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MODULATION BY CYCLOSPORIN A OF MURINE NATURAL RESISTANCE AGAINST HERPES SIMPLEX VIRUS INFECTION. I. INTERFERENCE WITH THE SUSCEPTIBILITY TO HERPES SIMPLEX VIRUS INFECTION

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Adult BALB/c mice which are medium-high resistant against intraperitoneal (i.p.) infection with herpes simplex virus type 2 (HSV-2) manifested a drastic increase in susceptibility to the virus when treated locally with cyclosporin A (CyA) during infection. Oral application of the drug had no effect on the natural resistance status. Mice appeared normal 2 weeks after CyA treatment with regard to their ability to resist i.p. infections. CyA did not interfere with established specific immune protection nor with the induction of immune responses to HSV-2.

cyclosporin A natural resistance herpes simplex virus

INTRODUCTION

The fungal metabolite cyclosporin A (CyA) has been demonstrated by us [1] and others (reviewed in ref. 11) to induce immunological tolerance in experimental animal systems. Preferential in vivo targets of the action of this drug were apparently T cells [1, 10, 11]. It was shown by us that CyA impairs antiviral cytotoxic T-cell functions [1]. However, this suppression did not interfere with the natural resistance of mice to influenza infection [1]. Induction of specific B-cell activity which is mostly responsible for immune protection in this animal species [3, 7] was not affected [1]. The drug was applied orally and mice were inoculated with the virus by the intravenous or intranasal route, thus demonstrating the systemic effects of the compound.

In order to elucidate the local effects of CyA on natural and acquired resistance, a different experimental system proved to be more appropriate. Certain inbred mouse strains are distinguishable from each other with regard to survival of intraperitoneal (i.p.) herpes simplex virus infection [6, 17]. We have chosen the medium-high resistant BALB/c strain for the studies presented here. Our results document that locally applied CyA decreases natural resistance of mice during treatment, without interfering with induction of specific immunity to the virus. Vaccinated mice were solidly protected against infection with HSV-2 even if they had been treated with CyA at the time of challenge.

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MATERIALS AND METHODS

Animals

Inbred BALB/c/A Bom mice were derived from Gl. Bomholtgård Ltd. (Ry, Denmark). Male mice aged 12-16 weeks were used.

Cell lines

Exponentially growing L929 and 3T3 tumor cells, expressing H-2^k and H-2^d cell surface antigens respectively encoded by the mouse major histocompatibility gene complex, were used as targets in the cytotoxicity assays. They were maintained in tissue culture.

Viruses

Herpes simplex virus type 2 strain G was grown on Vero cell monolayers. HSV-2 strain MS-CV was derived from strain MS by adaption to growth at 25°C by H.F. Maassab, (Ann Arbor, MI) [18] and proved to be apathogenic for mice. This strain was grown on MRC-5 cells at 34°C. Viruses were titrated by plaque formation on Vero cells under fluid overlay containing 0.1% rabbit hyperimmune serum against HSV-2. Viruses were stored in aliquots at -70°C.

Drug treatments

CyA (OL 27-400 N, lot No. 79809, Sandoz Ltd., Basel, Switzerland) was solubilized in commercially available olive oil (60°C for 20 min) immediately before injection. It was applied orally or i.p. at doses ranging from 0.05 to 1.0 mg per mouse (weight about 25 g) in 0.1 ml of olive oil. Treatment started 2 h (or in some experiments 2 weeks) before infection. Mice received four to five consecutive injections (one per day). Control mice were injected with olive oil alone.

Cyclophosphamide (Endoxan, Asta Werke AG, Linz, Austria) dissolved in saline was injected once (4 mg/mouse, i.p.) 24 h before infection with HSV.

Immunizations and infections

Virus was generally applied by the i.p. route. Pathogenic strain G was used for estimation of susceptibility to infection at doses ranging between 10^2 and 10^5 plaque-forming units (p.f.u.). Mortality was protocolled over a period of 21 days. Attenuated HSV-2 was used for induction of specific immunity and protection at a dose of 10^6 p.f.u./mouse. Spleen cells pooled from four to six mice were used as effector cells in the cytotoxic assays on day 6 after sensitization.

