Brief report

DNB: a partial D with anti-D frequent in Central Europe
Franz F. Wagner, Nicole I. Eicher, Jan R. Jørgensen, Cornelie B. Lonicer, and Willy A. Flegel

To improve routine D typing and define transfusion strategy, it is important to establish the frequency of partial D alleles and their susceptibility to anti-D alloimmunization due to transfusion or pregnancy. We identified the partial D DNB that was caused by an RH(D)355S allele associated with a CDe haplotype and whose phenotype presented a normal D in routine typing. The antigen density was about 6000 D antigens per red blood cell, and the Rhesus index was 0.02. Five anti-D immunization events with allo–anti-D titers up to 128 were observed. Twelve carriers of DNB were whites of Central Europe; the only Danish proband had Austrian ancestry. DNB was the most frequent partial D recognized so far in whites, occurring with frequencies of up to 1:292 in Switzerland. DNB was the underlying partial D phenotype in a relevant fraction of anti-D immunizations occurring in whites. (Blood. 2002;100: 2253-2256)

Introduction

Anti-D immunizations may occur in “partial D” carriers. This designation relates to more than 30 alleles differing in molecular structure, phenotype, population frequency, and lenience to anti-D production.1 The molecular bases were gene conversions,2 single missense mutations in the exofacial protein segments,3 and multiple missense mutations dispersed throughout the RhD protein.4 A partial D needs to be considered for transfusion strategies if its carriers are frequent, easily anti-D immunized, and likely to receive D-positive units in the case of transfusion. D category VI5 is the classical example. In several countries, typing methods6 were introduced that assured D-negative transfusion and anti-D prophylaxis for DVI mothers to prevent anti-D immunizations. Despite the large number of known partial D alleles, a relevant portion of anti-D immunizations is still occurring in individuals who did not carry any known partial D.7–9 Thus, the characterization of the unknown alleles underlying such case reports is remaining important.

Study design

Blood samples with anti-D

Two D-positive samples (RIR-2 from Bern, Switzerland; RIR-3 from Bavaria, Germany) with anti-D were referred to our laboratory to elucidate the cause of anti-D immunization. Later, 1 additional blood sample (RIR-41 from Denmark) and 2 DNA samples (RIR-7, RIR-8 from Austria) were referred to the Rhesus Immunization Registry (RIR) because of anti-D in these D-positive probands. The Danish proband RIR-41 had an Austrian grandfather.

RHD nucleotide sequencing

DNA was handled as described previously.10 The 10 RH(D) exons were sequenced by an RHD allele–specific method11 in the 2 index samples (RIR-2, RIR-3). In the remaining samples, exon 7 was sequenced only.

PCR-SSP

A polymerase chain reaction with sequence-specific priming (PCR-SSP) to confirm or detect the 1063G→A substitution in DNB was devised as modular extension of a PCR-SSP system previously developed for RHD typing.10,12 Specific primers were re77 (TCTCCACAGCTCCA TCA TGGG) and DNDb (CAGTGAACCACATGCCATTACCT) at a concentration of 3.5 μmol; HGH control primers were used at 0.75 μmol.

Immunohematology

Routine D typing was done in tube tests using commercial monoclonal anti-D (Seracline anti-D 226, clone BS226; and Seracline anti-D [232], clone BS232; Biotest, Dreieich, Germany; and ImmucClone anti-Dfast, clone D1-4E11; and ImmucClone anti-Drapid, clone RUM-1; Immucor, Norcross, GA). A commercial panel of monoclonal anti-D (D-Screen; Diagast, Loos, France) was tested in gel matrix technique (LISS-Coombs 37°C, DiaMed-ID Micro Typing System; DiaMed, Cressier sur Morat, Switzerland). D epitopes were determined by agglutination, and antigen density and Rhesus index were determined by flow cytometry as previously described.13 DIV type III13 and DNU14 controls derived from donors in Baden-Württemberg, Germany.

