Effect of Cyclosporin A and Zidovudine on Immune Abnormalities Observed in the Murine Acquired Immunodeficiency Syndrome

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Two therapeutic modalities, zidovudine (targeting retroviral replication) and cyclosporin A (targeting immunopathologic consequences of retroviral expression) were evaluated in a murine model of AIDS. In previous studies, cyclosporin A treatment (40 or 60 mg/kg/day) before and after infection with LP-BM5 murine leukemia viruses protected against the development of immunodeficiency disease. The present study extends these findings. First, a low dose of cyclosporin A (20 mg/kg/day) was ineffective, and treatment initiated 5 days after infection did not protect against virus-induced lymphoproliferation and hypergammaglobulinemia. Second, zidovudine added to drinking water (0.1 mg initiated 5 days after infection and continued for 8 weeks) was more effective than 0.2 mg/mL given day 5–12 after infection. This treatment reduced lymph node size, disease severity as determined histologically, retrovirus-induced gp70 expression, and IgE (but not IgM and IgG) levels. Third, combined treatment had an additive, protective effect on lymphocyte proliferative capacity. This successful dual therapeutic strategy in a mouse model has potential applicability for similar approaches in treating human immunodeficiency virus infection.

The LP-BM5 murine leukemia viruses (MuLV) induce an immunodeficiency disease in susceptible murine strains [1–6]. The disorder has a natural history in many ways comparable to human immunodeficiency virus (HIV-1)–induced AIDS in humans and has therefore been termed murine AIDS (MAIDS) [3]. Dysfunction of $CD4^+$ T cells is a key feature in both conditions and precedes a numerical reduction of this T cell subset even though the LP-BM5 viruses have no selective tropism for $CD4^+$ T cells [7].

Previous studies of mice with MAIDS demonstrated that interactions between $CD4^+$ T cells and B cells are central to the pathogenesis of this syndrome; infected mice depleted in vivo of $CD4^+$ T cells had phenotypically and functionally normal B cells [8], while infected mice depleted of mature B cells had phenotypically and functionally normal $CD4^+$ and $CD8^+$ T cells [9]. Indirect evidence suggests that B cell–dependent T cell activation through a mechanism involving a retrovirus-associated superantigen is crucial to the development of the disease [10]. Furthermore, inappropriate regulation of lymphokine expression, including genes encoding interferon (IFN)-α, -β, and -γ [11], interleukin (IL)-1 [12], -2 [7, 13], -4, -5, -6, and -10 [13], and tumor necrosis factor [12], may be of importance.

We have analyzed the therapeutic effect of cyclosporin A, which interferes with crucial steps in T cell activation [14]. Treatment with cyclosporin A (40 and 60/mg/kg/day) before and after infection with LP-BM5 MuLV significantly inhibited the development of immunologic and histopathologic changes characteristic of MAIDS [15]. This study extends our analysis by using cyclosporin A at lower doses either alone or combined with zidovudine, an inhibitor of retroviral reverse transcriptase [16].

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Materials and Methods

Mice and viruses. Inbred female C57BL/6 (B6; *H-2*^{b}; susceptible to LP-BM5–induced disease) mice were obtained from IFFA Credo (l'Arbresle, France). Mice were injected at the age of 4 weeks intraperitoneally with 0.1 mL of LP-BM5 MuLV stock containing a replication-defective component as described [2, 5, 6] and ~10 ffu/mL MCF (mink cell focus–inducing) virus and $10^{3.8}$–10^5 pfu/mL ectropic MuLV.

Drug treatments. Cyclosporin A was a gift of J.-F. Borel (Sandoz, Basel, Switzerland). The powder was dissolved in alcohol and tween 80, diluted with PBS, and injected daily intraperitoneally. Either 20 or 40 mg/kg/day was begun 1 day before

