Kx: Its Relationship to Chronic Granulomatous Disease and Genetic Linkage With Xg

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The relationship between neutrophil function and the neutrophil antigen, Kx, as well as the linkage of the gene, Xk, with Xg was examined in a kindred with X-linked chronic granulomatous disease. Four of the eight male siblings had chronic granulomatous disease by clinical history and tests of neutrophil function, and all four had Kx-negative neutrophils. The remaining four were in good health and had normal nitroblue tetrazolium reduction tests. However, one of these latter four had Kx-negative neutrophils that functioned normally. These data suggest that closely linked but distinct genes on the X chromosome code for chronic granulomatous disease and Kx. In addition, close linkage was demonstrated between Xk and Xg, a gene coding for an erythrocyte surface antigen.

CHRONIC granulomatous disease is a disorder of granulocyte function that is inherited primarily as an X-linked recessive trait, although a similar disorder is transmitted as an autosomal recessive trait in some kindreds. Affected individuals suffer from recurrent infections because their polymorphonuclear neutrophils have defective bactericidal activity against ingested microorganisms. This defect is due to the inability of these cells to generate metabolites of oxygen that are toxic to ingested bacteria. The dysfunction or absence of a membrane-bound oxidase, which catalyzes the one electron reduction of oxygen to superoxide ion, is believed to account for this defect in oxidative activity, but the precise biochemical abnormality has not been established. Recently, granulocytes from patients with X-linked chronic granulomatous disease have also been reported to lack an X-linked, Kell-related surface antigen, Kx. This antigen is present on granulocytes from normal individuals, suggesting that a causal relationship might exist between the absent surface antigen and the defective oxidase activity.

We investigated the association of these two abnormalities in a large kindred with X-linked chronic granulomatous disease. We also examined the possibility of linkage between Xk and Xg loci. Xk alleles code for the surface antigen, Kx, on both granulocytes and erythrocytes, while the Xg locus codes for Xg, the only other erythrocyte surface antigen that is known to have an X-linked mode of inheritance. The data indicate that the absence of Kx from neutrophils is strongly associated with chronic granulomatous disease, but does not preclude normal oxidative neutrophil function. In addition, the Xk and Xg loci appear closely linked on the X chromosome.

MATERIALS AND METHODS

Patients

The pedigree of this three-generation family together with details of the clinical features and neutrophil function studies of the four male siblings with chronic granulomatous disease have previously been reported. The diagnosis of chronic granulomatous disease was established by a compatible history of recurrent infections and the failure of neutrophils from these patients to reduce nitroblue tetrazolium or to exhibit other parameters of an oxidative burst of metabolic activity during phagocytosis.

Red Blood Cell Typing

Red blood cells from all family members were tested for antigens of the ABO, Rh, MN, Lutheran, Kell, Duffy, Kidd, and Xga blood groups using standard techniques.

Kx Typing

Erythrocyte Kx determinations were performed using a standard indirect antiglobulin technique with the result being scored for hemagglutination. Serum with an anti-Kx titer of 32 was obtained from a patient with absent erythrocyte and neutrophil Kx who had been inadvertently transfused with normal Kx-positive red cells. Equal volumes of a 3%-5% suspension of the test subject's red blood cells in isotonic saline and anti-Kx serum were incubated at 37°C for 30 min. The incubation mixture was then centrifuged and the red blood cells washed three times in isotonic saline. The final erythrocyte pellet was incubated with two drops of commercial polyspecific anti-human globulin and hemagglutination scored using the system described by Marsh. Appropriate controls were performed using Kell null (K0) erythrocytes, which have an excess of Kx antigen, and McLeod erythrocytes that lack Kx antigen.

The ability of the test subject's neutrophils to reduce the anti-Kx titer of a standard antiserum was used as a measure of Kx antigen on the neutrophil surface. Neutrophils from 15 ml of ACD-treated peripheral blood were obtained by sedimentation in 10% dextran. The neutrophil-rich plasma was removed, centrifuged (200 g, 10 min, 22°C), and freed of contaminating red blood cells by lysis in hypotonic (0.2%) saline. After washing twice in isotonic saline, the packed neutrophils were suspended in an equal volume of the standard anti-Kx serum, incubated for 1 hr at 37°C, and then
Neutrophil Function

Oxygen consumption, quantitative nitroblue tetrazolium reduction, hexose monophosphate shunt activity, myeloperoxidase-mediated iodination, and bactericidal activity were assessed as previously described using neutrophils isolated from normal volunteers, the four male siblings in this kindred with chronic granulomatous disease and Kx-negative neutrophils, and the one healthy male sibling with Kx-negative neutrophils. In addition, qualitative nitroblue tetrazolium tests were performed on neutrophils obtained from all family members.

