Adaptive Evolution of Simian Immunodeficiency Viruses Isolated from 2 Conventional-Progressor Macaques with Encephalitis

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Simian immunodeficiency virus–infected macaques may develop encephalitis, a feature more commonly observed in macaques with rapid progressive disease than in those with conventional disease. In this report, an analysis of 2 conventional progressors with encephalitis is described. Phylogenetic analyses of viruses isolated from the cerebrospinal fluid and plasma of both macaques demonstrated compartmentalization. Furthermore, these viruses appear to have undergone adaptive evolution to preferentially replicate in their respective cell targets of monocyte-derived macrophages and peripheral blood mononuclear cells. A statistically significant loss of potential N-linked glycosylation sites in Env of H160 was observed in viruses isolated from the central nervous system.

Similar to HIV infection in humans, simian immunodeficiency virus (SIV) infection can cause AIDS in macaques. The survival time for the majority of SIV-infected macaques that develop AIDS ranges from 1 to 3 years; however, a small percentage will develop rapid disease progression, with death occurring within 6 months [1]. This shortened survival time of rapid-progressor macaques has been correlated with the presence of SIV-induced encephalitis [2, 3]. Histopathological changes in the brain, such as the formation of multinucleated giant cells, microglial nodules, and perivascular infiltrates identical to those seen in humans with HIV encephalitis, have also been observed in macaques with SIV encephalitis. Indeed, SIV neuropathogenesis can recapitulate key elements of HIV-1 neuropathogenesis.

Recently, we have observed SIV encephalitis in 2 rhesus macaques with conventional progressive disease, surviving >1 year after inoculation. This situation is more likely to parallel that of HIV-infected patients who do not develop HIV encephalitis until they have late-stage AIDS than is the situation of rapid-progressor animals with SIV encephalitis. In the present study, we evaluated the sequential genetic and biological evolution of SIV in the plasma, cerebrospinal fluid (CSF), and brain from these conventional progressors with encephalitis.

Materials and methods. All macaques were rhesus macaques of Indian origin, housed in accordance with the National Research Council Guide for the Care and Use of Laboratory Animals [4]. To isolate virus, samples of cryopreserved CSF and plasma collected at week 76 after inoculation (H636) and at the time of euthanasia (week 116 for H631) were incubated with primary pigtail macaque peripheral blood mononuclear cells (PBMCs). Additionally, for H631, virus [5] was isolated from brain samples obtained during necropsy. Virus replication of these isolates was evaluated in rhesus macaque PBMCs or monocyte-derived macrophages (MDMs) as described elsewhere [5].

Synonymous/Nonsynonymous Analysis Program (SNAP) [6] was used to calculate the synonymous and nonsynonymous substitution rates. Potential N-linked glycosylation sites were analyzed by use of the N-Glycosite program [7]. The number of glycosylation sites in Env of H631 virus isolates and of H636 CSF virus isolates were compared with SIVsmE543-3 Env by use of a 1-sample, 2-tailed t test, whereas the number of sites in Env of H631 brain virus isolates and of H631 CSF or plasma virus isolates were compared by use of an unpaired, 2-sample, 2-tailed t test. P values <.05 were reported as significant.

Results. As part of ongoing studies, rhesus macaque H445 was inoculated intravenously with the molecular clone SIVsmE543-3 [8]. H445 developed a transient antibody and cytotoxic T lymphocyte response, had a high viral load, and rapidly progressed to AIDS; the macaque was euthanized at week 16 after inoculation [9]. Previous studies have suggested that passage of SIV in macaques may increase virulence; therefore, to evaluate the pathogenicity of SIV isolates from H445, uncloned
virus isolated from the mesenteric lymph node was inoculated intravenously into 6 rhesus macaques [5]. All 6 macaques became infected and had clinical and pathological symptoms characteristic of SIV-related disease [5] (figure 1). Only 1 of the 6 macaques, H635, progressed rapidly and was subsequently euthanized at week 9 after inoculation [5]. The survival time of the other 5 conventional-progressor macaques ranged from 52 to 116 weeks after inoculation [5].
Histopathological assessment of brains obtained at necropsy revealed that H631, H635, and H636 had SIV meningoencephalitis [5], as evidenced by the presence of multinucleated giant cells, consistent with increasing viral RNA levels in CSF (figure 1B). Cuffing of vessels with macrophages was observed in the brain of the rapid progressor, H635. In contrast, perivascular cuffs in the brains of the conventional progressors, H631 and H636, also included lymphocytes. Immunohistochemistry demonstrated that the infiltrating cells consisted of B and T cells and that these latter cells were predominantly CD8^+ T cells (figure 1C). Additionally, infiltrating macrophages and multinucleated giant cells were identified by HAM56, a macrophage marker, and these cells colocalized in lesions with SIV expression, as shown by in situ hybridization (figure 1C). Triple-label confocal microscopy using in situ hybridization for SIV RNA and immunofluorescence for HAM56 and anti-CD3 demonstrated that the SIV-expressing cells in the brain were macrophages (figure 1C).

