Wiskott-Aldrich Syndrome: Cellular Impairments and Their Implication for Carrier Detection

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A family in which two male siblings were affected with Wiskott-Aldrich syndrome (WAS) was studied using G-6-PD isoenzymes as an X-linked marker in order to investigate the nature of cellular abnormalities. Isolated peripheral blood cell types from the doubly heterozygous mother of the affected males seemingly failed to express the G-6-PD allele in cis position with the WAS allele while her cultured skin fibroblasts expressed both G-6-PD alleles. Additionally, a histogram analysis of platelet size revealed a single population of abnormally small platelets in the affected propositus, whereas the heterozygous mother had an appreciable small platelet subpopulation. In vitro culture of hemopoietic progenitor cells of the heterozygous mother showed that the majority of progenitor cells did not express the WAS allele. However, a small number of cells expressing the G-6-PD type linked with the WAS allele were detected. The proportion of the latter progenitors was significantly higher among more primitive progenitors (those giving rise to later appearing colonies). This observation suggests that selection against cells expressing the Wiskott-Aldrich defect takes place in the hemopoietic system of the heterozygous female and offers a possible means of carrier detection in some women. Linkage studies in this family revealed one example of probable recombination between the loci for WAS and G-6-PD among three informative subjects, suggesting that these two loci may not be closely linked on the X-chromosome.

WISKOTT-ALDRICH SYNDROME (WAS) is an X-linked, recessive disease characterized by immunologic abnormalities, thrombocytopenia, and abnormal platelet size. It has been recently demonstrated that the small platelet size observed in WAS subjects appears to be a secondary abnormality since it disappears after splenectomy. Affected patients suffer from frequent infections, hemorrhagic manifestations, and eczema. Additionally, some affected patients eventually succumb to lymphoma or leukemia. The exact metabolic lesion in WAS is not known; however, it has been suggested that aberrant metabolism of nucleotides may occur in this disorder. It has also been suggested that this defect may lead to abnormal platelet aggregation in females heterozygous for WAS, perhaps permitting detection of the carrier state.

Evidence has been presented that females heterozygous for G-6-PD (X-linked enzyme) and for Lesch-Nyhan syndrome (X-linked disease) have a decreased or nondetectable number of blood cells that express the Lesch-Nyhan gene. More recently it has been suggested that selection against cells expressing the gene for Incontinentia Pigmenti occurs among blood cells and skin fibroblasts of women heterozygous for this X-linked disorder. This report describes a black family in which two brothers had WAS and in which their mother was heterozygous for G-6-PD. We have undertaken this study to determine whether a similar phenomenon to that described in Lesch-Nyhan syndrome also occurs in WAS and, if so, the mechanism of this phenomenon. In addition, investigations designed to establish the respective positions of WAS, G-6-PD, and other X-linked genetic loci on the X chromosome were carried out.

MATERIALS AND METHODS

Subjects
A black male born in February, 1975, III-3 presented to Children's Hospital in Birmingham at age 6 mo with eczema; his platelet count ranged between 16,000 and 40,000/cu mm and he had iron deficiency anemia. A sickle cell preparation was positive and hemoglobin electrophoresis revealed an AS pattern. At age 24 mo, he had hemoglobinopathy, and expired shortly thereafter.

In June, 1978, after a febrile episode accompanied by GI, GU, and pulmonary hemorrhage, the patient was admitted to the hospital and expired shortly thereafter.

The propositus, III-4, a black male born in January, 1977, had bronchiolitis and a skin rash when first admitted to the Children's Hospital in Birmingham at age 1 mo. Peripheral blood counts, including platelet and reticulocyte counts were normal. At 11 mo of age, fever, anemia, thrombocytopenia (platelet count 26,000 to 47,000/cu mm), eczema, and possible sepsis were noted. Ten subsequent hospital admissions ensued for fever of unknown origin...
or secondary to documented pneumonia or septicemia. On several other occasions, emergency room treatment was given for febrile episodes. At age 20 mo, this child’s IgA was elevated to 300 mg% (normal level for this age is 9-143 mg/100 ml) and there were no detectable isohemagglutinins to red cell A and B antigens (patient was blood group B). More recently, this patient has developed iron deficiency anemia secondary to repeated gastrointestinal bleeding.

