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Immunology

Acquired immunodeficiency syndrome in a colony of macaque monkeys

(monocyte/opportunistic infection/T-cell subset/tumor)

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ABSTRACT **A naturally occurring immunodeficiency syndrome has been seen in a captive colony of macaque monkeys. This syndrome is seen primarily in the species *Macaca cyclopis*. Affected animals died with lymphomas (a rare disease in macaques) or such opportunistic infections as *Pneumocystis carinii* and noma (necrotizing gingivitis). These *M. cyclopis* exhibited anemia, neutropenia, and a circulating bizarre immature monocyte. In addition, liver function tests suggested hepatitis. Pokeweed mitogen-, concanavalin A-, and xenogeneic cell-stimulated proliferative responses by lymphocytes of animals with the syndrome were dramatically diminished. The T4 (helper, inducer)/T8 (suppressor, cytotoxic) ratio in the peripheral blood mononuclear T-cell populations of *M. cyclopis* in this colony are decreased when compared with those from either *Macaca mulatta* in the same colony or normal humans. Epidemiologic evidence implicates a common source agent in this syndrome. The similarity of this syndrome in macaques to human acquired immunodeficiency syndrome suggests that it may provide an important model for studying the human syndrome.**

The immune system plays a crucial role in surveillance against tumor growth and the development of infections. Breakdowns in this surveillance system do occur, most commonly in humans with congenital immunodeficiency syndromes or during immunosuppressive chemotherapy. In those settings, individuals can develop rare tumors and opportunistic infections (1). A naturally acquired breakdown of this surveillance system can also occur. This is usually seen in settings of severe malnutrition or as a complication of the anergic state associated with such infectious diseases as tuberculosis. The emerging acquired immunodeficiency syndrome (AIDS) in man is an alarming example of such an acquired breakdown in normal immune function (2, 3). Diseases of this kind provide important models for elucidating the role of the immune system in protecting individuals against infections and tumors.

In this report, we describe a naturally occurring immunodeficiency syndrome in macaque monkeys and document clinical and epidemiologic aspects of the illness and lymphocyte function in affected animals. This syndrome is a striking example of the susceptibility of the immune system to overwhelming dysfunction and the ramifications of that dysfunction for the organism. Furthermore, it provides an important model for the study of acquired immunodeficiency syndromes.

MATERIALS AND METHODS

Animal Selection. The New England Regional Primate Research Center breeds and maintains a colony of 1,200 primates representing 14 different species. They are housed in both individual and gang cages; some of these cages are indoors and others are in outdoor facilities. The center maintains 780 animals of the genus *Macaca*, comprised mostly of the species *M. mulatta* (rhesus), *M. fascicularis* (crab-eating macaque), and *M. cyclopis* (Formosan or Taiwanese rock macaque). Detailed clinical records are maintained on each animal and autopsies are performed on all the animals that die at the center. These records served as a portion of the data base for this study.

Animals used in this study were maintained in accordance with the guidelines of the Committee on Animals of the Harvard Medical School and those prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources of the National Research Council.

Clinical Laboratory Studies. The routine hematologic studies reported here were carried out at the clinical laboratory at the center. The serum chemistry studies were done at a nearby commercial laboratory.

Staining and Analysis of Peripheral Blood Mononuclear Cells. Peripheral blood mononuclear cells (PBM) were prepared from heparinized venous blood of the macaques by density gradient centrifugation using a 9% Ficoll (Sigma)/34% sodium diatrizoate (Sterling Drug, New York) solution having a specific gravity of 1.076 g/ml. The cells were treated with 0.15 M NH_4Cl to lyse erythrocytes and washed with Hanks' balanced salt solution/2.5% newborn calf serum; aliquots of 1×10^6 cells were incubated with monoclonal antibodies at 4°C for 30 min. The characterization of the antibodies has been described (4). These cells were then washed twice with Hanks' balanced salt solution/2.5% pooled human AB serum and incubated with fluorescein isothiocyanate-conjugated goat anti-mouse Ig (TAGO, Burlingame, CA) for 30 min at 4°C. Each sample was washed twice with phosphate-buffered saline, and the cells were then examined by fluorescence microscopy or analyzed on a fluorescence-activated cell sorter (FACS I; Becton Dickinson).

