ISOLATION OF RETROVIRUSES FROM TWO PATIENTS WITH "COMMON VARIABLE" HYPOGAMMAGLOBULINAEMIA

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Summary Retroviruses related to human T-lymphotropic virus III/lymphadenopathy-associated virus (HTLV-III/LAV) have been isolated from peripheral-blood mononuclear cells of two patients with "common variable" hypogammaglobulinaemia who were being treated with intravenous gammaglobulin. One has had three different opportunistic infections. In both patients hypogammaglobulinaemia developed within 6 years of a longstanding undiagnosed viral-like illness in adolescence, and it is suggested that the virus causing that illness also gave rise to the hypogammaglobulinaemia. However, intravenous infection from intravenous gammaglobulin cannot be ruled out.

Introduction "Common variable" hypogammaglobulinaemia (CVH) presents at all ages, although the peak incidence is in the third decade. The disease is heterogeneous, although subgroups can be recognized. Most patients have some circulating B lymphocytes, which can be induced in vitro to make IgM and IgG, although immunoglobulin production in vivo is impaired. In general these patients are prone to certain bacterial and mycoplasma infections but not to opportunistic viral, protozoal, and fungal infections, such as are seen in patients with severe defects in cellular immunity. Nevertheless, at least 30% of CVH patients have a T-cell lymphopenia and fail to show delayed-hypersensitivity skin reactions. In some patients the disease appears to start with a viral-like illness, with malaise, fever, splenomegaly, and lymphadenopathy.

We describe the isolation of retroviruses from two patients with CVH on intravenous gammaglobulin therapy who first presented in adolescence with a viral-like illness. Opportunistic infections characteristic of the acquired immunodeficiency syndrome (AIDS) have developed in one patient; the other remains relatively well.

Case reports

Table 1 outlines the initial illness and present clinical status of the two patients. Both patients presented at our clinic with recurrent upper and lower respiratory infections. Neither abused drugs. Pneumocystis carinii pneumonia developed in patient 1 4 months after starting intravenous gammaglobulin therapy ("Sandoglobulin", 12 g every 3 weeks). She responded to cotrimoxazole, but 5 months later generalised cutaneous Herpes zoster developed, and this responded to acyclovir. She is now gaining weight and has an unexplained fever. She had sexual intercourse for the first time after the development of the Pneumocystis infection. Her partner is well and is seronegative for human T-lymphotropic virus type III/lymphadenopathy-associated virus (HTLV-III/LAV) by enzyme-linked immunosorbent assay (ELISA) (Burroughs Wellcome). In August, 1983, non-A, non-B hepatitis developed in patient 2, a married man who denied homosexual contact, during a trial of intravenous gammaglobulin. He had previously been on regular intramuscular gammaglobulin therapy for 5 years. His liver function tests returned to normal within a year. He had had about 6 months' treatment with another intravenous preparation ("Gamuline", Cutter) before a retrovirus was isolated from his blood in August, 1984: he has since been receiving regular intravenous "Sandoglobulin" and remains well apart from episodes of diarrhoea due to chronic salmonella enteritis.

Methods

Immunological Tests (Table 1)

Patient 1 was profoundly lymphopenic before treatment, with very low relative numbers of T cells and a reversed T4/T8 ratio. Patient 2 was mildly lymphopenic with a normal percentage of T cells. Lymphocyte transformation with phytohaemagglutinin (PHA), concanavalin A, and irradiated allogeneic cells (mixed

TABLE 1—CLINICAL DATA

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Hypogam-</th>
<th>Present age</th>
<th>Initial illness</th>
<th>Recent clinical status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>19</td>
<td>20</td>
<td>Age 18 yr; 5 mo.; illness with malaise, cervical lymphadenopathy, and fever</td>
<td>Pneumocystis carinii pneumonia, August, 1983; severe herpes zoster, September, 1983; recurrent bronchitis</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>21</td>
<td>33</td>
<td>Healthy at 15 yr followed by idiopathic polyarthopathy and Compos</td>
<td>Milder, chronic salmonella enteritis for past year</td>
</tr>
</tbody>
</table>

* Both patients are currently receiving intravenously gammaglobulin (200 mg/kg/week).

