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LENTIVIRUS INFECTIONS AND MECHANISMS OF DISEASE RESISTANCE IN CHIMPANZEES

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1. ABSTRACT

One year after the human immunodeficiency virus (HIV) was pinpointed as the etiological agent of the acquired immunodeficiency syndrome (AIDS) in humans, chimpanzees were identified as one of the few living species also capable of sustaining persistent HIV-1 infection. During the mid to late 1980s, as the AIDS epidemic spread globally in humans, the chimpanzee was eagerly looked to for answers concerning effective AIDS therapies and a possible HIV vaccine. Neither an effective vaccine nor a therapy has emerged probably because of the complicated inter-relationship of the AIDS virus with the human immune system. Nevertheless, one remarkable observation is that, unlike humans, chimpanzees are relatively resistant to the development of AIDS. In the meantime, HIV-1 vaccine and therapy research has moved to SHIV/SIV_{mac} infection in rhesus macaques as a model of AIDS for which disease intervention studies can be better performed. Chimpanzees are very rarely used in applied HIV-1 research anymore. However, pertinent questions about the mechanisms of resistance to AIDS in this species beg to be answered. Furthermore, after more than twenty years of intense search for the origin of the AIDS epidemic, the spotlight has recently been turned once again on to the chimpanzee. Here we review the history of HIV-1 infection in this species as well as the observations that have led to some of the current leading hypotheses regarding the resistance to AIDS in naturally infected African primates.

2. INTRODUCTION

With an estimated 97 to 98% DNA homology with that of humans (1, 2), chimpanzees are humankind's closest living relative. However, the species is highly endangered, and in various regions of Africa certain subspecies of the common or pygmy chimpanzee are severely threatened with extinction due to habitat destruction, poaching and disease. Chimpanzee populations are fragmented and are found throughout equatorial Africa in various regions of West, Central and East Africa (Figure 1). Although debated, it is estimated that there may be up to four different subspecies of the common chimpanzee in addition to the Bonobo (pygmy chimpanzee). Chimpanzees are highly susceptible to a number of human viral infections, and outbreaks of Polio and/or Ebola have led to loss of life in the animals' natural habitats. Despite their close resemblance to humans, however, chimpanzees are obviously significantly different. (It should be remembered that even the mouse has approximately 90% DNA homology with humans.) These chimpanzee similarities and differences are the focal point of the following discussion. What are the natural immunological and non-immunological differences that chimpanzees possess, which allow them to successfully combat the AIDS virus and to avoid development of the disease despite their susceptibility to infection? Here we document the historical progression of studies of HIV-1 and related lentiviruses in chimpanzees and the similarities to and differences from humans with respect to specific humoral immune responses and to innate mechanisms. We review the discovery of the natural SIV_{cpz} infection in chimpanzees, and compare HIV-1 and SIV_{cpz} infection in *Homo sapiens* and *Pan troglodytes,* respectively.

3. EARLY STUDIES WITH HUMAN LENTIVIRUS ISOLATES

In the late 1970s and early 1980s, in San Francisco, Amsterdam and other cities around the world, an epidemic of Kaposi's sarcoma in homosexual men emerged, which raised intense concern that an unidentified

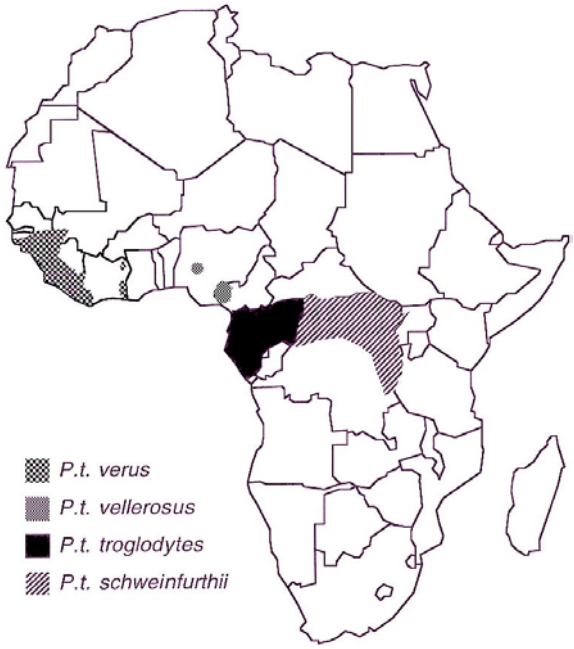

Figure 1. Distribution of wild common chimpanzee populations in Africa. Chimpanzees are widely spread throughout sub-Saharan Africa. Each subspecies has a well-distinguished habitat. SIV_{cpz} occurs in the two species that live closely together in the Gabon / Zaire region (*schweinfurthii and troglodytes*), whereas the more northern- living species (*verus and vellerosus*) have not yet been identified as carriers of a natural SIV_{cpz} infection. Most animals used for HIV-1 studies have been *P.t. verus.*