Functional T-Lymphocyte Studies. PBM were assayed for their proliferative response to pokeweed mitogen (PWM) and concanavalin A (Con A) as described (5). Mixed lymphocyte reactions (MLR) using mitomycin C-treated human PBM as stimulator cells were carried out as described (5).

RESULTS

Epidemic of Deaths in *M. cyclopis* Colony Related to Immunosuppression. It was the impression of workers at the New England Regional Primate Research Center that there had been an increase in the number of deaths in its macaque colony. A

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Abbreviations: AIDS, acquired immunodeficiency syndrome; Con A, concanavalin A; PBM, peripheral blood mononuclear cells; PWM, pokeweed mitogen; CMV, cytomegalovirus.

Table 1. Mortality rate in macaque colony

Year	Mortality, %			
	All species*	*M. mulatta*	*M. fascicularis*	*M. cyclopis*
1981	13.9 (104/758)	12.1 (38/315)	12.5 (43/344)	29.0† (18/62)
1980	18.0 (130/726)	15.0 (45/300)	17.6 (56/318)	33.8† (24/71)
1979	12.9 (95/734)	9.6 (33/343)	14.8 (40/271)	13.3 (11/83)
1978	11.9 (75/631)	8.8 (26/294)	19.1 (38/199)	8.0 (7/87)

Numbers in parentheses represent total number of deaths in species for year/number of species at mid-year (June 1) census.

*Includes *M. mulatta*, *M. fascicularis*, and *M. cyclopis* as shown, as well as a small number of *Macaca arctoides*, *Macaca nemestrina*, and *Macaca radiata*.

†Significantly greater than annual rate for *M. mulatta* or *M. fascicularis* and also greater rate than for *M. cyclopis* in 1979 and 1978; $P < 0.005$ by χ^2 table.

retrospective study of autopsy records was initiated to delineate the extent of this problem. Examination of the species-specific annual mortality rates of macaques at the center during the previous 4 yr showed a significant increase in deaths in 1980 and 1981 in the species *M. cyclopis* (Table 1). The mortality rate in this species rose both in comparison with the *M. cyclopis* death rates in previous years and in comparison with death rates in *M. mulatta* and *M. fascicularis* in 1980 and 1981. One-third of the *M. cyclopis* colony died in 1980.

The autopsy records and laboratory studies of 1981 were reviewed to determine the cause of this increased mortality in the *M. cyclopis* colony. A significant number of the *M. cyclopis* that died in 1981 had a similar hematologic profile: anemia, neutropenia, and monocytosis (Table 2). A similar hematologic picture was also identified in *M. mulatta*. To increase the number of cases for further study, an additional eight cases with this hematologic picture were identified by a review of laboratory studies and autopsy reports of *M. cyclopis* deaths from 1980. These 15 cases (13 *M. cyclopis* and 2 *M. mulatta*) provided a cohort of similar cases for analysis.

The causes of death noted at autopsy on these 15 cases (Table 3) include noma (necrotizing gingivitis), *Pneumocystis carinii*, and systemic cytomegalovirus (CMV) infections. These are all diseases normally seen in human and nonhuman primates only in the setting of marked immunosuppression. Three cases of lymphoma were also documented in this population. This is an unusual disease in macaques. Three animals from this cohort died after a prolonged course of wasting and diarrhea.

Table 2. Causes of death of macaques in 1981

Autopsy diagnosis	*M. mulatta*	*M. fascicularis*	*M. cyclopis*
Anemia, neutropenia, and monocytosis*	2	0	5
Infectious disease			
Bacterial enteritis	3	1	2
Hepatitis	1	0	3
Measles	0	5	0
Pneumonia	5	2	0
Other	2	2	0
Fat macaque syndrome†	2	0	3
Experimental	8	11	1
Accidental	2	4	1
Insufficient data	4	5	2
Total	29 (38)	30 (43)	17 (18)

Causes of 1981 deaths were determined retrospectively on animals on which complete autopsies had been performed. Numbers in parentheses represent number of macaques of species that died in 1981.

*Attribution made when hematologic data showed anemia, neutropenia, and abnormal circulating monocyte.

†See ref. 6.