Other family members had no record of frequent febrile episodes, bleeding episodes, or eczema. The boy’s maternal uncle, II-5, has sickle cell anemia. Their two-half-sisters, III-1 and III-2, 9 and 7 yr old, respectively, are healthy as is their mother, II-3, and their grandmother, I-2. The family pedigree is depicted in Fig. 1.

Cell Separation

Fibroblast cultures from the propositus’ mother (II-3) and from one of his sisters (III-2) were established from skin biopsies using a 3 mm punch, and blood cells were separated as described previously.17

In Vitro Culture of Hematopoietic Cells

Mononuclear cells from marrow and blood were obtained from the interphase of a Ficoll-Hypaque gradient.18 The cells were then cultured as previously described.19

G-6-PD Electrophoresis

G-6-PD electrophoresis of cultured skin fibroblasts and of separated blood cells was performed on cellulose acetate strips as previously described.19 The G-6-PD electrophoresis on in vitro cultured colonies was performed using a Helena cellulose acetate system (Helena Laboratories, Beaumont Tex.). Individual colonies were placed on Titan III cellulose acetate plates, presoaked in an electrophoretic buffer (pH 8.2) that contained saponine. The lysis of cultured colonies was performed using a Helena cellulose acetate strip electrophoretic system (Helena Laboratories, Beaumont Tex.).

Platelet Histograms

A histogram analysis of platelets was performed (using EDTA anticoagulated peripheral blood) by a Coulter-Counter Model S+ (Coulter Electronics, Hialeah, Fla.). These histograms graphically evaluated the proportion of particles that ranged in size from 2 to 20 cuμ.

Fig. 1: Pedigree of the WAS family. The two affected males are represented by darkened squares. Apparent G-6-PD genotypes are indicated on the pedigree, with the exception of I-2 where only phenotype was determined. I-1, I-3, II-1, and II-2 were unavailable for study.

Results

Separation of G-6-PD deficient red cells from red cells having normal G-6-PD activity. The separation of G-6-PD deficient erythrocytes from normal erythrocytes was performed using blood from females who were either known carriers or potential carriers of Wiscott-Aldrich syndrome (II-3, III-1, III-2). This separation is dependent upon the presence of S hemoglobin along with non-S hemoglobin as seen with sickle cell trait, a hemoglobinopathy present in all female family members. The details of this method have been previously described.2

Typing for Other X-Linked Genetic Markers

Erythrocyte Xg phenotype determination. This X-linked red cell marker was assayed in most of the family members by Dr. Ruth A. Sanger and the MRC Blood Group, London, as previously described.22

Thyroxin-binding globulin phenotype determination. The recently described X-linked marker in the serum, thyroxin-binding globulin, was assayed by Dr. L. L. Cavalli-Sforza, Stanford University, according to his originally described method.23

Color vision testing. Five family members underwent color vision testing. The four subjects old enough for psychophysical testing (II-3, II-5, III-1, and III-2) had color vision testing performed by the Farnsworth-Munsell 100 Hue and Farnsworth Panel D-15 tests.24 The fifth patient, III-4, because of his young age, had his color vision evaluated by recording visually evoked potentials over the occipital cortex, elicited in response to his viewing alternating checker boards of varying colors.

