Vet Pathol 29:53-59 (1992)

A Comparative Study of Natural Killer Cell Activity, Lymphoproliferation, and Cell Phenotypes in Nonhuman Primates

G. RApPOCCIOLO, J. S. ALLAN, J. W. EICHBERG, AND T. C. CHANH

Department of Virology and Immunology and Center for AIDS Research, Southwest Foundation for Biomedical Research, San Antonio, TX

Abstract. Three different species of nonhuman primates (baboons *[Papio hamadryas]*, rhesus monkeys *[Macaca mulatta]*, and African green monkeys *[Cercopithecus aethiops])* were evaluated for their natural killer cell activity, and for the ability of their peripheral blood mononuclear cells to proliferate in response to known mitogens (concanavalin A, phytohemagglutinin, and pokeweed mitogen) and to react with a panel of mouse monoclonal antibodies directed against human leukocyte surface antigens. Rhesus monkeys displayed the highest natural killer cell cytotoxic activity (185.7 ± 33 lytic units) compared with those of baboons (83.8 ± 19 lytic units) and of African green monkeys from West Africa (39.08 ± 8 lytic units) and from the Caribbean basin (37.9 ± 9 lytic units). No correlation was observed between the natural killer cell cytotoxic activity and the percentage of CD 16' natural killer cells among the three species studied. High spontaneous proliferative capacity was observed in African green monkeys obtained from West Africa compared with those of the other species studied. Although no significant differences were noted in T and B cell mitogen-induced in vitro proliferation, baboon mononuclear cells were less responsive to concanavalin A (stimulation index of 16 ± 3 [$\bar{x}$ ± standard error of mean]) than to phytohemagglutinin (stimulation index of 47 ± 12). However, rhesus and African green monkey cells proliferated more efficiently in response to concanavalin A. Unlike in human beings where the ratio between helper-inducer (CD4+) and cytotoxic-suppressor (CD8+) T-lymphocytes is generally> I, the CD4+/CD8ₜ ratios in baboons and rhesus and African green monkeys were 0.58, 0.69, and 0.35, respectively. Basic information on normal immune functions in these primates is important because of their increased use as experimental animal models for the study of human diseases such as acquired immunodeficiency syndrome (AIDS).

Key words: Acquired immunodeficiency syndrome (AIDS); Iymphoproliferation; natural killer cell; nonhuman primates.

During the past several years, the use of nonhuman primates as animal models for the study of human diseases has become increasingly important. Some human viruses, such as HIV-1 and hepatitis A, B, and non-A non-B (C) viruses, can infect only primates.[1,9,11,24] Furthermore, the relatively recent discovery of a simian lentivirus, simian immunodeficiency virus (SIV), with biological properties similar to those of HIV-1[7,14] and capable of inducing an AIDS (acquired immunodeficiency syndrome)-like disease in rhesus monkeys, has made the nonhuman primate model important for the understanding of virus-induced acquired immunodeficiency diseases.

Very few studies on the basic immunological parameters in nonhuman primates have been reported. Information on the phenotypic expression of some lymphocytic antigens in primates has been used to establish phylogenetic distances among different species'<!? and to compare the ability of different monoclonal antibodies to identify homologous subsets ofleukocytes in human beings and in nonhuman primates.>'-" Natural killer cells represent an important first line of defense against viral infections, against neoplastic cell growth, and in the control of hematopoiesis." A few studies have examined the natural killer cell activity in some primate species (e.g., rhesus monkeys *[Macaca mulatta])*, and the cells responsible for the observed cytotoxic activity have been, in some instances, characterized.[4,22,27,30] In this report, we describe the reactivity of a panel of murine monoclonal antibodies specific for human leukocytic antigens against peripheral blood lymphocytes obtained from rhesus monkeys, African green monkeys *(Cercopithecus aethiops)*, baboons *(Papia hamadryasi*, and healthy human volunteers. We have also evaluated the normal natural killer cell activity and the proliferative responses of peripheral blood lymphocytes to mitogens to provide normal background values that may be useful when virus-induced

alterations of these immunological functions are studied. For example, the onset of human AIDS correlates with an impairment of natural killer cell functions--w> and of T and B cell proliferative capacity':" and with a sharp decrease in the absolute number of cells belonging to the helper-inducer T cell (CD4+ cells),6,15 among other clinical abnormalities. Information on these immune functions in nonhuman primates is therefore important, considering their role as animal models for the study of potential therapies and vaccines directed toward the control of human retrovirus infections.

