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LESSONS LEARNT FROM STUDIES OF THE IMMUNE CHARACTERIZATION OF NATURALLY SIV INFECTED SOOTY MANGABEYS

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1. ABSTRACT

The vast number of African non-human primates species that are naturally infected with the simian immunodeficiency viruses (SIV) but have not shown any signs of lentivirus associated disease as compared to the Asian non-human primate species that do not demonstrate any detectable signs of lentiviral infection but who upon experimental infection with select SIV isolates from the African species develop clinical signs and laboratory based findings similar to human HIV-1 infection provide a powerful model to define virus-host relationships. It is our belief that unraveling those differences which are specifically associated with disease resistance and/or disease susceptibility culled out from those that are species specific differences unrelated to disease outcome may provide some important insights which maybe fruitful for the formulation of vaccine strategies. The purpose of this chapter is to provide the reader with a summary of the findings from our laboratory from the past decade using the naturally SIV infected sooty mangabey model aimed at ferreting out some of these differences. Some very important paradoxes exist with this naturally infected lentivirus model. Thus, it is difficult to determine why these species demonstrate highly effective immune responses but yet maintain very high viral loads. Why do these naturally SIV infected species not demonstrate the plethora of clinical symptoms ascribed to select proteins of the SIV such as 'tat', 'nef', and other viral proteins as do the rhesus macaques and humans to HIV? Most of the targets of such proteins are highly conserved and yet no detectable pathology? We submit that the naturally infected species has evolved over time with a highly regulated immune system (a perfect host/parasite relationship) that is sufficient to prevent pathology and not in the order to exhaust the immune system. In addition, the quality of the

anti-viral immune response is of interest. Thus, the mangabeys demonstrate a clear skew in their cytokine based immune response towards a predominantly TH2 bias which we believe is the reason why this species of mangabeys among the many studied, is perhaps is the only species that is susceptible to M. leprae infection (see Dr. B. Gormus's chapter). Some of our working hypotheses that are aimed to provide explanations for some of these paradoxes are provided herein.

2. INTRODUCTION

Although the primary focus of HIV/AIDS research with the use of nonhuman primates has been and continues to be to utilize this species to either screen for potential vaccine formulations, testing for anti-retroviral drugs or to define potential mechanisms of pathogenesis of disease, studies of how certain species of nonhuman primates which are naturally infected with the simian immunodeficiency virus (SIV) and remain disease resistant throughout their life has been to a large extent been paid little attention. This is ironic because viral isolates from these species when experimentally injected into Asian macaques invariably leads to the same sequence of pathologic events characteristic of the human immunodeficiency virus (HIV-1) in humans which include $CD4^+$ T cell loss, hemogram abnormalities, diarrhea, wasting, susceptibility to opportunistic infection and eventually death (1). Thus, nature has provided us with an extremely valuable model for the study of virus/host relationships/adaptation with viruses that infect at least some 26 different old world African nonhuman primate species (2) and do not cause any detectable pathology or disease. Select viral isolates, however, cultured from these African species have been shown to cause pathology and disease in other mostly Asian nonhuman primate species, very similar to HIV induced pathology and disease in man. The reasons for this relative lack of interest are not clear but are likely due to a number of reasons. These reasons include a) the relatively real or perceived difficulties in access to the naturally SIV infected disease resistant species of nonhuman primates b) the finding of considerable diversity in the host/virus relationships in each of these naturally infected disease resistant nonhuman primates (making it difficult to chose which model to study) c) the prejudice among some within the scientific community that the mechanisms of disease resistance in these naturally infected species are not likely to provide insights which will be helpful for vaccine design and d) the predominant importance being placed on deriving a potential vaccine against SIV with obvious implications for the preparation of candidate vaccines against HIV. Some of these reasons germinated some time ago when rightfully or wrongfully, emphasis was being placed on deriving a "sterilizing" vaccine against HIV-1 (3-5) and thus the concept that if one could derive a vaccine that could lead to disease prevention without complete elimination of the virus, as observed in the naturally infected disease resistant nonhuman primates, was not being entertained. It is now becoming increasingly clear that such a concept of deriving a vaccine formulation that could lead to "sterilizing immunity" was based on poor scientific thought and reasoning. Thus, it would seem that such a realization would provide a new surge of interest in defining the potential mechanisms of disease resistance in the naturally SIV infected nonhuman primate species. This renewed interest has yet to occur and one of the reasons for the idea of getting together the present monograph is to provide the scientific community with the intellectual incentive to explore these SIV infected disease resistant models of nonhuman primates which in our minds may in fact provide some valuable clues that can be exploited for vaccine formulations against HIV.

Our laboratory initiated studies in attempts to define the potential mechanisms that contribute to disease resistance in one of these species called sooty mangabeys (Cercocebus atys), a species from West Africa that has been bred in captivity at the Yerkes Regional Primate Center of Emory University since 1968. It is important to note that although this species bred in captivity has been known to be naturally infected with SIV for nearly 2 decades (6), it is still not fully clear how the virus is transmitted in this colony. It is generally reasoned that the virus is transmitted in this species via the sexual route since SIV specific seroconversion appears to coincide with the achievement of sexual maturity. It is also possible that virus is transmitted via bites and scratches and via mother to infant during delivery and/or nursing. Puzzling exceptions have been nonetheless noted. Thus, infants born to seronegative mothers have been found to be seropositive and infected (7). The fact that the Yerkes Primate Center has been successful in deriving and maintaining a SIV seronegative and presumably uninfected colony of sooty mangabeys suggest that clearly not all mangabeys are infected at least with the level of sensitivity currently available. What is also clear is that all the sooty mangabeys that become infected with SIV, seroconvert with readily detectable titers against the same spectrum of SIV proteins as do the disease susceptible rhesus macaques following experimental infection. An exception to the preceding statement has been the finding in one study that shows that African green monkeys (AGM), who are also a species from Africa that is naturally infected with SIV but remains disease resistant, are tolerant to their own SIV native Gag protein (8). Our laboratory isolated SIV from 3 individual sooty mangabeys and performed Western Blots with sera from the same 3 mangabeys. Interestingly sera from all 3 sooty mangabeys reacted with the SIV Gag protein from each of the 3 virus isolates. Thus, the previous finding in AGM's did not appear to be observed in sooty mangabeys suggesting different mechanisms maybe operating in the tolerance/response seen in AGM's and sooty mangabeys. Most of the studies being performed by our laboratory on the naturally SIV infected nonhuman primates have involved sooty mangabeys and, as such, the present report will focus and summarize our findings on studies performed with tissues and blood samples from this species. Thus, the concepts of the mechanisms of disease resistance of the naturally SIV infected nonhuman primates forwarded in the present review is biased with the belief that the findings are generalizable to all naturally SIV infected nonhuman primates, which clearly may not be the case. Below is presented in a summary form what we know

with regards to the phenotypic subsets, functional innate and acquired immune responses against nominal antigens, role of viremia, humoral and cellular SIV specific immune responses and finally conclusions that we have drawn from all these studies.

3. PHENOTYPIC ANALYSIS OF PBMC SUBSETS

One of the early lessons learnt from studies attempting to define PBMC subsets in nonhuman primates was that not only do we have to screen monoclonal antibody reagents defined against human cell surface markers for cross reactivity against the nonhuman primate species being studied, but more importantly we need to make sure that the antibody in question is recognizing the appropriate homologue and furthermore that such subset performs function similar to that associated with the human subset. This concern is highlighted by the finding by the laboratory of Dr. C. Zink (9) on the problems associated with the expression of CD56 in rhesus macaques. Thus CD56 appears to be expressed by the monocyte/macrophage lineage of hematopoietic cells in monkeys unlike humans, which express this marker predominantly on the NK cell lineage. Thus, investigators are cautioned in their interpretation of data in the nonhuman primate literature that describe results with the use of antibodies that have not been properly screened and defined. Before providing a summary of the findings from our laboratory, it is important to note that several other laboratories have screened a laundry list of monoclonal antibodies that are important sources of information (10-14). One of the hallmarks of lymphocyte subset analysis of nonhuman primates is the observation that the ratio of CD4: CD8 subset of T cells is usually below 1.0 unlike human in which the ratio is > 1.0. However, it is of interest that the ontogeny of the $CD4^+$ T cell lineage in nonhuman primates is very similar to that seen in humans with 80% of the T cells expressing CD4 at birth (15). Our laboratory initiated studies on the phenotypic characterization of non-human primate PBMC subsets sometime ago (16). These characterizations of rhesus macaque and sooty mangabey PBMC subsets continue to be conducted and database updated as new reagents are identified and become available.

3.1. Major subset analyses

Some of the major differences in the frequency and absolute number of PBMC subsets that our lab has previously documented and continue to observe are described under Table 1. For the analysis of all the data, our lab has chosen to compare data obtained on uninfected rhesus and SIV seronegative mangabeys in efforts to highlight species specific differences since values for PBMC subsets in SIV infected rhesus macaques tend to vary based on stage of disease, virus isolate utilized, etc. In addition, we have also attempted to analyze and highlight differences that we have observed between SIV seropositive versus SIV seronegative sooty mangabeys in efforts to determine the effect of asymptomatic SIV infection on PBMC subsets. As seen, whereas there were no differences in the frequency of total $CD3^+$ T cells in sooty mangabeys and rhesus macaques, there was always a consistent lower frequency of $CD3^+$ $CD4^+$ cells in SIV seropositive mangabeys as compared with either seronegative sooty mangabeys or uninfected rhesus macaques ($p < 0.001$). Thus, SIV infection does result in a relatively lower frequency of $CD4^+$ T cells in infected sooty mangabeys but clearly such lower $CD4^+$ T cell levels do not appear to lead to disease susceptibility. When one examines the frequency of $CD45RA^+$ cells (naïve cells), there is clearly a difference between rhesus macaques and sooty mangabeys. The uninfected rhesus macaques have a much higher frequency of both $CD3^+$ $CD4^+$ $CD45RA^+$ and the $CD3^+$, $CD8\beta^+$, $CD45RA^+$ cells than either the SIV seronegative and seropositive sooty mangabeys ($p< 0.01$). Although there was a trend for an increased frequency of $CD8\beta^+$, $CD45RA^+$ in the SIV seronegative mangabeys than the seropositive mangabeys, this was not as marked a statistical significance. Several other significant differences were noted as far as phenotypic subsets. First of all, most of the rhesus macaques consistently demonstrated a noticeable frequency of $CD3^+/CD4^+/CD8^+$, the so-called double positive (DP) T cells. However, the CD8 molecule expressed by such DP cells was always CD8 α/α and hence the data is presented separately for CD8α v/s CD8β expressing cells. Secondly, rhesus macaques also appeared to have a significant number of CD3- cells that expressed CD8α alone. The functional significance of these differences is yet to be determined. The $CD3^+$ T cells that expressed the CD8 molecule always appeared to express this molecule in the heterodimeric form (CD α/β). We believe that such CD8 α/β+ T cells are the true cytotoxic T cells and the CD8 α/α expressing cells are likely to represent a NK cell lineage. It is also important to note that the SIV seropositive mangabeys have a higher frequency of $CD3^+$ $CD8\beta^+$ cells than the seronegative sooty mangabeys indicating that SIV infection may lead to an expansion of this lineage in the sooty mangabeys.

