The Pattern of Genetic Transmission of the Leukocyte Defect in Fatal Granulomatous Disease of Childhood

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ABSTRACT The leukocyte-phagocytic function test which was found to be abnormal in boys with fatal granulomatous disease of childhood has been found to be abnormal to an intermediate extent in their mothers. Nine of nine mothers were shown to be abnormal, whereas none of eight fathers and none of five healthy brothers exhibited a defect. 10 of 16 female siblings were abnormal to the same degree as their mothers, as were all three maternal grandmothers available for study. Assuming that this intermediate functional defect represents the heterozygous state, the nine family pedigrees are entirely compatible with the concept that the trait is transmitted on the X-chromosome.

A tetrazolium dye-phagocytosis histochemical test was also abnormal in the carrier females and provided independent confirmation of the selection of the female siblings suspected of being carriers for the trait. In addition, this procedure gives indirect evidence that the gene in question is subject to the random inactivation that appears to affect many X-linked genes in mammalian females.

The family members were also studied with two of the metabolic assays that have been shown to be abnormal in the cells of affected boys. One assay, the oxidation of the first carbon of glucose-$1^{-14}$C by the isolated leukocytes, was significantly abnormal in the cells of carrier females. The other assay, the oxidation of formate-$14$C by leukocytes of heterozygotes was not significantly different from control values.

The practical problem of diagnosing patients would appear to be best solved with a tetrazolium dye procedure, whereas the more subtle abnormality in carrier females is best detected with the leukocyte function test. Improved methods for the function test are being developed.

INTRODUCTION

Peripheral blood leukocytes of children with the granulomatous disease of childhood have recently been shown to be defective in their capacity to kill certain bacteria in vitro. Furthermore, these leukocytes have been found to have abnormalities in the known metabolic concomitants of actively phagocytizing cells.

The disease in which the abnormal leukocytes are found has been reported from several areas in the United States (for review, see reference 1) and recently from Europe (2, 3). The initial manifestations are an eczematous dermatitis and fluctuant cervical or inguinal adenopathy, developing in male children during the first 18 months of life. Chronic infiltrative pulmonary disease with superimposed acute episodes, hepatosplenomegaly, and osteomyelitis are the more serious continuing problems, and pericarditis, stomatitis, and abscesses in a variety of locations are frequent. Various bacteria usually considered to be of low virulence can often, but not always, be cultured from supplicative lesions and granulomas. Studies

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Received for publication 16 August 1967 and in revised form 30 October 1967.

1026 The Journal of Clinical Investigation Volume 47 1968
of immune capacity yield normal findings and the patients react appropriately to known virus agents, although MacFarlane, Speirs, and Sommerville (2) found abnormalities in the capacity of these leukocytes to reduce the number of Vaccinia or Herpes simplex organisms in vitro.

Berendes, Bridges, and Good (4) and Bridges, et al. (5) suggested that this syndrome was familial. Subsequent recognition of affected brothers of patients studied in our clinic has strengthened this possibility as has the study of Carson, Chadwick, Brubaker, Cleland, and Landing (6).

Early in our studies of polymorphonuclear leukocyte function, it became evident that the leukocytes of the mothers of affected boys were also abnormal in the in vitro test. Further, three male maternal cousins of patients were found to have developed early signs of granulomatous disease. Thus it became probable that this defect was genetically determined and X-linked.

It is the purpose of this paper to report that studies of the polymorphonuclear leukocyte defects indicate that they are transmitted in an X-linked manner, and that the carrier state in female siblings can be predicted. Our original term “fatal” seems justified, since the gene exhibits a high degree of lethality for the affected boys.

METHODS

53 members of nine families were studied. One family includes affected half brothers, each from a different father. All first degree family members except 3 of 11 fathers and 4 of 15 diseased boys were examined. Three maternal grandmothers representing four families and one maternal grandfather were also studied. This population was analyzed using three different approaches; (a) a leukocyte function test, (b) two metabolic analyses, and (c) a combined histochemical-phagocytosis procedure.