Materials and Methods

Animals

The three species of nonhuman primates used in this study included 24 rhesus monkeys, nine African green monkeys, and ten baboons. The African green monkeys were divided into two groups, according to their geographic origin; group I included four monkeys obtained from West Africa, and group 2 included 5 monkeys from the Caribbean basin. At the time of the studies, all animals tested seronegative for simian T-Iymphotropic virus type-I, type D retroviruses (SRV-I and SRV-2), and simian immunodeficiency virus. Serologic tests for two other common viruses in these species, foamy virus and Epstein-Barr virus, were not done. All animals were housed at the Southwest Foundation for Biomedical Research. Health human laboratory workers were used as controls (n = 7).

Separation of peripheral blood lymphocytes

Peripheral blood lymphocytes were separated on a Ficoll-Hypaque (Sigma Chemical Co., St. Louis, MO) density gradient as previously described.' The cells obtained were resuspended in complete medium, which included RPMI-1640 (Cell Culture Laboratories, Cleveland, OH) supplemented with 100 V/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine, I M Hepes buffer, I% nonessential amino acids, and 10% heat inactivated fetal bovine serum (Hazelton Biologics, Lenexa, KS).

Cell lines

The mycoplasma-free human cell lines K562 and U937 (obtained from the American Type Culture Collection, Rockville, MD) were cultured in RPMI-1640 complete medium. The cell lines were determined mycoplasma-free by lack of hybridization with a mycoplasma DNA probe (Mycoplasma T.C. Detection Kit, Gen-Probe, San Diego, CA).

Lymphocytic subset characterization

The relative percentages of different subsets of lymphocytes were evaluated by viable membrane immunofluorescence.!" Murine monoclonal antibodies directed against human lymphocytic antigens included anti-Leu-4-FITC (total CD3+ T cells), anti-Leu-5b-FITC (total CD2+ T cells), anti-Leu-3a-PE and OKT4a-FITC (helper-inducer CD4+ T cells), anti-Leu-2a-PE (cytotoxic-suppressor CDS+ T cells), and anti-Leu-I la-FITC (CDI6+ NK cells). The Leu monoclonal antibody series was obtained from Becton-Dickinson (Mountain View, CA), and the OKT4a-FITC was purchased from Coulter Immunology (Hialeah, FL). A solution of 2 x 10^6 cells in 50 μl of phosphate-buffered saline supplemented with 5% fetal bovine serum and 0.1% sodium azide (washing solution) was incubated at 4 C with with 20 μl of the appropriate monoclonal antibody for 30 minutes. After the incubation, the cells were washed twice and fixed in 4% paraformaldehyde for flow cytometric analysis using a FACS-Scan (Beeton-Dickinson). The scatter gate was set on the lymphocyte peak, and 5 x 10^3 cells were counted. The percentage of positive cells was calculated as the percentage of cells that stained more intensely than the threshold defined by the negative control cells (cells without the monoclonal antibody or cells stained with a combination of irrelevant IgG I-FITC and IgG2a-PE to evaluate nonspecific staining; Simultest Control, Becton-Dickinson).

