Prematurity, hypogammaglobulinemia, and neuropathology with human immunodeficiency virus (HIV) infection

(Acquired immunodeficiency syndrome/human immunodeficiency virus antibody/intracranial calcification)

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ABSTRACT Infection with the human immunodeficiency virus (HIV) is characteristically associated with hypogammaglobulinemia in both adult and pediatric cases. We report herein four infants who had an HIV infection in association with severe hypogammaglobulinemia and did not exhibit antibodies against HIV. HIV was isolated antemortem or postmortem in all four infants from either peripheral blood, cerebrospinal fluid, or body tissues. HIV infection could be presumed to be acquired transplacentally in two infants and by way of infected blood transfusions during the neonatal period in the other two. Each infant became symptomatic within the first year of life and developed rapidly progressive manifestations of the disease. Features that were common to all four infants include premature birth, failure to thrive, hepatomegaly, and progressive neurological abnormalities that were associated with intracranial calcifications. We conclude that, when infection occurs early in development either by transplacental exposure to the virus or from blood transfusion in small premature infants, hypogammaglobulinemia and deficiency of antibody production leading to the absence of antibody responses on which diagnosis is usually based can occur. Furthermore, progressive central nervous system disease may be a frequent finding in such infants, and this may lead to cerebral calcifications that must be attributed to the HIV infection itself and not to complicating infections—e.g., toxoplasmosis. It is suggested that patients with hypogammaglobulinemia, antibody deficiency syndrome, and central nervous system disease have an extremely bad prognosis.

Infection with the retrovirus, human T-lymphotropic virus type III/lymphadenopathy-associated virus (HTLV-III/LAV), now known as human immunodeficiency virus (HIV), results in a wide spectrum of clinical manifestations, ranging from asymptomatic states to the severest disease forms associated with the acquired immunodeficiency syndrome (AIDS) (1–12). This virus infects preferentially T lymphocytes bearing the T4 antigen and also seems to exhibit preference for cells, especially macrophages, of the central nervous system (13, 14). Among the immunological disturbances resulting from infection with HIV, hypogammaglobulinemia appears to be a consistent finding in adults as well as in most children (5, 15–17). The presence of antibodies to HIV in serum is considered to be a hallmark of this infection. In this report four infants are described in whom HIV infection was documented by positive virological cultures; they also exhibited profound hypogammaglobulinemia and did not produce HIV antibodies. These infants were born prematurely and had illnesses characterized by severe and progressive neurological disease. Although the protective role of HIV antibodies has not been established, these observations suggest that severe hypogammaglobulinemia that develops in infants with HIV infection may be a bad prognostic feature. Furthermore, neurological complications occur and appear to be severe in such hypogammaglobulinemic infants.

MATERIALS AND METHODS

Study Subjects. The four patients described herein were each born prematurely between 26 and 30 weeks of gestation (Table 1). Two infants (patients 1 and 2) were born to HIV antibody-positive, asymptomatic mothers; the first was an intravenous drug user, and the second probably had sexually acquired the infection. The other two infants (patients 3 and 4) were born to healthy mothers. The HIV serology was negative on both parents in each of the latter infants who had, however, been given repeated transfusions in the neonatal period prior to availability of HIV antibody screening. These infants were presumed to be infected by HIV-infected blood transfusions; the blood donor of patient 3 was subsequently proven to have an HIV infection.

The clinical manifestations in the four patients are recorded in Table 2. Patient 1 became symptomatic between 7 and 8 months of age. Patient 1 was followed from birth, and patients 2, 3, and 4 were followed from the ages of 5, 8, and 10 months, respectively. Patient 3, who had radiological evidence of interstitial pneumonitis, was found by biopsy to have lymphoid interstitial pneumonitis negative for Pneumocystis carinii pneumonia, bacteria, fungi, and all other identifiable viruses. All infants had progressive retardation of their growth and development, and each exhibited clear evidence of central nervous system (CNS) involvement. Evidence of brain atrophy and CNS calcification was present in the computerized tomographic scans of each of the four patients; examples of the CNS calcifications are illustrated in Fig. 1. Cytomegalovirus was repeatedly isolated from the urine and saliva of only one infant, patient 2. Severe and recurrent bacterial infections resulting in pneumonia, meningitis, or bacteremia ultimately occurred in each of the patients. All patients were treated with intravenous gamma globulin because they exhibited hypogammaglobulinemia. The dose of gamma globulin was gradually increased over a 4-week period from 100 to 300 mg/kg and was given at this dose every 2–4 weeks, depending upon compliance. Patients 1 and 2 were started on intravenous injections of gamma globulin at 6 months of age, whereas patients 3 and 4 first received injections at 13 months of age. Only patient 2 had adverse reactions to the gamma globulin, which often precluded administration of the desired dose. All patients died of sepsis at 7, 22, 21, and 17 months of age, respectively. Autopsy was performed on patient 1. This analysis revealed generalized

Abbreviations: HIV, human immunodeficiency virus; AIDS, acquired immunodeficiency syndrome; CNS, central nervous system. To whom reprint requests should be addressed: All Children’s Hospital, 801 6th Street South, St. Petersburg, FL 33701.
lymphocyte depletion and the presence of microglial nodules, calcification, and atrophy of cerebral cortex of the brain. Parents refused to permit autopsies on the other three patients.