Results

G-6-PD Isoenzymes of Separated Peripheral Blood Cells and Fibroblasts

The erythrocytes from II-3 expressed G-6-PD isoenzymes A and B in a visually estimated ratio of 3:1 whereas her buffy coat exhibited only type A (Fig. 2A). Her separated granulocytes, platelets, monocytes, B-lymphocytes, and T-lymphocytes had only the A isoenzyme detectable (Fig. 2B), whereas repeated study of her erythrocytes revealed isoenzymes A and B in a 3:1 ratio. Cultured skin fibroblasts from II-3 expressed G-6-PD types A and B in equal proportions. The G-6-PD electrophoresis studies of red blood cells and the buffy coats of I-2, II-4, II-5, III-1, III-2, and III-4 revealed the G-6-PD type B isoenzyme only. In addition, cultured skin fibroblasts from III-2 were G-6-PD type B. The erythrocytes and the buffy coat isolated from III-1 contained G-6-PD isoenzymes A and B. Both isoenzymes were also present in her isolated granulocytes, platelets, and B- and T-enriched lymphocyte preparations in a visually estimated ratio of 4:6 (A:B).

Platelet Histograms

Figure 3 shows histograms of III-4 (WAS propositus) and II-3, II-4, III-1, and III-2. The abnormal configuration III-4 is apparent and confirms the reported presence of small platelets, which is one
characteristic of this disease. On the other hand, the histograms of an obligate heterozygote (II-3) and those who might be heterozygous for WAS (III-1, III-2) are not appreciably different from the histogram of the father (II-4), who, since he does not carry the WAS gene, serves as a normal control.

Culture of Hemopoietic Progenitors

Hemopoietic colonies originate from a single cell and are thus, in a G-6-PD heterozygote, type A or B but not AB. Hemopoietic cultures were established in subject II-3 (WAS heterozygote, G-6-PD AB heterozygote) and maintained for 29 days. The proportion of G-6-PD A and B types of individual neutrophil/macroage and erythroid colonies harvested on day 8–29 is shown in Table 1. Only a small number of erythroid colonies were grown from peripheral blood. A majority of the erythroid colonies grown from bone marrow grew in the first half of the study. The comparison of A/B ratios of the colonies (regardless of the type) revealed no significant difference (p > 0.9) between marrow and peripheral blood. There were 949 A and 53 B colonies in the marrow and 594 A versus 35 B colonies grown from peripheral blood. Similarly, comparison of G-6-PD A/B ratios between erythroid colonies and those colonies growing neutrophil/macrophages did not reveal any significant difference with respect to the cell type (p > 0.01). A significant difference (p < 0.005) was found when a comparison was made between A/B ratios of colonies harvested early and late (Table 2). For the purpose of this analysis the data were arbitrarily divided into first and second 2-wk periods. There were relatively more B colonies in the second half of the study; thus, there were more hemopoietic colonies carrying G-6-PD isoenzyme B (in this case a presumed marker for WAS colonies) among colonies appearing later in the culture. These late colonies likely originate from more primitive hemopoietic precursors.

Separation of G-6-PD Deficient Red Cells From Those Having Normal G-6-PD Activity

The G-6-PD electrophoretic variant A may represent either an A* or an A- isoenzyme, each the product of a different gene. The fact that females potentially or actually heterozygous for WAS in this kindred may also be heterozygous for G-6-PD makes the actual maternal contribution of specific G-6-PD isoenzymes of crucial importance for the analysis of the linkage of the G-6-PD and WAS gene loci. Table 3 presents the data indicating that the genotype of II-3 is likely A-B and III-1 is A-B. Different proportions of methemoglobin in the filtered cells of III-2 suggested that a minor population of A- erythrocytes may have been present. This possibility, however, appeared to be ruled out by the fact that her fibroblasts contained only G-6-PD type B.

The older of the WAS boys (III-3) was not alive at the time of this study, however, a quantitative G-6-PD analysis of his erythrocytes prior to his death revealed normal enzyme activity. Since his mother’s (II-3) genotype was G-6-PD A-B, it can be assumed that III-3 was G-6-PD type B.
PLATELET HISTOGRAMS

Fig. 3. Histogram analysis of platelets from II-3, II-4, III-1, III-2, and III-4. Each histogram graphically evaluates the proportion of particles that ranged in size from 2 to 20 µm.