infectious agent with serious public health consequences was circulating. In 1982 blood was transmitted from one of Amsterdam's first (Kaposi's sarcoma) AIDS patients to a chimpanzee at the TNO primate centre (now Biomedical Primate Research Centre); however, no evidence of disease could be detected. In 1984 Alter and co-workers published the first report on HIV-1 infection in chimpanzees, by a virus, which, at that time, was called HTLV-III (3). It was this study that heightened the world's concern about the problem of contaminated blood supplies. Two of three animals given plasma from patients with acquired immunodeficiency developed evidence of HIV-1 infection ten to twelve weeks after transmission. One of these animals developed lymphadenopathy and a transient decrease in the CD4+ T lymphocytes. In this one animal, mild to moderate lymphadenopathy persisted for 32 weeks, during which antibodies to the virus developed (3). In 1986, it was documented that the virus could also be transmitted via vaginal secretions to this primate species (4), demonstrating that heterosexual transmission in the human population was also an important risk factor. Interestingly, however, studies in 1987 of chimpanzees in group housing with infected HIV-1 carriers documented a lack of transmission of HIV from infected to uninfected animals (5). Later that year it was noted that chimpanzees carrying a primary HIV-1 infection could be super-infected with a second, different strain of human immunodeficiency virus (6). Studies examining the early events of HIV entry and integration into peripheral blood mononuclear cells revealed that, despite the difference in a panel of HIV-1 isolates, there was no impairment of HIV entry, expression and production from chimpanzee cells. Both human and chimpanzee cells were reported to be similar with respect to pre- and post-entry replication events of the virus (7).

4. HUMORAL IMMUNE RESPONSES TO HIV-1 IN CHIMPANZEES

It was quickly noted that the serological IgG response to HIV-1 infection in infected chimpanzees was very similar to that in humans (8). In 1987 Nara and co-workers reported on the specific characteristics of the serological responses and the property of re-isolated viruses in infected animals. Interestingly, they found a highly type-specific neutralising activity in the serum from the infected animals (9, 10). Antibody titres against viruses continued to increase more than two years after exposure, but had limited ability to neutralise both homologous and heterologous virus from other individuals. It was in these studies that early type-specific and group-specific antibody-mediated and complement-dependent cytolysis of HIV-infected cells was identified (9, 10). Such studies on neutralising antibody responses induced by the HIV-1 envelope precipitated efforts to develop a gp120 or gp160 envelope-based vaccine. A primary attempt to develop a prophylactic vaccine against HIV-1 infection was made, and in 1987 the initial report of chimpanzees immunised with the first candidate recombinant subunit HIV vaccine was published (11).

Subsequently, a focus on HIV vaccines began with studies to evaluate the efficacy of early vaccine candidates to protect chimpanzees from infection. One of the first HIV vaccine efficacy trials evaluated recombinant vaccinia virus expressing the HIV-1envelope. Despite the induction of HIV-specific antibodies and T-cell responses by immunisation, virus could still be isolated from all vaccinated animals after exposure (12). Use of the HIV-1 envelope as vaccine antigen was justified by the perception that such a subunit vaccine could elicit broad neutralising antibodies against diverse HIV strains. Proof of principle studies, undertaken by Prince *et al.*, showed that pooled inactivated human immunoglobulin from HIV-infected individuals could neutralise virus *in vitro* (13). However, primary efforts with this 'HIVIG' preparation were ineffective in preventing infection of chimpanzees. Studies to raise even higher titres of antibodies to the envelope proteins of HIV-1 (gp120/ gp160) continued. Subunit gp120 expressed in eukaryotic CHO-cells was successful in inducing neutralising antibodies to HIV-envelope pseudotyped homologous virus as well as several closely related, but heterologous, HIV-1 isolates. However, the immune responses induced by the recombinant gp120 were not effective in preventing infection after exposure to HIV-1. It soon became obvious that the specificities of the neutralising antibodies were so narrow that prevention of infection with closely related but different virus isolates was unlikely.

The studies on chimpanzees infected with HIV-1 were fundamental in characterising the precise epitopes of

the envelope, which bound to certain specific neutralising antibodies (14). In this era the race for an HIV vaccine accelerated. The number of vaccine efficacy trials in chimpanzees involved various forms of recombinant gp120 expressed in different prokaryotic or eukaryotic systems. One notable study reported the protection of chimpanzees after immunisation with gp120, but not gp160 (15). This team later went on to perform studies that demonstrated a key role for highly specific neutralising antibodies in HIV vaccination. Emini and co-workers (16) showed that a high dose of chimpanzee polyclonal anti-HIV immunoglobulins could protect chimpanzees from infection. An important observation was that a particular monoclonal antibody directed to the V3 principal neutralising domain was capable of reducing viral load. During this period the specific mapping of neutralising antibody determinants was begun by peptide screening (17). Meanwhile, other investigations defined additional types of protective humoral responses. Antibody-dependent cellular cytotoxicity (ADCC) responses were identified in chimpanzees immunised with different inactivated or recombinant vaccinia virus vaccine formulations. However, anti-HIV ADCC activities in infected chimpanzees were found to be different from well-characterised anti-gp120 ADCC present in HIV-infected patients. ADCC was first reported to be relatively rare and a late occurring event in chimpanzees (18). Several years later Broliden convincingly demonstrated the presence and differences of broad ADCC in infected chimpanzees (19).

