RAPID COMMUNICATION

Maternal Genomic Neutrophil FcRIII Deficiency Leading to Neonatal Isoimmune Neutropenia

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The healthy mother of a child with transient immune neutropenia was found to be "NA-null." The mother's neutrophils did not react with anti-NA1 and anti-NA2 antibodies (polyclonal human alloantibodies and mouse monoclonal antibodies). A healthy donor was discovered during routine neutrophil antigen typing whose neutrophils were also "NA-null." This NA-phenotype was due to the absence of FcRIII (CD16 antigen) on neutrophils as demonstrated with anti-FcRIII monoclonal antibodies. The neutrophils of these two individuals were not able to bind dimeric immunoglobulin G. However, their cells had a normal expression of other phosphatidylinositol (PI)-linked membrane glycoprotein (CD24, CD67, and CLB grad/5 antigens), ruling out the existence of a PI-linkage defect, such as paroxysmal nocturnal hemoglobinuria. The mother (propositus) had isoantibodies in her blood against neutrophil-FcRIII without allospecificity, apparently produced during pregnancy and responsible for the neutropenia of her child. The expression of FcRIII on natural killer lymphocytes of both individuals was normal. FcRIII is encoded by two separate genes, one (FcRIII-1) for the neutrophil-PI-linked receptor, another (FcRIII-2) for the natural killer cell and macrophage-transmembrane receptor. By messenger RNA and DNA analysis (with an FcRIII-cDNA probe and restriction endonucleases) the neutrophil-FcRIII deficiency appeared to be due to deletion of the FcRIII-1 gene in both individuals, while the FcRIII-2 gene was normally present. The parents of the propositus were found to be heterozygous for this defect. Thus, FcRIII-1 gene deficiency of the mother may be a cause of (iso)immune neutropenia of the newborn. Whether this deficiency may have other clinical consequences has to be studied.

CASE REPORTS

The blood of a healthy dysmature neonate was routinely screened. The hemoglobin level and platelet count were normal. The total white blood cell (WBC) count was 7,100/mm³, with 6% segmented neutrophils, 4% band forms, 57% lymphocytes, and 33% monocytes. This child was the third child of the family and was born after a pregnancy of 38 weeks, with a birth weight of 2,350 g. The only remarkable event during the pregnancy was growth retardation, for which the pregnancy was terminated at 38 weeks. The second blood count was performed on the fourth day postpartum and showed a total WBC count of 9,500/mm³, with 1% granulocytes. To prevent infections, the neonate was treated with cotrimoxazol.

Further studies on the neutropenia showed a normal hematopoiesis as judged by normal erythroid, myeloid, and megakaryocytic cellularity. The neonate demonstrated a high incidence of circulating immune complexes.

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series in the bone marrow. The presence of IgG antibodies in the serum of the child and that of the mother against the neutrophils of the father, detected by neutrophil immunofluorescence, was indicative for immune-mediated destruction of the neutrophils of the neonate. Because the neutropenia persisted, the neonate was treated with intravenous IgG. After 5 doses of 0.4 g/kg, the neonate still had only 2% neutrophils, but after 1 week (3 weeks postpartum), the neutrophil count began to increase. Five weeks postpartum, the number of neutrophils was above 1,000/mm³ and cotrimoxazol prophylaxis was stopped.

Immunohematologic analysis showed that the neutrophils of the father were NA(1,-2,-), NB(1,+), whereas those of the mother were NA(1,--), NB(+). This difference prompted us to investigate whether the mother had the carrier molecule of the NA antigens, the FcRIII (CD16 antigen). Binding studies with a panel of CD16 MoAbs failed to detect FcRIII on her neutrophils (see Results and Discussion). The expression of three other PI-linked proteins (CD67, CD24, and the CL.Bgran/5 antigen) on her neutrophils was normal, and no abnormalities were found with her red blood cells (RBC) (acid-Ham test and acetylcholine-esterase activity). All other tests (see Results and Discussion) showed an isolated FcRIII deficiency on her neutrophils. Despite this deficiency, the mother was healthy. The only remarkable event was an osteomyelitis of unknown origin in a leg when she was 8 years old. The two other children were healthy, as were her father, mother, and brother. The propositus refused further analysis of the WBC of the children.