Population screens for DNB

Ethylendiaminetetraacetic acid (EDTA)– or citrate-anticoagulated blood samples were collected from blood donors in Baden-Württemberg (Germany), 1118 donors of CcDe phenotype, 1010 donors of ccDe phenotype, Ticino (Italian-speaking Switzerland, 500 D-positive donors), Bern region therein. The RIR is sponsored by the German Society for Transfusion Medicine and Immunohematology (DGfI) and the German Red Cross (DRK) Blutspendedienst Baden-Württemberg–Hessen gGmbH. Reprints: Willy A. Flegel, Abteilung Transfusionsmedizin, Universitätsklinikum Ulm, and DRK-Blutspendedienst Baden-Württemberg–Hessen, Institut Ulm; Helmholtzstrasse 10, D-89081 Ulm, Germany; email: waf@ucsd.edu.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked “advertisement” in accordance with 18 U.S.C. section 1734.

© 2002 by The American Society of Hematology
(German-speaking Switzerland, 693 donors of CcDee phenotype), and
Denmark (768 donors of CcDee phenotype). The donors were tested with
the immunoglobulin G (IgG) monoclonal anti-D LOR17-6C7 (Germany
and Denmark) or HIRO-7 (Switzerland).13 Suspected DNB samples were
confirmed by sequencing of exon 7 or by PCR-SSP. Haplotypes frequencies
used for calculation of DNB allele frequencies were Cde 0.431, cde 0.394,
cDe 0.021, and Cde 0.011 in Germans16; 0.4236, 0.3972, 0.0145, and
0.0059 in Danes17; and 0.4442, 0.3617, 0.0232, 0.0113, and cDe 0.049 in
Swiss.17 Phenotype frequencies for CcDee were compared using the Fisher
exact test for a 4 × 2 contingency table; for this calculation the 500
D-positive samples in Ticino were inferred to comprise 187 CcDee samples.

Nomenclature

The name DNB derived from DNU-like and Bayern (Bavaria, Germany) or
Bern, because DNB was similar to DNU,14 whose point mutations were
closely adjacent.

Results and discussion

Molecular structure

The molecular structure of 2 D-positive samples with anti-D
(RIR-2, RIR-3) was determined by RHD-specific sequence from
genomic DNA. In RHD exon 7, a single G to A exchange at
position 1063 was detected that resulted in a Gly to Ser substitution
at codon 355. The affected amino acid was located in the exofacial
loop 6, adjacent to the mutations observed in DII (Ala to Asp at
position 354)14 and DNU (Gly to Arg at position 353).14 The
nucleic acid and amino acid sequence data were deposited in
EMBL/GenBank/DDBJ under accession number AJ417868.

Immunohematology

In routine D typing, both DNB samples typed as CcDee without
noticeable weakening of the antigen D. All 9 monoclonal anti-Ds of
a commercial partial D classification kit (D-Screen) were reactive.
An antigen density of 5908 antigens per cell and a Rhesus index 13,18
(RI) of 0.02 were determined in RIR-2. These results were comparable
to those obtained with a DNU sample (8073 antigens
per cell, RI 0.06) and a CcDee DIV type 3 sample (4544 antigens
per cell, RI 0.06) and a CcDee DIV type 3 sample (4544 antigens
per cell, RI 0.06). The D epitope pattern was determined for both
index samples and was unique (Table 1): 6 of 83 anti-Ds tested
did not agglutinate DNB, indicating a loss of epitopes epD6 and
epD31 as well as part of epD18 and epD23. The crossmatch with
DNB red cells was faintly positive; there are no DII red cells
available anymore.

Population frequencies

We screened random samples of CcDee blood donors in 4
European populations (Table 2). With the exception of Danes, DNB
was found frequently. The highest frequency was observed in the
Ticino population. DNB was the most frequent partial D in whites
described so far; its frequency even exceeded that of DVII.19
Further testing of other populations may be of potential benefit.

Including the probands with anti-D, we presented 12 unrelated
carriers of DNB who were whites of Central Europe; the only
Danish proband had an Austrian grandfather, from whom he may
have inherited the DNB allele. All DNB probands were of CcDee
phenotype. The CDe haplotype association was further corrobora-
ted by testing the parents of the Danish proband RIR-41, who
inherited his DNB allele from his CcDee father.