About this time, data from a sequential series of studies on chimpanzees revealed that both vaccination and/or natural infection led to very type-specific and narrow neutralising responses. Subsequent investigations illustrated the need for various types of immune responses for protection of vaccinated chimpanzees. Studies by Gibbs (20) suggested that immunity could be induced in the absence of strong neutralising antibodies. Furthermore, not only were humoral responses in blood studied, but mucosal immune responses as well. Again differences were noted between infected chimpanzees and humans. In chimpanzees, mucosal studies suggested that HIV-1-specific antibodies at the mucosal surfaces of infected animals were of the IgG and not of the IgA subtype (21).

Insight was also gained into the development of neutralising antibodies together with other cellular immune responses (22). Chimpanzee studies have been important in revealing the fine specificity of gp120-directed neutralising anti-serum. It was observed that the presence of all five hypervariable regions (V1 to V5) was required for optimal neutralisation, and that these epitopes were highly conformational (23). Notably, Bruck and co-workers demonstrated a correlation between high neutralising antibody titres and protection from infection (22). Most importantly, not only did they find a correlation of titres with protection from infection, but they also demonstrated, for the first time that animals with higher neutralising antibodies were able to suppress virus load. Strong cellular immune responses were also found in particular animals that were either protected from infection and/or suppressed virus load, suggesting a key role of certain types of specific cell-mediated immune responses and in particular T-helper responses in vaccine protection.

5. CELLULAR IMMUNE RESPONSES AND APPARENT ABSENCE OF CTL

Only after several years did reports begin to emerge describing the cell-mediated immune (CMI) responses mounted against the virus by HIV-1-infected chimpanzees. It was found that chimpanzees had intact T-cell proliferative responses to a number of different recall as well as HIV antigens. This finding was in contrast to observations in HIV-infected humans, where the ability of T-cells to proliferate *in vitro* to both antigens and mitogens slowly deteriorates after infection (24). Over time, strong proliferative responses were found against the purified whole virus, as well as to the recombinant proteins gp120, gp41 and p24. Cell-mediated proliferative responses were also detected in chimpanzees immunised with first-generation HIV-1 candidate vaccines expressed by vaccinia viruses (25). In 1990, Zarling reported that HIV-infected humans, but not chimpanzees, developed circulating cytotoxic T-lymphocytes (CTL) that lysed uninfected CD4+ T cells (26). The cytotoxic cells in humans were characterised as CD8+Tcells and not NK cells. It was hypothesised that chimpanzees may not develop AIDS because of the lack of detectable CTL, which lyse uninfected T cells.

Not all CD8+ T-cell responses are cytotoxic, however. In 1991 Castro *et al.* reported that CD8+ T cells from uninfected chimpanzees were capable of suppressing HIV-1 replication in the absence of direct cell-to-cell contact (27). It was proposed that this CD8+ cell-mediated suppression of viral replication was the possible explanation of the chimpanzee's natural resistance to AIDS. Subsequent studies comparing infected chimpanzees and humans revealed that antigen-presenting cell function in chimpanzees was intact and that T cells had normal proliferative capacity (28).

In the late 1990s DNA vaccine studies indicated that immunisation with naked DNA could lead to CD8+ T-cell-mediated killing of targets (29). In 1999 a series of papers emerged that clearly demonstrated that chimpanzees also developed CTL responses to HIV-1 with specific characteristics. Balla *et al.* were the first to reveal that infected, non-vaccinated chimpanzees developed CTLs, highly specific for conserved HIV epitopes, and they were found at low levels in all animals studied (30). Interestingly, some of the epitopes identified by chimpanzee CTL were highly conserved and were restricted by HLA-B27 and HLA-B57 molecules, which were predominant in human long-term survivors. Subsequently they reported that CTL were also induced in chimpanzees following gp120 immunisation with a potent Th-1/Th-2 adjuvant (31). Later that year an independent group confirmed the reports from Balla *et al.* (32). An important *in situ* study later revealed that HIV-1-infected chimpanzees did not accumulate CTL in germinal centres of lymph-nodes (33). This was in contrast to infected humans, who had a high number of infected

Table 1. Human Th-cell tropic retroviruses and Th-cell diseases

Characteristic	Mechanism in AIDS
Underlying lesion	Loss of Th cells
Virus	HIV-1
Characteristic in host	Persistent intracellular and extracellular viremia; high viral load
Frequency of disease	Frequent; approximately > 98% of infected develop AIDS
Activation	Antigen-antibody complexes in germinal centres; infection of APC; aberrant cytokine production by APC and altered cytokine production by Th cells
Apoptosis	Increase in frequency and in susceptibility to Tat
CD3/TCR triggering	Increase in HIV infection

Characteristics of Th -cell tropic virus infection in human patients (43, 85).