Analysis of the maternal antibodies showed no allospecificity when tested with neutrophils from a large panel of donors. Immunoprecipitation on 125I-labeled neutrophils of an NA/NA2 donor using maternal antiserum isolated a protein of 45 to 80 kD with a pattern identical to that precipitated by anti-FcRIII MoAb CLBgran1. The antibodies did not react with the neutrophils of a 27-year-old blood donor, in whom an isolated FcRIII deficiency was found (see Results and Discussion). This second FcRIII-deficient individual was healthy, had no history of repeated or protracted infections, and no clinical features indicating autoimmune or immune-complex pathology. Siblings and parents of this "donor 2" were healthy, although two of five siblings of his mother had died in their fifties from recurrent infections and heart disease and one sibling had died at birth from an unknown cause.

MATERIALS AND METHODS

**Immunohematologic analysis**. Leukocyte suspensions were prepared from venous blood by centrifugation over Ficoll-Hypaque (1.077 g/cm³). Lymphocytes and monocytes were isolated by counterflow centrifugal elutriation of the mononuclear leukocytes, as described by Roos and de Boer. Neutrophils were isolated by lysis of the RBC of the pellet in isonic NH4Cl. These cell suspensions contained greater than 95% neutrophils. Neutrophils were fixed with 1% (wt/vol) paraformaldehyde, washed in phosphate-buffered saline (PBS), and incubated with human anti-NA sera, with anti-FcRIII MoAbs (BL-LGL1, CLB-FcRgran1, BW209/2, YFC120.5, 3G8, VEP13), with the allele-specific MoAbs CLB-gran1 (anti-NA1-FcRIII), GRM1 (anti-NA2-FcRIII) and with control MoAbs. Subsequently, the cells were washed and incubated with a fluorescein isothiocyanate (FITC)-labeled sheep-anti-human-lg or goat-anti-mouse-lg conjugate. The cells were then washed and examined by fluorescence microscopy and/or flow cytometry.

**Binding assay**. Dimeric IgG complexes were made as described. In short, Ig myeloma proteins were incubated with 125I-labeled anti-light-chain F(ab'), in a molar ratio of 1:1. These complexes were separated from unbound IgG by Biogel-A.5M gelfiltration (Biorad Laboratories, Richmond, CA) chromatography. Cells (10⁴/mL) and complexes were mixed and incubated on ice for 30 minutes, layered on a dibutyphthalate/dinonylphthalate oil mixture, and centrifuged to separate unbound complexes from cells with bound complexes. The pellet and the supernatant were counted separately in a multi-gamma counter.

**RNA/DNA analysis**. RNA was isolated from 5 x 10⁶ purified neutrophils, 30 x 10⁶ purified lymphocytes (these lymphocytes contained 15% CD16-positive lymphocytes) or 5 x 10⁶ cells of the FcRIII-negative cell line OVCAR4 (an ovarian carcinoma cell line) as described. DNA was isolated from mononuclear cells as described. Aliquots of the DNA were digested overnight with the indicated restriction enzymes. Hybridization of blots was performed with a 32P-labeled 2.2-kb BamHI-BamHI restriction fragment of pGP5. This fragment contains the entire coding region of FeRIII, including the 5' untranslated region.

RESULTS AND DISCUSSION

In Table 1, it is shown that the neutrophils of the propositus and donor 2 did not react with CD16 MoAb. Neither did these cells react with anti-NA MoAbs or human anti-NA sera. The binding of these antibodies to control neutrophils is also shown in Table 1. In both individuals, the expression of other PI-linked proteins on their neutrophils was normal, as was the expression of FeRIII on their NK cells and on their cultured monocytes. These results indicate an isolated and total absence of the CD16 antigen (FeRIII) on neutrophils, because the various CD16 MoAbs react with different epitopes. Moreover, we also measured the binding of dimeric IgG complexes to neutrophils, because this binding is mediated via FeRIII. Control neutrophils bound approximately 50,000 to 150,000 IgG dimers per cell, but binding of the dimeric IgG complexes to the neutrophils of the two individuals was undetectable. Therefore, we conclude that FeRIII is absent from the neutrophils of these individuals.

