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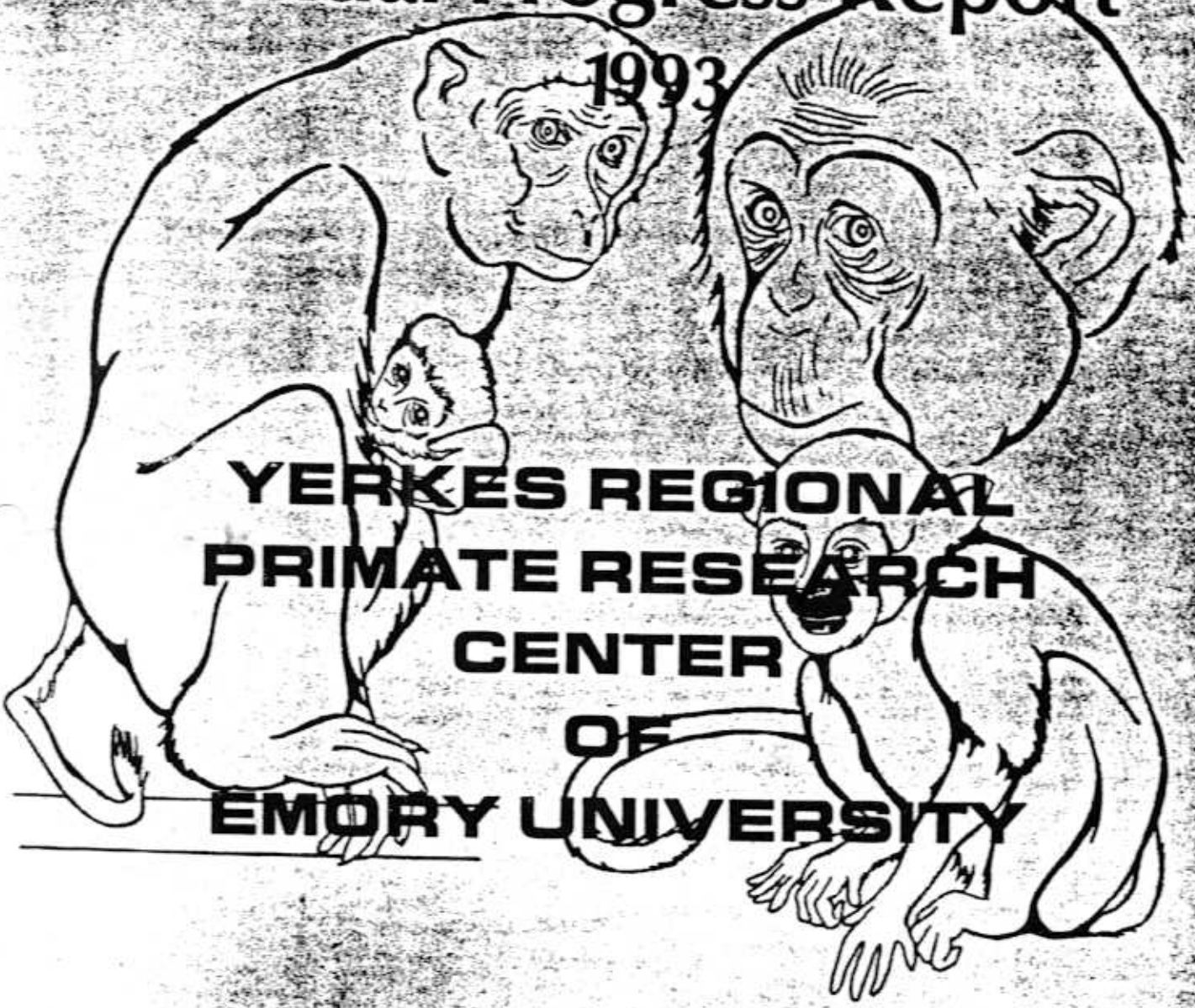
Annual Progress Report

1993



YERKES REGIONAL PRIMATE RESEARCH CENTER OF EMORY UNIVERSITY

January 1, 1993 — December 31, 1993

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

NATIONAL CENTER FOR RESEARCH RESOURCES
COMPARATIVE MEDICINE PROGRAM
REGIONAL PRIMATE RESEARCH CENTERS PROGRAM (RPRC)
ANNUAL PROGRESS REPORT

1. PHS GRANT NUMBER: P51RR00165-33

2. NAME OF RECIPIENT INSTITUTION: Yerkes Regional Primate Research Center

3. HEALTH PROFESSIONAL SCHOOL (If applicable): Emory University Woodruff Medical Center

4. REPORTING PERIOD:

   A. FROM (Month, Day, Year): 01-01-93

   B. TO (Month, Day, Year): 12-31-93

5. CENTER DIRECTOR:

   A. NAME: Frederick A. King, Ph.D.

   B. TITLE: Director and Professor, Yerkes Regional Primate Research Center; Professor, Department of Anatomy; Adjunct Professor, Department of Psychology; Associate Dean, Emory University School of Medicine

   C. SIGNATURE: Frederick A. King

6. DATE SIGNED (Month, Day, Year): April 8, 1994

7. TELEPHONE (Include Area Code): (404) 727-7707

span" for each stimulus parameter. On the recognition span task, the intermediate aged monkeys were significantly impaired relative to the young adult group on both the spatial and color conditions of the tasks, while no difference was found between the performance of the intermediate and very old monkeys. This impairment of recognition memory span in our intermediate aged monkeys parallels that seen in both cross-sectional and longitudinal studies of normal human aging.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Seasonal Control of Behavior in Male Rhesus Monkeys

AXIS I: 1a, 2, 15

AXIS II: 36, 92, Neuroendocrinology

PRC UNIT: Neurobiology and Vision

INVES1: Herndon, James G.
DEGREE1: Ph.D.
DEPT1: Neuropsychobiology/Neurobiology and Vision
STAFF1: C

INVES2: Turner, Jane J.
DEGREE2: Ph.D.
DEPT2: Neurobiology and Vision
STAFF2: O

SPECIES1: Macaca mulatta
NUM1: 22

NON-HOST INST: NA

ABSTRACT: In recent years we studied several factors which may contribute to seasonality of reproduction in the male rhesus monkey. Previous work demonstrated that (1) shortening daylength is not required for the onset of seasonal mating in the male and (2) exposure to females is not required for the display of physiological indications of seasonality. One question addressed during the present project year is whether exogenous melatonin, administered during the non-mating season, might induce or accelerate the mating season. In order to investigate this question, we implanted 7 male rhesus monkeys with melatonin-containing capsules in May, at the end of the normal period of reproductive activity. An additional group of 7 males received blank implants. There were no differences between the two groups of animals; both showed normal onset of the mating season at the appropriate time. In a related study we examined the influence of prolongation of the short-daylength phase of the photoperiod. Again, two groups of males were examined. One group was maintained on the normal photoperiod, while the daylength of the other group was locked, beginning on the winter solstice, on the short daylength prevalent on that day at this latitude. In this way we were able to determine importance of the spring lengthening of photoperiod, both in the offset of the reproductive period and in the timing of the subsequent period of recrudescence. Results of the photoperiod portion of the project are still being analyzed, but appear consistent with the idea that an endogenous process within the male monkey drives the timing of the annual reproductive rhythm. There are few cyclic phenomena in primates which are as clear-cut as the annual reproductive rhythm. An understanding of its mechanisms will help clarify mechanisms for less absolute rhythmic events present in humans.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Cognitive Performance in Aging Chimpanzees

AXIS I: 1a, 21, 28 (Behavior)

AXIS II: 30, 36, 41

PRC UNIT: Neurobiology and Vision

INVES1: Herndon, James G.
DEGREE1: Ph.D.
DEPT1: Neuropsychobiology/Neurobiology and Vision
STAFF1: C

INVES2: Turner, Jane J.
DEGREE2: Ph.D.
DEPT2: Neurobiology and Vision
STAFF2: O

SPECIES1: Pan troglodytes
NUM1: 4

NON-HOST INST: NA

ABSTRACT: The first aim of this pilot project is to modify the spatial condition of the Delayed Recognition Span Test (DRST) task and the Delayed Non-Matching to Sample (DNMS) tasks for use in tests of memory and cognitive ability in chimpanzees. These tasks are in use in our studies of aging in rhesus monkeys. A second aim is to pre-train 2 aged (>40 years) and two mid-aged (<30 years) chimpanzees for use of the test apparatus, and to conduct DRST and DNMS tests in these animals.

The immediate goal of these studies is to demonstrate feasibility of such tests, and to gain practical experience in carrying them out. The use of two old and two mid-age chimps was done to provide some idea of the range of problems we might expect in developing a larger study. Ultimately, we hope to be able to examine cognitive aspects of aging and the correlation of neural changes with age-related behavioral deficits through the use of relatively non-invasive imaging techniques. The conduct of such studies in chimpanzees is particularly relevant to age-related changes in humans because the chimpanzee is the primate species most closely resembling the human.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Correction of Neonatal Monocular Aphakia with IOLS

AXIS I: 1a, 25b

AXIS II: 86

PRC UNIT: Neurobiology and Vision

INVES1: Lambert, Scott R.
DEGREE1: M.D.
DEPT1: Neurobiology and Vision
STAFF1: O

INVES2: Boothe, Ronald G.
DEGREE2: Ph.D.
DEPT2: Neuropsychophysics/Neurobiology and Vision
STAFF2: C

SPECIES1: Macaca mulatta
NUM1: 76

NON-HOST INST: NA

ABSTRACT: The objective of this study was to determine if implanting an intraocular lens increases the incidence of complications following a lensectomy and anterior vitrectomy in a neonatal nonhuman primate eye. Between 1985 and 1992, seventy-six monkeys underwent a lensectomy and anterior vitrectomy during the first 16 days of life, 21 of which had an IOL implanted into the posterior chamber. The eyes were examined at regular intervals using biomicroscopy, applanation tonometry, and ophthalmoscopy until the aphakic monkeys were 80±42 weeks of age and the pseudophakic monkeys were 92±26 weeks of age. Five eyes were studied histopathologically. These studies revealed that pupillary membranes and lens regrowth obscuring the visual axis occurred more often in the pseudophakic eyes. As a result, the pseudophakic eyes required more reoperations than the aphakic eyes in order to keep the pupillary opening clear. There was no significant difference in the incidence of glaucoma between the pseudophakic and aphakic eyes. Pupillary capture of the IOL optic occurred in 52% and haptic breakage in 24% of the pseudophakic eyes. All of the eyes with broken haptics had prominent Soemmerring ring cataracts varying in maximum thickness from 0.6-2.0 mm. Seven of the haptics from these 5 eyes had eroded into the iris, one into the ciliary body and one into the anterior chamber. As a result of these studies, we concluded that implanting an IOL into a neonatal eye after a lensectomy and anterior vitrectomy increases the likelihood of a reoperation being necessary. Haptics frequently erode into the iris and ciliary body and may break due to a stress placed on the optic haptic junction.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: ICG Angiography for Choroidal Bloodflow Measurement

AXIS I: 1a, 25b

AXIS II: 50b, 63j

PRC UNIT: Neurobiology and Vision

INVES1: Lim, Jennifer I.
DEGREE1: M.D.
DEPT1: Neurobiology and Vision
STAFF1: O

SPECIES1: Macaca mulatta
NUM1: 2

NON-HOST INST: NA

ABSTRACT: ICG angiography is a relatively new technique for imaging the choroidal vasculature. The use of the ICG dye combined with infrared light enables the vasculature to be visualized through blood, pigment, turbid fluid and exudates. The technique, when coupled to video systems, can capture dye filling rates. Thus, the bloodflow rates can be measured.

Pentoxifylline is a hemorrheologic agent that is used in humans to improve the circulation. To this date, there has been no direct measurement of the effect of bloodflow. Over the past few months, the ICG videoangiography unit has been used in rhesus monkeys pre- and post-pentoxifylline injection. The video imaging systems capture the filling rate at 15 frames per second. Using computer algorithms, the topographical representation of the dye filling rates can be determined. Thus, the effect of the drug on the circulation can be determined.

Two monkeys have had this performed using general anesthesia. The results have shown that pentoxifylline can alter the choroidal bloodflow. Thus, pentoxifylline appears to have a useful effect on the vasculature.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Neural Substrates of Cognitive Decline in Aging Monkeys

AXIS I: 1a, 21

AXIS II: 30, 41

PRC UNIT: Neurobiology and Vision

INVES1: Moss, Mark B.
DEGREE1: Ph.D.
DEPT1: Neurobiology and Vision
STAFF1: O

INVES2: Rosene, Douglas L.
DEGREE2: Ph.D.
DEPT2: Neurobiology and Vision
STAFF2: O

INVES3: Herndon, James G.
DEGREE3: Ph.D.
DEPT3: Neurobiology and Vision
STAFF3: C

SPECIES1: Macaca mulatta
NUM1: 10

NON-HOST INST: Boston University School of Medicine (MBM, DLR)

ABSTRACT: This ongoing project focuses on the behavioral changes which accompany aging in the rhesus monkey. Behavioral tasks in this study are designed to assess cognitive flexibility or "executive function" and memory. In prior years of the study, we have demonstrated an age difference in abilities of animals in our battery of behavioral tasks, which are derived from those used to assess geriatric patients. The brain areas thought to underlie cognitive flexibility and memory are, respectively, the prefrontal association cortex and the temporal lobe limbic system. We hypothesize that changes in these areas will involve synaptic dysfunction rather than an actual reduction in the number of cells. Accordingly, changes in behavioral performance will be correlated with anatomical changes as well as with neurochemical and metabolic measures. While behavioral changes in our monkeys have clearly been demonstrated, the correlation of these changes with morphological and physiological characteristics within the same individual are still underway. All aspects of this work are designed to shed light on aspects of human aging. Thus, many of our behavioral tests are derived from tests which reveal cognitive deficits in human, and one of the tests developed in our laboratory is now widely used as an early means of early memory dysfunction in aged adults with suspected dementia.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Neural Substrates of Cognitive Decline in Aging Monkeys

AXIS I: 1a, 21

AXIS II: 30, 36, 41, 46

PRC UNIT: Neurobiology and Vision

INVES1: Peters, Alan
DEGREE1: Ph.D.
DEPT1: Neurobiology and Vision
STAFF1: 0

INVES2: Rosene, Douglas L.
DEGREE2: Ph.D.
DEPT2: Neurobiology and Vision
STAFF2: 0

INVES3: Moss, Mark B.
DEGREE3: Ph.D.
DEPT3: Neurobiology and Vision
STAFF3: 0

INVES4: Abraham, Carmela
DEGREE4: Ph.D.
DEPT4:
STAFF4: 0

INVES5: Hyman, Brad
DEGREE5: M.D., Ph.D.
DEPT5:
STAFF5: 0

INVES6: Kemper, Thomas
DEGEEE6: M.D.
DEPT6:
STAFF6: 0

INVES7: Tigges, Johannes
DEGREE7: Ph.D.
DEPT7: Neuroanatomy/Neurobiology and Vision
STAFF7: C

INVES8: Volicer, Lavislav
DEGREE8: M.D., Ph.D.
DEPT8:
STAFF8: 0

SPECIES1: Macaca mulatta
NUM1: 22

NON-HOST INST: Boston University School of Medicine (AP, DLR, MBM, CA, BH, TK, LV)

ABSTRACT: We are testing monkeys of various ages to see if there is any memory loss or change in executive function. A group of young adults, 5-10 years of age, is being compared to a group over 25 years of age. The animals are first tested behaviorally. The brains of the monkeys are then examined to ascertain if there are any features that can be correlated with the behavioral results. We have just completed the third year of funding of this program project, which is being supported by NIA. Our results to date demonstrate that although some senile plaques develop with age, there are no losses of neurons with age in the visual, motor and prefrontal cortices, or in the hippocampus. The brain stems are now being examined and assessments are also being made of alterations in the levels of neurotransmitters, and of amyloid.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Relative Age-related Preservation of Synaptic Parameters in Dentate Gyrus of Adult Rhesus Monkeys

AXIS I: 1a, 21

AXIS II: 30

PRC UNIT: Neurobiology and Vision

INVES1: Tigges, Johannes
DEGREE1: Ph.D.
DEPT1: Neuroanatomy/Neurobiology and Vision
STAFF1: C

INVES2: Herndon, James G.
DEGREE2: Ph.D.
DEPT2: Neuropsychology/Neurobiology and Vision
STAFF2: C

INVES3: Rosene, Douglas L.
DEGREE3: Ph.D.
DEPT3: Neurobiology and Vision
STAFF3: O

SPECIES1: Macaca mulatta
NUM1: 10

NON-HOST INST: Boston University School of Medicine (DLR)

ABSTRACT: A light and electron microscopic examination was carried out on the dentate gyrus of 10 rhesus monkeys (4-35 years) to determine the effects of age on axon terminals. A total of 100 electron micrographs for each monkey was taken in the outer portion of the molecular layer; counts and measurements were made on enlarged prints. Statistical analysis revealed no age-associated loss of synapsing axon terminals or shrinkage of their cross-sectional areas. Further, there was no loss in the total number of synapses or change in the length of their postsynaptic membrane density. Only when considered separately did the axodendritic (shaft) synapse show a significant age-associated loss; this loss may have a small effect, as the axodendritic synapses constitute only 13% of the total synapse population. Also, there was no detectable shrinkage of the thickness of the molecular layer. Regarding non-quantified observations, a moderate number of dystrophic myelinated axon and corpora amylacea located in astrocytic processes were seen in the outer portion of the molecular layer of older monkeys. Furthermore, glial cells and pericytes showed age-associated accumulation of lipofuscin-like inclusions. A singular occurrence of a structural inclusion body in a dendrite was noted.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Apolipoprotein E4 and Beta-amyloid in Senile Plaques and Cerebral Blood Vessels of Aged Rhesus Monkeys

AXIS I: 1a, 21

AXIS II: 30

PRC UNIT: Neurobiology and Vision

INVES1: Poduri, Annapurna
DEGREE1: M.D.
DEPT1:
STAFF1: O

INVES2: Gearing, Marla
DEGREE2: Ph.D.
DEPT2:
STAFF2: O

INVES3: Rebeck, G. William
DEGREE3: Ph.D.
DEPT3:
STAFF3: O

INVES4: Mirra, Suzanne S.
DEGREE4: M.D.
DEPT4: Pathobiology & Immunobiology
STAFF4: O

INVES5: Tigges, Johannes
DEGREE5: Ph.D.
DEPT5: Neuroanatomy/Neurobiology and Vision
STAFF5: C

INVES6: Hyman, Bradley T.
DEGREE6: M.D., Ph.D.
DEPT6:
STAFF6: O

SPECIES 1: Macaca mulatta
NUM1: 9

NON-HOST INST: VA Medical Center (SSM, MG); Massachusetts General Hospital (AP, GWR, BTH)

ABSTRACT: Recent studies of late onset familial and sporadic Alzheimer's disease (AD) show a genetic disequilibrium between inheritance of the $\epsilon 4$ allele of the apolipoprotein E (ApoE) gene and development of AD. β-amyloid(Aβ)-positive senile plaques and blood vessels in AD are immunoreactive for ApoE, suggesting that ApoE plays a role in amyloid

deposition. We examined the brains of nine rhesus monkeys (Macaca mulatta) to determine the immunohistochemical distribution of ApoE and to investigate the association of ApoE with Aβ in this species. Antibodies to ApoE and Aβ labeled senile plaques and vessels in the brains of aged monkeys, indicating cross-species homogeneity of the association of these two proteins. PCR/restriction enzyme analysis of the ApoE (3/ε4 allelic site (residue 112) in the rhesus monkey revealed that the rhesus has an arginine at this site, like the human ε4 allele, the cynomolgus monkey, baboon, cow, pig, mouse, and rat, but unlike the human ε3 allele and the rabbit. These results emphasize the value of aged nonhuman primates as animal models for Aβ deposition and ApoE4-Aβ interactions in AD and aging.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Does Atropine Prevent Deprivation-Induced Excessive Axial Eye Elongation?

AXIS I: 1a, 21, 25b

AXIS II: 60, 62

PRC UNIT: Neurobiology and Vision

INVES1: Tigges, Margarete
DEGREE1: Ph.D.
DEPT1: Neural Ultrastructure/Neurobiology and Vision
STAFF1: C

INVES2: Iuvone, P. Michael
DEGREE2: Ph.D.
DEPT2: Neurobiology and Vision
STAFF2: O

SPECIES1: Macaca mulatta
NUM1: 13

NON-HOST INST: NA

ABSTRACT: We are continuing to use newborn rhesus monkeys as models for visual system dysfunctions in human infants. The emphasis of the current project is on the prevention of myopia by pharmacological means. Atropine, a relatively non-selective antagonist of muscarinic cholinergic receptors, prevents the development of deprivation-induced myopia in chicks, but not in eyelid-sutured rhesus monkeys. We have shown previously that occlusion with opaque occluder lenses causes excessive postnatal axial eye elongation and, thus, myopia. In 13 newborn rhesus monkeys, the right eye was occluded with an occluder lens. Seven monkeys received 1 drop of 1% atropine per day in the occluded eye and 1 drop of vehicle solution in the fellow eye. Six control monkeys received vehicle solution in both eyes. Five of the 7 atropine-treated eyes were similar in axial length compared to their fellow eyes, while 2 atropine-treated eyes grew excessively compared to their fellow eyes. At the end of the experiment, ocular tissue including retina, choroid, iris, sclera, lens and vitreous will be examined for alterations in a variety of neurotransmitter/neuromodulator substances, muscarinic receptor subtypes and levels of growth factors IGF-1 and bFGF, in comparison with the same ocular tissues from the controls.

Pharmaca that interact with specific ocular substances to prevent myopia may be relevant clinically. In humans, myopia is a common vision disorder and, in extreme cases, can even result in the loss of vision. Since the visual system of infant rhesus monkeys and human infants are comparable in structure and functional development, information about pharmacological eye growth regulatory mechanisms obtained from the monkey model may be useful in developing therapeutic strategies for ocular growth abnormalities in humans.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: The Effect of Pirenzepine on Postnatal Axial Eye Elongation

AXIS I: 1a, 21, 25b

AXIS II: 60, 62

PRC UNIT: Neurobiology and Vision

INVES1: Tigges, Margarete
DEGREE1: Ph.D.
DEPT1: Neural Ultrastructure/Neurobiology and Vision
STAFF1: C

INVES2: Iuvone, P. Michael
DEGREE2: Ph.D.
DEPT2: Neurobiology and Vision
STAFF2: O

SPECIES1: Macaca mulatta
NUM1: 7

NON-HOST INST: NA

ABSTRACT: We are examining whether pirenzepine, an M1-selective muscarinic antagonist, can prevent deprivation-induced myopia in newborn rhesus monkeys, as it does in chicks. Seven newborn rhesus monkeys were raised with an occluder lens in the right eye; they received 1 drop of 5% pirenzepine twice a day in the occluded eye and vehicle solution in the unmanipulated fellow eye. Six monkeys from another project served as controls. In 4 of the pirenzepine-treated monkeys, both eyes elongated similarly while in 3 monkeys the occluded eyes elongated excessively. At the end of the experiment, we will examine ocular tissues, including retina, choroid, iris, sclera, lens and vitreous, for possible alterations in a variety of neurotransmitter/neuromodulator substances, muscarinic receptor subtypes, and levels of IGF-1 and βFGF, in comparison with the same ocular tissues from the controls.