cells and accumulated antigen trapped in germinal centres and developed follicular fragmentation associated with infiltrating CD8+ T cells. This data suggested that CD8+ CTLs in humans were associated with destruction of normal lymphoid tissue architecture, whereas in chimpanzees anti-HIV CTL were associated with control of viral infection and possibly with resistance to AIDS. Additional evidence then revealed that CTL may also play an important role in protecting immunised animals from subsequent infection (34). However, in contrast to HIV-infected humans, chimpanzees were able to maintain strong and potent CD4+ T-cell responses against HIV (35). It has been noted that both humans and chimpanzees may experience lysis of HIV-envelope glycoprotein-expressing cells by CD4+ T lymphocytes (36), so in this regard no differences exist between human and chimpanzee.

6. MECHANISMS OF CHIMPANZEE RESISTANCE TO AIDS CIRCA 1993

Almost ten years after the first chimpanzees were infected with HIV-1, no evidence was apparent that these animals would develop AIDS, unlike the situation with HIV-infected humans. Indeed, clear differences were beginning to emerge, which indicated how chimpanzees could maintain a persistent HIV infection without developing the disease. In the period around 1993, the apparent absence of CTL in chimpanzees was thought to be one of the leading explanations. However, the other unusual property was the ability of chimpanzee CD8+ T cells to secrete a soluble factor capable of suppressing HIV-1 replication (CAF for CD8 anti-HIV factor) (27). Interestingly, when CD8 T cells were depleted *in vivo* from HIV-infected chimpanzees, these animals still did not develop disease (AIDS) (37), suggesting that other factors may also play a role. Another consideration was the possible resistance of chimpanzee monocyte/macrophages to HIV infection (28). It was believed that this APC function was preserved in chimpanzees because they were not susceptible to monocytotropic HIV variants. However, two independent groups revealed that *in vivo* passage of HIV-1 in chimpanzees resulted in HIV-1 variants that could infect monocyte/macrophages (38, 39). Chimpanzees infected with these passage variants did not go on to develop AIDS. At about this time two separate groups reported that the development of apoptosis occurred at very high levels in HIV-infected patients. Subsequently we and others demonstrated that HIV-infected chimpanzees had very low or normal levels of apoptotic CD8+ and CD4+ T cells (28, 40). A study of secondary lymphoid tissues revealed that HIV-infected chimpanzees were able to preserve the lymphoid microenvironment in lymph-nodes, and that these observations correlated with the ability of APC to present antigen to intact and functional CD4+ T cells (33). We proposed that it was the ability of infected chimpanzees to maintain normal CD4+ T-cell function in the absence of abnormal levels of activation, anergy, and apoptosis which allows CD4+-dependent effector function (CTL and neutralising antibodies) to be maintained (40).

7. IMPAIRED RENEWAL HYPOTHESIS

During our long-term follow-up study of a cohort of HIV-1-infected chimpanzees we made a number of observations. Loss of chimpanzee peripheral CD4+ T cells does occur in HIV-1 or SIV_{cpz}-infected chimpanzees *in vivo*. However, despite this loss the vast majority of chimpanzees are able to maintain relatively normal levels of peripheral and tissue CD4+ T cells. Furthermore, chimpanzees are able to maintain normal T-helper-cell functions and responses to specific antigens including HIV-1. This is in contrast to HIV-infected humans (Table 1). To address this question we undertook a detailed analysis of the possible causes of the loss of T-helper cell function and/or numbers. Unlike HIV-1-infected humans, persistently infected chimpanzees did not have increased levels of apoptosis or anergy or a shift to a Th-2-like imbalance which was associated with the progression to AIDS in humans (41, 42). It was clear that HIV-1 was cytopathic for chimpanzee CD4+ T cells *in vitro,* but their cell population *in vivo* kept their normal function and numbers. We concluded that chimpanzees maintained the ability to replace infected CD4+ T cells that were either destroyed by the virus or cytotoxic T cells or NK cells, in such a way that these cells were immunologically functional and competent (Table 2). Detailed analysis of secondary lymphoid tissue from infected animals indicated that normal lymphoid architecture and the microenvironment for APC CD4+ T cell interaction was maintained. We proposed that the principal mechanism of AIDS resistance was the ability of chimpanzees to preserve the lymphoid microenvironment necessary for the functional replacement of lost CD4+ T cells (43).

