Dr Gunson

UCH D4 supernatant has now been used in our blood typing laboratory in parallel with the conventional anti-D reagent to type over 100 consecutive samples. UCH D4 antibody reacted strongly with all the D-positive red blood cells, weakly with D^u red blood cells, and gave negative results with all the D-negative red blood cells tested.

Discussion

The results show that the cell line UCH D4 is monoclonal with respect to its immunoglobulin heavy and light chain expression (IgG_1, κ), and that the antibody secreted into the supernatant culture medium is specific for rhesus D antigen. The antibody in the unconcentrated culture supernatant reacts to a titre of 1:256 in the indirect Coombs' test, with papain-treated red blood cells, and in albumin (table). As expected for an IgG rhesus D antibody it does not agglutinate D-positive cells in saline alone. The antibody reacted strongly with all D-positive cells tested and less strongly with D^u cells. It will therefore be useful as a red blood cell typing reagent and after purification may also be useful for prevention of rhesus disease of the newborn.

There have been two previous reports of EB-virus-transformed cell lines which produce rhesus D antibody[17,18] and the establishment of our line confirms that such specific antibody-producing cell lines can be produced. However, both the previously reported cell lines were apparently uncloned, and uncloned EB-virus-transformed cell lines eventually lose the ability to produce specific antibody, presumably due to the overgrowth of non-producing cells within the culture.[12,15] The cloned cell line reported here has now been growing for 9 months in continuous culture and has maintained a stable level of antibody production of around 20 μg/ml. Another such cloned cell line producing anti-X31-influenza-virus nucleoprotein has now been stable and producing antibody for 2 years in our laboratory.[15] This level of antibody production compares favourably with that of the rodent and human hybridoma systems.

Successful production of our stable antibody-producing cell line was probably the result of refinements made to the technique used by other workers. Firstly, the use of papain-treated-D-red blood cells to preselect the antibody-producing cells may have increased the number and the stability of the rosettes formed since D-red blood cells contain appreciably more surface D antigen sites than do ordinary D-positive cells.[22] Secondly, the initial culturing of EB-virus-infected cells at low cell numbers (10^3/well) on an X-irradiated feeder layer reduces the number of contaminating non-producer cells and in this case may have led to the growth of an initially cloned cell line. That this was indeed so is suggested by the finding that at the first cloning all the wells with cell growth also produced antibody. Finally, early cloning of antibody-producing wells is essential to maintain antibody production.

These results indicate that EB-virus transformation of antibody-producing cells is a useful method for the production of human monoclonal antibodies and that a rhesus D antibody at least suitable for blood grouping has been produced.

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References at foot of next column

Preliminary Communication

EPIDEMIC OF ACQUIRED IMMUNODEFICIENCY IN RHESUS MONKEYS

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Summary A syndrome closely resembling acquired immunodeficiency syndrome (AIDS) in man has been identified in a group of 64 rhesus monkeys

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(*Macaca mulatta*) maintained outdoors at the California Primate Research Center. The syndrome is characterised by generalised lymphadenopathy, severe opportunistic infections including cytomegalovirus, chronic wasting, and high mortality. In 1 animal a multifocal cutaneous fibrosarcoma developed. This syndrome in monkeys may provide an animal model for human AIDS.

INTRODUCTION

SINCE 1979, a syndrome of acquired immunodeficiency has reached epidemic proportions in young male homosexuals and has also been recognised in heterosexual intravenous-drug abusers, haemophiliac patients, and Haitian nationals and refugees.[1-3] This acquired immunodeficiency syndrome (AIDS) has early clinical signs of fever, weight-loss, diarrhoea, and lymphadenopathy which is characterised histopathologically as a reactive hyperplasia.[4] Later signs may include *Pneumocystis carinii* pneumonia,[5,6] other severe opportunistic infections such as *Mycobacterium avium intracellulare* and cytomegalovirus (CMV).[7,8] A previously rare form of Kaposi's sarcoma is common in AIDS patients.[9] Those affected have a poor response to therapy and high mortality. The immune dysfunction is characterised by cutaneous anergy, lymphopenia, suppressed T helper to T suppressor cell ratio, and decreased lymphocyte response to mitogen stimulation.[5,6,10,11]