Cytotoxicity assays

Cytotoxic responses were assayed by the ^{51}Cr-release method [12] with modifications detailed previously [5]. Briefly, killer T-cell activity was assayed on H-2-compatible HSV-2-infected targets (3T3 for BALB/c). B-cell responses are indicated by the lysis of

H-2-incompatible target cells (L929 for BALB/c) in the presence of complement [5]. Cytotoxic cells induced after i.p. infection in the spleen do not lyse L929 and 3T3 tumor cells which are non-infected or infected with viruses other than HSV at the time of the assay neither in the absence nor the presence of complement (data not shown, to be published). Results are expressed as percent specific ^{51}Cr-release using the formula: [E–LC)/HC–LC) X 100] [5]. E represents the isotope released from the targets in the presence of sensitized effector cells. LC is determined by the activity of cells from normal mice. HC is the amount of ^{51}Cr released after incubation with 10% Triton X-100 for 15 min at 37°C. Standard error of the mean between replicas was less than 0.05. Cytolysis in the presence of non-sensitized cells was less than 30%.

All experiments were performed at least three times.

RESULTS

Effects of CyA on susceptibility of mice to i.p. infection with HSV-2

Orally applied CyA (or olive oil alone) at a dose of 1 mg per mouse per day (4 times) did not change the resistance status of BALB/c mice to i.p. HSV-2 infection (Fig. 1). In contrast, local application (i.p.) of CyA increased mortality significantly when the

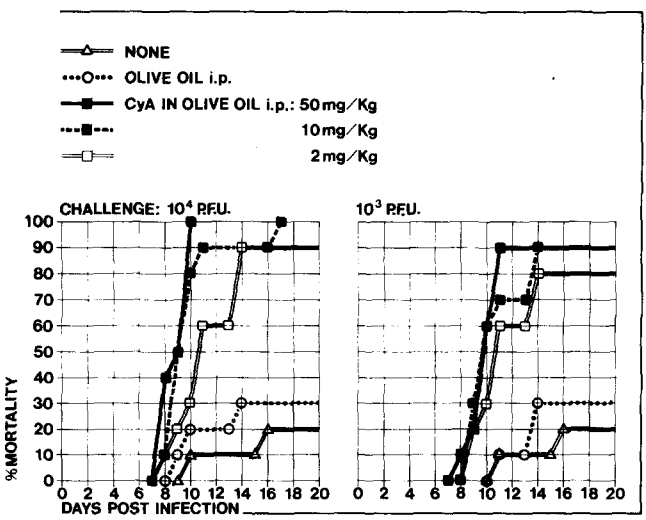

Fig. 1. Effect of oral and intraperitoneal application of CyA on susceptibility of BALB/c mice to i.p. infection with HSV-2. Mice received four daily injections of either CyA in olive oil (1 mg/mouse/day), olive oil alone or were left untreated. All animals were challenged 2 h after onset of drug treatment by i.p. infection with either 10^3 or 10^4 p.f.u. of pathogenic HSV-2. Ten mice per group. *The effectiveness of i.p.-applied CyA as compared to oil or no treatment was significant according to contingency table analysis ($P < 0.001$).*

mice were challenged with 10^3 or 10^4 p.f.u. of HSV-2. Again, olive oil alone had no significant effect. Impairment of natural resistance by local administration of CyA was also achieved with much lower doses (0.05 and 0.25 mg/mouse/day) of the drug (Fig. 2).

Decrease of natural resistance was only temporarily expressed, since mice treated with CyA 2 weeks before resisted 10^2 and 10^3 p.f.u. HSV-2 as well as untreated control animals (Table 1). Nevertheless, there was still a tendency to increased mortality of CyA-treated mice at higher challenge doses in this and other experiments (data not shown).

CyA does not interfere with specific immunity to HSV-2

Mice vaccinated with attenuated HSV-2 exhibit long-lasting, specific and solid protection against lethal infection with pathogenic virus (to be published). In the experiment presented here (Table 2), protection was already established 7 days after vaccination. Most importantly, CyA treatment did not influence this type of resistance, whereas – as before – non-vaccinated mice showed increased susceptibility to HSV infection after drug treatment.