8. ROLE OF INNATE-LIKE IMMUNE RESPONSES AND SOLUBLE INHIBITORY FACTORS IN RESISTANCE

The mechanism of maintaining an asymptomatic HIV-1/SIV_{cpz} infection in chimpanzees has often been attributed to one or more innate immune responses. Although classical concepts of innate immunity are rapidly changing, textbooks still lump together gamma-delta T cells (also known as NKT cells), NK cells and various

Table 2. Th-cell dysfunction and loss in HIV pathogenesis

Loss of Th function	Anergy:	CD4 Cross linking	
		Inappropriate signaling	
Loss of Th cells	Cell loss		
	Direct, as a result of HIV infection		
		Single cell lysis	
		Syncitia formation	
		Immunological removal	
		(CTL/ADCC/NK/Auto T-cells)	
		Apoptosis	
	Indirect		
		Apoptosis	CD4 cross linking
			SA triggering
			APC dysfunction
		Anergy	
Loss of renewal capacity	Anergy		
	Loss or impairment of precursor pool		
	Loss of maturation environment		

HIV infection causes CD4+ T-cell dysfunction and a transient decrease in number. This effect on helper T cells leads to development of characteristic auto immune disease pathogenesis (43, 85).

types of interferon-producing cells. Investigations to date in chimpanzees have only focused superficially on the number and phenotype of innate-type cells in blood of infected versus non-infected animals, and in most cases have not examined in any specific detail the functions of these cells. One study has revealed differences in NK-cell numbers, which were noted to fluctuate inversely with plasma SIV_{cpz} load (44), suggesting that certain NK-cell populations may play a significant role in SIV_{cpz} infection. The activity of NK cells is also correlated with interferon-gamma and TNF-alpha production and specific apoptosis of lymphocytes in humans. It was noted that in chimpanzees these cells do not exhibit high activity (45). These early findings suggested relatively little NK-cell activity in HIV-infected chimpanzees, and were later supported by data from the same group (46), who suggested low NK induced anti-HIV antibody-dependent cellular cytotoxicity (ADCC) in infected chimpanzees as compared to humans. More recent chimpanzee-ADCC studies revealed a relatively low but consistent response to a very broad panel of viruses, a finding that was different from that involving infected humans (19).

In the late 1980s Walker *et al.* (47) described how virus replication in human PBMC cell cultures was increased by the depletion of CD8+ T cells. Notably, virus replication could be inhibited in these cultures by adding CD8+ T cells from infected individuals. There was an absence of HIV-suppressing activity of CD8 cells from seronegative humans. Later, chimpanzee CD8+ T cells were found to produce this factor irrespective of infection (27). Husch *et al.* (48) also reported on an anti-HIV effect of CD8+ T cells from seropositive chimpanzees, but this was only observed in the first 3 weeks after infection, in contrast to findings in humans. Further investigations (27, 49) confirmed the antigen aspecificity of this CD8 effect and also proved that the reduction of viral replication was induced by a soluble factor produced by these cells. In their search for the identity of the CD8 anti-HIV factor, many groups tested large amounts of cytokines and chemokines on their antiviral effect, and used known blocking antibodies in an effort to block the effect in CD8 cultures (50, 51). However, no exclusive conclusions could be drawn from these experiments. Despite the lack of specific identity of the factor, this line of research has continued. Barker and co-workers (52) found suppression against an extremely broad spectrum of HIV virus variants. In chimpanzees, natural killer cells also express the CD8 marker. Thus Ondoa *et al.* (53) performed studies with chimpanzee PBMC, which led them to the conclusion that the CD8 factor was only secreted by CD8+ T cells and not by NK cells. Most recently, a new group of proteins has been proposed as possible CAFs. Zhang *et al.* (54) reported CAF-like HIV inhibitory activity to be due to alpha-defensins in human long-term non-progressors. It remains to be seen whether the alpha-defensins are instrumental in the control of lentivirus in chimpanzees. With the new developments in innate immunology and the advent of proteomics and genomics, insights into the role of innate mechanisms in lentivirus control in chimpanzees are anticipated.

The discovery of this HIV inhibitory activity secreted by CD8+ cells opened up a new direction in HIV research (49). Some years previously it had been revealed that there were different populations of HIV-1 virions, which circulated in the tissues of HIV-infected individuals. Some of these HIV variants had the ability to induce syncytia and cause CD4+ T-cell lysis, while others induced neither syncitia nor cell lysis, and were primarily tropic for monocyte/macrophages. These were referred to as syncytia-inducing (SI) and non syncytia-inducing (NSI) phenotypes of HIV-1 (55, 56). It was later shown that these two phenotypic populations of viruses used specific co-receptors to enter their respective target-cell populations. In addition to CD4, these co-receptors were identified as CCR5 (R5) for NSI variants and CXCR4 (X4) for SI variants (57-59). The search for the soluble HIV-1 inhibitory factor was thought to have ended with the discovery that the beta-chemokines, RANTES, MIP-1alpha and MIP-1beta bound to CCR5 and thus inhibited entry of NSI HIV-1 variants (60, 61). Similarly, another factor

called SDF-1 was identified as the ligand for CXCR4 and found to inhibit the entry of SI variants (62). It was later discovered that these factors could be generated by vaccination and were correlated with vaccine protection (63, 64). It does not appear that the chimpanzee HIV inhibitory factor which we term (CHIF) are the beta-chemokines we reported to block HIV-1 in chimpanzee cultures, since the addition of excess blocking anti-beta-chemokines antibodies did not block HIV infection in this species (65, 66). Furthermore, these beta-chemokines were not found to be elevated in infected chimpanzees, but, surprisingly, were elevated in infected humans, suggesting that these were not resistance factors associated with the protection of chimpanzees from AIDS (53). It is now apparent the beta-chemokines are not the same as the CAF factor and that other soluble molecules must be contributing to this protective effect. Only further rigorous study will ultimately determine whether the relative resistance of chimpanzees to AIDS can be attributable to a single active soluble factor or to a family of them. Separately in this series, K.K. Murthy expands on the role of these soluble factors and lentivirus infections.

