at 20,000 × g for 20 min and the supernatant fraction used in subsequent procedures.

Immune complexes in the detergent-soluble membrane fraction were isolated by incubation, with constant mixing for 90 min at room temperature and overnight at 4 °C, with protein A-Sepharose. For cells exposed to the murine monoclonal antibody, anti-K14, a donkey anti-mouse antibody coated on cellulose beads or a rabbit anti-mouse IgG coupled to Sepharose 4B replaced protein A-Sepharose. The beads were washed with the buffer Triton X-100 and deoxycholate solution (4 × 10 volumes) and once in distilled water. Immune complexes were eluted from the protein A-Sepharose or cellulose beads with 8 M urea and 1% sodium dodecyl sulfate (SDS) in 0.47 M Tris buffer, pH 6.7. Some samples were reduced with 0.26 M 2-mercaptoethanol. The mixture was heated in boiling water for 3 min and the proteins separated by SDS-polyacrylamide gel electrophoresis using the buffer system described by Maizel (14). The proteins were detected by autoradiography or by staining with Coomassie Blue.

**Antibodies—**Monospecific, human alloimmune anti-K1, anti-K2,

*The abbreviations used are: PBS, phosphate-buffered saline; SDS, sodium dodecyl sulfate; TPCK, L-1-tosyl-amido-2-phenylethyl chloromethyl ketone; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.
anti-K7, anti-K22, and anti-K23 all having strong IgG components were used. Anti-K14 is a mouse monoclonal antibody raised by injection of human red cells into BALB/c mice and fusion with cells of the NS-1 myeloma (15). Sheep antibody to a 43,000-dalton fragment of human band 3 was a gift from Drs. J. Letsinger and V. Marchesi of Yale University.

A specific antibody to Kell protein was prepared by isolating the 93,000-dalton Kell protein, as described above, excising the pertinent area from the polyacrylamide gel, homogenizing it in complete Freund’s adjuvant, and injecting rabbits, subcutaneously, with the mixture. At weekly intervals for 3 weeks and then every subsequent month for up to 6 months, the rabbits were injected with similar material prepared in incomplete Freund’s adjuvant.

Radiolabeling of Proteins on SDS-Polyacrylamide Gel Slices—The procedure of Elder et al. (16) was followed with a few modifications. In brief, the proteins were stained with 0.05% Coomassie Blue in 25% isopropl alcohol and 10% acetic acid and then destained in 25% isopropl alcohol and in 10% acetic acid. The protein bands were excised, placed in siliconized tubes, and extensively washed with 25% isopropl alcohol and 10% methanol to remove SDS. The dried gel slice was then radiolabeled with 125I using the chloramine-T method (17). The gel slices were again extensively washed and dialyzed against 10% methanol, dried, and used for peptide mapping.

Peptide Mapping—One ml of trypsin-TPCK (50 µg/ml) or α-chymotrypsin (50 µg/ml) in 50 mM NH4HCO3 buffer, pH 8.0, was added to the dry slices. After 24 h of incubation at 37 °C, the digests were decanted and lyophilized. The digests were dissolved in 20 µl of electrophoresis buffer (acetic acid/formic acid/H2O; 15:5:80, v/v), and approximately 100 cpm/dalton of radioactive protein was spotted on 5 x 10-cm cellulose-coated TLC plates. The proteins were electrophoresed at 300 V at 0 °C for approximately 1 h (or until a spot of basic fuchsin dye migrated 9.5 cm from the origin). After drying, the plates were run in the second dimension (TLC) in the following buffer system: butanol/pyridine/acetic acid/H2O (32:55:25:20, v/v). The plates were dried and the separated peptides detected by autoradiography. This procedure is essentially that described by Elder et al. (16) as modified by Markowitz and Marchesi (18).

Phosphorylation—Washed red cells (5 ml packed volume) were incubated with gentle shaking in 60 µl of 120 mM NaCl, 5 mM KCl, 1 mM MgCl2, 10 mM dextrose, 20 mM NaHCO3, 5 mM Hepes, pH 7.4, 1 mg/ml bovine serum albumin, 0.1 mg/ml kanamycin, 10 units/ml penicillin, and 1.1 mCi [32P]Pi for 21 h at 37 °C (20).

Western Blots—Western immunoblots were performed by the method of Burnette (21) using 125I-protein A as a second reagent. The radioactive areas were detected by autoradiography.

