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MODULATION BY CYCLOSPORIN A OF MURINE NATURAL RESISTANCE AGAINST HERPES SIMPLEX VIRUS INFECTION. II. INFLUENCE ON THE HSV-INDUCED NATURAL KILLER CELL RESPONSES, MACROPHAGE ACTIVITIES AND INTERFERON LEVELS

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Cyclosporin A (CyA) interfered locally at the site of injection with several resistance functions which are of potential importance in experimental herpes simplex virus (HSV) infections of mice. HSV-induced stimulation of macrophage phagocytosis was reduced by CyA when the mice were infected 5 days before the assay. The in vivo replication of the virus in macrophages, however, was enhanced. Natural killer (NK) cell responses were severely impaired. To some extent this could be attributed to the induction of suppressive macrophages by the drug treatment. Interferon levels induced by HSV were not diminished but rather enhanced in some experiments. Inhibitory effects ceased after termination of CyA treatment and could be prevented by presensitization of the mice with attenuated HSV type 2.

cyclosporin A natural resistance herpes simplex virus

INTRODUCTION

The fungal peptide cyclosporin A (CyA) has already been shown [5] to increase susceptibility to experimental herpes simplex virus type 2 (HSV-2) infections in inbred mice without interfering with induction of virus-specific immunity when both drug and virus were applied by the interperitoneal (i.p.) route. The aim of the present study was to investigate the influence of CyA on various non-antigen-specific cellular functions which might be associated with natural resistance. The results show that in fact various cell activities with potential importance in antiviral defence were changed during drug treatment. The most crucial effect appeared to be the suppression of natural killer (NK) cell responses.

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MATERIALS AND METHODS

Animals

Inbred BALB/c/A Bom mice were derived from Gl. Bomholtgård (Ry, Denmark). C3H/HeJ mice were obtained from Jackson Laboratories (Bar Harbour, ME). Male mice aged 12–16 weeks were used.

Cell lines

Exponentially growing L929, P815, Neuro 2A, 3T6 and YAC-1 murine tumor cells were used as targets in the cytotoxicity assays. Cells were maintained in tissue culture. Vero cells were grown to confluency in six well plates (35 mm diameter; Costar, Cambridge, MA) and used in the infectious centre assays.

Viruses

HSV-2 strain G and HSV type 1 strain Brand were 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) [17] and proved to be apathogenic for mice. This strain was grown on MRC-5 cells at 34°C. Virus was titrated by plaque formation on Vero cells under fluid overlay containing 0.1% rabbit hyperimmune serum against HSV-2. Virus was stored at -70°C. Influenza strain A/Hong Kong/68 (H3N2) and A/PR8/34 (H0N1) were grown in 10-day embryonated eggs and stored as allantoic fluid at -70°C.

CyA treatments

CyA treatments were performed as described in the preceding paper [5]. Deviations from the protocol are indicated in the tables.

Stimulation of macrophage phagocytosis

The preparation of cells and stimulation of macrophage phagocytic activity were performed as detailed previously [2]. Briefly, mice were infected i.p. with 10^3 or 10^5 plaque-forming units (p.f.u.) of HSV-2. Adherent peritoneal exudate cells (PEC) (pooled from 5–12 mice per group) were tested 5 days after infection with respect to their ability to engulf opsonized, ^{51}Cr-labelled sheep red blood cells. Stimulation index (SI) was calculated as follows:

$$SI = \frac{\text{c.p.m. of macrophages from virus inoculated mice.}}{\text{c.p.m. from resident (control) macrophages}}$$

Infectious centre assay

The assay was described in detail in a previous publication [2].

Induction of NK cells

NK cell responses were generated by i.p. injection of 10^4 or 10^5 p.f.u. of HSV-2.

PEC (pooled from 5–12 mice) were assayed 2 days later. The characteristics of HSV-induced NK cells and our assay system have been described in detail [6].

