Australia Antigen and Antibody in Transfused Children with Thalassaemia*

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Vierucci, A., London, W. T., Blumberg, B. S., Sutnick, A. I., and Ragazzini (1972). Archives of Disease in Childhood, 47, 760. Australia antigen and antibody in transfused children with thalassaemia. As a consequence of frequent transfusions, 10% of 169 Italian patients with thalassaemia developed Au(1) and 20% anti-Au(1). Au(1) persisted in the children in whom it was detected for the duration of the study (2–7 years) or until the patient died. Anti-Au(1) was less persistent.

In these children, Au(1) or anti-Au(1) was detected, but not both, suggesting that patients with persistent Au(1) and antibody formers represent two distinct subgroups of the thalassaemia population. Au(1) was more common in males and in patients less than 7 years old, and was associated with earlier death; whereas antibody was more common in females, and in children older than 7 years, and was associated with longer survival.

Australia antigen was discovered as a result of a systematic search for human serum protein polymorphisms (Blumberg, 1964). Epidemiological, morphological, biochemical, and tissue culture data strongly support the hypothesis that Australia antigen Au(1) is (or is located on) an infectious agent which can cause hepatitis in man (Sutnick, London, and Blumberg, 1967; Blumberg, Sutnick, and London, 1970c; Shulman, Hirschman, and Barker, 1970; Gerin et al., 1969).

In acute viral hepatitis Au(1) appears early in the disease, often before any clinical signs or symptoms, lasts a few days or weeks, and then becomes undetectable. Some people, however, are chronic carriers of Au(1). They have little or no evidence of liver disease but transfusion of their blood can transmit hepatitis to recipients. Studies have shown that 1 to 20% of apparently normal persons among certain (mostly tropical) populations have Au(1) in their blood (Blumberg et al., 1970c). Family studies by our group and in Ceppellini’s laboratory (Ceppellini et al., 1970) are compatible with the hypothesis that susceptibility to become an Au(1) carrier is inherited as an autosomal recessive trait.

On systematic follow-up studies of 189 recipients of Au(1) blood, 131 developed hepatitis (as denoted by serum glutamic pyruvic transaminase (SGPT) elevation), 65 developed Australia antigen, and 29 antibody to Australia antigen. Of 345 recipients of only Australia antigen negative blood, 93 developed SGPT elevation, 14 developed Au(1), and 2 anti-Au(1). Some of the people who developed Au(1) or anti-Au(1) had no apparent clinical illness. Thus, the recipient of blood containing Au(1) is likely to develop hepatitis, Au(1), or anti-Au(1) (Goese et al., 1970; Okochi et al., 1970; Gocke, Greenberg, and Kavey, 1970; Goldfield, 1970).

Anti-Au(1) antibodies (detected by immunodiffusion) have been found primarily in sera from transfused patients with haemophilia, aplastic anaemia, and thalassaemia. They have also been detected in patients who have never been transfused; some of these have had clinical hepatitis, but others have had no apparent illness. The observations are consistent with the hypothesis that antibodies form in patients ‘infected’ with Au(1) either by transfusion or by other routes. The frequency of anti-Au(1) antibodies in sera from thalassaemic

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patients was much lower (2·1%) than in sera from
haemophiliac patients (28·6%) (Blumberg, Alter, and
Visnich, 1965), perhaps because the patients with
aemophilia received fresh frozen plasma, whereas the
patients with thalassaemia received
packed red cells and whole blood.

We have found that there is an enormous vari-
dation in host response to infection, including the
development of acute viral hepatitis, chronic and
persistent active hepatitis, chronic anicteric hepatitis,
and chronic infection (carrier state) with Au(1)
without evidence of hepatitis (Blumberg et al.,
1970c). From this it is clear that host factors
must be taken into account in evaluating the effects
of transfusion.

