Comparison of the Effects of 3'-Azidothymidine with Those of Neutralizing Antibodies on Simian Immunodeficiency Virus Infection in Macrophages

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We previously showed that simian immunodeficiency virus-infected macaque macrophages contacting uninfected CD4⁺ lymphocytes caused extensive cell fusion and synthesis of phlogistic cytokines. In this study, macaque macrophage cultures inoculated with SIV_{mac}251 and treated simultaneously with 10 μ M 3'-azidothymidine (AZT) became infected and produced small amounts of viral antigen (p27) but failed to fuse with CD4⁺ CEM174 cells. When AZT was added 1 to 3 days after virus inoculation, the infected cells caused fusion and the release of tumor necrosis factor and produced increasing amounts of p27. In contrast, neutralizing antibodies prevented infection when added at the time of virus inoculation, and they were much more effective than AZT in limiting virus replication, fusion cytopathic effect, and cytokine production when added up to 3 days postinoculation. Neutralizing antibodies may be more effective than AZT in reducing the virus load in the macrophage population and in preventing both cell fusion and the production of potentially pathogenic cytokines.

Human immunodeficiency virus and simian immunodeficiency virus (SIV) are related lentiviruses (1) that cause similar disease syndromes in their respective hosts characterized by immunodeficiency (AIDS) with a loss of CD4⁺ lymphocytes and/or virus-induced inflammatory lesions in the central nervous system and other locations (8, 9). Disease manifestations typically develop after an extended period of subclinical infection, and they are associated with an increased virus load in plasma, peripheral blood mononuclear cells, and tissue macrophages (2, 6, 7, 16). Macrophages are believed to play a key role in the pathogenesis of the virus-induced inflammatory lesions, and they may provide a reservoir for the virus within the infected host (4, 8, 15, 19, 20, 22). We have shown previously that macaque macrophages infected in vitro with the 251 strain of SIV_{mac} fused with and lysed uninfected CD4⁺ lymphocytes (13) and, during this process, released tumor necrosis factor alpha (TNF- α) and interleukin-6 into the culture medium (12). These phenomena could contribute to some of the pathologic effects caused by these viruses. We have also shown previously that neutralizing antibodies (NAb) produced by infected macaques restricted SIV replication in infected macrophage cultures and prevented these cultures from reaching their full lymphocyte-lytic potential (11, 14), thereby controlling potentially pathogenic expression of this viral infection. In this report, we show that the antiretroviral drug 3'-azidothymidine (AZT) had a similar effect on SIV replication in macaque macrophages but was substantially less

effective than NAb in controlling virus replication and expression in these cells.

Initial experiments determined the concentrations of AZT and NAb required to inhibit SIV-induced fusion cytopathic effect (CPE) in 50% of inoculated macrophage cultures. Macrophages were derived from peripheral blood mononuclear cells of uninfected rhesus macaques and cultivated in 10% human AB serum and granulocyte macrophage-colony-stimulating factor and macrophage-colony-stimulating factor as previously described (13). Differentiated macrophages were incubated at 37°C with serial twofold dilutions of AZT (Sigma, St. Louis, Mo.) or NAb. Dilutions were made with medium containing SIV_{mac} 251 such that the multiplicity of infection would be approximately 0.05 (i.e., five infectious particles per 100 cells) when the various dilutions were placed on the cells. After 7 days of incubation, the medium was removed and uninfected CEM174 cells were added; cocultures were examined 24 h later to detect viral CPE (13). In repeated experiments, fusion CPE developed in at least two out of four cultures treated with 0.15 to 0.30 μ M AZT or with 1:1,200-to-1:3,600 dilutions of neutralizing macaque antiserum to SIV_{mac} 251. In all subsequent experiments, we used a 1:50-to-1:100 dilution of this serum and 10 μ M AZT in order to achieve comparable (approximate 50-fold excess) antiviral activity with either NAb or the drug.

In order to determine the relative rates of viral replication, cytopathicity, and cytokine production under different treatment conditions, we measured SIV p27 concentrations and TNF activities in supernatant fluids of AZT- or NAb-treated cultures, as previously described (11, 12), and quantitated CPE (13). Macrophage cultures were inoculated with SIV at a multiplicity of infection of 0.05 and treated with AZT either simultaneously or at 1, 2, or 3 days after virus inoculation. By using the same protocol, parallel cultures were treated with NAb. Cultures were rinsed 24 h after inoculation, and supernatant fluids were sampled for p27 at 5-day intervals;

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TABLE 1. Production of viral protein and CPE by macrophages treated with AZT or NAb^a

Treatment and day	% p27 production ^b on day:			CPE ^c on day 16
	5	10	15	
AZT				
0	2.8 ± 0.1	1.8 ± 0.3	1.5 ± 0.6	—
1	14 ± 4	12 ± 0.3	15 ± 4.6	+
2	28 ± 10	44 ± 18	54 ± 9	++
3	61 ± 21	41 ± 10	58 ± 7	++
NAb				
0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	—
1	2.5 ± 0.7	1.3 ± 0.2	1.0 ± 0.2	—
2	3.7 ± 0.4	2.8 ± 0.3	2.2 ± 0.8	—
3	5.6 ± 1.4	5.0 ± 0.3	3.2 ± 0.1	—
None	100	100	100	++++

^a p27 and CPE were measured either at the time of virus inoculation (day 0) or up to 3 days after inoculation. Results (expressed or means ± standard deviations) represent data from four separate experiments. Day 0 p27 levels reflect residual from the inoculum.