**TABLE 2—IMMUNOLOGICAL TESTS

<table>
<thead>
<tr>
<th>Patient</th>
<th>Total lymphocytes (×10⁶)</th>
<th>T cells (%)</th>
<th>T4/8 ratio</th>
<th>IgG (µg/ml)</th>
<th>IgA (µg/ml)</th>
<th>Mixed lymphocyte reaction (in vitro)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>400-600</td>
<td>19</td>
<td>0-7</td>
<td>C1</td>
<td>0.9-1</td>
<td>&lt;18</td>
</tr>
<tr>
<td>2</td>
<td>600-1200</td>
<td>41</td>
<td>2-5</td>
<td>C1</td>
<td>0.4-0.5</td>
<td>&gt;10</td>
</tr>
<tr>
<td>Normal</td>
<td>1500-2000</td>
<td>40-60</td>
<td>0-7</td>
<td>&lt;2</td>
<td>0.5-0.7</td>
<td>&gt;18</td>
</tr>
</tbody>
</table>

* Before treatment.

† Patient's lymphocytes were mixed with irradiated lymphocytes from a normal subject. Figures given are stimulation indices; e.g. of patient's normal cells.

‡ Stimulation indices for PHA and concanavalin A were 2 and C1, respectively.

†† Stimulation indices for PHA and concanavalin A were 2 and C1, respectively.
lymphocyte reaction was severely depressed in patient 1 but within the normal range in patient 2. Both patients' sera were negative for HTLV-III antibodies when tested by means of immunofluorescence against HTLV-III-infected cell lines or by competition ELISA (Burroughs Wellcome).

Virus Isolation Procedures

Peripheral blood mononuclear cells were separated by "Ficoll-Hypaque" gradient centrifugation. The cells were grown in RPMI supplemented with 20% fetal calf serum and stimulated with PHA for 24-72 h, after which the cultures were supplemented with recombinant interleukin-2 (IL-2, Biogen). Subsequently, intervals of 2-3 days, samples from these cultures were cocultivated with CD4-positive cell lines. Polybrene (4 μg/ml) was added to the co-cultures from patient 1. Reverse transcriptase was measured in the pooled supernatants from co-cultures 1-7. Syncytium formation was observed in all co-cultures, which on electron microscopy showed a retrovirus morphologically indistinguishable from HTLV-III/LAV-1 (see figure) and minimal lentiviruses. Supernatant from this co-culture was positive for reverse transcriptase, and the cells were positive by immunofluorescence with serum from patient 1 with AIDS and with the anti-HTLV-III monoclonal antibodies, 6e24 and 6e-p19 (from Dr. R. C. Gallo). Southern blot of restricted DNA from infected cells was probed with AB1-10 (from Dr. R. C. Gallo). 10 indicated that the viral genome showed homology to HTLV-III/LAV but with restriction enzyme sites distinct from the prototype isolates, HTLV-III/LAV-1. Reverse transcriptase, renaturation, and immunofluorescence with AIDS sera were also found in the co-culture cells from patient 2.

Discussion

There are obvious parallels between the clinical and laboratory abnormalities in some patients with CVH and those with AIDS. Lymphopenia, absent delayed hypersensitivity skin reactions, and a tendency to auto-immune blood dyscrasias, particularly thrombocytopenic purpura, are common to both disorders. At least 10% of CVH patients also have a relative excess of circulating T8 positive lymphocytes. The major laboratory difference between CVH and AIDS is that AIDS patients are usually hypergamaglobulinemic, although specific antibody production is impaired in many.

