Decrease of the Major High Molecular Weight Surface Glycoprotein of Human Granulocytes in Monosomy-7 Associated With Defective Chemotaxis

By Carl G. Gahmberg, Leif C. Andersson, Petri Ruutu, Timo T.T. Timonen, Auli Hänninen, Pekka Vuopio, and Albert de la Chapelle

By use of the galactose/NaB₃H₄ surface labeling technique followed by polyacrylamide slab gel electrophoresis, it is shown that the major labeled surface glycoprotein (GP130) of normal human blood granulocytes is markedly reduced in granulocytes from three patients with a chromosomal abnormality in all or most bone marrow mitoses. The abnormality consisted of monosomy-7 in two and deletion of the distal half of the long arm of chromosome-7 in the third. The granulocytes from these patients showed reduced chemotaxis. These results suggest that the expression of GP130, as well as the chemotactic ability of the cells, are at least in part controlled by one or several genes on chromosome-7. The GP130 protein may be involved in normal granulocyte chemotaxis.

Most if not all cell surface proteins are glycoproteins with their carbohydrate located on the external part of the plasma membrane. Alterations in nonreducing terminals of the carbohydrate moieties may dramatically affect antigenicity and intercellular recognition. Analysis of these components has been greatly facilitated with the recent introduction of radioactive labeling methods specific for cell-surface glycoproteins and glycolipids. These methods make it possible to detect subtle changes in the molecular organization and structure of plasma membrane glycoconjugates. The molecular mechanism of granulocyte chemotaxis is not understood, but it is reasonable to assume that cell-surface structures are involved (for a recent review see ref. 8). The finding that monosomy-7 is associated with defective chemotaxis suggested the possibility of changes in surface membrane components in granulocytes from patients with this abnormality.

In this work we have labeled surface glycoproteins of granulocytes from normal persons and from patients with monosomy-7 by the galactose oxidase/NaB₃H₄ labeling technique and studied them by polyacrylamide slab gel electrophoresis. We report that the major labeled surface glycoprotein of normal granulocytes with an apparent molecular weight of 130,000 is greatly reduced in patients with monosomy-7.

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MATERIALS AND METHODS

Cells
Blood was drawn into heparin-containing tubes from normal adults, from three patients with monosomy-7 of bone marrow cells, from patients with refractory sideroblastic anemia without any chromosomal abnormality, and from patients with chronic myeloid leukemia. Monosomy-7 patient 1 had multiple myeloma and refractory sideroblastic anemia, and patients 2 and 3 had refractory sideroblastic anemia. The isolation of granulocytes has been described previously. Briefly, it involved Ficoll-Isopaque centrifugation and purification of the granulocytes from the cell pellets after sedimentation of erythrocytes with dextran followed by lysis of remaining erythrocytes in 0.83% NH₄Cl-0.017 M Tris-HCl pH 7.45. The preparations obtained contained more than 98% granulocytes as judged from May-Gruenwald-Giemsa stained cytocentrifuged smears. More than 95% of the cells were alive as judged by the trypan blue dye exclusion test. Lymphocytes were prepared and cultured as described previously.

Chromosomal Analysis
Our methods for chromosome work have been described in detail previously.

Determination of Chemotaxis
Chemotaxis was studied by the method of Zigmond and Hirsch as previously described. The cell concentration was adjusted to 10⁶ mature neutrophils/ml. The cells were allowed to migrate into 3-μm Millipore filters in modified Boyden chambers for 55 min towards casein (5 mg/ml; Sigma Chemical Co., St. Louis, Mo.) in Hanks solution, or for 90 min towards Hanks solution, after which the position of the leading front of the cells was determined in 5 fields of each of 6 filters.

Radioactive Cell-Surface Labeling and Polyacrylamide Slab Gel Electrophoresis
Cells were surface labeled by reduction with NaB³H₄ (8.6 Ci/m mole, Radiochemical Centre Ltd., Amersham, U.K.) after treatment with Vibrio cholerae neuraminidase and galactose oxidase. The labeling conditions and the purities of the enzymes have been described in detail. After labeling, the cells were dissolved at 0°C in 0.15 M NaCl-0.01 M sodium phosphate (pH 7.4), containing 1% Triton X-100, 1% ethanol, and 2 mM phenylmethyl sulfonylfluoride (Sigma), and the nuclei pelleted by centrifugation at 3000 rpm for 5 min. The supernatant solutions were recovered and boiled for 1 min in the sample buffer of Laemmli, and aliquots containing similar amounts of radioactivities were electrophoresed on 8% acrylamide slab gels containing sodium dodecyl sulfate. After fixation in 20% sulfosalicylic acid, the gels were treated for fluorography according to Bonner and Laskey and exposed to RP X-Omat film (Kodak, Rochester, N.Y.) for 2–4 days at –70°C. The films were then scanned with a Joyce-Loebl Chromoscan apparatus. The apparent molecular weights of the proteins were determined according to Weber and Osborn with ^14C-labeled proteins as standards.

RESULTS

Chromosome Findings
The constitutional karyotype was normal in all three patients as judged by the study of phytohemagglutinin-stimulated lymphocytes from peripheral blood. In patients 1 and 2, bone marrow cells lacked one chromosome -7 on several occasions. Table 1 shows the chromosome counts at the time of cell labeling. It can be seen that 8 of 12 (patient 1) and all (patient 2) bone marrow cells were monosomic for chromosome-7. In patient 3, an apparent terminal deletion involving approximately half of the long arm of one chromosome-7 was seen in all cells studied on several occasions: 46,XX,del (7) (q22).
Table 1. Bone Marrow Chromosome Findings at the Time of Cell Surface Labeling

<table>
<thead>
<tr>
<th>Patient</th>
<th>Chromosome Counts</th>
<th>Most Prevalent Karyotype</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>&lt;45</td>
<td>45 46 47 &gt;47 Total</td>
</tr>
<tr>
<td>1</td>
<td>8 -</td>
<td>3* 1 12</td>
</tr>
<tr>
<td>2</td>
<td>18 -</td>
<td>18 45,XY,-7</td>
</tr>
<tr>
<td>3</td>
<td>- 1</td>
<td>15 1 2 19</td>
</tr>
</tbody>
</table>

*Cells containing two of chromosome – 7.