Immune protection against HSV-2 appears to be based exclusively on the presence of specific B-cell activities; as demonstrated in Table 3, this is the only specific effector cell which is detectable after in vivo HSV-2 infection. H-2-compatible virus-infected 3T3 targets were not lysed by spleen cells from specifically sensitized BALB/c mice unless

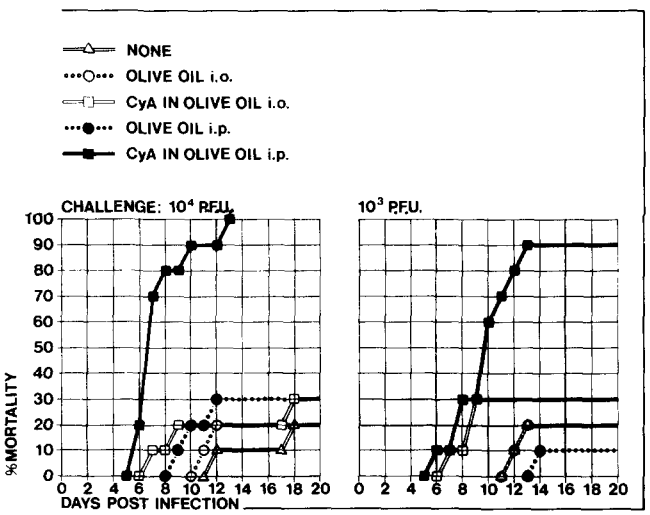

Fig. 2. Dose relationship of CyA-induced effects on susceptibility of BALB/c mice to i.p. infection with HSV-2. Mice received four daily i.p. injections of various doses of CyA in olive oil. Control mice were treated with injections of olive oil alone or were left untreated. All animals were challenged 2 h after onset of drug treatment by i.p. infection with either 10^3 or 10^4 p.f.u. of pathogenic HSV-2. Ten mice per group. *The effectiveness of all doses of CyA as compared to oil or no treatment was significant according to contingency table analysis ($P < 0.001$, except for mice treated with 2 mg CyA/kg after challenge with 10^3 p.f.u. of HSV-2. P was 0.02 in comparison with oil-treated mice).*

TABLE 1

Sensitivity of BALB/c mice to i.p. HSV-2 infection 2 weeks after cyclosporin A treatment

Treatment[a]	Challenge dose[b] (p.f.u. HSV-2)	Mortality on day 21	
		Total	%
None	10^4	3/10	30.0
	10^3	2/10	20.0
	10^2	2/10	20.0
4 X CyA, i.p., (1 mg/injection)	10^4	5/10	50.0
	10^3	2/10	20.0
	10^2	1/10	10.0

[a] Mice received daily injections of CyA or were left untreated.

[b] Mice were challenged i.p. 2 weeks after onset of the CyA treatment.

TABLE 2

Cyclosporin A has no effect on the resistance of presensitized BALB/c mice to HSV-2

Presensitization[a]	Cyclosporin A treatment[b]	Challenge[c] (p.f.u. HSV-2)	Mortality on day 21	
			Total	%
None	None	10^5	8/10	80.0
		10^4	2/10	20.0
		10^3	1/10	10.0
	5 X CyA, i.p.	10^4	10/10	100.0
		10^3	10/10	100.0
		10^2	8/10	80.0
Attenuated HSV-2	None	10^6	0/10	0.0
		10^5	0/10	0.0
	5 X CyA, i.p.	10^6	0/10	0.0
		10^5	1/10	10.0

[a] Mice were vaccinated by i.p. injection with 10^6 p.f.u. of HSV-2 7 days before onset of CyA treatment.

[b] Mice received daily injections of CyA (1 mg/mouse/day).

[c] I.p. challenge 2 h after the first CyA injection.

complement was added, indicating the absence of HSV-specific killer T cells, but the presence of antibodies reacting with viral determinants. H-2-incompatible HSV-infected L929 were not expected to be lysed by BALB/c T-effector cells. However, cytotoxic activities after addition of complement again suggest the presence of specific B lympho-

TABLE 3

Effect of cyclosporin A and cyclophosphamide on the induction of HSV-2 specific immune responses in BALB/c mice[a]