Induction of anti-influenza killer T cells

Influenza-specific cytotoxic T lymphocytes were induced by intravenous injection of about 150 international units of H3N2 5 days before the assay. Spleen cells pooled from four mice were used.

Cytotoxicity assays

For details and calculations see the accompanying paper [5] and refs. 3 and 6.

Assay for suppressive macrophages

Macrophages potentially suppressing NK cell responses of CyA-treated mice were removed by plastic adsorption for 2 h at 37°C in 10% fetal calf serum (FCS)-containing culture medium. The non-adherent cell population was then assayed either alone or in the presence of monolayers of adherent cells present in 1.3×10^5, 2.5×10^5 or 5×10^5 PEC respectively. Monolayers were prepared by culturing PEC from CyA-treated or untreated mice in flat-bottomed microtiter plates (microtest II, Falcon, Div. Becton Dickinson, GmbH, Heidelberg, F.R.G.) for 3 h in 10% FCS-containing culture medium. Non-adherent cells were removed by thorough agitation of the cultures and several washings with warm medium.

Interferon induction and interferon assay

Interferon was induced by i.p. injection of 10^7 p.f.u. HSV-1. Ice-cold culture medium was injected 5 or 18 h later and recovered within 30 min from the peritoneal cavity. Peritoneal fluid from five mice was pooled. Samples were cleared by centrifugation and filtration (0.45 μm, Millipore Corp., Bedford, MA). Interferon was assayed in 96-well microtiter plates on L929 cell monolayers by the inhibition of cytopathic effect after challenge with vesicular stomatitis virus [7].

RESULTS

Effect of CyA on macrophages

We have already demonstrated [2] a correlation of natural resistance to HSV infection with two different macrophage functions in a variety of inbred mouse strains: 1) stimulation of macrophage phagocytosis representing a late extrinsic activity; and 2) in vivo permissiveness for virus replication, which may be important early post-infection [2]. In a series of experiments we investigated the effects of CyA on both macrophage activities. As a source of macrophages we used plastic adherent peritoneal cells after i.p. drug treatment and/or i.p. HSV-2 infection.

Table 1 summarizes the results of one experiment showing the influence of CyA treatment on the HSV-dependent stimulation of macrophage phagocytosis of heterologous, opsonized erythrocytes. Control mice were treated with olive oil alone. It is obvious from the results that virus and oil were equally potent stimulants for macrophage phagocytosis. In general, there was no significant enhancement of phagocytic activity by HSV in oil-treated mice. Virus alone induced the highest activity when given 5 days before the assay. This is also the only group in which CyA treatment caused significant suppression of stimulation. Whether CyA interfered with the stimulus provided by HSV, or the oil, or both together could not be determined.

Whereas in the macrophage phagocytosis assay suppression by CyA was at most around 50%, the effects of the drug on the macrophage susceptibility to virus infection was much greater. This is shown in Table 2. It is clear from the results that i.p. treatment with CyA (starting 3 days before infection) drastically increased the number of virus-replicating macrophages in the peritoneum after i.p. infection. Olive oil alone had no such effect. In one of the experiments (experiment I) we tested whether oral application of CyA would exert similar effects when compared to i.p. injections. Although there was an increase in infectious centres when calculated per 10^6 PEC, no effect could be observed on the total number of HSV-producing macrophages per mouse. Further, i.p. admini-