**Laboratory Methods.** Serologic testing for HIV antibodies was performed by immunological blot analysis (18). Specifically, the presence of the anti-p41 antibody to HIV was considered as evidence of HIV infection. HIV cultures were performed on peripheral blood lymphocytes of all four patients and on cerebrospinal fluid of patient 4. For these studies, lymphocytes from peripheral blood or cerebrospinal fluid were cultured with phytohemagglutinin, grown in the presence of interleukin 2-stimulated lymphocytes, and subsequently cocultivated with an uninfected clone of the H-9 cell line, as previously described (19). Virus production in the infected cell clones was monitored by assaying for reverse transcriptase activity in culture supernatants and by electron microscopy. In patient 1, tissues obtained at autopsy from brain and lung were cultured for HIV; the virus was isolated from primary cultures of lung tissue and was propagated in peripheral blood-derived macrophages of normal donors (20). Virus expression was tested by an immunofluorescence assay using a monoclonal antibody to p17 and by measurement of reverse transcriptase activity (20).

T and B lymphocytes, and cells of the major T-cell subsets that bear T4 or T8 antigens on their surfaces, were quantified in whole blood using monoclonal antibodies (Ortho-immune and Coulter clone reagents) and flow cytometry (21). The functional capacity of peripheral blood lymphocytes was assessed by investigating the proliferative responses of the lymphocytes to phytoimotogens, namely phytohemagglutinin, concanavalin A, and pokeweed mitogen, as determined by incorporation of [14C]thymidine (New England Nuclear) (22).

Quantitative serum immunoglobulin levels were determined by radial immunodiffusion.

**RESULTS**

Serum immunoglobulin levels and results of HIV antibody testing are shown in Table 3 for all four infants. In patients 1 and 2, serum IgG levels were within normal limits for age at 1 and 5 months, respectively, but they were decreased at 3 months of age in patient 1 and at 7 months of age in patient 2. Patients 3 and 4 had reduced IgG levels at the time of presentation (i.e., at 8 and 10 months of age, respectively). Patients 1 and 2, who were initially positive for HIV antibody, became negative after 6 months of age. It is presumed that the antibodies in the infants’ serum were passively transferred to the infants from their HIV-positive mothers. Patients 3 and 4 were negative for HIV antibodies when they were first tested at 8 and 15 months of age, respectively. Serum IgA levels were ultimately low in all four patients. Serum IgM levels were elevated in patients 2 and 3 at the time of initial diagnosis and became low after 14 months of age. Sometime during the course of the disease, three of the patients had low levels of IgM. The fourth patient, patient 2, had elevated or normal IgM levels early in life; these levels dropped in subsequent months, but they were still in the normal range prior to death.

All of the children were treated with intravenous gamma globulin (IGG) for their hypogammaglobulinemia as described above.

HIV was isolated from peripheral blood lymphocytes of patients 2, 3, and 4 and from cerebrospinal fluid of patient 4. In patient 1, virus was also isolated from autopsy material in lung macrophages as described above (20).

Table 4 summarizes the results of analyses of lymphocyte surface markers. Absolute lymphocyte counts were decreased in two patients, and a depression in the number of T4 cells, with absolute counts less than 400/mm³, was noted in three of the four patients. The T4/T8 ratios were less than 1.0 in all four infants. B-cell numbers, which are usually increased in HIV infection, were decreased in each of the three infants tested. Results of immune testing are summarized in Table 5. Functional lymphoproliferative responses to the phytoimotogens phytohemagglutinin, concanavalin A, and pokeweed mitogen were severely depressed in two infants (patients 1 and 2), and response to concanavalin A was depressed in patient 3. Normal proliferative responses were observed in patient 4 at the time of testing.

**DISCUSSION**

Documentation of HIV antibodies in serum has proved to be a reliable and practical means of establishing the presence of HIV infection (18). On rare occasions, a transient or persistent seronegative virus-infected state occurs and poses a potential problem in serodiagnosis of HIV infection (23). In
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Fig. 1. Computerized tomographs of the brain from patient 3. Cuts at various levels show cortical atrophy, ventricular dilatation, and intracranial calcifications.