Cytotoxicity assay

K562 target cells (I x 10^6) were labeled with 100 μCi of slCr ($Na_2{}^{51}CrO_4$) and incubated at 37 C for I hour. The labeled cells were washed three times in complete medium and resuspended at I x 105cells/ml in complete medium. Mononuclear cells obtained from the different species examined were used as effector cells. They were added to targets in triplicate wells of round bottom 96-well microtiter plates at effector: target ratios of 50: I, 25: I, 12.5: I, and 6.25: I to determine experimental 5lCr release. Controls included triplicate cultures of target cells in assay medium (spontaneous 5lCr release) and in I N NaOH (maximum SICrrelease). The plates were spun at 200 x $g$for 5 minutes and then incubated at 37 C in a 5% CO_2 humidified incubator for 4 hours. After incubation, radioactivity in 100 μl of supernatant fluid was determined with a gamma counter. The percentage of 51Cr_ specific release was determined using the following formula: {[(experimental release) - (spontaneous release)]I[(maximum release) ~ (spontaneous release)]} x 100. Spontaneous release of target cells incubated in the absence of effector cells was always less than 20%. The results were expressed in lytic units. One lytic unit was defined as the number of lymphocytes required for 20% specific "Cr release from 5 x 10^3 target cells, as estimated by exponential regression analysis for four effector: target cell ratios." The data were calculated as lytic units per 10^7 lymphocytes.

In vitro proliferative responses

Peripheral blood lymphocytes were resuspended at 2 x 10^6 cells/ml in complete medium and cultivated in triplicate wells in flat bottom microtiter plates in the presence of I μg/ml concanavalin A, I μg/ml phytohemagglutinin, or 10 μg/ml pokeweed mitogen. Cells cultivated in medium alone served as controls. The plates were incubated in a 5% CO_2 humidified incubator for 72 hours. After incubation, ^{3}H-thymidine was added at I μCi/well, and the plates were incubated for an additional 12 hours. The cells were then collected from the wells onto fiberglass filters, using a semiautomated microharvesting device (Brandel, Gaithersburg, MD), and the amount of radioactivity incorporated was measured in a scintillation counter.

The data were expressed either as mean counts per minute from unstimulated or mitogen-stimulated cultures or as stimulation index calculated by dividing the mean counts per minute of mitogen-stimulated cultures by the mean counts per minute of unstimulated cultures. Student's t-test was used to compare mean values.

Results

Natural killer cell activity in nonhuman primates

The natural killer (NK) cell activity in normal healthy human volunteers, baboons, rhesus monkeys, and African green monkeys was evaluated using the erythroleukemia cell line K562 as target. Among the various species examined, different degrees of NK cell cytotoxic activity were observed (Fig. 1). Baboons and both groups of African green monkeys had significantly lower NK cell activity compared with that of normal human volunteers (300 ± 37 lytic units [LU] in human beings compared with 83.3 ± 19 LU in baboons, and 39.0 ± 8 LU and 37.9 ± 9 LU in African green monkey group 1 (AGM 1) and group 2 (AGM 2), respectively, $P < 0.05$). The NK cell activity of the 24 rhesus monkeys (185.7 ± 33 LU) studied was also lower than that of the human controls, but the difference was not statistically significant. When NK cell activity among primates was compared, there was a significant difference ($P < 0.05$) between the NK cell activity of rhesus monkeys and those of both groups of African green monkeys and baboons. Similar results were obtained with another NK cell-sensitive human cell line (U937) as target cells in the cytotoxicity assays (data not shown).

When peripheral blood lymphocytes (PBL) obtained from these species were stained with anti-Leu-lla monoclonal antibodies (mAb) directed against the surface marker CD16 associated with cells belonging to the NK cell lineage," there were no significant differences in the relative percentages of cells bearing the CDl6 phenotype (Table 1). Although baboon PBL showed the highest mean percentage of cells expressing the CD16 surface marker, they displayed a relatively lower NK cell activity compared with those of human and rhesus PBL.