Typing for Other X-linked Genetic Markers

Samples from all available family members (I-2, II-3, II-4, II-5, III-1, III-2, III-4) were typed for Xg erythrocyte antigens. All blood samples typed were Xg(a+).

Serum samples from the same individuals were typed for heterogeneity of thyroxin-binding globulin. All were of a common phenotype, c, with the exception of III-1 who had, in addition, a product of another allele, s, which must have been inherited from her father.

Color Vision Testing

The unaffected uncle (II-5) of the WAS propositus was not evaluable due to poor cooperation. On Farnsworth-Munsell 100 Hue testing, the sisters of the propositus (III-1 and III-2) made total error scores of 267 and 139, respectively. Errors were widely distributed with no clear protan, deutan, or tritan axis. The mother (II-3), an obligate carrier of a gene for Wiskott-Aldrich syndrome, had a total error score of 199, with no particular axis demonstrable by this test or by the Panel D-15 test. The mean total error score of women homozygous for normal color vision is 40 ± 10. The distribution and total of errors in our three female subjects (II-3, III-1, III-2) are entirely consistent with heterozygous carrier performance on this test.26

The propositus (III-4) demonstrated good visual electroencephalograms to checkerboards of alternating black-white and alternating blue-yellow, but no

Table 1.

<table>
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<tr>
<th>Day of Harvest</th>
<th>8</th>
<th>9</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>15</th>
<th>18</th>
<th>20</th>
<th>22</th>
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<tr>
<td></td>
<td>E</td>
<td>9:0</td>
<td>27:0</td>
<td>43:1</td>
<td>2:0</td>
<td>44:1</td>
<td>13:0</td>
<td>9:0</td>
<td>—</td>
<td>—</td>
<td>—</td>
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<td>4:0</td>
<td>1:0</td>
<td>1:0</td>
<td>—</td>
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<td>—</td>
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</table>

The numbers of G-6-PD type A and G-6-PD type B colonies (A:B) grown in bone marrow (BM) and peripheral blood (PB). Granulocyte/macrophage colonies (C) and erythroid colonies (E) are defined in the text. The day of harvest of the colonies dated from the day of initiation of the cultures is indicated in the upper column.
Table 2. Numbers of G-6-PD Type A and Type B Colonies in First 2 wk and Second 2-wk Periods of Study

<table>
<thead>
<tr>
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<th>Day 8-15</th>
<th>Day 16-29</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-6-PD A</td>
<td>520</td>
<td>1,023</td>
</tr>
<tr>
<td>G-6-PD B</td>
<td>15</td>
<td>73</td>
</tr>
<tr>
<td>Total all colonies (C and E from BM and PB)</td>
<td>535</td>
<td>1,096</td>
</tr>
</tbody>
</table>

Abbreviations: C, granulocyte/macrophage colonies; E, erythroid colonies; BM, bone marrow; PB, peripheral blood.

detectable response could be obtained to alternating red-green checks, indicating a red–green color defect.

DISCUSSION

Selection Against WAS Cells

The G-6-PD locus is on the X-chromosome and is subject to the X-chromosome inactivation process.27 Thus, in females heterozygous for G-6-PD isoenzymes A and B, two populations of cells are present, those expressing type A and those expressing type B. Likewise, females heterozygous for WAS might be expected to have two populations of cells, those which express the WAS defect and those which do not (e.g., small and normal size platelets). In females heterozygous for WAS, heterozygosity for G-6-PD isoenzymes could provide a readily detectable genetic marker of cells expressing the WAS mutation in all tissues.

