Macaques with Rapid Disease Progression and Simian Immunodeficiency Virus Encephalitis Have a Unique Cytokine Profile in Peripheral Lymphoid Tissues

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Received 20 October 2000/Accepted 24 January 2001

The influence of host cytokine response on viral load, disease progression, and neurologic lesions was investigated in the simian immunodeficiency virus (SIV)-infected macaque model of AIDS. Cytokine gene expression (interleukin-1 β [IL-1 β], IL-2, IL-6, IL-10, gamma interferon [IFN- γ], and tumor necrosis factor alpha [TNF- α]) and viral loads were evaluated by semiquantitative reverse transcription-PCR in lymph nodes of 5 control animals and 28 animals infected with SIVmac251 at the terminal stages of AIDS. Infected animals showed higher expression of IFN- γ , IL-6, and IL-10 mRNAs compared with controls. Levels of all cytokines were comparable between animals with rapid (survival, <200 days) or slow/normal (survival, >200 days) disease progression. However, among rapid progressors, the eight animals with SIV encephalitis had a unique cytokine profile (increased IL-2, IL-6, and IFN- γ) that was associated with higher viral loads. These observations provide evidence that host cytokine responses may influence SIV neuropathogenesis independent of disease progression.

Dysregulation of the cytokine network is postulated to play a role in the pathogenesis of human immunodeficiency virus type 1 (HIV-1) infection. It has been proposed that the progression of HIV infection is accompanied by a shift in the cytokine expression profile from a predominant T helper type 1 (Th1) cytokine profile (production of interleukin-2 [IL-2] and gamma interferon [IFN- γ]) to a T helper type 2 (Th2) cytokine profile (production of IL-4 and IL-10). While evidence from several studies with adults (5, 6) and children (27) supports this notion, not all data fit the proposed Th1/Th2 switch in HIV disease progression (8, 25, 33).

Cytokines can influence disease pathogenesis through a variety of direct and indirect mechanisms. Cytokines that induce cellular activation and/or proliferation have been shown to promote infection and replication of HIV in vitro (1, 2, 4). Inflammatory cytokines may influence viral pathogenesis by modulating expression of chemokines in monocytes (23) and chemokine receptors in CD4⁺ T cells (2, 35), which are important in leukocyte recruitment and function as coreceptors for HIV.

A number of studies have addressed early cytokine responses in peripheral blood and lymph nodes during primary simian immunodeficiency virus (SIV) infection, focusing on events during the first several weeks to months following infection (3, 4, 13, 14). However, few reports have investigated the role of host cytokine responses in disease pathogenesis and the subsequent development of pathology. To explore the as-

sociation of cytokine expression with disease progression, viral burden, and neuropathologic status, we examined cytokine profiles in peripheral lymph nodes from subgroups of rhesus macaques infected with SIVmac251.

Thirty-three juvenile and adult rhesus macaques (*Macaca mulatta*) were evaluated retrospectively. Twenty-eight animals were inoculated intravenously with uncloned SIVmac251 and sacrificed at the terminal stages of AIDS. Five age-matched uninfected control animals were also evaluated. Virus stocks and doses have been described previously (15, 26, 32). Survival time among infected animals ranged from 111 to 854 days postinoculation (dpi) with an average survival of 333 days. Sixteen animals showed a rapid disease course with progression to AIDS by 200 dpi (mean survival, 157 days), with the remaining 12 animals having slow/normal disease progression with survival longer than 200 days (mean survival, 567 days).

The diagnosis of SIV encephalitis (SIVE) in infected animals was based on the presence of perivascular accumulations of macrophages and multinucleated giant cells and by virus infection within the central nervous system (CNS) demonstrated by in situ hybridization as previously described (14–16, 31). Riboprobes for virus localization were kindly provided by Vanessa Hirsch and Charles Brown, National Institute of Allergy and Infectious Diseases, Rockville, Md., and have been described elsewhere (11). Using these methods we found that 9 of 28 (32.1%) infected animals showed histologic evidence of SIVE (Fig. 1). The majority of these animals (8 of 9) were rapid progressors (mean survival, 155 days), in accordance with previously published data (30).