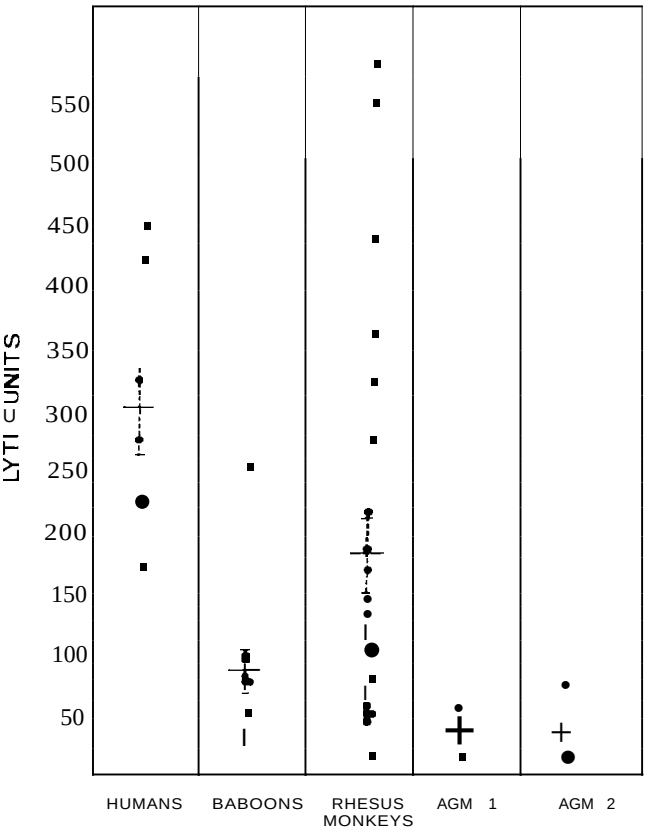

Fig. 1. Natural killer cell activity in human beings (n = 7), baboons (n = 10), rhesus monkeys (n = 24), African green monkey group I (AGM I) (n = 4), and African green monkey group 2 (AGM 2) (n = 5) using K562 cells as targets. Each dot represents individual values. Horizontal bars represent mean lytic units ± standard error of the mean.

In vitro proliferative responses

The proliferative responses of PBL to the T-cell mitogens concanavalin A and phytohemagglutinin and to the B-cell activator pokeweed mitogen were evaluated

Table 1. Percentage of peripheral blood Iymphocytes* from human beings and nonhuman primates that reacted with a variety of monoclonal antibodies.

Species	n	Monoclonal Antibodies					
		Anti-Leu-a	Anti-Leu-5b	Anti-Leu-2b	Anti-Leu-3a	OKT4a	Anti-Leu-II a
Human beings	7	60.6 ± 4.2	73.4 ± 2.9	21.5 ± 2.2	42.4 ± 3.0	55.7 ± 2.8	15.0 ± 4.0
Baboons	10	32.7 ± 8.4	82.4 ± 2.0	65.2 ± 2.5	37.9 ± 2.7	32.3 ± 3.4	23.4 ± 1.8
Rhesus monkeys	24	51.8 ± 3.2	78.4 ± 2.4	37.2 ± 1.8	25.9 ± 3.1	35.1 ± 2.7	12.9 ± 1.6
AGM lt	4	37.4 ± 7.3	68.0 ± 3.8	74.7 ± 1.3	26.2 ± 4.0	34.9 ± 5.4	11.7 ± 1.9
AGM 2‡	5	ND§	53.9 ± 5.9	43.9 ± 3.5	ND	40.6 ± 1.6	ND

* Mean percentage positive cells ± standard error of the mean.
t African green monkey group I.
‡ African green monkey group 2.
§ NO = not done.

Table 2. Proliferative response of peripheral blood lymphocytes from human beings and nonhuman primates in control and mitogen-stimulated cultures.