Subject II-3 was heterozygous for WAS and for G-6-PD. Among her nucleated blood cells and platelets analyzed either as buffy coat or individual cell isolates only G-6-PD type A was detected. In contrast, among erythrocytes both A and B types were detected. This latter finding was probably caused by the fact that erythrocytes that express the A⁺ isoenzyme have a much lower G-6-PD activity than the G-6-PD A⁻ nonerythroid cells. Consequently, the visually estimated ratio of erythrocyte G-6-PD A to G-6-PD B (3:1) in the electrophoretic study represents in reality a much higher proportion of erythrocytes expressing the A⁺ isoenzyme, as opposed to those expressing the B isoenzyme. Since her cultured skin fibroblasts expressed G-6-PD isoenzymes A and B in equal proportions, the presence of only a single detectable G-6-PD isoenzyme phenotype in the peripheral blood leukocytes and platelets of II-3 (and predominance of her erythrocytes expressing the same G-6-PD isoenzyme) indicated that selection, either for or against some X-linked gene product (probably the WAS mutant) was taking place in her hemopoietic cells. The platelet histogram of II-3 appeared normal (without a small platelet subpopulation characteristic of WAS and indeed found in her son); thus, it would seem that selection favored normal, G-6-PD type A⁻ cells. Thus, her hemopoietic cells that expressed the WAS trait were presumed to be G-6-PD type B. If this selection is a consistent feature among women who are carriers of the WAS mutation, the presence of only one G-6-PD isoenzyme in all or some hemopoietic cell lines allows the possibility of carrier detection in those females who are also heterozygous for G-6-PD isoenzymes.

Recently, another black family with WAS was studied using G-6-PD isoenzymes.28 The authors concluded that selection against the WAS allele took place among only platelets and T-lymphocytes of their doubly heterozygous subject, however, it is possible that in their WAS carrier selection also occurred to a lesser degree in the other peripheral blood types as well.29

One daughter (III-1) of the WAS heterozygote (II-3) was heterozygous for G-6-PD types A⁺ and B. Unlike her mother, the peripheral blood cells of III-1 expressed both G-6-PD isoenzymes. Two interpretations of this finding were considered. (1) III-1 inherited the genes for G-6-PD type B and for WAS from her mother; however, selection against cells expressing the WAS defect (and G-6-PD type B) did not take place among her hemopoietic cells. (2) III-1, as the result of meiotic recombination between the WAS and G-6-PD gene loci, inherited G-6-PD type B from her mother but did not inherit the WAS gene. This latter explanation is likely the correct one since III-1 had no significant proportion of small platelets detectable in

Table 3.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>G-6-PD Before Study</th>
<th>G-6-PD After Filtration in Cells Not Passing Filter</th>
<th>G-6-PD in Filtrate</th>
<th>MeHb% Prior to Gassing</th>
<th>MeHb% After Filtration in Cells Not Passing Filter</th>
<th>MeHb% in Filtrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>II-3</td>
<td>1.08</td>
<td>1.81</td>
<td>0.47</td>
<td>52.65</td>
<td>21.55</td>
<td>62.72</td>
</tr>
<tr>
<td>III-1</td>
<td>12.71</td>
<td>11.12</td>
<td>12.23</td>
<td>37.1</td>
<td>28.03</td>
<td>27.47</td>
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<tr>
<td>Normal control</td>
<td>(Caucasian male)</td>
<td>8.44</td>
<td>6.7</td>
<td>7.18</td>
<td>34.4</td>
<td>20.3</td>
</tr>
</tbody>
</table>

*Mean of 3 different experiments.

Separation of G-6-PD deficient erythrocytes from G-6-PD nondeficient erythrocytes. G-6-PD activity is expressed in international units per gram of hemoglobin. The percentage of methemoglobin (MeHb) is expressed in percent of the total hemoglobin concentration.
her platelet histogram as might be expected if the G-6-PD type B platelets were also WAS cells. It was concluded that III-1 was not a carrier of WAS. Since neither I-2 nor III-2 were heterozygous for G-6-PD isoenzymes, no conclusions regarding their status as possible WAS heterozygotes could be made.

It is noteworthy that previous investigations have shown that selection favoring normal cells over cells that are deficient in hypoxanthine-guanine-phosphoribosyl-transferase occurs in the hemopoietic system of females heterozygous for Lesch-Nyhan syndrome, another X-linked disorder. Interestingly, it has been suggested that WAS may also be characterized by abnormal purine metabolism.

