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Immunologic Alterations in Monkeys with Simian Acquired Immunodeficiency Syndrome (SAIDS) (42091)

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Abstract. Levels of lymphocyte responsiveness to T- and B-cell-specific mitogens and expressions of Ia, T_4, T_8, and T_{11} surface markers were monitored during the course of Simian acquired immunodeficiency syndrome (SAIDS) in four *Rhesus* macaques that either died or became ill and survived. The monkey that died showed progressively suppressed responses to concanavalin A (Con A), phytohemagglutinin (PHA), pokeweed mitogen (PWM), and *Staphylococcus aureus* Cowan I strain (SAC) through the time of death ($5\frac{1}{2}$ weeks). For the three animals that survived, the responses of peripheral blood mononuclear cell (PBMC) to the same mitogens were decreased significantly during the period 4–6 weeks after inoculation. Levels of Ia-bearing cells in the PBMC population were markedly reduced in the moribund monkey but were not significantly decreased in the three survivors. There was no significant change in the percentage of T_{11}-bearing cells in any of the study animals. The ratio of T_4- and T_8-positive cells did not vary significantly during the 18 weeks of observation in any of the animals. The infected animals showed other evidence of immunosuppression including neutropenia, lymphopenia, and depletion of lymphocytes in lymph nodes. The animal that had progressive disease and death also had Kaposi-like lesions and staphylococcal septicemia. These results indicated that *in vitro* evidence of immunosuppression due to SAIDS appears within a few weeks after infection and this may progress in animals that die.

Simian acquired immunodeficiency syndrome (SAIDS) can be experimentally transmitted to *Rhesus* monkeys (*Macaca mulatta*) by tissue homogenate, serum, or the SAIDS type D retrovirus isolated from infected animals (1, 2). The disease has many of the findings which are seen in human acquired immunodeficiency syndrome (AIDS) (3). Animals inoculated with the SAIDS type D retrovirus develop lymphadenopathy, splenomegaly, weight loss, anemia, lymphopenia, and may die with anemia, opportunistic infections, and malignancies. Lymph nodes show progressive depletion of both T and B cells and immunoglobulin levels are decreased (4). Previous studies reported an impairment of cellular and humoral immunity of monkeys infected with SAIDS at a late stage of disease (2, 3, 5).

We have studied the *in vitro* parameters of the T- and B-cell immune disfunction in fatal and nonfatal SAIDS and have found suppression of mitogen responses and changes in Ia levels which may progress through death.

Materials and Methods. *Animals.* Normal 3-year-old Rhesus monkeys which weighed approximately 3 kg and were reared at the Infectious Diseases Branch of the National Institute of Neurological and Communicative Disorders and Stroke were used in these studies. All animals were examined clinically and evaluated by performing total and differential blood cell counts and establishing serum chemistry profiles before and periodically after infection.

Animal inoculation. Four monkeys were each inoculated intravenously with 0.9 ml of a pool of filtered plasma (Pool A, first passage from naturally infected monkeys) from moribund donor animals with experimentally transmitted SAIDS (animals numbered 88, 90, 104, and 105) (2). The clinical history and pathology of the donor animals were described previously (6). Four control animals were also studied (numbered 87, 89, 103, 608).

Blood. Blood was drawn from the femoral vein of monkeys anesthetized with 20 mg/kg of Ketamine·HCl (Parke Davis, Morris

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Plains, N.J.). Blood was collected in Vacutainer Tubes (Becton Dickinson, Rutherford, N.J.) according to manufacturer specifications.

Mitogens. Concanavalin A (Con A), phytohemagglutinin (PHA), pokeweed mitogen (PWM), and formalinized *Staphylococcus aureus* Cowan strain I (SAC) were purchased from Sigma Chemical Company (St. Louis, Mo.) and used for tests of lymphocyte responsiveness to B- and T-cell-specific mitogens. All mitogen solutions were prepared in RPMI 1640 medium containing 50 IU penicillin and 50 μg streptomycin/ml (M.A. Bioproducts, Walkersville, Md.).

Cell preparations. Peripheral blood mononuclear cells (PBMC) were purified by density gradient centrifugation of citrated blood through a solution of Percoll of density 1.077. The PBMC were then washed and finally resuspended at 1×10^6 viable, trypan-blue-excluding cells per milliliter in RPMI 1640 supplemented with 2% heat-inactivated fetal bovine serum (Microbiological Associates, Walkersville, Md.).