In this paper we present results of a study of
patients with thalassaemia living in northern Italy
who have received large numbers of transfusions.
This investigation has the epidemiological advan-
tage that there is no problem of sampling; the
entire sample group is included and the follow-up
is nearly complete. We report our observations on
the occurrence of Au(1) and anti-Au(1) antibody
in 169 patients with thalassaemia. An average of
three serum samples was tested annually during a
period of 2 to 7 years. The largest number was 20.
Age, sex, immunoglobulin levels, mortality rate,
and splenectomy have been considered in their
possible relation to the presence of Au(1) antigen
and antibody.

Materials and Methods

A total of 3180 specimens from 169 thalassaemic
patients collected over a 7-year period were studied.
These patients included 143 from the Centro della
Microcitemia of Ferrara; 21 from the Paediatric
Clinic of the University of Ferrara; 3 from the Paediatric
Clinic of the University of Siena; and 2 from the
Paediatric Clinic of the University of Florence. This
represents essentially the entire thalassaemia population
attending these clinics during the period of observation.

Test for Au antigen and antibody.

(A) Immunodiffusion. The test was carried out in
8 × 10 cm plates, in 1·1% agarose in phosphate
buffered saline (pH 7·4) (Blumberg et al., 1970a). The
method of Piazzl (1969) was also employed in the samples
collected during 1969 and 1970 to determine the optimal
conditions for the antigen/antibody reaction.

(B) Cross-electrophoresis. Australia antigen and anti-
body were also investigated in the sera collected during
1969 and 1970 by a modification of the reaction electropho-
resis method of Lang (1966). 8 × 10 cm lantern
slides were layered with 1% agarose (1·3% agar noble
for the screening of antibodies) in barbitone buffer
pH 8·2, 0·05 M. Two parallel rows of 2 mm diameter
holes were punched in the gel at a distance of 5 mm from
each other. When the test is used for the detection of
Au(1) antigen, the sera to be tested are placed in the
wells punched in the coidcic portions of the slide.
Antibody to Au(1) is placed in each opposite well.
When detection of anti-Au(1) is desired, the unknown
sera are placed in the anodic part of the slide, and a
known positive Au(1) in the opposite wells. A current
of 60 volts is then applied to the extremes of the slide,
for a period of 75 minutes.

Quantitation of IgA, IgG, IgM, and IgD. The 18
thalassaemia patients with Au(1) in their serum were
matched by sex in age order with 18 patients with anti-
Au(1), and with 18 patients who had not developed
either the antigen or the antibody. The age range for
most triplets was three years, the maximum range for
any triplet was six years. All sera were tested for
immunoglobulin levels (IgA, IgG, IgM, IgD) by the
method of Mancini, Carbonara, and Heremans,
1965, using commercial immunodiffusion plates
(Melpar).

Statistical methods. The immunoglobulin values
in the three groups of patients were compared by the
Wilcoxon matched pair signed rank test. The relation
between mortality, splenectomy, sex, and age, and
the distribution of Au(1) antigen and antibody were tested
by the χ² method, and Fisher’s exact test. The sex
distribution of Au(1) and anti-Au(1) was also evaluated
using tests for the difference between two cor-
related proportions. When a total sample (n) is divided into
several categories (x₁, x₂, etc.), we can observe the
proportions \[ x₁/n = p₁, x₂/n = p₂, \ldots \] etc. To test the
null hypothesis \[ p₁ = p₂ = p₃ \ldots \] we expect \[ p₁\ldots pₖ \]
to be normally distributed with large enough n, and use the
2 statistics, with \[ Z = (p₁\ldots pₖ)/\sqrt{2/n} \]. The Mann-
Whitney test was used for evaluating mortality differences
between the groups (Siegel, 1966).

Results

Frequency of Au(1) and anti-Au(1). Australia
antigen was detected in 18 (10·6%) of the
169 thalassaemia children and the antibody in 35
(20·7%). In one case the antigen was observed
before transfusion. The results with cross electropho-
resis and the technique of Piazzl (1969) were
the same as immunodiffusion, except that (1)
antibody was detected in an additional 2 patients
(35 instead of 33), and (2) in 9 patients, who had
had Au(1) or anti-Au(1) detected by immunodiffu-
sion in early samples; some follow-up samples
were positive by cross electrophoresis though
negative by immunodiffusion.