3.2. $CD4^+$ T cell subsets

A long list of the expression of cell activation and immunoregulatory markers were also analyzed with the hope that the differential expression of these cell surface markers could lead us to define potential mechanisms that may form the basis for the disease resistance phenotype of the SIV infected sooty mangabeys. The cell surface markers studied included standard cell activation markers such as CD25 (IL-2R), CD69, HLA-DR, the co-stimulation molecules CD28, CTLA-4 and its ligands CD80/86 (B7-1/2), CD152 (CTLA-4), other co-stimulation molecules such as CD40L and ICOS, cytokine receptors known to play a role in T cell activation and regulation such as CD127 (IL-7R), CD122 (IL-2Rβ/IL-15R), CD100 (semaphorin R),and the recently described molecule CRTH2 (expressed by Th2 cytokine prototype synthesizing cells). The data for the expression of these molecules by $CD4^+$ T cells is shown in Table 2 and those expressed by $CD8^+$ T cells is shown in Table 3. As alluded to above, there are at least 2 issues deemed important for the analyses of these data. The first concerns whether the reactivity seen is truly against the non-human primate homologue of the human molecule since the monoclonal antibodies being utilized were defined against human cells. The second issue

Table 1. Comparative analysis of PBMC subsets in uninfected rhesus macaques, SIV seronegative and SIV seropositive mangabeys

	Frequency (% Positive) mean +/- S.D.		
Phenotype	Unifected Rhesus Macaque	SIV seronegative Sooty Mangabey	SIV seropositive Sooty Mangabey
$CD3^+$	60.9 +/- 4.6	63.7 +/- 7.4	65.2 +/- 8.2
$CD3^+$. $CD4^+$	34.7 +/- 4.9	31.4 +/- 2.8	19.6 +/- 3.5 [2]
$CD4^+$, $CD45RA^+$	61.4 +/- 10.8 [2]	10.8 +/- 2.8	4.9 +/- 2.1
$CD4^+$, CD8 α/α^+	5.24 +/- 1.4 [2]	<0.1	<0.1
$CD3^+$, CD8 α/β^+	16.4 +/- 1.2	18.9 +/- 6.4	37.6 +/- 7.2 [2]
$CD3^+$, CD8 α/α^+	9.4 +/- 4.2	5.2 +/- 1.4	4.9 +/- 1.1
CD3-, CD8 α/α^+	25.3+/- 4.2 [2]	15.7 +/- 3.9	16.8 +/- 3.1
$CD8^+$, $CD45RA^+$	82.6 +/- 7.4 [2]	37.2 +/- 9.9	18.4+/- 6.2

[1] mean +/- standard deviation, [2] Statistically significant from the other 2 values ($p<0.01$)

Table 2. Comparative analysis of the expression of cell activation, co-stimulatory and immunoregulatory molecules by $CD3^+$ $CD4^+$ Tcells from uninfected rhesus macaques, SIV seronegative and seropositive mangabeys

	Frequency (% Positive) mean +/- S.D.		
Phenotype	Uninfected Rhesus Macaque	SIV seronegative Sooty Mangabey	SIV seropositive Sooty Mangabey
$CD4^+$ $CD25^+$	5.9 +/- 1.4	10.9 +/- 4.2 [2]	8.7 +/- 3.9 [2]
$CD4^+$ $CD69^+$	0.4+/- 0.1	0.5 +/- 0.2	0.5 +/- 0.1
$CD4^+$ HLA-DR^+	3.5 +/- 1.4	7.3 +/- 3.4 [2]	8.6 +/- 2.8 [2]
$CD4^+$ $CD80^+$	1.6 +/- 0.4	2.5 +/- 0.2	1.7 +/- 0.2
$CD4^+$ $CD86^+$	0.5 +/- 0.3	0.4+/- 0.1	0.4 +/- 0.1
$CD4^+$ $CD28^+$	35.5 +/- 2.3 [2]	22.1 +/- 8.4	16.9 +/- 4.8
$CD4^+$ $CD40L^+$	<0.1	<0.1	<0.1
$CD4^+$ $1COS^+$	89.4 +/- 6.9	88.3 +/- 7.4	82.4 +/- 5.3
$CD4^+$ $CD152^+$	0.5 +/- 0.1	1.9 +/- 0.5	0.2 +/- 0.1
$CD4^+$ $CD127^+$	3.8 +/- 2.1	1.8 +/- 0.3	6.4 +/- 3.9
$CD4^+$ $CD122^+$	0.2 +/- 0.1	0.1 +/- 0.04	0.2+/- 0.1
$CD4^+$ $CD100^+$	0.5 +/- 0.3	N.D.	N.D.
$CD4^+$ $CD95L^+$	2.9 +/- 1.2	3.1 +/- 1.4	2.6 +/- 1.2
$CD4^+$ $CRTH2^+$	0.4 +/- 0.1	1.7 +/- 0.3	1.9 +/- 0.2

[1] Mean +/- standard deviation, [2] $p< 0.01$

Table 3. Comparative analysis of the expression of all activation; co-stimulatory and immunoregulatory molecules by $CD3^+$ $CD8^+$ T cells from uninfected rhesus macaques, SIV seronegative and seropositive sooty mangabeys

	Frequency (% Positive) mean +/- S.D.		
Phenotype	Uninfected Rhesus Macaque	SIV seronegative Sooty Mangabey	SIV seropositive Sooty Mangabey
CD8+ $CD25^+$	3.4 +/- 0.3	5.5+/- 2.1	7.2 +/- 1.4 [2]
CD8+ $CD69^+$	2.9 +/- 1.4	12.4 +/- 4.1 [2]	11.9+/- 2.4 [2]
CD8+ HLA-DR^+	8.9 +/- 4.2	19.4 +/- 4.5	55.6+/- 11.2
CD8+ $CD80^+$	7.8 +/- 1.9	15.9 +/- 6.2 [2]	19.6+/- 4.2
CD8+ $CD86^+$	0.3 +/- 0.1	0.5 +/- 0.1	0.4 +/- 0.2
CD8+ $CD28^+$	13.4 +/- 2.3	12.8+/- 1.9	15.6 +/- 3.4
CD8+ $CD40L^+$	<1.0	<1.0	<1.0
CD8+ $1COS^+$	42.4+/- 10.8	38.6+/- 5.9	39.5+/- 4.3
CD8+ $CD152^+$	1.9 +/- 0.4	1.6 +/- 0.3	1.1 +/- 0.4
CD8+ $CD127^+$	4.2 +/- 1.2	4.2+/- 1.4	7.8+/- 1.4 [2]
CD8+ $CD122^+$	2.7 +/-0.4 [2]	0.6+/- 0.1	0.6+/- 0.1
CD8+ $CD100^+$	2.9+/- 0.1	N.D.	N.D.
CD8+ $CRTH2^+$	2.2+/-0.2	2.9+/- 0.3	2.6 +/- 0.2

[1] Mean +/- standard deviation, [2] $p< 0.01$

concerns the relative density of expression of the given molecules. The latter does provide concern when the frequency of positive events is < 5% and the density is such that it is difficult to draw clean gates and is more easily observed by a small right shift using a histogram display. Clearly immunoprecipitation with the monoclonal antibody under question, followed by microsequencing of the appropriate precipitated molecule is the best way to resolve such a concern. As seen in Table 2, there did not appear to be any marked differences in the expression of most of the cell surface markers expressed by $CD4^+$ T cells from mangabeys and macaques. The only notable differences were the increased expression of CD25 and HLA-DR and a marked decrease in the expression of CD28 by sooty mangabey $CD4^+$ T cells as compared with $CD4^+$ T cells from rhesus macaques ($p < 0.01$). SIV infection per se, did not appear to differentially affect the differences noted in the expression of the molecules by $CD4^+$ T cells from the mangabeys except for a slight trend for a further decrease in CD28 expression seen in $CD4^+$ T cells from SIV infected as compared with negative mangabeys ($p< .05$). The CD100 monoclonal antibody appeared to react with cells from rhesus macaques but not sooty mangabeys.

3.3. $CD8^+$ T cell subsets

When the $CD8^+$ T cell subsets were examined, as seen in Table 3, the sooty mangabeys appeared not only to have an increased frequency of $CD25^+$, $CD69^+$ cells, CD80 expressing $CD8\beta^+$ cells but a clearly marked increase in HLA^-DR^+ $CD8\beta^+$ T cells as compared to rhesus macaques, which basically exemplifies an expanded and activated $CD8^+$ T cell population. Other cell surface markers did not show much of a difference in their expression pattern. Besides a major difference in the frequency of CD8 cells that express HLA^-DR^+ ($p < 0.001$) , there also was not much of a difference between the values obtained from SIV^+ v/s SIV^- sooty mangabeys, except for a slight increase in the frequency of $CD25^+$ cells. This again appears to indicate that following SIV infection there appears to be a chronic presence of activated $CD8^+$ T cells in sooty mangabeys.