Materials—Trypsin-TPCK, α-chymotrypsin, and lactoperoxidase were purchased from Sigma, β-glucose oxidase from Boehringer Mannheim, Na125I and 125I-protein-A from New England Nuclear, protein A-Sepharose-Cl 4B from Pharmacia, and nitrocellulose paper from Schleicher & Schuell. Anti-mouse IgG, coupled to Sepharose 4B, was purchased from Cooper Biomedical, Malvern, PA, and donkey anti-mouse IgG coated on cellulose beads (Sac-cell) from Wellcome Research Laboratories, Beckenham, Kent, England. Thin layer chromatography plates, (10 x 10-cm) precoated with cellulose (layer thickness 0.1 mm), without fluorescent indicator, were purchased from E. M. Laboratories, Elmsford, N.Y.

RESULTS

Immunoprecipitation of 93,000-Dalton Protein with Antibodies of Different Kell Specificities—Two sets of red cells, K7,14 and Ko (null), were surface-labeled with 125I and treated either with human alloimmune antibody to K7 or with a mouse monoclonal antibody to K14. The isolated radioactive immune complexes were reduced with mercaptoethanol, separated by SDS-polyacrylamide gel electrophoresis, and detected by autoradiography. Both the K7,14 and Ko cells were surface-labeled to approximately the same extent, with major incorporation of radioactivity into three proteins, two of which migrate closely together, as wide bands, at the 85,000-95,000-dalton range (Fig. 1, lanes 1 and 2). These proteins have been reported to be band 3 and the glycoporphins (13, 22). Treatment of Ko cells with either human alloimmune antibody to K7 or mouse monoclonal antibody to K14 did not immunoprecipitate radioactive proteins (Fig. 1, lanes 3 and 4). However, treatment of K7,14 cells, with either of these Kell antibodies, yielded a single radioactive protein of about 93,000 daltons (Fig. 1, lanes 5 and 6). About 0.05-0.1% of the surface-labeled protein radioactivity was immunoprecipitated, as determined by measuring the radioactivities in total trichloroacetic acid precipitable proteins and in the immunoprecipitates.

Chymotryptic Maps of 93,000-Dalton Proteins Isolated with Antibodies to K7 and K14—A previous study suggested that different Kell antibodies (K7 and K22) reacted with the same 93,000-dalton protein since a mixture of these antibodies yielded a single surface-labeled protein, and the radioactivity of the proteins isolated by the mixture was not additive (11). If K7 and K22 were markers on different proteins, each antibody would separate its own protein, and the radioactivity of the proteins isolated would be additive. To further compare the 93,000-dalton proteins immunoprecipitated by different Kell antibodies, the proteins isolated by a human alloimmune antibody (anti-K7) and a mouse monoclonal antibody (anti-K14) were analyzed by peptide mapping.

 Autoradiography of the peptide maps showed similar patterns for both proteins, indicating that both the human alloimmune antibody to K7 and the mouse monoclonal antibody to K14 immunoprecipitate the same protein (Fig. 2).
Fig. 2. Chymotryptic peptide maps of 93,000-dalton proteins immunoprecipitated with antibodies of K7 and K14. Kell protein (93,000 daltons) was isolated from K7,14 red cells by immunoprecipitation with either a human alloimmune antibody to K7 or a mouse monoclonal antibody to K14 and by SDS-polyacrylamide gel electrophoresis as described in Fig. 1. The protein was iodinated with 125I by the chloramine-T method, digested with chymotrypsin, and peptide mapping performed as described under “Experimental Procedures.” The panel on the left shows an autoradiogram of the radioactive chymotryptic peptides of the Kell protein isolated with anti-K7 and that on the right of that isolated with anti-K14.

Band 3 was separated from other red cell membrane proteins by SDS-polyacrylamide gel electrophoresis. Although other proteins coelectrophoresed in the band 3 region (23, 24), two-dimensional isoelectric focusing and SDS-polyacrylamide gel electrophoresis indicates that band 3 is the predominant component (25). The appropriate portions of the gel were excised and then proteins were labeled in situ with 125I by the chloramine-T method, digested with trypsin or chymotrypsin, and autoradiograms of the peptide maps compared.

The chymotryptic and tryptic peptide maps of Kell protein were markedly different from those obtained from band 3 protein (Fig. 3). Treatment of band 3 with either trypsin or chymotrypsin produced more radioactive peptides than when Kell protein was treated with these enzymes. In addition there was little similarity, with the possible exception of some tryptic peptides at the positive end of the plate, between the peptides obtained from band 3 and those from the Kell protein (Fig. 3). This indicates that, although Kell protein has a similar size to band 3, it is a different protein.