Species	*n*	Control*	Mitogens			
			Phytohemagglutinin		Concanavalin A	
			Counts per Minute*	Stimulation Indexj	Counts per Minute	Stimulation Index
Human beings	7	1,452 ± 497	22,166 ± 4,231	72 ± 39	34,742 ± 7,299	66 ± 29
Baboons	6	1,236 ± 769	17,374 ± 839	47 ± 12	9,331 ± 3,476	16 ± 3
Rhesus monkeys	24	378 ± 52	47,786 ± 8,900	170 ± 22	67,362 ± 6,257	231 ± 27
AGM 1‡	4	27,563 ± 10,164	135,757 ± 31,325	9±4	149,368 ± 24,460	9 ± 3
AGM 2§	5	139 ± 46	15,641 ± 6,896	261 ± 130	16,724 ± 3,834	239 ± 78

* Mean counts per minute ± standard error of the mean of triplicate cultures.
t Mean stimulation index ± standard error of the mean (stimulation index = $\bar{x}$ counts per minute of mitogen-stimulated cultures/x counts per minute of unstimulated [control] cultures).
‡ African green monkey group I.
§ African green monkey group 2.

(Table 2), Although all the species studied exhibited a proliferative response against these mitogens some differences were noted, particularly between AGM 1 and AGM 2. The proliferation of AGM 1 PBL was high even in the absence of any stimulus (medium alone), Proliferative capacity of PBL from AGM I was higher than that of AGM 2 when the mean net counts per minute (mean counts per minute in the presence of mitogen − mean counts per minute in the absence of mitogen) were compared, but when the data were calculated as stimulation index, the AGM I had lower proliferative capacity, Although the phytohemagglutinin-induced proliferation of baboon PBL was comparable to that of human and rhesus monkey cells, proliferation in response to concanavalin A was lower than that of the other species examined. In contrast, concanavalin A seemed to be more effective in inducing T-cell proliferation, both in rhesus monkeys and in African green monkeys, than phytohemagglutinin.

Phenotypic characterization of primate PBL

Mouse mAb directed against human lymphocytic surface antigens react with nonhuman primate peripheral mononuclear cells.[20,21] These mAb have been useful in the identification of conserved epitopes on the surface of primate mononuclear cells and in the determination of the phylogenetic relatedness among these species. Because we observed differences in NK cell activity and proliferative responses in the primates studied, we examined the relative percentages of T-lymphocytes and NK cells in the peripheral blood. For T-lymphocyte identification, anti-Leu-4 and anti-Leu-5b mAb, which recognize the CD3 and CD2 determinants, respectively, were used; CD2 is highly conserved among primates. The data showed that the relative percentage of cells expressing the Leu-5b determinant was significantly higher than the percentage of cells binding the anti-Leu-4 mAb in each of the species studied ($P < 0.05$) (Table I).

An important parameter in the study of T-lymphocyte responses in immunodeficiency diseases is the proportion of cells belonging to the two main subsets of lymphocytes, the inducer-helper (CD4+) and the cytotoxic-suppressor (CD8+) T-lymphocytes. In healthy human beings, the ratio between CD4+ cells and CD8+ cells is always> I, whereas in the nonhuman primate species in this study, the ratio of CD4+: CD8+ was < I. Two different mAb specific for the CD4 receptor, anti-Leu-3a and OKT4a, were used. Although OKT4a recognized a greater percentage of T-lymphocytes than did anti-Leu-3a, the CD4+: CD8+ ratios were < 1 in each of the nonhuman primate species examined (baboons = 0.5, rhesus monkeys = 0.9, AGM 1 = 0.4, AGM 2 = 0.9), whereas the CD4+: CD8+ ratio in normal human volunteers was 2.5.

Discussion

This report details a comparative study in human beings and nonhuman primates (baboons, rhesus monkeys, and African green monkeys) of some of the immunological parameters of importance in the study of the pathogenesis of immunodeficiency diseases induced by viral infections. The significance of natural killer (NK) cells in the surveillance against infectious agents and in the control of neoplastic growth has been widely investigated, particularly in the mouse system." In the present study, higher NK cell activity was observed in rhesus monkeys as compared with that of the other primates studied. This observation is consistent with a previous report in which the cytotoxic capacity of rhesus monkey peripheral blood lymphocytes (PBL) was evaluated against human NK cell-sensitive and NK cell-resistant cell lines as targets.' An unusually high NK cell activity was observed in rhesus monkeys against the Epstein-Barr virus-transformed

Table 2. Extended.