Hematologic and Chemistry Profile of This Cohort of Macaques. The hematologic profile of these animals was characterized by the presence of a circulating large bizarre mononuclear cell and anemia, neutropenia, and monocytosis. A unique circulating mononuclear cell was identified in the peripheral blood smear of virtually every animal in this cohort (Fig. 1). Every macaque in this group also showed an absolute monocytosis. A significant number of these monocytes were large immature-appearing cells with vacuolated cytoplasm. The range of blood hemoglobin values in this group of animals was 6.1–11.7 g/dl with a median value of 8.5 g/dl. The corpuscular indices were low on every tested specimen. The animals also showed marked neutropenia with 9 of 15 macaques having absolute neutrophil counts of $<2{,}000/mm^3$. Recurrent episodes of neutropenia followed by some recovery of circulating neutrophils were documented in two of the three animals in the cohort on which multiple determinations had been made over a period of time. These 15 animals also showed a lymphocytosis early during the course of their illness.

In all the animals in this cohort on whom serum chemistries were checked, liver function tests were abnormal (Table 4). These studies indicate that a hepatitis was ongoing in these macaques. That these reflect a chronic process is suggested by the decreased serum albumin seen in many of these animals.

Case History of a *M. cyclopis* with this Syndrome. An asymptomatic macaque with what we presume to be an early stage of this syndrome was found on a routine hematologic examination. Four months prior to diagnosis this 6-yr-old asymptomatic female *M. cyclopis* had a leukocyte count of $8{,}300/mm^3$ with a differential of 41% neutrophils, 57% lymphocytes, and 2% basophils and a hemoglobin value of 11.7 g/dl. At the time of diagnosis, her weight was 8.17 kg, normal for such an animal. She had no palpable adenopathy or abdominal organomegaly. Her leukocyte count was $1{,}500/mm^3$ with a differential of 1% neutrophils, 65% lymphocytes, 11% large and atypical lymphocytes, 16% monocytes, 7% large and atypical monocyte-like cells, and a hemoglobin value of 9.7 g/dl. Her serum chemistries included sodium, 152 meq/liter; potassium, 4.3 meq/li-

Table 3. Causes of death in cohort of macaques

Attribution of death at autopsy	Animals, no.
Noma	4
P. carinii	3
Lymphoma	3
Diarrhea, wasting	3
CMV mononucleosis	1
Bacterial pneumonia	1
Total	15

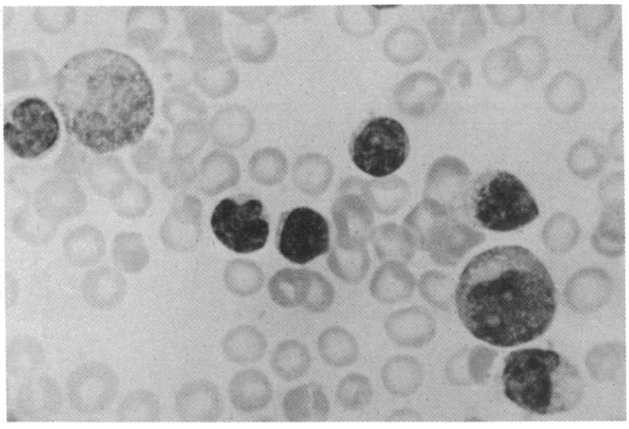

FIG. 1. Wright–Giemsa-stained cytospin preparation of PBM from the neutropenic *M. cyclopis*.

ter; chloride, 112 meq/liter; CO_2, 23 meq/liter; serum glutamic-oxaloacetic transaminase, 46 units/liter; lactate dehydrogenase, 652 units/liter; alkaline phosphatase, 579 units/liter; serum glutamic-pyruvic transaminase, 43 units/liter; total protein, 6.8 g/dl; albumin, 3.2 g/dl; globulin, 3.6 g/dl; amylase, 457 Somogyi units/dl; lipase, 0.3 units/liter. Serum protein electrophoresis showed a hypogammaglobulinemia with α-1, 0.3 g/dl; α-2, 0.5 g/dl; β, 0.9 g/dl; γ, 0.3 g/dl. Serum immunoelectrophoresis showed a decrease in all classes of immunoglobulin: IgA was <55 mg/dl, IgM was <30 mg/dl, and IgG was 325 mg/dl. A bone marrow aspirate showed normal erythroid precursors, an absence of granulocyte precursors, and a large number of bizarre mononuclear cells similar in appearance to those seen in the peripheral blood.