Treatment[b]	% Specific ^{51}Cr-release											
	3T3-HSV-2 + C′			3T3-HSV-2, no C′			L929-HSV-2 + C′			L929-HSV-2, no C′		
	100 : 1	50 : 1	25 : 1	100 : 1	50 : 1	25 : 1	100 : 1	50 : 1	25 : 1	100 : 1	50 : 1	25 : 1
None	46.1	25.5	10.9	< 1.0	< 1.0	< 1.0	40.9	21.9	18.6	4.9	4.5	2.5
5 × CyA, i.p.	38.5	24.8	10.5	< 1.0	6.3	< 1.0	42.0	29.4	11.3	3.7	5.6	4.9
1 × CyP, i.p.	*1.0*[c]	*< 1.0*	< 1.0	< 1.0	1.1	< 1.0	*18.7*	*2.2*	*1.1*	7.7	3.2	5.9

[a] Mice were immunized by i.p. infection with 10^6 p.f.u. of attenuated HSV-2. Spleen cells were assayed on day 6. There was no lysis of non-infected targets plus or minus complement.

[b] Cyclophosphamide (CyP, 4 mg/mouse) was given 1 day before infection. Cyclosporin treatment (1 mg/mouse/day) was started 2 h before infection. Mice received one injection per day.

[c] Values in italics are significantly lower as compared to the untreated controls ($P < 0.01$ by Students t test).

cytes in the test culture. These results were reproduced in our laboratory in a great number of experiments using different HSV-2 strains, virus doses, target cells and other mouse lines [2]. It is evident from the data presented here that CyA does not affect induction of B-cell immunity under the conditions employed. In contrast, the cytostatic drug cyclophosphamide completely prevented generation of B-cell immunity to HSV-2.

DISCUSSION

We demonstrated here that the immunosuppressant CyA impairs natural resistance against HSV-2 infections in a mouse model system. This effect was only detectable when CyA and virus were applied via the same route (i.p.). In a previous publication [1] we reported that, analogous to the HSV system, no increase in susceptibility to the lethality of influenza could be observed when CyA and virus were administered by different routes. We also documented a preferential suppression of anti-influenza killer T-cell responses in mice infected during drug treatment [1]. Our data regarding the effects of CyA on specific immunity to HSV are in agreement with these original observations in the influenza system. Since HSV-2 does not induce significant levels of virus-specific cytotoxic T cells in our hands, we could only investigate the influence of the fungal compound on B-lymphocyte responses. There was no inhibitory effect of CyA on the induction of humoral immunity. This finding also corroborates reports of Borel et al. [10, 11] and of Kunkel and Klaus [16], demonstrating that CyA does not interfere with functions of certain B-cell subsets. Thus, in our system mice exhibited increased susceptibility to virus infection, despite the fact that they were capable of generating specific immune responses. However, this weakening of resistance could be prevented by prevaccination of the animals with attenuated virus. Once specific immunity was established no impairment of the acquired resistance status by CyA was detected. Hence CyA should allow one to discriminate between effector mechanisms involved in natural and induced resistance to HSV-2.

In recent publications we demonstrated [6, 9] that early non-virus-specific cellular functions are the most important ones for the natural defence against HSV infections. Among those were natural killer cell responses and macrophage activities. In a similar experimental system, Engler et al. [14] demonstrated that interferon production represents another essential resistance function against HSV infection. In further studies, documented in a subsequent paper [8], we will present evidence that CyA impairs several of these resistance functions.

Since CyA is a drug designed for human use [13] one has to be aware of the possibility that it might affect the natural barrier against infectious agents in man as it does in mice. In particular, those infections against which no specific immunity had been established before drug treatment might represent a potential danger. However, CyA still appears preferable to other immunosuppressants, because it seems to act on certain cellular subsets only and leaves others intact which are capable of compensating possible

detrimental effects. Furthermore, a high local concentration of CyA is apparently necessary for detection of the effects on natural resistance as described here. Such concentrations might not be reached after oral application of the compound. In the mouse, orally administered CyA does not aggravate i.p. HSV or intranasal influenza [1] infections at doses used in clinical trials. In addition, mice returned to a normal resistant state 2 weeks after termination of the drug treatment.

The cytostatic compound CyP has also been reported to diminish natural resistance functions [19] and to cause death of mice against normally apathogenic viruses [15]. The latter findings are in agreement with those of our own laboratory regarding HSV infections in the mouse (to be published). In the present publication we have demonstrated suppression of B-cell responses by this compound. Thus CyP obviously interferes with both inborn and acquired defence mechanisms.

We conclude that CyA is a drug which will be most useful in studying the role of particular cellular subsets and cell functions in acquired and inborn resistance to infectious and other life-threatening diseases.

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