Mitogens	
Pokeweed Mitogen	
Counts per Minute	Stimulation Index
18,983 ± 2,273	38 ± 13
17,404 ± 4,003	33 ± 13
6,126 ± 473	20 ± 2
164,216 ± 4,318	10 ± 3
7,875 ± 2,558	64 ± 24

cell line Raji. The cells mediating this cytotoxicity belonged to a particular effector cell subpopulation bearing the CD16+/CD2+/CD8+ phenotype. This high NK cell activity may explain the resistance of rhesus monkeys to infection with Epstein-Barr virus. The NK cell activity in our cohort of healthy human volunteers was consistent with that of previous reports on NK cell lytic activity in human beings." Although significant differences were observed in the NK cell lytic activity among the species studied, there were no differences in the relative percentages of lymphocytes expressing the CD 16 NK cell phenotype (Table 1). There was no positive correlation between the NK cell activity and the percentage of CD 16+ lymphocytes; PBL from the baboons studied had the highest mean percentage of lymphocytes expressing the CD 16 phenotype yet displayed a relatively lower NK cell lytic activity compared with that of human and rhesus monkey PBL. These observations suggest the possible involvement of CD16- subpopulations in the non-MHC-restricted NK cell cytotoxic activity in nonhuman primates, especially in the rhesus monkeys, which had a relatively low percentage of CD 16+ cells and the highest NK cell activity among the primates studied. The high NK cell cytotoxicity observed in the rhesus monkey PBL may be due in part to CD 16+ NK cells and in part to cells expressing a phenotype different from CD 16 or to cells belonging to the T-lymphocyte lineage." The NK cell activity shown by PBL was different for rhesus monkeys and African green monkeys. Some African green monkeys caught in the wild are naturally infected with a simian lentivirus, simian immunodeficiency virus (SIV), with no apparent pathologic effects.t" whereas rhesus monkeys infected with a similar retrovirus displayed a symptomatology similar to that of human AIDS.18 The apparent resistance of African green monkeys to diseases induced by SIV in rhesus monkeys may result from a different ontogeny of immune cells and not from a higher NK cell activity, which may allow the existence of an asymptomatic but viremic state, in contrast to the immunosuppressed state observed in SIV-infected monkeys. A comparison of the NK cell cytotoxic activity in the SlV-seronegative African green monkeys in our study with the activity in this species upon infection with SIV would be of interest, particularly regarding the phenotypic characteristics of the subpopulation(s) of NK cells mediating the response.

In the mitogen-induced proliferation studies, PBL from African green monkey group 1 had a higher mean net counts per minute compared with that of the African green monkey group 2; however, when the stimulation indices were compared, group 1 proliferative capacity was lower than that of group 2. This discrepancy is explained by the high spontaneous proliferation observed in group 1. The use of stimulation indices for evaluating proliferative responses in individuals with either normal or altered immune functions is not very satisfactory, particularly when the baseline incorporation of 3H-thymidine may be due to factors independent of the ability of the lymphocytes to respond to a stimulus." Although a difference in spontaneous proliferation was observed, there was no significant difference in NK cell activity between groups 1 and 2.

The ratios of CD4+: CD8+ lymphocytes in all nonhuman primates studied were < 1, whereas that of normal human beings was approximately 2.5. Thus, in the primates studied, there appears to be a "suppressed" immunological status with regard to T-cell phenotypes, although no experimental evidence exists to support this notion. The greater number of CD8+ cells in these species is not reflected, however, in a truly suppressed immunological status, as indicated by the functional tests performed.

These observations on the normal immunological functions of nonhuman primates should be useful in the study of immune alterations induced by viral diseases in these animal models for human viral diseases.