RESULTS

Red Blood Cell Typing

None of the family members' sera contained atypical blood group antibodies and direct anti-human globulin tests on all erythrocyte samples were negative. There were no unusual reactions when the erythrocytes were examined for the blood group antigens described in the methods, and none of these results suggested nonparentage. The erythrocytes from all family members were Kx-positive and Kell type K-k+, Kp(a-b+). No weak or unusual reactions with Kell system antisera were observed. Both parents in generation I had Xg(a+) red blood cells, but only five of the eight male siblings in generation II had Xg(a+) on their red blood cells (Fig. 1), indicating that the mother was heterozygous at the Xg locus, i.e., she is genotypically Xg+aXg. The only female sibling in the second generation had Xg(a+) erythrocytes, as would be expected since her father's erythrocytes were also Xg(a+).

Neutrophil Kx Typing

As shown in Fig. 1, all four male siblings in generation II with chronic granulomatous disease lacked Kx on their neutrophils. However, neutrophils obtained from the last born male sibling who did not have a clinical history suggestive of chronic granulomatous disease and whose qualitative nitroblue tetrazolium test was normal, also lacked Kx when separate samples were examined on two occasions. The three remaining healthy male siblings all had Kx-positive neutrophils. Therefore, the mother must also be heterozygous at the Xk locus.

Neutrophil Function

Since the absence of neutrophil Kx antigen has previously only been recognized in patients with chronic granulomatous disease, we studied the neutrophil function of the single male in this family who had Kx-negative neutrophils, but who did not have a history of repeated infection (Table I). Neither a quantitative defect, as measured by the quantitative nitroblue tetrazolium test, hexose monophosphate shunt, or iodination, nor a kinetic defect, as measured by continuously monitored oxygen consumption and bactericidal activity, could be demonstrated.

DISCUSSION

The Kell blood group is a system of autosomally determined allelic antigens present on red, but not white, blood cells. It has been proposed that Kx is a precursor substance that is converted to the appropriate Kell antigens by an enzyme coded for on the autosome determining Kell type. In contrast to the Kell antigens, Kx is normally present on both red cells and granulocytic white cells, although lesser amounts are detected on red cells, presumably due to the conversion of Kx to Kell system antigens. It has further been suggested that Kx results from the
expression of an X-linked structural gene, Xk. Erythrocytes and granulocytic white cells may possess Kx independent of one another, depending on which of four recognized Xk alleles occupies the Xk locus. Thus, the Xk allele codes for Kx on both cell types (normal phenotype), but Xk results in the absence of Kx from both cell types (chronic granulomatous disease, McLeod phenotype). The Xk allele codes for Kx on red cells but not granulocytes (chronic granulomatous disease phenotype), while the reverse is true for Xk (McLeod phenotype). In the family reported here, all members had Kx-positive red cells. However, five of the males in the second generation had Kx-negative granulocytes and three had Kx-positive granulocytes. These data indicate that the mother must be heterozygous at the Xk locus and must have the Xk allele codes for Kx on red cells but not granulocytes (chronic granulomatous disease phenotype), while the reverse is true for Xk (McLeod phenotype). In the family reported here, all members had Kx-positive red cells. However, five of the males in the second generation had Kx-negative granulocytes and three had Kx-positive granulocytes. These data indicate that the mother must be heterozygous at the Xk locus and must have the Xk allele codes for Kx on red cells but not granulocytes (chronic granulomatous disease phenotype), while the reverse is true for Xk (McLeod phenotype). In the family reported here, all members had Kx-positive red cells. However, five of the males in the second generation had Kx-negative granulocytes and three had Kx-positive granulocytes. These data indicate that the mother must be heterozygous at the Xk locus and must have the Xk allele codes for Kx on red cells but not granulocytes (chronic granulomatous disease phenotype), while the reverse is true for Xk (McLeod phenotype).

The results of Kx and Xg typing were analyzed in the eight male siblings. All five males with Kx-negative neutrophils had Xg(a−) red cells, while all three with Kx-positive neutrophils were Xg(a+). These data provide strong evidence for genetic linkage between Xk and Xg. The lod score calculated for the linkage between the Xk and Xg loci in this family is +2.104 at a recombination fraction of zero. A lod score of +2.0 or greater is customarily accepted as the level of significance for linkage of X-borne characters.