I. Leukocyte function test

The leukocyte function test developed by Holmes, Quie, Windhorst, and Good (7) was used. The major difference between this procedure and that of Quie, White, Holmes, and Good (8) is the addition of antibiotics to the culture at 20–30 min to eliminate extracellular bacteria. That most of the bacteria which can be cultured at the 2-hr time interval are intracellular is inferred from the fact that cultures set up without cells, but which are otherwise identical to the test system, will not have culturable bacteria present at that time (9 and footnote 1).

That the carrier females' leukocytes are intermediate in capacity to kill staphylococci is demonstrable without adding antibiotics; however, this addition accentuates the abnormality and provides increased reliability in recognition of the carrier state. 1,2 Antibiotics make it possible to obtain reliable results with a shaking water bath as the culture method, if end-over-end tube mixers are not available. We found that a compact, inexpensive tilter Lab-Tech aliquot mixer, (Lab-Tech Instrument Co., Westmont, Ill.) gives excellent results in the procedure either with or without antibiotics. Substitution of sterile, disposable, plastic equipment (such as culture tubes) for siliconized glassware has proved feasible.

Because of the variability in the leukocyte function test, each day's study was made complete in itself. A culture using normal cells was always included as a negative control and one using cells from one of the affected boys was usually included as a positive control. In eight families, this control was their own family member.

II. Metabolic studies

Holmes, Page, and Good (1) have found the peripheral leukocytes of affected boys to be abnormal in 4 of 12 major metabolic parameters which they examined. Two of these, (a) the capacity of the phagocytizing cells to increase the oxidation of the first carbon of glucose and (b) the oxidation of formate were selected for family screening studies. The methods used were those of Holmes et al.

III. Histochemical procedure

For these studies, an adaptation of the original method of Baehner and Nathan (10) was developed. This test is based on their observation that phagocytizing leukocytes of the affected boys do not reduce tetrazolium dyes as do normal leukocytes. Our modification was developed to examine the carrier female's cells in an effort to demonstrate that two populations of leukocytes, normal and defective, may be present. The cells were isolated, incubated, and smears were prepared as previously described (11). The nitro-blue tetrazolium appears to vary considerably from batch to batch, and a new lot should first be tested with normal cells to determine its capacity to give positive results. Duplicate cultures were prepared from each collection of cells, and all cultures were coded so that the smears could be examined without knowledge of the source of the cells.

Cells from normal individuals show reduction of the tetrazolium dye, most noticeably in cells that have phagocytized the latex particles. Differential counts were made on smears by counting 100 cells containing 10 or more phagocytized latex particles. Cells were positive if any deep-blue granular dye precipitate could be seen. A faint homogenous blue tinge of the cell cytoplasm was not considered a positive finding.


RESULTS

I. Results of the leukocyte function test

Bacterial counts were plotted as a function of time on five-cycle semilogarithmic paper. A representative family study is illustrated in Fig. 1. It is evident that bactericidal capacity of cells from the father, a normal brother, and one sister was similar to that of the control. However, the mother's cells and those of another sister functioned at an intermediate level. A partial abnormality has been present without exception in multiple examinations of each of the nine mothers. Three maternal grandmothers have shown the same pattern. An example is presented in Fig. 2. None of the fathers or the one maternal grandfather has been abnormal. Five healthy brothers were all entirely normal in this test.

Since interpretation of the plots of the leukocyte function tests has been so consistent for recognition of carrier mothers, we used this method to select the suspected carriers among the female siblings. Thus, 10 of the 16 sisters have been categorized as carriers.

![Figure 1](image-url)  
**Figure 1** Surviving intracellular bacteria in leukocytes of the members of the S family (broken lines) examined on the same day and plotted in relation to that day's control cells (solid lines). The father, the normal brother, and one sister have cells which function well, whereas the mother's cells and those of the other sister kill bacteria at a rate intermediate between that of the cells from the patient and that of the control.

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cells from a known normal, unrelated individual were determined. Thus, the ratios were based on the control cells used for the given day’s study, and a figure of one or less would be indicative of bactericidal function which was as good as that of the control cells.