Chemotaxis

Table 2 shows that the random migration of granulocytes of all three patients was normal. However, there was a strongly reduced migration towards casein in all three cases.

Surface Glycoprotein Patterns

Figure 1 shows the scanning curves obtained from the fluorography patterns of surface-labeled granulocyte glycoproteins separated by polyacrylamide slab gel electrophoresis. The major labeled band of normal granulocytes (GP130) was clearly reduced in granulocytes from patients 1 and 2 with monosomy-7 (Fig. 1B and C) as compared to normal cells (Fig. 1A). In the granulocytes of patient 3 with a deletion of one of chromosome-7 [del (7)q(22)], the decrease was less obvious, but the GP130 level was below the normal range when the GP155/GP130 ratio (2.65) was calculated from the peak areas by triangulation. The normal range was 3.9–6.6. The surface glycoprotein patterns of granulocytes from patients with chronic myeloid leukemia appeared essentially normal (Fig. 1E), possibly with an accentuation of GP130. No change was observed in the surface glycoprotein patterns of granulocytes from patients with refractory sideroblastic anemia without chromosomal abnormalities (Fig. 1F and G). We have studied the surface glycoprotein patterns of granulocytes from more than 20 normal persons of different ages and from 8 patients with chronic myeloid leukemia but have never seen much variation in the labeling intensities of GP130.

Also, the GP97 band was relatively weaker in the patients with monosomy-7, but the change was less obvious than for the GP130.

DISCUSSION

The mechanism(s) for granulocyte chemotaxis is probably complex and is not understood at present. It is assumed that surface receptors are involved that
Fig. 1. Scanning patterns of labeled granulocyte surface glycoproteins separated by polyacrylamide slab gel electrophoresis and visualized by fluorography. (A) Pattern of normal granulocytes; (B) pattern of granulocytes from patient 1; (C) pattern of granulocytes from patient 2; (D) pattern of granulocytes from patient 3; (E) pattern of granulocytes from a patient with chronic myeloid leukemia; (F and G) patterns of granulocytes from patients with refractory sideroblastic anemia without chromosomal abnormalities. GP155: glycoprotein with an apparent molecular weight of 155,000 etc.
GRANULOCYTE GLYCOPROTEINS IN MONOSOMY-7

mediate the information to the contractile elements of the cell through cyclic nucleotides. One or more gene products of chromosome-7 must be important in granulocyte chemotaxis, and the present work describes one approach to the problem.

GP130 is the major labeled surface glycoprotein of normal granulocytes and monocytes. As shown here, its expression correlates with the presence of chromosome-7. However, we do not know whether the decrease of GP130 seen in the patients with monosomy-7 is due to a lower level of the GP130 polypeptide or whether the defect only involves its carbohydrate portion. The decrease of GP130 is probably not due to proteolysis, because we have shown earlier that the expression of GP130 in normal cells is constant and proteolytic activity acting on cell surface proteins is not observed during the labeling procedure.

Because patients with monosomy-7 often later develop myeloid leukemia, we also studied granulocytes from patients with this disease but never saw any reduction of GP130. The surface glycoprotein patterns of patients with refractory sideroblastic anemia without chromosomal abnormalities also appeared normal. Cells from patients with acute leukemia and sideroblastic anemia have normal chemotaxis.

The rationale for using blood cells from patients with abnormal bone marrow karyotypes for gene mapping has been discussed previously. In patients whose bone marrow is dominated by a cytogenetically abnormal clone, mature erythrocytes and polymorphonuclear cells are derived from precursors with the abnormal karyotype, while lymphocytes are not. For example, the glutathione reductase activity is increased in the erythrocytes from patients with trisomy-8 of the bone marrow, confirming the assignment of the gene for glutathione reductase to chromosome-8. Since blood lymphocytes are derived from different precursors, and since they have normal karyotypes, it is not surprising that we were unable to detect any surface glycoprotein abnormality in the lymphocytes of the present patients (data not shown). On the other hand, erythrocytes from the present patients are descendants of cells lacking a chromosome-7, or part thereof, and should therefore show alterations in the quantities of any protein coded for by genes on chromosome-7. However, GP130 does not evidently occur on the surface of normal erythrocytes, and the red cells from these patients showed a normal glycoprotein pattern (data not shown).

Deletion mapping of human genes is an established procedure. We feel justified in claiming assignment for the gene affecting chemotaxis (and decrease in GP130) not only to chromosome-7, but more specifically to the region 7q22→7qter. The latter claim, being based on findings from only one patient, must of course await confirmation.

In this connection, it is interesting to note that Ford et al., using human–mouse hybrid cells, recently found that chromosome-7 codes for a fibroblast surface protein with an apparent molecular weight of 165,000. The physiologic function of only very few surface proteins is known. We cannot say whether GP130 actually is involved in chemotaxis, but we suggest that the approach of correlating changes in surface-associated functions with the surface glycoprotein patterns may become useful. Specific functions for these poorly understood molecules may thus be detected.
ACKNOWLEDGMENT

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REFERENCES


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