Brief Report

Chromosome-21 and Paroxysmal Nocturnal Hemoglobinuria

By E. Beutler, E. W. Goldenburg, S. Ohno and M. Yettra

Although great strides have been made in the past decade in both cytogenetics and biochemical genetics, the location of genes on the 44 autosomes has remained unknown. The experiments of nature in which an additional chromosome-21 is present and where a portion of this chromosome has been deleted have provided, for the first time, a clue to the possible location of some autosomal genes. The studies of Alter and his associates were the first of a series of investigations suggesting that a gene regulating the formation of leukocyte alkaline phosphatase was located on this chromosome. A flurry of reports has followed suggesting that genetic control of ABO blood group substances, but not the D antigen, may also be located on this chromosome.

Like Alter et al., we were struck by the fact that paroxysmal nocturnal hemoglobinuria (PNH) is associated with low leukocyte alkaline phosphatase activity. It also occurred to use that the clinical course of this disease, with its onset in the second, third, or fourth decade of life, its chronicity, and its involvement of several cell lineages, all are compatible with the hypothesis that this disorder, like chronic granulocytic leukemia (CGL), may be due to proliferation of a clone of cells with a deletion of genetic material from chromosome-21. It is particularly interesting, in this regard, that the blood of patients with paroxysmal nocturnal hemoglobinuria appears to consist of a mixture of normal and abnormal erythrocytes. The highly variable course of the disease might be due to the results of competition—with the ecologic sense—between the normal and abnormal clones of cells.

A further intriguing possibility seemed to be that the hitherto unexplained decrease in acetylcholinesterase activity could be due to the presence of the gene controlling the formation of this enzyme on the deleted chromosomal segment. The latter possibility seemed to be strengthened by the unpublished observation of Samuels that the red cell cholinesterase activity in treated chronic granulocytic leukemia in relapse was also diminished, particularly in the more severely anemic patients studied. Sawitsky et al. had previously reported a slightly lowered red cell cholinesterase activity.
in chronic granulocytic leukemia, but had found the difference from normal not statistically significant. The loss of a red cell enzyme could well occur as a result of chromosomal deletion in chronic granulocytic leukemia, since Trujillo and Ohno\textsuperscript{15} have shown that in chronic granulocytic leukemia erythroblasts may also contain the Philadelphia chromosome. As Alter et al. point out, a normal karyotype has been reported in a single case of paroxysmal nocturnal hemoglobinuria\textsuperscript{19} and one peripheral blood karyotype has recently been reported to be normal in abstract.\textsuperscript{29} We have been able to obtain three bone marrow and four peripheral blood specimens from four patients with well-authenticated, classical paroxysmal nocturnal hemoglobinuria. All patients had diminished leukocyte alkaline phosphatase activity. Although it was thought that in some instances a borderline shortening of one of the small acrocentric chromosomes could be seen, no distinct abnormality in the karyotype was noted. Although the Y-chromosome was larger than normal in one male subject, this was not the case in the others.

In a further investigation to determine whether the gene for red cell acetylcholinesterase was located on chromosome-21, studies of red cell acetylcholinesterase were carried out in children with Down's syndrome. Using Michel's\textsuperscript{21} method for the determination of this enzyme we have measured the cholinesterase activity of the red cells of adults, children, PHN patients, and mongols. These studies are summarized in table 1. The difference between the limited number of Mongol and normal children studied is not statistically significant. If the activity of this enzyme were strictly dosage-related and the gene controlling its synthesis were on chromosome-21, one would anticipate that the average enzyme activity of the red cells of Mongol children would be one and one-half times normal, approximately 1.20 $\Delta$ pH/hr. units.

Thus, although it is attractive to suppose that paroxysmal nocturnal hemoglobinuria may be due to a chromosomal deletion, we have been unable to find any strong morphologic or biochemical support for this concept. These negative findings do not, of course, rule out the presence of a small deletion which cannot be detected morphologically and which gives rise to both acetylcholinesterase deficiency and leukocytic alkaline phosphatase deficiency.

The concept that PNH represents the result of a somatic mutation, whether demonstrable by cytogenetic technics or not, may have important therapeutic implications. Thus, treatment with cytotoxic agents, e.g., 6-mercaptopurine or busulfan, in the attempt to suppress the undue proliferation of the abnormal cells, might be helpful in the therapy. Clinical studies with such drugs are underway.

**Summario in Interlingua**

Es reportate le resultatos negative de effortios de documentar per datos morphologic o biochimic que hemoglobinuria paroxysmal nocturne resulta de un deletion chromosomal. Remane le possibilitate que tanto le carentia de
Table 1

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Number Studied</th>
<th>Age</th>
<th>Source</th>
<th>Red Cell Cholinesterase Activity pH/hour</th>
<th>Mean</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>12</td>
<td></td>
<td>Michel2\textsuperscript{3}</td>
<td>.753</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>10</td>
<td>Adults</td>
<td>This study</td>
<td>.763</td>
<td>.023</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>6</td>
<td>Children</td>
<td>This study</td>
<td>.795</td>
<td>.022</td>
<td></td>
</tr>
<tr>
<td>Mongols</td>
<td>5</td>
<td>Children</td>
<td>This study</td>
<td>.846</td>
<td>.012</td>
<td></td>
</tr>
<tr>
<td>PNH patients</td>
<td>3</td>
<td>Adults</td>
<td>This study</td>
<td>.530</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

de acetylcholinesterase como etiam le carentia leucocytic de phosphatase alcalin es de facto associate con un morphologicamente non detegibile micre deletion chromosomal. Es signalate que studios clinic es in progresso que visa a evalutar le tractamento de hemoglobinuria paroxysmal nocturne con agentes cytotoxici del tipo de 6-mercaptopurina o busulfano.

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