TABLE 1

Effect of cyclosporin A on the HSV-2-induced macrophage phagocytosis activity

Treatment[a]	HSV-2 (i.p.)		Macrophage-erythrophagocytosis on day 5			
	Day	Dose (p.f.u.)	C.p.m.	S.E.M.[b]	Stimulation indices[c]	
					HSV-induced	Total
None	–	None	86.6	1.03	–	–
	0	10^3	815.9	1.01	9.4	–
	3	10^5	195.9	1.02	2.3	–
	0 + 3	$10^3 + 10^5$	464.5	1.03	5.4	–
5 × Olive oil	–	None	795.0	1.02	–	9.2
	0	10^3	1143.2	1.03	1.5	13.2
i.p., days 0–4	3	10^5	633.9	1.04	0.8	7.3
	0 + 3	$10^3 + 10^5$	1125.1	1.02	1.4	13.0
5 × Olive oil	–	None	441.2	1.01	–	5.1
	0	10^3	453.0	1.04	1.0	5.2
plus CyA, i.p.,	3	10^5	658.5	1.02	1.5	7.6
5 × 1 mg, days 1–4	0 + 3	$10^3 + 10^5$	904.5	1.02	2.1	10.4

[a] BALB/c mice received one injection of olive oil or CyA in olive oil per day or were left untreated. Adherent PEC were tested.

[b] Standard error of the mean. Four replicas per group.

[c] PEC from untreated mice served as control for calculation of total stimulation.

TABLE 2

Effect of cyclosporin A on the in vivo permissiveness of macrophages for HSV-2 replication[a]

Experiment	Treatment	Vaccination[b]	PEC yields/ mouse (× 10^6)	Infectious centres	
				Per 10^6 PEC	Total (per mouse)
I	None	None	17.0	6.7	113.9
	Olive oil, orally		9.5	5.2	49.4
	CyA in olive oil, orally		3.6	33.3	119.9
	None	None	7.2	10.0	72.0
	Olive oil, i.p.		3.5	18.4	64.4
	CyA in olive oil, i.p.		8.2	75.3	617.5[c]
II	None		24.0	3.5	84.0
	Olive oil, i.p.	None	18.0	9.0	162.0
	CyA in olive oil, i.p.		28.0	39.6	1108.8[c]
	None		15.6	1.3	19.8
	Olive oil, i.p.	Attenuated HSV-2	26.3	0.3	7.9
	CyA in olive oil, i.p.		14.4	2.3	33.1

[a] BALB/c mice received one injection of olive oil or CyA in olive oil per day or were left untreated. Duration of treatment: 4 days. CyA dose: 1 mg/mouse/injection. Mice were infected on day 4 with 10^6 p.f.u. HSV-2. Adherent PEC were assayed 24 h later.

[b] 10^6 p.f.u./mouse, i.p., 8 days before challenge.

[c] Increase of infectious centres after CyA treatment in comparison to oil treatment was significant according to contingency table analysis ($P < 0.001$).

stration of CyA was ineffective when mice were vaccinated with attenuated HSV-2 8 days before challenge (experiment II). There was a drastic reduction of infectious centres in the assays when PEC from untreated mice were compared to those from vaccinated animals.

Suppression of local NK cell responses during CyA treatment

Early NK cell activity after HSV-2 infection is another cell function which correlates with resistance in most mouse strains [4]. As demonstrated in Table 3 medium-high resistant BALB/c mice exhibit good local NK responses assayed on three different tumor cell targets 24 h after i.p. infection. CyA treatment, four daily injections, starting 3 days before infection, completely inhibited the cytolytic activities against two targets (3T3, L929 infected with HSV-2) and drastically decreased cytotoxicity against the highly sensitive YAC-1 cells. This inhibitory effect was transient, since mice appeared normal with respect to their capability to generate NK cell responses at about 3 weeks after termination of drug treatment. Olive oil alone had no effect on induction of NK activity.

TABLE 3

Kinetics of the suppressive effect of cyclosporin A on the local induction of NK cell responses by HSV-2[a]