Persistence of antigen and antibody. Once
present the antigen persisted for the duration of
this study (2 to 7 years) or until the patient died
(Fig. 1). The only exception was the failure to detect Au(I) in patient PT in 1966 and 1968. Follow-up studies of the 35 patients with antibody are shown in Fig. 2. Antibody, in general, was persistent, but in several cases it disappeared, usually to return again. There was one case in which a weak antibody was found in a single sample, and antigen was subsequently detected in later samples. Other than this, the thalassaemic children had either antigen or antibody but not both.

**Sex, age, mortality, and splenectomy.**

Au(I) is more frequent in males (14·3%) than in females (6·4%) (Table I). This difference is not statistically significant ($\chi^2 = 1·972$, $P > 0·10$), but it is consistent with findings in other healthy and disease populations (London, Sutnick, and Blumberg, 1969; Sutnick et al., 1968; Blumberg et al., 1970b; Blumberg et al., 1972). Antibody is more frequent in females (30·0%) than in males (22·9%), but this difference is also not significant ($\chi^2 = 0·26$, $P > 0·50$). However, when the differences between two correlated proportions are tested we found that the proportion of females with anti-Au(I) was significantly greater than the proportion of females with Au(I) ($P = 0·00126$), whereas there was no significant difference between the proportion of males with Au(I) and those with anti-Au (I).

![Fig. 1.—Australia antigen in 18 patients with thalassaemia. Initials identify individual patients. Au(0) = absence of Australia antigen.](image)

![Table I](image)

![Fig. 2.—Antibody to Australia antigen in 35 patients with thalassaemia. Initials identify individual patients.](image)
Australia Antigen and Antibody in Transfused Children with Thalassaemia

The average life span of patients with thalassaemia is 15 years. In order to determine the relation of age to the presence of Au(1) antigen and antibody, the study group was divided into those less than 7 years and those greater than 7 years (Table II).

| TABLE II |

Distribution of Au(1) Antigen and Anti-Au(1) Antibodies in Thalassaemic Children Divided in Two Groups According to Age

<table>
<thead>
<tr>
<th>Age</th>
<th>With Au(1)</th>
<th>With Anti-Au(1)</th>
<th>Without Au(1) or Anti-Au(1)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;7 yr</td>
<td>10</td>
<td>6</td>
<td>61</td>
<td>77</td>
</tr>
<tr>
<td>&gt;7 yr</td>
<td>8</td>
<td>29</td>
<td>55</td>
<td>92</td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>35</td>
<td>116</td>
<td>169</td>
</tr>
</tbody>
</table>

Au(1) vs. anti-Au(1) with respect to age: \( \chi^2 = 14.428, P < 0.001. \)

| TABLE III |

Distribution of Au(1) and Anti-Au(1) in Thalassaemic Children by Age at First Blood Transfusion ( Reliable data only )

<table>
<thead>
<tr>
<th>Age at First Transfusion</th>
<th>With Au(1)</th>
<th>With Anti-Au(1)</th>
<th>Without Au(1) or Anti-Au(1)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;12 mth</td>
<td>8</td>
<td>15</td>
<td>48</td>
<td>71</td>
</tr>
<tr>
<td>&lt;12 mth</td>
<td>9</td>
<td>14</td>
<td>47</td>
<td>70</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>29</td>
<td>95</td>
<td>141</td>
</tr>
</tbody>
</table>

Median age (and ranges) at 1st transfusion (yr) 9/12 (3/12—7) 1 (3/12—5) 1 (3/12—20)

<table>
<thead>
<tr>
<th>Au(1) vs. Anti-Au(1)</th>
<th>P(Mann-Whitney)</th>
<th>( \chi^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Au(1) vs. Au(0)</td>
<td>0.803</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Anti-Au(1) vs. Au(0)</td>
<td>0.754</td>
<td>&gt;0.90</td>
</tr>
</tbody>
</table>

There is a higher frequency of antibody in the older patients and a higher frequency of antigen in the younger ones, and this joint difference is significant \( \chi^2 = 17.410, P < 0.001. \) At the time of the first blood transfusion, there were no differences between the ages of the patients who eventually developed Au(1), and anti-Au(1), or neither (Table III).