To evaluate viral load and levels of IL-1 β , IL-2, IL-6, IL-10, IFN- γ , and tumor necrosis factor alpha (TNF- α) mRNA in lymph nodes of rhesus macaques infected with SIVmac251, we used semiquantitative reverse transcription-PCR. Total RNA was extracted from 30 mg of snap-frozen axillary lymph node

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FIG. 1. Representative histologic lesion in the CNS of an SIV-infected macaque with SIVE. (A) Hematoxylin and eosin-stained section of brain demonstrating a typical lesion of SIVE consisting of perivascular accumulations of mononuclear cells and multinucleated giant cells. (B) RNA in situ hybridization for SIV transcripts demonstrating abundant viral replication within mononuclear cells and multinucleated giant cells within a lesion. Original magnification, $\times 400$.

using the SV Total RNA Isolation Kit (Promega, Madison, Wis.), and reverse transcription-PCR was performed as described previously (17). The optimal number of PCR cycles was determined initially by using a variable number of cycles to identify a linear range of amplification for each transcript. β -Actin cDNAs were amplified using primers modified from those previously described (23), and cytokines (IL-1 β , IL-2, IL-6, IL-10, IFN- γ , and TNF- α) were detected using published sequences (28). Levels of SIV *env* transcripts were also evaluated using the following primers: 5'-GGAATCAGCTGCTTATCG-3' and 5'-AGCTTTCTGTAAACATTAAGG-3'. Twenty microliters of PCR products was electrophoresed through a 2% agarose gel, stained with ethidium bromide, and then visualized under ultraviolet light. Images were captured and band densitometry was assayed using the Gel Doc 2000 PCI system and Quantity One software (Bio-Rad, Hercules, Calif.). All densities were normalized against respective β -actin signals obtained from the same sample, and the data were expressed as the ratios of signal obtained from the mRNA of interest over the signal obtained from β -actin mRNA. Control and SIV-infected groups were compared using Student's *t* tests, and a *P* value of <0.05 was considered statistically significant.

Overall, levels of IFN- γ , IL-6, and IL-10 mRNA expression were significantly increased ($P < 0.003$, $P < 0.04$, and $P = 0.001$, respectively) in lymph nodes from SIV-infected animals ($n = 28$) compared to uninfected controls ($n = 5$) (Fig. 2). Levels of IL-2 mRNA were increased slightly in infected animals but this was not significant ($P = 0.18$). Expression of TNF- α and IL-1 β mRNA was unchanged following infection. To determine if rapid disease progression in SIV-infected animals was associated with a specific cytokine response, cytokine mRNAs were compared for animals with rapid ($n = 16$) and slow/normal ($n = 12$) progression. We were surprised to find that there were no significant differences between the two groups of animals in any of the cytokines evaluated (Fig. 3). Levels of IL-2 mRNA tended to be higher for rapid progressors, but this was not significant ($P = 0.06$). These data suggest

that rapid disease progression, in general, is not associated with a unique host cytokine response in the SIV-infected macaque, but rather there appears to be a common cytokine profile present in animals at the terminal stages of disease.

Previous studies in our laboratory have shown that approximately 25% of all macaques infected with pathogenic SIV develop SIVE. However, among rapid progressors the incidence of SIVE was increased with approximately 50% of these animals demonstrating characteristic CNS lesions (30). We were therefore interested in examining cytokine profiles in this subpopulation of rapid progressors. Although cytokine profiles were indistinguishable among rapid and slow/normal progressors, when rapid progressors were divided into those with SIVE ($n = 8$) and those without ($n = 8$), it became clear that encephalitic animals had a unique cytokine profile. Rapid progressors with SIVE showed a predominant Th1 cytokine response (Fig. 4) with significantly higher levels of IFN- γ and IL-2 ($P = 0.045$ and $P = 0.004$, respectively). Increased expression of IL-6 mRNA ($P = 0.008$) was also observed in rapid progressors with encephalitis. The overall cytokine profile for rapid progressors without encephalitis did not differ significantly from that observed for animals with slow/normal progression.