Pharmaca that interact with specific ocular substances to prevent deprivation-induced excessive eye elongation may be relevant clinically. In humans, excessive axial eye elongation also causes myopia, a common vision disorder affecting a large percentage of the US population. In extreme cases, myopia can even result in the loss of vision. The etiology of myopia is still not well understood. The visual system of infant rhesus monkeys and human infants are comparable in structure and functional development. Therefore, information about eye growth regulatory mechanisms obtained from the monkey model may be useful in developing therapeutic strategies for ocular growth abnormalities in humans.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Circuitry of the Dorsal Lateral Geniculate Nucleus in the Cat and Monkey

AXIS I: 1a, 21, 25b

AXIS II: 92, Neuroscience

PRC UNIT: Neurobiology and Vision

INVES1: Wilson, James R.
DEGREE1: Ph.D.
DEPT1: Neurophysiology/Neurobiology and Vision
STAFF1: C

SPECIES1: General monkeys and domestic cats
NUM1: 0

NON-HOST INST: NA

ABSTRACT: This is a review of the last 20 years on anatomical studies carried out in primate and cat LGNs. The dorsal lateral geniculate nucleus (dLGN) is a prominent thalamic region for processing visual information. It has the major research advantages of clear anatomical separation from other thalamic nuclei with direct, separate, and easily manipulated inputs from each eye, plus a wealth of anatomical and physiological data on it. Approximately 2500 studies concerning the dLGN have been published since 1966. Following the 1971 review by Guillery, pathway tracing using horseradish peroxidase (HRP), immunohistochemical staining techniques, and other markers have greatly expanded our knowledge of the inputs to the dLGN and ability to selectively label neurons and their processes. The present review provides an update on efforts to analyze the circuitry of the cat's and monkey's dLGN beyond that of Guillery, 1971. New data on the neurons and circuitry of the visual part of the reticular nucleus of the thalamus (RNT) also is covered because of the clear relationship and importance to the dLGN. No attempt has been made to be exhaustive, and those areas already covered by Guillery's review will not be referenced, i.e., those prior to 1971. As prerequisites to this review, the general features of the dLGNs of cats and primates can be found in reports by Guillery (1970) and Kaas et al. (1978). Other reviews that have emphasized different species, physiology, development, or the effects of abnormal visual inputs should be consulted for those aspects of the dLGN.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Morphological/Physiological Relationships in Primate LGN

AXIS I: 1a, 21, 25b

AXIS II: 92, Neuroscience

PRC UNIT: Neurobiology and Vision

INVES1: Wilson, James R.
DEGREE1: Ph.D.
DEPT1: Neurophysiology/Neurobiology and Vision
STAFF1: C

SPECIES1: Saimiri sciureus
NUM1: 10

NON-HOST INST: NA

ABSTRACT: This is a continuation of a 4-year grant from NIH/NEI to study the anatomy of the squirrel monkey's lateral geniculate nucleus using electron microscopic, immunohistochemical, and behavioral methods. The research in 1993 concerned the evaluation and final analyses of deprived and non-deprived neurons in the LGN. Intracellular injections of horseradish peroxidase were used to label cells in the dLGNs of normal and monocularly lid-sutured squirrel monkeys. Their synaptic inputs were then examined with the electron microscope. Four deprived and four normal cells from the parvocellular laminae were studied. The total dendritic lengths were not significantly different between the two groups although there was a tendency for those of the deprived cells to be shorter (normal avg. = 5500 μm versus deprived avg. = 3900 μm). The distribution of types, numbers, or densities of synapses on the dendrites was also not different between the two groups. However, a much greater number of synapses was found on the somata of the deprived cells compared with those of the normal cells (dep. avg. = 24 vs. normal avg. = 6). Nearly all of the synapses made onto the normal cells were of the F type whereas all types contacted the deprived somata. These results indicate that the synaptic inputs to the dendrites of monocularly deprived dLGN neurons is normal, but there are abnormal inputs to the somata. This abnormal synaptic pathway could cause improper visual signals to be sent to the cortex.

with similar, although less extensive, brainstem lesions in the rhesus infant. Both infants were euthanatized because of the potential risk to personnel. Although Herpesvirus simiae infection is prevalent in adult macaques, it usually does not cause significant lesions and has not previously been reported in pig-tailed macaques or in newborn or neonatal macaques. Because of the serious consequence of the infection in man, the finding of infection in neonates or in infant macaques underscores the importance of considering all macaques, including infants, as potentially infected and stresses the need to handle all nonhuman primates with caution.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Streptococcus zooepidemicus Infection in Rhesus Monkeys

AXIS I: 1a, 7b

AXIS II: 66

PRC UNIT: Pathobiology & Immun

INVES1: Anderson, Daniel C.
DEGREE1: D.V.M.
DEPT1: Pathobiology and Immunobiology
STAFF1: C

INVES2: Klumpp, Sherry A.
DEGREE2: D.V.M., M.S.
DEPT2: Pathobiology and Immunobiology
STAFF2: O

INVES3: McClure, Harold M.
DEGREE3: D.V.M.
DEPT3: Pathobiology and Immunobiology
STAFF3: C

INVES4: Paul, Katherine S.
DEGREE4: D.V.M.
DEPT4: Pathobiology and Immunobiology; and
Animal Resources and Veterinary Medicine
STAFF4: O

SPECIES1: Macaca mulatta
NUM1: 11

NON-HOST INST: NA

ABSTRACT: Between October 27 and December 12, 1993, a fulminate, rapidly fatal outbreak of Streptococcus zooepidemicus infection resulted in the death of 11 rhesus macaques over a 46 day period with most of the deaths occurring in the first 10 days after the initial infection was noted. These animals were part of an outdoor housed colony of 119 animals. The first case had extensive, multiple wounds over the body and death was initially considered to be due to trauma associated with fight wounds although an acute peritonitis which was characterized by a few thin fibrinous tags over the spleen and liver was also found at necropsy. Subsequent deaths occurred very acutely with the animals, which were often noted to be active and apparently healthy one day, found dead the next morning. Clinical signs were usually limited to short-term respiratory distress and nasal hemorrhage at or near the time of death. Most of the animals appeared to be in an advanced state of autolysis and gross lesions were nonspecific; lesions were generally limited to reddish discoloration of the skin and mild pulmonary edema with occasional epistaxis

and hematuria. Histologic lesions were similar in virtually all animals and consisted predominantly of acute pharyngitis/tonsillitis and multifocal necrotizing hepatitis with gram positive cocci easily found in vessels throughout the body. A mild acute suppurative meningoencephalitis was observed in several animals; a single animal with severe CNS lesions lacked evidence of hepatitis suggesting that she survived an initial septicemic episode. A Lancefield group C streptococcus, Streptococcus zooepidemicus was isolated from multiple tissues from all animals. The epizootic was controlled by treatment of the entire colony with antibiotics. Lancefield group C streptococcal infections are well known in laboratory animals, particularly in guinea pigs, and have been isolated from many domestic animals. Such infections, particularly those caused by S. zooepidemicus, are considered to be rare in humans. These cases are only the second report of S. zooepidemicus infection in nonhuman primates and resemble a toxic shock-like syndrome associated with severe group A streptococcal infections in man. The first report of S. zooepidemicus infection in nonhuman primates occurred in a zoo setting in animals fed horse meat. The source of infection in the animals in this outbreak has not been determined. However, the available and prompt clinical pathology and necropsy evaluation of these cases greatly assisted the clinical veterinarians in developing a therapeutic regimen for this naturally occurring disease outbreak. The prompt pathology work-up and prompt and vigorous treatment of animals in this colony by the clinical veterinarians greatly curtailed the morbidity and mortality associated with this spontaneously occurring disease problem.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: CD8+ T-cell Regulation of SIV Replication

AXIS I: 1a, 1d, 7b, 19

AXIS II: 31, 64, 66

PRC UNIT: Pathobiology & Immun

INVES1: Ansari, Aftab A.
DEGREE1: Ph.D.
DEPT1: Pathobiology and Immunobiology
STAFF1: C

INVES2: Knuchel, M.
DEGREE2: D.V.M.
DEPT2: Pathobiology and Immunobiology
STAFF2: O

INVES3: Villinger, Francois
DEGREE3: D.V.M., Ph.D.
DEPT3: Pathobiology and Immunobiology
STAFF3: O

SPECIES1: Cercocebus atys
NUM1: 6

NON-HOST INST: NA

ABSTRACT: Our laboratory has previously shown that CD8+ T cells from SIV-infected rhesus macaques and sooty mangabeys have potent regulatory function on the replication of SIV *in vitro*. Of interest is the observation that this regulatory population of CD8+ T cells is gradually lost post-SIV infection in rhesus macaque but not in sooty mangabeys. This loss of CD8+ T-cell function is not secondary to a depletion of CD8+ T cells but due to a functional loss of the CD8+ T cells which occurs prior to SIV-induced disease. We hypothesize that such a loss of CD8+ T-cell function may be one mechanism for the progression of SIV-induced disease. It is thus reasoned that the mechanism by which such CD8+ T cells mediate their regulatory role on SIV replication is important and hence the rationale for the present studies. Our laboratory has chosen to study in depth the molecular mechanisms that are involved in such CD8+ T-cell function, using the sooty mangabey as a model.

First of all, we demonstrated that CD8+ T cells from naturally infected disease-resistant sooty mangabeys (*Cercocebus atys*) secrete a soluble factor which inhibits the *in vitro* replication of the simian immunodeficiency virus (SIV). To gain further insight on the mechanism(s) involved, CD8+ effector T cells as well as target cells from sooty mangabeys were immortalized and cloned. The target cells were then stably transfected with an SIV-LTR-CAT

construct or with the parental CAT plasmid as a control. A quantitative RT-PCR method, providing the necessary sensitivity, was developed to monitor the influence of the cloned CD8+ T cells on the CATmRNA contained in the target cells. It could be demonstrated that a soluble factor was secreted by the cloned CD8+ T cells from sooty mangabeys, which appeared to regulate CATmRNA activity in a dose-dependent and reversible manner. Kinetic experiments showed that the CATmRNA transcriptional activity was initially augmented at 30 minutes post coculture and was followed by a marked decrease in transcriptional activity after a few hours. The immediate early response could be mitigated utilizing H7, Calmodulin, or PDTC, suggesting that the pathway was protein kinase-dependent and that the NF-κB site may be involved. The inhibitory effect could also be overcome using a protein synthesis inhibitor, suggesting that protein synthesis was needed to negatively regulate CATmRNA activity and hence SIV promoter activity.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Chronic Immunologic Activation and SIV-Induced Disease

AXIS I: 1d, 2, 3, 7a, 7b, 19

AXIS II: 31, 64, 66, 77

PRC UNIT: Pathobiology & Immun

INVES1: Ansari, Aftab A.
DEGREE1: Ph.D.
DEPT1: Pathobiology and Immunobiology
STAFF1: C

INVES2: McClure, Harold M.
DEGREE2: D.V.M.
DEPT2: Pathobiology and Immunobiology
STAFF2: C

INVES3: Villinger, Francois
DEGREE3: D.V.M.
DEPT3: Pathobiology and Immunobiology
STAFF3: O

INVES4: Folks, Thomas M.
DEGREE4: Ph.D.
DEPT4: Pathobiology and Immunobiology
STAFF4: O

SPECIES1: Macaca mulatta
NUM1: 18

NON-HOST INST: Centers for Disease Control and Prevention (TMF)

ABSTRACT: Three groups of 3 rhesus macaques were inoculated with an infectious dose of SIVmac251 sufficient to induce infection, seroconversion and disease. Animals of group a) received SIV + keyhole limpet hemocyanin (KLH) and tetanus toxoid, allogeneic cells from a sibling animal, or KLH at monthly intervals thereafter . Animals of group b) were stimulated with allogeneic PBMC from a sibling at monthly intervals starting from the SIV inoculum date and group d) received SIV only. Three animals (group c) were injected with the same regimen as group a) but without SIV inoculation.

All animals from group a) and 2 of 3 animals of group b) died 4 to 7 months pi, the third animal of group b) is currently showing signs of disease, 12 months pi. Only 1 out of the 3 macaques that received SIV without further immune activation (group d) died at 5 months pi, the other 2 survived past 12 months pi. All monkeys that died within 5 months pi showed low or no seroconversion for SIV, despite an initial rise in titer against KLH (group a). The animals of the stimulated but non infected group remained healthy during the same period.

These results strongly suggest that chronic stimulation of the host's immune system is upregulating the kinetics of the SIV induced disease and immune deficiency.

Pending experiments include the addition of 8 rhesus macaques to the study. These animals will be inoculated with SIVsmm. Following confirmed seroconversion, 4 of them will be hyperactivated at monthly intervals as per above with Freund's incomplete adjuvant, soluble antigens, and allogeneic lymphocytes. These monkeys will be monitored for CD4/CD8 cell numbers and ratios and clinical disease. Similar studies will also be done in SIV seropositive, asymptomatic mangabeys to determine if immune activation results in the development of disease in this species.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Development of an Assay to Quantitate CATmRNA

AXIS I: 1a, 1d, 7b

AXIS II: 31, 66, 39

PRC UNIT: Pathobiology & Immun

INVES1: Ansari, Aftab A.
DEGREE1: Ph.D.
DEPT1: Pathobiology and Immunobiology
STAFF1: C

INVES2: Knuchel, M.
DEGREE2: D.V.M.
DEPT2: Pathobiology and Immunobiology
STAFF2: O

INVES3: Villinger, Francois
DEGREE3: D.V.M., Ph.D.
DEPT3: Pathobiology and Immunobiology
STAFF3: O

INVES4: Bednarik, D.P.
DEGREE4: Ph.D.
DEPT4:
STAFF4: O

INVES5: Folks, Thomas M.
DEGREE5: Ph.D.
DEPT5: Pathobiology and Immunobiology
STAFF5: O

SPECIES1: Cercocebus atys
NUM1: 4

NON-HOST INST: Centers for Disease Control and Prevention (DPB, TMF)

ABSTRACT: Our laboratory has been involved in defining molecular mechanisms of the regulation of SIV replication in target cells mediated by soluble factor(s) secreted by CD8$^+$ T cells from SIV-infected sooty mangabeys. We have utilized a transient transfection assay of an LTR-CAT construct whereby CAT activity serves as a readout.

Such plasmid constructs which contain the CAT reporter gene have been utilized extensively for a variety of *in vitro* studies designed to elucidate intracellular pathways and mechanisms of gene regulation. These constructs have been especially useful in identifying sequences in the long terminal repeat (LTR) region of lentiviruses that play a role in modulating viral

transcription and in identifying nuclear factors that bind to these sequences. The conventional strategy has been to transiently transfect host cells with an appropriate plasmid containing the sequence of interest spliced with the CAT reporter gene. Subsequently, CAT activity is measured as a readout by thin layer chromatography (TLC), or the levels of CAT protein are determined using an ELISA. With most CAT construct transfected cells, only low constitutive CAT levels are observed, making these cells ideal for studies of parameters that upregulate gene expression. However, studies that are focused on defining negative regulatory elements using these plasmid constructs suffer from this low basal CAT readout. Alternative strategies to overcome this problem have been utilized and include the use of cell activation agents such as PMA, PHA, IL-2, and TNFα, or cotransfection with a second enhancing plasmid (e.g., in the case of HIV-1 LTR, the enhancer plasmid expressing *tat*), leading to the synthesis of elements that will upregulate transcription of the CAT gene. However, use of such signal enhancers renders the evaluation of molecular mechanisms difficult, since most of these agents have pleiotropic effects, some of which may interfere with the pathway(s) being studied and therefore provide erroneous results. In addition, current assays to quantitate CAT rely on the appearance of the end product and therefore not only reflect transcriptional regulation but depend also on eventual translational modulation events.

The above thoughts prompted our laboratory to develop a more sensitive quantitative RT-PCR assay which allows for direct monitoring of CAT gene expression at the transcriptional stage by measuring levels of CAT mRMA. This quantitative assay, which does not require limiting dilution analysis of the samples or use of competetive PCR templates, is less labor-intensive and diminishes the risk of sample contamination. The RT-PCR assay is relatively easy to perform, highly sensitive, and reproducible.

The ability of this assay to detect CAT mRNA but not CAT DNA demonstrates its specificity and is achieved using a tailed oligoprimer for the reverse transcription step. This assay is able to measure the equivalent of as few as eight copies of CAT mRNA, is reproducible, and relatively easy to perform. The quantitative capability of the assay relies on a constant production of CAT mRNA, which is achieved using permanently transfected and cloned cell lines bearing a defined number of CAT DNA copies per cell. This assay provides a tool to monitor events at the transcriptional level and thereby complements the currently utilized CAT ELISA and thin-layer chromatography assays.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Subset Analysis of PBMC in Macaques and Mangabeys

AXIS I: 1a, 1d, 7b, 19

AXIS II: 31, 64, 66

PRC UNIT: Pathobiology & Immun

INVES1: Ansari, Aftab A.
DEGREE1: Ph.D.
DEPT1: Pathobiology and Immunobiology
STAFF1: C

INVES2: Villinger, Francois
DEGREE2: D.V.M.
DEPT2: Pathobiology and Immunobiology
STAFF2: O

INVES3: Folks, Thomas M.
DEGREE3: Ph.D.
DEPT3: Pathobiology and Immunobiology
STAFF3: O

SPECIES1: Macaca mulatta
NUM1: 20

SPECIES 2: Cercocebus atys
NUM2: 20

NON-HOST INST: Centers for Disease Control and Prevention (TMF)

ABSTRACT: Analysis of cytokines that are secreted by individual cloned T-cell lines from mice and humans has led to the discovery that such clones can be distinguished into at least three major subsets termed TH0, TH1, and TH2. The TH0 clones are the precursors for TH1 and TH2 subsets. Evidence from various laboratories indicates that (a) TH1-type clones predominantly secrete IL-2, IFN-γ, and TNF-β; (b) TH2 clones predominantly secrete IL-4, IL-5, IL-6, and IL-10; and (c) TH0 appears to synthesize cytokines with overlapping TH1 and TH2 profiles. Several important observations have been made since the discovery of such subsets. First of all, it appears clear that the Th1 type of subset is involved in pro-inflammatory cellular responses such as those involved in delayed-type hypersensitivity (DTH) reactions, whereas the Th2 subset appears to be involved in the regulation of humoral immune response. Of importance is the observation that the cytokines secreted by TH1 inhibit TH2 cellular responses and vice versa. Such cross-regulation is clearly important in health and disease because a productive TH1 versus TH2 response could dictate disease outcome. The predominant TH1 versus TH2 response has already been shown to occur in a number of infectious diseases, such as

schistosomiasis, filariasis, and tuberculoid versus lepromatous leprosy. Of relevance to HIV infection, there now appears clear evidence that a shift from TH1 to TH2 type of T cells characterizes the development of progressive disease. These observations prompted our laboratory to establish techniques for identifying similar subsets in nonhuman primate models of HIV infection. The goal of our study was to determine whether such a shift from TH1 to TH2 accompanies experimental SIV infection in rhesus macaques, which progressively develop disease, and to determine the type of T-cell response in sooty mangabeys, which are naturally infected with SIV but to a large extent appear to remain asymptomatic. Cloned T-cell lines were established from these two species. Each cloned T-cell line was phenotyped and, using RT-PCR assays, was analyzed for a number of cytokines. Results, to date, indicate that normal uninfected rhesus macaques have a predominant frequency of T cells that show a TH1 profile. In contrast, sooty mangabeys appear to demonstrate T cells in their PBMC which are predominantly of the TH2 type, based on cytokine profile. The data, first of all, demonstrate that nonhuman primates, similar to humans, have T cells that can be readily distinguished into TH0, TH1, and TH2 subsets based on cytokine analysis. Second, of importance is our finding that normal rhesus macaques have a dominant TH1 profile, whereas sooty mangabeys have a dominant TH2 profile. In addition, such subsets exist not only for $CD4^+$ T cells but also for $CD8^+$ T cells.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Perflubron as a Blood Pool Contrast Agent for CT Angiography

AXIS I: 1a, 9, 16d, 19

AXIS II: 63a

PRC UNIT: Pathobiology & Immun

INVES1: Bernardino, Michael E.
DEGREE1: M.D.
DEPT1: Pathobiology and Immunobiology
STAFF1: O

INVES2: Swenson, R. Brent
DEGREE2: D.V.M.
DEPT2: Animal Resources
STAFF2: C

SPECIES1: Macaca mulatta
NUM1: 5

NON-HOST INST: NA

ABSTRACT: Perflubron (perfluorooctyl bromide [PFOB]) emulsion has been used in the past for liver and spleen imaging due to blood pool and RES uptake. As a blood pool agent it may also have uses as a vascular contrast agent for CT angiography. We studied the ease of vessel visibility (celiac, SMA, renal arteries) in rhesus monkeys after the intravenous administration of a low dose of perflubron emulsion using spiral CT and 3D reconstructions.

Five rhesus monkeys were examined under general anesthesia. Perflubron emulsion, 90% w/v PFOB (Imagent® BP, Alliance Pharmaceutical Corp., San Diego, CA) was administered intravenously at a dose of 1.5 ml/kg at a rate of 30 ml/min. Spiral CT with 1 mm thick slice reconstructions (pitch = 1) were performed immediately after injection and repeated five hours after injection. Three dimensional reconstructions of the aorta at the level of the celiac, SMA and renal arteries were performed. The 3D images were then blindly rated 0-4 (not seen - excellent) by two observers and averaged. All of the vessels had the best ratings immediately after injection: celiac 2.8, SMA 2.7, left renal 2.1, right renal 1.9. The five hour delay ratings were: celiac 1.3, SMA 1.5, left renal 1.5 and right renal 1.2.

This study demonstrated that immediately after injection the larger vessels were better visualized with the 3D angiograms but all vessels were seen. The ratings at 5 hours were lower due to continued contrast agent clearance with time. This study with low-dose perflubron emulsion as a vascular blood pool contrast agent for CT angiography is encouraging. Further work to optimize time and dose may make contrast assisted CT angiography a feasible alternative to conventional angiography.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Perflubron Emulsion as a CT Contrast Agent: Enhancement of Liver, Spleen and Great Vessels Following Rapid Intravenous Infusion

AXIS I: 1a, 9, 16d, 19

AXIS II: 63a

PRC UNIT: Pathobiology & Immun

INVES1: Bernardino, Michael E.
DEGREE1: M.D.
DEPT1: Pathobiology and Immunobiology
STAFF1: O

INVES2: Swenson, R. Brent
DEGREE2: D.V.M.
DEPT2: Animal Resources
STAFF2: C

SPECIES1: Macaca mulatta
NUM1: 5

NON-HOST INST: NA

ABSTRACT: Prior studies of perflubron (perfluorooctylbromide [PFOB]) have concentrated on enhancement at two points - immediately and 24-48 hrs after contrast administration. The window for clinically useful hepatosplenic imaging following a contrast bolus has not been established. We studied hepatosplenic enhancement in rhesus monkeys during five hours after rapid administration of a reformulated version of perflubron emulsion in an attempt to determine a clinically useful imaging window.

Five rhesus monkeys were examined under general anesthesia. Perflubron emulsion, 90% w/v (Imagent® BP, Alliance Pharmaceutical Corp., San Diego, CA) was administered IV at a dose of 1.5 ml/kg and rate of 30 ml/min. Helical CT examination of the abdomen was obtained prior to the contrast bolus; and 5-10 min, 30 min, 1, 2, 3, 4 and 5 hrs post contrast. Mean density of liver, spleen, and aorta was measured at each time interval.

Aorta density peaked after 5-10 min at 53±6HU (148%) above baseline and decreased to 45±2HU (124%) by 5 hr. Liver density also peaked after 5-10 min at 19±4 HU (37%) above baseline and was 22±6HU (43%) greater than precontrast level after 5 hr. Spleen density was 35±8HU (89%) greater at 5-10 min and increased in density for four hours to a peak of 113±20 HU (294%) above baseline.

This study demonstrated that the liver enhances rapidly to a level which then remains relatively unchanged over five hours. Blood pool enhancement peaks immediately with a small decrease over time. This should allow

differentiation of small hepatic tumors from hepatic veins for at least 5 hours post-contrast. Splenic enhancement may be exaggerated by anesthetic-induced splenic sequestration but appears to increase gradually to a peak at 4 hours. This combination of characteristics suggests a wider window for hepatosplenic imaging with perflubron than currently available with iodinated contrast agents.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Schering Iopromide-Carrying Liposomes CT Contrast Agent: Optimum Length of the Imaging Window

AXIS I: 1a, 9, 16d, 19

AXIS II: 63a

PRC UNIT: Pathobiology & Immun

INVES1: Bernardino, Michael E.
DEGREE1: M.D.
DEPT1: Pathobiology and Immunobiology
STAFF1: O

INVES2: Swenson, R. Brent
DEGREE2: D.V.M.
DEPT2: Animal Resources
STAFF2: C

SPECIES1: Macaca mulatta
NUM1: 5

NON-HOST INST: NA

ABSTRACT: Since non-contrast, contrast-enhanced, and angiographically assisted computed tomography do not detect all the lesions within a liver, there is a need to develop a hepatic specific CT contrast agent. Recently, a liposomal agent with iopromide as its iodinated contrast has been developed. The purpose of this study was to evaluate hepatic enhancement in primates (pre-clinical evaluation) using these iopromide-carrying liposomes.

Four male rhesus monkeys received iopromide-carrying liposomes (Schering AG, Berlin, Germany) at a dose of 200 mg of iodine per kg of body weight. The dose was administered at 100 mg iodine/minute/kg. The monkeys were examined on a GE "High Speed Spiral" CT scanner (Milwaukee, WI). All studies were done in the spiral mode. Five mm continuous columation was used. The entire liver of the monkey was examined prior to and at 5, 10, 15, 30 and 45 minutes, and 1, 2, 3, 4 and 5 hours post intravenous administration of the contrast agent. Three user defined regions of interest (excluding vessels) were obtained per hepatic slice. ROI's were obtained from three separate adjacent slices. Thus, nine hepatic ROI's per animal were evaluated at each time point and a mean Hu density obtained per time point. Percentage of enhancement was calculated: (liver density post-enhancement minus liver density pre-enhancement)/(liver density pre-enhancement) for each time point.

The spleen was evaluated in four of the animals. Splenic enhancement was seen in all spleens after 30 minutes and percentage of enhancement was calculated in two of the four animals. Splenic data was obtained by taking three ROI's of a single splenic mid organ slice. These splenic ROI's were then averaged.

Percentage enhancement of the spleen was calculated in the same fashion as the liver.