The mortality rate during the 7-year study period in patients with Au(1), and anti-Au(1) and with neither is shown in Table IV. It is higher in the patients with Au(1) (22.2%) than in those of the other groups (anti-Au(1) = 11.4%, neither Au(1) nor anti-Au(1) = 9.5%), and the median age at death is younger (Au(1) = 5 years, anti-Au(1) = 10.5 years, neither Au(1) nor anti-Au(1) = 7 years). However, the longer survival among the patients with anti-Au(1) is significant compared with the patients with Au(1) \( P=0.014), and is marginally significant compared with those without antigen or antibody \( P = 0.086). The relation of splenectomy to Au(1) and anti-Au(1) is shown in Table V. Treatment with

| TABLE IV |

Mortality Rate in Thalassaemic Patients Observed Over a 5-year Period

<table>
<thead>
<tr>
<th>Patients</th>
<th>No.</th>
<th>Dead</th>
<th>%</th>
<th>Median Age at Death (yr)</th>
<th>Age at Death Range (yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>With Au(1)</td>
<td>18</td>
<td>4</td>
<td>22.2</td>
<td>5.0</td>
<td>(4-6)</td>
</tr>
<tr>
<td>With anti-Au(1)</td>
<td>35</td>
<td>4</td>
<td>11.4</td>
<td>10.5</td>
<td>(8-16)</td>
</tr>
<tr>
<td>Without Au(1) or Anti-Au(1) [Au(0)]</td>
<td>116</td>
<td>11</td>
<td>9.5</td>
<td>7.0</td>
<td>(1-12)</td>
</tr>
</tbody>
</table>

Comparison of patients with Au(1) or anti-Au(1) vs. those with neither antigen nor antibody.

\( \chi^2 = 7.149, p. <0.05. \)
splenectomy was most frequent in patients with anti-Au(1) and was more common in patients with Au(1) or anti-Au(1) than in the remaining patients with neither the antigen nor the antibody and the difference is significant \( \chi^2 = 7.149, d.f. = 2, \)
P <0·05). However, splenectomy was performed in many instances after antibody was already present and, in general, on older children.

Immunoglobulins. Children with Au(1) or anti-Au(1) had significantly lower IgG immunoglobulin levels than those with neither antigen nor antibody. IgA levels were significantly lower in those with antigen than with antibody. No other significant differences were found, but levels of all immunoglobulins were slightly lower in those with Au(1) than with anti-Au(1) (Table VI).

Discussion

Patients with thalassaemia are subjected to repeated exposure to Australia antigen as a consequence of repeated blood transfusions. We have observed persistent antigen and antibody in them. We have not studied antigen/antibody complexes since the methods hitherto available for this measurement are either insensitive or indirect. The recent development by Coller et al. (1971) of a highly sensitive radioimmunoassay coprecipitation method which can apparently detect antigen/antibody complexes will now permit the biological study of significance of this reaction.

The frequency of Au(1) is relatively high in this thalassaemia patient group (10·6%), but not significantly higher ($\chi^2 = 2·445$, $P <0·10$) than that reported for American thalassaemia patients (Blumberg et al., 1965) from whom only single or small numbers of sera from each individual were tested. The antigen once present in these patients is nearly always persistent; there were only two inconsistencies in 61 patient years. The results are similar to those previously reported for apparently asymptomatic individuals living in Rongelap (Blumberg et al., 1966), Down's syndrome patients (London et al., 1969), lepromatous leprosy patients living in Cebu (Blumberg and Melartin, 1970), and leukaemia patients (Sutnick et al., 1970).

The persistence of Au(1) antigen in these patients contrasts with the situation in acute viral hepatitis in which the presence of the antigen is transient and may persist for only days or weeks (London et al., 1969; Shulman and Barker, 1969). We have made the hypothesis that the persistence of the antigen is related to an immune impairment present in some patients (i.e. Down's syndrome, leukaemia) which may be associated with cellular immunity (Blumberg et al., 1970c). It is not known if the persistence is due to recurrent infection of patients living in an environment where they are continually exposed to 'infection' with Australia antigen, or to the persistence of a single infection. Since these thalassaemic patients are transfused repeatedly they are continually re-exposed to the infection, and hence it is not possible to distinguish reinfection from persistent infection from these data.

