Determination of the N-Terminal Sequence of Human Red Cell Rh(D) Polypeptide and Demonstration That the Rh(D), (c), and (E) Antigens Are Carried by Distinct Polypeptide Chains

By Christian Bloy, Dominique Blanchard, Wolfgang Dahr, Konrad Beyreuther, Charles Salmon, and Jean-Pierre Cartron

Human monoclonal antibodies (MoAbs) directed against the blood group Rh(D), (c), and (E) antigens produced by Epstein-Barr virus (EBV)-transformed lymphoblastoid cell lines have been used to characterize the Rh components of human red cell membranes and to determine whether the Rh(D), (c), and (E) epitopes are carried by distinct polypeptides. After immunoprecipitation with the anti-Rh(D) antibody and preparative gel electrophoresis, a homogeneous preparation of the Rh(D) protein was obtained from two different erythrocyte samples (Blo and D−/D−), which have an increased density of Rh(D) antigen. Both preparations exhibited the same N-terminal sequence (S)-(S)-K-Y-P-R-S-V-R-R-(L)-L-P-L-X-A, indicating that different Rh(D)-positive red cells are carrying a similar Rh(D) protein. Comparative immunoprecipitation studies with the human monoclonal anti-Rh(D), (c), and (E) antibodies have also shown that Rh components from intact and papain-treated erythrocytes have similar apparent mol wt of 30 to 32 Kd and are buried in the lipid bilayer and are not readily available to the proteolytic enzyme. Further investigations by indirect affinity chromatography and one-dimensional peptide mapping of the Rh(D), (c), and (E) molecules immunopurified from a single red cell sample demonstrate that a common Rh haplotype encodes three distinct polypeptide chains carrying the Rh(D), (c), and (E) epitopes, respectively.

MATERIALS AND METHODS

Chemicals and isotopes. Reagent grade chemicals were used throughout and, unless stated, were from Merck (Darmstadt, FRG). Protein A-Sepharose CL-4B (2 mg/mL of gel) was obtained from Pharmacia (Uppsala, Sweden) and Avidin-agarose (37 U/mL of gel) from Sigma Chemical (St. Louis). Iodo-gen (1,3,4,6-tetrachloro-3-aminophenyl) was obtained from Pierce (Rockford, IL) and biotin-N-hydroxysuccinimide ester (BNHS) from Zymed Laboratory, Inc (San Francisco). 125INa (650 mol/L, Bu2/µg iodine) was from the Radiochemical Centre (Amersham, Bucks, England). Trypsin (32 U/mg), chymotrypsin (53 U/mg), papain (10 U/mg), and bovine serum albumin (BSA) were obtained from Serva (Heidelberg, FRG). Triton X-100 was from Boehringer (Mannheim, FRG). Enzyme beads and reagents for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) were from Bio-Rad (Richmond, CA).

Antibodies. Human monoclonal antibodies (MoAbs) against Rh(D), (c), and (E) antigens were obtained from EBV-transformed lymphoblastoid cell lines11 and purified by Protein A-Sepharose CL-4B chromatography.28 The IgG fractions were 125I-labeled by the method of Ikenberry and Celander.29 Labeling efficiency ranged from 80 to 90%.

From the Unité Inserm U76 and Institut National de Transfusion Sanguine (INTS), the Institut für Immunologie und Serologie der Universität Heidelberg, and the Zentrum für Molekularbiologie der Universität Heidelberg, FRG.

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Address reprint requests to Jean-Pierre Cartron, PhD, Unité Inserm U76, INTS, 6, rue Alexandre Cabanel, 75015 Paris, France.

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iodo-gen procedure at a specific activity ranging from 33.4 to 66.8 kBq/µg protein and were occasionally further biotinylated with the BNHS reagent according to Guesdon et al., except that the BNHS:protein ratio was 1:5 and the reaction carried out for two hours at room temperature.