Individuals of the Xk or Xk genotype lack Kx on their red blood cells, and their red cells are morphologically abnormal. This abnormality of the red cell surface is associated with a mild compensated hemolytic anemia. Individuals of the Xk or Xk genotype lack Kx on their granulocytes. This lack of Kx has shown a high degree of association with X-linked chronic granulomatous disease, a disorder believed to result from the dysfunction of a membrane oxidase. The rare patients with the Xk genotype have both abnormal red and white cells. At the present time, we are unaware of any individual with X-linked chronic granulomatous disease whose neutrophils have been Kx positive. That all persons with Kx-negative neutrophils have chronic granulomatous disease does not appear to be true, since one male sibling in this kindred has Kx-negative neutrophils but does not have either clinical or laboratory evidence of chronic granulomatous disease. The clinical expression of chronic granulomatous disease in this kindred appears to be relatively mild in that the four affected males did not experience recurrent infections until the age of 5, they have all survived into their 30s, and appear to have overcome their susceptibility to recurrent infection. Given the relatively mild clinical course in the affected brothers with Kx-negative neutrophils, we investigated the unaffected male sibling with Kx-negative neutrophils for the possibility of a partial defect in the function of his neutrophils. We were unable to identify any defect in neutrophil oxidative function. These results demonstrate that while the absence of Kx from neutrophils is closely associated with chronic granulomatous disease, the presence of this antigen is not required for normal neutrophil function. Our data are consistent with the interpretation that separate genes on the X chromosome code for chronic granulomatous disease and Xk. If this is the case, then the normal brother with Kx-negative neutrophils reflects a recombination between the chronic granulomatous disease and Xk loci. However, the recombination did not separate the Xk and Xg loci, suggesting that the order of these three genes on the X

### Table 1. Neutrophil Function Studies

<table>
<thead>
<tr>
<th>Function*</th>
<th>Normal</th>
<th>Patient†</th>
<th>CGD‡†</th>
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<tbody>
<tr>
<td>Qualitative NBT†§% reduction (%)</td>
<td>92 ± 2.7</td>
<td>91 ± 4.1</td>
<td>2.8 ± 1.0</td>
</tr>
<tr>
<td>Quantitative NBT reduction (%)</td>
<td>100</td>
<td>82.5 ± 18.5</td>
<td>29.8 ± 13.1</td>
</tr>
<tr>
<td>Hexose monophosphate shunt (%)</td>
<td>100</td>
<td>80.0 ± 3.0</td>
<td>26.3 ± 2.0</td>
</tr>
<tr>
<td>Iodination§ (cpm/106 PMN)</td>
<td>24,063 ± 956</td>
<td>26,035 ± 1,658</td>
<td>8,546 ± 226</td>
</tr>
<tr>
<td>Oxygen consumption§ (µl O2/hr/106 PMN)</td>
<td>9.04 ± 1.92</td>
<td>7.27 ± 1.20</td>
<td>0.43 ± 0.15</td>
</tr>
<tr>
<td>Bactericidal activity§ (% bacteria killed)</td>
<td>96.6 ± 2.8</td>
<td>89.6</td>
<td>12</td>
</tr>
</tbody>
</table>

*Mean ± SEM.†Except for bactericidal activity, each test was performed on the patient or on the 4 siblings with CGD 2–3 times.‡CGD, chronic granulomatous disease; NBT, nitroblue tetrazolium; PMN, polymorphonuclear neutrophils.§Patient but not CGD is within 2 SD of the mean for normal.
Kx, CHRONIC GRANULOMATOUS DISEASE, AND Xg

chromosome may be chronic granulomatous disease–
Kx–Xg. It should be noted that this interpretation does
not explain the high degree of association (linkage
disequilibrium) of chronic granulomatous disease with
Kx-negative granulocytes.

Other possible explanations for the separation of
Kx-negative neutrophils and chronic granulomatous
disease must be considered. The procedure for detect-
ing Kx on granulocytes is an indirect method that
measures the residual hemagglutination titer in a
standard anti-Kx antiserum after it has been absorbed
by the test granulocytes. Although giving reproducible
results, the method has low sensitivity. It is possible
that the neutrophils of this sibling have a reduced
amount of Kx antigen that is sufficient for functional
activity but which is below the level of detection by the
present technique. Another possibility is that some
compensatory process operating at the phenotypic
level on the neutrophil membrane results in partial or
full protection from the disease. Further studies of the
Kx antigen on neutrophils from normal individuals as
well as from individuals with chronic granulomatous
disease are needed to distinguish among these possibil-
ities. Evaluation of the descendants of males with
Kx-negative neutrophils may prove to be particularly
rewarding.

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