Characterization of the Abnormal Cell in this Macaque. Immunofluorescence staining techniques were used to characterize the abnormal circulating cell in this *M. cyclopis*. We used the fact that many monoclonal antibodies prepared against subsets of immunologically active cells in humans recognize analogous structures on such cells in nonhuman primates (7, 8). Cells from this *M. cyclopis* were stained with monoclonal antibodies

Table 4. Liver function tests performed on macaques from cohort with AIDS

	Value	Normal range	Abnormal*
Serum glutamic-oxaloacetic transaminase	139 ± 96 units/liter	(0–41)	9/9
Serum glutamic-pyruvic transaminase	125 ± 104 units/liter	(0–45)	6/7
Alkaline phosphatase	459 ± 182 units/liter	(30–115)	8/8
Lactate dehydrogenase	1,173 ± 952 units/liter	(100–225)	9/9
Total protein	5.7 ± 0.9 g/dl	(6.0–8.0)	5/9
Albumin	2.5 ± 0.5 g/dl	(3.0–5.0)	5/9
Globulin	2.8 ± 1.2 g/dl	(1.0–3.5)	5/6

Results represent mean ± SD.
* Number of animals with abnormal value/number of animals for which determinations were made.

specific for B lymphocytes (B1), T lymphocytes (T11), monocytes and natural killer cells (Mo1), monocytes only (Mo2), monocytes and granulocytes (MY4), and an antibody that recognizes a framework Ia determinant (4). These cell populations were then examined by microscopy to determine the presence of fluorescence on the morphologically abnormal cell population. The large bizarre cells stained positively with Mo1, Mo2, MY4, and the framework Ia antibody. These data indicate that the abnormal circulating cell is of the monocyte lineage.

Lymphocyte Function in this *M. cyclopis*. The susceptibility of these macaques to tumors and opportunistic infections suggested that the animals might have depressed T-cell function. Therefore, the responsiveness of PBM from this same asymptomatic macaque to lectins and xenogeneic cells was assessed. As shown in Fig. 2*A*, PBM from this *M. cyclopis* showed a marked diminution in proliferation after stimulation with PWM when compared with the proliferative response of PBM from three normal juvenile and three normal adult *M. cyclopis*. Similarly,