Red cells. Common and rare red cells (D-/D-, Gou) typed for Rh antigens were freshly collected at the Centre National de Transfusion Sanguine (Paris) and used within a few days. Intact washed erythrocytes, either native or papain-treated (1 vol of red cell incubated for one hour at 37°C with 4 vol of 10 mM/L phosphate buffer [pH 7.2], 0.15 mol/L NaCl (NaCl/P04) containing 0.25 mg/mL papain, 1 mM/L EDTA, and 2 mM/L cysteine hydrochloride), were 125I-labeled by the lactoperoxidase procedure and the membranes prepared as described.

Immunoprecipitation. Immunoprecipitation of Rh antigens from the membrane preparations were carried out as published by Gahmberg. Alternatively, immunoprecipitation with anti-Rh(c) and (E) was performed from Triton X-100 lysates prepared as described below. The immunocomplexes were solubilized in a buffer containing 1% (wt/vol) Triton X-100 and purified on Protein A-Sepharose. The radioactive material was eluted by heating the gel beads for 15 minutes at 60°C and for three minutes at 100°C in 100 µL of 10 mM/L Tris-HCl, 1 mM/L EDTA, pH 6.8, containing 5% (wt/vol) SDS.

Indirect affinity chromatography. The fractionation of immunocomplexes by indirect affinity chromatography was performed with the purified monoclonal anti-Rh antibodies carrying either one or two of the 125I and biotin labels. In a typical experiment 1.5 x 10^6 washed cDE/cDE red cells were incubated for one hour at room temperature with saturating amounts of 125I-labeled antibodies, either with or without the biotin label (4 µg of anti-Rh(D) and 2 µg of anti-Rh(c) or - (E) MoAbs) in 100 µL of NaCl/P04 containing 2% (wt/vol) BSA and 1 mg/mL of D-glucose. Coated red cells were washed three times with 500 µL of NaCl/P04-PO4-BSA, twice with NaCl/P04, and then lysed in 200 µL of cold NaCl/P04 containing 2% (wt/vol) Triton X-100. About 85% of the radioactivity was recovered in the lysis supernatants after centrifugation at 80,000 g during 30 minutes. The clear lysates were then incubated for one hour at room temperature with 25 µL of Avidin-agarose (1 U of avidin) previously washed in NaCl/P04. The Avidin-agarose beads were then washed four times with 200 µL of NaCl/P04. Washed beads and combined supernatant washes (1 mL final volume) were counted in a LKB Gamma counter (model 120 A, Applied Biosystems, Foster City, CA) equipped with an on-line PTH analyzer (model 120 A, Applied Biosystems), using 0.5 to 1.0 nmol of protein. For o-phthalaldehyde-2-mercaptoethanol treatment, 10 µL each of 0.1% (vol/vol) 2-mercaptoethanol in pyridine and 0.1% (wt/vol) o-phthalaldehyde in ethanol were applied 15 times to the filter over a period of two hours.

RESULTS

Characterization of Rh(D), (c), and (E) antigens. The blood group specificity and the immunochemical properties of human MoAbs against Rh(D), (c), and (E) antigens have been described in detail elsewhere. Scatchard analyses, using purified IgG preparation, showed that these antibodies bind to a similar number of Rh(D), (c), and (E) antigens on cDE/cDE erythrocyte membranes (range 0.32 to 0.43 x 10^6/cell) with functional affinity constants of 0.6, 0.035, and 2.0 x 10^6 mol/L^-1, respectively. On native erythrocyte membranes the anti-Rh(D), (c), and (E) anti-