Next, we investigated whether this deficiency was caused by a failure in posttranslational processing of FeRIII or by a genetic defect resulting in a lack of mRNA-encoding FeRIII. We did not detect any mRNA-encoding FeRIII in the isolated neutrophils of the propositus, whereas FeRIII-encoding RNA was detected in her lymphocytes (donor 2 was not tested for FeRIII mRNA). To determine the genetic defect that produced this absence of mRNA, we performed restriction-fragment analysis of the region encoding the neutrophil-FcRIII and NK-FcRIII gene. Studies of the locus encoding these two genes have shown that it is possible to distinguish restriction-enzyme fragments of the gene encoding neutrophil FeRIII from those of the gene encoding NK FeRIII (see Fig 1B, the unique restriction sites in each gene are indicated with an arrow). The restriction-fragment analysis of the DNA digested with Tag I, Dra I, and Bam HI, showed that the fragments specific for the gene encoding neutrophil FeRIII (6.5-kb Tag I, 0.9-kb Dra I, 5-kb Bam HI) were absent in the propositus without appearance of any additional fragments (Fig 1A, lanes 4, 8, and 12). DNA digestion with other restriction enzymes (HindIII, NciI, or...
NEUTROPHIL FcRII DEFICIENCY

Table 1. Antibody Binding to Neutrophils

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Propositus</th>
<th>Donor 2</th>
<th>NA1NA1</th>
<th>NA1NA2</th>
<th>NA2NA2</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD16 MoAb</td>
<td>45 ± 90</td>
<td>15 ± 20</td>
<td>30 ± 20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BL-LGL/1</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CLB-FcRgran1</td>
<td>745 ± 250</td>
<td>690 ± 265</td>
<td>650 ± 95</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BW209/2</td>
<td>195 ± 110</td>
<td>240 ± 165</td>
<td>285 ± 86</td>
<td></td>
<td></td>
</tr>
<tr>
<td>YFC120.5</td>
<td>180 ± 100</td>
<td>250 ± 130</td>
<td>175 ± 86</td>
<td></td>
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</tr>
<tr>
<td>3G8</td>
<td>760 ± 285</td>
<td>680 ± 245</td>
<td>690 ± 260</td>
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<tr>
<td>VEP13</td>
<td>350 ± 90</td>
<td>330 ± 70</td>
<td>315 ± 170</td>
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<tr>
<td>Allele-specific CD16 MoAb</td>
<td></td>
<td></td>
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<tr>
<td>CLB-gran11</td>
<td>750 ± 380</td>
<td>410 ± 140</td>
<td>0 ± 5</td>
<td></td>
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<tr>
<td>GRM1</td>
<td>27 ± 24</td>
<td>520 ± 230</td>
<td>550 ± 150</td>
<td></td>
<td></td>
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<tr>
<td>Human antisera</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Anti-NA1</td>
<td>995 ± 193</td>
<td>NT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-NA2</td>
<td>1,125 ± 250</td>
<td>NT</td>
<td>909 ± 131</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD24</td>
<td>420 ± 288</td>
<td>(non-NA-typed controls)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD67</td>
<td>210 ± 143</td>
<td>(non-NA-typed controls)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CLB-gran/5</td>
<td>375 ± 207</td>
<td>(non-NA-typed controls)</td>
<td></td>
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</tbody>
</table>

Mean fluorescence on a FACSCAN. Fluorescence is expressed in arbitrary units, scale 0 to 10,000. The background fluorescence of neutrophils incubated with an isotype-matched control MoAb was subtracted from all values. Data of six controls are given as mean ± SEM.

Abbreviation: NT, not tested.

EcoRI) showed no abnormalities. These results, in combination with the lack of mRNA in the neutrophils, suggest that the gene encoding neutrophil FcRIII is partially or completely deleted. This implies that the phenotype of the propositus is actually "NA-null." DNA analysis of donor 2 revealed the same abnormalities (not shown).

Figure 1 also shows the restriction fragment analysis of the parents of the propositus, who both had the phenotype NA(1-2+). To investigate whether the FcRIII deficiency is caused by a genetic defect, we compared the genotype of the parents with that of control NA2 homozygous donors by measuring the intensity of the 6.5-kb fragments specific for neutrophil FcRIII versus that of the 2.2-kb fragments specific for NK FcRIII. The ratio of the radioactivity in the 6.5-kb and 2.2-kb Taq 1 restriction fragments in normal NA(1-2+) control donors was about 0.5. This result suggests that the parents had one chromosome with a gene encoding neutrophil FcRIII and one chromosome that is deleted for this gene. The parents of donor 2 were not available for studies.

Both the phenotype frequencies in the Japanese population and a report by Clay and Kline21 provide indications that the "NA-null" phenotype exists. Clay and Kline21 reported an NA1 homozygous child born from an NA2 homozygous mother. A possible explanation is that the actual genotype of the mother is NA2NA0 and that of the child NA1NA0. Moreover, Lalezari et al22 suggested the possible existence of a third allele.