Table I shows these data plotted as means with 95% confidence intervals for the means. It is seen that, (a) the ratios for normal family members approached unity, (b) the ratios for carrier sisters (mean of ratios = 95) were very similar to the ratios of their mothers (mean = 100), and (c) the ratios of these carrier groups were different not only from those ratios of the normal family members but also from those of the diseased boys (mean of ratios = 674). The probability that the mean of the grandmother-mother group is in the same population as the mean of the abnormal boys is 0.042 and a similar figure for this known carrier group compared with the normal family members is less than 0.0001. There is no overlapping of the 95% confidence intervals for normals, affected boys, and carrier females.

Because statistics are of little value in the actual prediction of the status of a given individual, the range of individual determinations and the range of means for individuals from which Table I was derived are shown in Fig. 4. It will be noted that only one determination is given for a number of individuals. All persons in the study were given multiple tests and these showed consistent findings; however, as this work progressed, modifications were made in the procedure and only those studies actually performed, as described in Methods, were included in the statistical analysis.

In the range of the means, as well as the ranges of individual determinations, a considerable amount of overlap can be seen. The wide variability in the patients’ values is unexplained, but may be related to the extremes of conditions under which these boys were studied. Of more immediate interest is the critical problem of predicting the carrier status of females. The mother (Mrs. B.) whose mean of ratios was 10 is also an aunt of the affected boys; hence, she is surely a carrier. For this reason, we feel that a technically adequate examination in which a girl’s ratio is found to be greater than 10 can be taken as strong evidence for the carrier state. Ratios less than 10 in potential carriers must be confirmed by repeat examina-

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**Figure 2** Surviving intracellular bacteria in leukocytes of members of the L family. These are plotted to illustrate that the maternal grandmother’s cells exhibit an abnormality comparable to that of the mother’s.

The pedigrees constructed on the basis of this evaluation are recorded in Fig. 3. It should be noted that 9 of the 15 diseased boys can be regarded as probands, and that the oldest of the 9 living affected boys is only 11 yr. The J family and the L family were represented by case 3 and case 4 in the report by Bridges et al. (5).

One carrier sister has had a diagnosis of ulcerative colitis since the age of 11, and one mother has had chronic furunculosis. Several of the mothers and carrier sisters have recurrent aphthous stomatitis, and one mother is said to have had rheumatic fever.

Despite findings suggestive of disease in the carrier state, the three maternal grandmothers are in their 70s and two have outlived their husbands.

**Reliability of the interpretation of the leukocyte function test.** For the purpose of statistical analysis, the bacterial counts at the 2–3 hr sampling point were utilized. The ratios of the remaining viable bacteria in the culture of the experimental cells to those remaining in the culture containing...
Figure 3 Pedigrees of the nine families diagrammed on the basis of the leukocyte function test. Note the affected maternal half brothers in the L-B family. Note also that maternal uncles in the L, N, and W families died in infancy with probable sepsis. There are no living affected maternal uncles.

Table I
Ratios of Viable Intracellular Bacteria at 2–3 Hr:
Family Members/Same Day Controls

<table>
<thead>
<tr>
<th></th>
<th>Means of ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patients</strong></td>
<td></td>
</tr>
<tr>
<td>25 determinations on 11 patients</td>
<td>674 ± 464</td>
</tr>
<tr>
<td><strong>Mothers and grandmothers</strong></td>
<td>100 ± 52</td>
</tr>
<tr>
<td>21 determinations on 8 mothers and 1 grandmother</td>
<td></td>
</tr>
<tr>
<td><strong>Carrier sisters</strong></td>
<td></td>
</tr>
<tr>
<td>15 determinations on 9 sisters</td>
<td>95 ± 79</td>
</tr>
<tr>
<td><strong>Fathers, brothers, and normal sisters</strong></td>
<td></td>
</tr>
<tr>
<td>29 determination on 8 fathers, 6 sisters, and 5 brothers</td>
<td>1.5 ± 0.8</td>
</tr>
</tbody>
</table>

Consistent findings of ratios of 5–10 require a guarded prognosis.

II. Results of the metabolic studies

Normal neutrophils increase their oxidation of the first carbon of glucose almost 10-fold when they are presented with particles to phagocytize. This oxidation is a function of glucose-6-phosphate dehydrogenase (G-6-PD) and is in consequence of an increased over-all activity of the hexose monophosphate (HMP) shunt (12). Cells of patients with fatal granulomatosis have reduced HMP shunt activity during the resting state and fail to show stimulation of the shunt during phagocytosis.