Levels of IL-2 and IL-6 in rapid progressors without encephalitis and in slow/normal progressors were not significantly different from those of control animals. However, IL-6 was increased in all infected animals compared to controls (Fig. 2). Thus, the elevation of IL-6 we observed in infected animals overall was due almost entirely to the contribution of rapid progressors with SIVE. Although IL-10 was increased from that of controls for all groups of SIV-infected animals, levels were not significantly different among the three groups of infected animals (Fig. 4). Expression of IL-1 β and TNF- α was not significantly different among the three groups of SIV-infected animals and controls.

In general, two distinct patterns of cytokine expression were evident in lymph nodes of macaques infected with SIV. The

FIG. 2. Cytokine gene expression in axillary lymph nodes of macaques infected with SIVmac251 and sacrificed at the terminal stages of disease. Bars represent mean values $\pm$ standard error of the mean for uninfected control animals (Uninfected; $n = 5$) and SIV-infected animals (Infected; $n = 28$). Results are expressed as a ratio of cytokine mRNA/ β -actin mRNA. *, $P < 0.05$.

predominant profile, which was present in slow/normal progressors and rapid progressors without SIVE (20 of 28 infected animals; 71%), was typified by overexpression of IFN- γ and IL-10 mRNAs. In these animals, levels of IL-2 and IL-6 were comparable to those of controls. The less prevalent profile, which was seen in animals with rapid disease progression and SIVE (8 of 28 infected animals; 29%), was also characterized by overexpression of IFN- γ and IL-10. However, lymph nodes

from these animals also contained higher levels of IL-2 and IL-6 mRNA compared to control animals and other groups of SIV-infected animals (Fig. 4).

Levels of IFN- γ and IL-10 transcripts were increased from control values in all groups of infected animals, although there was some degree of variation in the magnitude of response among the groups. Similar increases in IL-10 and IFN- γ have also been reported in intestinal lymphocytes from chronically

FIG. 3. Evaluation of cytokine gene expression in axillary lymph nodes of SIV-infected macaques grouped by disease progression. Animals with a survival period of ≤ 200 dpi were grouped together as rapid progressors (Rapid; $n = 16$; mean survival, 155 days) and animals with a survival period of ≥ 200 dpi were grouped together as slow progressors (Slow; $n = 12$; mean survival, 567 days). Bars denote mean values $\pm$ standard error of the mean. Results are expressed as a ratio of cytokine mRNA/ β -actin mRNA.

FIG. 4. Evaluation of cytokine gene expression in axillary lymph nodes of SIV-infected macaques grouped by disease progression and pathologic outcome. Animals with a survival period of ≤ 200 dpi were first grouped together as rapid progressors and then subgrouped into those with SIV encephalitis (Rapid SIVE; $n = 8$) and those without SIV encephalitis (Rapid no SIVE; $n = 8$). Animals with a survival period of ≥ 200 dpi were grouped together as slow progressors (Slow; $n = 12$), and the fourth group was uninfected controls (Controls; $n = 5$). Bars denote mean values $\pm$ standard error of the mean. Results are expressed as a ratio of cytokine mRNA/ β -actin mRNA. *, $P < 0.05$, Rapid no SIVE versus Rapid SIVE.

SIV-infected animals (24). From this we may conclude that induction of IFN- γ and IL-10 mRNAs is a generalized response to SIV infection and that overexpression of these two cytokines alone is not directly related to disease progression or neuropathologic outcome of infection. In contrast, IL-2 and IL-6 mRNAs were increased only in the subpopulation of infected animals with rapid disease progression and histologic lesions of SIVE. These findings suggest that this particular cytokine profile is not a universal response to SIV infection but is limited to a subpopulation of infected macaques that develop SIVE.