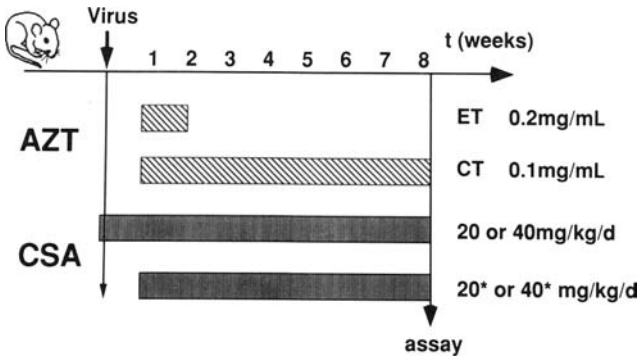

Figure 1. Experimental protocol. B6 mice were infected with LP-BM5 murine leukemia virus at 4 weeks old. Zidovudine (AZT) was added to drinking water beginning 5 days after infection, either at 0.2 mg/mL for 1 week (ET, early treatment) or at 0.1 mg/mL until end of experiment at 8 weeks (CT, continuous treatment). Cyclosporin A (CSA) was given at 20 or 40 mg/kg/day intraperitoneally; treatment was begun 1 day before infection or 5 days after (*).

infection or 5 days after infection until the end of the experiment 8 weeks later.

Zidovudine was supplied by Wellcome Research Laboratories (Beckenham, UK) and administered orally in the drinking water at a concentration of 0.2 mg/mL days 5–12 after infection (early treatment) or at 0.1 mg/mL from day 5 until the end of the experiment (continuous treatment). The doses used did not induce toxicity, and mouse weight, hematocrit, and histology of major organs remained unaffected.

Treatment regimens are shown in figure 1. Two independent sets of experiments were done, and representative results from one set are reported.

Histopathology. Samples of all major organs were obtained at autopsy, and histologic sections were stained with hematoxylin-eosin. The histopathologic staging was done by an immunopathologist blinded for the experimental protocol according to criteria previously described [3]. Briefly, splenic histology is defined as reactive (R) by hyperplasia of periarterial lymphoid sheaths and follicles due to accumulation of immunoblasts and plasmocytoid cells as well as plasma cells without distortion of their round shape. Stage I is distinguished by an eccentric hyperplasia of the periarterial sheaths with hourglass shapes. The mantle zone is still intact and there is no compression of the red pulp. In stage II, there is extensive enlargement of periarterial sheaths, loss of the mantle zone, and compression of the red pulp. Lymphoid masses are still separated by stretches of red pulp. In stage III, nodular confluent masses of lymphoid tissue are associated with obliteration of the red pulp.

Serology. Serum immunoglobulin levels were determined by ELISA with immunoglobulin class- and subclass-specific reagents and standards as detailed elsewhere [17, 18]. The determination of serum gp70 levels has been described [19].

Proliferative responses. Proliferative responses to mitogens were induced in cultures of spleen cells (2×10^5/well) in 0.2 mL of Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal calf serum, 2'-mercaptoethanol, and antibiotics in 96-well microtiter plates (Costar, Cambridge, MA). Concanavalin A (2 μg/mL; Pharmacia, Uppsala, Sweden) and lipopolysaccharide (20 μg/mL; *Escherichia coli* O55:B5; Difco, Detroit) were added at the beginning and cultures were pulsed overnight with [^{3}H]thymidine before harvesting after 48 h.

Statistical analysis. The Wilcoxon signed rank test was used.

Results

Effect of zidovudine and cyclosporin A treatment on development of lymphadenopathy and histopathologic changes. Infection with the LP-BM5 viruses is associated with subsequent lymphoproliferation due to polyclonal T and B cell expansion. Disease progression is paralleled by typical changes in splenic histomorphology.

Table 1 summarizes the effect of cyclosporin A and zidovudine on lymphoid organ size and on histopathology. The higher dose of cyclosporin A (40 mg/kg/day) was effective in reducing lymph node size, but only when treatment was initiated before infection ($P < .05$). Treatment with zidovudine was more efficacious when given continuously than when given during days 5–12 only. Combining zidovudine and cyclosporin A resulted in a further significant reduction of lymph node size compared with treatment with either continuous zidovudine ($P < .05$) or cyclosporin A ($P < .05$) alone.

Disease severity, quantitated by morphologic criteria, was attenuated by cyclosporin A treatment initiated before and continued after infection. Infected mice treated with cyclosporin A at 40 mg/kg/day showed mostly reactive changes irrespective of whether zidovudine was coadministered. Zidovudine alone (continuous treatment), however, did attenuate histologic disease severity. Cyclosporin A initiated 5 days after infection reduced disease severity to a lesser but statistically significant degree ($P < .05$).