Most CVH patients are clinically distinguishable from those with AIDS, since they are not prone to life-threatening opportunistic fungal, viral, and protozoal infections. However, the opportunistic infections seen in AIDS have, rarely, been reported in CVH patients. Some of these patients have had an associated thymoma. Cutaneous Herpes zoster infection is also common in CVH, but it is usually self-limited and relatively mild.

We looked for retroviruses in CVH patients whose disease had developed in adolescence after a possible prodromal viral-like illness. Retroviruses, morphologically indistinguishable from HTLV-III/LAV, have been isolated from their peripheral blood mononuclear cells, and no opportunistic infections characteristic of AIDS have developed in one patient. The viruses were isolated at a time when both patients were 'receiving intravenous gamaglobulin treatment' and it could be argued that this was the source of infection, particularly in view of a recent report that HTLV-III/LAV may survive the ethanol precipitation used to produce Cohn fraction II material. However, about 30 other CVH patients on regular intravenous gamaglobulin therapy have not shown any clinical signs of AIDS, despite receiving gamaglobulin known to contain anti-HTLV-III, and there have been no reports of AIDS in the many hundreds of other patients receiving this treatment throughout the world. Furthermore, patients with hypergamaglobulinemia who received the same batches of gamaglobulin given to patient 1 have been traced, and none has clinical features of AIDS.

Other possible modes of transmission of HTLV-III/LAV need to be considered. Infection through sexual contact is one possibility, particularly since women have contracted AIDS through heterosexual contact. However, neither of our patients was promiscuous. Patient 1 had never had sexual intercourse until after the development of her first opportunistic infection, and her partner is seronegative for HTLV-III/LAV. It is possible that retrospective analysis of these patients are particularly prone to HTLV-III/LAV infection through trivial exposure, but there is no evidence that our patients were in contact with anyone considered to be in a high-risk group for AIDS.

The retroviruses isolated from the two CVH patients are clearly related to HTLV-III/LAV, but further analysis is required to establish their identity. It remains to be seen whether this virus is causally related to CVH or is a rare invertebrate infection.

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GUT TRANSFER OF ENVIRONMENTAL PLUTONIUM AND AMERICIUM

Sixty-one-year-old man with mild haemophila A was transferred to the emergency room at North Carolina Memorial Hospital on June 30, 1985, with a large haematoma in his left leg, and an acute compartment syndrome. He had been treated with cryoprecipitate in his youth but had not had plasma, cryoprecipitate, or other blood products since 1975. He admitted to previous drug use, including intravenous drugs, but denied use of any depressants. At no time had he had any risk factors for AIDS.

An emergency four-compartment fasciotomy was done after administration of heat-treated factor VIII concentrate. Packed red blood cells from two female donors who were HTLV-II negative by ELISA were administered during the operation. Over the 20 day convalescent period, the patient received 99 960 units of heat-treated concentrate from five different kits obtained from a single manufacturer. Each lot was confirmed to have been heat-treated by the manufacturer.
HTLV-III reactivity by western blot analysis.

HTLV-III related antibodies were detected using SDS-disrupted HTLV-III (right panel) and recombinant peptide 121 (left panel) as antigen. Serum from successive bleed were tested at a 1:50 dilution and binding antibody was detected with 125I-labelled protein A. Sample 6-30-85 was obtained 30 days after treatment with factor VIII; 6-30-85, from day of admission with factor VIII; 8-30-85, second sample from admission with factor VIII; 8-25-85, from follow-up clinic visit. Molar weight of viral polypeptide are on right. Recombinant peptide 121 was a gift from Centocorr Laboratories.