9. DISCOVERY OF NATURAL LENTIVIRUS INFECTIONS IN CHIMPANZEES

In 1989 Peeters *et al.* reported on two cases of chimpanzees positive for HIV-1-related lentiviruses in Gabon (67). Virus from one of these animals was partially characterised, revealing antigenic similarity to HIV-1. This new lentivirus was designated $SIV_{cpz\text{-}Gab1}$ (67). Molecular characterisation revealed that $SIV_{cpz\text{-}Gab1}$ possessed a *vpu* gene, a gene characteristic of human immunodeficiency viruses; however, its sequence was different enough to be considered another subtype of HIV-1-related lentiviruses (68). The hypothesis that SIV_{cpz} transmitted between chimpanzees sparked a debate on the origin of the HIV-1 / AIDS epidemic which was met with considerable scepticism because of the lack of other chimpanzee isolates. Two years later a survey of forty-four animals revealed an additional SIV_{cpz} isolate, termed $SIV_{cpz\text{-}ant}$. Interestingly, more HIV-1 sera cross-reacted with $SIV_{cpz\text{-}Gab1}$ than with $SIV_{cpz\text{-}ant}$, suggesting that the latter variant may be a substantially different genetic variant (69). This new virus was isolated from a *P.t. schweinfurthii* chimpanzee called Noah (ch-No), which had been shipped illegally as a gift to Belgium by a former ruler of Zaire. Thought to be a risk to animal caretakers at local zoos, Noah and his cage-mate were sent to the TNO primate centre in the Netherlands. The two chimpanzees joined a larger cohort of HIV-1-infected asymptomatic chimpanzees that were carefully monitored to ensure that there was no evidence of progression to AIDS (40). Follow-up of a naturally infected animal was now possible and infection could be compared with this cohort of HIV-1-infected animals. A number of properties distinguished the SIV_{cpz}-infected animal from those infected with HIV-1. SIV_{cpz} could be routinely cultured from plasma, while HIV-1 was very difficult to culture from material from the HIV-1-infected animals. Furthermore, SIV_{cpz} did not induce syncytia, similar to the NSI variants of HIV-1. SIV_{cpz} plasma virus loads were higher, but were noted to fluctuate inversely with neutralising antibody titres. Nyambi and co-workers investigated the neutralisation antibody kinetics in sera from ch-No against sequential isolates of $SIV_{cpz\text{-}ant}$. They detected a pattern of emergence of $SIV_{cpz\text{-}ant}$ neutralisation escape variants followed by successful host neutralisation responses. On average, escape mutants emerged every 15 months, and it took up to 8 months for a new neutralisation response to develop. Complex changes in envelope regions V1, V2, C3, V4, V5 and gp40 were correlated with this series of events (70). Curiously, the V3 loop sequence (the principal neutralising domain of HIV-1) of $SIV_{cpz\text{-}ant}$ was found to be constant (71). Detailed analysis of lymphocyte subsets of these animals indicated that CD4 T-cell levels remained stable over a 49-month follow-up period. Interestingly, in this study, chimpanzee CD8+ T cells were demonstrated to suppress virus production *in vitro* (44).

In 1995 the genome sequence of a new SIV_{cpz} variant ($SIV_{cpz\text{-}us}$) was determined that clustered with the Gabon SIV_{cpz} isolates. Mitochondrial DNA analysis of the host animal revealed a distinction between the virus genotype and the subspecies of the chimpanzee infected, *P.t. troglodytes* of central Africa and *P.t. schweinfurthii* of Eastern Africa (to which ch-No belongs). Interestingly, Hahn and co-workers concluded that the $SIV_{cpz\text{-}gab}$ and $SIV_{cpz\text{-}US}$ (both from *P.t. troglodytes*) were more related to each other and to all HIV-1 groups than to $SIV_{cpz\text{-}ant}$ from *P.t. schweinfurthii*. They noted that HIV-1 group N was a mosaic of SIV_{cpzUS}- and HIV-1- related sequences. In addition, they proposed that *P.t. troglodytes* was the primary reservoir for HIV-1-related viruses and the source of at least three zoonotic introductions into the human population (72). This publication and a controversial book called “The River” by E. Hooper escalated the debate on the origins of the human AIDS epidemic. After several convincing publications (73-76) ruled out the possibility of contamination of human polio-vaccine stocks as proposed by Hooper, all eyes turned back to the chimpanzees, and to the possibility of chimpanzee bushmeat consumption being the source of human infection (77, 78). However, in 2000 we questioned whether indeed chimpanzees were a direct source or whether an as yet unknown reservoir existed that could have been a common source to both humans and chimpanzees. In the meantime, a number of molecular clock models that range from the early 1900s to the 1950s (79, 80) have been used in an attempt to date the origin of the human epidemic. All indicate that HIV-1 infection in humans is a relatively recent event. Since then, efforts to identify SIV_{cpz} prevalence by assays that would not disturb already threatened wild chimpanzee populations have been successfully undertaken (81), and additional isolates have been identified.