Tryptic Maps of Surface-labeled Kell Protein and Band 3—To compare the surface-exposed fragments of band 3 and Kell protein, intact red cells were surface-labeled with 125I, and Kell protein and band 3 were isolated as described above. Both proteins were excised from the gels, digested with trypsin, and the autoradiograms of the peptide maps compared. Kell protein only yielded two radioactive peptides, which migrated toward the cathode, while band 3 gave several radioactive peptides. The two radioactive peptides produced by trypsin digestion of surface-labeled Kell protein migrated differently than those obtained from band 3 (Fig. 4).

Reaction of Kell Antibodies by Immunoblotting with Membrane Proteins from Kell Positive and Kell Null Red Cells—Total membrane proteins from Kell antigen positive red cells and from Ko cells were separated by SDS-polyacrylamide gel electrophoresis, transferred to nitrocellulose paper and probed by immunoblotting using various Kell antibodies. The proteins from both cell samples gave similar SDS-polyacrylamide gel patterns when stained with Coomassie Blue (Fig. 5, lanes 1 and 2).

None of the human alloimmune antibodies (anti-K1, anti-K7 and anti-K22) nor the mouse monoclonal antibody (anti-K14) reacted with the separated red cell membrane proteins when tested by Western blotting. These antibodies, which react with the Kell proteins of intact cells, also did not react when the Kell protein was isolated either by reducing or...
nonreducing conditions, separated by SDS-polyacrylamide gel electrophoresis, and transferred to nitrocellulose paper.

However, rabbit antibody to the 93,000-dalton Kell protein gave a positive reaction both with the isolated Kell proteins (not shown) and with a 93,000-dalton protein from Kell antigen positive red cells (Fig. 5, lane 4) but not from Ko red cells (Fig. 5, lane 3). Iodinated Protein-A was used as the second reagent to localize immune complexes on the nitrocellulose paper, and some nonspecific radioactive bands appeared at the high molecular mass region (200,000 daltons) and in the location of the heavy chain of IgG (at 52,000 daltons). These radioactive areas were present when either Kell positive or Ko red cell membrane proteins were analyzed (Fig. 5, lanes 3 and 4).

Antibody to Band 3 Does Not React with Isolated Kell Protein—Further evidence that the 93,000-dalton Kell protein is distinct from band 3 protein was obtained by Western immunoblot analysis of isolated Kell protein with a sheep antibody to a 43,000-dalton fragment of human band 3. This antibody reacted with band 3 from red cell membrane proteins separated by SDS-polyacrylamide gel electrophoresis but did not react with Kell protein which had been isolated by immunoprecipitation with anti-K7 and SDS-polyacrylamide gel electrophoresis.

Phosphorylation of Membrane Proteins—To determine whether Kell protein is phosphorylated, red cells were incubated for 21 h with H\textsubscript{3}\textsuperscript{[32P]}PO\textsubscript{4}, and Kell protein was isolated by immunoprecipitation with anti-K7 and SDS-polyacrylamide gel electrophoresis. Under these conditions marked phosphorylation of band 3 and of the β band of spectrin are noted (20, 26). Coomassie Blue-stained Kell protein, immunoprecipitated from 0.6 ml of packed red cells which contained 1.2 × 10\textsuperscript{9} cpm, was not radioactive, indicating that in contrast to band 3, the Kell protein is not phosphorylated when intact red cells are incubated with H\textsubscript{3}\textsuperscript{[32P]}PO\textsubscript{4}.

Association of Kell Proteins with Other Red Cell Membrane Components—A previous study showed that if surface-labeled Kell protein is isolated by immunoprecipitation and SDS-polyacrylamide gel electrophoresis in nonreduced conditions, several radioactive areas are obtained (11). Some of the radioactive proteins do not enter the 7.5% polyacrylamide gel and others migrate as broad radioactive areas slightly above and below the 200,000-dalton protein standard, at 115,000 daltons and at 85,000–90,000 daltons (Fig. 6, lane 4). These radioactive fractions all contain the 93,000-dalton Kell protein since only a single radioactive 93,000-dalton protein is obtained when the sample is reduced prior to electrophoresis (Fig. 6, lane 1). The higher molecular mass radioactive species are probably not oligomers of the 93,000-dalton protein since their molecular sizes are not multiples of 93,000. These data suggest that Kell protein is in association, by disulfide linkages, with other red cell membrane components which are not accessible to lactoperoxidase catalyzed iodination. The association of Kell and other proteins may occur during the isolation procedure. To minimize disulfide interactions during isolation of Kell protein, red cells were treated with 0.1 M iodoacetamide prior to treatment with the antibodies. Red cells treated with iodoacetamide were still capable of reacting with antibodies to Kell reactive groups (anti-K7), and under reducing conditions a single 93,000-dalton radioactive protein was obtained (Fig. 6, lanes 1 and 2). Treatment of red cells with iodoacetamide changed the patterns of radioactive proteins obtained under nonreducing conditions. Less of the very high molecular mass radioactive proteins were obtained, and there was a relative increase in protein radioactivity at 115,000 daltons (Fig. 6, lane 5). This indicates that Kell protein does interact with other membrane protein components during isolation but that blocking of free sulfhydryl groups with iodoacetamide does not completely abolish the isolation of proteins larger than 93,000 daltons, since a 115,000-dalton