The frequency of anti-Au(1) is very high in the patients reported here (20·7%). In thalassaemia patients transfused in Greece it is still higher (40·2%) (Edonomidou et al., 1970), which might be due to a higher frequency of Au(1) among Greek blood donors than among Italian donors. Anti-Au(1) is sometimes persistent but less so than the antigen (Fig. 2). Individuals who develop persistent antibody are distinct from those who develop persistent antigen; that is, the ability to form antibody or antigen appears to be an inherent quality of the individual.

There are other characteristics that can define the differences between individuals who form persistent antibody, and those who develop persistent antigen. Males have a higher frequency of Australia antigen and females have a higher frequency of antibodies. The former observation is

| TABLE VI |

Comparison of Immunoglobulins in Respect of Australia Antigen and Antibody in Thalassaemia Patients (Wilcoxon Matched Pair Signed Ranks Test) (Mean Values Shown in mg/ml)

<table>
<thead>
<tr>
<th></th>
<th>IgG (mg/ml)</th>
<th>P</th>
<th>IgM (mg/ml)</th>
<th>P</th>
<th>IgA (mg/ml)</th>
<th>P</th>
<th>IgD (mg/ml)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Au(1)</td>
<td>13·7</td>
<td></td>
<td>1·27</td>
<td></td>
<td>1·54</td>
<td>&lt;0·05</td>
<td>0·078</td>
<td></td>
</tr>
<tr>
<td>Anti-Au(1)</td>
<td>16·9</td>
<td></td>
<td>1·62</td>
<td></td>
<td>2·19</td>
<td></td>
<td>0·158</td>
<td></td>
</tr>
<tr>
<td>Au(1)</td>
<td>13·7</td>
<td>&lt;0·01</td>
<td>1·27</td>
<td></td>
<td>1·54</td>
<td></td>
<td>0·078</td>
<td></td>
</tr>
<tr>
<td>Au(0)*</td>
<td>20·0</td>
<td></td>
<td>1·58</td>
<td></td>
<td>2·40</td>
<td></td>
<td>0·0195</td>
<td></td>
</tr>
<tr>
<td>Anti-Au(1)</td>
<td>16·9</td>
<td>&lt;0·05</td>
<td>1·62</td>
<td></td>
<td>2·19</td>
<td></td>
<td>0·158</td>
<td></td>
</tr>
<tr>
<td>Au(0)</td>
<td>20·0</td>
<td></td>
<td>1·58</td>
<td></td>
<td>2·40</td>
<td></td>
<td>0·0195</td>
<td></td>
</tr>
</tbody>
</table>

*Au(0) = patients without Australia antigen or antibody.
consistent with our earlier observations that Au(1) is more common in males than in females in 22 of 23 populations studied (London et al., 1969; Sutnick et al., 1968; Blumberg et al., 1970b; Blumberg et al., 1972). The higher frequency of antibody in females has not been observed previously. Persistent antigen is more common among the younger patients, confirming our earlier observations in other populations (Blumberg, Sutnick, and London, 1968; Blumberg et al., 1970c). Persistent antibody is commoner in older patients; this has not been studied previously.

The decreased immunoglobulin values may be related in part to the high frequency of splenectomy. We plan to test the hypothesis that transfused patients with thalassaemia may develop antigen/antibody complexes, and the decreased levels of immunoglobulin could be associated with this.

We had previously reported (Vierucci et al., 1968) that the formation of anti-Ag (lipoprotein) antibodies contribute to the mortality in thalassaemic children. The increased mortality among the patients with Australia antigen is small and should be confirmed, but its validity is supported by the increased survival of the patients with anti-Au(1). If it is sustained, then we have identified a second factor which contributes to early mortality in thalassaemic children.

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References


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