PAG AND PURIFICATION OF RH PROTEINS. PAGE in the presence of SDS was carried out on slab gels with a 10% (wt/vol) polyacrylamide separating gel (1.5 mm thick; 90 mm long) using a discontinuous buffer system. After electrophoresis the gels were fixed, dried, and revealed by autoradiography on Cronex 4 film with intensifying screens (Dupont de Nemours, Wilmington, DE). For preparative purposes the slab gels (3 mm thick) were cast 48 hours before used, unshorted, and washed twice in Tris 50 mM/L, boric acid 60 mM/L buffer containing 0.1% (wt/vol) SDS. The gels were then put back against the glass plates and a 3% (wt/vol) polyacrylamide stacking gel was cast on top. A 2-ml sample containing up to 1 nmol of Rh protein was electrophoresed with trace amount of radioactive Rh material in the Tris-borate buffer. After electrophoresis the unfixed gels were packed in Saran wrap and exposed to Cronex 4 x-ray film overnight at -80°C. The 30- to 32-Kd band corresponding to the monomeric form of Rh proteins was then excised from the gel and electroeluted. The electroeluted protein solution (vol = 10 mL) was concentrated down to 200 µL in a Centriplus 10 system (YM 10, Amicon, Danvers) pretreated with 0.1 mol/L NaOH and washed several times with distilled water. The concentrated protein solutions (aliquots of 100 µL) were further freed from contaminants by three washings with 1 mL heptane:ethylacetate (2:1, by vol) followed by one wash with 1 mL butylacetate. The washed protein was finally dried under vacuum without heating (Speed Vac, Savant Instruments, NY) and redissolved in a small volume of 0.5% (wt/vol) highly purified SDS. The purity of the preparations was checked by PAGE in the presence of SDS as described above.

One-dimensional peptide maps. Purified Rh polypeptide preparations were 125I-labeled by the lactoperoxidase and glucose oxidase procedure using Enzymobeads (Bio-Rad) as described by the manufacturer. Briefly, 20 µg of Rh proteins were incubated with 0.2 mCi of 125I Na and 150 µL of Enzymobeads. After a 15-minute incubation at room temperature the free iodine was separated on a Sephadex G-25 column (PD 10, Pharmacia, Uppsala, Sweden) eluted with 0.5% (wt/vol) SDS. The radioactive Rh(D), (c), and (E) polypeptides (30- to 32-Kd bands) were further subjected to electrophoresis in a 10% (wt/vol) denaturating polyacrylamide gel and submitted to proteolytic digestion in 50 mM/L ammonium bicarbonate within the gel according to Elder et al. The iodopeptides released from the gel (70% to 80% of the total input radioactivity) were desalted by repeated evaporation under vacuum, separated by HPTLC on silica gel plates in CHCl3/CH3OH/NH4OH (2/2/1, by vol), and detected by autoradiography.
bodies bind to a 30- to 32-Kd component, which was identified by SDS-PAGE of immunocomplexes isolated on Protein A-Sepharose. As shown in Fig 1, immunoprecipitation studies carried out with membranes prepared from papain-treated erythrocytes indicate that the Rh proteins are not altered by extensive proteolysis of the intact red cells. Indeed, the Rh(D), (c), and (E) polypeptides from these cells have the same mobility as those that were immunoprecipitated from untreated red cells, suggesting that the Rh protein is buried in the phospholipid bilayer and is not readily available to proteases. These findings are in agreement with the known protease resistance of Rh antigens on red cells. In the authors’ experiments the electrophoretic mobilities of the Rh(D), (c), and (E) polypeptides, which migrate as rather diffuse bands, were identical, even using longer (140 mm) gels or gradient (7% to 12%) gels (data not shown). This is in agreement with recent data from others indicating an apparent mol wt of 32 Kd for the Rh(c) and (E) polypeptides and 32 Kd for the Rh(D) protein. Many factors, however, related either to the different preparation or to the electrophoretic separation procedure or both, may account for this apparent discrepancy. Moore and Green have postulated that the small difference in apparent mol wt might be related to the protein structure rather than to the extent of glycosylation of the Rh molecules. Clarification of this hypothesis will require the isolation and characterization of the Rh molecular species. As a first approach the authors have used the anti-Rh(D) antibody, which was available in large amounts for the purification and structural analysis of the Rh(D) protein.

**Purification of Rh proteins and N-terminal sequence of Rh(D) molecule.** The main step of the purification protocol, carried out with red cells from one of the authors (Blo) and with those from a D--/D-- donor (Gou) that have more Rh(D) antigen sites than common erythrocytes, includes the immunoprecipitation of the Rh(D) polypeptide followed by a preparative PAGE. A yield of about 50 pmol protein/mL packed red cells was obtained. The purified material was homogeneous by PAGE in the presence of SDS as judged from Coomassie blue staining and autoradiography (not shown). The amino acid compositions of these preparations were similar to that described previously. Sequence analysis performed on three different preparations from the two donors (Blo and Gou) yielded the following identical N-terminal sequence of 16 amino acid residues:

(S)-(S)-K-Y-P-R-S-V-R-R-(L)-L-P-L-X-A

(1 = provisional determination)

Presence of contaminating free amino acids, predominantly residual glycine, caused difficulties in the unambiguous identification of the two N-terminal residues. However, the presence of a histidine residue at the N-terminus, as suggested by the work of Sinor et al., could be ruled out. The yield of phenylthiohydantoin (PTH) derivatives was rather low (about 5% to 10%). These technical problems made it difficult to obtain reliable sequence information, even after \( \alpha \)-phthalaldehyde treatment before Pro at position 5. Since low sequencing yields were also noticed for other proteins purified in the authors’ laboratories by PAGE in the presence of SDS, it is likely that the N-terminus became partially blocked during isolation of the protein. It is less likely that the N-terminus of the Rh(D) molecule is blocked in vivo and that the authors have determined the sequence of a contaminating protein in the two different Rh(D) preparations. Moreover, the same sequence has been found after purification of the Rh proteins from a detergent extract of Rh(D)-positive red cell membranes by hydroxylapatite gel chromatography (P. Agre, personal communication).

Although the purification yield is rather low (5% to 8%, based on the known number of antigen sites per single erythrocyte), it allows the preparation of 10 to 12 nmol of Rh(D) protein/blood donation provided that enough purified monoclonal IgG anti-Rh(D) is available (20 to 25 mg). Preliminary investigations carried out with the monoclonal anti-Rh(c) and (E) antibodies indicated that the Rh(c) and (E)-active polypeptides can also be immunopurified from cDE/cDE erythrocytes by the same procedure. The yield with anti-Rh(E) was rather similar (40 pmol of protein/milliliter packed red cells), but that with anti-Rh(c) was much lower (13 pmol of protein/milliliter packed red cells), probably resulting from the low binding constant of this antibody (see above). One crucial point was to determine whether the molecules that the authors have immunopurified are different from each other.

**How many different Rh molecules are present on erythrocytes?** The question of whether the Rh(D), (c), and (E) epitopes are present on separate molecules or whether they are present together on a single molecule was investigated in the following way. The red cells from a single donor were coated with pairs of antibodies (e.g., anti-Rh(c) and anti-Rh(D)), both members of the pair being labeled with \( \text{I}^{125} \) but only one with biotin; the antigen-antibody complexes were then solubilized with Triton X-100. If the epitopes are on
separate molecules, then it should be possible to separate the biotin-labeled complex away from the other complex using an Avidin-agarose column. On the other hand, this separation would not be possible if the epitopes were present on the same molecule. The results shown in Table 1 demonstrate that this separation was possible. Indeed, the immunocomplexes formed with antibodies carrying only the 125I-label were not retained on the Avidin-agarose beads, whereas those formed with antibodies carrying the 125I and biotin labels bound strongly to the affinity gel (Table 1, top). When cDE/cDE red cells were coated simultaneously with two radiolabeled antibodies, one of which was biotinylated, the red cells were coated with two cDE/cDE agarose gel beads, whereas the 125I-labeled polypeptide attached to unlabeled anti-Rh(c) or (E) antibodies were always found on the Protein A-Sepharose gel, whereas the 125I-labeled polypeptide attached to the biotinylated antibodies were found on the Avidin-agarose gel (Table 1, bottom). When the anti-Rh(c) and (E) were used together for immunoprecipitation, the Rh(c) and (E) polypeptides were present in the Protein A-Sepharose or Avidin-agarose eluates, depending on which antibody was biotinylated. Furthermore, a quantitative effect was observed, at least with the native antibodies, since, for example, the radioactivity recovered in the 30 to 32-Kd components following immunoprecipitation with both anti-Rh(c) and anti-Rh(E) corresponded approximately to the sum of the radioactivity precipitated by the antibodies used separately. This was not always found, since the technical conditions for quantitative immunoprecipitations are difficult to define. However, the data reported in Table 1 argue for a clear separation of the Rh(D), (c), and (E) polypeptides on human red cells.