Kell Blood Group Protein

FIG. 5. Western immunoblots of Kell protein from Kell positive and Ko red cells. Membrane proteins from Kell positive and Ko red cells were separated on 7.5% SDS-polyacrylamide gels. Lane 1 shows the Coomassie Blue pattern of the proteins from Ko cells and Lane 2 that from Kell positive cells. Standard protein markers are listed on the left side. Another identical set of proteins was transferred to nitrocellulose paper, reacted with rabbit antibody to Kell protein, and the immune complex localized with 125I-protein-A. Autoradiograms are shown in lanes 3 and 4. Lane 3 shows the pattern obtained with Ko cells and lane 4 that with Kell positive cells.

FIG. 6. Effect of iodoacetamide on the isolation of Kell protein: SDS-polyacrylamide gel electrophoresis under reduced and nonreduced conditions. Red cells were surface-labeled with 32P and treated with either antibody to K7 or with inert serum. One set of cells was treated with 0.1 M iodoacetamide. Kell protein was isolated by immunoprecipitation and SDS-polyacrylamide gel electrophoresis. Autoradiograms are shown. Lanes 1 and 2 show the patterns obtained with antibody to K7 and proteins separated under reducing conditions. In lane 1 the cells were not treated with iodoacetamide, and in lane 2 the cells were treated with iodoacetamide. Lanes 3 to 6 show the patterns obtained when separation was performed under nonreducing conditions. The proteins in lanes 3 and 4 are from cells not treated with iodoacetamide. Lane 3 contains proteins isolated by treatment with anti-K7 and lane 4 with inert serum. Lanes 5 and 6 show the patterns obtained when cells were treated with iodoacetamide. Lane 5, that obtained with anti-K7 and lane 6, that with inert serum. The + and − signs at the top of the gel indicate that cells were either not treated (−) or were treated (+) with iodoacetamide. The numbers (kDa) on the right side show the location of standard proteins.
protein is a prominent component of the immunoprecipitate. This suggests that in situ Kell may be in association with other membrane proteins.

**DISCUSSION**

Kell blood group antigens, on human red blood cells, are part of a surface-exposed 93,000-dalton glycoprotein (11). Several lines of evidence establish that Kell antigens are not a component of band 3 protein. The 93,000-dalton glycoprotein, immunoprecipitated with monospecific antibodies to Kell antigens, yields different tryptic and chymotryptic peptide maps to band 3. In addition the surface-exposed portions of band 3 and Kell protein, when labeled by lactoperoxidase-catalyzed iodination of intact red cells, produce a different set of radioactive chymotryptic peptides. Kell protein and band 3 are immunologically different. An antibody to a 43,000-dalton fragment of human band 3 does not react, on Western immunoblots, with isolated Kell protein; conversely a rabbit antibody to isolated denatured Kell protein does not react with band 3 protein from Ko red cells. Band 3 is phosphorylated (27, 28), and the Kell protein is not. These experiments establish that the 93,000-dalton Kell protein and band 3, the anion transport protein, are different proteins.

The finding that a rabbit antibody to isolated, reduced, Kell protein reacts with a 93,000-dalton membrane component of Kell antigen positive cells but not from those of Ko cells indicates that Kell cells either lack the Kell protein or that this protein is only present in small quantities and cannot be detected by immunoblotting. Since the antibody was raised against the entire denatured protein it is unlikely that Ko cells contain Kell protein but that the antigenic sites are occluded.

Antibodies to different Kell surface antigens (antibodies to K1, K2, K7, K14, K22, K23) all immunoprecipitate a 93,000-dalton protein. Mixing experiments indicate that different Kell antibodies react with the same protein, since the amount of surface-labeled Kell protein which is immunoprecipitated by mixtures of antibodies is not additive (11). In this study we also show that two different Kell antibodies, a human alloimmune antibody to K7 and a mouse monoclonal antibody to K14, immunoprecipitate proteins which yield nearly identical chymotryptic peptide maps. This indicates that both K7 and K14 antigenic sites reside on the same protein. The factors that determine the differences in Kell antigens are not known. Differences in Kell antigen specificity may be due to differences in the oligosaccharide moiety, to minor differences in the amino acid sequence of the surface-exposed portion of the Kell protein, or to a combination of differences in both sugar and amino acid composition or sequence.