To determine whether specific SIV variants were associated with the development of encephalitis, we examined the Env sequence of viral RNA amplified directly from the CSF and plasma of H631, H635, and H636. The SIVsmH445 inoculum showed few changes in the gp120 region of Env, compared with the parental SIVsmE543-3 [5]. The majority of these changes were characteristic rapid progressor–specific mutations [5, 11], including the loss of a highly conserved potential N-linked glycosylation site in V2 (N158D/S or S160N/G), substitutions in the V3 analog (P337T/S/H/L), and substitutions in the highly conserved GDPE motif (G386R and D388N/V). These variant clones represented the minority population. Analyses of sequences obtained directly from the CSF (data not shown) and plasma [5] of H635 at death showed that those rapid progressor–specific mutations predominated in both compartments, consistent with the specific selection of this minority variant from the inoculum. In contrast, sequencing of CSF and plasma from H631 and H636 did not reveal rapid progressor–specific mutations in any of the clones analyzed at any time point (figure 2 and data not shown). Consequently, we have used the parental SIVsmE543-3 sequence for comparisons.

Phylogenetic analyses were performed to study the evolution of SIV in H631 and H636 throughout the disease course. During primary viremia, very little evolution had occurred, as reflected by the fact that CSF and plasma sequences were closely related to one another as well as to SIVsmE543-3. However, by week 76, divergence from SIVsmE543-3 could be observed in both tissue compartments (figure 3).

To study the biological consequences of the observed mutations, we isolated virus from the CSF and plasma of both macaques at the last available time point. Additionally, in H631, virus was isolated from the brain. Viruses isolated from the plasma of both macaques were found to replicate in PBMCs much more efficiently than did the corresponding virus from the central nervous system (CNS) of the same macaque (figure 4A). On the basis of our earlier observation of SIV-infected macrophages in the brain, we evaluated replication in MDMs (figure 4B). In contrast to what was observed in the PBMC infections, in MDMs the infectivity of virus isolated from the CNS of both macaques was much higher than that of virus isolated from plasma.

To ensure that there were no donor biases [9], this infectivity assay was repeated, using an additional 3 different donor macaques. The same donor macaques were used in both the PBMC and MDM experiments. Data from the 4 different infection assays were then combined to obtain an average, and the experiment was independently repeated a second time. These experiments (data not shown) confirmed the initial results and demonstrated a marked trend in cell tropism that is dependent on the compartment of origin of the virus.

On the basis of the observed differences in biological phenotypes, we evaluated the phylogenetic relationships based on gp160 sequences obtained from viral RNA. Additionally, bootstrapping support tests were done to examine the reliability of the branching order of the tree. With the exception of 1 clone from plasma virus, all sequences from H631 brain, CSF, and plasma clustered within their respective groups (figure 4C). Although the viruses were quite distinct and formed their own clades, bootstrap values did not support compartmentalization of the viruses. This is not surprising, because we expected brain and CSF viral sequences to be closely related and therefore not to show compartmentalization supported by bootstrap analyses. However, when sequences from brain were excluded, bootstrap values did support compartmentalization between viruses isolated from CSF and viruses isolated from plasma (bootstrap values, 722 and 943, respectively; data not shown). H636 viruses from the CSF and plasma compartments were also clearly compartmentalized (bootstrap values, 916 and 736, respectively) (figure 4C). In contrast, gp160 sequences amplified directly from the CSF and plasma viral RNA of the rapid progressor, H635, showed that these sequences were indistinguishable from one another. Phylogenetic analysis demonstrated that there was in-
termingling of virus between compartments, with no tissue-
specific localization (data not shown), indicating a difference in
selection pressure in this rapid progressor versus the 2 conven-
tional progressors.