3.4. Expression of cell surface markers following cell activation

While the above represent constitutive expression of cell surface markers on PBMC and its subsets, the same sets of markers have also been examined on experimentally activated PBMC's from macaques and mangabeys. The agents utilized to activate the cells include PHA, monoclonal antibody anti-CD3 alone (signal 1 alone) and in combination with anti-CD28 (signal 1 and 2). Overall, the pattern of expression is not much different between mangabeys and macaques. The only salient differential features included the mean density of expression of the activation and co-stimulatory molecules and the kinetics of expression. Thus, HLA-DR, CD25, CD80, CD86, CD40L and ICOS all appeared at a much higher mean density on $CD4^+$ and $CD8^+$T cells from mangabeys than rhesus macaques and in most if not all cases these markers appeared much more rapidly on cells from mangabeys than macaques. The general feeling one obtained from analyses of these data is that a certain fraction of each subset of cells from mangabeys appear to already be activated and others poised to be activated, a reflection of the kinetics by which these markers were shown to be expressed. While the data on CD40L have been published (17), the data on the expression of other activation markers and co-stimulatory molecules such as ICOS, CD100 and the cytokine receptors is currently in progress.

3.5. NK cell markers

Our lab was the first to examine Natural Killer (NK) and Lymphokine Activated Killer (LAK) cell function in the SIV infected sooty mangabeys and for comparison rhesus macaques (18). As outlined above, we were unaware at the time of the problems associated with the use of CD56 as a marker for NK cells in the nonhuman primates (9). Since then we clearly recognize that CD56 is expressed by the macrophage lineage in the PBMC of rhesus macaques. Of importance in our studies was the finding that there was a marked increase in the level of total functional NK and LAK cell activity in the PBMC of sooty mangabeys as compared with rhesus macaques, which is discussed in more detail below. Phenotypically, while NK cells and LAK precursor cells from sooty mangabeys predominantly do express CD8, and the predominant NK cells from rhesus macaques do not appear to express CD8, the predominant LAK cell precursors from rhesus do express CD8. Thus, it appears that multiple lineages mediate NK/LAK cell function in rhesus macaques whereas the lineage from sooty mangabeys is more restricted. In this context, it is important to note that rhesus macaques have a higher frequency of resting CD3- and CD8 α/α^+ cells than the mangabeys. Following SIV seroconversion against both rhesus macaques and sooty mangabeys have a major expansion of $CD3^+$ CD8 α/β^+ cells.

3.6. Chemokine receptors

It is now well known that chemokine receptors expressed by $CD4^+$ cells (T cells and monocyte/macrophages) serve as important co-receptors for the entry of HIV and SIV into respective lymphoid cell subsets (19-22). Of the family of HIV-1 co-receptors so far catalogued, the CCR5 co-receptor appears to function predominantly for the entry of SIV into non-human primate lympho-hematopoietic cells (23). Our lab has studied the expression of both CCR5 and CXCR4 by $CD4^+$ and $CD8^+$ T cells from uninfected rhesus macaques and SIV seropositive and seronegative mangabeys. Table 4 summarizes our findings to date. Consistent with previously published data, it appears that while CCR5 is expressed primarily by both CD4 and CD8 $CD45RA^-$ (there is no reagent for CD45RO for monkeys and thus data reflects cells that were negative for CD45RA) subsets, CXCR4 appears to be expressed predominantly by CD4 $CD45RA^+$ cells (24-26). Previous studies have documented a marked decrease in the expression of CCR5 by $CD4^+$ T cells from SIV infected as compared to uninfected sooty mangabeys (24). However, in our studies, while a similar decrease in the frequency of $CD4^+$ T cells that expressed $CCR5^+$ from sooty mangabeys following infection was also noted in the $CD45RA^-$ population, the differences were not as profound in the $CD45RA^+$ cell population. The reason

Table 4. Relative expression of the chemokine receptors by $CD4^+$ and $CD8^+$ T cells from uninfected rhesus macaques, SIV seronegative and SIV seropositive mangabeys

	Frequency (% Positive) mean +/- S.D.		
Phenotype	Uninfected Rhesus Macaque	SIV seronegative Sooty Mangabey	SIV seropositive Sooty Mangabey
$CD4^+$, $CCR5^+$	5.1 +/- 2.1	2.4 +/- 1.3	3.9 +/- 2.1
$CD4^+$, $CCR5^+$	4.9 +/- 1.7	3.5 +/- 1.1	2.2 +/- 1.3
CD4, $CCR5^+$, $CD45RA^-$	14.2 +/- 4.3	7.9+/- 4.2	2.5 +/- 1.0
$CD8^+$, $CCR5^+$	8.4+/- 5.9	1.7 +/- 0.4	4.2 +/- 1.5
$CD8^+$, $CCR5^+$, $CD45RA^+$	6.2 +/- 2.5	5.3 +/- 3.1	4.8+/-2.1
$CD8^+$, $CCR5^+$ $CD45RA^-$	13.7 +/- 3.3	10.4 +/- 4.1	9.2+/-3.9
CD4, $CCR4^+$	84+/- 12.5	78.3 +/-8.4	75.6 +/- 9.2
$CD4^+$, $CXCR4^+$, $CD45RA^+$	68.9 +/- 10.4	55.3 +/- 6.5	48.4 +/- 6.6
$CD4^+$, $CXCR4^+$, $CD45RA^-$	34.2 +/- 5.8	36.9 +/- 4.2	30.9 +/- 5.1
$CD8^+$, $CXCR4^+$	57.6 +/- 11.1	60.2 +/- 7.4	58.9 +/- 9.2
$CD8^+$, $CXCR4^+$, $CD45RA^+$	N.D.	N.D.	N.D.
CD8, $CXCR4^+$, $CD45RA^-$	N.D.	N.D.	N.D.
$CD4^+$, $CXCR3^+$	8.5+/- 2.4	6.2 +/- 2.3	9.1 +/- 3.4
$CD8^+$,$CXCR3^+$	4.5 +/- 1.9	10.4 +/- 2.2	6.2 +/- 2.7

Table 5. Frequencyof HLA-E $tetramer^+$ cells in rhesus macaques and SIV seronegative and seropositive mangabeys

	Frequency of HLA-E $tetramer^+$ cells (Mean +/- S.D.)
Uninfected rhesus macaques (n=12)	6.7 +/- 0.5
SIV seronegative mangabeys (n=6)	7.4 +/- 0.2
SIV seropositive mangabeys (n=14)	15.9 +/- 0.5 [1]

[1] Mean +/- standard deviation, [2] $p < 0.001$

for this difference is not known at present. No other major differences were noted. In vitro activation of $CD4^+$ T cells using anti-CD3 + anti-CD 28 conjugated immunobeads from both rhesus macaques and sooty mangabeys appeared to lead to a marked decrease in CCR5 expression (27). Since $CD4^+$ T cells from sooty mangabeys have a higher constitutive frequency of activated cells, we interpret this increased frequency as one of the reasons for the decrease noted in the constitutive low expression of CCR5 in the $CD45RA^-$ (presumably memory) cell population.

3.7. Use of HLA-E tetramers for identifying NK and NK-T cell subsets

The Natural killer cell receptors (NK-R) have been a subject of intense study during the past decade. Their study was prompted by the paradoxical finding that whereas NK cells readily kill tumor cells and certain virus infected target cells, they do not normally kill otherwise normal non-malignant and/or the parent non-virus infected cells. Results of the studies aimed at resolving this paradox revealed that NK cells express receptors on their cell surface that have specificity for a number of MHC class I molecule and engagement of these receptors by the MHC molecules turns the killer function off and hence such receptors were named Killer cell inhibitory receptors or KIRs. These family of receptors include those that react with HLA-C (p58.1/p58.2), HLA-B (p 70 and CD94) and those react with HLA-A (p140) (28, 29). There has also been the finding of NK-R on a subset of $CD8^+$ T cells (30) which appear to recognize HLA-E (31). These NK-T cells are unique in that their NK-R appears to bind to HLA-E that contain within their peptide binding cleft portions of the MHC class I molecules and viral peptide molecules (31). There are 5 major alleles of the HLA-E molecule that have so far been identified (32) with 3 being considered as being the more acceptable alleles. HLA-E has been recently shown to be the primary if not exclusive ligand for the heterodimeric CD94/NKG2A receptors expressed by NK cells and by a subset of T cells. The diversity of NK cell Ig-like receptors expressed by rhesus macaques have also been recently described (33). Our lab has been interested in NK and NK-T cell lineages in rhesus macaques and mangabeys and has utilized the HLA-E tetramer reagent. Of interest is our preliminary findings that whereas 6-7 % of the rhesus macaques and SIV seronegative mangabeys PBMC's bind the HLA-E tetramer reagent, > 15 % of SIV seropositive mangabeys have HLA-E tetramer positive cells (see Table 5). This is significantly higher than SIV seronegative mangabeys and rhesus macaques ($p < 0.01$). The precise lineages and sub-lineages of PBMC's that bind the HLA-E tetramer reagent are currently being studied as is the correlation of the various phenotypes with NK and NK-T cell function. The mechanism(s) that lead to selective expression (and/or expansion) of these HLA-E tetramer positive cells following SIV infection is clearly important as it may shed light on potential disease protective mechanisms.

4. FUNCTIONAL IMMUNE RESPONSES

As stated above, characterization of innate and acquired humoral and cell mediated immune responses in rhesus macaques have been conducted by a number of laboratories including ours. However, limited studies of a similar nature have been conducted on naturally SIV infected non-human primate species such as sooty mangabeys, and, much has yet to be learnt. Below is a summary of the results we have obtained so far on our

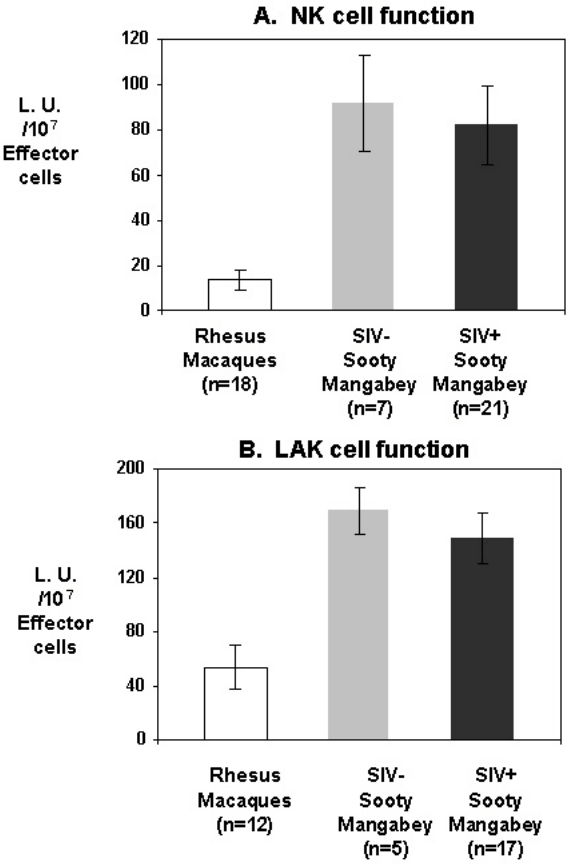

Figure 1. Relative NK and LAK cell activity in the PBMC from Rhesus Macaques, SIVseronegative and seropositive Sooty Mangabeys. PBMC from rhesus macaques (n=18), SIV seronegative mangabeys (n=7) and SIV seropositive mangabeys (n=21) were assayed for the quantitative levels of NK activity against K-562 target cells utilizing standard assay. Results are expressed as lytic units per 10^6 PBMC's. A) NK activity B) IL-2 expanded and activated PBMC's termed lymphokine activated killer (LAK) cells from rhesus macaques (n=12) SIV seronegative mangabeys (n=5) and SIV seropositive mangabeys (n=17) were assayed for lytic activity.

successes and failures to measure and quantitate innate and acquired immune responses in sooty mangabeys and rhesus macaques.