Further evidence that the Rh(D), (c), and (E) antigens immunopurified as described above are carried by distinct molecular species was provided by one-dimensional peptide mapping. Iodopeptides released from the gel slices during the digestion with trypsin or chymotrypsin represented about 80% of the total radioactivity incorporated in Rh(D), (c), and (E) polypeptides. As shown in Fig 2, a different separation pattern of the tryptic or chymotryptic iodopeptides were clearly detected after high performance thin layer chromatography (HPTLC) and autoradiography. Whereas six major peptides were resolved for the Rh(c) and Rh(D) tryptic fragments (Fig 2, lanes 1 and 2, respectively), much less were obtained for Rh(E) (Fig 2, lane 3). Chymotryptic fragmentation gave the same number of peptides migrating with a different pattern (Fig 2, lanes 4 to 6). Examination of the maps indicated that some bands are common to the different enzymatic supernatant. Among them the major one, representing 26% of the radioactivity recovered (as judged by counting the iodopeptides eluted from the silica), is composed of at least three peptides ranging from 7 to 12 Kd that were separated in a 10% to 20% gradient polyacrylamide gel in the presence of SDS (data not shown). Although similar peptide maps were obtained from three different blood samples, it cannot be formally excluded that polymorphisms could account for these results. Further studies by two-dimensional peptide mapping indicate a structural homology between the Rh polypeptides and will greatly help to discriminate among the properties of these molecules (Blanchard et al, manuscript in preparation). It is suggested,

### Table 1. Indirect Affinity Chromatography of Immunocomplexes Containing Rh-Specific Components

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
<th>Experiment 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Radioactivity (cpm × 10^3)</td>
<td>Coating Red Cells With Labeled MoAbs*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>c D^b c + D^b c^+ E E c^+ E D E^b D + E^b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bound to Avidin-agarose</td>
<td>1.6 35.8 38.4 130.8 6.5 123.1 2.8 69.8 53.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unbound to Avidin-agarose</td>
<td>19.4 5.7 21.9 2.1 104 135.8 52.0 11.0 64.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Note:** The MoAbs used in these experiments were 125I-labeled and were biotinylated or not, as indicated. The presence of the biotin label is noted by the superscript b (e.g., D^b, c^b, or E^b). The immunocomplexes obtained from cDE/cDE red cells incubated with one antibody alone (D, c, E, D^b, c^b, etc.) or with a mixture of two antibodies (c + D, c + E, or D + E) were separated on Avidin-agarose beads, and the radioactivity bound and unbound was determined.

†The MoAbs used in these experiments were either unlabeled or biotinylated, as noted by the presence of a superscript b (e.g., E^b). The immunocomplexes obtained from 125I-labeled cDE/cDE erythrocytes incubated with one antibody alone (c, E, E^b) or with a mixture of two antibodies (c + E, c^+ E, c^+ E^b) were separated on Avidin-agarose or Protein A-Sepharose, and the proteins bound to the gel beads were analyzed by SDS-PAGE. Only the radioactivity of the Rh components (30 to 32 Kd) was determined.
from a single cDE/cDE erythrocyte sample by preparative immuno precipitation. The purified polypeptides were separated by HPTLC obtained from the Rh(c), Rh(D), and Rh(E) antigens. The radioactive Rh components (mol wt 30 to 32 Kd) were determined from SDS gels. The radioactive Rh components (mol wt 30 to 32 Kd) were obtained from the Rh(c), Rh(D), and Rh(E) antigens. The radioactive Rh components (mol wt 30 to 32 Kd) were obtained from the Rh(c), Rh(D), and Rh(E) antigens.