Four separate outbreaks of apparent acquired immunodeficiency have been observed in non-human primates at the California Primate Research Center (C.P.R.C.). During 1969–75, 42 cases of malignant lymphoma were diagnosed in rhesus monkeys (*Macaca mulatta*).[12,13] *M. avium intracellulare* infection (11 cases), *Herpesvirus simiae* infection (7 cases), and progressive multifocal leucoencephalopathy (5 cases) were also diagnosed in these animals.[14] Cellular immune functions were abnormal in the animals examined.[15]

The second and third outbreaks occurred simultaneously in two groups of macaque monkeys maintained outdoors. One of these outbreaks occurred in a group of 54 stump-tailed macaques (*Macaca arctoides*). 44 of these animals died during a two-and-a-half-year period, 1976–78, from various illnesses in which *M. avium intracellulare* (18 cases), encephalitis (6 cases), and oral candidiasis (4 cases) were diagnosed (unpublished).

The other outbreak occurred over a five-year period in a group of 42 rhesus monkeys, housed in a cage similar to the one housing the stump-tailed macaques. Illness seen in this group included generalised lymphadenopathy, severe anaemia and lymphopenia, severe infections, and high mortality (unpublished). In August, 1981, all but 9 juvenile females were removed from this cage. 55 rhesus monkeys were added to this group of 9. This report describes the fourth outbreak of immunodeficiency-related diseases, which has occurred over the last 15 months in this new group of animals.

MATERIALS AND METHODS

Animals

In August, 1981, a group of 64 rhesus monkeys was established by placing 55 animals into a 15 m×30 m outdoor cage containing 9 apparently healthy females remaining from a previous group. Of the 6 males in the group, 2 had been caught wild and imported. The other 4 males and 58 females were colony-born. Most of the animals were juveniles under 3 years of age. Six adjacent cages held 558 healthy rhesus monkeys with a 15:1 ratio of females to males. We used these animals as controls.

Immunological Testing

Sequential blood-samples were taken during the course of the illness and complete blood-counts were routinely performed. Lymphocyte stimulation in 2 affected and 2 control animals was measured in vitro by incorporation of [^{3}H]thymidine after exposure to concanavalin A (Con A).[16] A stimulation index representing the [^{3}H]thymidine incorporated in the presence of mitogen to that incorporated without mitogen was determined. T-cell function in 7 affected and 5 control animals, previously vaccinated for tetanus, was measured in vivo by intradermal injection of tetanus toxoid (Wyeth Laboratories).

IgG, IgM, and IgA concentrations were measured by radial immunodiffusion (Hyland Laboratories) in serum collected before entry into the cage and serum taken during acute and terminal phases of the illness. Antibody titres to rhesus cytomegalovirus (CMV), and herpes simplex group-human-type 1, were determined by enzyme-linked immunosorbent assay (ELISA).[17] The immunofluorescent assay test was used to determine antibody titres to Epstein-Barr virus (EBV), toxoplasmosis, and SV-40.[18]

Pathology

Complete necropsies were performed on all 24 animals that died or were killed during the final stages of illness. Tissues were fixed in 10% neutral buffered formalin and routinely processed for paraffin embedding. Sections cut at 5 μm were stained with haematoxylin and eosin.

RESULTS

Epidemiology

Since establishment of the colony, 24 of the 64 animals have died; a mortality rate of 37·5%. Age and sex matched mortality in six other identical cages housing 558 animals for the same period of time was 5·5%. All the 24 that died were females. 22 were juveniles (0·5–3·5 years) and 2 were adults (older than 3·5 years). With the exception of 1 animal found dead in the cage, all others were in hospital for periods ranging from 2 weeks to 2 months during the course of their illness. Peak mortality occurred 6 months after introduction into the cage.

Clinical and Laboratory Findings

The following clinical signs and laboratory findings were observed in the 24 animals dying or killed because of serious illness: 71% had diarrhoea, 67% had anaemia (haematocrit <30%, normal; 35–41%),[19] 63% had peripheral or mesenteric lymphadenopathy, 63% had hypoproteinaemia (total plasma protein ⩽6 g/dl; normal, 7·1–8·0 g/dl),[19] 50% had lymphopenia (lymphocytes <2000 cells/μl; normal, 2500–6800 cells/μl),[19] 42% had splenomegaly, 33% had cutaneous abscesses, 29% had fever (temperature 39·5°C; normal 36·7–38·9°C),[19] 29% had arthritis, and 25% had bacteraemia. Other findings which occurred in 2 or more animals included necrotic gingivitis, granulocytopenia, thrombocytopenia, and oral oro-oesophageal candidiasis. In 1 animal a multiple cutaneous fibrosarcoma developed.