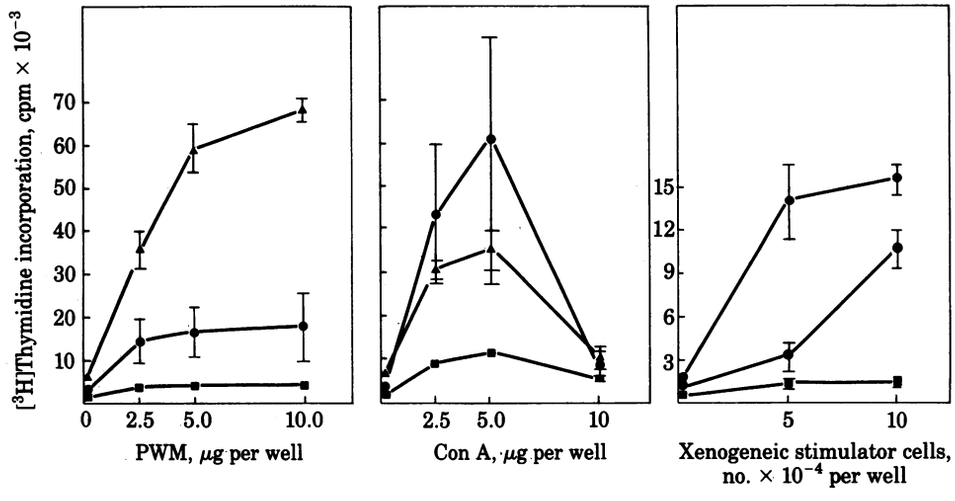

FIG. 2. PBM from a neutropenic *M. cyclopis* exhibit diminished T-cell proliferation after stimulation with lectins or xenogeneic cells. Proliferation after stimulation of PBM from the neutropenic *M. cyclopis* (■) is compared with proliferation of PBM from three normal adult (●) and three normal juvenile (▲) *M. cyclopis*; 2×10^5 responder cells were incubated with the indicated concentrations of lectin or stimulator cells in 0.2-ml volumes. (*A*) Proliferation after PWM stimulation of PBM. (*B*) Proliferation after Con A stimulation of PBM. (*C*) Proliferation after stimulation of PBM by mitomycin C-treated human cells. A similar mixed lymphocyte reaction using PBM from two normal age-matched *M. cyclopis* (●) is also shown. Results represent mean ± SD.

Table 5. Cage location of *M. cyclopis* that died with AIDS in 1980 and 1981

	Animals dying, %	
	Target	Elsewhere
All	23 (11/47)	5 (2/39)
Adults	9 (3/34)	3 (1/30)
Juveniles	62 (8/13)	11 (1/9)

Numbers in parentheses represent number of *M. cyclopis* housed where noted that died with AIDS during 2-yr period/number of *M. cyclopis* housed in that location during 2-yr period.

such cells showed a diminished proliferative response to Con A (Fig. 2*B*). Mitomycin C-treated human PBM stimulated no proliferative response in PBM from this macaque while stimulating a normal xenogeneic mixed lymphocyte reaction in PBM from two normal age-matched *M. cyclopis*. These findings suggest a defect in lymphocyte function in this animal.

Risk for Disease Development. *M. cyclopis* of the cohort of macaques were then studied to determine whether possible risk factors predisposing the animals to the development of this syndrome could be identified. The location in which the *M. cyclopis* were caged appears to correlate with the risk for development of this illness (Table 5). The *M. cyclopis* animals more than 1 yr old are housed in three outdoor facilities, each containing a number of gang cages. Eleven of the 47 *M. cyclopis* animals housed in one of these three facilities died with this disease in 1980 and 1981, while only 2 of 39 *M. cyclopis* housed in the other two facilities died with this syndrome. The risk of disease associated with caging in that particular building was even greater for juveniles (animals <4 yr old), with 8 of 13 juveniles housed there in 1980 and 1981 dying with this syndrome. Previous diagnosed illnesses, sex, animal origin (imported or colony born), or the particular genealogy of the animal did not constitute a risk for *M. cyclopis* dying with this syndrome in 1980 and 1981 (data not shown). These findings all suggest a common source or infectious risk for the development of this illness. They further suggest that the juvenile *M. cyclopis* are at greater risk for death due to this syndrome than are the adults. The probable infectious nature of this illness and the evidence of profound immune suppression in these animals suggests that this illness represents an acquired immunodeficiency syndrome (AIDS) in macaques.

Helper/Suppressor Ratio in *M. cyclopis*. The data given in Table 2 indicate that *M. cyclopis* have a higher incidence of this AIDS than do other macaque species. We sought to establish an immunologic correlation of this observation. It has recently been shown that the normal circulating T4 (helper, inducer T cell)/T8 (suppressor, cytotoxic T cell) ratio of 2:1 is reversed in human disease states in which a depression in T-cell function is seen—e.g., after herpesvirus infections (9) and in humans with AIDS (10). We determined such ratios for circulating PBM in *M. mulatta* and *M. cyclopis* of various ages. As shown in Fig. 3, PBM from adult macaques have somewhat lower ratios than do PBM from juveniles. Furthermore, the T4/T8 ratio in both juvenile (<4 yr old) and adult *M. cyclopis* are somewhat depressed compared with juvenile and adult *M. mulatta*. No regular pattern of T4/T8 ratios has emerged in *M. cyclopis* with AIDS. Some have shown very low ratios and others have very high ratios.