DISCUSSION

Human MoAbs against the Rh(D), (c), and (E) antigens were used to characterize and purify the Rh(D), (c), and (E) polypeptides from human erythrocytes. Enough material was obtained from Rh-positive cells of two different individuals for a determination of the N-terminal sequence of the Rh(D) protein. In the authors' hands the Rh(D), (c), and (E) polypeptides have a similar apparent molecular mass of 30 to 32 Kd on SDS-PAGE. The combined data from immunoprecipitation, indirect affinity chromatography, and one-dimensional peptide map analyses of the immunopurified Rh(D), (c), and (E) antigens clearly demonstrate that these epitopes are carried by distinct polypeptides. The same conclusion was deduced from flow cytometry analyses and radioimmunooassays (RIAs) performed with the same antibodies. The authors' findings are in agreement with the genetic model proposed by Fisher and Race, indicating that the inheritance of Rh antigens is under the control of three allelic pairs of closely linked structural genes (Dd, Cc, and Ee) segregating together. It is reasonable to assume that all the Rh antigens identified so far are distributed within the three molecular species described in this study, but a more complex model generating even more Rh molecules cannot be ruled out at present. The three different proteins that the authors identified might be encoded by three adjacent, presumably homologous, genes. However, one difficulty of this model, in terms of classical genetics, is the interpretation of so-called "compound antigens" such as cc, Ce, CE, and cE, which are produced by the interaction of two adjacent C or c and E or e alleles in cis (i.e., on one chromosome) but not in trans position. These compound antigens might be readily explained by assuming that the C or c and E or e antigens are represented by two adjacent amino acid substitutions on a single polypeptide chain that are both recognized by certain antibodies (anti-Ce, etc.). It is conceivable that the genetic mechanisms discussed below may provide a satisfactory explanation of these phenomena.

The model of Fisher and Race was revisited some years ago by Rosenfield et al., who proposed, on the basis of bacterial genetic concepts, that the Rh locus is composed of three structural loci under the control of four regulatory genes. Since then it was shown that a large number of eukaryotic genes have a mosaic structure consisting of a combination of exons and introns. Accordingly, a speculative model for the Rh blood group system based on a discontinuous gene structure has been proposed, but it is not yet possible to predict which factors or gene regions might regulate the production of Rh antigens.

That three polypeptides are produced by the Rh locus is not incompatible with a two-gene model or even a one-gene model, since a single transcription unit can generate multiple messenger RNAs (mRNAs) and therefore several proteins. This may occur by several mechanisms, such as the use of different promoters, alternative splicing of exons, and choice of different polyadenylation signals. The proteins translated from these messages may have common as well as specific antigenic properties. Therefore these mechanisms might account for the wide diversity of Rh variants identified so far. Post-translational processing or modification might also contribute to the complexity of the Rh system.

The existence of a complex transcription unit for blood group antigens has recently received some support, since after gene cloning and genomic DNA studies it was proposed that glycosphorin C and D, which carry different antigens of the Gerbich blood group system, are encoded by a single gene. This level of analysis is now a reasonable goal for the Rh system because significant progress has been made in purification and protein sequencing of Rh polypeptides, and therefore oligonucleotide probes will become available for gene cloning. It is likely that the molecular definition of the Rh system both at the protein and gene level will develop rapidly, but the crucial question of the function of Rh molecule in the red cell membrane remains unresolved, although a role as Na"/H" antiport and in the regulation of intracellular pH has been suspected. It has also been suggested that the Rh molecules serve to stabilize the membrane by forming complexes with different integral membrane proteins. Clarification of the functions will therefore require the availability of highly purified proteins. All this
information should also greatly help our understanding of the clinical significance of the Rh system and of abnormalities occurring in the Rh-deficiency syndrome.\textsuperscript{2,36,37}

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REFERENCES

5. Moore S, Woodrow CF, McClelland DBL: Isolation of membrane components associated with human red cell antigens Rh(D), (c), (E), and Fy\textsuperscript{a}. Nature 295:529, 1982
7. Gahmberg CG: Molecular characterization of the human red cell Rh(D) antigen. EMBO J 2:223, 1983
8. Gahmberg CG, Karihi KK: Association of Rh(D) polypeptides with the membrane skeleton in Rh(D)-positive human red cells. J Immunol 133:334, 1984
14. Fraker PJ, Speck JC: Protein and cell membrane iodinations with a sparingly soluble chloroamide 1,3,4,6-tetrachloro-3a,6a-diphenylglycoluril. Biochem Biophys Res Commun 80:849, 1978
18. Laemmli UK: Cleavage of structural proteins during the assembly of the head of bacteriophage T\textsubscript{4}. Nature 227:680, 1970
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