This study demonstrated that iopromide-carrying liposomes enhanced the liver and spleen in rhesus monkeys. Hepatic enhancement was not as significant as has been previously noted in lower phylogenetic animals. The amount of hepatic enhancement may or may not be sufficient for focal liver lesion detection and further evaluation of humans would be necessary to make this determination. There was significant splenic enhancement. Both hepatic and splenic enhancement were noted within five minutes after the injection. The contrast enhancement lasted for a prolonged period of time indicating that this agent would have a long window of opportunity and thus could represent a significant improvement over dynamic sequential bolus CT.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Induction of Plasmodium Infections to Support Malaria Vaccine Studies

AXIS I: 1a, 3, 4, 7c, 17

AXIS II: 64, 66

PRC UNIT: Pathobiology & Immun

INVES1: Collins, William E.
DEGREE1: Ph.D.
DEPT1: Pathobiology and Immunobiology
STAFF1: O

INVES2: McClure, Harold M.
DEGREE2: D.V.M.
DEPT2: Pathobiology and Immunobiology
STAFF2: C

SPECIES1: Pan troglodytes
NUM1: 9

NON-HOST INST: Centers for Disease Control and Prevention (WEC)

ABSTRACT: Short-term malaria infections were induced in chimpanzees to obtain blood-stage parasites for (1) preparation of genomic libraries, (2) extraction of m-RNA for genetic engineering studies, (3) antigen for serologic tests, (4) infection of mosquitoes through membrane feeding to produce sporozoites for (a) genetic engineering studies, (b) production of monoclonal antibodies, and (c) to infect Aotus and Saimiri monkeys and to test the efficacy of experimental vaccines, and (5) production of immune sera. The following parasites and animals were inoculated during the past year: Plasmodium ovale - animals C-0516, C-400, C-0475, and C-505; Plasmodium vivax - animals C-0516, C-0475, and C-505; Plasmodium malariae - animal C-0C6. These studies will continue in support of the development of vaccines for human malarias.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Primates as Hosts for Onchocerca volvulus

AXIS I: 1a, 7c, 14,17

AXIS II: 64, 66, 77, 91

PRC UNIT: Pathobiology & Immun

INVES1: Eberhard, Mark L.
DEGREE1: Ph.D.
DEPT1: Pathobiology and Immunobiology
STAFF1: O

SPECIES1: Pan troglodytes
NUM1: 6

SPECIES2: Cercocebus atys
NUM2: 8

NON-HOST INST: Centers for Disease Control and Prevention

ABSTRACT: The purpose of this project was to characterize the primate model for the study of onchocerciasis, a blinding disease of people in Africa and Central America. Chimpanzees, and to a much lesser extent mangabey monkeys, continue to be the only experimental hosts available for study, and the responses seen in these primates mimic closely that seen in man. We demonstrated that smaller inocula of L3 were sufficient to initiate infection as were larger inocula, and that the immunological and parasitological outcome will be the same. These studies in experimental primates, have demonstrated that there are antibody responses to several native and recombinant antigens which appear early in infection and can be used as diagnostic assays in human infections in epidemiologic studies. The standardization of survival, growth, and molting of O. volvulus larvae in implantable diffusion chambers has permitted us to begin vaccine trials. We were not able, despite extensive evaluation using ultrasound and MRI techniques, to document the location or occurrence of nodules in infected animals. This will preclude the evaluation of either the worms or nodules following chemotherapy or vaccine trials. It does raise the question of whether nodules always form around adult Onchocerca worms and whether even in people, there is a population of worms that are not encapsulated in nodules. The implications of this in field studies are not fully understood at this time. However, the characterization of the immunologic and parasitologic responses in nonhuman primates provides a much clearer picture for our understanding of human infections in people residing in endemic areas.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Succession of Putative Peri-implant Pathogens at Implant Sites After Periodic Scaling

AXIS I: 1a, 2, 3, 7, 22

AXIS II: 48, 52, 63, 77, 86

PRC UNIT: Pathobiology & Immun

INVES1: Eke, Paul I.
DEGREE1: Ph.D.
DEPT1: Pathobiology and Immunobiology
STAFF1: 0

INVES2: Braswell, Laura D.
DEGREE2: D.D.S.
DEPT2: Pathobiology and Immunobiology
STAFF2: 0

SPECIES1: Macaca mulatta
NUM1: 40

NON-HOST INST: NA

ABSTRACT: Levels of putative peri-implant pathogens are elevated in partially edentulous mouths early after implant placement and prosthetic reconstruction. The purpose of this study was to ascertain the effects of monthly periodic scaling on levels of putative peri-implant pathogens after dental implantation in partially edentulous monkey mouths. In nine monkeys, 8 teeth sites and ten implant sites were monitored microbiologically by selective and non-selective culture, darkfield microscopy and primary phenotype characterization of culture isolates. Each site was studied over nine months. At implant sites, there were no significant changes in levels of the capnophilic flora, A.a., Haemophilus sp. and Campylobacter sp., while significant decreases towards pre-implant levels were detected for spirochetes, F. nucleatum, F. corrodens and P. intermedia. At teeth sites, a significant drop in capnophilic flora, A. a, F. nucleatum, F. corrodens and Campylobacter sp. was detected after nine months. In contrast, levels of P. intermedia at teeth sites increased over the period. No significant differences in levels of any microbial groups were detected between implant and tooth sites in the same mouth. Levels of Porphyromonas sp. and spirochetes correlated positively with gingival inflammation. Levels of P. intermedia, Porphyromonas sp. and spirochetes correlated positively with probing depth. This study demonstrates that monthly scaling routine is effective in reducing levels of putative peri-implant pathogens at implant sites.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Engraftment of Human Stem Cells into Fetal Macaques

AXIS I: 1a, 1d, 4, 17

AXIS II: 60, 88

PRC UNIT: Pathobiology & Immun

INVES1: Fleming, William H.
DEGREE1: M.D., Ph.D.
DEPT1: Pathobiology and Immunobiology
STAFF1: O

INVES2: Baum, Charles M.
DEGREE2: M.D., Ph.D.
DEPT2: Pathobiology and Immunobiology
STAFF2: O

INVES3: Swenson, R. Brent
DEGREE3: D.V.M.
DEPT3: Animal Resources
STAFF3: C

SPECIES1: Macaca nemestrina
NUM1: 5

NON-HOST INST: NA

ABSTRACT: The growth of human hematopoietic stem cells (HSCs) in vitro does not result in the regulated production of normal blood cells or functional immune cells . As a result, progress in the isolation and purification of human HSCs has been impeded by the absence of a model system with which to study the development of HSCs. The developing fetus is known to be immunologically naive with a well documented tolerance to alloantigens. The phylogenetic similarity of macaques to man would be expected to produce a hematopoietic microenviroment favorable to human HSC development. This pilot study was undertaken in order to evaluate the potential of fetal macaques as a model system to study the developmental potential of human HSCs. A total of 5 early to mid-gestation fetal macaques were injected intraperitoneally with human CD34+ bone marrow cells. Two of these animals died in utero, one due to a Listeria infection (a common cause of spontaneous abortion in this species), and another due to indeterminate causes. All 3 remaining fetuses were carried to term and appeared healthy at the time of delivery. The bone marrow and peripheral blood from the newborn animals was tested for the presence of human cells by flow cytometry, however no human cells were detected. The small sample size, the number of CD34+ human HSCs injected and the gestational age at the time of injection are variables which need to be further evaluated to determine the usefulness of this model system. Model systems for studying the treatment of human genetic and infectious diseases may evolve from this experimental approach if the sustained engraftment of high numbers of circulating human cells can be accomplished.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: SIV_{MU} Pathogenicity Studies

AXIS I: 1a, 1d, 7d

AXIS II: 31, 66, 91

PRC UNIT: Pathobiology and Immun

INVES1: Folks, Thomas M.
DEGREE1: Ph.D.
DEPT1: Pathobiology and Immunobiology
STAFF1: O

INVES2: Hart, Clyde
DEGREE2: Ph.D.
DEPT2:
STAFF2: O

INVES3: Heneine, Walid
DEGREE3: Ph.D.
DEPT3:
STAFF3: O

INVES4: Switzer, William
DEGREE4: M.P.H.
DEPT4:
STAFF4: O

INVES5: Novembre, Francis J.
DEGREE5: Ph.D.
DEPT5: Pathobiology and Immunobiology
STAFF5: C

INVES6: McClure, Harold M.
DEGREE6: D.V.M.
DEPT6: Pathobiology and Immunobiology
STAFF6: C

SPECIES1: Macaca mulatta
NUM1: 7

NON-HOST INST: Centers for Disease Control and Prevention (TMF, CH, WH, WS)

ABSTRACT: Serologic evidence of occupational exposure to simian immunodeficiency yirus was discovered in a research laboratory worker. Blood from the worker was inoculated into a rhesus macaque in an effort to isolate the virus or observe seroconversion due to infection. In addition, the worker's PBLs were cultured and periodically tested for RT activity and SIV p27 antigen. The rhesus macaque did not seroconvert to SIV, however, a virus, termed SIV_{MU} was ultimately isolated from the worker's PBL cultures.

This isolate was determined to have a truncated *nef* gene, which may alter its pathogenicity. The isolate provides a unique opportunity to answer a question that is critical to the development of an HIV vaccine: can the simian virus isolated from a human revert to a pathologic strain? To answer this question, we inoculated 3 rhesus macaques with the SIV_{HU} isolate and 3 rhesus macaques with SIV_{B670}. An additional 3 rhesus macaques will be inoculated with an infectious molecular clone of SIV_{HU}, contingent on its development by investigators at CDC and Yerkes. $SIVB_{670}$ is the parental virus strain of SIV_{HU}, and is pathogenic to the monkeys. The pathogenicity of the SIV_{HU} isolate is unknown. It will be important to HIV vaccine studies to know if a subspecies of the SIV_{HU} that does not have the truncated *nef* will prevail evolutionarily in the monkeys. Observation of the *nef* genes from these viruses after *in vivo* replication will provide information that will be crucial to the development of a safe, effective HIV vaccine.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Implant, Prosthetic and Periodontal Studies in Monkeys

AXIS I: 1a, 2, 3, 6, 7, 22

AXIS II: 48, 52, 63, 77, 86

PRC UNIT: Pathobiology & Immun

INVES1: Fritz, Michael E.
DEGREE1: D.D.S., M.S., Ph.D.
DEPT1: Pathobiology and Immunobiology
STAFF1: O

INVES2: Braswell, Laura D.
DEGREE2: D.D.S.
DEPT2: Pathobiology and Immunobiology
STAFF2: O

INVES3: Eke, Paul I.
DEGREE3: Ph.D.
DEPT3: Pathobiology and Immunobiology
STAFF3: O

INVES4: Lemons, Jack E.
DEGREE4: Ph.D.
DEPT4:
STAFF4: O

SPECIES1: Macaca mulatta
NUM1: 36

NON-HOST INST: University of Alabama, Birmingham (JEL)

ABSTRACT: In 18 consecutively treated nonhuman primates (Macaca mulatta) (as part of a balanced block design study of 36 animals), osseointegration was examined in root form and plate-form implants prepared by atraumatic preparation of bone. Clinical measurements around selected teeth and digital radiology were utilized to monitor periodontal disease and bone deposition around the unloaded implants. Once monthly scaling procedures were utilized as a means of preventing further advance of periodontal disease. Results indicate that once monthly regime of scaling and root planing can prevent attachment loss of natural teeth and will not interfere with the healing of either type of implant; once monthly scalings produce significant reduction in redness ($P<.05$) and reduced probing depths ($P=<.01$). A second finding is that both root form and blade implants show radiographic evidence of osseointegration in this primate model. The quantitative analysis demonstrates bone gain not being stabilized until six months after healing. The data may indicate that occlusal loading of mandibular implants at three months may be premature.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: In Vivo Platelet Interactions with Adhesive Glycoproteins

AXIS I: 1a, 2, 3, 9, 13, 17

AXIS II: 48, 50b, 63f, 86

PRC UNIT: Pathobiology & Immun

INVES1: Hanson, Stephen R.
DEGREE1: Ph.D.
DEPT1: Pathobiology and Immunobiology
STAFF1: O

INVES2: Harker, Laurence A.
DEGREE2: M.D.
DEPT2: Pathobiology and Immunobiology
STAFF2: O

INVES3: Kelly, Andrew B.
DEGREE3: D.V.M.
DEPT3: Pathobiology and Immunobiology
STAFF3: C

SPECIES1: Papio cynocephalus
NUM1: 5

NON-HOST INST: NA

ABSTRACT: We have completed in baboons studies of an antithrombotic protein, designated VCL, which consists of amino acids 504-728 of native von Willebrand factor (vWF) plus an N-terminal methionine. The VCL protein blocks the interaction of circulating von Willebrand factor with platelet glycoprotein Ib. Using collagen coated tubular segments inserted for up to 3 hours into arteriovenous shunts under controlled flow conditions, the VCL fragment of vWF inhibited platelet thrombus formation only under conditions where fluid mechanical shear forces exceed those found in the normal vasculature, but which may be associated with stenotic vessel disease. The full dose-response relationship for intravenous VCL has been established, and indicates no significant inhibition of platelet-dependent thrombosis at doses < 1 mg/kg, and maximal inhibition at doses > 4 mg/kg, where the higher doses also produce a significant but transient bleeding tendency as assessed by the measurement of standard template bleeding times. Interestingly, the ineffective dose of VCL, when combined with an ineffective dose of oral aspirin (35 mg/kg) produces striking inhibition of shear-dependent thrombus formation, without significant bleeding. Since aspirin usage is routinely encountered in cardiovascular patients clinically, these results suggest that platelet inhibition with the combination of VCL/aspirin could represent a promising pharmacologic strategy for arterial thrombosis. That this approach may merit serious consideration is further suggested by recent findings that

small peptide inhibitors of the platelet glycoprotein IIb/IIIa receptor for fibrinogen, which also effectively inhibit thrombosis, may produce a serious bleeding tendency when combined with aspirin (which may often be unavoidably encountered clinically). In addition, targeting of other platelet receptors with monoclonal antibodies or synthetic peptides, such as the platelet thrombin receptor, has proven to be of only modest benefit for inhibition of thrombus formation in the baboon model. Hence, this latter approach is expected to produce little clinical benefit.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Evaluation of Small Vessel Prostheses

AXIS I: 2a, 2, 3, 9, 13, 17

AXIS II: 48, 50b, 63f, 86

PRC UNIT: Pathobiology and Immun

INVES1: Hanson, Stephen R.
DEGREE1: Ph.D.
DEPT1: Pathobiology and Immunobiology
STAFF1: O

INVES2: Harker, Laurence A.
DEGREE2: M.D.
DEPT2: Pathobiology and Immunobiology
STAFF2: O

INVES3: Kelly, Andrew B.
DEGREE3: D.V.M.
DEPT3: Pathobiology and Immunobiology
STAFF3: C

INVES4: Lumsden, Alan
DEGREE4: M.D.
DEPT4: Pathobiology and Immunobiology
STAFF4: O

SPECIES1: Papio cynocephalus
NUM1: 12

NON-HOST INST: NA

ABSTRACT: Small caliber vascular grafts used in arterial reconstruction frequently fail due to acute thrombotic occlusion or later occlusion secondary to stenotic tissue ingrowth (anastomotic intimal hyperplasia). This program has been directed towards understanding how graft characteristics of texture, porosity, and surface chemistry mediate graft healing phenomena. Since we previously showed that the healing of conventional expanded polytetrafluoroethylene (ePTFE) grafts was unaffected by coating the grafts with ultra-thin (glow discharge deposited) polymer layers which selectively modified the graft surface chemistry but not porosity, in a recent series of studies we chose to selectively modify graft texture while maintaining a uniform chemical composition. Expanded ePTFE (Gore-Tex) grafts (4 mm i.d. x 6 cm in length) were coated lumenally on one end with a smooth layer of silicone rubber polymer; the entire graft blood flow suface was then coated with an ultra-thin layer of plasma polymer based on hexafluoroethane (HFE). The grafts were then surgically placed in baboons as bilateral aorto-iliac

implants for periods of 1 and 3 months. Retrieval of the 1 month implants showed no pannus tissue migration or intimal hyperplasia at the anastomoses with the smooth end of grafts, while grafts treated with the HFE polymer only showed a normal hyperplastic response with a mean thickness of neointima within the graft averaging 0.2-0.3 mm. The 3 month implants are presently being evaluated morphometrically. These results challenge the commonly held view that vascular grafts must be porous to permit stable healing, and suggests a simple approach for limiting graft failure due to tissue infiltration, a problem which continues to plague the use of grafts for dialysis access and other applications.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Vascular Lesion Formation in Baboon Models

AXIS I: 1a, 2, 9, 13, 17

AXIS II: 48, 50b, 52, 63i, 77, 86

PRC UNIT: Pathobiology and Immun

INVES1: Hanson, Stephen R.
DEGREE1: Ph.D.
DEPT1: Pathobiology and Immunobiology
STAFF1: O

INVES2: Harker, Laurence A.
DEGREE2: M.D.
DEPT2: Pathobiology and Immunobiology
STAFF2: O

INVES3: Wilox, Josiah, N.
DEGREE3: Ph.D.
DEPT3: Pathobiology and Immunobiology
STAFF3: O

INVES4: Kelly, Andrew B.
DEGREE4: D.V.M.
DEPT4: Pathobiology and Immunobiology
STAFF4: C

INVES5: Lumsden, Alan
DEGREE5: M.D.
DEPT5: Pathobiology and Immunobiology
STAFF5: O

SPECIES1: Papio cynocephalus
NUM1: 12

NON-HOST INST: NA

ABSTRACT: Vascular cell proliferation after arterial injury, induced by clinical procedures including coronary balloon angioplasty and carotid endarterectomy, produces early restenotic vessel occlusion in a significant number of patients. Therefore, our studies in primates are designed to test the hypothesis that restenosis is mediated by factors associated with thrombus formation which occurs on the injured vessels, and especially the procoagulant enzyme thrombin, as well as by specific vessel-derived growth factors including basic fibroblast growth factor (bFGF) and platelet derived growth factor (PDGF). To facilitate these studies, a local infusion device has been developed which permits very high drug concentrations to be maintained at sites of vessel injury while minimizing circulating drug levels, overall drug

requirements, and systemic side effects. Using this approach, sites of carotid artery balloon angioplasty in 5 normal baboons were treated for 3 days with the potent antithrombin hirudin. As compared to untreated control (contralateral) arteries, hirudin therapy reduced intimal smooth muscle cell (SMC) from approximately 20% to 4% as determined by staining for cyclin/proliferating cell nuclear antigen. These studies strongly imply an important role for thrombin/thrombosis in the restenotic process. Conversely, local infusion of bFGF increased early SMC proliferation to nearly 100%. In preliminary studies we have also infused periodate-oxidized heparin into baboons continuously for 30 days. This material retains ~20% of the anticoagulant activity of standard heparin and produced a 3-4 fold prolongation of clotting times in the treated animals. After 30 days, the size of vascular lesions at sites of carotid endarterectomy, femoral artery and brachial artery angioplasty, were all reduced by 60-80%. While this heparin preparation may have produced effects on SMC unrelated to its anticoagulant properties, these studies represent the first demonstration of pharmacologic inhibition of vascular lesion formation in normal primates.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Antithrombotic Therapy in Experimental Thrombosis: Effects of Direct Antithrombins

AXIS I: 1a, 2, 3, 9, 13, 17

AXIS II: 48, 50b, 63f, 86

PRC UNIT: Pathobiology and Immun

INVES1: Harker, Laurence A.
DEGREE1: M.D.
DEPT1: Pathobiology and Immunobiology
STAFF1: O

INVES2: Hanson, Stephen R.
DEGREE2: Ph.D.
DEPT2: Pathobiology and Immunobiology
STAFF2: O

INVES3: Kelly, Andrew B.
DEGREE3: D.V.M.
DEPT3: Pathobiology and Immunobiology
STAFF3: C

SPECIES1: Papio cynocephalus
NUM1: 19

NON-HOST INST: NA

ABSTRACT: The importance of thrombin in high blood-flow, platelet-dependent thrombotic and hemostatic processes is evident from studies in baboons measuring the relative anti-thrombotic and antihemostatic effects of hirudin. We have shown results for the series of antithrombin III-independent antithrombins including recombinant hirudin, the irreversible antithrombin peptide D-Phe-Pro-Arg chloromethylketone (D-FPRCH$_2$Cl), the competitive antithrombin peptide D-Phe-Pro-boroArginine (D-FPRBOH), the bifunctional antithrombin peptide (BAP) combining the catalytic site inhibitor sequence D-FPR and the carboxy-terminal dodecapeptide of hirudin, benzamidine-based and arginine-based (argipidine) synthetic direct antithrombins. All of these direct antithrombins interrupt platelet and fibrin deposition and thrombotic occlusion in a dose dependent manner that is profound at the highest doses for all thrombogenic surfaces tested.

However, all of these direct antithrombins concurrently inhibit platelet hemostatic function in concert with their antithrombotic effects (bleeding times show intermediate prolongation by doses that reduce thrombus formation by half (ID_{50}). We conclude that platelet-dependent thrombotic and hemostatic processes are thrombin mediated and that direct antithrombins produce a potent dose dependent inhibition of arterial thrombus formation that greatly exceeds the minimal antithrombotic effects produced by heparin (even ten fold the

therapeutic dose), but cause an equivalent impairment of hemostatic function with corresponding risks of abnormal bleeding. Thus, while direct antithrombins exhibit antithrombotic efficacy for heparin resistant thrombosis, they achieve this benefit with an equivalent hemostatic burden.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Antithrombotic Therapy in Experimental Thrombosis: Inhibition of Thrombin Production

AXIS I: 1a, 2, 3, 9, 13, 17

AXIS II: 48, 50b, 63f, 86

PRC UNIT: Pathobiology and Immun

INVES1: Harker, Laurence A.
DEGREE1: M.D.
DEPT1: Pathobiology and Immunobiology
STAFF1: O

INVES2: Hanson, Stephen R.
DEGREE2: Ph.D.
DEPT2: Pathobiology and Immunobiology
STAFF2: O

INVES3: Kelly, Andrew B.
DEGREE3: D.V.M.
DEPT3: Pathobiology and Immunobiology
STAFF3: C

SPECIES1: Papio cynocephalus
NUM1: 19

NON-HOST INST: NA

ABSTRACT: Protein C undergoes catalytic activation by thrombin when complexed with thrombomodulin. Activated protein C (APC) inhibits subsequent thrombin production by inactivating the cofactor activities of fVa and fVIIIa in the autoamplification pathway. We have shown that infusions of human plasma-derived or recombinant APC (rAPC) inhibit VTF in a dose dependent manner without significantly impairing primary hemostasis in baboon models of carotid endarterectomy and thrombogenic segments incorporated into chronic AV shunts. As opposed to the direct antithrombins, antithrombotic doses of APC prolong the bleeding time only at the highest dose studied. Consequently, surgical bleeding is substantially less for APC than for equivalent antithrombotic doses of the antithrombin D-FPRCH$_2$Cl, although APC significantly increases surgical bleeding compared with untreated control endarterectomies. APC also enhances the antithrombotic effects of urokinase, but fails to increase circulating plasma markers of fibrinolysis when administered alone. Thus, exogenous APC interrupts heparin resistant VTF while sparing HPF. Since exogenous APC produces useful and safe antithrombotic effects, we have also examined the possibility that useful antithrombotic effects will result from inducing activation of endogenous PC in baboons. Endogenous APC was generated by infusing purified human α-thrombin intravenously at doses of 1 or 2

U/kg/min for 1 hr (concentration in the main pulmonary artery estimated to be 0.08 nM). These doses did not affect circulating concentrations of either platelets or fibrinogen. Circulating APC, measured by an enzyme immunocapture assay, attained antithrombotic levels of 280 ± 44 ng/mL ($P < 0.01$), and 613 ± 159 ng/ml ($P < 0.01$) for 1 and 2 U/kg/min, respectively. Thrombus formation was assessed using the low-flow thrombogenic device consisting of a platelet-rich Dacron graft component and a fibrin-rich chamber of disturbed flow incorporated into chronic AV shunts for 1 hr. Thrombus formation in the fibrin-rich chamber was abolished by both doses of infused thrombin, and was significantly decreased in the platelet-rich component of the device with the higher dose of thrombin. The antithrombotic effects are attributable to the elevation of APC because inhibiting the activation of protein C by prior injection of the monoclonal antibody HPC-4 (5 mg/kg bolus) eliminated the elevation in circulating APC and abolished the antithrombotic effects in the device. Bleeding times remained within the normal range (3-5 min). At 1 hr, APTT values were prolonged by 29 sec at 1 U/kg/min and by 117 sec at 2 U/kg/min doses of thrombin. Thus, the infusion of low-dose thrombin produces an antithrombotic state in vivo by inducing endogenous activation of protein C. These results imply that thrombin analogs may be useful in generating antithrombotic levels of endogenous APC for preventing and treating thrombosis. In preliminary studies we have also evaluated the capacity of protein S, the cofactor for APC, to enhance the antithrombotic effects of APC. Recombinant protein S, provided by Dr. Brian Grinnel, was infused by the boundary infusion device in combination with equimolar APC in a calcium-containing buffer, to achieve a local concentration of 5 nM for 1 hr in baboons. Whereas 5 nM APC detectably decreased thrombus formation, the addition of protein S significantly enhanced the antithrombotic effect. Protein S alone at 3 orders of magnitude greater concentration (5μM) exhibited no detectable antithrombotic effect; APC at 5μM abolishes thrombus formation. Baboon and human protein S activity and antigenic concentrations and C4b binding protein (C4BP) levels are comparable and readily measurable using the same assay systems.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Antithrombotic Therapy in Experimental Thrombosis: Safe Interruption of Thrombus Formation

AXIS I: 1a, 2, 3, 9, 13, 17

AXIS II: 48, 50b, 63f, 86

PRC UNIT: Pathobiology and Immun

INVES1: Harker, Laurence A.
DEGREE1: M.D.
DEPT1: Pathobiology and Immunobiology
STAFF1: 0

INVES2: Hanson, Stephen R.
DEGREE2: Ph.D.
DEPT2: Pathobiology and Immunobiology
STAFF2: 0

INVES3: Kelly, Andrew B.
DEGREE3: D.V.M.
DEPT3: Pathobiology and Immunobiology
STAFF3: C

SPECIES1: Papio cynocephalus
NUM1: 19

NON-HOST INST: NA

ABSTRACT: The relative antithrombotic efficacy and hemostatic safety of inhibiting fXa have been examined by comparing the dose response effects for two different natural potent and specific polypeptide inhibitors of fXa, antistasin and tick anticoagulant peptide (TAP), and contrasting their effects with hirudin, a comparably potent and specific natural inhibitor of thrombin. Acute arterial thrombus formation in baboons was measured in vivo as ^{111}In-platelet deposition and ^{125}I-fibrin accumulation onto segments of Dacron vascular graft, collagen-coated tubing, and homologous endarterectomized aorta, interposed as extension pieces into exteriorized arteriovenous shunts under arterial flow conditions using gamma camera imaging. Platelet hemostatic function was assessed by determining template bleeding times and coagulation was evaluated by measuring coagulation tests. Antistasin or TAP were administered intravenously for 60-120 min. Platelet deposition and fibrin accumulation were interrupted in concert in a dose-dependent manner with a half-maximal inhibitor dose (ID_{50}) of 2 μg/kg per min and 6 μg/kg per min, respectively; and corresponding inhibitor concentrations (IC_{50}) of 1.2 ± 0.04 μg/mL and 4.3 ± 0.39 μg/mL, respectively. Bleeding times remained normal (4.3 ± 0.4 min and 4.0 ± 0.6 respectively). APTT's were 199 ± 37 sec and 31 ± 2 secs. Thrombus formation on the segments of vascular graft was prevented for at least 24 hrs despite clearance of antistasin and TAP from the blood (t_{50}=2.3 hrs and 48 min, respectively). By contrast, hirudin infused for an

equivalent time inhibited thrombus with an ID_{50} of 6.6 mg/kg per min, IC_{50} of 5.75 μg/mL and bleeding times of 13 ± 3 min and APTT's of 130 ± 2 sec. Moreover, thrombus formed after discontinuing hirudin infusion after 1 hr, resulting in shunt occlusion. TAP (18 μg/kg per min) also abolished thrombus formation at sites of endarterectomy with markedly reduced surgical blood loss compared with hirudin.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Endarterectomy: Prevention of Thrombosis and Restenosis: Development of Baboon Models of EA, Characterization of VLF and Local Drug Infusion

AXIS I: 1a, 2, 3, 9, 13, 17

AXIS II: 48, 50b, 63f, 86

PRC UNIT: Pathobiology and Immun

INVES1: Harker, Laurence A.
DEGREE1: M.D.
DEPT1: Pathobiology and Immunobiology
STAFF1: 0

INVES2: Hanson, Stephen R.
DEGREE2: Ph.D.
DEPT2: Pathobiology and Immunobiology
STAFF2: 0

INVES3: Kelly, Andrew B.
DEGREE3: D.V.M.
DEPT3: Pathobiology and Immunobiology
STAFF3: C

INVES4: Lumsden, Alan
DEGREE4: M.D.
DEPT4: Pathobiology and Immunobiology
STAFF4: 0

SPECIES1: Papio cynocephalus
NUM1: 11

NON-HOST INST: NA

ABSTRACT: During the current period the required and relevant models have been developed to accomplish the proposed objectives. The detailed description of the endarterectomy procedure, its thrombogenicity with respect to imagable platelet deposition, and responsiveness of the thrombotic response to antithrombotic interventions are now well established. The methodology for documenting the initial proliferative responses of medial SMCs, accumulation of mononuclear leukocytes and the extent and composition of the subsequent mature vascular lesion formed at sites of EA are also well in hand. In addition, the ICC and ISH techniques for characterizing the growth factors, receptors, and modulating molecules and cells in VLF are in place in the laboratory.