Preliminary studies of lymphocyte stimulation with Con A indicated a depressed stimulation index (2 and 7) in 2 clinically affected animals and 2 controls (23 and 43). 5 of 7 affected animals tested with intradermal tetanus toxoid showed decreased or absent reactions. All 5 controls reacted positively.

CMV antibody titres were ⩾1:320 in all controls and in 7 of 12 sera taken from animals before entry into the cage and which subsequently became ill after being placed in the cage. In these 7 ill animals the titres to CMV declined progressively through the course of the disease to <1:40. This was paralleled by declines in serum IgG and IgA concentrations. Similar trends in SV-40 titres were not observed. Titres to herpes simplex group human-type 1, toxoplasmosis, and EBV were negative in affected animals and controls.

Pathology

Splenomegaly (two to five times normal weight) and generalised lymphadenopathy were common at necropsy. Lymphoid tissue demonstrated mild to severe, post-reactive, depletion of germinal centres and paracortical areas with paramyloid deposition. In all animals, there was variable, usually extensive sinus histiocytosis with erythrophagocytosis. Plasma cells were infrequent. Microscopic examination of lymph-node biopsy specimens revealed pronounced follicular lymphoid hyperplasia in 2 animals which were mildly affected clinically.

All but 1 animal had lesions in more than one organ. Weight-loss of 11% to 33% had occurred in 19 animals. In addition to the clinically recognised lesions, other organs with inflammatory lesions included: gastrointestinal tract (80%), kidney (62%), liver (50%), lung (38%), skin (33%), salivary glands (21%), pericardium (12%), and meninges (4%). 71% of the animals had multiple infections. Bacterial agents isolated, in order of decreasing frequency included *Campylobacter fetus* ss *jejuni, Klebsiella pneumoniae, Shigella flexneri* type 4, *Staphylococcus aureus, Yersinia pseudotuberculosis, Streptococcus pneumoniae, Streptococcus viridans, Yersinia enterocolitica, Corynebacterium renale, Pseudomonas maltophila,* and *Acinetobacter* sp. Protozoans present included *Entamoeba histolytica, Balantidium coli, Cryptosporidium* sp. and *Trichomonas* sp. 4 animals (17%) had CMV-type inclusions in the spleen, lymph-nodes, or liver.

The cutaneous tumours were composed of multiple invasive nodules of plump spindle cells arranged in short interlacing bundles with variable cleft formation. The tumour was diagnosed as a fibrosarcoma.

DISCUSSION

Rhesus monkeys in one outdoor cage at the C.P.R.C. are experiencing a striking outbreak of disease with a relentlessly progressive clinical course ending in the death of all animals affected. The mortality rate in animals in this cage is seven times greater than that observed in other groups of rhesus monkeys of comparable age and sex distributions housed in adjacent cages. There is evidence of immunodeficiency in affected animals. This is based on histopathology of spleen and lymph-nodes, pronounced lymphopenia, cutaneous anergy, declining CMV titres and immunoglobulin concentrations, and decreased mitogen stimulation. The increased number of infections with organisms normally found in monkey colonies also accords with immunodeficiency.

The clinical picture in monkeys parallels that observed in human AIDS.[1,2,4] Shared features include lymphadenopathy and splenomegaly, fever, diarrhoea, weight-loss, infections with organisms such as *Shigella* sp., *S. aureus, Candida* sp., *K. pneumoniae,* and *Pseudomonas* sp. The appearance of *E. histolytica, Cryptosporidium* sp., and CMV infections strengthens this comparison.

The case of multifocal cutaneous fibrosarcoma, a tumour not previously seen in the colony, invites comparisons with Kaposi's sarcoma. Previous reports of outbreaks of disease in the C.P.R.C. colony such as malignant lymphomas, progressive multifocal leucoencephalopathy, and avian tuberculosis, suggest recurrent episodes of immunosuppression. The aetiology of such episodes remains obscure but similar mechanisms may be at work in the outbreaks occurring at the C.P.R.C. and the current epidemic of human AIDS.

This outbreak may serve as a valuable model for AIDS by providing insight into immune function, transmission of the disease, and the role of viral or toxic agents that might contribute to the disease process.

ADDENDUM

A further cutaneous fibrosarcoma has been diagnosed in a 3-year-old female rhesus monkey which died. In another animal which is still alive, a mass has been found in the right triceps. Biopsy results suggest an early fibrosarcoma. Both of these animals are from the group of 64 rhesus monkeys described in the article.

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