DISCUSSION

We have described an AIDS in a captive colony of macaque monkeys. Affected animals died with lymphomas or such opportunistic infections as *P. carinii* and noma. These macaques

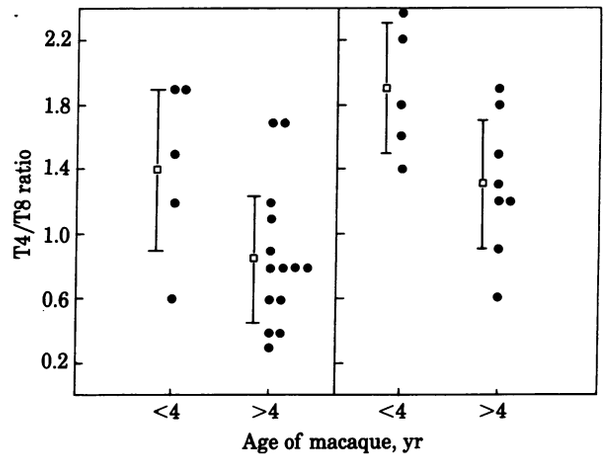

FIG. 3. T4/T8 ratios of PBM from various healthy *M. cyclopis* (*Left*) and *M. mulatta* (*Right*) shown in groupings according to age.

exhibited anemia, neutropenia, and a circulating bizarre immature monocyte. In addition, liver function tests suggested hepatitis. PWM, Con A, and xenogeneic cell stimulated proliferative responses by lymphocytes of animals with the syndrome were dramatically diminished. Epidemiologic evidence implicates a common source agent in this syndrome.

The parallels of this disease to human AIDS are striking. The human syndrome is a recently emerging illness seen predominantly in promiscuous homosexual men and intravenous drug abusers (2, 3). These individuals are predisposed to developing recurrent viral infections, opportunistic infections, and such unusual tumors as Kaposi sarcoma and Burkitt lymphoma. The etiologic agent(s) and mechanism accounting for the profound T-cell defect in these individuals remain unknown. The disease in macaques is similar in many ways to this human syndrome. A common source agent in this macaque disease, the deaths of these animals from unusual tumors and opportunistic infections, and the documented depressed responses of lymphocytes from these animals to lectins all parallel the syndrome in humans.

The differences, though, between the macaque and the human AIDS are significant. The most obvious difference seen between the macaque and the human disease is the presence in the macaques of a bizarre early monocyte form in the bone marrow and peripheral blood. This cell may be transformed by an as yet unidentified viral agent that may be playing a crucial etiologic role in the disease process. It may, on the other hand, reflect reactive changes by the monocytes to an infectious process in the same way that the atypical lymphocytes circulating in individuals with infectious mononucleosis have undergone morphologic changes in reacting to Epstein–Barr virus-infected B cells.

The evidence of an ongoing hepatitis in the macaques with AIDS also indicates that this clinical picture differs from that seen in human AIDS. This finding is consistent with a systemic viral mononucleosis, similar to that seen in Epstein–Barr virus or CMV infections. CMV has been readily isolated from lymph nodes and secretions from apparently healthy macaques and macaques with AIDS in this colony (M. Daniel, personal communication).

The hematologic profile of the macaques with AIDS also differs from that of humans with AIDS. Humans are lymphopenic and have normal or even elevated circulating immunoglobulin levels (2). The macaques are neutropenic with normal total lymphocyte counts and are hypoglobulinemic. While the macaques

with AIDS have shown no regular pattern of T4/T8 ratios, the depressed T4/T8 ratio in the *M. cyclopis* colony as a whole parallels that finding in humans with AIDS. This abnormality in the colony may reflect an endemic viral infection. It may, on the other hand, be "normal" for that species but predispose the species toward developing AIDS. This would explain the higher incidence of AIDS in *M. cyclopis* than in *M. mulatta*.

The high incidence of human AIDS among promiscuous male homosexuals suggests that the etiologic agent in that disease can be venereally transmitted. While we have no evidence pertaining to this issue, bisexual activity among captive macaques is well documented (11). Thus, a mode of disease transmission similar to that in human AIDS is possible in macaques with that syndrome.

The different clinical pictures seen in the human and macaque syndromes may simply reflect species-specific differences in responses to the same agent(s). It may, on the other hand, indicate that the etiologic agent(s) in these analogous syndromes is not the same. In either case, this AIDS in macaques presents an important instance in which an acquired depression of immune function predisposes animals to a variety of lethal opportunistic infections and tumors. Further study of this disease process promises to yield important insights into the mechanisms by which immune surveillance can break down.

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