Treatment	Time of assay (days after onset of treatment)	% Specific ^{51}Cr release								
		3T3			L929-HSV-2			YAC-1		
		100 : 1	50 : 1	25 : 1	100 : 1	50 : 1	25 : 1	100 : 1	50 : 1	25 : 1
None	–	22.1	18.4	14.5	33.9	23.5	19.5	87.3	81.2	73.3
4 × CyA, i.p.	4	*< 1.0*[b]	*< 1.0*	*< 1.0*	*< 1.0*	*< 1.0*	*< 1.0*	*25.4*	*15.5*	*8.4*
	12	*12.1*	*1.9*	*< 1.0*	*21.1*	17.2	9.5	*59.9*	*42.9*	*27.2*
	19	28.2	11.3	5.4	*21.4*	15.9	13.3	*54.1*	*47.5*	*37.7*
	26	28.7	21.7	20.7	36.7	27.9	20.2	85.1	61.3	*48.1*

[a] BALB/c mice were treated with four daily injections of CyA (1 mg/mouse/day) or left untreated. Mice were infected 2 days before the assay (i.p.) with 10^5 p.f.u. of HSV-2. PEC were assayed.

[b] Values in italics are significantly lower as compared to the untreated controls ($P < 0.02$ by Students t test).

The data were therefore omitted from the results shown (Table 3).

In the accompanying paper [5] it was demonstrated that only i.p. application of CyA caused increased susceptibility of mice to i.p. HSV-2 infection. Above, enhancement of macrophage permissiveness for virus replication was also associated with i.p. drug treatment. Table 4 shows that oral administration of CyA did not affect i.p. NK responses. Tested in parallel, however, a systemic effect of the compound is indicated by the complete suppression of killer T-cell responses against influenza, in accordance with earlier findings in our laboratory [1]. Suppression of activation of NK cells by CyA could be prevented, as shown in Table 5, by two inoculations of pathogenic virus during the drug-treatment course: one (low dose) was given 2 h after the first CyA injection, the other 3 days later. The assay was performed 2 days thereafter. A similar protective effect was seen after presensitization with attenuated HSV-2 (data not shown).

Presence of adherent PEC suppressive for NK activity in CyA-treated mice

Suppression of NK activity can be explained by the presence of inhibitory cells in the assay system interfering with cytotoxicity or by the in vivo inhibition of activation of NK cells or both. We considered the macrophage as the most likely candidate for a suppressor cell. We therefore investigated the hypothesis that removal of adherent cells would increase the cytolytic responses of PEC from CyA-treated mice to a greater extent than those from untreated animals. Further, admixture of adherent cells – in particular those from drug-treated mice – to non-adherent PEC should suppress NK activity. The results of one such experiment are shown in Table 6. As demonstrated before (Table 4) significant residual NK activity was only detectable when YAC targets were used in the assay. Removal of plastic adherent cells caused an increase of the ^{51}Cr-release from 11.3% to 20.6% at an effector target ratio of 50 : 1. In the respective control group from untreated mice, plastic adsorption did not enhance cytolytic activities significantly. However, this procedure did not reveal cytolytic responses of PEC from CyA-treated mice against Neuro 2A or other less NK-sensitive targets (data for the latter not shown). Admixture of adherent cells from non-treated mice did not significantly affect the activities of non-adherent PEC from either untreated or CyA-treated animals. In contrast, similar cell populations from drug-treated mice exerted significant, but low, inhibitory effects on the cytotoxic activities of NK cells from both mouse groups. Inhibition of the responses of non-adherent PEC from non-CyA-treated mice was only detectable when Neuro 2A targets were used, not with YAC cells. Thus, NK cells from CyA-treated mice appear more sensitive to the suppressive effects of adherent cells activated by the same drug than the respective cytotoxic cells from non-manipulated animals.