10. COMPARATIVE VIRUS LOADS BETWEEN SIV_{CPZ} AND HIV-1 STRAINS

During the first decade, most HIV-1 chimpanzee studies involved the laboratory-adapted strain of HTLV-III, later known as $HIV\text{-}1_{IIIB}$. Laboratory isolates were often selected because they grew well in transformed human CD4+T-lymphoma cell-lines, a characteristic of SI HIV-1

Table 3. Markers of progression to disease

	Humans	Chimpanzees
Serological markers		
Hypergammaglobulinemia	↑	-
B_2 microglobulin	↑	-
Neopterin	↑	-
$_S$CD8	↑	-
$_S$IL-2r	↑	-
$_S$TNF-alpha –r	↑	-
Cytokines		
TNF-alpha	↑	-
IL-6	↑	-
Phenotypic markers		
DR-CD8	↑	-
CD38	↓	-
CD28-CD8	↑	-
CD57	↑	Not det.
CD25	↑	-

In humans, numerous correlates with disease progression are known. In 20 years of research, however, no chimpanzees (with one exception) have exhibited any of these signs, which corresponds to the absence of clinical manifestations. (↑ = elevated, ↓ = decreased, - = no change and Not. Det.= not detectable in chimpanzee) (85).

variants. The HIV-1_{IIIB} strain was a laboratory contaminant of an isolate called LAI, originally isolated by Montagnier and co-workers (82, 83). This isolate had been sent to the Gallo laboratory for further study. However, *in vitro* propagation led unwittingly to specific adaptations that were not representative of the primary isolates cultured directly from patients on normal PHA-stimulated PBMC. Furthermore, most of the variants found early in HIV-infected individuals were of the NSI phenotype, thus macrophage tropic. Subsequently, since one of the early hypotheses of chimpanzee resistance to AIDS was based on this animal's resistance to monocyte/macrophage infection, it became important to re-address this issue.

Firstly, in the 1980s there were several important exceptions to the use of HTLV-IIIB or HIV-1_{IIIB}. Probably the first chimpanzee to be infected was in 1982 in the Netherlands, with the animal receiving uncultured blood directly from a Kaposi's sarcoma patient in Amsterdam (40). Other animals had received human plasma (3), and together these represented a small subset of animals which had become directly infected with uncultured, unmodified human viruses which did not cause the human AIDS in chimpanzees.

With the advent of the SI/X4 and NSI/R5 classification of HIV variants, a number of chimpanzees were exposed to prototypic NSI/R5 (i.e. HIV-1_{BAL}) or dual tropic (R5/X4) variants, such as HIV-1_{5016} or HIV-1_{HAN2}. The development of a highly sensitive pan-clade quantitative PCR made it possible to compare the *in vivo* virus load kinetics of chimpanzees infected with SIV_{cpz} to different well-characterised HIV-1 strains. Notably, the naturally occurring NSI SIV_{cpz} caused persistent plasma viremia with virus loads fluctuating between $1x10^4$ and $1x10^5$ RNA eq./ml. Classical SI HIV-1 strains such as IIIB or SF2 often had lower peak levels immediately following infection, and remarkably, as in the case of SF2 (and less rapidly with IIIB), declined precipitously to levels below 50 RNA eq/ml over the course of four to six months (84). Interestingly, this was in contrast to the NSI/R5 (BAL) or the dual tropic R5/X4 (5016) HIV-1 strains, which tended to persist at levels comparable to SIV_{cpz} (84). While suggesting a role of co-receptor use and/or cell tropism in persistence of high virus load, these data need to be extended with samples from additional animals infected with different SIV_{cpz} variants that have been characterised for cell tropism and co-receptor usage, in order to be confirmed.

11. ACTIVE INFECTION IN THE ABSENCE OF IMMUNE ACTIVATION

In the early 1990s, evidence began to accumulate that certain differences in lymphocyte markers for subsets of CD4 and CD8 T-cells existed between chimpanzees and humans. For instance, CD8+ T cells in HIV-1-infected humans possessed increased cell surface expression of MHC class II, whereas chimpanzee CD8+ cells had low levels of MHC class II (discussed by Dr Murthy in this issue). We were prompted to determine whether this was due to the activation state of chimpanzee CD8+ cells or whether MHC-II was differentially expressed in subsets of T-cells different from those in humans. To eliminate the role of immune activation we examined a long list of diverse activation markers that had been reported to occur in human HIV-1-infected individuals (reviewed by Copeland and Heeney (85)). A surprisingly large number of immune activation markers were up regulated in infected humans but not in chimpanzees (Table 3). These findings were confirmed in a more limited fashion in specific studies focused on individual FACS- and CMI-based analyses. Gougeon *et al.* described differences in apoptosis or programmed cell-death in (infected) CD4+ cells, which did not occur in HIV-1 and SIV_{cpz}-infected chimpanzees (86, 87).