We have also developed a local drug delivery system for infusing inhibitors of thrombin activity or its production into the blood fluid boundary layer at

sites of vessel wall injury. This method achieves very high drug concentrations locally while minimizing total drug requirements and circulating drug levels, and allows for the efficient design and interpretation of studies in primates with agents that are available in limited amounts or that might produce cardiovascular side-effects. We have now completed: a) a formal theoretical analysis of the convective diffusion problem for the local infusion flow geometry; b) in vitro studies with measurements of wall drug concentrations distal to infusion sites; c) studies with a baboon ex vivo shunt system using the local delivery device to block distal thrombus formation; and d) in vivo studies with local infusion of hirudin at sites of carotid artery angioplasty. The experimental studies document that the local infusion devices are remarkably efficient, deliver agents uniformly, and can be successfully used to block in vivo thrombus formation and modulate vascular healing. These studies document the reproducibility and efficiency of the method.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Endarterectomy: Prevention of Thrombosis and Restenosis: Immediate Reconstitution of Confluent Autologous Endothelium at Sites of EA

AXIS I: 1a, 2, 3, 9, 13, 17

AXIS II: 48, 50b, 63f, 86

PRC UNIT: Pathobiology and Immun

INVES1: Harker, Laurence A.
DEGREE1: M.D.
DEPT1: Pathobiology and Immunobiology
STAFF1: O

INVES2: Hanson, Stephen R.
DEGREE2: Ph.D.
DEPT2: Pathobiology and Immunobiology
STAFF2: O

INVES3: Kelly, Andrew B.
DEGREE3: D.V.M.
DEPT3: Pathobiology and Immunobiology
STAFF3: C

INVES4: Lumsden, Alan
DEGREE4: M.D.
DEPT4: Pathobiology and Immunobiology
STAFF4: O

SPECIES1: Papio cynocephalus
NUM1: 11

NON-HOST INST: NA

ABSTRACT: We have established the capability of forming confluent endothelium at sites of endarterectomy using cultured endothelial cells derived from mature arteries and veins. In baboons autologous EC (>10^6 cells) are harvested from surgically removed segments (5-6 cm each) of autologous cephalic vein by collagenase treatment, and expanded as much as 100 fold in culture. In situ incubation of fresh 1-cm carotid EA sites with 10^6 EC in suspension and rotational repositioning for 30 min results in acute attachment of EC in saturation density (3 x10^5 cells/cm^2). After restoring flow through the operated artery the attached ECs undergo subsequent spreading to confluence, despite exposure to arterial shear rates. This acute restoration of confluent endothelium abolishes subsequent ^{111}In-platelet deposition and maintains confluence when examined by SEM one week later. Thus, we have confirmed the feasibility of immediately restoring confluent EC at sites of mechanical vascular injury using autologous cultured cells.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Endarterectomy: Prevention of Thrombosis and Restenosis: Transduction of Tissue Plasminogen Activator (tPA) Gene Constructs into Cultured EC

AXIS I: 1a, 2, 3, 9, 13, 17

AXIS II: 48, 50b, 63f, 86

PRC UNIT: Pathobiology and Immun

INVES1: Harker, Laurence A.
DEGREE1: M.D.
DEPT1: Pathobiology and Immunobiology
STAFF1: 0

INVES2: Hanson, Stephen R.
DEGREE2: Ph.D.
DEPT2: Pathobiology and Immunobiology
STAFF2: 0

INVES3: Kelly, Andrew B.
DEGREE3: D.V.M.
DEPT3: Pathobiology and Immunobiology
STAFF3: C

INVES4: Lumsden, Alan
DEGREE4: M.D.
DEPT4: Pathobiology and Immunobiology
STAFF4: 0

SPECIES1: Papio cynocephalus
NUM1: 11

NON-HOST INST: NA

ABSTRACT: In collaboration with Dr. David Dichek, NIH, Bethesda MD, we have studied EC gene transfer experiments. Cultured baboon cephalic vein EC are transduced with tPA construct, using the techniques reported previously (Dichek, DA: Retroviral vector-mediated gene transfer into endothelial cells. Mol Biol Med 8:257-266, 1991). The transduced cells express high levels of plasminogen activator activity in the supernatant media (Lee SW, Kahn Ml, Dichek DA: Expression of an anchored urokinase in the apical endothelial cell membrane. J Biol Chem, in press). We have evaluated these cells for their antithrombotic effects in the baboon by attaching cells at subconfluent density of 2.5 and 5.0 x10^4 EC/cm^2 onto collagen-coated segments of vascular graft. The segments are then incorporated as extension pieces into exteriorized chronic femoral AV shunts in baboons with flow rates controlled at 40 mL/min, and ^{111}In-platelet deposition is measured on the EC-treated segments over 1 hr. Whereas, segments bearing normal non-transduced cells

accumulate platelets at intermediate values on the segments with somewhat greater deposition in the propogated tail extending downstream, the segments in 3 sets of paired studies at two different densities of attached transduced cells showed substantially reduced platelet deposition on the graft (<1 x10^5 plat/cm) for both the graft and tail components. Thus, we have demonstrated the feasibility of performing experiments with autologous cultured ECs that have been transvected with the gene construct of tPA resulting in the secretion of the gene product in sufficient amounts to interrupt thrombus formation.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Mechanisms of Damage Caused by Extracorporeal Circulation: ECC-Induced Platelet Embolic Dysfunction

AXIS I: 1a, 3, 9, 13, 17, 24

AXIS II: 48, 50b, 52, 63f, 86

PRC UNIT: Pathobiology & Immun

INVES1: Kelly, Andrew B.
DEGREE1: D.V.M.
DEPT1: Pathobiology and Immunobiology
STAFF1: C

INVES2: Harker, Laurence A.
DEGREE2: M.D.
DEPT2: Pathobiology and Immunobiology
STAFF2: O

INVES3: Hanson, Stephen R.
DEGREE3: Ph.D.
DEPT3: Pathobiology and Immunobiology
STAFF3: O

INVES4: Cornish, Devn
DEGREE4: M.D.
DEPT4: Pathobiology and Immunobiology
STAFF4: O

SPECIES1: Papio cynocephalus
NUM1: 12

NON-HOST INST: NA

ABSTRACT: Using baboons with previously infused autologous ^{111}Indium labeled platelets, platelets accumulate in the artificial lung throughout the ECC period. Sites of high shear blood flow conditions in the ECC produce predictable accumulation on the oxygenator and at points of disturbed blood flow. Platelet deposition in the ECC device peaks 20 to 40 min. after initiating flow, followed by some clearance from the ECC circuit by 90 min. However, platelets progressively accumulate at headers and circulatory sites of disturbed flow. Platelet accumulation in the circuit is associated with a reciprocal irreversible decrease in platelet count (379 ± 43 vs 230 ± 35; $p < 0.01$), implying that the subsequent loss of platelets from the ECC apparatus represents a process of microembolization. To determine whether microembolization from the circuit might be important in producing end-organ dysfunction, whole body imaging of radiolabeled platelet activity was carried out in 3 animals throughout 6 hour period of ECC. Transient localization of ^{111}In-platelets was observed in the pulmonary circulation during vena caval

veno-venous bypass, suggesting that unstable platelet aggregates embolize from the ECC circuit into the pulmonary vascular bed and subsequent aggregate disruption. Final increase radiolabeled platelet activity in hepatic and splenic circulation probably represent RE-system removal of disrupted platelet microemboli.

To quantify ECC-induced embolization, two additional sets of experiments have been performed. In the first set of experiments, modified ECC circuits were interposed into femoral arterio-arterial shunts in baboons previously labeled with ^{111}In-platelets. The legs were positioned over the gamma camera to document the accumulation of embolic material in the calf and foot. Clearly, embolic showering occurred in the limb bearing the A-A shunt during interposition of the ECC circuit, with a peak of activity corresponding to the disappearance of radiolabeled platelet activity from the ECC lung. Similar to lung images, ^{111}In-platelet activity in the distal limb, peaks at 40 min. clearance of most of the embolic material by 2 hours following cessation of ECC, although larger emboli were resistant.

In a second experimental group of 5 baboons, transient embolization into the brain was performed using thrombogenic segments of vascular graft as the generator. Embolic generation and showering of a single cerebral hemisphere was accomplished by incorporating a segment of vascular graft into a carotid A-A shunt while brain function was monitored using somatosensory evoked potentials (SEP's). Platelet emboli arising from the thrombogenic vascular graft were retained in the ipsilateral cerebral hemisphere. Despite rapid clearance of embolic material after removal of the vascular graft, evoked potentials were depressed significantly during embolization.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Mechanisms of Damage Caused by Extracorporeal Circulation: Inflammatory Response to ECC

AXIS I: 1a, 3, 9, 13, 17, 24

AXIS II: 48, 50b, 52, 63f, 86

PRC UNIT: Pathobiology & Immun

INVES1: Kelly, Andrew B.
DEGREE1: D.V.M.
DEPT1: Pathobiology and Immunobiology
STAFF1: C

INVES2: Harker, Laurence A.
DEGREE2: M.D.
DEPT2: Pathobiology and Immunobiology
STAFF2: O

INVES3: Hanson, Stephen R.
DEGREE3: Ph.D.
DEPT3: Pathobiology and Immunobiology
STAFF3: O

INVES4: Cornish, Devn
DEGREE4: M.D.
DEPT4: Pathobiology and Immunobiology
STAFF4: O

SPECIES1: Papio cynocephalus
NUM1: 12

NON-HOST INST: NA

ABSTRACT: ECC initiates an acute inflammatory response in baboons. Neutrophils decrease during the first hour (5.9 ± 2 vs. 2.7 ± 1 x 10^3/uL), while immature neutrophils become the prominent circulating leukocyte (<5 vs 43 ± 8%). Additionally, flow cytometric studies reveal significant increases in numbers of cells expressing CD11b adhesion molecules but lacking L-selectin. Circulating monocytes become detectably activated transiently over 1 hour.

Cytospin evaluation of bronchoalveolar lavage solution (BAL) show invasion of large numbers of macrophage-type cells present in the aspirates at 24 hours compared to aspirates at baseline. While tumor necrosis factor (TNF) and interleukin 1 (IL1B) are undetectable in peripheral blood, complement (C3a) levels peak early in the first hour (150 ± 27 vs. 450 ± 35 ng/mL). Neutrophil granular release byproduct, lactoferrin, increases rapidly during the first

hour (<50 vs 600 ± 200 ng/mL), but neutrophil elastase levels are not present in circulation or bronchoalveolar lavage fluid at the end of ECC and 24 hours following ECC.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Mechanisms of Damage Caused by Extracorporeal Circulation: Impairment of Pulmonary Function

AXIS I: 1a, 3, 9, 13, 17, 24

AXIS II: 48, 50b, 52, 63f, 86

PRC UNIT: Pathobiology & Immun

INVES1: Kelly, Andrew B.
DEGREE1: D.V.M.
DEPT1: Pathobiology and Immunobiology
STAFF1: C

INVES2: Harker, Laurence A.
DEGREE2: M.D.
DEPT2: Pathobiology and Immunobiology
STAFF2: O

INVES3: Hanson, Stephen R.
DEGREE3: Ph.D.
DEPT3: Pathobiology and Immunobiology
STAFF3: O

INVES4: Cornish, Devn
DEGREE4: M.D.
DEPT4: Pathobiology and Immunobiology
STAFF4: O

SPECIES1: Papio cynocephalus
NUM1: 12

NON-HOST INST: NA

ABSTRACT: The time course and degree of alveolar epithelial permeability has been determined using nebulized 99mTc labeled DTPA. In subjects anesthetized for the same period as those having ECC plus anesthesia, no significant changes in DTPA clearance from the lungs is seen. However, after 6 hours of ECC, DTPA is more rapidly cleared from the lungs, indicating significant disruption of alveolar integrity by 6 hours; clearance is impaired at 24 hours. Static images suggest that this is due in part to ventilation abnormalities.

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TITLE: Mechanisms of Damage Caused by Extracorporeal Circulation: Effects of Inhibitors on ECC Function

AXIS I: 1a, 3, 9, 13, 17, 24

AXIS II: 48, 50b, 52, 63f, 86

PRC UNIT: Pathobiology & Immun

INVES1: Kelly, Andrew B.
DEGREE1: D.V.M.
DEPT1: Pathobiology and Immunobiology
STAFF1: C

INVES2: Harker, Laurence A.
DEGREE2: M.D.
DEPT2: Pathobiology and Immunobiology
STAFF2: O

INVES3: Hanson, Stephen R.
DEGREE3: Ph.D.
DEPT3: Pathobiology and Immunobiology
STAFF3: O

INVES4: Cornish, Devn
DEGREE4: M.D.
DEPT4: Pathobiology and Immunobiology
STAFF4: O

SPECIES1: Papio cynocephalus
NUM1: 12

NON-HOST INST: NA

ABSTRACT: The importance of inhibiting thrombin generation to prevent consumption of platelets and fibrinogen during heparin resistant, platelet dependent thrombotic processes is well documented. Platelet disappearance and dysfunction during ECC are important causes of hemorrhage. ECC studies with the specific antithrombin D-FPRCH$_2$Cl infusing during ECC, demonstrate the importance of thrombin during ECC. Because of the potent antihemostatic effects, as measured by activated coagulation time (APTT) and bleeding times (BT) of antithrombins during full antithrombotic dosing, hemorrhagic consequences are not solved. To evaluate agents that interrupt thrombin generation and arterial thrombotic processes and at the same time determine hemostatic risk, we compared the direct antithrombin hirudin with the direct inhibitor of factor X, tick anticoagulant peptide (TAP) with respect to potency of inhibition of arterial thrombus, hemostatic risk and blood loss during clinically relevant surgical procedure and full antithrombotic dosing. Surgically implanted arteriovenous shunts were used for ex vivo determination of antithrombotic potential for inhibiting platelet thrombus onto vascular

graft during one hour of infusion of hirudin and TAP. Additionally, coagulation times and bleeding times were performed to determine hemostatic risk during infusion. Finally, using a maximally effective dose of each agent, carotid endarterectomy was performed. Thrombus was measured and blood loss during the infusion was determined by measuring total hemoglobin released into the wound. TAP provides potent antithrombotic activity with minimal hemostatic risk to the patient when compared to hirudin.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Encephalomyelitis Due to a Sarcocystis neurona-Like Protozoan in a Rhesus Monkey Infected with SIV

AXIS I: 1b, 7c, 21

AXIS II: 31, 66, 92 (Pathology)

PRC UNIT: Pathobiology & Immun

INVES1: Klumpp, Sherry A.
DEGREE1: D.V.M.
DEPT1: Pathobiology and Immunobiology
STAFF1: O

INVES2: Anderson, Daniel C.
DEGREE2: D.V.M.
DEPT2: Pathobiology and Immunobiology
STAFF2: C

INVES3: McClure, Harold M.
DEGREE3: D.V.M.
DEPT3: Pathobiology and Immunobiology
STAFF3: C

INVES4: Dubey, Jitender P.
DEGREE4: Ph.D.
DEPT4:
STAFF4: O

SPECIES1: Macaca mulatta
NUM1: 1

NON-HOST INST: Zoonotic Diseases Laboratory, United States Department of Agriculture (JPD)

ABSTRACT: A captive born rhesus monkey experimentally infected with SIVmac251 was used in a study to determine the effects of hyperimmunization on SIV disease progression. FACScan analyses obtained on a monthly basis throughout the experiment were similar to the baseline FACScan analysis. The animal was euthanatized at 7.5 months post-inoculation due to the development of progressive neurologic abnormalities. A chronic necrotizing encephalomyelitis with intralesional protozoal schizonts was diagnosed by light microscopy. The protozoa was identified as Sarcocystis neurona based on the morphologic characteristics by light and electron microscopy and immunocytochemical techniques. Although Sarcocystis neurona may be confused with Toxoplasma gondii by light microscopy, the former lacks rhoptries and parasitophorous vacuoles, and replicates by endopolygeny. The life cycle of Sarcocystis neurona is unknown. Only schizonts in various stages of development have been identified within the central nervous system of infected animals. The source of infection is also unknown. While Sarcocystis neurona

is a primary pathogen in horses, all cases of Sarcocystis neurona in raccoons have been associated with concomitant infection with distemper virus. The affected monkey had a poor antibody response to SIV which is often associated with rapid disease progression. Although FACScan analyses were not indicative of immunodeficiency, hyperimmunization most likely resulted in generalized immune activation and subsequent immunologic dysfunction of T lymphocytes. This is the first documented case of Sarcocystis neurona infection in a nonhuman primate.

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TITLE: Intravesical Injection of Teflon for Vesicoureteral Reflux

AXIS I: 1a, 27

AXIS II: 48, 62, 86

PRC UNIT: Pathobiology & Immun

INVES1: Malizia, Anthony A.
DEGREE1: M.D.
DEPT1: Pathobiology and Immunobiology
STAFF1: 0

INVES2: Woodard, John R.
DEGREE2: M.D.
DEPT2: Pathobiology and Immunobiology
STAFF2: 0

INVES3: Newton, Nancy E.
DEGREE3: M.D., Ph.D.
DEPT3: Pathobiology and Immunobiology
STAFF3: 0

INVES4: Anderson, Daniel C.
DEGREE4: D.V.M.
DEPT4: Pathobiology and Immunobiology
STAFF4: C

INVES5: Wyly, J. Bradley
DEGREE5: M.D.
DEPT5: Pathobiology and Immunobiology
STAFF5: 0

SPECIES1: Macaca mulatta
NUM1: 1

NON-HOST INST: NA

ABSTRACT: Polytef paste injections (intravesical/subureteric) have been used to treat vesicoureteral reflux in children, although only limited animal studies have been performed, and our previous studies in monkeys demonstrated distant migration of Polytef particles and the development of large foreign body granulomas at all injection sites. The results of our previous studies in monkeys, through three years post-injection of the Polytef paste, have been reported and included in previous annual reports. As an adjunct to these studies, monitoring of one Polytef injected monkey has been continued to document changes in the granulomatous reaction and to monitor the potential carcinogenic effects of Polytef paste. This animal is being followed radiographically by CT scanning and magnetic resonance imaging. Plans are to monitor this animal for up to 15 years. Continued monitoring of this animal revealed no additional adverse findings during 1993.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: HIV-2 Infection in Macaque Monkeys

AXIS I: 1a, 7b, 19

AXIS II: 31, 64, 66, 77

PRC UNIT: Pathobiology & Immun

INVES1: McClure, Harold M.
DEGREE1: D.V.M.
DEPT1: Pathobiology and Immunobiology
STAFF1: C

INVES2: Novembre, Francis J.
DEGREE2: Ph.D.
DEPT2: Pathobiology and Immunobiology
STAFF2: C

INVES3: Klumpp, Sherry A.
DEGREE3: D.V.M.
DEPT3: Pathobiology and Immunobiology
STAFF3: O

SPECIES1: Macaca mulatta
NUM1: 5

SPECIES2: Macaca nemestrina
NUM2: 5

NON-HOST INST: NA

ABSTRACT: The major objective of this study was to develop an animal model for HIV-2 infection that could be used to study the pathogenesis of HIV-2 infection, as well as for testing drugs and vaccines designed to prevent or treat infection with this type of human AIDS virus. Consequently, two rhesus macaques and two pig-tailed macaques were inoculated intravenously with 1 x 10^4 $TCID_{50}$ of HIV-2 (LAV-2) in June 1987.

Following virus inoculation, on rhesus and both pig-tailed macaques seroconverted within 4 to 6 weeks with low level antibody titers. One pigtail subsequently reverted to seronegative at 3 months post-infection. This animal and the seronegative rhesus received a second HIV-2 inoculation in June 1989. Both animals seroconverted, although the rhesus subsequently reverted to seronegative. Virus was not recovered from PBMC cultures of this animal at any time during a period of 6.5 years. Virus was recovered only sporadically from PBMC cultures of the other three animals over the course of 6.5 years. Both pig-tailed macaques showed a slowly rising antibody titer after July 1989 (up to titers of 1:25,600 and 1:51,600). During the latter half of 1993, one pig-tailed macaque developed persistent, chronic diarrhea and experienced a 26% weight loss over a six month period. Due to its progressively deteriorating clinical condition, this animal was sacrificed in December 1993 for autopsy examination and virus isolation attempts. Gross findings at

autopsy included severe emaciation, mild dehydration and moderate lymphadenopathy of the mesenteric, colonic and periaortic lymph nodes. Histologically, there was follicular lymphoid hyperplasia of the mesenteric and colonic lymph nodes with sinus histiocytosis and mild erythrophagocytosis, a moderate chronic active colitis and multifocal lymphoid aggregates in the bone marrow. Although cultures of the PBMC and mesenteric lymph nodes were virus negative, a lentivirus was isolated from cultures of the spleen from this animal. Subsequent PCR evaluation of this isolate confirmed that it was HIV-2. This HIV-2 isolate was subsequently inoculated intravenously into an additional three rhesus macaques and three pig-tailed macaques. These animals will be monitored by periodic culture of PBMC and serology to determine if the pathogenicity is increased following long-term infection in a macaque monkey.

This study demonstrates that it is possible to produce persistent, long-term (up to 6.5 years) infections in pig-tailed macaques inoculated with HIV-2. Although the relationship between the chronic HIV-2 infection and the chronic wasting disease seen in this macaque is unknown at this time, subsequent animal-to-animal passage of this virus may result in the development of an animal model in which HIV-2 infection results in immunosuppression and clinical disease.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Maternal Transmission of SIVsmm in Rhesus Macaques

AXIS I: 1a, 7b

AXIS II: 31, 64, 66, 77

PRC UNIT: Pathobiology & Immun

INVES1: McClure, Harold M.
DEGREE1: D.V.M.
DEPT1: Pathobiology and Immunobiology
STAFF1: C

INVES2: Novembre, Francis J.
DEGREE2: Ph.D.
DEPT2: Pathobiology and Immunobiology
STAFF2: C

INVES3: Anderson, Daniel C.
DEGREE3: D.V.M.
DEPT3: Pathobiology and Immunobiology
STAFF3: C

INVES4: Klumpp, Sherry A.
DEGREE4: D.V.M.
DEPT4: Pathobiology and Immunobiology
STAFF4: O

SPECIES1: Macaca mulatta
NUM1: 44

NON-HOST INST: NA

ABSTRACT: Due to the increasing importance and magnitude of HIV infection and AIDS in the human pediatric population, these studies were initiated to evaluate the perinatal/postnatal transmission of SIVsmm in experimentally infected rhesus macaques and to determine the feasibility of using experimentally infected rhesus macaques as a model system for the study of perinatal HIV infection. In the initial studies, 15 timed pregnant rhesus monkeys were infected with SIVsmm during various stages of gestation and their offspring were monitored for evidence of virus infection. Three groups of 5 animals were infected with SIVsmm during early (day 28-35), mid (day 71-78) and late (day 146-150) gestation. Offspring delivered by these experimentally infected macaques included 2 stillbirths and 13 livebirths; one liveborn infant died at 3 days of age. There was no evidence of virus infection in the stillbirths or neonatal death. The remaining infants and their mothers were evaluated within a week of parturition and at quarterly intervals thereafter by serology and virus culture of PBMC; milk samples were also collected from the mothers at each examination for virus culture. All infants were virus negative at birth; all infants in the early and mid-gestation groups and one infant in the late gestation group had low levels of maternal antibodies to SIVsmm. These maternal antibodies decreased to undetectable levels prior to 3

months of age in 4 of 9 infants, and between 3 and 6 months in the other 5 infants. Three infants subsequently seroconverted and became virus positive at 9-15 months of age. Milk samples from all mothers were virus negative at parturition, but milk samples from 4 animals were virus positive at 9 and 12 months postpartum. All three virus-positive infants died between 16 and 32 months of age (7, 13 and 20 months after seroconversion). Observations in one or more of these infants included diarrhea and weight loss, anemia, generalized lymphadenopathy, thymic atrophy, splenomegaly, pneumonia, lymphopenia, decreased numbers of $CD4^+$ cells, decreased $CD4^+/CD8^+$ cell ratios, CMV infection, cryptosporidiosis, bacterial infections, adenovirus pancreatitis, Pneumocystis carinii pneumonia, progressive multifocal leukoencephalopathy of the spinal cord due to SV40 infection, and hypergammaglobulinemia. These studies demonstrated maternal-infant transmission of SIVsmm in the experimentally infected macaque model and suggest that transmission most likely occurred by breast-feeding.