In this report, we describe four infants with HIV infection, who developed severe hypogammaglobulinemia and were HIV antibody-negative to this virus, although they were clearly infected with HIV. Presumably this immunity status is a consequence of the hypogammaglobulinemia. The features of all four of these infants included premature birth and progressive development of severe neurological abnormalities. The latter consisted of intracranial calcifications, cerebral atrophy, and clinical evidence of cerebral palsy.

B-lymphocyte dysfunction as an early manifestation of HIV infection is usually characterized by profound polyclonal hypergammaglobulinemia (5, 15–17, 24). In a previous study (5), hypergammaglobulinemia was consistently noted in HIV-infected infants, ranging in age from 3 months to 1 year, who had typical clinical features of AIDS or AIDS-related complex and were seropositive for HIV antibodies. Hypogammaglobulinemia occurring in association with HIV infection represents a new phenomenon, and its relationship to those situations in which HIV-infected patients are seronegative despite normal or increased levels of immunoglobulins needs to be investigated. Already, however, it has been shown that the levels of IgG2 and IgG4 subclasses may be low in patients with HIV infection, while hypergammaglobulinemia attributable to increased levels of IgG1 and IgG3 occurs in the same patient (25). Furthermore, hypogammaglobulinemia has been encountered in association with selective T-helper cell immunodeficiency in patients with severe combined immunodeficiencies or common variable immunodeficiencies (26, 27). Thus, it seems quite possible that the hypogammaglobulinemia seen in these infants may be attributable to profoundly defective functions of T-helper cells, defective T cell–B cell interactions, or even deficiencies in the function of B cells themselves (24).

In regard to the B-cell pathology in HIV infection, it has been shown already that proliferating B cells infected with

### Table 3. Immunoglobulin levels and HIV infection

<table>
<thead>
<tr>
<th>Patient and age</th>
<th>IgG, mg/dl</th>
<th>IgA, mg/dl</th>
<th>IgM, mg/dl</th>
<th>HIV-Ab*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 mo.</td>
<td>382</td>
<td>9</td>
<td>33</td>
<td>ND</td>
</tr>
<tr>
<td>3 mo.</td>
<td>133</td>
<td>&lt;7</td>
<td>20</td>
<td>+</td>
</tr>
<tr>
<td>6 mo.</td>
<td>25</td>
<td>8</td>
<td>14</td>
<td>–</td>
</tr>
<tr>
<td>7 mo.†</td>
<td>1220</td>
<td>&lt;7</td>
<td>35</td>
<td>–</td>
</tr>
<tr>
<td>Patient 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 mo.</td>
<td>333</td>
<td>26</td>
<td>158</td>
<td>+</td>
</tr>
<tr>
<td>7 mo.†</td>
<td>181</td>
<td>34</td>
<td>127</td>
<td>ND</td>
</tr>
<tr>
<td>11 mo.†</td>
<td>149</td>
<td>25</td>
<td>97</td>
<td>ND</td>
</tr>
<tr>
<td>14 mo.†</td>
<td>169</td>
<td>14</td>
<td>37</td>
<td>–</td>
</tr>
<tr>
<td>18 mo.†</td>
<td>374</td>
<td>&lt;7</td>
<td>55</td>
<td>–</td>
</tr>
<tr>
<td>Patient 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 mo.</td>
<td>147</td>
<td>16</td>
<td>52</td>
<td>–</td>
</tr>
<tr>
<td>9 mo.</td>
<td>72</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>10 mo.</td>
<td>42</td>
<td>ND</td>
<td>ND</td>
<td>–</td>
</tr>
<tr>
<td>14 mo.†</td>
<td>704</td>
<td>&lt;7</td>
<td>18</td>
<td>–</td>
</tr>
<tr>
<td>Patient 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 mo.</td>
<td>100</td>
<td>11</td>
<td>23</td>
<td>ND</td>
</tr>
<tr>
<td>13 mo.†</td>
<td>364</td>
<td>&lt;4</td>
<td>26</td>
<td>ND</td>
</tr>
<tr>
<td>15 mo.†</td>
<td>250</td>
<td>&lt;2</td>
<td>10</td>
<td>–</td>
</tr>
</tbody>
</table>

*Antibodies to HIV in serum, determined by immunological blot.
†Patient on intravenous gamma globulin infusions.

