Anti-i cold agglutinins in choriocarcinomatosis: trophoblastic i antigen

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SUMMARY The fetal antigen i has been demonstrated by indirect immunofluorescence on cord erythrocytes and placental trophoblast. A patient with disseminated choriocarcinoma developed high-titre anti-i cold agglutinins, and minor elevation of anti-i titres was seen in four out of six further patients with treated choriocarcinoma. In normal pregnant women, 6% showed similar increases in anti-i titres.

The i and I erythrocyte antigens have recently been described as ceramide hepta and deca saccharides (Hakamori, 1977). The fetal i character of erythrocytes changes after birth, and by 18 months of age the red cells carry mainly I (Marsh, 1961). Detection of i substance has been reported in saliva, milk, amniotic fluid, ovarian cyst fluid, and serum (Race and Sanger, 1975). In the following report, i antigen is demonstrated in placental trophoblast, and the development of an anti-i cold agglutinin is reported in a case of disseminated choriocarcinoma.

Case history

A 40-year-old woman had experienced abnormal postpartum vaginal bleeding for two years. She had passed blood clots and tissue fragments for eight months and had lost 5 kg in weight.

She appeared pale, and the uterus was enlarged to the size of an 18-week pregnancy. The liver and spleen were slightly enlarged.

A Prognosticon® pregnancy test was positive on urine diluted 1/10, and uterine curettings showed choriocarcinoma. Uterine ultrasound showed no pelvic wall invasion, and liver/spleen scans showed hepatosplenomegaly but without visible filling defects. Biochemical screening showed elevated alkaline phosphatase and lactate dehydrogenase, however, and a chest x-ray revealed multiple metastases in both lungs.

HAEMATOLOGY AND SEROLOGY
The Hb was 5·5 g/dl, and the blood smear showed a hypochromic microcytic picture and many red cell agglutinates. The WBC count and differential count were normal, and no atypical mononuclear cells were seen. A haemolytic process was excluded since the serum bilirubin was 0·2 mg/dl, urinary urobilinogen was negative, reticulocytes were 1·9% (76 × 10^6/l), and plasma haemoglobin and methaemalbumin were within normal limits.

The patient’s serum prepared at 37°C showed marked and specific haemagglutination of red cells carrying i antigen (adult i cell, cord i cells, and Cynomolgus monkey i red cells but little activity against adult I cells). Normal sera were unreactive to all red cells under these conditions.

<table>
<thead>
<tr>
<th>Test serum</th>
<th>Adult i</th>
<th>Adult i</th>
<th>Adult i</th>
<th>Cord i</th>
<th>Cynomolgus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td>&lt; 1/4</td>
<td>1/2048</td>
<td>1/256</td>
<td>1/128</td>
<td>1/8192</td>
</tr>
<tr>
<td>2 Normal (× 3)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3 IgMk monoclonal anti-i</td>
<td>1/8</td>
<td>1/256</td>
<td>1/512</td>
<td>1/64</td>
<td>1/128</td>
</tr>
<tr>
<td>4 Glandular fever</td>
<td>1/16</td>
<td>1/16</td>
<td>1/4</td>
<td>1/32</td>
<td></td>
</tr>
</tbody>
</table>

Samples 1 and 3 had shown some diminution in antibody strength by the time these final checks were made. Haemagglutination was read macroscopically after 2 hours' incubation at 4°C. The patient's serum, the IgMk antibody, and a glandular fever anti-i show high titre activity against adult i, cord i, and Cynomolgus monkey i red cells but little activity against adult I cells. Normal sera were unreactive to all red cells under these conditions.

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Pregnancy sera
Serum was prepared at 37°C from 36 women between 10 and 41 weeks of pregnancy. These were tested for 12 hours at 4°C, for anti-i and anti-I activity, against group O cord and adult red cells. Using undiluted sera, visual agglutination was absent in all 36 cases. Significant microscopic agglutination of cord red cells occurred in two instances, however, up to serum dilutions of 1/32 and 1/64.

**FLUORESCENCE MICROSCOPY**

**Material**

1. **Serum:** a human IgMκ monoclonal antibody with 22°C anti-i and anti-I titres of 1/2048 and 1/4 was used. Its specificity for anti-i is shown in Table 1.

2. **Eluates:** the above serum was incubated with group O cord red cells at 4°C for 30 minutes, and the cells and serum were then separated at 4°C. Antibody was then eluted at 40°C and gave anti-i and anti-I titres similar to the original serum.

3. **Adsorbed serum and eluate:** serum or eluate from (1) and (2) were adsorbed three times at 4°C for 30 minutes using equal volumes of 50% suspensions of group O cord or adult red cells.

4. **Placenta:** fresh full-term placenta was snap-frozen in liquid nitrogen, and 5 μm unfixed, cryostat sections were prepared.

5. **Blood films:** cord and adult group O erythrocytes were washed three times in normal saline, and air-fixed smears were made on glass slides.