Although 12 of the 15 original adult females have died due to an AIDS-like disease, the breeding program has been continued with the remaining females and 14 additional SIV infected females. This continued breeding program has resulted in an additional 13 pregnancies. These additional pregnancies have resulted in 5 live births and one stillbirth; seven animals are due to deliver in early 1994. One liveborn infant died at 6 days of age; this infant and the stillbirth did not show any evidence of SIV infection. The other four liveborn infants have been monitored since birth by serology and virus culture of PBMC. All infants had maternal antibodies at birth; these persisted through three months of age but had decreased to undetectable levels by six months of age. Cultures of PBMC have been negative for all infants through six months of age. Although these infants will continue to be monitored for evidence of virus infection, these observations support our earlier findings which suggested that intrauterine transmission of SIV occurs infrequently, if at all.

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TITLE: Natural SIV and STLV-1 Infection in Sooty Mangabeys

AXIS I: 1a, 7b

AXIS II: 31, 56, 64, 66, 77

PRC UNIT: Pathobiology and Immun

INVES1: McClure, Harold M.
DEGREE1: D.V.M.
DEPT1: Pathobiology and Immunobiology
STAFF1: C

INVES2: Novembre, Francis J.
DEGREE2: Ph.D.
DEPT2: Pathobiology and Immunobiology
STAFF2: C

INVES3: Gordon, Thomas P.
DEGREE3: M.S.
DEPT3: Behavioral Biology
STAFF3: C

SPECIES1: Cercocebus atys
NUM1: 182

NON-HOST INST: NA

ABSTRACT: A T-lymphotropic lentivirus, SIVsmm, has been isolated from a high percentage of mangabeys in the Yerkes mangabey breeding colony. In initial serologic evaluation in this colony, 72 of 118 (62%) mangabeys were seropositive and culture positive for SIVsmm and 45 of 104 (43%) were positive for antibodies to STLV-1. The frequency of SIVsmm infection in the mangabey colony appeared to increase with age, with infection documented in 94% (34 of 36) of mangabeys 9 years of age or older, in 83% (5 of 6) of animals 7-8 years of age, in 73% (11 of 15) of 5-6 year old animals, in 49% (17 of 35) of 3-4 year old animals, and in 23% (6 of 20) of animals 1-2 years of age. The high infection rate in mature animals and the occurrence of occasional infections in infants suggest that transmission of SIVsmm is comparable to the transmission of HIV (i.e., by sexual activity and perinatally).

In order to more definitively characterize the mode of virus transmission within the mangabey colony, and document the age at time of seroconversion, studies have been continued on a prospective serologic survey of seronegative mangabeys in the colony. Infants born in the colony are evaluated within the first month of life and at quarterly intervals thereafter until seroconversion occurs. During the current year, serologic monitoring was continued on 99 seronegative or newborn mangabeys. These included 13 animals born in 1993, 22 born in 1992, 28 born in 1991, and 36 animals born between 1984-1990 that continue to be seronegative for antibodies to SIV and/or STLV-1. In addition, 18 stillbirths, neonatal or infant deaths have been available for evaluation. Although cultures (PBL, spleen, lymph node) are pending on a number of the latter cases, none, to date, have been culture positive.

Eighteen animals born between 1984-1990 (3 to 9 years of age) continue to be seronegative (9 females and 9 males). Seven animals in this age group (1 male and 6 females) seroconverted between 15 months and 65 months of age; five of the seven seroconversions occurred between 15 and 22 months of age. Forty-seven infants delivered by SIV seropositive mothers had maternal antibodies when first checked at approximately one month of age. The maternal antibodies had decreased to undetectable levels by 4 months of age in 16 animals, by 6 months of age in 28 animals and by 7 months of age in 42 animals. Maternal antibodies had decreased to undetectable levels in all infants by 9 months of age. Four infants, seropositive at birth, did not serorevert and were virus positive at 4, 8, 10 an 13 months of age. In addition, 14 of 47 (30%) infants seroconverted between 7 and 21 months of age. Overall, 18 of 51 (35%) infants were seropositive and/or virus positive before two years of age. One male mangabey with a seronegative mother was noted to seroconvert at 28 months of age. These data indicate a significant level of SIV infection in neonatal and infant mangabeys, suggesting that maternal-infant transmission, by some mechanism, is occurring within the colony. The incidence of 35% infection in mangabey infants is similar to the expected rate of maternal transmission of HIV.

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TITLE: Serologic Survey of Nonhuman Primates in Kenya

AXIS I: 1a, 7b

AXIS II: 31, 64, 66, 77

PRC UNIT: Pathobiology and Immun

INVES1: McClure, Harold M.
DEGREE1: D.V.M.
DEPT1: Pathobiology and Immunobiology
STAFF1: C

INVES2: Novembre, Francis J.
DEGREE2: Ph.D.
DEPT2: Pathobiology and Immunobiology
STAFF2: C

INVES3: Isahakia, Mohamed
DEGREE3: Ph.D.
DEPT3: Pathobiology and Immunobiology
STAFF3: O

SPECIES1: Cercopithecus mitis
NUM1: 28 (feral)

SPECIES2: Cercopithecus aethiops
NUM2: 2 (feral)

SPECIES3: Papio cynocephalus
NUM3: 43 (feral)

SPECIES4: Cercopithecus neglectus
NUM4: 13 (feral)

SPECIES5: Cercopithecus ascanius
NUM5: 7 (feral)

SPECIES6: Cercopithecus albogularis
NUM6: 11 (feral)

NON-HOST INST: Institute of Primate Research, Kenya (MI)

ABSTRACT: Serological surveys are being conducted on serum samples from feral nonhuman primates (Papio and Cercopithecus species) in Kenya to determine the incidence of infection with HIV-1, HIV-2/SIV and HTLV-1/STLV-1 in various nonhuman primate species from different geographical regions of Kenya. Previous analyses showed a high prevalence of antibodies to SIV in Sykes (59% seropositive) and African green monkeys (51% seropositive). A somewhat lower prevalence of antibodies to STLV-1 was observed (30% of Sykes monkeys and 43% of African green monkeys). These observations in feral Sykes

and African green monkeys are similar to seroprevalence rates detected in the Yerkes mangabey breeding colony, suggesting that at least in these three African species of nonhuman primates, SIV infection is widespread. In these initial studies, the incidence of antibodies to SIV and STLV-1 was considerably lower in baboons; 0.2% of baboons were found to have antibodies to SIV and 5% had antibodies to STLV-1.

During the current year, serum samples were received from an additional 104 feral nonhuman primates. These included 43 baboons, 28 Blue Sykes monkeys, 7 Red Tail monkeys, 13 DeBrazza monkeys, 2 vervets, and 11 Highland Sykes monkeys. These serum samples were evaluated by HIV-2 and HTLV ELISA kits, with positive samples confirmed by Western blot. The results were as follows:

	SIV Positive	STLV-1 Positive
Baboon	0/43 (0%)	3/43 (7%)
Blue Sykes	10/28 (36%)	0/28 (0%)
Red Tails	1/7 (14%)	0/7 (0%)
DeBrazza	0/13 (0%)	1/13 (8%)
Vervets	0/2 (0%)	0/2 (0%)
Highland Sykes	3/11 (27%)	5/11 (45%)

In addition to the serologic survey of these feral Kenyan primates, serologic evaluation was also done on 30 baboons imported from Ethiopia. In contrast to a recent report of a high seropositivity rate for antibodies to SIV in Ethiopian baboons, we found all 30 of these Ethiopian baboons to be seronegative for antibodies to both SIV and STLV-1.

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TITLE: Transmission of SIVsmm by Feeding Virus-Containing Infant Formula

AXIS I: 1a, 7b

AXIS II: 31, 66, 77

PRC UNIT: Pathobiology & Immun

INVES1: McClure, Harold M.
DEGREE1: D.V.M.
DEPT1: Pathobiology and Immunobiology
STAFF1: C

INVES2: Novembre, Francis J.
DEGREE2: Ph.D.
DEPT2: Pathobiology and Immunobiology
STAFF2: C

INVES3: Anderson, Daniel C.
DEGREE3: D.V.M.
DEPT3: Pathobiology and Immunobiology
STAFF3: C

INVES4: Klumpp, Sherry A.
DEGREE4: D.V.M.
DEPT4: Pathobiology and Immunobiology
STAFF4: O

SPECIES1: Macaca mulatta
NUM1: 9

NON-HOST INST: NA

ABSTRACT: This study was initiated to determine whether SIVsmm can be transmitted to infant rhesus monkeys be ingestion of virus-containing infant formula. The ultimate goal is to provide additional information on the potential importance of breast feeding in the transmission of HIV or SIV. Any infections that result would be monitored to document the characteristics and pathogenesis of SIV infection in the rhesus neonate to further develop and characterize the SIV-infected macaque as a model for pediatric AIDS.

In the initial study reported last year, six infant rhesus macaques (approximately 30 days of age) were moved to cages in an SIV containment area and placed on an SMA formula diet. Each of these infants received 1,000 $TCID_{50}$ of cell-free SIVsmm once daily for a period of one month. This was administered by adding the virus to the morning SMA feeding. Prior to initiating virus administration by this route, studies were done in the laboratory to show that SMA did not inactivate the virus. In addition, to demonstrate viability of the virus used for these feeding experiments, three juvenile rhesus monkeys were inoculated intravenously with the same virus dose (1,000 $TCID_{50}$) at different time points during the feeding studies.

Each animal in the study was examined at 6 and 12 weeks after the initial virus exposure, and at quarterly intervals thereafter. At each examination, blood was collected for a CBC, immunology and virus serology and culture. Each of the three juvenile macaques became virus-positive following intravenous inoculation. However, the infant rhesus exposed to virus in the SMA formula have failed to show any evidence of infection after an 18 month follow-up period. These findings suggest that SIV is not readily transmitted by oral ingestion of cell-free virus. Studies are now underway to determine the outcome of oral ingestion of cell-associated SIV.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: SIVsmm Infection via Amniotic Fluid Inoculation

AXIS I: 1a, 7b

AXIS II: 31, 66, 77

PRC UNIT: Pathobiology and Immun

INVES1: McClure, Harold M.
DEGREE1: D.V.M.
DEPT1: Pathobiology and Immunobiology
STAFF1: C

INVES2: Novembre, Francis J.
DEGREE2: Ph.D.
DEPT2: Pathobiology and Immunobiology
STAFF2: C

INVES3: Klumpp, Sherry A.
DEGREE3: D.V.M.
DEPT3: Pathobiology and Immunobiology
STAFF3: O

INVES4: Anderson, Daniel C.
DEGREE4: D.V.M.
DEPT4: Pathobiology and Immunobiology
STAFF4: C

SPECIES1: Macaca mulatta
NUM1: 14

NON-HOST INST: NA

ABSTRACT: Since preliminary studies in our laboratory failed to demonstrate intrauterine transmission of SIV in experimentally infected rhesus macaques, studies were designed to determine the effects of SIV inoculation of the amniotic fluid of rhesus macaques in various stages of pregnancy. The ultimate goal was to develop a model that could be used to design treatment strategies for intrauterine lentivirus infection or to study the pathogenesis of SIV infection that occurred during various stages of fetal development. Subsequently, SIVsmm was inoculated into the amniotic fluid (guided by ultrasound) of two rhesus macaques at 80 days gestation, two at 100 days gestation, two at 120 days gestation, and one at 147 days gestation. Four of the seven females became virus infected and all seven females subsequently delivered clinically normal, term infants. Three of the adult females continue to be virus negative at more than a year post-amniotic fluid inoculation. Four of the seven infants delivered by these females developed SIV infection; three of these were seropositive when first checked within the first week after birth, and one additional infant was seropositive at six weeks of age. The other three infants have remained seronegative through 12 months of age. One seronegative infant has a virus positive mother, whereas the mothers of the other two seronegative infants are also seronegative. One

seropositive and virus positive infant has a seronegative mother. The virus positive infants were delivered by mothers who received amniotic fluid inoculations of SIV on gestation days 80, 100, 120 or 147. One infant, seropositive and virus positive at birth, developed a progressive disease characterized by anemia, hypergammaglobulinemia and immunosuppression and died at 15 months; the other three infants are currently alive at 20-21 months of age.

These data indicate that intrauterine infection with SIV can be accomplished by inoculation of virus into the amniotic fluid in over 50% of the inoculated animals. The reasons why three of the seven infants did not become infected are unknown at the present time. This method of infection should prove to be an appropriate model for pediatric AIDS that can be used for studies of the pathogenesis of lentivirus infection of fetuses and neonates, and for the development of treatment regimens for pediatric AIDS.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Immunological and Molecular Studies of Primate Antigens

AXIS I: 1a, 2, 3, 4, 6, 9

AXIS II: 1be, 39, 60, 64, 74ah, 76ab, 91

PRC UNIT: Pathobiology & Immun

INVES1: Metzgar, Richard S.
DEGREE1: Ph.D.
DEPT1: Pathobiology and Immunobiology
STAFF1: O

SPECIES1: Pan troglodytes
NUM1: 3

NON-HOST INST: Duke University Medical Center

ABSTRACT: The overall goals of this project are to continue to define selected antigens of human cells and to use chimpanzees for evaluating potential human tumor vaccines. The study utilizes the immunologic perspectives of chimpanzees to recognize epitopes of human antigens that may not be seen by non-primate mammalian species. The antigenic focus during the current and continuation year is on peptide determinants of human tumor and normal cell apomucins. Recombinant mucin apoproteins and peptides selected from rodent studies, which have stimulated good antibodies to various regions of tumor mucin and which show restricted specificity for normal or malignant mucin producing cells are being evaluated for immunogenicity and specificity in chimpanzees. Preclinical and clinical studies currently being conducted at various cancer centers have indicated that mucin peptides may be important antigenic molecules for active immunotherapy of pancreatic cancer patients.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Lentivirus Replication in Host Macrophages is a Prerequisite for Disease

AXIS I: 1a, 7b

AXIS II: 31, 64, 66, 77

PRC UNIT: Pathobiology and Immun

INVES1: Narayan, Opendra
DEGREE1: D.V.M., Ph.D.
DEPT1: Pathobiology and Immunobiology
STAFF1: O

INVES2: McClure, Harold M.
DEGREE2: D.V.M.
DEPT2: Pathobiology and Immunobiology
STAFF2: C

SPECIES1: Macaca mulatta
NUM1: 6

NON-HOST INST: University of Kansas (ON)

ABSTRACT: During the neuroadaptation of lymphocyte-tropic SIVmac239 and follow-up of rhesus macaques chronically infected with SIVmac239, we obtained viruses that caused severe encephalitis and interstitial pneumonia. Unlike virus 239, the new agents were highly macrophage tropic. Tissue homogenates containing these viruses caused severe and rapid disease in new animals. However, since tissue homogenates contain the virus swarm, we used the PCR procedure to amplify consensus viral env gene from DNA of pathologically affected lung and brain. These env genes were substituted for the env gene in the molecular clone of SIVmac239 DNA and the viruses examined for cell tropism and virulence. Chimeras containing the env gene of both brain and lung viruses were macrophage tropic. Comparison of the replication of parental virus 239 and the chimeric viruses in macaque macrophages, using pulse chase and immunoprecipitation of virus proteins within and on the surface of infected macrophages showed that whereas the chimeric viruses replicated efficiently in the macrophages, parental virus 239 replicated defectively. Neither the envelope precursor protein gp160 nor the gag precursor protein p57 were cleaved. Inoculation of the chimeras (brain) into new animals resulted in typical persistent infection, but surprisingly, the animals did not develop disease. Unlike the L-tropic virus 239, the macrophage tropic viruses did not activate T-cells, they failed to induce viremia and also failed to invade brain despite their neurotropic potential.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Determinants of Pathogenesis in SIVsmmPBj Infection

AXIS I: 1a, 1d, 7b

AXIS II: 31, 39, 66, 77

PRC UNIT: Pathobiology & Immun

INVES1: Novembre, Francis J.
DEGREE1: Ph.D.
DEPT1: Pathobiology and Immunobiology
STAFF1: C

INVES2: Hirsch, Vanessa M.
DEGREE2: D.V.M., Ph.D.
DEPT2: Pathobiology and Immunobiology
STAFF2: O

INVES3: Johnson, Philip R.
DEGREE3: M.D.
DEPT3: Pathobiology and Immunobiology
STAFF3: O

INVES4: McClure, Harold M.
DEGREE4: D.V.M.
DEPT4: Pathobiology and Immunobiology
STAFF4: C

SPECIES1: Macaca nemestrina
NUM1: 9

NON-HOST INST: National Institute of Allergy and Infectious Diseases, NIH (VMH) and Children's Hospital Research Foundation, Columbus, Ohio (PRJ)

ABSTRACT: The SIV/macaque system is an excellent model for investigating the pathogenesis of infection and disease caused by AIDS viruses. A variant SIV from sooty mangabeys (SIVsmm), termed SIVsmmPBj, uniformly induces an acutely lethal disease when inoculated into pig-tailed macaques. Utilizing a molecularly cloned virus, PBj6.6, which reproduces the disease, we have started to map important pathogenic determinants of this atypical virus. Chimeric molecular clones generated between PBj6.6 and other non-acutely pathogenic clones have facilitated dissection of important viral genetic elements associated with acute disease. The major genetic changes, a duplicated NF-kB site in the LTR, and a 5 amino acid insertion in the envelope gene, may not play a role in the increased virulence. However, multiple determinants, including elements in gp40, gag, and the central regulatory genes appear to be involved in the acute pathogenesis of PBj-induced disease. One of the unusual characteristics of this virus is the ability to induce proliferation of macaque PBMC in vitro. Analysis of our chimeric clones

reveals that this feature is directly associated with the ability of a virus to induce acute disease in vivo. Key to the development of disease may be a dual effect on lymphocytes that the virus exhibits in vivo. While inducing severe lymphopenia in the peripheral blood, SIVsmmPBj also induces a lymphoid hyperplasia in regional lymph nodes and in lymphoid tissue in the mucosa of the intestinal tract. The induction of lymphopenia appears to be linked to viral determinants in the central regulatory gene region. Studies using additional chimeric molecular clones will enable an understanding of the molecular mechanisms involved in the acutely lethal disease induced by SIVsmmPBj.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Molecular Cloning of SIV from Stump-tailed Macaques

AXIS I: 1a, 1d, 7b

AXIS II: 31, 39, 66, 77

PRC UNIT: Pathobiology & Immun

INVES1: Novembre, Francis J.
DEGREE1: Ph.D.
DEPT1: Pathobiology and Immunobiology
STAFF1: C

INVES2: Hirsch, Vanessa M.
DEGREE2: D.V.M., Ph.D.
DEPT2: Pathobiology and Immunobiology
STAFF2: O

INVES3: McClure, Harold M.
DEGREE3: D.V.M.
DEPT3: Pathobiology and Immunobiology
STAFF3: C

SPECIES1: Macaca nemestrina
NUM1: 10

SPECIES2: Macaca mulatta
NUM2: 10

NON-HOST INST: National Institute of Allergy and Infectious Diseases, NIH (VMH)

ABSTRACT: SIV from stump-tailed macaques represents a unique lentivirus because of its high rate of transmission when compared to other SIVs. In an effort to understand the basis of pathogenicity of SIVstm at the genetic level, infectious molecular clones of SIVstm were derived. Analyses of one of these clones (SIVstm/37.16) showed that SIVstm is a member of the SIVsmm/SIVmac/HIV-2 group of primate lentiviruses. An interesting characteristic of SIVstm is that it is equidistantly related to SIVsmm and SIVmac. Virus derived from two molecular clones and an uncloned virus stock were used for inoculation of rhesus and pig-tailed macaques to investigate the pathogenesis of SIVstm in related subspecies of macaque. In contrast to the rapid development of AIDS in naturally-infected stump-tailed macaques, SIVstm demonstrated an overall lower pathogenicity for rhesus and pig-tailed macaques. To date, approximately three years after inoculation, only seven of sixteen animals have developed AIDS-like disease.

Three of these animals received molecularly cloned virus and four were inoculated with uncloned virus stock. Of the surviving animals, four are showing signs of immunosuppression (decreased levels of CD4+ cells in the

peripheral circulation). The continued monitoring of these animals for development of AIDS and subsequent retrospective genetic and virologic studies should enable an examination of factors leading to the progression to AIDS in these animals.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Determination of Infection Status of Sooty Mangabey Infants

AXIS I: 1a, 1d, 7b

AXIS II: 31, 39, 66, 77

PRC UNIT: Pathobiology & Immun

INVES1: Novembre, Francis J.
DEGREE1: Ph.D.
DEPT1: Pathobiology and Immunobiology
STAFF1: C

INVES2: McClure, Harold M.
DEGREE2: D.V.M.
DEPT2: Pathobiology and Immunobiology
STAFF2: C

SPECIES1: Cercocebus atys
NUM1: 11

NON-HOST INST: NA

ABSTRACT: A vast majority of sooty mangabeys in captivity and a number in the wild are naturally infected with SIV. While these animals usually do not show any clinical signs of disease development throughout their lives, they remain seropositive and virus can very easily be isolated from PBMC of these animals. When this virus is transferred to Asian macaques, a disease process remarkably similar to human AIDS develops. The underlying enigma is why do mangabeys not develop disease. One of the first questions to address is when do mangabeys get infected. To answer this question, we have designed a study to examine infant mangabeys and their infection status. Infant mangabeys (identified at birth) are divided into three groups: 1) Infants separated from their mothers and nursery reared; 2) Infants kept with their mothers, but isolated from the mangabey colony; and 3) Infants kept with their mothers in the colony. These animals are then analyzed for the presence of SIV by serology, virus isolation, and PCR amplification of SIV-specific sequences. To date, group 1 contains seven infants, including two from seronegative mothers. Group 2 has two infant-mother pairs, and no animals have yet been assigned to group 3.

Observations to date include the following: Group 1--Five of seven mothers of group 1 infants are seropositive for SIV and virus has been isolated from four of these five animals. At birth, the infants' serology status corresponded to the mothers', i.e. five of seven were seropositive, presumably from maternal antibody. At four months after birth, only two infants remain seropositive. PCR studies are pending. Group 2--Both mothers are seropositive for SIV and their infants were both seropositive at birth and negative for virus isolation. PCR studies are pending.