The ratios of rates of nonsynonymous to synonymous
changes (dN/dS ratios) were calculated for viruses isolated from
H631 and H636, to determine whether the observed changes
were due to random selection or to positive selection from im-

Figure 4. Biological and phylogenetic characterization of isolated viruses from H631 and H636. A, Representative replication of H631 and H636 isolates in peripheral blood mononuclear cells (PBMCs). B, Representative replication of H631 and H636 isolates in monocyte-derived macrophages (MDMs). E543-3 was used as control (gray), and replication was monitored by RT activity of culture supernatants (Y-axis). Days after infection are shown along the X-axis. Solid black lines represent virus isolated from plasma, and solid red lines represent virus isolated from the central nervous system. Br, brain; cpm, counts per minute; CSF, cerebrospinal fluid; Pl, plasma; RT, reverse transcriptase. C, Phylogenetic analyses of viruses isolated from H631 and H636. Nucleotide alignment of the gp160 region of Env sequences obtained from viral RNA was used to generate a maximum likelihood tree incorporating site-specific nucleotide substitution rates. The tree was constructed using PHYLP [12] and DNArates (version 1.1.0) [13] and rooted on the parental SIVsmE543-3 sequence (GenBank accession no. U72748). Maximum likelihood bootstrapping support values were also calculated with PHYLP. Bootstrap support values are based on 1000 replicates. Only values >70% at the major nodes are shown. The scale shows the number of substitutions per site.
mune pressure. For all cases, the dN/dS ratio was >1, indicating that positive selection was responsible for the evolution of these viruses (data not shown).

Sequencing analyses revealed that there were changes in the potential N-linked glycosylation site pattern that resulted in a statistically significant loss of potential N-linked glycosylation sites in gp160 of H631 brain, CSF, and plasma viruses, as well as H636 CSF viruses, compared with E543-3 (P < .0001 to P < .0054). Furthermore, the number of potential N-linked glycosylation sites in Env of H631 brain virus was found to be lower than that of H631 CSF and plasma virus (P < .0001 and P < .0005, respectively; data not shown).

Discussion. We studied the evolution of actively replicating viruses and determined that, during the acute phase of infection, viruses from CSF and plasma were phylogenetically very similar to one another. This indicated a systemic spread, with seeding from the periphery to the CNS, because the macaques were inoculated by the intravenous route. However, immune selection pressures continued to drive the evolution of SIV in these macaques, such that divergent evolution between the different compartments was observed. Viruses isolated from the CSF of both H631 and H636 showed compartmentalization from viruses isolated from the plasma of the respective macaques. In contrast, there was no evidence of compartmentalization in the rapid progressor, H635. In addition, the types of mutations observed in H635 were typical of rapid-progressor variants, in contrast to the predominance of V1/V2 and V4 changes observed in H631 and H636.

The lack of compartmentalization in H635 is consistent with rapid disease progression and with major dysfunction of the blood-brain barrier in rapid-progressor macaques with SIV encephalitis in which SIV can be found within macrophages in tissues throughout the body [10, 14]. In contrast, SIV was primarily localized to the perivascular regions in H631 and H636. Furthermore, the pathology of the brain of these 2 conventional progressors was significantly different from that of H635 (which did not have lymphocytic infiltrates), with the presence of prominent perivascular mononuclear infiltrates containing both T and B cells. To our knowledge, this is the first report of the presence of B cell infiltrates in SIV-infected monkeys. The presence of lymphocytes is more similar to the pathology in HIV encephalitis [15, 16]. The compartmentalization and lack of rapid progressor–type variants, as well as the presence of lymphocyte infiltrates, support an SIV/macaque model for encephalitis using conventional progressors rather than rapid progressors with SIV encephalitis.

The data from our phylogenetic analysis strongly suggest that the inoculum evolved over time in different compartments under different local selective pressures, resulting in divergent evolution of virus. Consequently, virus from brain/CSF adapted to preferentially infect MDMs, and virus from plasma was better suited to infect PBMCs. Perivascular macrophages have been shown to be the primary cell type productively infected by HIV-1 and SIV in the brains of humans and macaques with lentivirus-induced encephalitis. Thus, our observations extend and support the notion that viruses isolated from the CNS would evolve to selectively infect macrophages.

Given that levels of CD4 expression on human and macaque macrophages are low to undetectable, it is possible that this adaptation may be a result of the selection of viral strains that have less dependence on using CD4 for entry. Indeed, some variants of SIV and HIV have been shown by infectivity assay to be CD4 independent [17, 18]. Moreover, CD4 independence has been associated with a loss of potential N-linked glycosylation sites [19, 20]. Our finding that there was a statistically significant loss of potential N-linked glycosylation sites in H631 brain and CSF and H636 CSF virus isolates supports adaptive evolution.

In summary, our data demonstrate the importance of the environment in the selection of particular genotypes from quasi species and suggest that the biology of encephalitis in conventional progressors may more closely model HIV encephalitis than do models relying heavily on macaques with rapid progression.

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References