Effect of zidovudine and cyclosporin A treatment on development of hypergammaglobulinemia. LP-BM5 infection induces rapid and sustained polyclonal B cell activation resulting in hypergammaglobulinemia [20, 21]. Treatment of infected mice with cyclosporin A resulted in a dose-dependent reduction of all immunoglobulin isotypes tested when treatment was initiated before infection (table 1). No significant effect was observed when the drug was started 5 days after infection. Zidovudine treatment did not significantly affect hypergammaglobulinemia of the IgM and IgG isotype observed in infected mice, nor did it add to the effect of cyclosporin A when the drugs were used together. However, IgE levels were reduced by continuous zidovudine treatment compared with those seen in infected untreated mice ($P < .05$).

Effect of zidovudine and cyclosporin A treatment on serum gp70 expression. Serum gp70 levels can be used as a means

Table 1. Effect of zidovudine and cyclosporin A treatment on development of lymphadenopathy, histopathologic alterations, and hypergammaglobulinemia.

Treatment regimen, infection	Cervical lymph node weight, mg	Histopathologic stage	Serum immunoglobulin levels		
			IgM, μg/mL	IgG, mg/mL	IgE, ng/mL
None					
Uninfected	17 (6)	ND	296 (224)	7.9 (2.6)	<20
Infected	224 (82)	II (I–III)	1556 (297)	32.9 (3.5)	572 (475)
Cyclosporin A before infection					
20 mg/kg/day					
Uninfected	12 (4)	ND	260 (187)	8.6 (1.9)	ND
Infected	220 (101)	ND	1295 (612)	35.7 (3.1)	ND
40 mg/kg/day					
Uninfected	10 (0)	ND	464 (193)	9.6 (2.3)	43 (17)
Infected	90 (14)	R (N–R)	452 (139)	19.9 (3.8)	136 (145)
Cyclosporin A after infection					
20 mg/kg/day, infected	335 (107)	ND	1224 (593)	31.3 (3.6)	ND
40 mg/kg/day, infected	494 (242)	I (R–II)	816 (230)	27.3 (5.1)	395 (559)
Zidovudine, early treatment					
Uninfected	10 (0)	ND	147 (46)	7.1 (1.5)	ND
Infected	213 (106)	II (I–II)	1302 (252)	28.6 (3.2)	875 (322)
Zidovudine, continuous treatment					
Uninfected	10 (0)	ND	103 (25)	5.4 (1.7)	ND
Infected	130 (49)	I (R–II)	1117 (304)	30.1 (3.9)	161 (81)
Cyclosporin A, 40 mg/kg/day, plus zidovudine					
Early treatment, infected	67 (28)	R (R)	303 (63)	15.2 (2.8)	21 (3)
Continuous treatment, infected	66 (34)	R (N–R)	856 (312)	21.6 (6.6)	94 (81)

NOTE. Intraperitoneal treatment with cyclosporin A (groups of 6 B6 mice) was begun at age 4 weeks, 1 day before or 5 days after infection with LP-BM5 murine leukemia viruses, and continued for 8 weeks. Zidovudine was added to drinking water 5–12 days after infection at 0.2 mg/mL (early treatment) or from day 5 after infection for 8 weeks at 0.1 mg/mL (continuous treatment). Histopathologic staging, given as median (range): N, normal; R, reactive; I–III, discrete and progressive stages of disease severity. Other data are mean (SD). ND = not done.

to quantify retroviral expression, as shown previously for LP-BM5 MuLV [15]. Figure 2 shows increased gp70 levels for infected compared with uninfected mice. These increases were not affected by treatment with cyclosporin A alone. However, chronic administration of zidovudine lowered gp70 expression. When infected mice were compared, differences between those untreated and continuously zidovudine treated were significant ($P < .03$), as were differences between mice treated with cyclosporin A (40 mg/kg/day) and with the combination of cyclosporin A (40 mg/kg/day) and zidovudine ($P < .03$ for early or continuous treatment). In other words, zidovudine (early treatment) combined with cyclosporin A was more effective than zidovudine (early treatment) alone or cyclosporin A alone.