The results from these studies should provide information on the transmission of SIV in the mangabey colony at Yerkes. In addition, these types of analyses will enable us to begin to examine why mangabeys do not become ill from SIV infection.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Evidence for SIV Infection in a Baboon

AXIS I: 1a, 1d, 7b

AXIS II: 31, 39, 66, 77

PRC UNIT: Pathobiology & Immun

INVES1: Novembre, Francis J.
DEGREE1: Ph.D.
DEPT1: Pathobiology and Immunobiology
STAFF1: C

INVES2: Chege, Gerald
DEGREE2: B.V.M.
DEPT2: Pathobiology and Immunobiology
STAFF2: O

INVES3: McClure, Harold M.
DEGREE3: D.V.M.
DEPT3: Pathobiology and Immunobiology
STAFF3: C

INVES4: Isahakia, Mohamed
DEGREE4: Ph.D.
DEPT4: Pathobiology and Immunobiology
STAFF4: O

SPECIES1: Papio
NUM1: 1

SPECIES2: Macaca nemestrina
NUM2: 4

NON-HOST INST: Institute of Primate Research, Kenya (GC, MI)

ABSTRACT: The Yerkes Regional Primate Research Center and the Institute of Primate Research in Kenya have an ongoing collaboration to examine the prevalence of lentiviral infections in feral nonhuman primates. A previous serosurvey revealed that 2 of 106 (2%) baboons were seropositive; one of these (animal #1621) confirmed by Western blot. Baboon 1621 was further evaluated after importation to the Yerkes Center. After confirmation of the seropositive status of this baboon (also seropositive for STLV-I), attempts were made at SIV isolation. All attempts thusfar have been negative. These virus isolations included depletion of CD8+ cells and co-culturing with a number of cell types (Baboon PBMC, CEMss, Molt4 Clone 8, U937, CEMx174, and Hut 78). Subsequently, a blood transfusion was performed between baboon 1621 and two pig-tailed macaques to try and transfer the SIV infection. Results to date are negative. In addition, a lymph node biopsy was obtained from 1621

for: 1) virus isolation; 2) macaque inoculation; and 3) genomic DNA preparation. Virus isolation was negative. Macques inoculated with lymph node cells have not seroconverted to SIV, but have seroconverted to STLV-I. DNA obtained from PBMC and lymph node cells was used in Southern blot hybridization and PCR experiments to detect SIV-specific sequences. Positive results were obtained with Southern blot and with PCR using conserved SIV LTR primers. Sequence analysis of amplified products revealed similarities to the HIV-2/SIVsmm/SIVmac group of primate lentiviruses. Efforts are continuing to obtain an SIV isolate from this baboon.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Molecular Cloning of SIV_{MU}

AXIS I: 1a, 1d, 7b

AXIS II: 31, 39, 66, 77

PRC UNIT: Pathobiology & Immun

INVES1: Novembre, Francis J.
DEGREE1: Ph.D.
DEPT1: Pathobiology and Immunobiology
STAFF1: C

INVES2: Folks, Thomas
DEGREE2: Ph.D.
DEPT2: Pathobiology and Immunobiology
STAFF2: O

INVES3: Hart, Clyde
DEGREE3: Ph.D.
DEPT3: Pathobiology and Immunobiology
STAFF3: O

INVES4: McClure, Harold M.
DEGREE4: D.V.M.
DEPT4: Pathobiology and Immunobiology
STAFF4: C

SPECIES1: NA
NUM1: NA

NON-HOST INST: Centers for Disease Control and Prevention (TF, CH)

ABSTRACT: Because SIV grows well in human cells in the laboratory, it has always been assumed that SIV could potentially infect humans. SIV has now been isolated from an exposed laboratory worker. This virus has subsequently been termed SIV_{MU}. In an effort to more clearly examine the genetics of this virus that may be associated with pathogenicity, we are beginning to generate infectious molecular clones of SIV_{MU}. To date we have been able to amplify both halves of the SIV_{MU} genome by PCR. The 3' half has been successfully cloned and we are awaiting results for the 5' half. Once both halves have been cloned, they will be combined to generate a full-length molecular clone. Genetic studies of this virus will be valuable for comparison to other SIV isolates.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Mucosal and Systemic Immunity to HTLV and STLV

AXIS I: 1a, 1d, 7b

AXIS II: 39, 64, 66, 77, 91

PRC UNIT: Pathobiology and Immun

INVES1: Novembre, Francis J.
DEGREE1: Ph.D.
DEPT1: Pathobiology and Immunobiology
STAFF1: C

INVES2: Compans, Richard W.
DEGREE2: Ph.D.
DEPT2: Pathobiology and Immunobiology
STAFF2: O

INVES3: Yamschikov, Vladimir
DEGREE3: Ph.D.
DEPT3: Pathobiology and Immunobiology
STAFF3: O

INVES4: McClure, Harold M.
DEGREE4: D.V.M.
DEPT4: Pathobiology and Immunobiology
STAFF4: C

SPECIES1: Macaca mulatta
NUM1: 12

NON-HOST INST: NA

ABSTRACT: Human T-cell lymphotropic virus type I (HTLV-I) is associated with the development of leukemia/lymphoma and tropical spastic paraparesis/HTLV-1-associated myelopathy in humans. The development of disease may be related to the extent of the immune response to this virus--certain immune responses (ADCC and CTLs) have been documented in symptomatic patients, but not in seropositive, asymptomatic individuals. The simian counterpart, STLV-I, is remarkably similar to HTLV-I at the genetic level. STLV-I infection of macaques provides an excellent model system for investigating the development of immunity (mucosal and systemic) and for vaccine development. We have initiated such studies by attempting to establish a challenge system in rhesus macaques for both HTLV-I and STLV-I. For these experiments, animals were exposed by iv inoculation or by vaginal inoculation to either an HTLV-I-producing cell line or an STLV-I-producing cell line. Results show that all animals receiving HTLV-I as an iv inoculation have seroconverted. Two of three animals receiving STLV-I as an iv inoculation seroconverted, but have reverted to a seronegative status. No animals receiving vaginal inoculations have seroconverted. Animals will continue to be monitored and non-infected

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Cognitive Development and Temperament in Young Nursery-Reared Pan troglodytes

AXIS I: 1a, 21, 25

AXIS II: 36, 41, 60, 71

PRC UNIT: Reproductive Biology

INVES1: Bard, Kim A.
DEGREE1: Ph.D.
DEPT1: Reproductive Biology
STAFF1: O

INVES2: Platzman, Kathleen A.
DEGREE2: Ph.D.
DEPT2: Reproductive Biology
STAFF2: O

INVES3: Suomi, Stephen J.
DEGREE3: Ph.D.
DEPT3: Reproductive Biology
STAFF3: O

INVES4: Swenson, R. Brent
DEGREE4: D.V.M.
DEPT4: Veterinary Medicine
STAFF4: C

SPECIES1: Pan troglodytes
NUM1: 11

NON-HOST INST: NICHD, Laboratory of Comparative Ethology (SJS)

ABSTRACT: Each nursery-reared chimpanzee infant was tested once a month, from the age of 3 months to 12 months, using the Bayley Scales of Infant Development. Tests were conducted on 11 infants, 5 infants began testing during this period and 6 infants were given continuing assessments. Comparisons were made between standard care infants and responsive care infants. This human-based test is used to assess temperamental responsiveness and manipulative abilities. This research is ongoing. Two scores were obtained from each chimpanzee's test: a mental development index (MDI) and an infant behavior record (IBR). Chimpanzees, 3-7 months of age, performed at higher cognitive levels than humans at the same age, whereas the same chimpanzees at 8-12 months of age performed at lower levels than humans. No differences in MDI were found between standard care and responsive care infants. The groups did not differ (1) in responsiveness to the demands of the task amount (2) amount or (3) coordination of physical activity, or (4)

auditory-visual reactivity but responsive care infants exhibited more positive affect and less negative emotional reactions compared with standard care infants. The value of this study is to provide species comparisons of cognitive competence, to provide data on individual difference in emotional responsivity, and to provide a normative database to evaluate the effectiveness of behavioral interventions.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Attachment in Nursery-Reared Pan troglodytes: Ainsworth Strange Situation

AXIS I: 1a

AXIS II: 36, 60, 71

PRC UNIT: Reproductive Biology

INVES1: Bard, Kim A.
DEGREE1: Ph.D.
DEPT1: Reproductive Biology
STAFF1: O

INVES2: Swenson, R. Brent
DEGREE2: D.V.M.
DEPT2: Veterinary Medicine
STAFF1: C

SPECIES1: Pan troglodytes
NUM1: 6

NON-HOST INST: NA

ABSTRACT: The quality of attachment in six additional nursery-reared chimpanzees was assessed using the Ainsworth Strange Situation. This research is ongoing. Each individual was tested with his or her favorite caregiver as the 'mother', and a completely unknown female as the stranger. The distribution of major attachment classifications in previously tested nursery-reared chimpanzees was similar to that found in human infants by Ainsworth. During this year, reliability in the scoring system applied to Strange situation behavior was assessed in 30 human infants. Training is continuing. Attachment mechanisms in chimpanzee infants parallel those in human infants. Research from other cultures suggest that minimal exposure to novelty, frequency of separations from caregivers, and multiple attachments may all influence attachment at 1 year. Knowledge obtained from this study can be used to design environments that can maximize behavioral competence.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Development of Self-Recognition in Pan troglodytes

AXIS I: 1a

AXIS II: 36, 41, 60

PRC UNIT: Reproductive Biology

INVES1: Bard, Kim A.
DEGREE1: Ph.D.
DEPT1: Reproductive Biology
STAFF1: 0

SPECIES1: Pan troglodytes
NUM1: 11

NON-HOST INST: NA

ABSTRACT: The goals of this study are to determine the age at which chimpanzees recognize their mirror image and to document mirror-directed behavior that many develop concurrently with this ability. Chimpanzees tested with the Gallup self-recognition paradigm exhibited clear evidence for self-recognition at 2 1/2 years of age. There was no compelling evidence for self-recognition in any of the four 2-year old chimpanzees. In the current year an additional 4 subjects have been videotaped in front of the mirror. Analysis suggests that mirror self-recognition develops between 28 and 30 months of age. Although only 1 of 5 28 month-olds exhibited evidence of self-recognition, all four of the 30-month-olds exhibited clear evidence for mirror self-recognition. These results highlight the developmental parallels in chimpanzee and human cognition.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Salivary Cortisol Levels

AXIS I: 1a

AXIS II: 36, 41

PRC UNIT: Reproductive Biology

INVES1: Bard, Kim A.
DEGREE1: Ph.D.
DEPT1: Reproductive Biology
STAFF1: 0

SPECIES1: Pan troglodytes
NUM1: 16

NON-HOST INST: NA

ABSTRACT: Baseline salivary cortisol values were obtained on 14 individuals ranging in age from 2 days to 12 months. One goal of this research is to document the physiological basis for emotional responsiveness in chimpanzees. Baseline cortisol levels in chimpanzees are similar to the levels found in human infants. Cortisol samples were obtained before and after neonatal neurobehavioral assessments for 5 subjects. Cortisol samples were obtained before and after tests of cognitive/manipulative ability (i.e., the Bayley Scales of Infant Development) for 11 subjects. Significant correlations were found between emotional responses (happiness, fear, and tension) and changes in cortisol levels during the months of BSID testing. This is research in progress. Future research designed to maximize behavioral competence can be evaluated according to individual changes in cortisol levels.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Ontogeny of Emotional Expression

AXIS I: 1a

AXIS II: 36, 41

PRC UNIT: Reproductive Biology

INVES1: Bard, Kim A.
DEGREE1: Ph.D.
DEPT1: Reproductive Biology
STAFF1: 0

SPECIES1: Pan troglodytes
NUM1: 40

NON-HOST INST: NA

ABSTRACT: Facial and vocal emotional expressions of 40 chimpanzees were studied as a part of a long-term study of captive care of chimpanzees. Emotional expressions were recorded during the NBAS, administered every other day from 2 days after birth through 30 days of age. All infants were given standard nursery care but 10 of the 40 neonates were given responsive caregiving 20 hours per week in addition. Facial expressions of happiness ("playface" and smiles), interest, anger (bulging and compressed lips), and distress (pout face and scream face) were given in response to contextual cues, i.e., animated examiners face, environmental stimuli, physical manipulations, lack of contact and elicitation of reflect items. In fact, many of the same NBAS items were responded to with the same emotional expression by both chimpanzee and human newborns. Vocal expressions of activity (effort grunt), distress (hoo, whimper, and scream), greeting, anger (threat bark), and alarm were also found. Individual differences were noted. The emotional expressions that were found in neonatal chimpanzees are similar both in form and in emotional tone to those found in adult chimpanzees. In addition, most emotions are expressed in the same context and in response to the same type of stimuli for both neonates and adults. Learning and experience appear to be required in order for infant chimpanzees to use alarm calls and threats in the appropriate situations. Finally, the emotional tone or quality of expression found in neonatal chimpanzees is strikingly similar to the emotional tone of expressions found in human neonates.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Behavioral Interventions

AXIS I: 1a

AXIS II: 36, 41

PRC UNIT: Reproductive Biology

INVES1: Bard, Kim A.
DEGREE1: Ph.D.
DEPT1: Reproductive Biology
STAFF1: 0

SPECIES1: Pan troglodytes
NUM1: 15

NON-HOST INST: NA

ABSTRACT: Chimpanzee management programs can be evaluated according to a protocol of behavioral assessments. Standard nursery-rearing for chimpanzees at the Yerkes Research Center consists of groups of three to six peers formed as early as 6 weeks of age given human contact during caregiving activities. Normative data have been collected on infants raised under these standard nursery conditions. A behavioral intervention project, conducted during the last 3 years, was designed to (1) provide responsive caregiving to nursery-reared chimpanzees, which more closely approximates species-typical rearing; (2) to provide monitored and protected access to younger individuals, an experience that more closely approximates that which older siblings receive in species-typical family groups. Preliminary analyses indicate that the behavior and neurobehavioral integrity of infants, 2 to 30 days of age, does not differ in the two different rearing conditions. Differences are found, however, in early emotional expressiveness. Changes in emotional responsiveness also appear to effect subsequent performance on standardized tests of cognitive and manipulative performance given to infants from 3 to 13 months of life. The second intervention has proven successful in providing older infants with hands-on experience with younger infants. We predict enhanced maternal competence in those individuals who had hands-on experiences as infants. The implications of this research relate to long-term effects of early experiences and early learning.

animals will be re-challenged. These experiments, once successful, will provide an animal model for examining the specific immunity to these viruses that may or may not be protective.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Synthesis and Biotransformation of Anti-HIV Prodrugs: Biotransformations of 2'-F-ara-ddI Analogues

AXIS I: 1a, 2, 28 Pharmacokinetics

AXIS II: 31

PRC UNIT: Pathobiology & Immun

INVES1: Schinazi, Raymond
DEGREE1: Ph.D.
DEPT1: Pathobiology and Immunobiology
STAFF1: O

INVES2: McClure, Harold M.
DEGREE2: D.V.M.
DEPT2: Pathobiology and Immunobiology
STAFF2: C

SPECIES1: Macaca mulatta
NUM1: 3

NON-HOST INST: Veterans Administration Hospital, Atlanta, GA (RS)

ABSTRACT: 2'-F-ara-ddI is undergoing clinical trials at the NIH as a stable analogue of ddI, and 2'-F-ara-ddA is expected to undergo clinical trials soon. Extensive studies of these novel prodrugs already synthesized in our laboratory have been performed. Studies in rodents and monkeys have included determination of toxicity, brain targeting, oral bioavailability, and half-life. This work is based on a new strategy which utilizes xanthine oxidase mediated biotransformation in designing anti-HIV prodrug 2'-F-ara-ddI. 2'-F-ara-ddI is a stable analogue of ddI, which was reported to be as effective as ddI in vitro. We have developed an efficient synthetic method for the prodrug, 2'-F-ara-ddP and evaluated its in vitro stability and pharmacokinetics in mice and monkeys.

In vitro study indicated that 2'-F-ara-ddP is stable in acid (pH 2) and mouse serum, while in mice liver homogenate 2'-F-ara-ddP was almost completely converted to 2'-F-ara-ddI within 20 min ($t_{1/2}$ = 3.5 min). The prodrug was also biotransformed to 2'-F-ara-ddI by xanthine oxidase in vitro, which was inhibited by allopurinol, a xanthine oxidase inhibitor. The biotransformation was also detected in brain homogenate, although the rate of conversion from 2'-F-ara-ddP to 2'-F-ara-ddI was slow. Interesting results have been observed from in vivo studies regarding the concentration of 2'-F-ara-ddI following oral administration of 2'-F-ara-ddP. The level of parent drug was close to the detection limit, while 2'-F-ara-ddI was detected at significantly higher concentrations in the brain after oral administration of 2'-F-ara-ddP. From this study, we have demonstrated for the first time the enhanced brain delivery of anti-HIV nucleosides utilizing the xanthine oxidase mediated

biotransformation system. Therefore, it was of interest to study 2'-F-ara-ddP as a potentially useful prodrug for 2'-F-ara-ddI in rhesus monkeys, an animal model which is the closest to humans.

Our recent preliminary studies in rhesus monkeys indicated that significant amounts of 2'-F-ara-ddP and 2'-F-ara-ddI could be detected in the CSF after oral administration of 2'-F-ara-ddP. Again, we have demonstrated that we can deliver the ddI analogue to the CNS using the biotransformation system mediated by xanthine oxidase. Further in-depth studies in monkeys with 2'-F-ara-ddP as well as comparative studies with 2'-F-ara-ddI itself are required to fully assess the potential of 2'-F-ara-ddP as a clinically improved anti-HIV prodrug for 2'-F-ara-ddI.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Synthesis and Biotransformation of Anti-HIV Prodrugs: Antiviral Purine Dioxyolanes

AXIS I: 1a, 2, 28 Pharmacokinetics

AXIS II: 31

PRC UNIT: Pathobiology & Immun

INVES1: Schinazi, Raymond
DEGREE1: Ph.D.
DEPT1: Pathobiology and Immunobiology
STAFF1: O

INVES2: McClure, Harold M.
DEGREE2: D.V.M.
DEPT2: Pathobiology and Immunobiology
STAFF2: C

SPECIES1: Macaca mulatta
NUM1: 3

NON-HOST INST: Veterans Administration Hospital, Atlanta, GA (RS)

ABSTRACT: Recently, we discovered that several enantiomerically pure β-D-dioxolane-purine nucleosides have potent and highly selective anti-human immunodeficiency virus type 1 (HIV-1) and anti-hepatitis B virus (HBV) activity. We are currently studying their in-depth virological properties and biochemical pharmacology in vitro and in vivo, including rhesus macaques, to determine their potential clinical usefulness. Hence, we plan to continue studying these prodrugs of (-)-β-D-dioxolane-guanine (DG), to improve the efficacy and pharmacokinetic properties.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Metabolism of 3'-Deoxy-2', 3'-Didehydrothymidine

AXIS I: 1a, 2, 17

AXIS II: 31, 50a, 74c

PRC UNIT: Pathobiology & Immun

INVES1: Sommadossi, Jean-Pierre
DEGREE1: Pharm. D., Ph.D.
DEPT1: Pathobiology and Immunobiology
STAFF1: O

INVES2: McClure, Harold M.
DEGREE2: D.V.M.
DEPT2: Pathobiology and Immunobiology
STAFF2: C

SPECIES1: Macaca mulatta
NUM1: 2

SPECIES2: Macaca fascicularis
NUM2: 4

NON-HOST INST: University of Alabama at Birmingham (J-PS)

ABSTRACT: The metabolism of 3'-deoxy-2', 3'-didehydrothymidine (D4T) was evaluated in isolated hepatocytes and in nonhuman primates. Rapid formation of thymine and β-aminoisobutyric acid (BAIBA) occurred following incubation of hepatocytes with 10 μM [5-^{3}H]D4T. Substantial levels of tritiated water were also detected. Exposure of cells to D4T in the presence of either 1 mM thymine or 10 μM benzyloxybenzyluracil, an inhibitor of dihydropyrimidine dehydrogenase, decreased intracellular BAIBA levels by approximately 89 and 63% respectively. Concurrently [^{3}H]thymine levels increased two- to fivefold. These results are consistent with D4T being cleaved to thymine, which is then degraded to BAIBA. A similar metabolic disposition was observed in monkeys following administration of 25 mg of [5-^{3}H]D4T per kg of body weight. BAIBA, thymine, and tritiated water were identified in plasma and urine. Approximately 50% of the administered dose was recovered in urine within 24 hours, with the majority of the radioactivity representing unchanged drug. After administration intravenously or orally of 25 mg of [4-^{14}C]D4T per kg of body weight to monkeys, a novel metabolite, designated X, in addition to unchanged D4T, thymine, and BAIBA, was also detected. The sum of the three metabolites and unchanged drug accounted for virtually all of the radioactivity in plasma and urine. Thymine and X exhibited kinetic profiles similar to that of D4T, with plasma elimination half-life of 2 to 3 h, whereas BAIBA levels remained constant for extended periods and declined slowly; this metabolite could be detected 24 h after intravenous drug administration. Mean oral bioavailability of D4T was high at approximately 70%. As observed in the [5-^{3}H]D4T study performed in monkeys, approximately half of the administered

[4-^{14}C]D4T was recovered unchanged. The remainder was not recovered in urine or feces collected up to 30 days after administration. These data suggest that D4T metabolites are further metabolized by salvage pathways and/or converted to biological macromolecules.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Detection, Quantification and Characterization of Nonhuman Primate Cytokines

AXIS I: 1a, 13, 2, 9, 15

AXIS II: 39, 58, 64

PRC UNIT: Pathobiology & Immun

INVES1: Villinger, Francois
DEGREE1: D.V.M.
DEPT1: Pathobiology and Immunobiology
STAFF1: O

INVES2: Ansari, Aftab A.
DEGREE2: Ph.D.
DEPT2: Pathobiology and Immunobiology
STAFF2: C

SPECIES1: Cercocebus atys
NUM1: 6

SPECIES2: Macaca mulatta
NUM2: 6

SPECIES3: Macaca nemestrina
NUM3: 4

NON-HOST INST: NA

ABSTRACT: Efforts have been devoted at expanding the number of nonhuman primate cytokines that can be detected and quantified to address their potential relation to disease resistance or susceptibility in lentivirus infections.

Oligoprimers and probes were designed and tested for the detection of IL-12α, IL-12β, IL-13, GM-CSF, TGFβ from human, macaque and mangabey origin, using a sensitive reverse transcription/polymerase chain reaction amplification assay.

In addition, cDNAs were cloned and sequenced from mangabeys and macaques that code for IL-2, IL-4, IL-5, IL-6, IL-10, IL-12α, IL-12β and γIFN, to determine differences accounting for antigenic detection and activity levels of these cytokines from nonhuman primates.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Role of Nef in SIVsmm9 Infection

AXIS I: 1a, 1d, 7b

AXIS II: 31, 59, 66

PRC UNIT: Pathobiology & Immun

INVES1: Villinger, Francois
DEGREE1: D.V.M.
DEPT1: Pathobiology and Immunobiology
STAFF1: O

INVES2: Ansari, Aftab A.
DEGREE2: Ph.D.
DEPT2: Pathobiology and Immunobiology
STAFF2: C

SPECIES1: Cercocebus atys
NUM1: 2

SPECIES2: Macaca mulatta
NUM2: 6

SPECIES3: Macaca nemestrina
NUM3: 1

NON-HOST INST: NA

ABSTRACT: Based on the finding that the Nef gene is indispensable for the development of SIV induced disease in macaques, and our finding that asymptomatic mangabeys that are naturally infected with SIV harbor essentially SIV isolates with an open Nef gene, the objective in this study was to investigate a possible role for Nef in long term survivor macaques that were inoculated with uncloned SIVsmm9 or its PBj derivative.

All SIV Nef clones obtained from macaques with a rapid disease course were as expected all open. All Nef clones however, obtained from SIVsmm9 inoculated but asymptomatic mangabeys were also open, as well as all Nef clones isolated from a healthy pigtail macaque that survived SIVsmmPBj infection for 7 years. SIV Nef clones from 2 healthy rhesus macaques (4 and 6 years pi respectively) infected with uncloned SIVsmm9 were all truncated and showed over 17% sequence divergence with i) the published SIVsmm9 sequence or ii) Nef clones from other SIVsmm9 infected animals. Taken together, these data confirm that Nef is required for induction of disease in macaques infected with SIVsmm but Nef does not appear to be the sole determinant of virulence in these isolates. The data further suggest that a minor (avirulent?) SIVsmm isolate is included in the original uncloned SIVsmm9 stock, a variant that appears to have selective growth advantage in a low percentage of infected animals.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Thrombosis and Vascular Lesion Formation

AXIS I: 1a, 13, 17

AXIS II: 39, 48, 86

PRC UNIT: Pathobiology & Immun

INVES1: Wilcox, Josiah N.
DEGREE1: Ph.D.
DEPT1: Pathobiology and Immunobiology
STAFF1: O

INVES2: Kelly, Andrew
DEGREE2: D.V.M.
DEPT2: Pathobiology and Immunobiology
STAFF2: C

INVES3: Harker, Laurence A.
DEGREE3: M.D.
DEPT3: Pathobiology and Immunobiology
STAFF3: O

INVES4: Hanson, Stephen
DEGREE4: Ph.D.
DEPT4: Pathobiology and Immunobiology
STAFF4: O

SPECIES1: Papio cynocephalus
NUM1: 15

NON-HOST INST: NA

ABSTRACT: Angioplasty has achieved wide acceptance for the treatment of coronary artery disease but remains complicated by the development of a proliferative restenosis within 3-6 months. The rate of human post-angioplasty restenosis varies, but typically 30-40% of those individuals undergoing this procedure will experience recurrence of symptoms and require another interventional procedure. The rat and rabbit have traditionally been used as models for human restenosis. Unfortunately, these models have not been predictive of efficacy of drugs to prevent this significant clinical problem. The studies proposed in the present grant on vascular lesion formation after arterial injury in nonhuman primates represent the first examination of lesion formation in vessels that more closely resemble humans. Our studies this year have yielded new information regarding the role of a protein normally involved in blood coagulation, thrombin, and its action to directly promote the growth of cells in the artery wall. The involvement of neutrophils, an inflammatory cell circulating in the blood, has never been demonstrated in rat and rabbit models for post-angioplasty restenosis. However in the nonhuman primate we have found that neutrophils migrate to the

site of injury after angioplasty, where they may stimulate the proliferative response. Additional studies indicate that thrombin may cause the migration of neutrophils to the angioplasty site. It has been hypothesized that after angioplasty cell proliferation begins in the media, the vessel wall underneath the atherosclerotic plaque. Studies in the nonhuman primate now suggest that this assumption may be incorrect and point instead to cell growth beginning in the adventitia, the tissue around the vessel, which was not previously thought to respond at all. Together, these findings have implicated new cells and molecules not previously identified with post-angioplasty restenosis which may be potential targets for therapeutic intervention.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Cytokine Effects on Post-Chemotherapy Immunohematopoietic Regeneration Using a Nonhuman Primate Model

AXIS I: 1a, 1d, 2, 17

AXIS II: 50a, 76b, 88

PRC UNIT: Pathobiology & Immun

INVES1: Winton, Elliott F.
DEGREE1: M.D.
DEPT1: Pathobiology and Immunobiology
STAFF1: O

SPECIES1: Macaca mulatta
NUM1: 9

NON-HOST INST: NA

ABSTRACT: The purpose of these studies was to define the effects of recombinant hematopoietic (rh) growth factors on marrow regeneration after high-dose, stem cell toxic chemotherapy. The chemotherapy employed was hepsulfam given as a single injection, followed by rh growth factor(s) administered by subcutaneous injection, once or twice daily for 21 days. The animals received supportive care during the period of marrow aplasia which included platelet and red cell transfusions, prophylactic antimycotic and antibacterial agents. Recombinant growth factors employed included IL-3, IL-6, G-CSF and GM-CSF. All of these factors have been used as single agents, and in the following combinations: IL-6 + G-CSF; IL-6 + GM-CSF; IL-3 + GM-CSF; IL-3 + IL-6. Regeneration of the hematopoietic system is monitored by Monday, Wednesday, Friday complete blood counts, weekly marrow exam for progenitor cell and CD34 cell quantification.