The gene mapping of the FcRIII genes has shown two greater than 99% identical genes.2 Therefore, a deletion of the gene encoding neutrophil FcRIII might originate from unequal crossing over between the two FcRIII genes after mispairing of homologous chromosomes during meiosis. Such unequal crossing overs have been described for other genes, such as the α-globin genes.23

We conclude that we have identified a new genomic defect in one of the receptors for IgG: FcRIII on neutrophils. During this study, Schnell et al24 reported another case of severe neonatal alloimmune neutropenia in an NA(1-2-) and therefore presumably FcRIII-deficient mother. The identification of the defect that causes the "NA-null" phenotype and subsequently causes isoimmune neutropenia in the FcRIII-positive children is clinically important. While this manuscript was in preparation, Clark et al25 reported a similar FcRIII-1 gene abnormality in a patient with systemic lupus erythematosus, suggesting that FcRIII-1 deficiency is not an extremely rare phenomenon found only by chance in two Dutch individuals. The 27-year-old blood donor was identified after screening of 60 donors. While this manuscript was under review, we identified a third, presumably FcRIII-1-deficient individual. This individual was a 37-year-old woman, whose blood was sent to our institute to exclude paroxysmal nocturnal hemoglobinuria. Her neutrophils did not express FcRIII, whereas normal expression of other PI-linked antigens was found. Moreover, she did not have any soluble FcRIII in her plasma, a characteristic shared with the patient reported by Schnell et al24 (unpublished observation, T.W.J. Huizinga) and the two individuals described in the present study.26 This woman did not suffer from infections but suffered from anemia, caused by increased RBC destruction in combination with a fatty liver possibly caused by chronic alcoholism. These individuals provide new insights into the relevance of PI-linked FcRIII. Although neutrophil FcRIII is one of the most abundant receptors for IgG, the other FcRs (such as FcRII) apparently possess enough capacity to induce antibody-mediated destruc-

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Fig 1.  (A) Identification of FcRIII sequences in human genomic DNA. DNA isolated from an NA2 homozygous control donor (lanes 1, 5, and 9), the father of the propositus (lanes 2, 6, and 10), the mother of the propositus (lanes 3, 7, and 11), and from the propositus herself (lanes 4, 8, and 12) were digested with *BamH*I (lanes 1 through 4), *Dra*I (lanes 5 through 8), and *Taq*I (lanes 9 through 12). Note that the ratio of the 5.0-kb fragment versus the 18-kb fragment in lane 4 is different from lane 1 or lane 2 and 3. Moreover, the 0.9-kb fragment in lane 8 and the 6.6-kb fragment in lane 12 are missing. The altered ratio between the 5-kb band and the 18-kb band in the *BamH*I digest is indicative for the absence of the neutrophil-FcRIII gene, because the extra *BamH*I site in this gene causes two fragments of 5 kb from the neutrophil-FcRIII gene, whereas *BamH*I digestion of the NK FcRIII yields fragments of 5 kb and 18 kb (see B for the map of the FcRIII genes). The 0.9-kb *Dra*I restriction fragment is indicative for neutrophil FcRIII, because an additional *Dra*I site in the NK gene for FcRIII causes small fragments of 0.55 kb and 0.35 kb (fragments too small to be seen in this blot). Two additional *Taq*I sites in the gene encoding NK FcRIII compared with the gene encoding NA2 FcRIII result in a 2.2-kb fragment from the gene encoding NK FcRIII and a 6.5-kb fragment from the gene encoding neutrophil NA2 FcRIII. (B) Physical map of the FcRIII genes. Restriction map of the NA2 allotypic variant of neutrophil FcRIII and NK FcRIII (obtained from ref 2). *BamH*I; *Dra*I; and *Taq*I. Restriction sites specific for neutrophil FcRIII or NK FcRIII are indicated with an arrow.
tion of pathogens. Ceuppens et al.\textsuperscript{17} have described a family lacking FcRI on their phagocytes. These persons were also healthy. The clinical condition of these people might lead to the assumption that FcRII or the transmembrane form of FcRIII is the most essential Fc receptor in antibody-mediated destruction. However, detailed studies on larger groups of FcRIII-1 gene-deficient individuals are needed to settle whether such individuals may develop diseases in due time (infections, autoimmune diseases, or cancer).

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REFERENCES

Isolation of complementary DNA (cDNA) clones and chromosomal localization. Science 228:1401, 1985


Maternal genomic neutrophil FcRIII deficiency leading to neonatal isoimmune neutropenia [see comments]

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