Mitogen stimulation assays. All cell cultures were prepared in flat-bottom wells of sterile, plastic 96-well microculture plates (Costar, Cambridge, Mass.) and each test was conducted in triplicate. Each well contained a volume of 0.15 μl, consisting of 100 μl of PBMC suspension, and 50 μl of RPMI 1640 medium alone or containing either PHA, Con A, PWM, or SAC. Con A and PHA were added at three different final concentrations. These concentrations were 10 μg/ml, 2 μg/ml, and 0.4 μg/ml. Pokeweed mitogen was added at final dilution of 1:400, 1:2000, and 1:4000. SAC was added at dilutions of 1:2000, 1:8000, and 1:20,000. After incubation at 37°C for 66 hr in a 5% CO_2-in-air atmosphere saturated with water vapor, each culture received 1 μCi of tritiated thymidine (sp act 2 Ci/mmole, New England Nuclear, Boston, Mass.) in 50 μl of RPMI 1640 medium and was further incubated under the same conditions for an additional 6 hr. The culture fluids were then collected with an automated harvester (Titer Tek Cell Harvester, Flow Labs, McLean, Va.) and processed for measurement of incorporated radioactivity into synthesized DNA. PBMC from normal monkeys and monkeys with SAIDS were always tested in paralleled assays. The results obtained with normal or infected PBMC were used to calculate the corresponding stimulation indices by the equation:

$$\text{stimulation index} = \frac{\text{mitogen-induced response}}{\text{background response}}.$$

The mitogen-induced and background responses represent the values obtained with cells in the presence and absence of mitogen, respectively. Background response was never above 1000 cpm.

Detection of surface PBMC markers. The presence of Ia, T_4, T_8, and T_{11} antigens on PBMC was detected by indirect immunofluorescence, using a fluorescence-activated cell analyzer (Ortho FC 200/4800 Cytoflurograph, Boston, Mass.) and mouse monoclonal antibodies specific for the corresponding human cell markers (Ortho Diagnostic Systems, Raritan, N.J.), which are known to cross-react with *Rhesus* monkey lymphocytes (7). A fluorescein-labeled goat anti-mouse immunoglobulin serum (Cappel, Malvern, Pa.) was used in the second step of the test. In all determinations, channels were selected so that only those cells with the scattering characteristics of viable lymphocytes would be analyzed.

Results. *Patterns of disease demonstrated by monkeys inoculated with SAIDS.* All monkeys inoculated with the SAIDS plasma developed lymphadenopathy, splenomegaly, leukopenia, and neutropenia 2–4 weeks past inoculation. Early leukocyte levels (2–4 weeks after inoculation) were between 25 and 30% of total white cells (normal range 23–50%). Three inoculated animals (numbered 88, 104, 105) returned to "normal" levels in 6–8 months after inoculation. One inoculated animal (number 90) had progressive disease and died $5\frac{1}{2}$ weeks after inoculation. This animal had lymphadenopathy, splenomegaly, severe neutropenia (1–10% range), anemia, and severe weight loss. Weight loss was associated with protracted diarrhea. Hypoproteinemia, staphylococcal septicemia, and Kaposi-like lesions on the abdomen area were observed in this severely affected animal.

Altered lymphoproliferative responses by PBMC monkeys with SAIDS. Monkeys with

SAIDS showed markedly impaired responses to the T-cell-specific mitogens PHA, Con A, as well as to the T-cell-dependent B-cell stimulant PWM between 4 to 6 weeks post-infection. One of the four animals with SAIDS monitored in this study died after $5\frac{1}{2}$ weeks without showing any signs of recovery in PBMC responsiveness, whereas the other three displayed improved and eventually near-normal responses after 12 and 18 weeks and they survived (Figs. 1A, B, and C). Qualitatively, similar observations were made when the B-cell mitogen SAC was used to stimulate PBMC responses (Fig. 1D).