To understand the relationship of host cytokine response with the control of virus replication in vivo, viral loads were evaluated in lymph nodes and then correlated with cytokine profiles. In comparing viral burdens in lymph nodes of macaques with rapid and slow/normal disease progression, we found that there was no significant difference between these two groups of infected animals (Table 1), although there was a trend towards higher viral loads in rapid progressors ($P = 0.07$). Similarly, rapid progressors without SIVE and slow/nor-

mal progressors showed no difference in viral loads. This was not entirely surprising based on the finding that overall cytokine profiles were indistinguishable between these groups of infected animals. However, when we analyzed viral loads in rapid progressors with SIVE, we found that the distinct cytokine profile in lymph nodes of macaques in this group was associated with significantly higher viral burdens ($P = 0.016$) compared to rapid progressors without SIVE. Higher viral loads in cerebrospinal fluid and brain tissues have been correlated with the presence and severity of CNS lesions in pigtailed macaques infected with SIV (34), substantiating the association of higher levels of viral RNA with the presence of SIVE.

The concurrent increases in viral loads with IL-2 and IL-6 mRNAs in rapid progressors with SIVE suggest a direct relationship between cytokines and viral replication in lymph nodes. The association of altered cytokine expression with higher viral burden in lymph nodes may reflect the inductive effects of IL-2 and IL-6 on virus replication, as has been reported with HIV replication in vitro (1, 4, 20, 29). Alternatively, IL-2 may increase CCR5 expression in CD4⁺ T cells as has been recently reported with HIV infection in vivo (35), potentially increasing the number of mononuclear cells susceptible to infection with HIV and SIV.

The presence of perivascular infiltrates of macrophages in the CNS is a key feature of encephalitis with SIV and HIV-1 infections, and an increased number of brain macrophages has been correlated with dementia in people with AIDS (10). HIV-infected patients with dementia also show expansion of a unique subset of peripheral blood monocytes with an activated phenotype (21), and it is possible that these activated monocytes migrate into the brain parenchyma resulting in increased numbers of brain macrophages (19). It is likely that alterations

TABLE 1. Viral loads in lymph nodes of macaques infected with SIVmac251

Type of disease progression	<i>n</i>	Relative mRNA levels (mean $\pm$ SEM) ^a
Slow/normal	12	0.85 $\pm$ 0.11
Rapid	16	1.03 $\pm$ 0.07
Rapid with SIVE	8	1.17 ^b $\pm$ 0.03
Rapid without SIVE	8	0.89 $\pm$ 0.11

^a Expressed as a ratio of SIV *env* mRNA/ β -actin mRNA.

^b $P < 0.05$, Rapid with SIVE versus Rapid without SIVE.

in cytokines in the periphery influence CNS disease pathogenesis via activation of monocytes/macrophages and/or brain microvascular endothelial cells, although the exact mechanisms remain unclear. IL-2 has been shown to have monocyte/macrophage-activating properties (7) and leads to activation of brain microvascular endothelium (9) resulting in elevations in cell adhesion molecules (18). Thus, it is possible that IL-2 plays a role in the neuropathogenesis of SIV infection via cellular activation mechanisms.

This study clearly demonstrates that the cytokine profile in SIV-infected macaques with rapid disease progression and SIVE reflects a distinct and unique host response to virus infection. Here we extend previous observations correlating rapid disease progression with the presence of SIVE to include specific host determinants in rapidly progressing animals that influence neuropathogenesis.

We gratefully acknowledge the pathology staff at the NERPRC for tissue collection and histopathology. We also thank Daniel Shvetz, Brendon Thompson, Kristen Toohey, and Douglas Pauley for assistance.

This work was supported in part by Public Health Service grants RR00168, NS30769, NS37654, and NS35732. A. Lackner is the recipient of an Elizabeth Glaser Scientist Award.

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