Table 1. Reactivity patterns of monoclonal anti-D

<table>
<thead>
<tr>
<th>Pattern*</th>
<th>Anti-D tested, n</th>
<th>Partial D</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-9</td>
<td>1-37</td>
<td>DNB</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>+</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>11</td>
<td>+</td>
</tr>
<tr>
<td>6/7</td>
<td>12</td>
<td>+</td>
</tr>
<tr>
<td>6/7</td>
<td>13</td>
<td>+</td>
</tr>
<tr>
<td>6/7</td>
<td>15/16</td>
<td>+</td>
</tr>
<tr>
<td>6/7</td>
<td>17</td>
<td>+</td>
</tr>
<tr>
<td>6/7</td>
<td>18</td>
<td>+</td>
</tr>
<tr>
<td>6/7</td>
<td>20/21</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>22</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>23</td>
<td>+</td>
</tr>
<tr>
<td>N/A</td>
<td>31</td>
<td>+</td>
</tr>
<tr>
<td>N/A</td>
<td>32</td>
<td>+</td>
</tr>
<tr>
<td>N/A</td>
<td>33</td>
<td>+</td>
</tr>
<tr>
<td>N/A</td>
<td>34</td>
<td>+</td>
</tr>
<tr>
<td>N/A</td>
<td>35</td>
<td>+</td>
</tr>
<tr>
<td>N/A</td>
<td>36</td>
<td>+</td>
</tr>
<tr>
<td>N/A</td>
<td>37</td>
<td>+</td>
</tr>
</tbody>
</table>

N/A indicates not applicable; +, a normal or weak positive result; and −, a
negative result.

*Pattern is as described previously by Lomas et al12 (1-9) and Scott15 (1-37).
†A complete listing of the antibodies was published previously13 (Table 2). The
following antibodies failed to agglutinate DNB: LOR17-6C7 (epD6), P3F20 (epD18),
HIRO-7, HIRO-8 (epD23), NAU6-1G6, and NOU (epD31). In addition, the reactivity of
the following anti-Ds differed from that of the majority assigned to the respective
epitope: D-90/7, SAL20-1D5, LHMM528, BIRMA-DG3 (epD15/16), as well as
VOL-3F6 (epD20/21) did not agglutinate DIV type 3; LHM77/64, BIRMA-D6 (epD23),
and NAU8-4D5 (epD36) did not agglutinate DNU.
‡The DNB pattern was established with samples RIR-2 and RIR-3 and the DNU
pattern with red cells of the original DNU donor.14 The DIV type 3 data are a
revaluation of the original pattern using a CcDee DIV type 3 donor from our
local population.
§The results depicted for D category II were determined by Scott21 and are
shown for comparison.

Anti-D immunization

Including the 2 index probands, 5 cases of allo–anti-D immuniza-
tion submitted to the Rhesus Immunization Registry occurred in
DNB probands (RIR-2, RIR-3, RIR-7, RIR-8, and RIR-41).20
These cases represented a sizable fraction of the 32 confirmed
allo–anti-D immunizations reported to this registry until February
2002. Although any Rhesus-positive blood sample with anti-D—
with or without known Rhesus variant—may be sent to the registry
for analysis, no other RHD allele was involved in a similar number
of cases. Two additional carriers of DNB with anti-D were from
Slovakia (Dr Martin Pisacka, personal communication, 1999) and
from Germany (submitted as RIR-49 after we had finished the
data analysis).

Anti-D titers ranged from 4 to 128, indicating that DNB carriers
were generally able to produce strong anti-D. However, no clinical
data like signs of transfusion reactions or hemolytic disease of
the newborn were reported. In concordance with the assumption that
Table 2. Population frequencies of DNB

<table>
<thead>
<tr>
<th>Population</th>
<th>Phenotype checked</th>
<th>Samples tested</th>
<th>DNB detected, n</th>
<th>Phenotype frequency among CcDeDe</th>
<th>Phenotype frequency among all phenotypes</th>
<th>Haplotype frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Switzerland (Lugano)</td>
<td>All D-positive</td>
<td>500</td>
<td>2</td>
<td>N/A†</td>
<td>1:292</td>
<td>1:220</td>
</tr>
<tr>
<td>Switzerland (Bern)</td>
<td>CcDeDe</td>
<td>693</td>
<td>4</td>
<td>1:173</td>
<td>1:538</td>
<td>1:389</td>
</tr>
<tr>
<td>Germany (Ulm)</td>
<td>CcDeDe</td>
<td>1118</td>
<td>2</td>
<td>1:559</td>
<td>1:1644</td>
<td>1:1295</td>
</tr>
<tr>
<td>ccDeDe (control)</td>
<td></td>
<td>1010</td>
<td>0</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Denmark (Aarhus)</td>
<td>CcDeDe</td>
<td>768</td>
<td>0</td>
<td>&lt;1:2.56§</td>
<td>&lt;1:1.798§</td>
<td>&lt;1:6.005§</td>
</tr>
</tbody>
</table>