The results of the studies on the families of the granulomatous boys are summarized in Table II.
The mean resting levels of $^{14}$CO$_2$ production from glucose-1-$^{14}$C for all carrier females was intermediate between and significantly different from the mean values for patients and for normal family members. This was true for the stimulated rate of $^{14}$CO$_2$ production and for the per cent of stimulation during phagocytosis as compared to the resting state. Thus, the female carriers have a defect in regulation of the HMP shunt. However, the overlap of individual determinations in control and carrier groups precludes using this procedure as a reliable means of detecting the carrier state in any one person.

The oxidation of formate-$^{14}$C has been used as an indicator of H$_2$O$_2$ production by neutrophils (13). The neutrophils from the granulomatous patients have normal resting levels of formate oxidation, but these cells fail to show increase in formate oxidation during phagocytosis (1).

Table III gives the formate studies on family

### TABLE II

Oxidation of Glucose-1-$^{14}$C by Peripheral Blood Leukocytes

<table>
<thead>
<tr>
<th></th>
<th>Resting rate</th>
<th>Stimulated rate (phagocytosis of polystyrene particles)</th>
<th>% Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11 determinations on</td>
<td>504</td>
<td>815</td>
<td>82</td>
</tr>
<tr>
<td>7 patients</td>
<td></td>
<td>$P &lt; 0.025$</td>
<td></td>
</tr>
<tr>
<td>Carriers</td>
<td>886</td>
<td>4827</td>
<td>463</td>
</tr>
<tr>
<td>44 determinations on</td>
<td></td>
<td>$P &lt; 0.01$</td>
<td></td>
</tr>
<tr>
<td>9 mothers and 10</td>
<td></td>
<td>$P &lt; 0.0005$</td>
<td></td>
</tr>
<tr>
<td>sisters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family controls</td>
<td>1307</td>
<td>9475</td>
<td>748</td>
</tr>
<tr>
<td>30 determinations on</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>19 family members:</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>8 fathers, 5 brothers,</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>and 6 sisters</td>
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</table>

Results expressed as cpm of $^{14}$CO$_2$ collected during 10 min incubation of $5 \times 10^6$ cells in 1 ml of Krebs-Ringer phosphate buffer containing 1 μC and 1 μm of glucose.
and the diseased boys show 9.9% positive cells. This provides independent evidence that the selection of the female siblings who are carriers was correct.

**DISCUSSION**

The findings presented here indicate that predisposition to fatal granulomatous disease of childhood is transmitted on the X-chromosome. Of 18 patients on whom this diagnosis had been made at the University of Minnesota hospitals, since 1956, only eight are living, and the oldest of these is chronically ill at the age of 11. Carson et al. noted the same high fatality rate, although one of their patients had lived to the age of 16 before death occurred (6). Three mothers in our series (N and W sisters, and L family) had brothers who died in early childhood with diseases similar to those of their sons. These facts, plus the absence of living affected maternal uncles in our families, support the concept that the consequences of this mutation are fatal for the hemizygous male.

On the other hand, because new diseases are first defined in terms of their most severe manifestations, less severe forms of this trait may eventually be found. The hemizygous male could then transmit the abnormal gene, and females manifesting the disease would be a possibility.

Of more immediate interest with regard to females is that the heterozygous females in our study have leukocytes with capacity to kill bacteria intermediate between those of the boys with disease and those of normal persons. The consistency with which this is true and the equal consistency with which the normal females, fathers, and normal brothers do not show significant deviation from controls indicate that the function test measures a phenotypic response which reflects the presence of both normal and mutant genes. The intermediate abnormality which carrier females exhibit in the oxidation of the first carbon of glucose can also be regarded as indicating the heterozygous state.

That this biochemical defect can be detected in the heterozygote suggests that the defective oxidation of glucose-1-14C is not subject to enough modifying influences to obscure the abnormality even in the partially normal heterozygote.

Such a quantitatively intermediate function of
the female carriers' cells could be due to a partial defect in all cells or to a mixed population of cells in which some are completely normal and others fully abnormal. The latter situation would obtain if random inactivation of one X-chromosome normally occurs in female cells as hypothesized by Lyon (14, 15) and Beutler, Yeh, and Fairbanks (16).