4.1. Innate immune function

An important role for innate immune effector mechanisms in particular during the early events following infection is being realized (34). Thus, prior to the development of virus specific humoral and cellular immune responses, it is reasonable to assume that such innate immune effector mechanisms must play an important role in eliminating virus infected cells and setting the proper environment for the development of acquired antigen specific immune responses. However, limited studies have been performed to define the role of these innate immune mechanisms for reasons that are not clear. Below are outlined some of the findings that have so far been documented.

4.1.1. NK/LAK cell function

Our lab studied the NK and LAK cell function of sooty mangabeys and rhesus macaques (18). Basically, the sooty mangabeys appeared to have significantly ($p < 0.01$) higher levels of functional NK and LAK cell activity as compared to rhesus macaques (see Figure 1). Interestingly, no differences were noted in the relative NK/LAK cell levels in the PBMC from SIV seronegative as compared with SIV seropositive sooty mangabeys suggesting that such increased function was not likely to be secondary to SIV infection per se. Since these previous studies, our lab has attempted to define whether the increased level of NK and LAK activity was due to increased frequencies of such cells or increased intrinsic ability of the cells from sooty mangabeys to lyse NK sensitive target cells. Cloned cell lines with the appropriate phenotypic cell surface markers (outlined above) were established from rhesus macaques and sooty mangabeys utilizing limiting dilution techniques. Analysis of NK and LAK cell function using varying ratios of cloned effector cells from rhesus macaques and sooty mangabeys against a fixed number of the same NK sensitive target cells failed to demonstrate any significant difference in the level of NK cell activity. These data appear to indicate that the differences in the quantitative levels of NK cell activity in the 2 species is not due to an intrinsic increased functional activity at least as measured by such a method. It is likely that the increased NK cell function in the PBMC of sooty mangabeys is due to the relative levels of activated NK effector cells. It is also possible that there are multiple lineages mediating such function and the use of cloned cell lines does not permit appropriate quantitative evaluation of such function.

4.1.2. Inhibition of virus replication by a non-cytolytic effector cell function

The ability of $CD8^+$ T cells to inhibit virus replication by a non-cytolytic effector mechanisms was initially reported by the laboratory of Dr. Levy in HIV infected humans (35) and by Dr. Letvin in SIV infected rhesus macaques (36). These findings were supported by the practical observation that it was relatively easier to isolate HIV and SIV from infected humans and monkeys if one first deleted $CD8^+$ cells. It was of interest to us to determine whether such functional activity was also present in the PBMC from naturally SIV infected sooty mangabeys. Indeed, this turned out to be the case (37). Of importance was our finding that whereas such $CD8^+$ T cell function was low to undetectable levels in PBMC from normal uninfected rhesus macaque, such activity was clearly present in both the seropositive and the seronegative sooty mangabeys (37). As a matter of fact the level of activity seen in seronegative mangabeys was similar to that seen in seropositive mangabeys. This finding was a surprise since it was assumed that this function is acquired following SIV infection. Our lab was also the first to demonstrate that such activity was mediated at the cellular level of virus transcription. Data derived with the use of wild type and NF-kB mutant LTR driven CAT-reporter gene transfected target cells, suggested that the inhibition was likely to involve cell derived NF-kB binding proteins. Our lab went further on to show that in fact this inhibition was biphasic, was protein kinase dependent and required

protein synthesis (38). While a number of molecules have been thought to mediate such anti-viral effects including molecules such as the chemokines MIP-1 α, MIP-1 β, RANTES (by binding and blocking their cognate receptors such as CCR5 which of interest also serve as co-receptors for lentviruses) and SDF-1 (by binding to its cognate receptor CXCR4, also a co-receptor for lentiviruses) (20, 22, 39)and more recently the α-defensins (40), the jury is still out as to whether these are in fact the molecules described by Dr. Levy's lab as $CD8^+$ cell anti-viral fact (CAF). CAF has been characterized as a factor that involves protease activity or a protein that interacts with protease inhibitors (41). A couple of additional issues with regards to these $CD8^+$ anti-viral factors are important to note. First of all, since our lab found such anti-viral factors to be present in SIV seronegative sooty mangabeys, it was important for us to determine the cellular requirements for the synthesis and release of this factor. PBMC samples from rhesus macaques were collected prior to and at varying time intervals following experimental SIV infection. The $CD4^+$, $CD8^+$ enriched populations and the non-CD4/CD8/CD20 expressing cells (presumably enriched for monocytes/macrophages and other cell lineages, heretofore referred to as M cells) were separated prior to cryopreservation from each PBMC sample from each monkey using negative selection techniques (i.e. removal of the unwanted cell lineage (s)) using appropriate immunobeads. Thereafter, $CD8^+$ T cell from each monkey collected prior to and post SIV infection were co-cultured with autologous M cells collected prior to infection, shortly post infection during the acute viremia stage and during the chronic infection period and the mixture activated with PHA-P and then tested for their ability to influence SIV replication. Results of these extremely labor intensive experiments revealed several important clues. a) $CD8^+$ cells collected from monkeys post SIV infection when co-cultured and activated with M cells either post or pre-SIV infection were highly capable of suppressing SIV replication, although the M cells from post SIV infection appeared to be more optimal b) $CD8^+$ cells collected prior to SIV infection were highly capable of suppressing SIV replication when they were co-cultured and activated with M cells collected post but not pre-SIV infection and c) $CD8^+$ cells collected pre-SIV infection when activated either without M cells or with M cells pre-SIV infection did not suppress SIV replication, as previously published. These findings (42) appeared to indicate that whatever were the cell lineages in the fraction termed M cells must consist of a function that is only acquired post SIV infection which is able to confer (educate) $CD8^+$ cells irregardless of their exposure to the lentivirus or lentiviral proteins the ability to synthesize anti-viral factors that were functional in suppressing viral replication. The problem with these studies is clearly that the precise nature of the anti-viral factor was not studied and it remains to be determined whether such a factor(s) are the known chemokines, alpha defensins or yet to be defined anti-viral factors. It is reasonable to assume that there are likely several biologically active molecules that can manifest such function. What role each plays in regulating viral replication and/or disease resistance in the naturally infected sooty mangabey remains to be established. The fact that $CD8^+$ T cells from sooty mangabeys acquire this function prior to seroconversion but the rhesus macaques only acquire at least the M cell function post infection and seroconversion appears to suggest that either such function is unrelated to SIV and is due to an indirect effect of a viral infection that is genetically present in sooty mangabeys (a generalized response to any viral infection), or that M cells from sooty mangabeys are intrinsically programmed to synthesize such factors. Limited studies on the ontogeny of the acquisition of such function in sooty mangabeys have been conducted utilizing two different approaches. Firstly, $CD8^+$ T cells were acquired from a set of 3 sooty mangabeys prior to seroconversion and at varying times post seroconversion and assayed for their ability to influence SIV replication (43). As published, sooty mangabeys appeared to acquire this function not only prior to seroconversion but the level of function is just as potent prior to as post seroconversion (43). Secondly attempts were made to obtain blood samples from sooty mangabeys of varying ages. This study has been hampered by the fact that < 3 month old mangabeys much like other non-human primates (15) have a predominance of $CD4^+$ T cells and given the small volume of blood that can be obtained from the neonates, such studies were not deemed feasible. The youngest sooty mangabey tested was 13 months old and this monkey had already acquired $CD8^+$ T cell anti-viral function. Since a seronegative colony of sooty mangabeys has been established at the Yerkes Primate Center, it is our aim to now conduct studies on these monkeys at varying ages and at varying time intervals post experimental infection with SIV in efforts to define the ontogeny more appropriately. It is also important to note that recent studies have also identified an anti-viral factor that is synthesized by $CD4^+$ T cells that is distinct from the β-chemokines, α-interferon, α-defensins and CAF (Dr. Tanaka, personal communication). Thus, as stated above, there must exist multiple redundant and/or overlapping pathways involved in the control of viral infection and replication.

4.1.3. Role of defensins

Mammalian defensins are small (~3-6 kD) cationic microbicidal peptides containing characteristic disulfide bonds (44-53). Defensins are categorized into two main families – the α and β defensins, based on the positioning of the six cysteine residues and spacing of the disulfide bonds of these molecules. The third family – cyclic θ defensins - has been recently identified in polymorphonuclear leukocytes from non-human primates (54). They are synthesized as inactive pre-pro-peptides, that require the removal of the signal and the pro-segment to release an active peptide (55-59). Defensins are an important microbicidal component of macrophage granules. However they are synthesized also by other cells in the skin and mucosal tissues and secreted into the lumen of the gastrointestinal tract (GIT), respiratory and genitourinary systems (46, 60-70). As such, defensins form a first line of immune defense against pathogenic micro-organisms. An overview of human defensins is provided in Table 6.