Normally, X-chromosome inactivation in a female who is heterozygous for an X-linked gene will result in a mixture of two cell populations. One would predict that a female who carries an X-linked gene that leads to production of small platelets would have two platelet populations characterized by a different mean platelet size. The hemizygous propositus who had WAS (III-4) did indeed have an abnormal small platelet population. The WAS obligate heterozygote (II-3), her possibly heterozygous mother (I-2), and her potentially heterozygous daughters (III-1, III-2) had platelet histograms indistinguishable from those of the normal control (II-4). This finding does not rule out a minute, but by this method, undetectable, subpopulation of small platelets, however, it does not support the recent contention that it is possible to detect WAS heterozygotes by studies of platelet function. Our data suggest that the absence of a significant proportion of abnormal platelets, at least in our WAS heterozygous subject, would not be expected to materially contribute to an overall abnormal platelet function.

Our investigation directed to elucidation of the mechanism of loss of WAS peripheral blood cells utilized in vitro studies of hemopoietic cell proliferation. The obligate heterozygote for WAS (II-3) was shown to have a small but detectable number of G-6-PD type B colonies (a marker of the WAS mutation). Furthermore, a higher proportion of G-6-PD type B colonies was found in those colonies appearing late in culture. This finding is compatible with the hypothesis that the WAS metabolic defect may be detrimental to the more committed hemopoietic precursors or that the WAS metabolic defect is associated with a delayed proliferation/maturation of those hemopoietic cells expressing the WAS mutation.

Genetic Linkage

Providing the locus of G-6-PD was in close proximity to the WAS locus on the X-chromosome, prenatal diagnosis of WAS might be made in a female doubly heterozygous for both of these X-linked genes. Analysis of the family studied indicated that, in II-3, the genes for G-6-PD type B and WAS were in cis arrangement on the same X-chromosome. This assumption was based on several independent lines of evidence: (1) the apparent hemizygous G-6-PD type A phenotype of the leukocytes and platelets of II-3; (2) in vitro culture of hemopoietic cells for II-3 that demonstrated a predominance of G-6-PD type A cells; (3) platelet histograms revealing a single population of small platelets in III-4 but an absence of any significant population of small platelets in II-3.

Typing of our family for heterogeneity of other X-linked genes (specifically thyroxin-binding globulin and the Xg blood group), provided no information relevant to the position of the WAS locus on the X chromosome. Based on qualitative and quantitative G-6-PD studies it appeared that all four offspring of II-3 inherited their mother's gene for G-6-PD type B. The color vision data from those family members who were tested supported this notion. The loci for G-6-PD and deutan colorblindness are known to be closely linked on the X chromosome. Consequently, recombination between genes at these loci is infrequent. Since III-4 was G-6-PD type B and colorblind, it follows that the genes for both traits were in cis arrangement on the same X chromosome in his mother (II-3). Since color vision analysis indicated that both III-1 and III-2 were heterozygous carriers of colorblindness, it is likely that both also inherited their mother's gene for G-6-PD type B.

Recombination between two genes on the same chromosome is more likely to occur when the loci for the genes are not in close proximity to each other. In III-3 and III-4 the genes for G-6-PD type B and WAS were nonrecombinant. III-1 inherited her mother's gene for 6-6-PD type B, but apparently did not inherit the gene for WAS, therefore, recombination has occurred. It is not possible to accurately assess from information on a single small kindred the degree of linkage between the loci for WAS and G-6-PD on the X-chromosome. Interestingly, a study of another black family demonstrated one example of recombination between the loci for WAS and G-6-PD among two informative individuals. These data and ours showing one example of probable recombination among three informative subjects suggests that the two loci may not be closely linked.

ACKNOWLEDGMENT

We are indebted to Dr. Ruth A. Sanger and the MRC Blood Group, London, and to Dr. L. L. Cavalli-Sforza, Stanford University, for providing some data for this investigation and to Drs. Alexander R. Lawton and Max D. Cooper, University of Alabama in Birmingham, for their thoughtful criticism of the manuscript.
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