Acknowledgements

We thank D. L. Maltos, S. R. Trevino, and T. Newton for their excellent technical assistance, K. Meads for the FACS analysis, and J. Fletcher for her help in preparing this manuscript. This study was supported in part by grant numbers Al 26893-02 and Al 28696 from the National Institutes of Allergy and Infectious Diseases.

References

Alter HJ, Eichberg JW, Masur H, Saxinger WC, Gallo R, Macher AM, Lane HC, Fauci AS: Transmission of HTLV-lII infection from human plasma to chimpanzees: an animal model for AIDS. Science 226:549-552, 1984

2 Bonavida B, Katz 1, Gottlieb M: Mechanism of defective NK cell activity in patients with acquired immunodeficiency syndrome (AIDS) and AIDS-related complex. I. Defective trigger on NK cells for NKCF production by target cells, and partial restoration by II. 2.1 Immunol 137: 1157-1163, 1986
3 Boyum A: Separation ofleucocytes from blood and bone marrow. Scand 1 Clin Lab Invest 21(Suppl 97):1-109, 1968
4 Carver FM, Thomas 1M: Natural killer cells in rhesus monkeys: properties of effector cells which lyse Raji targets. Cell ImmunoI1l7:56-69, 1988
5 Ciobanu N, Welte K, Kruger G, Venuta S, Gold 1, Feldman SP, Wang CY, Koziner B, Moore MAS, Safai B, Mertelsmann R: Defective T-cell response to PHA and mitogenic monoclonal antibodies in male homosexuals with acquired immunodeficiency syndrome and its *in vitro* correction by interleukin 2. 1 Clin ImmunoI3:332-340, 1983
6 Dalgleish AG, Beverley PCL, Clapham PR, Crawford DH, Greaves MF, Weiss RA: The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus. Nature 312:763-767,1984
7 Daniel MD, Letvin NL, King NW, Kannagi M, Sehgal PK, Hunt RD, Kanki Pl, Essex M, Desrosiers RC: Isolation of T-cell tropic HTLV-III-like retrovirus from macaques. Science 228: 1201-1204, 1985
8 Dean lH, Connor R, Herberman RB, Silva 1, McCoy 11., Oldham RK: The relative proliferation index as a more sensitive parameter for evaluating lymphoproliferative responses of cancer patients to mitogens and alloantigens. Int 1 Cancer 20:359-370, 1977
9 Deinhardt F: The use of nonhuman primates in human viral hepatitis. Monogr Primatol 2: 135-148, 1983
10 Eichberg lW, Montiel MM, Morale BA, King DE, Chanh TC, Kennedy RC, Dreesman GR: Lymphocyte subsets in chimpanzees. Lab Anim Sci 38: 197-198, 1988
II Fultz PN, McClure HM, Swenson RB, McGrath CR, Brodie A, Getchell lP, Jensen FC, Anderson DC, Broderson lR, Francis DP: Persistent infection of chimpanzees with human T-Iymphotropic virus type III/lymphadenopathy-associated virus: a potential model for acquired immunodeficiency syndrome. 1 Virol 58: 116-124, 1986
12 Haynes BF, Dowell BL, Hensley 1.1., Gore I, Metzgar RS: Human T cell antigen expression by primate T cells. Science 215:298-300, 1982
13 Kanki Pl, Kurth R, Becker W, Dreesman G, McLane MF, Essex M: Antibodies to simian T-Iymphotropic retrovirus type III in African green monkeys and recognition ofSTLV-III viral proteins by AIDS and related sera. Lancet 1:1330-1332,1985
14 Kannagi M, Yetz 1M, Letvin NL: *In vitro* growth characteristics of simian T-Iymphotropic virus type III. Proc Natl Acad Sci USA 82:7053-7057, 1985