The biochemical structure of defensins allows for their insertion into phospholipid membranes of the bacteria

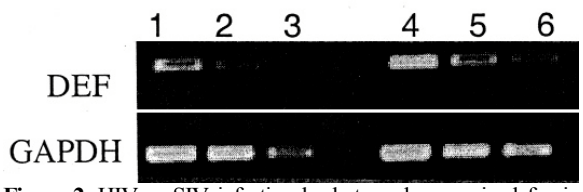

Figure 2. HIV or SIV infection leads to a decrease in defensin specific mRNA in small intestine. Small intestinal tissue from uninfected and HIV infected humans and SIV infected animals was enriched for Paneth cells and RNA was isolated using standard protocols. RNA was reverse transcribed and the resulting cDNA used as a template in PCR reaction with defensin specific primers (5'-CCCAGCCATGAGGACCATCG-3' and 5'-TCTATCTAGGAAGCTCAGCG-3'). Analysis of the amplification products revealed specific signal in uninfected humans (lane 1) and non-human primates (lane 4) and a marked diminution of the defensin specific message in HIV infected humans (lanes 2 and 3) and SIV infected rhesus macaques (lanes 5 and 6).

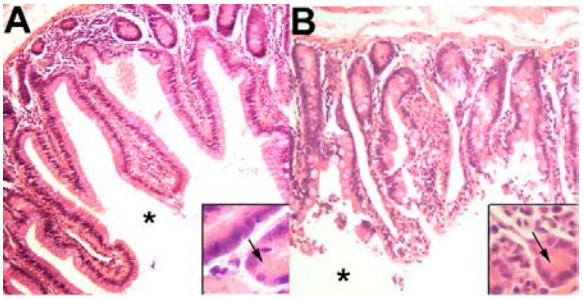

Figure 3. Paneth cells are maintained in SIV infected rhesus macaques. Sections from the small intestine from A) SIV naïve rhesus macaque and B) SIV infected rhesus macaque with high virus load and presence of O.I.s were stained with H-E and evaluated for morphological changes. In both groups of animals, Paneth cells (insets, indicated by arrows) were present in similar numbers and did not exhibit detectable differences in morphology.

that leads to pore formation, depolarization of the membrane and bacterial killing (71-75). The α-defensins exhibit individual spectra of antibacterial activity against both gram-positive and gram-negative bacteria; antiviral activity against herpetic viruses and HIV; and antifungal activity targeting Candida albicans (76-83). Other defensin sensitive microorganisms include the protozoan Giardia spp. and the spirochete treponema (84-86). Several β-defensins have been shown to be active against gram positive and gram-negative bacteria, C. albicans and Aspergillus fumigatus (46, 87-90). It has been shown that while some defensins are constitutively expressed (hBD1) (47, 67, 91, 92), expression of other defensins is inducible (hBD2) (87). Inflammation, bacteria (probably through LPS) and the pro-inflammatory cytokines IL1β and TNF-α are factors that have been shown to induce defensin production (93, 94). The expression of some defensins has been shown to be developmentally regulated. The mRNA coding for HD5 and HD6 appears in the small intestine of the fetus at 14 – 17 weeks of gestation, but in much lower quantities than in adults (69). This low level of expression of the enteric defensins was suggested as one of the factors that may contribute to the immature state of the local immune system and predispose premature born infants to infections with intestinal micro-organisms (95). Differential regulation of the transcription of certain α-defensins such as HNP defensins (see Table 6) leads to the selective activation and defensin synthesis at the pro-myelocytic stage of granulocyte development (96, 97). Several studies have provided data that implicate an important role for regulators of defensin transcription in the synthesis of these molecules. Among these include NF-κB which was implicated in LPS induced transcription (45), while PU.1 and other as yet unidentified factors were implicated in pro-myelocyte specific regulation of HNP (98). Another important factor that regulates the concentration of active defensins is the local availability of enzymes responsible for the cleavage of inactive pre-prodefensins into active mature peptides. It has been shown that the intestinal tracts of mice, which are defective in matrilysin (an enzyme responsible for the cleavage of mouse intestinal defensin precursors) do not contain any mature defensins and intestinal peptides from these mice showed decreased microbicidal activity accompanied by a marked increased susceptibility to GIT infections (99). In addition to their direct antibiotic effect, defensins have also been shown to have other effects on the immune system that suggest their involvement in immune regulation (44, 100, 101). In mice, when co-administered with antigen, HNP enhanced the production of IFN-γ, interleukin 5, 6 and 10 and systemic IgG (44). HBD2 was shown to interact with the chemokine receptor CCR6 and exhibit chemotactic activity for dendritic cells and memory T cells (102). These systemic effects of defensins on the immune system suggest that they most likely play an important role as a link between innate and acquired immunity. In GIT, defensins are produced by Paneth cells (along with lysozyme and phospholipase A2) and secreted into crypts where their high concentrations form a protective environment for stem cells that are important for the regeneration of GIT epithelia.

Our laboratory hypothesized that such defensins could play an important role in the initial events following SIV infection of non-human primates. This concept was based on the previous findings from a number of labs that the G.I. tissue is the major site of viral replication, $CD4^+$ T cell depletion and dysfunction following SIV infection of rhesus macaques (103-106). Our preliminary data from biopsy tissues of the small intestine from HIV-1 infected humans and SIV infected rhesus macaques show that both humans (n=2) and these monkeys (n=6) demonstrate a marked decrease in mRNA transcription of defensin in the gut during AIDS. A representative RT-PCR profile of a HIV-1 infected human and SIVmac251 infected non-human primate is illustrated in Figure 3. This decrease was seen in a series of tissues from the small intestine at autopsy from the human patients and from biopsies obtained both during the asymptomatic and disease phase in the SIV infected rhesus macaques. While there was decreased synthesis in tissues during the asymptomatic phase, there was a further decrease (complete absence) was noted during AIDS associated with severe diarrhea and weight loss.

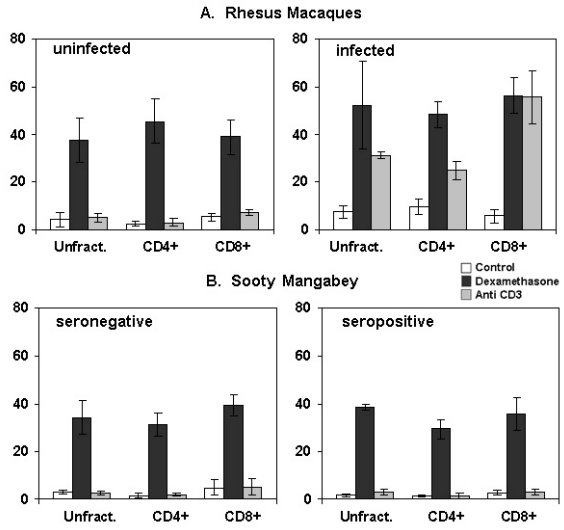

Figure 4. Relative susceptibility to undergo apoptosis *in vitro* by unfractionated and enriched populations of $CD4^+$ and $CD8^+$ subsets from uninfected and infected Rhesus Macaques and SIV seronegative and seropositive Sooty Mangabeys

However, it was reasoned that the absence of defensin mRNA maybe due to the physical absence of Paneth cells. Thus, pathophysiologic changes induced in the intestinal epithelia or selective destruction of Paneth cells by HIV and SIV or opportunistic infections may be a trivial reason for the inability to detect defensin mRNA. Therefore we obtained histological sections of the small intestines from HIV infected humans at autopsy and a series of uninfected (Figure 3A) and SIV infected rhesus macaques at autopsy who had documented evidence of severe diarrhea and O.I.'s (Figure 3B) and evaluated the gut epithelia morphologically.

Microscopic evaluation of stained slides revealed superficial damage of epithelial cells in both human HIV infected tissue samples and SIV infected animals. However, the number and appearance of Paneth cells (insets in Figure 3 A and B) was similar in both infected and uninfected humans and rhesus macaques. These preliminary data seem to indicate that the absence of defensin RNA detected in HIV infected humans and SIV infected rhesus macaques is not due to the damage or destruction and/or absence of secreting cells. The fact that the patients and rhesus macaques died following HIV and SIV infection and O.I.'s but yet their Paneth cells appear morphologically similar to normal, provides strong evidence that the decrease noted in the level of defensin mRNA during the asymptomatic phase cannot be due to destruction of Paneth cells. A similar study of tissues from SIV infected sooty mangabeys failed to reveal any decreases in the level of defensins at least at the mRNA (i.e. same approximate copy numbers as normal rhesus macaques by real time PCR) (Miller RS, in progress). The precise mechanisms that lead to depletion at the mRNA levels in SIV infected rhesus macaques but not SIV infected sooty mangabeys is currently not known. Similarly, the physiological and clinical relevance of these findings is not clear at present. It is possible that the mechanisms of disease resistance of SIV infected sooty mangabeys is due in part to the functional preservation of G.I. tissues of this species. Knowledge of these mechanisms may provide important clues to the role of defensins in the pathology of human HIV and rhesus macaque SIV infections.

4.1.4. Relative susceptibility to undergo apoptosis

It is now generally understood that direct cytolysis of $CD4^+$ T cells by HIV and SIV does not alone account for the magnitude and ultimately complete loss of this cell lineage in both HIV infected humans and SIV infected disease susceptible rhesus macaques (107, 108). It is thought that indirect mechanisms that lead to progressive immunodeficiency that is characteristic of HIV infection in humans and SIV infection in rhesus macaques is secondary to loss of uninfected $CD4^+$ T cells by apoptotic mechanisms and due to impaired regenerative capacity of the lymphoid compartment. One view that appears to gain wide acceptance within the scientific community is that there is an initial $CD4^+$ T cell loss due to direct virus induced cytolysis, followed sequentially by an expansion of $CD8^+$ cells with subsequent increased susceptibility to apoptosis of both uninfected CD4 and $CD8^+$ T cells and associated $CD4^+$ T cell dysfunction, destruction of the thymus and lymph node architecture, hematopoietic exhaustion and failure and increased susceptibility to opportunistic infections and death. Levels of immune activation appears to be more closely related to the levels of $CD4^+$ T cell depletion than viral loads (109). Our laboratory obtained PBMC's from a group of uninfected and SIVmac251 infected rhesus macaques and for purpose of comparison PBMC's from SIV seropositive and seronegative sooty mangabeys and examined the major PBMC subsets for their relative susceptibility to undergo apoptosis in vitro. Thus, PBMC's were either cultured with immobilized anti-CD3 alone (clone FN18), with 5 X 10-5 dexamethasone (positive control) or media (negative control) overnight. The frequency of apoptotic cells was then measured using the APOPTAG kit. Several interesting data emerged (see Figure 4). First of all, the PBMC's from SIV infected rhesus macaques were relatively more susceptible to undergo apoptosis than cells from uninfected rhesus macaques. Secondly, the relative increased susceptibility of the PBMC's from SIV infected rhesus macaques to undergo apoptosis was unrelated to the level of plasma viremia. Thirdly, the $CD8^+$ T cells from the SIV infected rhesus macaques were more susceptible to undergo apoptosis than the $CD4^+$ T cells from the same animal. Fourthly, the PBMC's from SIV^+ sooty mangabeys did not demonstrate such susceptibility since the frequency of apoptotic cells was similar to that seen with SIV- sooty mangabeys and uninfected rhesus macaques. The relative resistance of PBMC's from uninfected rhesus macaques and the sooty mangabeys to undergo apoptosis was not secondary to an intrinsic resistance of the cells since the values obtained with PBMC's incubated with dexamethasone were essentially similar from rhesus macaques and sooty mangabeys. In addition, the resistance of SIV^+ sooty mangabey PBMC's to undergo apoptosis was not secondary to a failure to undergo proliferation upon

