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EFFECT OF CYCLOSPORIN A ON THE PRODUCTION OF INTERFERON BY HUMAN PERIPHERAL BLOOD LEUKOCYTES IN VITRO

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Cyclosporin A (CsA) was assessed for its effect on the production of antiviral activity by human peripheral blood leukocytes (PBL). CsA markedly reduced the production of interferon-γ (IFN-γ) in response to stimulation with lectin mitogens, bacterial products, alloantigens, or Epstein-Barr virus (EBV)-transformed lymphoblastoid cell lines (LCL). CsA-mediated suppression of IFN-γ secretion was dose-dependent and did not result from a shift of kinetics of the production of antiviral activity. The production of IFN-α in response to stimulation with *Corynebacterium parvum* (CP), viruses, and synthetic polynucleotides was not affected by the addition of CsA. These findings confirm earlier observations that CsA predominantly acts on T lymphocyte function. CsA may prove a valuable agent to study the role of IFN-γ in the pathogenesis of virus-associated malignant lymphoproliferative disease.

cyclosporin A interferon

INTRODUCTION

Cyclosporin A (CsA) is a biologically active fungal metabolite that acts as a potent immunosuppressive agent in clinical and experimental transplantation [3,4,6,7]. CsA affects predominantly T lymphocyte-dependent responses. Thus, it has been shown that CsA inhibits the proliferative response of lymphocytes to T cell mitogens [12] and to alloantigens in the mixed leukocyte culture (MLC) [11,15]. Recent studies have demonstrated that, in vitro, this agent decreases the induction of cytotoxic T lymphocytes in the MLC [11,15]. Clinical trials have confirmed the potential usefulness of CsA as a selective immunosuppressive drug [7]. However, there have been disturbing reports of lymphomas in renal allograft recipients treated with CsA [9,14]. In one reported case lymphoma followed asymptomatic infectious mononucleosis [14] and in another Epstein-

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Barr virus (EBV)-associated nuclear antigen was present in the lymphomatous B cells [9]. Defects of immune interferon (IFN) production have been described in patients with fatal primary infection with EBV or with EBV-associated lymphoma [20]. In view of the possible role of deficient synthesis of IFN in the development of EBV-associated malignant disease, we have studied the effect of CsA on the production of antiviral activity by human PBL in vitro. We report that CsA markedly decreases the synthesis of IFN-γ in response to stimulation with mitogens, alloantigens, and EBV-transformed lymphoblastoid cell lines (LCL), but has no significant effect on the production of IFN-α induced by treatment of PBL with viruses or synthetic polynucleotides.

EXPERIMENTAL

Mononuclear cells were separated from heparinized human peripheral blood by sedimentation on Ficoll-Isopaque gradients. Cells harvested from the interface were washed and resuspended in RPMI 1640 (Flow Laboratories, Bonn, Germany) tissue culture medium supplemented with 10% heat-inactivated fetal calf serum (FCS) and antibiotics. Adherent cells were largely removed by incubation for 1 h in plastic tissue culture flasks. Nonadherent cells (2.5×10^6/ml) were induced to produce IFN by the addition of 2.5 μg/ml purified phytohemagglutinin (PHA-P) (Wellcome, Grossburgwedel, Germany), 50 μg/ml protein A of *Staphylococcus aureus* (SpA) (Pharmacia, Freiburg, Germany), 0.1 μg/ml staphylococcal enterotoxin B (SEB) (Serva, Heidelberg, Germany), 5 μg/ml *Corynebacterium parvum* (CP) (Wellcome), 100 hemagglutinating units (HAU)/ml Sendai virus (SV) (Flow), 100 HAU/ml influenza A/X31 virus (kindly provided by Dr. J. Skehel, Mill Hill, England), 100 μg/ml polyinosinic-polycytidylic acid (poly I:C) (kindly provided by Dr. M. Hilleman, Merck, Sharp and Dohme, West Point, U.S.A.), or 2.5×10^5 heat treated (56°C, 45 min) EBV-transformed human LCL. Supernatants were harvested after 24 h and tested for antiviral activity.

Primary mixed lymphocyte cultures (MLC) were established by co-culturing 10×10^6 responding cells with an equal number of mitomycin C-treated (50 μg/ml, 45 min), stimulating cells in 10 ml RPMI-FCS in polystyrene tissue culture flasks. After 12 days of incubation the alloantigen-primed cells were harvested, washed and resuspended in RPMI-FCS. For secondary MLC, 2.5×10^6 primed responder cells were co-cultured with an equal number of fresh mitomycin C-treated stimulating cells (autologous to those used for sensitization) in 1 ml of RPMI-FCS. Supernatants of 48-h secondary MLC were harvested and tested for antiviral activity.