12. MECHANISMS OF CHIMPANZEE RESISTANCE TO AIDS IN CIRCA 2003

Since 1990, when our group and others began to address questions concerning natural resistance to HIV-1-related lentiviruses in chimpanzees, a number of significant facts have become apparent. To begin with, chimpanzees are only relatively resistant to AIDS (as depicted in Figure 2). The development of an AIDS-like disease in a chimpanzee (88, 89) indicates that some individuals are susceptible and may represent the left-hand side of a bell-shaped population curve. In contrast to humans, only a very small percentage of the current chimpanzee population may lack the correct complement of inherited genes necessary to confer an individual animal protection from developing AIDS. Most animals, however, appear to be resistant, and as a population, have acquired over time a susceptibility/resistance curve to HIV-1/SIV_{cpz} lentiviruses, which has been moved to the right of the bell shaped human curve (Figure 2).

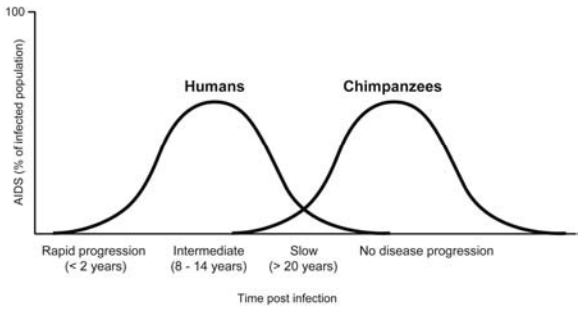

Figure 2. Patterns of disease in outbred populations. Progression to AIDS can be categorised in four stages; rapid progressors will develop AIDS within 2 years after infection. Most HIV-infected humans will develop disease in 8-14 years, whereas the long-term non-progressors tend to live symptom-free for more than 20 years. Most chimpanzees are considered non-progressors, because in more than 20 years of observation only one animal has ever developed AIDS-like disease.

Indeed, it appears that not only the host but also the virus variants are critical factors in the outcome. The HIV-1 that triggered the disease in one animal was an unusual recombinant capable of causing cell death / apoptosis as supported by the evidence of immune activation in histopathological analysis of tissues of recipient animals (90). Another important observation has been that the disease resistance naturally seen in HIV-1 infected chimpanzees is not due to relatively low viral loads since chimpanzees do not control viral infection efficiently, as was observed in early studies (43). Thus, plasma virus loads can be persistent in chimpanzees and maintained at relatively high levels above $1x10^4$ RNA copies /ml. These levels are sufficient to precipitate an AIDS-like disease in susceptible primate species (91). The persistence of high virus loads to date appears to correlate with NSI R5 or X4/R5, but not human SI isolates (84). These moderately high plasma loads are observed in the absence of increased immune activation (43) and elevated beta-chemokines, as found in humans (92).

Moreover, a proposed tolerance-like balance between HIV1/SIV_{cpz} and the chimpanzee host likely plays an even more vital role in sooty mangabeys, African green monkeys and other naturally SIV-infected African primate species. Chimpanzees are not truly tolerant to HIV-1/SIV_{cpz} lentiviruses. They generate neutralising antibodies that appear to be only partially effective, since SIV_{cpz} mutates and escapes (70, 71). We have demonstrated that chimpanzees generate effective CTL responses to highly conserved regions of the virus (30), and believe this is a selective advantage that chimpanzees have acquired. Evidence for this has recently emerged after chimpanzee MHC introns were sequenced. Data generated by de Groot and co-workers have placed this MHC selection before the subspeciation of chimpanzees (94).

Most importantly, chimpanzees are able to maintain an intact CD4+ T-helper response in the face of active HIV-1/ SIV_{cpz}-infection (95). We feel this is due to their maintenance of an intact MHC class II APC T-helper cell micro-environment (33), which allows the effective replacement (43) of any CD4+ T cells that may be lost due to the viral infection. This critical difference between humans and chimpanzees has probably tilted the balance in favour of the vast majority of chimpanzees. The preservation of a competent T-helper immune response allows the effector arms of the immune system to continuously adapt and to maintain full functional integrity. Unlike infected humans, in which the T-helper population becomes functionally and numerically compromised, and compounded by the loss of the MHC class II microenvironment, chimpanzees preserve this environment, necessary for competent renewal of fully functional T-helper cells. The consequence for infected humans is the loss of fully functional and mature effector cell responses such as CD8+ CTL, which in infected humans are immature (96). Clearly, additional differences distinguish humans and chimpanzees and their abilities to control lentivirus infections. Chimpanzee PBMC in general, appear to be less permissive to lentivirus infection *in vitro* (93). This suggests that NK or other cell types probably produce innate-like factors, such as the homologues of human defensins or interferons that have yet to be discovered in chimpanzees.

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