culture with immobilized anti-CD3 since the level of proliferation was in fact higher in cells from mangabeys than macaques (see below). While considerable data has accumulated on the various pathways and mechanisms that underlie $CD4^+$ T cell loss by apoptosis in HIV infected humans which include cross linking of CD4 by gp120, aberrant T cell signaling, cytokines, Fas/Fas-L interactions, superantigen activity and the potential role of accessory cell induced signals (110-123), relatively little is known as to why such apoptosis does not occur in the hematopoietic lineages from SIV infected sooty mangabeys. Our lab attempted to address this issue. First of all, the role of cytokines in promoting apoptosis of PBMC's from SIV infected rhesus macaques and resistance of PBMC's from SIV infected sooty mangabeys was studied. Results of this study showed that whereas overnight incubation of PBMC's from SIV infected rhesus macaques with immobilized anti-CD3 in the presence of varying concentrations of IL-2, IL-4, IFN-g and anti-TNF-a had no measurable and/or reproducible effect on the frequency of apoptotic cells, the addition of IL-10, IL-12 and anti-IL-4 led to a significant reduction on the frequency of apoptotic cells. A similar study using PBMC's from SIV seropositive and seronegative sooty mangabeys failed to reverse resistance to anti-CD3 induced apoptosis. Secondly, we reasoned that whereas SIV infected rhesus macaques have a range from 0.1 to 10 in 10,000 PBMC's that are infected, SIV infected sooty mangabeys demonstrate a somewhat lower frequency of pro-virus infected PBMC's. In addition, the apoptosis resistance could be due to the gradual depletion of apoptosis sensitive cells in sooty mangabeys following infection and the PBMC population obtained from mangabeys may represent a "filtered" population. To address this issue, we obtained PBMC's from 3 SIV seronegative mangabeys, stimulated them with either PHA or con-A and infected them in vitro with SIVmac239 and following infection assessed their susceptibility to undergo apoptosis by incubating them with media alone, immobilized anti-CD3 alone or immobilized anti-CD3 + anti-CD28. Uninfected cells cultured in the same fashion served as controls. The frequency of apoptotic cells following overnight incubation was assessed by flow cytometry. No increased susceptibility to undergo apoptosis due to acute SIV infection was noted in these studies (data not shown). It has been previously shown that SIV infection of rhesus macaques induces severe depletion of $CD4^+$ $CD8^+$ (DP) thymocytes, an increase in the apoptosis of thymocytes, and down regulation of MHC class I molecules (124). The increased levels of apoptosis was associated with an increase in Fas and decrease in the levels of Bcl-2 (124). Unfortunately such studies utilizing surgically obtained thymocytes are difficult to perform on sooty mangabeys due to their endangered species status, otherwise it would be of great interest to determine the role SIV infection plays on thymic architecture of sooty mangabeys.

4.2. Role of acquired immunity

The acquired humoral and cell mediated immune response profile of sooty mangabeys as compared with rhesus macaques following immunization with nominal antigens and SIV is provided below.

4.2.1. Humoral immune responses

Our lab has experimentally immunized normal rhesus macaques and SIV infected sooty mangabeys with a number of nominal antigens such as KLH, tetanus toxoid, allogeneic cells and an attenuated influenza virus in efforts to determine if there is any quantitative and/or qualitative differences in the antigen specific immune responses of these 2 species (43). With regards to the antigen specific humoral immune responses, SIV infected sooty mangabeys mounted the same levels (EIA titers) of KLH, T.T., anti-alloantibody and flu specific IgG responses as rhesus macaques. The only difference noted was in the kinetics. Thus peak antibody responses occurred much faster in sooty mangabeys than rhesus macaques. This is reasoned to be likely due to the constitutively immune activated state of SIV infected sooty mangabeys. Unfortunately levels of plasma viremia and pro-viral DNA loads were not determined in samples from these monkeys to allow for any correlation between level of viremia and immune responses. Of importance is to note that immunization with the same nominal antigens leads to marked decreases in antigen specific memory responses in rhesus macaques following SIV infection (125). Thus, whereas experimental SIV infection of rhesus macaques leads to a marked reduction in antigen specific memory immune responses, no such decrease was noted in the naturally SIV infected sooty mangabeys. A limited study was also performed by our lab in efforts to determine the immunoglobulin isotype specific immune responses against the same nominal antigens. We first attempted to screen the available monoclonal antibodies previously characterized as having specificity against human Ig isotypes for their potential cross reactivity against rhesus macaque and sooty mangabey serum Ig isotypes based on data published (126, 127). Such a study has led to the identification of monoclonal antibodies that clearly react with the major immunoglobulin isotypes IgM, IgG and its subclasses IgG1, IgG2, IgG3 and IgG4, IgA and to a lower extent IgD from rhesus macaques and mangabeys. Reactivities against rhesus macaque and sooty mangabey IgE were difficult to ascertain in these studies. It is important to note that the study from the lab of Dr. S. McDougal (126) utilized a capture assay. If one utilized the monoclonal antibodies as detection reagents, some of the low affinity monoclonal antibodies, which showed little or no reactivity by the capture assay, did seem to react with rhesus macaque and sooty mangabey sera. Thus monoclonal antibody clones HP 6002 (IgG 2) and clone 6083 (IgM) do show reactivity as a detection but not as capture reagents. Use of reactive isotype specific monoclonal antibodies revealed no major distinction in either the titer or isotype preference in the response of rhesus macaques or sooty mangabeys against KLH, T.T or influenza virus. The only difference noted was the relatively faster kinetics of the response in mangabeys and of great interest a significantly prolonged antibody titer in mangabeys as compared to macaques. Thus, anti-KLH, T.T. and flu specific circulating antibody responses could readily be detected in the immunized mangabeys at 2 years following immunizations when the similarly immunized rhesus macaques showed titers of < 1:50. Thus, it appears that memory humoral immune responses appear to be relatively more long lived in mangabeys than rhesus macaques.

With regards to SIV specific antibody responses, once again sera from both SIV infected rhesus macaques and sooty mangabeys appear to contain high titers of antibodies against SIV. The patterns on Western Blots of sera from SIV infected macaques and mangabeys were indistinguishable. Isotype specific titers against SIV were performed and some interesting differences emerged. Thus, sera from SIV infected sooty mangabeys appeared to have significantly lower titers of IgG 2 anti-SIV specific response than SIV infected rhesus macaques ($p< 0.01$). Conversely, shortly following acute infection, the sera from rhesus macaques had significantly higher IgG 1 and IgG 2 responses than the sera from the SIV infected sooty mangabeys. The significance of these differences is not readily apparent at present and could be due to the cytokine milieu differences in the 2 infected species. Autologous virus specific neutralizing antibodies were present in the sera from both SIV infected rhesus macaques and sooty mangabeys and the titers varied from animal to animal with no clear picture emerging. The breadth of the neutralizing antibody pattern in sera from these 2 species was also performed given the fact that broader SIV specific neutralizing antibody responses are known to correlate with slow disease progression (128). However aliquots of sera from the SIV infected rhesus macaques and SIV infected sooty mangabeys gave inconsistent data using a number of SIV isolates. The reasons for these inconsistencies are not clear at present. There also did not appear to be any correlation between anti-SIV specific antibody titers and level of plasma viremia in both SIV infected species. In addition, we have recently utilized a synthetic 50-mer peptide termed streptococcal IgA-binding peptide (AP) that selectively binds the Fc portion of IgA (129) and have affinity purified SIV infected rhesus macaque and sooty mangabey IgA using this peptide immobilized to a column. Such affinity purified IgA specific antibodies were then analyzed for their reactivity to SIV. Sera from both SIV infected rhesus macaques and SIV seropositive mangabeys showed high titers of IgA isotype specific antibody reactivity against SIV. Such reactivity was not observed in similarly isolated IgA in sera from uninfected animals. Studies are currently in progress in efforts to determine whether such IgA specific antibodies have virus neutralization capacity and whether such IgA anti-SIV antibodies are present in saliva and vaginal washes of SIV infected macaques and mangabeys. The importance of HIV specific IgA antibodies has recently been identified (130).

One additional role of SIV specific antibody has recently been identified by our lab. Thus sera from Mamu-A01$^+$ SIV infected rhesus macaque monkeys appeared to contain anti-p55 gag specific antibodies. Such antibodies, interestingly, were shown to contribute to cross priming of cells from these animals. Thus, incubation of enriched populations of dendritic cells (DC) from these animals with recombinant p55 gag-affinity purified IgG but not normal IgG followed by co-culture of these DC's with autologus PBMC's led to a markedly enhanced CD8$^+$ CTL response against p11C-M peptide specific immune response (131). This report has been the first report for a novel function for SIV specific antibodies in the enhancement of cross priming. It remains to be seen if such cross priming of HIV specific immune response occurs in humans infected with HIV.

4.2.2. Cellular immune responses

Our lab has conducted intense study on the characterization of cellular immune responses using PBMC samples from SIV infected rhesus macaques and sooty mangabeys. Results from these studies are presented below and broken down into those related primarily to CD4$^+$ T cell function and those primarily related to CD8$^+$ T cell function.