15 Klatzmann D, Barre-Sinoussi F, Nugeyre MT, Dauguet C, Vilmer E, Griscelli C, Brun-Vezinet F, Rouzioux C, Gluckman lC, Chermann 1-C, Montagnier L: Selective tropism oflymphadenopathy associated virus (LAV) for helper-inducer T lymphocytes. Science 225:59-63, 1984
16 Lanier 1.1., I.e AM, Phillips lH, Warner NL, Babcock GF: Subpopulations of human natural killer cells defined by expression of the Leu-7 (HNK-l) and Leu-II (NK-15) antigens. 1 Immunol131: 1789-1796, 1983
17 Letvin NL, King NW, Reinherz EL, Hunt RD, Lane H, Schlossman SF: T lymphocyte surface antigens in primates. Eur 1 Immunol13:345-347, 1983
18 Letvin NL, Daniel MD, Sehgal PK, Desrosiers RC, Hunt RD, Waldron LM, MacKey 11, Schmidt DK, Chalifoux I.V, King NW: Induction of AIDS-like disease in macaque monkeys with T-cell tropic retrovirus STLV-III. Science 230:71-73,1985
19 Lew F, Tsang P, Solomon S, Solikoff 11, Bekesi lG: Natural killer cell function and modulation by alpha IFN and 11.2 in AIDS patients and prodromal subjects. 1 Clin Lab Immunol 14: 115-121, 1984
20 Murayama Y, Fukao K, Noguchi A, Takenake 0: Epitope expression on primate lymphocyte surface antigens. 1 Med Primatol 15:215-226, 1986
21 Ormerod LD, Osborn KG, Lowenstine LJ, Meyer PR, Parker lW, Smith RE, Taylor CR: Lymphoid immunohistochemistry of macaque primates. Clin Exp Immunol 74:435-442, 1988
22 Powell lD, McClure HM, Anderson D, Fultz PN, Sell KW, Ahmed-Ansari A: Phenotypic and functional differences in NK and LAK cells in the peripheral blood of sooty mangabeys and rhesus macaques. Cell Immunol 124: 107-118, 1989
23 Pross HF, Baines MG, Rubin P, Shragge P, Patterson MS: Spontaneous human lymphocyte-mediated cytotoxicity against tumor target cells. IX. The quantitation of natural killer cell activity. 1 Clin Immunol 1:51-63, 1981
24 Provost Pl, Conti PA, Giesa PA, Banker FS, Buynak EB, McAleer Wl, Hilleman MR: Studies in chimpanzees of live, attenuated hepatitis A vaccine candidates. Proc Soc Exp Bioi Med 172:357-363, 1983
25 Rappocciolo G, Toso IF, Torpey nr III, Gupta P, Rinaldo CR 1r: Association of alpha interferon production with natural killer cell lysis of U937 cells infected with human immunodeficiency virus. 1 Clin Microbiol 27: 41-48,1989
26 Rook AH, Masur H, Lane HC, Frederick W, Kasara T, Macher AM, Djen lY, Manischewitz IF, lackson I., Fausi AS, Quinnan GV: Interleukin-2 enhances the depressed natural killer and cytomegalovirus-specific cytotoxic activities of lymphocytes from patients with the acquired immunodeficiency syndrome. 1 Clin Invest 72: 398-403, 1983
27 Savary CA, Lotzova E, Gray KN, lardine lH: Natural killer cell-mediated anti-tumor reactivity of rhesus monkeys. Nat Immun Cell Growth Regul 4:328-339, 1985
28 Trinchieri G: Biology of natural killer cells. Adv ImmunoI47:187-376,1989
29 Wahren B, Morfeldt-Mansson I., Biberfeld G, Moberg

L, Sonnerborg A, Ljungman P, Werner A, Kurth R, Gallo R, Bolognesi 0: Characteristics of the specific cell-mediated immune response in human acquired immunodeficiency virus infection. J Virol 61:2017-2023, 1987

30 Yamada YK, Yabe M, Tatsumi M: Phenotypic characterization of cynomolgus monkey natural killer cells. Cell Immunol 122:524-533, 1989

Request reprints from Dr. T. C. Chanh, Department of Virology and Immunology, Southwest Foundation for Biomedical Research, PO Box 28147, San Antonio, TX 78228-0147 (USA).