Acknowledgments—This report is dedicated to Professor Abraham Mazur, on the occasion of his 75th birthday, with grateful thanks for many years of encouragement and advice. We also thank Alex Scarborough for excellent technical assistance during the latter part of this study and Tellervo Huima for help with photography.

**REFERENCES**

1. Coombs, R. R. A., Maurant, A. E., and Race, R. R. (1946) *Lancet* 1, 264-266
2. Marsh, W. L. (1981) in *Blood Groups of Human Red Cells in Clinical Practice of Blood Transfusion* (Petz, L.D., and Swisher, S.N., eds) pp. 79-130, Churchill-Livingstone, New York
3. Warn, B. M., Marsh, W. L., Taswell, H. F., and Galey, W. R. (1977) *Br. J. Haematol.* 36, 219-224
4. Symmons, W. A., Shepherd, C. S., Marsh, W. L., Øyen, R., Shobert, S. B., and Linehan, B. J. (1979) *Br. J. Haematol.* 42, 575-583
5. Hughes-Jones, N. C., and Gardner, B. (1971) * Vox. Sang.* 21, 154-158
6. Masoureda, S. P., Sudora, E., Mahan, L. L., and Victoria, E. J. (1980) *Hematologica* 13, 59-64
7. Branch, D. R., and Petz, L. D. (1982) *Am. J. Clin. Pathol.* 78, 161-167
8. Advani, H., Zamar, J., Judd, W. J., Johnson, C. L., and Maria, W. L. (1982) *Br. J. Haematol.* 51, 107-115
9. Dobert, D. G., Shapura, R., and Burnett, W. T. (1957) *J. Am. Chem. Soc.* 79, 5667-5671
10. Branch, D. R., Muench, H. A., Siok Hian, A. L. S., and Petz, L. D. (1983) *Br. J. Haematol.* 54, 573-578
11. Redman, C. M., Marsh, W. L., Mueller, K. A., Avellino, G. P., and Johnson, C. L. (1983) *Transfusion* (Phila) 24, 176-178
12. Moore, S., Woodrow, C. F., McClelland, D. B. L. (1982) *Nature* 295, 529-531
13. Reichstein, E., and Blostein, R. (1975) *J. Biol. Chem.* 250, 6256-6263
14. Maelzel, J. V., Jr. (1971) in *Methods of Virology* (Maramosh, K., and Kiprowski, A., eds) pp. 179-246, Academic Press, New York
15. Goding, J. W. (1980) *J. Immunol. Methods* 39, 285-308
16. Eldon, J. H., Pickett, R. A., Il, Hampton, J., and Lerner, R. A. (1977) *J. Biol. Chem.* 252, 6510-6515
17. Greenoed, F. C., Hunter, W. M., and Glover, J. S. (1963) *Biochem. J.* 89, 114-123
18. Markowitz, S., and Marchesi, V. T. (1981) *J. Biol. Chem.* 256, 6463-6468
19. Yu, S., Sher, B., Kudryk, B., and Redman, C. M. (1984) *J. Biol. Chem.* 259, 10574-10581
20. Tang, L. L., Redman, C. M., Williams, D., and Marsh, W. L. (1981) *Vox. Sang.* 40, 17-26
21. Burnette, W. N. (1981) *Anal. Biochem.* 112, 195-203
22. Morrison, M., Mueller, T. J., and Huber, C. T. (1973) *J. Biol. Chem.* 249, 2668-2660
23. Phillips, D. R., and Morrison, M. (1971) *Biochem. Biophys. Res. Commun.* 45, 1103-1108
24. Knutlermann, H., Blakkki, S., Schmidt-Ulrich, R., and Wallach, D. F. H. (1973) *Biochim. Biophys. Acta* 330, 356-361
25. Harrell, D., and Morrison, M. (1979) *Arch. Biochem. Biophys.* 193, 158-168
26. Harris, H. W., Jr., Levin, N., and Lux, S. E. (1980) *J. Biol. Chem.* 255, 11521-11525
27. Stock, J. L. (1974) *J. Cell Biol.* 62, 1-19
28. Marchesi, V. T., Furthmayr, H., Tomita, M. (1976) *Annu. Rev. Biochem.* 45, 667-698