4.2.2.1. CD4$^+$ T cell function

Initially, our lab spent considerable time and effort to standardize assays for use with PBMC from the monkeys using as a template assays that were carried out routinely for human PBMC. These assays have been described by our lab in a number of publications. In general, some of the important issues to keep in mind include the fact that unlike human blood, monkeys that are bled are normally bled following anesthesia for obvious reasons. PBMC's isolated from such blood varies considerably from sample to sample based on the amount of endogenous levels of cortisol. Thus, if the assays to be performed are influenced by cortisol, levels of plasma cortisol should be monitored in the sample being studied so that results obtained can be better interpreted. Secondly, while most monkeys are screened for a number of agents such as STLV-1 (132, 133), lymphocryptovirus (134) etc., it is prudent to check on these issues since chronic infections with such agents may interfere with the study and test being performed. Finally, most non-human primates appear to get infected with agents that either induce diarrhea or some other infection at some time period during their captivity. This is normally always noted by attending veterinarians and a careful evaluation of this record should be included for the proper evaluation of data obtained on samples from such monkeys.

4.2.2.1.1. TH1/TH2 paradigm

Germane to the major thrust of the present review, our laboratory attempted to characterize the cytokine profile of naturally SIV infected disease resistant sooty mangabeys as compared to experimentally SIV infected disease susceptible rhesus macaques with the rationale that the cytokine profile may provide some clues to the diametrically opposing results of such infections in these 2 species. In addition, there were studies being conducted during this time period which appear to suggest that a skewing of the quality of the immune response predicted and/or was coincident with the development of disease in HIV-1 infected humans and SIV infected rhesus macaques. Thus, the view presented was that a skew from a TH1 to a TH2 prototype cytokine response in such infected humans and rhesus macaques predicted the onset of disease (135, 136). This general view and/or its precise interpretation is not without controversy (137), nonetheless, it does provide an interesting paradigm with which one can utilize for the monitoring of SIV infection. Thus, the prediction of the Clerici-Shearer hypothesis would be that the disease resistant sooty mangabeys would chronically maintain a Th1 skewed general or SIV specific immune

response which would protect them from the development of disease whereas rhesus macaques who present themselves with a TH2 prototype general or SIV specific immune response following experimental infection would lead to disease. This paradigm was examined in considerable detail by our lab using randomly cloned $CD4^+$, $CD8^+$, $CD4^+/CD8^+$ (DP), and CD4-/CD8- (DN) T cell lines from SIV infected rhesus macaques, uninfected rhesus macaques and SIV seropositive and SIV seronegative sooty mangabeys. Results of these studies (138) basically showed that uninfected rhesus macaques generally demonstrated a TH0 profile and following SIV infection appeared to have a predominantly TH1 skewed cytokine profile early post infection which gradually developed into a TH0 profile late post infection. In contrast, both SIV seropositive and SIV seronegative mangabeys predominantly demonstrate a TH2 prototype skewed profile. Later follow up studies of SIV infected rhesus macaques indeed appeared to confirm the thesis put forth by Clerici and Shearer that the TH1 profile of T cells does change to a predominant TH0 or TH2 profile. However, of great interest is the finding that the sooty mangabeys continuously maintain a TH2 prototype cytokine based profile and of further interest this is also seen in the seronegative mangabeys (43, 138). These data appear to suggest that either the naturally infected disease resistant sooty mangabeys are not a good model to study the role of cytokines in the pathogenesis of lentiviral infection or that it is not the cytokine profile per se but the indirect effects of the cytokines synthesized that is different in the 2 species. It should be noted that such a study was also conducted on enriched population of in vivo activated subsets of $CD4^+$ and $CD8^+$ T cells in these same monkeys using a novel protocol with the hypothesis that chronic SIV infection must lead to a substantial frequency of SIV specific T cells and thus the enrichment of such in vivo activated T cells could represent a significant frequency of SIV specific T cells (138). The findings from these studies were essentially similar to those with the randomly cloned T cell lines. Sooty mangabeys, which are SIV infected and are disease resistant, predominantly express a TH2 cytokine profile. Thus, if a TH2 cytokine profile leads to disease why does it not occur in sooty mangabeys? This issue remains to be resolved. More recently, our lab also showed that it is likely that the infection/disease resistant phenotype could in fact be a result of the mode of activation of $CD4^+$ T cells. Thus, similar to the results from the lab of Dr. Carl June (139) our lab shown that immobilized anti-CD3 + anti-CD28 immunobeads when cultured with $CD4^+$ T cells from rhesus macaques leads to resistance to in vitro infection with CCR5 tropic SIV (27). It was shown in fact that this was because the $CD4^+$ T cells activated in this fashion led to the synthesis of TNF-α which in turn led to the synthesis of significant amounts of MIP-1α, MIP-1 β and RANTES, the natural ligands for CCR5 (140). In addition, it led to an increased expression of CXCR4, which predicts a novel dual role for TNF–α.

4.2.2.1.2. MHC class II restricted immune responses

The second issue with regards to $CD4^+$ T cell function we studied was our ability to measure $CD4^+$ MHC class II restricted immune responses in the SIV infected sooty mangabey with the rationale that SIV infection may in some way compromise this lineage specific immune response at either the $CD4^+$ T cell level or at the level of the antigen presenting cells (APCs) since both these cell lineages serve as an excellent source for viral replication (141). We compared the $CD4^+$ T cell responses of 6 SIV infected sooty mangabeys with that of 4 SIV seronegative mangabeys and 6 uninfected rhesus macaques. Following immunization with KLH, T.T. and flu, PBMC's from each of the SIV^+ and SIV^- sooty mangabeys and rhesus macaques were analyzed for their ability to proliferate and compared the degree of $CD4^+$ T cell proliferation with similarly immunized rhesus macaques. Basically, plastic adherent cells from each monkey of each species were pulsed with varying concentrations of KLH, T.T. and the inactivated flu virus overnight, washed and then co-cultured with autologous T cells and the degree of proliferation determined using standard 3H-thymidine uptake assays. While varying degrees of antigen specific proliferation were noted in individual animals from each species (with no statistical difference in the magnitude of the delta immune response), the SIV^+ and SIV- sooty mangabeys gave a peak proliferative response either on day 3 or 4, the co-cultures from rhesus macaques gave peak proliferative response on days 5 to 6. Thus, the only major difference was the kinetics of the immune response. Similar studies were performed on the same group of monkeys at 6, 14 and 26 month following immunization. Once again the PBMC's from the SIV^+ and SIV- sooty mangabeys maintained their antigen specific immune response for a much longer time interval than the rhesus macaques. Thus, while PBMC from SIV+ and SIV- sooty mangabeys gave stimulation indices of 5.7 +/- 2.1, 3.4 +/- 0.9, and 4.3 +/- 1.1 (mean +/- S.D.) for KLH, T.T. and flu antigen at 26 month post last immunization, rhesus macaques similarly immunized gave stimulation indices of < 2.0 at this same time interval. These data appear to suggest that the homeostatic mechanisms that maintain antigen specific memory T cells are more optimal in sooty mangabeys than rhesus macaques. It is also not clear whether this increased longevity of antigen specific memory T cells is secondary to an increase in the magnitude of the antigen specific T cells at the time of immunization which is reflected by a higher frequency of clonal expansion, or the data are indeed a true reflection of an intrinsic long lived homeostatic mechanisms that keep these cells around for a longer time period. It is also important to note that Long Term Non-Progressor (LTNP) and Non- Progressor (NP) SIV infected rhesus macaques appear to maintain antigen specific memory CD4+ T cell responses against nominal antigens that have a high avidity for the antigen in question as compared with regular progressor or Rapid Progressor (RP) SIV infected rhesus macaques that appear to maintain only the low avidity antigen specific memory $CD4^+$ T cell responses in vivo (142). Similar studies of antigen specific avidity of the $CD4^+$ T cell response in sooty mangabeys has not so far been performed, but, given the above observation of longer term maintenance of memory in both SIV^+ and SIV^- sooty mangabeys, one could predict that the mangabeys should maintain high avidity antigen specific immune responses.

4.2.2.1.3. SIV specific $CD4^+$ T cell responses

Our lab has previously shown reasonable SIV specific $CD4^+$ T cell response in SIV infected sooty

mangabeys (142). This is of interest and importance since the presence of such virus specific helper T cell responses in naturally SIV infected non-human primates have been questioned by data from other labs. Our lab initially also had a difficult time demonstrating virus specific $CD4^+$ T helper responses by just culturing PBMC's from SIV infected mangabeys. The only way we could see some reliable data is when we pulsed APC's from the mangabeys with inactivated "whole" SIV preparations and then washed them and co-cultured them with autologous PBMC's. It should be kept in mind that such virus preparations contain a lot of proteins other than virus encoded protein and we agree that this issue needs to be re-addressed utilizing a large series of SIV peptides and more sensitive assays than just proliferation and the specificity of the $CD4^+$ T cell response needs to be carefully re-examined. Such studies are currently underway in our labs using pools of SIVmac239 based synthetic pools of overlapping peptides and ELISPOT or ICC assays.

4.2.2.1.4 .$CD4^+$ T cell and immunological anergy

During the course of studies related to defining the mechanisms of disease resistance of SIV infected sooty mangabeys, our lab reasoned that a detailed examination of the mechanisms by which $CD4^+$ T cells from this species maintains its immunological function (as described above) and does not demonstrate any detectable functional compromise, would be a good initial place to start. We initiated these studies by first examining the requirements for optimal activation of this cell lineage with the use of anti-CD3 and anti-CD3 + anti-CD28 immobilized beads. Much to our surprise, it appeared that $CD4^+$ T cells from SIV seropositive and for that matter SIV seronegative sooty mangabeys appeared to be relatively resistant to the induction of immunological anergy (125). We believe that the signals and the pathways that are activated by this cell lineage upon TCR ligation with and without co-stimulation may provide some unique insights on the functional properties of this cell lineage which in turn may provide some insights on disease resistance (143, 144). Such studies are in progress and additional details on this subject are provided in a different chapter in this monograph.