Values less than 2 SD for age are in boldface. ND, not done.
another virus can grow the HIV (28). It is possible that
different subpopulations of B lymphocytes react differently
to the influence of the virus; some are stimulated while others
are inhibited. Even in the absence of direct infection of B cells
by HIV, different components of the HIV might indirectly
exert either stimulatory and/or inhibitory influences (29). In
fact, two patients reported herein, in spite of having low
levels of serum IgG, had high levels IgM at some time during
the first year of life. This recalls the dysgammaglobulinemia
first described with virus infections in patients with the in utero
infections caused by the rubella virus (30, 31). The
infants described herein also had a paucity of B cells, which
is unusual in patients with HIV infection. This abnormality in
B-cell numbers was unrelated to the mode of acquisition of
the HIV infection—i.e., whether it occurred as a conse-
quence of in utero infection or after birth. It is possible that
in the premature infant infected with HIV the B lymphocytes,
which are characteristically immature in the newborn period
(32, 33), are more readily compromised in their development
than at a later time in life. Only further studies will ascertain
just how and in what cells the primary influence of the virus
to inhibit the development of a normal capacity for immu-
noglobulin production is exercised.

Accumulating clinical, pathological, and virological evi-
dence has firmly established the neurotropic potential of HIV
(3, 13, 14). In brain tissues of AIDS patients, HIV sequences
have been demonstrated by Southern blot analysis of host
cell DNA. Further, HIV messenger RNA has been detected
by in situ hybridization (13). In a recent study, Popovic and
coworkers (20) demonstrated selective tropism by HIV viral
isolates for macrophages obtained either from brain tissue of
an AIDS patient or from lung macrophages of an infant. It has
been suggested that in certain patients, such as patient 1, the
macrophages represent a primary target for transmission of
infection to the CNS. In agreement with this concept is
another report that shows that virus-positive cells in the brain
tissue of AIDS patients belong to the mononuclear phagocyte
series (34). As yet, an association between hypogammaglob-
ulinemia and tropism of the HIV for macrophages has not
been described. It is possible that monocyte dysfunction
might further contribute to the failure of the development of
a normal capacity to produce antibodies in such patients.

An additional concurrent viral infection might be respon-
sible for the CNS disease observed in these children. This is,
however, an unlikely possibility, but it is one that cannot be
conclusively ruled out in each of these patients because of the
hypogammaglobulinemia and consequent absence of sero-
logical responses that otherwise might be expected—for
example, toxoplasma, rubella, cytomegalovirus, or Epstein–
Barr virus. However, cerebrospinal fluid cultures in one
patient were negative for cytomegalovirus and also were
negative for other herpes or enteric viruses and, at the same
time, were positive for HIV. The presence of HIV in the
cerebrospinal fluid of patient 4, the autopsy findings of the
brain in patient 1, and the radiological and clinical abnormal-
ities of the brain in all these patients raise the likelihood that
a primary HIV infection involving the brain has occurred in
every one of these children. The mechanism whereby such an
infection would result in intracranial calcifications is as yet
unclear, but this finding should be included in the pathologic
features of HIV infection.

Thus, these patients who were infected with HIV virus
very early in life present evidence both of a threatening
neuropathology of the HIV virus, especially for very young
children, and challenging new evidence of the complexity of
the lymphoid cellular involvement in this disease, which now
must be recognized as potentially leading to hypogam-
moglobulinemia or agammaglobulinemia. Although a protec-
tive role for HIV antibodies has not been conclusively
established, the absence of such antibodies in the cases
described herein was associated in each instance with rapid
progressive clinical deterioration. A heightened awareness of
HIV seronegativity occurring as a consequence of hypogam-
moglobulinemia has important consequences for the serodi-
agnosis of HIV infection in children.

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1. Gallo, R. C., Salahuddin, S. Z., Popovic, M., Kaplan, G. M.,
Haynes, M., Falker, B. F., Redfield, T. J., Oleske, R., Safai,
224, 500–502.
1292–1297.

Table 4. Lymphocyte markers, percentages, and absolute numbers

<table>
<thead>
<tr>
<th>Patient and age</th>
<th>T3 %</th>
<th>T4 %</th>
<th>T8 %</th>
<th>T11 %</th>
<th>B1 %</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 mo. Patient 1</td>
<td>35</td>
<td>24</td>
<td>54</td>
<td>75</td>
<td>2.1</td>
</tr>
<tr>
<td>7 mo. Patient 2</td>
<td>68</td>
<td>34</td>
<td>43</td>
<td>198</td>
<td>76</td>
</tr>
<tr>
<td>15 mo. Patient 3</td>
<td>34</td>
<td>9</td>
<td>52</td>
<td>421</td>
<td>48</td>
</tr>
<tr>
<td>15 mo. Patient 4</td>
<td>38</td>
<td>10</td>
<td>25</td>
<td>511</td>
<td>36</td>
</tr>
<tr>
<td>9 73 52 421 48 389 ND ND</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values falling outside of the normal range for age are in boldface. For T8, boldface numbers indicate increased values; for all others, boldface numbers indicate decreased values. ND, not done.

![Image: Table 4. Lymphocyte markers, percentages, and absolute numbers](image-url)