^b Expressed as percentages of untreated controls.

^c — to +++++, no to high CPE, respectively.

supernatant fluids were replaced at these times with fresh medium containing AZT or NAb. CPE and TNF activities were evaluated at 7 and 15 days postinoculation.

Treatment of macrophage cultures with 10 μ M AZT at the time of virus inoculation resulted in a sustained low rate of p27 production (mean = 29 ± 6 pg/ml) throughout a 15-day period with no evidence of CPE (Table 1). Cultures that were rinsed and reincubated for 7 more days in AZT-free medium developed fulminant CPE (data not shown). Incubation of the macrophages with 200 μ M AZT (1,000 times the 50% inhibitory concentration) 24 h before virus inoculation and maintenance of the drug at this concentration in the culture medium for 7 days (≤ 200 μ M AZT did not affect cell viability; higher concentrations were not tested) still did not prevent the development of CPE in any culture when the cultures were rinsed, reincubated in AZT-free medium, and examined for fusion CPE 7 days later. These results showed that AZT in concentrations of up to 200 μ M did not completely prevent infection in macrophage cultures when applied before or at the time of virus inoculation but effectively suppressed the rate of virus replication and the development of CPE as long as the drug was maintained in the culture medium. In contrast, the addition of AZT to cultures 1, 2, and 3 days after virus inoculation failed to prevent the development of CPE and only partially reduced the rate of p27 production (Table 1). The amount of p27 produced and the intensity of the CPE generated by these infected cultures correlated with the amount of cytokines released following cocultivation with uninfected CEM174 cells (12); macrophage cultures treated with AZT 24 h after virus inoculation produced less TNF (4.5 ± 1.9 U/ml) and fusion CPE (Table 1) than untreated controls (TNF, 12.8 ± 0 U/ml) but more of these than cultures containing uninfected macrophages (TNF, 0 U/ml; TNF assay sensitive at ≥ 1 U/ml). Therefore, the addition of AZT to macrophage cultures after virus inoculation partially restricted viral replication, CPE, and cytokine production.

Treatment of cultures with a 1:50 dilution of NAb at the time of virus inoculation completely prevented antigen production, in contrast to that with AZT (Table 1), and the removal of NAb did not result in the development of CPE,

indicating that, as previously reported (11), NAb completely prevented infection under these conditions. Macrophage cultures treated with NAb 1 to 3 days after viral inoculation became infected, but they did not produce fusion CPE (Table 1) or TNF activity (0 U/ml) following cocultivation with CEM174 cells and, as indicated in Table 1, produced less p27 than parallel cultures treated with AZT.

These results showed that while AZT reduced viral replication, CPE, and cytokine production when added to macrophage cultures up to 3 days after virus inoculation, the drug did not prevent infection and became progressively less effective as the interval between virus inoculation and treatment increased. In contrast, NAb were much more effective in limiting virus expression when introduced after inoculation. We recently showed that NAb efficiently prevented replication of SIV in macrophages by inhibiting infection and reinfection of the cells with exogenous and/or progeny virus particles (14). The partial effectiveness of AZT could result from the failure of the drug to completely prevent reverse transcription of viral RNA. This would presumably be due to the failure to terminate DNA chain elongation in all instances because of competition with cellular deoxynucleoside triphosphates (17) and/or the kinetics of cellular phosphorylation of AZT to the active triphosphate (18), with the resultant formation of proviral DNA. Alternatively, it is possible that the differences in the effectiveness of NAb and that of AZT result from targeting of different stages in the virus's life cycle which bracket an event(s) critical to the establishment of fully productive-pathogenic infection in a cell or population of cells; i.e., whereas NAb inhibit viral entry, AZT inhibits reverse transcription after the virus has entered the cell.

The inflammatory lesions of human immunodeficiency virus and SIV infection and the progression of disease are thought to be associated with, if not caused by, the production of cytokines and increased virus loads. The relative in vitro ability of a chemical or biologic agent to limit virus replication and pathogenic expression in target cells may therefore be relevant to events occurring in the infected host during therapeutic intervention. Our results are compatible with those of clinical studies in which the induction of active (3) and passive (23) humoral immunity or AZT treatment (5, 10) reduces virus load and viral pathogenicity in infected monkeys and humans. It has also been shown previously that AZT becomes less effective in vivo as the time between the inoculation of virus and the initiation of treatment increases (5, 10, 21). It is unknown whether NAb could prevent infection or substantially alter disease progression in an infected individual when given soon after exposure of the individual to the virus.

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