We have shown that carrier females for the granulomatous disease do appear to have a mixed population of neutrophils, some abnormal and some normal by their ability to reduce tetrazolium dye. This is compatible with the assumption that X-chromosome inactivation is operating to affect the gene under consideration (11).

The practical clinical consequences of this assumption are of importance, since X inactivation is presumably random and may be capable of operating to produce a wide range of phenotypic findings in heterozygous females. Thus in G-6-PD deficiency, females whose enzyme levels are within the normal range, as well as women with completely abnormal levels of enzyme, have been demonstrated to be heterozygous on the basis of their normal and abnormal sons (17). Therefore, it seems reasonable to think that in the granulomatous trait, heterozygous females may be found who have the same clinical disease on this basis as the boys.

The analogy of fatal granulomatous disease to G-6-PD deficiency can be extended. Besides being X-linked, the two disorders affect closely related cell lines, neutrophils, and erythrocytes, respectively, which are circulating, highly specialized, nondividing cells. In such cells, major areas of metabolism might be expected to have evolved in the direction of serving the specialized function of the cell. For instance, the HMP shunt provides mechanisms for stabilization of the red cell membrane whereas in nonterminal dividing cells, the HMP shunt is involved in fatty acid turnover and nucleic acid synthesis, among other things. The role of the increased HMP shunt activity in phagocytizing neutrophils is unclear.

G-6-PD is the entering enzyme for the HMP shunt, and the over-all functioning of this shunt in the granulomatous carriers' and patients' leukocytes is abnormal. The first two steps of the HMP shunt generate reduced nicotinamide adenine dinucleotide phosphate (NADPH). The reduction of tetrazolium dyes is apparently a function of the oxidation of nicotinamide adenine dinucleotide (NADH) or NADPH by specific oxidases (18). NADH and NADPH apparently do not diffuse into intact cells and it is not possible to know which, if either, is involved in the dye reduction seen in normal leukocytes but not in the leukocytes of granulomatous patients. It is nevertheless tempting to speculate that the enzyme defect in the patients with granulomatous disease is in the electron transport system for the hydrogen ions, which are activated by G-6-PD and 6-phosphogluconate dehydrogenase.

Such speculation must not obscure the immediate practical problems of detection of new patients with fatal granulomatous disease and the estimation of carrier status in female relatives. These require evaluation of the diagnostic procedures now available. For simplicity and speed a tetrazolium dye test, employed with a simultaneous control of normal cells, is the procedure of choice for patient diagnosis. However, the difficulties in interpretation of the test for the intermediate results found in the carrier state are such that it is unsuitable for carrier diagnosis.

Simultaneously, the abnormalities observed in glucose-1-\(^{14}\)C metabolism in the carrier females as a group are widely variable in individual determinations; hence, this procedure is not applicable to the diagnosis of individual females. In fact, neither the dye test nor the metabolic test offer additional help to the leukocyte function test in the selection of a given individual as a carrier.

The technically more complicated leukocyte bactericidal test provides a solid indication as the the carrier status of a female within 24–48 hr of the initial venipuncture, particularly when abnormal control cells, as well as normal cells, are included in the day's run. Improved and simplified procedures for this test are being developed.

ACKNOWLEDGMENTS

The untiring and enthusiastic technical assistance of Miss Judy Sater was basic to this project.

Dr. D. B. Windhorst is an assistant professor and a recipient of special fellowship AM-22,103 from the National Institutes of Health. Dr. A. R. Page is an associate professor and a U. S. Public Health Service Career Development awardee. Dr. B. Holmes is a research ass-

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sistant and recipient of a U. S. Public Health Service Training grant (AI 90-06) in medical microbiology. Dr. P. G. Quie is a John and Mary R. Markle Scholar in academic medicine and a recipient of U. S. Public Health Service Career Development Award 5-K3-AI-10,109-05.

This work was supported by the Minnesotan Heart Association, the Graduate School of the University of Minnesota, U. S. Public Health Service grants AI-00798 and AM-05830, and The National Foundation.

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