Effect of zidovudine and cyclosporin A treatment on mitogen-induced proliferative responses. Eight weeks after infection, spleen cells were assessed for their capacity to respond to stimulation with the mitogens concanavalin A and lipopolysaccharide (table 2). Proliferative responses were almost totally abrogated in spleen cells from infected untreated mice compared with those from uninfected control mice, changes characteristic of MAIDS [1, 7, 20]. Treatment with cyclosporin A or zidovudine alone did not beneficially influence unresponsiveness of splenocytes, as previously reported, reflecting a more advanced stage of disease progression in the present study [15]. Combining both drugs, however, led to a significant increase in proliferative responses irrespective of whether zidovudine was given as early or continuous treatment ($P < .05$).

Discussion

Therapeutic strategies are generally designed to interfere with crucial steps in the pathogenetic sequence of a disease process. According to our working hypothesis, LP-BM5 MuLV–induced immunodeficiency can be viewed as a retrovirus-triggered superantigen-driven polyclonal stimulation of the immune system followed by exhaustion of immune function [10]. In spite of major differences in viral tropism and replication between MAIDS and AIDS, recent indirect evidence underlines a possible pathogenic role for a retrovirus-encoded superantigen in AIDS [22]. Others consider the disease a primary neoplasia with an acquired immunodeficiency syndrome as a paraneoplastic syndrome [23].

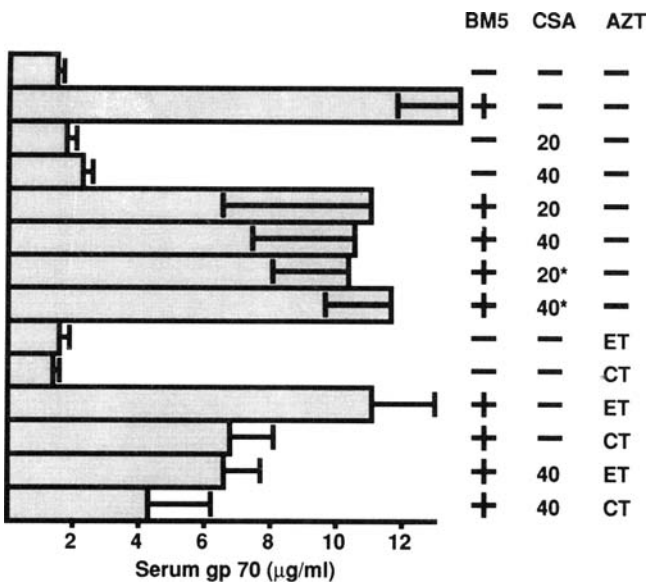

Figure 2. Effect of treatment with cyclosporine A (CSA) and zidovudine (AZT) on serum gp70 levels in LP-BM5-infected and uninfected B6 mice. gp70 levels of sera obtained after 8 weeks from 6 mice per group were determined by ELISA; results are means ± SDs. Treatment regimens are explained in legend to figure 1.

Two therapeutic modalities, zidovudine (targeted at retroviral replication) and cyclosporin A (aimed at the immunopathologic consequences of virus expression), were evaluated in this study.

The effect of cyclosporin A on MAIDS is dose dependent. A dose of 40 or 60 mg/kg/day is necessary to suppress the rate at which disease develops; 20 mg/kg/day is not effective. Results obtained in other studies using cyclosporin A in vivo suggest that doses in the range of 15–30 mg/kg abrogate self-Ia–restricted T helper function and that higher doses are required to suppress other T cell functions [24]. Treatment with cyclosporin A has to be initiated very early to influence the disease process; administration before viral infection protects mice against MAIDS, whereas a delay of 5 days renders the disease resistant to the action of cyclosporin A used at 40 mg/kg/day. This was not because of interference with helper retrovirus expression, since cyclosporin A–treated infected mice had similar levels of serum gp70, as shown here, and LP-BM5 ecotropic MuLV, as reported previously [15].