Antiviral activity was measured by inhibition of the cytopathic effect (CPE) of Vesicular Stomatitis virus (VSV) on human WISH amnion cells (Flow) as previously described [1]. Antiviral units were expressed as the reciprocal of the dilution corresponding to 50% inhibition of the CPE, one unit in this assay was equivalent to approximately one reference unit of the WHO Human Leukocyte Reference Interferon B 69/19. Antiviral activity induced by stimulation of PBL with PHA-P, SpA, SEB, or alloantigens in secondary MLC was characterized as IFN-γ by the following criteria. Antiviral activity was

sensitive to heat and pH 2; inhibition of the CPE was only demonstrated on homologous human cells; and the antiviral activity was not affected by incubation with antibody against human IFN-α or IFN-β (kindly provided by Dr. K. Cantell, Helsinki, Finland, and Dr. A. Billiau, Leuven, Belgium, respectively). Antiviral activity induced by stimulation of PBL with CP, SV, A/X31, and poly I:C shared several of the characteristics of IFN-α. It was insensitive to treatment at 56°C or pH 2; inhibition of the CPE was observed on both human and heterologous bovine cells; and the antiviral activity was neutralized by antibody against IFN-α but not by antibody against IFN-β. Culture supernatants of PBL stimulated with allogeneic EBV-LCL contained a mixture of IFN-γ and IFN-α.

In several experiments, shown in Fig. 1 and Tables 1 and 2, the effect of cyclosporin A (CsA) was tested by including the drug in the medium together with the inducers. Powdered CsA (Sandoz, Basle, Switzerland) was kindly provided by Dr. H. Rodt, Institute of Hematology, Munich, Germany. Solutions of CsA were made in 95% ethanol to a final solvent concentration in the culture medium of 0.2%.

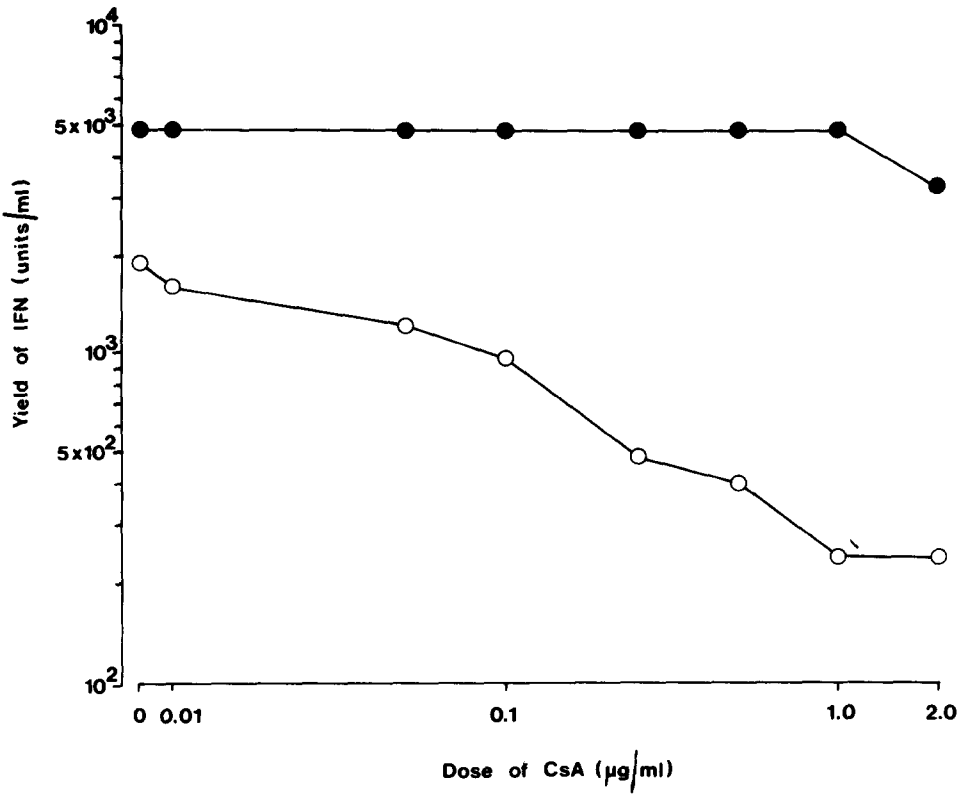

Fig. 1. Effect of CsA on production of IFN by PBL stimulated with influenza A/X31 virus (●—●) or SEB (○—○). The range of IFN yields observed in three separate experiments was within 28% of the mean value.