Levels of Ia- and T_{11}-positive cells during SAIDS. The results presented in Table I show that the monkey that succumbed had significantly lower levels of Ia-positive cells 4 weeks after infection. Table I also shows that the levels of Ia-positive cells in one of the surviving monkeys were not greatly different from those of the control, uninfected monkeys; similar results were obtained with the other two survivors (data not shown). The

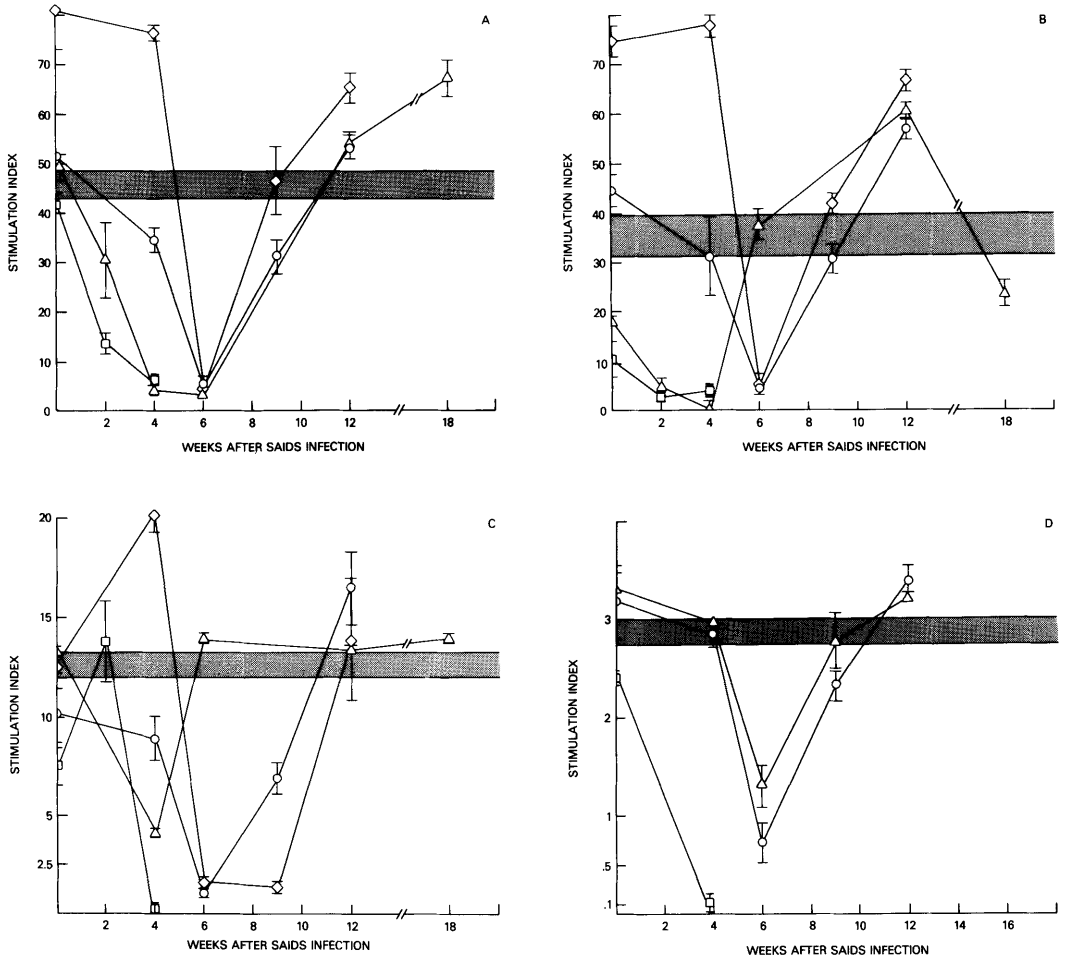

FIG. 1. Mitogenic responses at different periods before and after SAIDS infection in four monkeys (No. 88, △; 90, □; 104, ◇; and 105, ○). Each point represents the mean of three separate determinations and the vertical bar the standard error of the mean. The shaded area includes values obtained with four uninoculated animals (Nos. 87, 89, 103, 608, shaded area) tested several times along with the infected animals. (A) PHA, (B) Con A, (C) PWM, and (D) SAC. △ (88), ○ (105), and ◇ (104) responses by PBMC from monkeys that showed transient disease and recovered after 12 weeks. □, Responses by PBMC from the monkey which died (No. 90).