4.2.2.1.5. Role of $CD4^+$ T cells in immune reconstitution

One of the areas of active studies in our lab involve seeking methods for immune reconstitution of SIV infected monkeys with the aim that findings from such studies may provide important information that can be readily translated into studies of immune reconstitution of HIV infected humans. Our data appear to suggest that while there is substantial frequencies of SIV specific CTL's in SIV infected rhesus macaques late post infection as determined by MHC class I tetramer staining, these cells appear to be non-functional as determined by decreased CTL effector function, ability to synthesize IFN-γ by both ELISPOT and intracellular cytokine (ICC) assays. Addition of autologous $CD4^+$ T cells (collected prior to infection from these monkeys) to the non-functional PBMC collected post SIV infection in vitro appeared to replenish the SIV antigen specific CTL function. These findings coupled with our finding that anti-CD3 + anti-CD28 activated $CD4^+$ T cells from rhesus macaques appear resistant to SIV infection in vitro prompted us to examine the potential of such in vitro expanded $CD4^+$ T cells to provide immune reconstitution in vivo. Results of these studies although utilizing a small number of monkeys (n = 3/ group) appear to strongly suggest that such infusions of non-antigen specific $CD4^+$ T cells led to marked reductions in plasma viral loads, pro-viral DNA loads and of clinical interest, a marked prolongation of disease free survival (145). The mechanisms by which such antigen naïve $CD4^+$ T cells mediate such functional immune reconstitution is under study. Our working hypothesis is that such activated $CD4^+$ T cells must either provide non-specific helper function to the $CD8^+$ T cells in the form of cytokines etc. which rejuvenates the virus specific effector function or that such non-specific helper function must lead to induction of immune responses against newer SIV epitopes that are highly functional in reducing viral burdens. The reasons for inclusion of these findings in this paper are that we attempted a somewhat similar study with cells from sooty mangabeys. Thus, our lab has had difficulty in demonstrating SIV specific $CD8^+$ CTL functional activity in disease free seropositive sooty mangabeys for the past decade with exhaustive attempts. Our rationale was that perhaps that this lack of $CD8^+$ CTL function maybe due to the absence of effective $CD4^+$T helper cell responses since these monkeys are indeed infected and some of them with relatively high viral loads. Thus, studies were performed whereby $CD4^+$ T cells from SIV seropositive mangabeys were expanded in vitro by co-culture with anti-$CD3^+$ anti-CD28 conjugated immunobeads. Such activated $CD4^+$ T cells were then cultured with autologous $CD8^+$ T cells in the presence of autologous APC's that were previously infected with Vaccinia-env or Vacc-gag/pol as previously described (142) and the resulting cells analyzed for SIV env and gag/pol specific CTL activity. Such studies failed to lead to any reproducible levels of antigen specific CTL activity. Thus, our failure to detect such SIV specific CTL function in PBMC's from SIV infected sooty mangabeys was not likely secondary to a lack of $CD4^+$ T helper cell function. It is important to note that the laboratory of Dr. Kaur has on the other hand clearly shown the existence of SIV specific CTL's in sooty mangabeys (146) and thus there must be some technical issues that need to be examined carefully to address such paradoxical results. In this regard, it is important for our lab to utilize more optimal methods for the activation and expansion of $CD4^+$ T cells from rhesus macaques for their evaluation as potential immunotherapeutic strategies, as recently outlined by Zhang et al (147) .

4.2.3. $CD8^+$ T cell function

It is now the consensus opinion that one of the major contributors to effective anti-lentiviral immunity and disease protection from lentiviral infection in both HIV infected humans and SIV infected rhesus macaques are lentiviral specific $CD8^+$ T cell mediated CTL responses (148). The role of $CD8^+$ T cells in inhibiting viral replication in vitro by non-cytolytic mechanisms is described above under innate immune responses although one can clearly argue that it is possible that such function is acquired post infection and should therefore be discussed under this section. The narrative herein will focus primarily

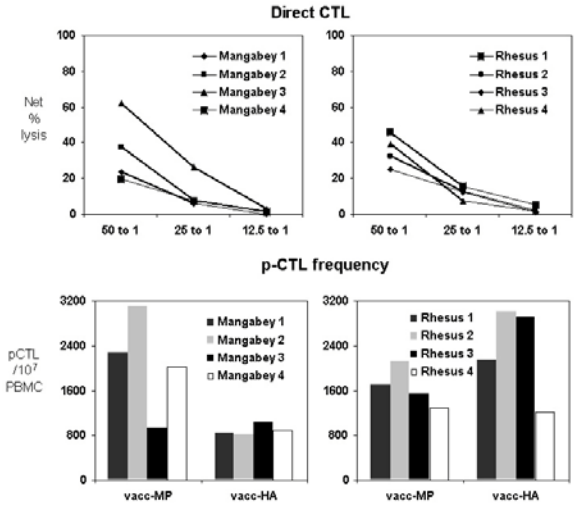

Figure 5. CTL effector function in Influenza virus immunized SIV infected Sooty Mangabeys and for comparison, uninfected Rhesus Macaques. PBMC's from 4 SIV seropositive mangabeys and 4 rhesus macaques who were immunized with influenza virus were assessed for direct CTL activity (Fig. 5A) or for p-CTL values using standard analysis. (Fig. 5B). Data represent net % activity.

on $CD8^+$ T effector CTL function. Several issues with regards to such function in sooty mangabeys and rhesus macaques have and continue to be addressed by our lab. These are outlined below.

4.2.3.1. Nominal antigen specific CTL function

First of all, can we readily detect non-SIV related nominal antigen such as influenza virus specific $CD8^+$ CTL effector function in SIV infected sooty mangabeys or is this function only present in SIV seronegative mangabeys and is compromised following SIV infection of this species? In efforts to address this issue, we first established herpes transformed cell lines from 4 SIV infected sooty mangabeys and 4 uninfected rhesus macaques and then experimentally infected this group of 4 SIV infected sooty mangabeys and uninfected rhesus macaques each with live attenuated influenza virus intranasally. Following a couple of booster doses with the same virus, standard CTL responses were assessed. The CTL assay was performed using vaccinia-MP and vaccinia-HA infected target cells both by a direct CTL assay and by a limiting dilution CTL assay that required re-priming in vitro. As seen in Figure 5 A and B, both sooty mangabeys and rhesus macaques possess readily detectable $CD8^+$ effector cells against flu-MP and flu-HA in their PBMC's. The PBMC were analyzed on day 7 for p-CTL values and day 12 for direct CTL values. There did appear to be more p-CTL's against flu-HA in rhesus macaques than sooty mangabeys for reasons that are not readily apparent. However, the important issue is that there is no detectable defect of CTL function (quantitative and/or qualitative) in the PBMC's of SIV infected sooty mangabeys. The CTL function being measured in these assays was shown to be MHC class I restricted (> 75 % blocking of CTL function by anti-MHC class I monomorphic antibody) and being mediated by $CD3^+$, CD8 α/β expressing T cells (by depletion of CTL activity by the removal of CD 8 α/β expressing cells) in both the assays (data not shown).

4.2.3.2. SIV specific $CD8^+$ CTL function

Clearly based on the evidence presented so far in both HIV infected humans and SIV infected rhesus macaques, virus specific CTL effector function play an important role in eliminating virus infected cells and maintenance of LTNP status. It therefore appears clear that SIV infected sooty mangabeys must possess such SIV specific effective CTL function. However, as described above briefly, our lab has been unable to demonstrate any reproducible SIV specific CTL effector function in SIV infected sooty mangabeys despite a decade of attempts. We utilize the same protocol as we have in the past for the measurement of CTL function in rhesus macaques and as described above for the measurement of flu-specific CTL's in sooty mangabeys. To a large extent, there is always a relatively higher background with mangabey effector cells, which we interpret to indicate that mangabey cells are usually always activated and the background could be a reflection of this chronic activation state. We have recently begun a study (similar to the one described above for the analysis of SIV specific $CD4^+$ T cell function in SIV infected mangabeys) utilizing a large number of overlapping SIV env, gag, pol, nef, tat synthetic peptides and we are analyzing the ability of $CD8^+$ T cells to synthesize IFN-γ by the ICC assay. Data from such studies may help resolve these differences.

5. THE PARADOX

The interpretation of all the data presented above needs to be analyzed in the context of the existing paradox. Thus, if SIV infection is readily contained by a very efficient and effective SIV specific humoral and cellular response in sooty mangabeys, why then is there such a high viral load in these monkeys and why is virus not eliminated or at least reduced to a level that is seen in LTNP rhesus macaques? The levels of plasma viral load and pro-viral DNA load in sooty mangabeys can approach and/or in select animals exceed those seen in rhesus macaques that develop disease and die. Thus it is not viral load that can account for the lack of disease in this species. If the virus in the mangabeys is not immunogenic and/or if the mangabeys have developed immunological tolerance to the viral antigens, why then do they demonstrate such high antibody titers and at least T helper cell responses? Sometime ago, our lab had forwarded the hypothesis (Jonathan Powell and A. Ansari) that perhaps sooty mangabeys have developed "SPLIT TOLERANCE". In other words, they develop humoral immune response but not CTL responses. This concept seems to suggest that perhaps CTL response are not the best to have in lentiviral infections and perhaps development of CTL responses is a 2-edged sword. It can eliminate virus infected cells but in the process lead to a robust level of immune activation with an associated increase in the level of virus replication and further destruction of target cells, which in the case of SIV means $CD4^+$ T cells and macrophages. In the case of mangabeys, the viral response is only against dead virus, which is processed and presented to the immune system for

the generation of anti-virus specific antibodies, but such antibodies do not cause any inflammation and/or immune system activation. It should be noted that while experimental infection of SIV seronegative mangabeys with SIVmac239 and SIVmac251 leads to readily detectable viremia and readily detectable humoral SIV specific immune responses, to date such experimentally infected mangabeys have not developed any detectable disease. Thus, if such "SPLIT TOLERANCE" was operational, how is this induced in SIV negative mangabeys since such T cell tolerance must have occurred in utero during thymic positive/negative selection? Is it possible that there is a pathway for the development of T cell tolerance during adult life? The naturally infected non-human primates provide a very interesting and we submit a very important PARADOX that needs some attention. It is also important to note that depletion of $CD8^+$ T cells in SIV infected mangabeys similar to the study performed by Dr. Letvin's lab has also not led to any detectable disease in these animals. The other issue that is always bothersome to our lab concerns the plethora of data that exists in the literature on how recombinant SIV proteins and peptides such as tat and nef (there are many others) cause a multitude of pathological effects on nearly every mammalian organ including heart, CNS, kidney, gut etc. If this is really true why do mangabeys, who have the same virus and to the same quantitative level, never demonstrate any of these effects? Are these studies utilizing recombinant SIV proteins in effect artifacts? For example, the effect of "tat" on the endothelial cells has long been recognized and thought to play an important role in the vasculopathies associated with SIV including the development of Kaposi's sarcoma like lesions. Why does "tat" not manifest such functions in the naturally infected species? Why is hematopoiesis basically normal in SIV infected sooty mangabeys? There are a multitude of issues that need to be addressed which appear as paradoxes.

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