Although the molecular mechanism of cyclosporin A action is still incompletely understood, its main mode of action both in vitro and in vivo is interference with lymphokine expression during T cell activation [14, 24–26]. Recent studies of cytokine production in MAIDS suggest at least two distinct phases of the disorder [13]. At 1 week after infection there is a burst of spontaneous proliferation and moderate- to high-level expression of IL-2, -4, -5, and -10 and IFN-γ by spleen cells, a pattern consistent with activation of Th0 or a mixture of Th1 and Th2 helper cells. However, at 2 weeks after infection and thereafter, cytokine expression by spleen was seen only after stimulation of cells with concanavalin A, and the pattern of expression was that of Th2 cells only (IL-4, IL-10). It is most likely the very early process of virus-dependent T cell activation and cytokine expression that is crucially interfered with by treatment with cyclosporin A at the time of infection. Treatment beginning at later times might be expected to be less effective, as Th2 cells are 10-fold more resistant to the effects of cyclosporin A than are Th1 cells [27].

Hypergammaglobulinemia encompassing IgM, IgG (all subclasses), and IgE reflects activation of both Th1 and Th2. The effect of cyclosporin A on hypergammaglobulinemia was not biased towards a particular isotype, suggesting impediment of both T helper pathways. This is in accord with in vitro and in vivo studies in which cyclosporin A affected expression of lymphokines belonging to both Th subsets, although, as noted above, Th2 cells are more resistant to the effects of cyclosporin A than are Th1 cells [24, 26, 27].

Zidovudine has previously been used alone or combined with other nucleoside inhibitors in the LP-BM5 mouse model [28–31]. Several groups reported an effect of oral zidovudine on the development of immune abnormalities; one used a higher dose (1 mg/mL) [29], one initiated the treatment on the day of viral infection and used a lower dose (30 mg/kg/day) [30], and another began 24 h after infection and used low doses (0.25 and 0.1 mg/mL) [28]. The dose used here, 0.1 mg/mL corresponds to ~25 mg/kg/day and was not toxic, even when given for prolonged periods of time, as

Table 2. Effect of zidovudine and cyclosporin A treatment on mitogen-induced proliferative responses of spleen cells in murine AIDS.

Treatment regimen, infection	Lipopolysaccharide	Concanavalin A
None		
Uninfected	103 (19)	106 (22)
Infected	6 (4)	2 (2)
Cyclosporin A, 40 mg/kg/day		
Uninfected	95 (24)	57 (29)
Infected	9 (2)	5 (4)
Zidovudine, early treatment		
Uninfected	93 (11)	97 (23)
Infected	6 (2)	4 (2)
Zidovudine, continuous treatment		
Uninfected	78 (15)	118 (27)
Infected	9 (3)	3 (2)
Cyclosporin A plus zidovudine		
Early treatment, infected	32 (14)	17 (3)
Continuous treatment, infected	18 (21)	14 (7)

NOTE. Treatment regimens are explained in footnote to table 1. Data are mean (SD) Δcpm × 1000. Δcpm = cpm of thymidine incorporation of mitogen-containing cultures − cpm of unstimulated cultures.

in these or other previously reported studies [28]. Low-dose zidovudine treatment of an established HIV infection is the clinical setting that we tried to mimic in our study design. We found zidovudine given continuously effectively reduced gp70 expression and, in addition, inhibited the development of lymphadenopathy and attenuated histopathologic signs of disease progression. No significant effect on hypergammaglobulinemia, except IgE, was observed. There was an additive effect when both zidovudine and cyclosporin A were used in preserving mitogen responsiveness of splenic lymphocytes.

Further studies are required before cyclosporin A can be considered as a candidate drug for the treatment of HIV-infected humans. A preliminary study of 11 patients with advanced HIV disease treated with a short course of cyclosporin A failed to show a beneficial effect on CD4 cell counts [32]. A recent report of 88 HIV-infected patients with solid organ transplants, most of whom were treated with immunosuppressants including cyclosporin A, found no effect, either beneficial or harmful, of cyclosporin A on the course of infection with HIV [33].

This study presents a successful attempt to develop a dual therapeutic strategy combining antiviral (zidovudine) and immunomodulatory (cyclosporin A) drugs in a mouse model of AIDS that will potentially form the basis for designing similar approaches for humans infected with HIV.

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