We have begun studies to develop a clinical protocol using rh blood cell growth factors for optimizing the number of primitive hematopoietic cells in the blood for collection for transplantation. The strategy for these studies involves administering 5 days of early acting cytokines (IL-3, IL-6 or the combination of both factors) followed by 5 days of G-CSF, a cytokine well established as effective in mobilizing primitive cells. The quantification of CD34 subsets includes determination of DR, Thy-1, CD38 and cell cycle status of these CD34+ subsets.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Neurobehavioral Responsivity of Neonatal Nursery-Reared Chimpanzees

AXIS I: 1a, 21, 25

AXIS II: 36, 60, 71

PRC UNIT: Reproductive Biology

INVES1: Bard, Kim A.
DEGREE1: Ph.D.
DEPT1: Reproductive Biology
STAFF1: O

INVES2: Platzman, Kathleen A.
DEGREE2: Ph.D.
DEPT2: Reproductive Biology
STAFF2: O

INVES3: Suomi, Stephen J.
DEGREE3: Ph.D.
DEPT3: Reproductive Biology
STAFF3: O

INVES4: Swenson, R. Brent
DEGREE4: D.V.M.
DEPT4: Veterinary Medicine
STAFF4: C

INVES5: Lester, Barry M.
DEGREE5: Ph.D.
DEPT5:
STAFF5: O

SPECIES1: Pan troglodytes
NUM1: 5

NON-HOST INST: NICHD, Laboratory of Comparative Ethology (SJS); Bradley Hospital and Brown University (BML)

ABSTRACT: From January, 1993 through December 31, 1993, 5 chimpanzee infants were placed in the nursery, due to inadequate maternal care. Their neurobehavioral integrity was assessed with the Neonatal Behavioral Assessment Scale (NBAS) (Brazelton, 1984). When comparisons are made with human infants striking similarities are found in the following areas: capacity for attention to visual and auditory, social and nonsocial stimuli; motor activity, coordination, and muscle control; autonomic nervous system stress. Significant differences between the species were found in two clusters of behavior related to infant state. Analyses reveal that significant

differences exist by the end of the neonatal period at least between the standard nursery-reared group and the biological mother-reared chimpanzees (data collected elsewhere). Higher, more human-like, levels of social responsiveness were found in the standard nursery-reared chimpanzees. That differential responses are not evident at 2 days of age but are evident at 30 days of age points to the very early effects of these different environments on social responsiveness in young chimpanzees. Further analyses comparing responsive nursery-reared with standard nursery-rearing will explore additional flexibility in neonatal chimpanzees' social responsivity.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Enhancement of Lumbar Spine Fusion with Bone Morphogenetic Protein (BMP)

AXIS I: 1a, 1d, 2, 26

AXIS II: 46, 48, 88, 89

PRC UNIT: Reproductive Biology

INVES1: Boden, Scott D.
DEGREE1: M.D.
DEPT1: Orthopaedic Surgery
STAFF1: O

INVES2: Gould, Kenneth G.
DEGREE2: Ph.D.
DEPT2: Reproductive Biology
STAFF2: C

SPECIES1: Macaca mulatta
NUM1: 26

NON-HOST INST: NA

ABSTRACT: Lumbar spinal fusion is commonly performed in humans but the failure rate of bone union is reported to range from 5-36%. Recently, osteoinductive growth factors isolated from bovine long bones have been shown to induce bone formation in heterotopic sites. A bovine derived bone protein (BP) has been effective in generating spine fusions in a rabbit model. To determine the appropriate dose of the growth factor for human use and to determine the speed of healing, a nonhuman primate model has been chosen. Two rhesus monkeys (as of 12/31/93) have undergone lumbar spine fusion and implantation of the BP extract delivered in a carrier of demineralized bone particles. The animals have tolerated the surgical procedure well and preliminary results indicate that the protein will form bone in a higher animal. Additional animals will be utilized in 1994. These studies are critical to providing the information needed for the next step which is human clinical trials. This treatment, if successful, will significantly impact on the care of spine patients and prevent multiple surgeries in the 5-35% of patients who do not heal their spine fusion on the first attempt.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Perineal Swelling of Pan troglodytes and Puerperal Pathology

AXIS I: 1a, 15, 21, 23

AXIS II: 36, 65, 71, 72, 93

PRC UNIT: Reproductive Biology

INVES1: Dahl, Jeremy F.
DEGREE1: Ph.D.
DEPT1: Reproductive Biology
STAFF1: O

INVES2: Gould, Kenneth G.
DEGREE2: Ph.D.
DEPT2: Reproductive Biology
STAFF2: C

SPECIES1: Pan troglodytes
NUM1: 44

NON-HOST INST: NA

ABSTRACT: It follows from the similarity between apes and humans that studies of ape reproduction and pathology are highly comparable to those of humans. Based on this premise, this work represents efforts to understand diseases that affect women by controlled study of Pan. There is evidence that the hormonal milieu of pregnancy may influence puerperal pathology in both human and non-human primates. In a preliminary evaluation of this association in chimpanzees, ano-genital swelling (AGS) was used as a marker for steroid concentration; high scores were assumed to indicate a Major Estrogenic Effect on the Perineum (MEEP). AGS scores were assessed for the occurrence of MEEP in 10 day intervals through 44 pregnancies with various outcomes. Outcome was assessed in terms of infant health and maternal competence. Eleven pregnancies had normal outcomes (infant healthy, adequate maternal competence); MEEP occurred during all 10 day periods in 65% of the females during the first trimester and 77.5% during the second. This contrasts with 20% (first) and 19% (second) in six stillborns. In 17 cases where a healthy infant had to be removed from an incompetent mother, the occurrence of MEEP was 40% and 30% for each trimester and significantly less than in the 11 normal cases. Outcome can be predicted from the AGS scores obtained during the first and second trimesters. These data are consistent with the notion that the hormonal milieu of pregnancy effects the behavior of the mother in the postpartum.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Contraceptive Behaviors of Pan troglodytes

AXIS I: 1a, 15, 18, 21, 23

AXIS II: 36, 71, 72, 93

PRC UNIT: Reproductive Biology

INVES1: Dahl, Jeremy F.
DEGREE1: Ph.D.
DEPT1: Reproductive Biology
STAFF1: O

INVES2: Gould, Kenneth.G.
DEGREE2: Ph.D.
DEPT2: Reproductive Biology
STAFF2: C

SPECIES1: Pan troglodytes
NUM1: 15

NON-HOST INST: NA

ABSTRACT: The resemblance between apes and humans in female reproductive endocrinology is a basis for understanding regulatory mechanisms and pathology; study of apes has implications for human birth control and infertility and our fundamental understanding of human endocrinological mechanisms. In female Pan, it is clear that nipple self-stimulation behaviors (NSBs) can cause a prolonged post-partum amenorrhea (PPAm) when the infant is absent. In attempts to understand and treat low fertility in captive Pan attention was focused on how NSBs might also disrupt ovulation and implantation as well as cause prolonged post-partum amenorrhea (PPAm). Observation of five subjects revealed: 1) Four levels of NSB occur; 2) Three of these patterns occur in subjects with PPAm and with menstrual cycles; 3) Levels of NSB change through the ovarian cycle. Additional observations of 65 minutes duration each (N = 300) were completed on 15 subjects and a mother with infant. Results show that the four levels of auto-NSB occur consistently across a large number of subjects that are infertile, and that at least two levels correspond closely with nipple stimulation during suckling by an infant. It is possible that different levels of NSB effect the appearance of distinctive hormonal profiles.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Prolactin and the Regulation of the Ovarian Cycle and Pregnancy

AXIS I: 1a, 15, 18, 21, 23

AXIS II: 36, 71, 72, 93

PRC UNIT: Reproductive Biology

INVES1: Dahl, Jeremy F.
DEGREE1: Ph.D.
DEPT1: Reproductive Biology
STAFF1: O

INVES2: Gould, Kenneth G.
DEGREE2: Ph.D.
DEPT2: Reproductive Biology
STAFF2: C

INVES3: Bonsall, Robert W.
DEGREE3: Ph.D.
DEPT3:
STAFF3: O

SPECIES1: Pan troglodytes
NUM1: 15

NON-HOST INST: NA

ABSTRACT: Our interest in the regulatory role of prolactin (PRL) arises from our attention to hyperprolactinemia during the postpartum of chimpanzees and extends to the way it may be implicated in the etiology of Late Luteal Phase Dysphoric disorder and premenstrual Molimena. Attention has been primarily focused on a method by which variation in PRL production can be meaningfully monitored. Sera from nine subjects were analyzed for prolactin (PRL), some of which were observed to self-stimulate their nipples, and were likely to exhibit a hyperprolactinemia, and others were treated with a dopamine receptor agonist that effectively constrains PRL production. A highly specific 3-site immunoradiometric assay (Nichols Institute Diagnostics), previously validated for use in the chimpanzee, was used to measure serum PRL. The assay detected clear differences in PRL concentration between different classes of subject sampled under anesthesia. Those with no NSB had mean PRL of 40 ng/ml (SE = 6 ng/ml), stimulators had mean values about 150 ng/ml, and subjects treated with a dopamine receptor agonist had mean PRL of 6 ng/ml (SE = 1 ng/ml). Subjects with menses and auto-NSB, which had either failed to conceive or aborted early in pregnancy, had elevated PRL. Exploratory application of an RIA to measure PRL in urine did not distinguish stimulators from non-stimulators. The nature of the behavior and the action of oxytocin (OT) as a PRL releaser, coupled with what is known of the luteolytic action of OT in women, implicates OT in both the proximate reinforcement of the behavior, and as a disruptive influence on the luteal phase and/or the establishment of pregnancy.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Conservation of Neotropical Forests and Populations of Ateles and Alouatta

AXIS I: 1a, 8, 11, 23

AXIS II: 34, 36, 54b

PRC UNIT: Reproductive Biology

INVES1: Dahl, Jeremy F.
DEGREE1: Ph.D.
DEPT1: Reproductive Biology
STAFF1: 0

SPECIES1: Alouatta pigra
NUM1: < 20 (wild)

SPECIES2: Ateles geoffroyi
NUM1: < 20 (wild)

NON-HOST INST: NA

ABSTRACT: This program continued studies in Belize, Central America, on a spider monkey (Ateles geoffroyi) and a howler monkey (Alouatta pigra), which have either vulnerable or endangered status consequent to their relatively large size and the preferences of hunters, on the one hand, and destruction or disruption of their forest habitat, on the other. Surveys of remaining habitat yield results that are critical for long-term management. Moreover, the development of appropriate and cost-effective patrolling of the forest is necessary to minimize disruption that can severely impact monkey populations. Current options for deploying Forest Guards rely on expensive 4-wheel drive vehicles. A field trip to Belize in May and June evaluated: A] The status of forest immediately above the confluence of the Upper Macal and Raspaculo Rivers below which a large dam was planned. The dam would inundate most of the riverine habitat of the Chiquebul Forest Reserve; B] The effectiveness of a bicycle to provide appropriate transport to and from relatively remote forest habitats. An area of 5 km^2 was surveyed by a three man team (Dahl, Laylor, and Howe) from elevations about the Upper Macal at about 380 meters elevation to above 650 m. Alouatta pigra were heard at about 500 m, and reports of Ateles were obtained from the nearby Raspaculo drainage and from further up the Upper Macal. The forest cover was patchy; quite high forest on some ridges included cedar, but dense thicket was frequent closer to the base of some drainages. The bird fauna was diverse in the riverine and lower lying forest; a partial listing totalled 65 species including two large woodpecker species and numerous scarlet macaw. **Much of the biodiversity in the area, including habitat that supports both Ateles and Alouatta as well as tapir, is limited to that part of the drainage which will be severely impacted by a dam.** Remnants of hardwood regrowth at higher elevation may not be so severely

impacted, but there will be insularization effects produced by the long, wide finger lakes that would be created by the dam so extinction rates over at least a third of the Chiquebul Forest are likely to be high. The bicycle was designed for rugged conditions, had 21 gear settings, and was fitted with a double pannier. It proved to be a most cost-effective and versatile form of transportation under relatively dry conditions. It was used: (a) To provide access to the Bermudian Landing monkey reserve from Belize City (round-trip, 83 kms, coastal plain); (b) To assess communication and transportation logistics between the Forest Station at Augustine and the survey site south of the Upper Macal (round trip 50 - 60 km., hilly); (c) Provide access for investigator and supplies to a howler monkey study site in the Rio On/Pinol area (round trip about 20 km). Bicycles can play a productive part of extensive patrolling activity at a relatively low cost.

P51RR00165-33 1/1/1993 - 12/31/93 Yerkes Regional Primate Research Center

TITLE: Primate Vision and Acuity Under Dim Light Conditions

AXIS I: 1a, 21, 25b

AXIS II: 34, 36, 54b, 85

PRC UNIT: Reproductive Biology

INVES1: Dahl, Jeremy F.
DEGREE1: Ph.D.
DEPT2: Reproductive Biology
STAFF2: 0

SPECIES1: Alouatta pigra
NUM1: > 100 (wild)

NON-HOST INST: NA

ABSTRACT: Documentation of arrhythmic activity patterns in both prosimian and anthropoid taxa raises questions about characterizing primates as nocturnal or diurnal. This has implications for understanding design features of the visual system of primates such as binocularity and color vision. In attempts to articulate hypotheses about selection for such design features and visual ability under dim conditions (e.g., twilight and moonlight), illumination in a primate habitat was assessed. A central question concerned how pervasive dim conditions are at times other than about sunrise and sunset. Illumination inside and outside the forest habitat of Alouatta pigra was measured in Belize, Central America, under both cloudy and clear skies using a Panlux meter (Gossen) with a range of 0.05 - 200,000 footcandles (fc). Measurements were made from before sunrise till after sunset during May and June, and at nights about the full moon. Illumination was reduced by the canopy to a twilight range (< 40 fc) for most of the day when it was cloudy, and for approximately half the day when skies were clear. Strong moonlight was estimated to be about 0.025 fc on the canopy. It is suggested that ability to see substrates and vegetable foods in these dim conditions was selected for in early primate or pre-primate ancestral stocks. It would allow arboreal foraging for buds, fruits, and leaves over discontinuous substrates underneath and at the lower levels of the canopy during the day as well as the upper strata of the canopy during moonlit parts of the night. Primate vision may have its origins in selection for visual ability in the dim.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: In Vitro Fertilization

AXIS I: 1a, 23

AXIS II: 60, 63h, 77

PRC UNIT: Reproductive Biology

INVES1: Gould, Kenneth G.
DEGREE1: Ph.D., DVM
DEPT1: Reproductive Biology
STAFF1: C

INVES2: Dahl, Jeremy F.
DEGREE2: Ph.D.
DEPT2: Reproductive Biology
STAFF2: O

SPECIES1: Pan troglodytes
NUM1: 12

NON-HOST INST: NA

ABSTRACT: As part of ongoing studies directed to development of improved breeding methods for endangered primates and development of primate models for understanding of SIV and HIV resistance, we are establishing IVF at the Yerkes Center. Six chimpanzees have undergone hormone stimulation for the purpose of oocyte recovery. It is apparent that stimulation of the chimpanzee with Metrodin and Pergonal is more effective when the female is previously downregulated by exposure to GnRH agonist for 21 - 30 days prior to stimulation. Successful response is associated with the recovery of 8 - 10 oocytes either by laparoscopy or by use of newly developed methods for transvaginal ultrasound guided follicle aspiration. Fertilization has been successful in each case, but reimplantation has not resulted in pregnancy. When successful in the common chimpanzee this work will lead to the use of Pan troglodytes females as surrogate mothers for Pan paniscus embryos also obtained by IVF.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Ultrasound Evaluation of Fertility Status in the Chimpanzee

AXIS I: 1a, 23

AXIS II: 63h

PRC UNIT: Reproductive Biology

INVES1: Gould, Kenneth G.
DEGREE1: Ph.D., DVM
DEPT1: Reproductive Biology
STAFF1: C

INVES2: Dahl, Jeremy F.
DEGREE2: Ph.D.
DEPT2: Reproductive Biology
STAFF2: O

SPECIES1: Pan troglodytes
NUM1: 28

NON-HOST INST: NA

ABSTRACT: As part of research programs in artificial breeding and ape reproductive biology, ultrasonography is being used to assess ovarian follicular growth, uterus size, and endometrial development in female chimpanzees (*Pan troglodytes*). A total of 28 subjects were examined during 87 ovarian cycles using 5 MHz abdominal and transvaginal transducers with a General Electric RT 3200 ultrasound system. Review of the images produced showed that the abdominal probe was most useful for assessing the uterus and endometrial development, while the vaginal probe enabled detailed views of the ovaries, follicles and early corpora lutea. Typically, several small follicles appeared early in the cycle, followed by emergence of the "dominant" follicle prior to ovulation. Follicle size and number were not significantly different between natural and clomiphene stimulated cycles ($n=102$, $p=0.84$ for size; $n=93$, $p=0.40$ for number). Cycles stimulated with human LH/human FSH (Metrodin, Pergonal) alone or in combination, produced multiple large follicles. Uterine pathologies such as endometrial hyperplasia were identified via the abdominal probe. Ultrasonography yields valuable information about the reproductive state of female chimpanzees.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Induction of Twinning

AXIS I: 1a, 9, 23

AXIS II: 60, 77

PRC UNIT: Reproductive Biology

INVES1: Gould, Kenneth G.
DEGREE1: Ph.D., DVM
DEPT1: Reproductive Biology
STAFF1: C

INVES2: Younis, Abdelmoneim I.
DEGREE2: Ph.D.
DEPT2: Reproductive Biology
STAFF2: O

SPECIES1: Mus musculus
NUM1: 240

NON-HOST INST: NA

ABSTRACT: In an effort to produce identical twins from nonhuman primates, we have pursued the use of microinjection and micromanipulation techniques to create double blastocysts from two cell mouse embryos. Solutions of sodium alginate and calcium chloride are introduced, in sequence, between the blastomeres of two cell mouse embryos. Development has been followed in vitro, and the establishment of two blastocysts within a single zona pellucida documented up to the time of blastocyst hatching. Current efforts are directed to measurement of the number of offspring developed after transfer of in vitro developed embryos into recipient, pseudopregnant, females. The availability of identical primate twins would significantly reduce the number of individuals needed for both cognitive and pharmaceutical studies as a large portion of genetic and behavioral variability would be removed from the study population.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Functional Assays of Sperm Fertilizing Capacity

AXIS I: 1a, 9, 23

AXIS II: 73, 92 (fertility)

PRC UNIT: Reproductive Biology

INVES1: Gould, Kenneth G.
DEGREE1: Ph.D., D.V.M.
DEPT1: Reproductive Biology
STAFF1: C

INVES2: Young, Leona G.
DEGREE2: Ph.D.
DEPT2: Reproductive Biology
STAFF2: O

SPECIES1: Pan troglodytes
NUM1: 14

SPECIES2: Mesocricetus auratus
NUM2: 50

NON-HOST INST: NA

ABSTRACT: Use of functional assays as measures of potential fertilizing capacity is especially pertinent when direct evaluation of fertilizing capacity using natural or artificial breeding of the female is logistically impractical, a situation which often pertains when endangered or threatened species are under study. Ejaculates were obtained from anesthetized chimpanzees using rectal probe electrostimulation and from conscious chimpanzees using an artificial vagina. Semen was collected between 0830-1030 h. Ejaculate collections from the same animal were separated in time by a minimum of 4 days. Computer assisted motion analysis was conducted on sperm recovered after 30 minutes of semen liquefaction at 37°C. Motion analysis was conducted on samples collected for this study (N/RPE=15; N/AV=25) and on previously stored videotape of samples collected under the same conditions (N/RPE=15; N/AV=36). The taped images were digitized using a Motion Analysis VP110 processor operating at 30 frames per second (Motion Analysis, Santa Clara, CA) and analyzed using CTS software, version 3.2. Calculation was performed for velocity (VCL), speed (VSL), linearity (LIN) and lateral head movement (ALH) for at least 100 sperm from each sample. Oocytes were cultured at 37°C for four or twenty-four hours in 0.25ml of Hams F10 with 15% HSA under a humidified atmosphere of 5% CO2, 5% O2 and 90% N2. The sperm concentration was adjusted to $5x10^6$/ml of total cells recovered from Hams F10 overlayed upon the sperm pellet obtained by centrifugation of the liquefied portion of the semen sample. The unstained eggs were observed on a Zeiss IM35 microscope using phase contrast illumination and the number of oocytes penetrated and the number of penetrations per oocyte recorded.

Table 1: Comparison of quantitative semen parameters related to method of collection: (Mean +/- S.E.)

Method	# Sperm used	Motile %	VSL (μ/sec)	VCL (μ/sec)	LIN (0-100)	ALH (μ)
RPE	113.1 (7.36)	83.97 (2.52)	35.37 (3.68)	93.77 (7.56)	37.13 (1.54)	9.23 (1.78)
AV	130.31 (5.5)	88.31 (2.09)	41.77 (2.15)	106.5 (3.61)	39.71 (1.49)	6.77 (3.86)

Table 2: Comparison of SPA with regard to method of semen collection: (Mean +/- S.E.)(N=5)

Method of Collection	Incubation Hours	Percent Penetrated	# Sperm per oocyte
A.V.	4	90(8.94)	1.6(0.55)
A.V.	24	87(7.43)	2.17(0.46)
A.V.	Total	88.5(5.56)	1.89(0.34)
R.P.E.	4	82.5(10.23)	1.3(0.17)
R.P.E.	24	69.4(5.24)	1.9(0.34)
R.P.E.	Total	75.2(5.12)	1.6(0.22)

There were no significant differences detected between any of the variables, collection method, time of incubation, percent penetration or number of sperm penetrating the oocyte. Further, there was no significant increase in the penetration rate or number of sperm penetrating the oocyte which corresponded with the increased length of incubation.

The results imply that sperm recovered by RPE retain their fertilizing capacity and that the lack of success in use of such sperm for artificial insemination in the chimpanzee and human resides with difficulties associated with the insemination procedure itself.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Evaluation of Vascular Graft Materials

AXIS I: 1a, 1d, 3, 17

AXIS II: 48, 50, 52, 63i

PRC UNIT: Reproductive Biology

INVES1: Harker, Laurence A.
DEGREE1: M.D.
DEPT1: Pathobiology and Immunobiology
STAFF1: 0

INVES2: Anderson, J.A.
DEGREE2: M.A.
DEPT2:
STAFF2: 0

SPECIES1: Papio
NUM1: 60

NON-HOST INST: NA

ABSTRACT: As part of ongoing studies to reduce the degree and extent of thrombus formation after organ transplantation or implantation of artificial devices, SEM has been used to evaluate the effect of various treatments of Goretex (R) vascular grafts on thrombus development. Grafts have been coated with collagen and seeded with cells from baboon jugular endothelium. The cells used for seeding have also been transfected with additional tPA genes to increase cell anti-thrombogenic activity. SEM is most valuable for determining the extent of cell coverage of the lumen, presence of thrombus and the condition of the underlying collagen matrix.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Neonatal Testosterone and Primate Sexual Development

AXIS I: 1a, 1d, 2

AXIS II: 60, 64, 92 (reproductive endocrinology)

PRC UNIT: Reproductive Biology

INVES1: Mann, David R.
DEGREE1: Ph.D.
DEPT1: Reproductive Biology
STAFF1: O

INVES2: Wallen, Kim
DEGREE2: Ph.D.
DEPT2: Behavioral Biology
STAFF2: C

INVES3: Gould, Kenneth G.
DEGREE3: Ph.D., D.V.M.
DEPT3: Reproductive Biology
STAFF3: C

INVES4: McClure, Harold M.
DEGREE4: D.V.M.
DEPT4: Pathobiology and Immunobiology
STAFF4: C

INVES5: Ansari, A.A.
DEGREE5: Ph.D.
DEPT5: Pathobiology and Immunobiology
STAFF5: C

SPECIES1: Macaca mulatta
NUM1: 40

NON-HOST INST: Morehouse School of Medicine (DRM)

ABSTRACT: We have been conducting an ongoing study designed to determine whether neonatal treatment of male rhesus monkeys with an LH-releasing hormone (LHRH) analogue (agonist or antagonist) alters sexual and behavioral differentiation and immune system development. As part of this investigation, we examined the effect of treatment with a LHRH agonist (Ag), antagonist (Ant), or Ant and androgen (Ant/And) for the first 4 months of postnatal life on lymphocyte subsets and cellular and humorally mediated immune responses in juvenile and adult male monkeys. We also determined the effect of 9 weeks of Ant treatment on lymphocyte subsets in adult male monkeys. Adult male monkeys

that had been treated neonatally with an Ag had increased levels of CD8-positive ($CD8^+$) T-cells and reduced levels of B-cells compared to vehicle-treated controls. Lymphocytes from these animals also showed an elevated proliferative response to a variety of mitogens compared to cells from control animals. Antibody production in response to tetanus toxoid was normal in treated animals. Other neonates treated with Ant/And exhibited subnormal levels of lymphocytes, $CD8^-$ T-cells, and B-cells at 4 months of age. Similar changes, but of lesser magnitude, were observed in animals treated with Ant alone. At 6 months of age, lymphocytes from both groups of Ant-treated monkeys exhibited an above normal proliferative response to streptolysin-O, but not to other mitogens. At 18 months of age, animals treated with Ant alone produced more antitetanus antibody in response to a tetanus toxoid booster than the controls or Ant/And-treated animals. Ant treatment was without major effect on lymphocyte subsets in adult monkeys. Serum LH and testosterone levels declined, and there was a small but significant increase in B-cells, lymphocytes expressing the interleukin-2 receptor, and the $CD4^+/CD8^-$ T-cell ratio during treatment, but these parameters normalized during the posttreatment period. The data suggest that chronic neonatal treatment with an Ag or Ant alters the development of immune system responses in male primates. The significance of these changes and their impact on the ability of these animals to respond to pathogenic agents is under investigation.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Behavior and Physiology of the Gibbon