TABLE 1

Effect of CsA on the production of IFN by human PBL

Inducer	CsA (1.0 μg/ml)	IFN yield (units/ml)[a]	Percent residual IFN activity in CsA-treated cultures
PHA-P	–	4267 ± 1478	
PHA-P	+	1067 ± 370[b]	25.0
SpA	–	5973 ± 3910	
SpA	+	1920 ± 2771[b]	32.1
SEB	–	2987 ± 739	
SEB	+	427 ± 185[b]	14.3
Allogeneic PBL[c]	–	587 ± 92	
Allogeneic PBL	+	67 ± 23[b]	11.4
CP	–	853 ± 370	
CP	+	640 ± 0[d]	75.0
SV	–	3200 ± 0	
SV	+	3200 ± 0[d]	100.0
A/X31	–	3733 ± 924	
A/X31	+	4267 ± 1838[d]	114.3
Poly I:C	–	1280 ± 0	
Poly I:C	+	1280 ± 0[d]	100.0
EBV-LCL	–	480 ± 160	
EBV-LCL	+	213 ± 231[b]	44.4

[a] Mean ± S.D. of three separate experiments.
[b] Significant difference from controls when analyzed by Wilcoxon rank test.
[c] Secondary MLC.
[d] No significant difference from controls.

The effect of graded quantities of CsA on the production of mitogen-induced or virus-induced IFN in human leukocyte cultures is shown in Fig. 1. Synthesis of IFN-γ in response to stimulation with SEB was suppressed by CsA in a dose-dependent fashion. Maximal suppression of the yield occurred at a CsA concentration of 1 μg/ml. Influenza A virus-induced production of IFN-α was not significantly affected.

Additional experiments (Table 1) showed that CsA markedly inhibited the production of IFN-γ in response to stimulation with various other inducers: lectin, bacterial products, or alloantigens in secondary MLC. In contrast, the production of IFN-α by leukocytes stimulated with CP, viruses, or a synthetic polynucleotide was not affected. The production of antiviral activity induced by EBV-LCL was decreased to approximately 50%. This appeared to be due to interference with IFN-γ production, because the remaining IFN activity in CsA-treated cultures could be completely neutralized by incubation with antibody against IFN-α (data not shown).

As demonstrated in Table 2, CsA-mediated inhibition of the production of IFN-γ may at least partly be due to a change in kinetics of IFN production. Suppression of the SEB-induced production of IFN-γ in one of the two experiments appeared most prominent

TABLE 2

Effect of CsA on the kinetics of IFN production by PBL in response to SEB or A/X31

Inducer	CsA (μg/ml)	IFN (units/ml) yield at		
		24 h	48 h	72 h
Exp. I				
SEB	–	2560	1280	1280
SEB	1.0	320	320	640
SEB	0.1	640	640	640
SEB	0.01	640	1280	1280
A/X31	–	3200	3200	1600
A/X31	1.0	3200	3200	1600
A/X31	0.1	3200	3200	1600
A/X31	0.01	3200	3200	1600
Exp. II				
SEB	–	2560	5120	5120
SEB	1.0	640	640	640
A/X31	–	6400	3200	3200
A/X31	1.0	6400	3200	3200

in leukocyte culture supernatants harvested after 24 h, while culture supernatants harvested after 48 or 72 h showed a partial or even full recovery of the production in response to SEB. Culture periods of up to 3 days did not reveal inhibitory effects of CsA on the virus-induced production of IFN-α.

In summary our results demonstrate that CsA inhibits the production of mitogen-, alloantigen-, or EBV-LCL-induced IFN-γ by human PBL, but has no effect on the secretion of IFN-α induced by stimulation of PBL with viruses, CP, or poly I:C.

Recent studies have demonstrated that CsA is an effective immunosuppressive agent in vivo and in vitro [3,4,6,7]. CsA inhibited the generation of cytotoxic T effector cells induced by allogeneic or EBV-infected autologous cells [11,17]. The inhibitory activity of CsA on the development of cytotoxic T cells appeared to be due to decreased production of interleukin 2 [5,16]. Our observations provide further evidence for the assumption that CsA acts predominantly on T lymphocyte-dependent immune responses [11,12,16]. It has been shown that both interleukin 2 and IFN-γ are produced by T lymphocytes of the helper cell subset [8,10,18]. Thus, inhibition of the production of T cell factors appears to be a general feature of CsA activity.

Lymphokines have been postulated to exert potent regulatory effects on the activity of cytotoxic cells. Thus, it has been shown that interleukin 2 is strictly required for the proliferation and maturation of cytotoxic T lymphocytes [10]. IFN-γ has strong stimulatory activity for the lytic potential of specific T effector cells and nonspecific natural killer (NK) cells [13,18]. Abrogation of cytotoxic effector cell control may explain the reported promotion of the spontaneous outgrowth of EBV-induced lymphoblastoid cell

lines by CsA in vitro [2]. In the presence of CsA, inhibition of effector mechanisms results in a polyclonal proliferation of EBV-infected B cells which in vitro form lymphoblastoid cell lines but in vivo manifest as the immunoblastic proliferation characteristic of immunosuppressed patients [19]. Further studies will be needed to clarify the possible contribution of CsA-mediated suppression of the production of interleukin 2 and IFN-γ to the development of EBV-associated malignant lymphoproliferative disease. Selective inhibition of the secretion of T cell factors by CsA provides an experimental system to investigate the role of the antiviral, antiproliferative and immunomodulatory effects of IFN-γ in the pathogenesis of virus-induced malignancies.

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