*Samples were checked for DNB using monoclonal anti-D LOR17-6C7 (Germany and Denmark) and HIRO-7 (Switzerland).
†The phenotype frequencies among CcDeDe were statistically significantly different, assuming that the 500 D-positive samples tested in Ticino corresponded to 187 CcDeDe samples (P < .0023, Fisher exact test; 4 × 2 contingency table).
‡Not applicable. The frequency among D-positive was 1:250; both carriers were of CcDeDe phenotype.
§Upper limit of the 95% confidence interval according to the Poisson distribution.

these anti-Ds were triggered by transfusion or pregnancies, 4 of the probands were female; the male and at least 2 of the female probands were transfused prior to the detection of the anti-D. The anti-D was still detectable up to 8 years following the latest possible anti-D immunization event.

Impact on typing strategies

The repeated observations of anti-D immunizations probably reflected the combination of a high population frequency with a phenotype that triggered D-positive transfusions and with a moderate anti-D immunization potential. Hence, we recommended D-negative transfusions if a recipient is known to carry a DNB phenotype.

A serologic recognition of DNB would be demanding because it has a normal antigen density and is agglutinated by most anti-Ds, including almost all commercial anti-D typing reagents. All tested high-affinity monoclonal IgM anti-Ds that did not bind DVI but that agglutinated most weak D phenotypes agglutinated DNB also (Table 1). Therefore, a serologic strategy for detecting DNB would have to rely on a separate anti-D especially introduced to discriminate DNB from normal D. By careful selection of this third antibody (eg, HIRO-7 or HIRO-8), the strategy could be tuned to discriminate also DII, DIV, and DII46 from normal D.13,21 Such a major change in D typing would be costly and might induce uncertainties that outweigh the benefit by far. The anti-D immunization risk in DNB carriers may safely be estimated to be lower than 1% per D-positive transfusion. This immunization index was less than known for anti-K and anti-c22 but may be comparable to that of anti-Fy(a) and anti-Jk(a).

Genotyping strategies are increasingly utilized for blood group23 and may be devised to meet predefined specificity criteria. For example, RHD PCR in whites may be tuned to a specificity of greater 0.9999 and allows the identification of D-positive units missed by routine serology.10 Likewise, DNB or DIIa alleles may be detected specifically without any limitations imposed by the lack of suitable monoclonal anti-D. Because the frequency of partial D with relevant anti-D immunization risk is low in whites, their D-negative transfusion would not compromise the D-negative blood supply. Hence, a specific recognition and D-negative transfusion strategy for DNB may be perceived advantageous and become feasible with genotyping strategies in the future.

Acknowledgments

We are indebted to the contributors of the Workshop on Monoclonal Antibodies against Human Red Blood Cells and Related Antigens in Nantes in 1996,2 who provided most of the monoclonal anti-Ds used in this study. The authors thank Letizia Carmazza, Servizio Trasfusionale della Svizzera italiana, fondazione CRS, Lugano, Switzerland, for performing the population survey in Ticino; Drs Christoph Gassner and Dieter Schönitzer, Innsbruck, Austria, for supplying RIR-7 and RIR-8 DNA samples; Dr Ernst-Markus Quenzel, Hagen, Germany, for supplying the RIR-49 sample; and Drs Martin Pisacka (supported by grant IGA MZ CR NM/5952-3), Reference Laboratory for Immunohaematology, Praha, Czech Republic, and Daniela Cupanikova, Clinic of Haematology and Blood Transfusion, Bratislava, Slovak Republic, for communicating one independent observation of DNB. We acknowledge the expert technical assistance of Marianne Lotsch, Anita Hacker, Katharina Schmid, and Sabine Zahn in Ulm as well as of Lene Christiansen in Aarhus.

References


From www.bloodjournal.org by guest on July 16, 2017. For personal use only.


DNB: a partial D with anti-D frequent in Central Europe

Franz F. Wagner, Nicole I. Eicher, Jan R. Jørgensen, Cornelia B. Lonicer and Willy A. Flegel