**Method**

Sections of placenta, or blood films, were incubated with anti-i preparations for 1 hour at 22°C. They were then washed three times in buffered saline and reincubated at 22°C for 30 minutes with fluorescein-labelled sheep anti-human IgM (Wellcome Reagents, Beckenham, Kent). Finally, they were again washed in buffered saline and mounted in glycerin. They were then examined under a Leitz ortholux II microscope, fluorescence intensity being graded 0 to 4+. Representative fields were photographed using Ektachrome 200 film.

**Results**

Table 2 shows that anti-i serum and its cord red blood cells (RBC) eluate both stained placenta and cord erythrocytes but not adult erythrocytes. When the eluate was adsorbed with cord RBCs, the placental staining was markedly reduced, whereas adsorption with adult erythrocytes had no such effect. The absence of significant fluorescence in the AB serum and saline controls excludes non-specific staining.

The Figure shows the fluorescent staining of placenta and cord RBCs using anti-i eluate. The majority of fluorescence is derived from the cytoplasm of the syncytiotrophoblastic cells. In the cord RBC preparation, there is a spectrum of cell membrane fluorescence from 0 to 2+.

**Discussion**

I and i erythrocyte antigens appear to be ceramide hepta and deca saccharides (Hakamori, 1977), which may be precursors of ABH and Lewis substances (Feizi et al., 1971). They are also present in various endodermal tissues and body fluids and are subject to alteration when malignancy occurs in these sites. Thus, endodermal tumours may show deficiency of ABH substances, or an increase in I/i antigens (Salmon, 1978). In some malignant disorders, a derepressed embryonic protein appears (carcinoembryonic antigen), and this is similar though not identical with i substance (Cooper et al., 1974).

Antibodies to i substance are uncommon, however, except in infectious mononucleosis where they are formed in 8-66% of cases (Jenkins et al., 1965; Rosenfield et al., 1965). Anti-i has also been reported in cirrhosis (Rubin and Solomon, 1967) and in lymphomas (Marsh, 1961). In these circumstances, it has been suggested that anti-i appears as a non-specific effect of intense synthetic activity in the R/E system, but in none of these cases has a source of i antigenic stimulation been identified.

The identification of i substance in normal placent al trophoblast was predictable, and a search for anti-i antibodies in normal pregnancy sera revealed normal levels in 34 cases and minor elevations in two (6%). Only one previous report of anti-i in pregnancy could be traced (Bell et al., 1967),

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Dilution</th>
<th>Placenta</th>
<th>Adult RBCs</th>
<th>Cord RBCs</th>
</tr>
</thead>
<tbody>
<tr>
<td>a IgMκ anti-i serum</td>
<td>1/32</td>
<td>+ + +</td>
<td>±</td>
<td>+</td>
</tr>
<tr>
<td>b Cord RBC eluate</td>
<td>1/32</td>
<td>±</td>
<td>±</td>
<td></td>
</tr>
<tr>
<td>c Eluate (b) adsorbed with group O cord RBCs</td>
<td>1/24</td>
<td>±</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d Eluate (b) adsorbed with group O adult RBCs</td>
<td>1/24</td>
<td>+ + +</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal AB serum</td>
<td>1/32</td>
<td>±</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Saline</td>
<td>-</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

The final dilution of each reagent and the results with relevant control reagents are included.

Table 2. Intensity of fluorescent staining of placenta, adult RBCs, and cord RBCs (graded 0 to 4+)
Figure (a) Anti-i eluate 1/32 fluorescent staining. Trophoblast cells lining normal placental villi. (b) Absent fluorescence in the saline control. (c) Anti-i eluate staining of cord erythrocytes. The saline control of this preparation was negative. All preparations are magnified × 145.
but the above findings suggest that it is not such a rarity.

In the case of disseminated choriocarcinoma, however, anti-i was present in high titre, and, despite its clinical insignificance, it produced autoagglutination of patient’s red cells at 22°C and caused minor blood grouping and crossmatching difficulties.

A preliminary survey of six further cases of choriocarcinoma showed increased anti-i activity against adult-i cells (4 cases) and Cynamolgus monkey cells (3 cases). A patient with ovarian dysgerminoma showed similar findings. In these cases, titres of antibody were in the range 1/16-1/32 and were therefore similar to the 6% normal pregnancies discussed above. Since these choriocarcinoma patients had received therapy for their malignancy, this may have reduced anti-i titres to some degree.

The usefulness of this new tumour marker needs to be pursued further, and the significance of anti-i formation in pregnancy and choriocarcinomatosis remains to be determined.

Help with the fluorescent microscopy was kindly given by Mr Tony Chaplin; Dr John Morton provided the IgM\textsubscript{K} anti-i antibody, and Mrs S. Brown typed the manuscript. Adult i cells were kindly donated by the Oxford Regional Blood Transfusion Centre, and Cynamolgus monkey cells by Shamrock Farms (Great Britain) Ltd. Professor K. Bagshaw, Charing Cross Hospital, kindly supplied sera from six further patients with choriocarcinoma.

References

Vox Sanguinis, 13, 4-6.


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