AXIS I: 1a, 15, 23

AXIS II: 36, 74e

PRC UNIT: Reproductive Biology

INVES1: Nadler, Ronald D.
DEGREE1: Ph.D.
DEPT1: Reproductive Biology
STAFF1: C

INVES2: Dahl, Jeremy F.
DEGREE2: Ph.D.
DEPT2: Reproductive Biology
STAFF2: O

INVES3: Gould, Kenneth G.
DEGREE3: Ph.D.
DEPT3: Reproductive Biology
STAFF3: C

INVES4: Collins, Delwood C.
DEGREE4: Ph.D.
DEPT4: Reproductive Biology
STAFF4: O

SPECIES1: Hylobates lar
NUM1: 9

SPECIES2: Pongo pygmaeus
NUM2: 17

NON-HOST INST: Univeristy of Kentucky (DCD)

ABSTRACT: The objectives of this research are 1) to determine whether a hypothesis regarding the ultimate (evolutionary) causation of differences in reproductive behavior, anatomy and physiology of the polygamous great apes, extends to the monogamous gibbon, and 2) to determine the relationship among sex hormone levels and female genital swelling and the proximate activation of reproductive behavior in this lesser ape. The hypothesis attributes reproductive differences to differences in intermale competition for estrous females (and female choice of a male at estrus). Research on the gibbon permits us to assess comparatively, the relevance of a monogamous sexual relationship to the regulation of reproductive behavior, anatomy and physiology. During the reporting period, articles were published on the serum and urinary concentrations of sex hormones and genital swelling during the menstrual cycle of female gibbon and on testis size in the orang-utan. The

results indicate that the relationship between sex hormone concentrations and genital swelling in the monogamous gibbon was comparable with that of polygamous female primates, such as the chimpanzee, which live in multimale groups. The genital swelling of the gibbon is a useful marker for monitoring progress of the menstrual and the presumptive time of ovulation, but it is more conspicuous than hypothesized on the basis of intermale competition. The data on testis size in orang-utans suggest that difference among male great apes is related to differences in size of the female reproductive tract. Our research suggests that the evolution of reproductive characteristics in the great and lesser apes is more complex than previously thought and that mechanisms as yet unclear contribute to these characteristics.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Behavioral Effects of an Oral Contraceptive in Chimpanzees

AXIS I: 1a, 15, 23

AXIS II: 36, 50b, 74e

PRC UNIT: Reproductive Biology

INVES1: Nadler, Ronald D.
DEGREE1: Ph.D.
DEPT1: Reproductive Biology
STAFF1: C

INVES2: Dahl, Jeremy F.
DEGREE2: Ph.D.
DEPT2: Reproductive Biology
STAFF2: O

INVES3: Gould, Kenneth G.
DEGREE3: Ph.D.
DEPT3: Reproductive Biology
STAFF3: C

INVES4: Collins, Delwood C.
DEGREE4: Ph.D.
DEPT4: Reproductive Biology
STAFF4: O

SPECIES1: Pan troglodytes
NUM1: 13

NON-HOST INST: University of Kentucky (DCD)

ABSTRACT: The behavioral and physiological effects of a combined oral contraceptive (OC) were studied in chimpanzees for comparative purposes related to 1) the ambiguity surrounding the effects of OCs on the sexuality of humans, 2) the close biological relationship between chimpanzees and humans, especially with respect to hormones and sexual behavior, and 3) the relatively greater behavioral sensitivity of the chimpanzee to changes in sex hormone levels such as those that accompany the use of OCs. Two different types of pair-tests were used to evaluate better separate effects on female and male behavior. During the reporting period, one article was published on the behavioral effects of the OC, one article on the regulation of sexual behavior during natural (control) cycles was accepted for publication and several review articles were accepted for publication. The results of the former study suggest that the OC inhibited sexual behavior differentially as a function of social relationships, profound in less compatible pairs, but less significant in compatible ones. The data likely have relevance to some subset

of human couples with high sensitivity to hormonal influences and/or those with pre-existing marital problems. The study of sexual behavior during the natural menstrual cycle suggests that female hormonal state interacts with the different sexual proclivities of males and females; social compatibility with the partner influences the female's sexual behavior, whereas, the males employ their dominance over the female to mate less discriminatingly. These data are similar to our earlier results on the other great apes and are comparable to data on human couples. They suggest that there are certain similarities in the regulation of sexual behavior among the great apes and that more focused studies of human couples may reveal comparable factors in our species.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Spermatogenesis in Rhesus Macaques Inoculated with SIV Prior to Puberty

AXIS I: 1d, 15, 23

AXIS II: 31, 60, 66, 83

PRC UNIT: Reproductive Biology

INVES1: Nadler, Ronald D.
DEGREE1: Ph.D.
DEPT1: Reproductive Biology
STAFF1: C

INVES2: McClure, Harold M.
DEGREE2: D.V.M.
DEPT2: Pathobiology and Immunobiology
STAFF2: C

INVES3: Anderson, Daniel C.
DEGREE3: D.V.M.
DEPT3: Pathobiology and Immunobiology
STAFF3: C

SPECIES1: Macaca mulatta
NUM1: 26

NON-HOST INST: NA

ABSTRACT: The objectives of the research were 1) to determine if the simian immunodeficiency virus (SIV) has a comparable effect on the reproductive tract of male rhesus macaques as that described for men who die of acquired immunodeficiency syndrome (AIDS), i.e., primarily azoospermia, and if so, 2) to determine if the condition develops as a direct result of (SIV) infection or as a result of the cachexia associated with the disease and 3) to determine the developmental and endocrine basis of the azoospermia. The experimental subjects were 13 rhesus macaques inoculated with SIV between 1.5 and 47 months of age and which died between the ages of 22 and 71 months of age. Thirteen control animals were selected on the basis of comparable age at death to the experimentals and noninvolvement in research on the endocrine system or SIV. During the reporting period, an article was reported on the results. The presence of sperm in testicular tissue of the rhesus macaques that died as a result of infection with SIV was related to age and body weight. Depressed levels of testosterone were not associated with elevated levels of LH, i.e., negative feedback regulation of gonadotropins was compromised. The data suggest that azoospermia in the SIV-infected macaques was due to cachexia and not a direct effect of virus on the testis. These results support a similar hypothesis regarding azoospermia in men infected with human immunodeficiency virus and suggest that the SIV-infected macaque is a useful model for the human in longitudinal studies on the relationship between hormone secretion and disease progression in HIV infection.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Reproductive Behavior in the Chimpanzee

AXIS I: 1a, 15, 23

AXIS II: 36, 74e

PRC UNIT: Reproductive Biology

INVES1: Nadler, Ronald D.
DEGREE1: Ph.D.
DEPT1: Reproductive Biology
STAFF1: C

SPECIES1: Pan troglodytes
NUM1: 6

NON-HOST INST: NA

ABSTRACT: The objective of the research is to determine the cause(s) of increased male sexual initiative and copulation, in relation to the natural habitat, that frequently occurs in laboratory pair-tests of sexual behavior in chimpanzees and other nonhuman primates and more generally, to determine the factors that influence male sexual behavior. The specific hypothesis to be tested states that "greeting rituals", which are a species-typical response to reunion following separation in chimpanzees, account, in part, for increased male sexual initiative in laboratory pair-tests. We use two different types of pair-test to test the hypothesis, 1) an immediate access test in which the door separating the male and female is opened immediately upon introduction of the male into the cage adjoining the female and 2) a delayed access test in which the door is opened after a 5-minute interval. During the reporting period, we used data from delayed access tests to assess penile erection as a measure of male sexual motivation. We hypothesized that penile erection scores and percent time erect 1) would be greater during the midcycle phase of maximal female anogenital swelling than during the early follicular or luteal phases due to maximal female attractivity, 2) would be greater during the early follicular phase than during the luteal phase due to greater female attractivity in the early follicular phase, 3) would decline over the course of a test due to either a) low female attractivity or b) ejaculation, early in the test, when females were attractive; cycle phase differences, therefore, would be most apparent during the initial minutes of the tests, 4) would exhibit the least intermale variability during the midcycle phase due to a ceiling effect and 5) would exhibit the most intermale variability during the early follicular phase due to differences in male responsiveness to subtle increases in female attractivity. The data supported most of the hypotheses; they suggest that penile erection is a useful measure of sexual motivation in the male chimpanzee under controlled laboratory conditions. These results, thereby, support the use of this measure in our continuing investigation of the regulation of male sexual initiative.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Studies in Cardiovascular Morphology

AXIS I: 1a, 1d, 2, 13, 17

AXIS II: 50, 63, 86

PRC UNIT: Reproductive Biology

INVES1: Robinson, Keith A.
DEGREE1: Ph.D.
DEPT1: Reproductive Biology
STAFF1: O

SPECIES1: Porcine
NUM1: 10

NON-HOST INST: NA

ABSTRACT: Various modes of scanning electron microscopy have been used to visualize deposition of In-111 labelled platelets on vascular grafts placed in an ex vivo arteriovenous shunt. This study, together with work directed to observation of colloidal gold particles, characterized by size and shape, used as markers and drug carriers for localized arterial wall perfusion using balloon catheters, is directed to improving our understanding of the changes in vascular lining associated with the success or failure of arterial stenting and balloon catheterization.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Metallic Stent Transplants into Arterial Walls

AXIS I: 1a, 3, 4, 13

AXIS II: 48, 70, 86

PRC UNIT: Reproductive Biology

INVES1: Robinson, Keith A.
DEGREE1: Ph.D.
DEPT1: Reproductive Biology
STAFF1: 0

SPECIES1: Homo sapiens
NUM1: 0 (cell cultures)

NON-HOST INST: NA

ABSTRACT: Studies are underway to determine whether cells grown on metallic stent coils (endovascular prosthesis) can be successfully transplanted into the artery wall. Cells will be cultured on a variety of materials; some will be permeabilized and immunolabelled for an intracellular antigen (SV40) for BSE with colloidal gold. Morphologic, immunospecific, and morphometric characterizations of the cells on these coils, especially those fixed in vitro or acutely transplanted, can best be imaged using SEM.

Additionally, collaborative work is being performed with other investigators for morphologic and immuno characterization of other cell lines: 1) infected with a variety of bacteria and viruses (currently meningitis bacteria on hepatoma cells); 2) EC-SMC co-culture on synthetic collagen matrix; and 3) culture HUVEC for VCAM-1.

P51RR00165-33 1/1/93 - 12/31/93 Yerkes Regional Primate Research Center

TITLE: The Effect of a Human Growth Hormone (hGH) Analogue on IGF-I in Male Rhesus Monkeys

AXIS I: 1a, 2, 15, 26

AXIS II: 50b, 60

PRC UNIT: Reproductive Biology

INVES1: Wilson, Mark E.
DEGREE1: Ph.D.
DEPT1: Reproductive Biology
STAFF1: C

INVES2: Jones, Andy
DEGREE2: Ph.D.
DEPT2:
STAFF1: O

SPECIES1: Macaca mulatta
NUM1: 12

NON-HOST INST: Genentech, Inc., San Francisco, California

ABSTRACT: Studies were initiated this year to test the effectiveness of new growth hormone (GH) preparations. Serum GH and insulin-like growth factor-I (IGF-I) concentrations were monitored in male rhesus monkeys given subcutaneous injections of depot GH preparations. Encapsulation of the injection site was also monitored. Biochemical parameters are still under analysis at Genentech, Inc. These studies will provide much needed information on the effectiveness of these compounds which may alleviate the need for daily injections for those children receiving treatment for growth disorders.

P51RR00165-33 1/1/93- 12/31/93 Yerkes Regional Primate Research Center

TITLE: Prolonged Lactational Infertility During Adolescence

AXIS I: 1a, 2, 15, 23

AXIS II: 36, 60, 71,

PRC UNIT: Reproductive Biology

INVES1: Wilson, Mark E.
DEGREE1: Ph.D.
DEPT1: Reproductive Biology
STAFF1: C

INVES2: Gordon, Thomas P.
DEGREE2: M.S.
DEPT2: Behavioral Biology
STAFF2: C

SPECIES1: Macaca mulatta
NUM1: 13

NON-HOST INST: NA

ABSTRACT: Adolescent rhesus monkey mothers experience a prolonged period of lactational infertility following their first parturition. The mechanisms responsible for this prolonged period of infertility is due, in part, to an increased sensitivity to estradiol negative feedback inhibition on LH secretion during the lactating months in adolescent mothers. This differential sensitivity to estradiol negative feedback is not related to differences in nursing behavior between adolescent and adult mothers. Biochemical analyses of previously collected data were conducted to determine if certain nutritional parameters may be responsible for this enhanced estradiol negative feedback. No difference was observed in milk content between adult and adolescent mothers and that all infants, regardless of the age of their mother, showed the same developmental patterns in food intake. Furthermore, no difference in serum lipids and indices of glucose metabolism were observed between adolescent and adult mothers. These data indicate that the observed differences in LH secretion between adolescent and adult mothers are not the consequences of differential nutritional demands imposed on these lactating mothers. In contrast these differences are likely due to maturational factors which directly affect how estradiol regulates LH-releasing hormone neurons. These studies will not only provide insight into basic mechanisms regulating the duration of lactational infertility but also what factors affect infant health.

P51RR00165-33 1/1/93 - 12/31/93 Yerkes Regional Primate Research Center

TITLE: Common Neuroendocrine Mechanisms for Growth and Puberty

AXIS I: 1a, 2, 15, 23, 26

AXIS II: 60, 71

PRC UNIT: Reproductive Biology

INVES1: Wilson, Mark E.
DEGREE1: Ph.D.
DEPT1: Reproductive Biology
STAFF1: C

INVES2: Gordon, Thomas P.
DEGREE2: M.S.
DEPT2: Behavioral Biology
STAFF2: C

INVES3: Tanner, James M.
DEGREE3: M.D.
DEPT3:
STAFF3: O

SPECIES1: Macaca mulatta
NUM1: 21

NON-HOST INST: Stentwood Auxological Institute, London, England (JMT)

ABSTRACT: Studies were continued this year to determine if insulin-like growth factor-I (IGF-I) regulates the tempo of puberty in females and if endogenous opioid peptides affect developmental changes in luteinizing hormone (LH) secretion. Females were ovariectomized at 15 months of age and either served as untreated controls (n = 6), received a constant subcutaneous infusion with IGF-I (125 μg/kg/day), or received a subcutaneous implant of the β-endorphin antagonist, naltrexone (2.2 mg/day). Developmental changes in LH in the absence of estradiol replacement and in response to varying doses of estradiol were monitored in all subjects as were estimates of bone age and measures of height. Although constant infusion with IGF-I did not affect developmental changes in LH absent estradiol, IGF-I treatment significantly accelerated the decrease in hypersensitivity to estradiol negative feedback inhibition on LH secretion. In addition, total height was increased by IGF-I treatment. These data suggest that IGF-I, under the regulation of GH, may affect the tempo of puberty and the rate at which females progress through the final stages of maturation prior to first ovulation. On the other hand, naltrexone administration had no effect on any developmental parameters of LH secretion, suggesting that, in female primates, endogenous opioid peptides do affect maturational changes in LH.

Additional studies of a subgroup of these animal (n = 12) assessed the effects of a somatostatin-analogue (Sandostatin®) on circulating IGF-I concentrations and pulsatile LH secretion in the absence of estradiol replacement. Analyses to date indicate that, in addition to a significant decrease in IGF-I levels, the frequency and amplitude of pulsatile LH is reduced in the face of an increased somatostatin tone.

These studies have described how reproductive maturation and skeletal growth are linked by a common neuroendocrine mechanism and have provided insight into how puberty may be affected in children with growth disorders.

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Comparison of Semen Parameters in Ejaculates Obtained Using an Artificial Vagina or Rectal Probe Electrostimulation

AXIS I: 1a, 9, 23

AXIS II: 60, 77

PRC UNIT: Reproductive Biology

INVES1: Young, Leona G.
DEGREE1: Ph.D.
DEPT1: Reproductive Biology
STAFF1: O

INVES2: Gould, Kenneth G.
DEGREE2: Ph.D., D.V.M.
DEPT2: Reproductive Biology
STAFF2: C

SPECIES1: Pan troglodytes
NUM1: 35

NON-HOST INST: NA

ABSTRACT: Ejaculates collected from adult chimpanzees using rectal probe electrostimulation (rpe) and using an artificial vagina (av) were compared. Ejaculate weight, semen volume, concentration of sperm and total number of sperm were significantly ($p<0.005$) lower and percentage liquefaction was significantly higher in ejaculates collected by rpe. Total amounts of protein and of α-glucosidase activity were significantly lower in seminal fluid from rpe samples. Percentages of motile sperm and of live sperm in semen and total amounts of fructose and citrate in seminal fluid did not differ significantly between the two collection methods. For ejaculates collected by rpe, semen volume correlated positively with protein ($r=0.8640$, $p<0.001$), fructose ($r=0.6976$, $p<0.001$) and citrate ($r=0.6976$, $p<0.001$); number of sperm correlated positively with α-glucosidase activity; and, protein correlated positively with fructose ($r=0.5906$, $p<0.002$), citrate ($r=0.5926$, $p<0.002$) and α-glucosidase activity ($r=0.6006$, $p<0.001$). For ejaculates collected by av, semen volume correlated positively with percentage liquefaction ($r=0.6058$, $p<0.001$), protein ($r=0.8055$, $p<0.001$), fructose ($r=0.6606$, $P<0.001$) and citrate ($r=0.8272$, $p<0.001$); percentage of motile sperm correlated positively with sperm number ($r=0.4196$, $p<0.004$) and with percentage of live sperm ($r=0.4388$, $p<0.002$); and, protein correlated positively with fructose ($r=0.6947$, $p<0.002$), citrate ($r=0.5926$, $p<0.002$) and α-glucosidase activity ($r=0.6202$, $p<0.001$).

P51RR00165-33 1/1/1993 - 12/31/1993 Yerkes Regional Primate Research Center

TITLE: Identification of a 27kD Androgen Dependent Epididymal Secretory Protein

AXIS I: 1a, 9, 23

AXIS II: 73, 92 (fertility)

PRC UNIT: Reproductive Biology

INVES1: Young, Leona G.
DEGREE1: Ph.D.
DEPT1: Reproductive Biology
STAFF1: O

INVES2: Gould, Kenneth G.
DEGREE2: Ph.D., D.V.M.
DEPT2: Reproductive Biology
STAFF2: C

SPECIES1: Pan troglodytes
NUM1: 2

NON-HOST INST: NA

ABSTRACT: We previously identified a 27kD cauda epididymal secretory protein which appears to affect sperm motility. Secretion of this protein is androgen dependent. The protein has been purified using SDS-PAGE and sequenced subsequent to electroblotting onto PVDF membrane. This protein appears to be homologous to a 16.570kD protein in the human. Antibodies have been generated to this protein which are being used to locate the site of synthesis within the epididymis. Should this protein be directly involved with sperm fertilizing capacity, then it is primary candidate for vaccine development related to a male contraceptive. The demonstrated homology to a human epididymal protein indicates that information obtained in this study will be readily transferred to the human male.

DIVISION OF ANIMAL RESOURCES AND VETERINARY MEDICINE

J. T. Bielitzki, M.S., D.V.M., Chief

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P51RR00165-33 1/1/93 - 12/31/93 Yerkes Regional Primate Research Center

TITLE: Establishment of A Chimpanzee Breeding and Research Program

AXIS I: 1a, 23

AXIS II: 36,60, 92 (Breeding)

PRC UNIT: Anim.Res. & Vet.Med.

INVES1: Swenson, R. Brent
DEGREE1: D.V.M.
DEPT1: Animal Resources and Veterinary Medicine
STAFF1: C

INVES2: Gould, Kenneth
DEGREE2: Ph.D., D.V.M.
DEPT2: Reproductive Biology
STAFF2: C

INVES3: Bard, Kim A.
DEGREE3: Ph.D.
DEPT3: Reproductive Biology
STAFF3: O

INVES4: Gordon, Thomas P.
DEGREE4: M.S.
DEPT4: Behavioral Biology
STAFF4: C

INVES5: Strobert, Elizabeth A.
DEGREE5: D.V.M.
DEPT5: Animal Resources and Veterinary Medicine
STAFF5: C

SPECIES1: Pan troglodytes
NUM1: 95

NON-HOST INST: NA

ABSTRACT: This breeding and research program is part of a cooperative program which includes four other institutions. The goal of the program has been to establish and maintain a self-sustaining population of chimpanzees which can also supply subjects for AIDS and other health-related research. Eleven live births were produced in 1993. A new housing area capable of holding up to 40 animals was completed at the field station and a new social group was established in the portion consisting of five indoor dens attached to a single 10,000 square foot outdoor compound. The group is a multi-male social group and includes some nursery-reared animals that had been integrated into it. A new outdoor exercise area and a system of overhead tunnels connecting separate housing sections at the main station is currently being assembled.

PART II, SECTION B1 GRANT NUMBER: P51RR00165-33

INVESTIGATORS WITH PUBLIC HEALTH SERVICE SUPPORT

CORE: XXX OTHER:

NAME	TYPE	AGENCY	GRANT/CONTRACT	TOTAL FUNDS	% RPRC USED
Ansari, Aftab A.	FED	NIH	AI-27057	$ 170,060	25
Boothe, Ronald G.	FED	NIH	EY-05975	137,836	100
Byrd, Larry D.	FED	NIDA	DA-01161	197,493	100
	FED	NIDA	DA-06264	269,485	100
de Waal, Frans B.M.	FED	NCRR	RR-05276	40,856	100
	FED	NIMH	MH-49475	49,065	100
Gould, Kenneth G.	FED	NIH	RR-03587	161,710	100
	FED	NIH	RR-03587-S1	20,644	100
	FED	NCRR	RR-05994	118,146	100
	FED	NIH	HD-26423 (Morehouse Subcontract)	21,874	100
Kelly, Andrew B.	FED	NIH	HL-47181	204,479	65
McClure, Harold M.	FED	NIH	RR-00165(S)	1,641,492	100
	FED	NIH	HL-42125 (UAB Subcontract)	133,531	100
	FED	NIH	RR-06753 (Univ. of Kansas Subcontract)	86,357	100
Swenson, R. Brent	FED	NIH	RR-03591	531,215	100
Tigges, Johannes	FED	NIH	AG-00001	99,444	100
Tigges, Margarete	FED	NIH	EY-09737	119,688	100
Wilson, James R.	FED	NIH	EY-04976	86,117	100
Wilson, Mark E.	FED	NICHD	HD-16305	111,280	100
TOTAL PHS SUPPORT			This page:	$ 4,200,772	
			Grand (Cumulative) Total:	$ 4,200,772	

PART II, SECTION B1 GRANT NUMBER: P51RR00165-33

INVESTIGATORS WITH PUBLIC HEALTH SERVICE SUPPORT

CORE: OTHER: XXX

NAME	TYPE	AGENCY	GRANT/CONTRACT	TOTAL FUNDS	% RPRC USED
Bakay, Roy A.E.	FED	NIH	NS-24340	151,442	70
Bard, Kim A.	FED	NCRR	RR-06158	159,417	100
Fritz, Michael E.	FED	NIDR	DE-08917	590,141	28
	FED	NIDR	DE-08917(S1)	34,239	100
Hanson, Stephen R.	FED	NIH	HL-31469	203,011	50
	FED	NIH	HL-31950	122,024	50
	FED	NIH	HL-48667	104,295	50
Harker, Laurence A.	FED	NIH	HL-41619	187,060	45
	FED	NIH	HL-31950	100,677	20
Harwerth, Ronald S.	FED	NIH	EY-01139	148,959	7
Hopkins, William D.	FED	NINDS	NS-29574	69,000	100
Howell, Leonard L.	FED	NIDA	DA-05346	74,141	100
Lambert, Scott R.	FED	NIH	EY-08544	132,786	90
Mann, David R. Morehouse School of Medicine	FED	NICHD	HD-26423	131,499	13
Moss, Mark B. Boston University	FED	NIH	AG-00001 (Subproject)	10,244	50
Peters, Alan Boston University	FED	NIH	NS-07152	185,834	5
	FED	NIH	NS-07016	154,220	35
	FED	NIH	AG-00001	103,436	25

TOTAL PHS SUPPORT This page: $ 2,662,425
Grand (Cumulative) Total: $ 3,372,043

INVESTIGATORS WITH PUBLIC HEALTH SERVICE SUPPORT (Continued)

CORE: OTHER: XXX

NAME	TYPE	AGENCY	GRANT/CONTRACT	TOTAL FUNDS	% RPRC USED
Schinazi, Raymond	FED	NIH	AI-25899 (Univ. of Georgia Subcontract)	126,470	10
Sommadossi, Jean-Pierre Univ. of Alabama	FED	NIH	HL-42125	356,661	1
Wilcox, Josiah	FED	NIH	HL-47838	226,487	60

TOTAL PHS SUPPORT

This page: $ 709,618
Grand (Cumulative) Total: $ 3,372,043

PART II, SECTION B1 GRANT NUMBER: P51RR00165-33

INVESTIGATORS WITH SUPPORT OTHER THAN PUBLIC HEALTH SERVICE

CORE: XXX OTHER:

NAME	TYPE	AGENCY	GRANT/CONTRACT	TOTAL FUNDS	% RPRC USED
Gouzoules, Harold	FED	NSF	IBN-92-09844	33,519	100
Herndon. James G.	FED	NSF	BNS 90-071701	27,357	100
Nadler, Ronald D.	FED	NSF	BNS 91-09441	62,000	100
Wallen. Kim	FED	NSF	BNS 89-19888	120,000	100

TOTAL NON-PHS SUPPORT This page:
Grand (Cumulative) Total:

PART II, SECTION B1 GRANT NUMBER: P51RR00165-33

INVESTIGATORS WITH SUPPORT OTHER THAN PUBLIC HEALTH SERVICE

CORE: OTHER: XXX

NAME	TYPE	AGENCY	GRANT/CONTRACT	TOTAL FUNDS	% RPRC USED
Bakay, R.A.E.	FED	VA	Merit Award	59,849	70