TABLE I. Ia MARKER ON PBMC OF MONKEYS WITH SAIDS

Monkey No.	Time of inoculation: Preinoculation	2 Weeks	4 Weeks	18 Weeks
87	45.8	25.2	25.6	29.3
89	40.7	34.2	26.8	32.6
88[a]	48.9	33.0	21.2	47.0
90[a]	33.6	11.3	4.5	Dead

Note. Monkey 90, which died, showed a very marked decrease in the percentage of cells carrying the Ia marker. Absolute numbers of Ia-positive cells were also markedly depressed.

[a] Infected.

percentage of T_{11} antigen-bearing cells did not vary during the course of disease (data not shown).

Ratios between T_4- and T_8-positive lymphocytes during SAIDS. Although the ratios between T_4- and T_8-positive lymphocytes fluctuated after inoculation, the changes shown by each animal did not appear to be consistently undirectional or of a significant magnitude (Table II). Similar results were obtained with another set of animals (2 infected and 2 controls, data not shown). The percentage of OKT_{4+} and OKT_{8+} cells was not significantly decreased during the course of disease.

Discussion. These studies provide *in vitro* evidence of immunological alterations occurring during SAIDS. The animal which died (number 90) showed progressive loss of T- and B-cell responses to mitogens. The three monkeys that survived (numbers 88, 104, 105) had similar decreased responses to PHA, Con A, PWM and SAC 4–6 weeks after inoculation but subsequently reverted to normal. Although the lymphoproliferative responses to T- or B-cell mitogens were markedly impaired in all of the infected animals, only the ones which survived (numbers 88, 104, 105) recovered to eventually mount normal proliferative responses. Previous studies have also reported lower proliferative responses to mitogens PHA, Con A, and PWM of PBM cells in monkeys infected with SAIDS in late disease stages (2, 3, 5). In this study sequential titrations during the course of the disease showed transient impairment of responses in three animals that survived infection and progressive deterioration in the animal that died. The disease in monkeys resembles human AIDS in respect to T-cell responses to mitogens. In both diseases these responses are markedly impaired (8, 9). B-Cell responses to SAC in human AIDS are augmented in contrast to our findings in SAIDS (10). Furthermore, no hypergammaglobulynemia has been observed in SAIDS monkeys as has been described in human AIDS (2, 10).

A striking difference between SAIDS and AIDS relates to the T_4 to T_8 levels of peripheral blood leukocytes. In the great majority of AIDS patients a reverse T_4/T_8 ratio has been reported (8). Those changes were not observed with the SAIDS inoculated monkeys which survived nor in the one (number 90) which had developed a fatal disease. Furthermore, no changes in the percentage of OKT_{11+} cells had been detected in infected monkeys indicating a functional defect on the T cells rather than a selective depletion of any population or subpopulation. The level of Ia-positive cells was significantly diminished during the course of disease. This could be due to lower levels of Ia^+ cells such as macrophages, B cells, and activated T cells in peripheral blood or to the presence of autoantibodies or immunocomplexes that interact with Ia^+ cells and block their surface staining characteristics. The latter possibility is less probable since unlike AIDS patients, SAIDS infected monkeys show a severe decrease in the level of circulating immunoglobulins. Ia^+ cells are known to function as antigen-presenting cells to helper T cells, and

TABLE II. RATIOS BETWEEN T_4- AND T_8-POSITIVE PBMC DURING SAIDS

	Monkey	Time after infection (weeks): 0[a]	2 Weeks	4 Weeks	18 Weeks
Uninfected	87	1.8	0.8	2.1	3.1
Uninfected	89	1.5	1.6	1.5	1.9
SAIDS[b]	88	2.0	2.0	1.4	1.8
SAIDS	90	1.3	1.0	1.7	Dead

[a] These test materials were collected 20 min prior to infection with SAIDS virus.

[b] Monkey No. 88 survived the infection.

an inhibitory activity on surface Ia expression of these cells could lead to an impaired OKT_{4+} cell (helper) function determining the final immunodeficiency. This has been previously postulated by for human AIDS (11). Patients with AIDS have lower Ia^+ Langerhans' cells in the skin (antigen-presenting cells) and this finding could be related to the high incidence of Kaposi's sarcomas in AIDS patients.

Our findings further demonstrate that SAIDS virus infection has effects on both T- and B-cell functions and this can proceed in severity until death. The animal that died had severe combined immune deficiency and developed opportunistic infections and malignancies in association with these altered T- and B-cell functions. The reasons the animal showed progressive disease and died in contrast with the animals that survived deserve further investigation.

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