16. Serum titers against Epstein-Barr virus capsid and early antigen were suggestive of recent infection. Antibody titers (in vivo) were: IgG >1600, capsid IgM 10, early antigen more than 20, nuclear antigen 5. Western blotting was negative. Liver-function tests were normal and he was hepatitis antigen negative. Anti-HBs was positive. The patient recovered fully with supportive therapy. ELISA for HTLV-III antibodies was not done. All HTLV-III antibody studies were done by western blot. The figure shows a western blot analysis of serum samples obtained at various times during the patient's postoperative course. The sample labeled "6-30-85" was taken before factor VIII infusion and the labeled "8-30-85" was taken several hours after infusion. Samples were tested on nitrocellulose strips containing SDS-disrupted HTLV-III as well as recombinant peptide 121. (p21) 121 contains about one-half the amino acid sequence of the viral transmembrane protein gp41 and has been shown to be reactive with most HTLV-III antibody positive sera. Specific antibody was only apparent in the last bleed sample (Aug 9) and this sample showed reactivity to both the recombinant envelope peptide 121 as well as to the internal core antigens of HTLV-III (p24) and the unchased precursor of the internal antigen (p30). The pattern of reactivity to gp41 on the strip containing disrupted HTLV-III despite binding to the analogous sequence in the recombinant peptide 121 is not unusual. This is thought to be related to increased detection efficiency of these antibodies when higher levels of the target antigen are loaded on the test strips. This pattern of reactivity is also characteristic of sera taken relatively early during the infectious process.

While the seroconversion encountered in this case appears to represent a specific immune response against HTLV-III antigens, the significance of this response is uncertain. Although a single attempt to detect live virus in the patient's lymphocytes at the time he was seropositive was unsuccessful, the possibility remains that in this case, heat treatment failed to inactivate virus and immunity was in response to infected virus. The finding of an increased T4/T8 ratio (0.62) at the time seroconversion was first noted suggests live virus transmission. Also, the febrile illness 25 days postoperatively could have been acute HTLV-III infection. Another possibility is that inactive virus or viral fragments provoked the immune response. If viral material is present in concentrates, heat treatment might inactivate virus, but viral antigen might still be present in amounts sufficient to elicit an immune response. Seroconversion in this case would not signify exposure to active virus. Another possibility is possible transfer of HTLV-I antibody, IgG is present in factor VIII concentrates but we have been unable to identify antibodies against HTLV-III proteins in the concentrates we have tested and one would have expected the samples of June 30 and July 25 to have been antibody positive. A final possibility is that the patient acquired HTLV-III through intravenous drug use, despite his denial of current activity. More recent specimens are not available; we have lost touch with this patient.

This case illustrates the need for further studies of heat-treated factor VIII and factor IX concentrates with the aim of determining as rapidly as possible how frequently HTLV-III seroconversion occurs after administration of heat-treated product and whether seroconversion reflects a response to live or attenuated virus. The urgency of these studies is obvious in view of the need to make reliable recommendations for safe treatment of haemophiliacs.

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RATIONAL FOR EARLY USE OF LEVODOPA IN PARKINSONISM

The use of levodopa in Parkinson's disease has been plagued by a variety of side effects. These side effects include fluctuations in clinical response, which can occur in patients who have been treated with levodopa. These fluctuations can lead to poor control of symptoms and a decrease in quality of life. There are several factors that contribute to fluctuations in clinical response:

1. Fluctuations are common in early stages of the disease. These fluctuations are thought to be due to changes in the levels of levodopa in the brain, which can affect dopamine levels and thus the overall response to the medication.

2. Fluctuations are also common in patients who are not taking their medications correctly, such as those who miss doses or take the medication at inappropriate times. This can lead to fluctuations in clinical response.

3. Fluctuations can also be caused by changes in the patient's environment, such as changes in diet or sleep patterns. These changes can affect neurotransmitter levels and thus the overall response to levodopa.

It is important to manage these fluctuations in response to levodopa in order to maintain adequate control of symptoms and improve quality of life. This can be done through various strategies, such as adjusting medication dosages, increasing the frequency of medication, or using adjunctive therapies. It is also important to monitor patients for these fluctuations and adjust therapy as needed to maintain adequate control of symptoms.