Effect of CyA on the local levels of HSV-1-induced interferon

Interferons have been widely accepted as the most important inducers of NK cells activities (reviewed in ref. 20). Inhibition of interferon production, e.g. by CyA, could

TABLE 4

Effect of orally applied cyclosporin A on the induction of cytotoxic T cells and NK cell responses

Treatment[a]	Sensitizing virus[b]	Effector cells[c]	% Specific ^{51}Cr release[d]								
			L929-HSV-2			L929 non-infected			Neuro 2A non-infected		
			100 : 1	50 : 1	25 : 1	100 : 1	50 : 1	25 : 1	100 : 1	50 : 1	25 : 1
None	HSV-2, i.p.	PEC	100.0	53.4	32.8	85.3	45.9	27.4	100.0	72.7	52.6
Olive oil			82.2	48.4	28.6	68.3	39.7	29.7	89.4	62.2	43.1
CyA			87.9	51.0	33.8	67.0	43.8	26.3	89.7	68.2	51.1
			P815-H3N2			P815-H0N1			P 815 non-infected		
			100 : 1	50 : 1	25 :1	100 : 1	50 : 1	25 : 1	100 : 1	50 : 1	25 : 1
None	H3N2, i.v.	Spleen cells	100.0	59.5	45.5	80.2	46.3	28.6	2.5	5.2	1.3
Olive oil			98.5	57.2	38.1	75.8	38.9	24.2	< 1.0	< 1.0	< 1.0
CyA			4.1	10.5	4.4	7.3	< 1.0	1.2	< 1.0	< 1.0	< 1.0

[a] BALB/c mice received five daily oral injections of CyA in olive oil (1 mg/mouse/day), olive oil alone or were left untreated.

[b] Virus doses per mouse; H3N2 = 150 international units, HSV-2 = 10^5 p.f.u.

[c] Assays were performed 5 days after onset of CyA treatment and/or sensitization.

[d] The assay measures NK activity in PEC and cytotoxic T-cell responses in spleen cells (see Materials and Methods).

TABLE 5

NK cell responses of BALB/c mice infected once or twice during CyA treatment[a]

Treatment	HSV-2, i.p. infection		% Specific ^{51}Cr release								
	Day	Dose (p.f.u.)	L929-HSV-2			3T6			Neuro 2A		
			100 : 1	50 : 1	25 : 1	100 : 1	50 : 1	25 : 1	100 : 1	50 : 1	25 : 1
None	0	10^4	52.5	24.4	13.6	100.0	71.3	34.7	37.0	19.2	10.0
	3	10^5	100.0	75.7	49.0	100.0	98.9	61.0	72.0	48.3	34.3
	0 + 3	$10^4 + 10^5$	52.0	22.2	9.7	100.0	66.4	34.6	34.5	21.6	4.8
5 × CyA, i.p.	0	10^4	*6.3*[b]	*3.1*	*< 1.0*	*12.0*	*6.2*	*1.1*	*3.5*	*< 1.0*	*< 1.0*
	3	10^5	*10.8*	*7.2*	*< 1.0*	*12.2*	*5.3*	*< 1.0*	*1.0*	*< 1.0*	*< 1.0*
	0 + 3	$10^4 + 10^5$	72.8	47.4	31.9	61.3	35.1	25.3	29.2	18.4	6.3

[a] Mice were treated from day 0 to day 4 (1 mg/mouse/day). PEC were assayed on day 5.

[b] Values in italics are significantly lower as compared to the respective control groups ($P < 0.01$ by Students t test).

TABLE 6

Presence of adherent PEC with suppressive activity for NK cell responses in cyclosporin A-treated mice

Cell groups	Effector cells[a]				% Specific ^{51}Cr release	
	Adherent PEC		Non-adherent PEC[b]			
	In vivo treatment	Cell number	In vivo treatment	Cell number	YAC[c]	Neuro 2A[c]
I	–	–	None	5.0×10^5	69.3	18.1
II	None	5.0×10^5	–	–	< 1.0	< 1.0
III	–	–	CyA	5.0×10^5	20.6	< 1.0
IV	CyA	5.0×10^5	–	–	< 1.0	< 1.0
I + II	None	5.0×10^5	None	5.0×10^5	64.9	19.7
		2.5×10^5			67.5	17.3
		1.3×10^5			74.3	19.6
I + IV	CyA	5.0×10^5	None	5.0×10^5	54.0	5.7[d]
		2.5×10^5			62.7	8.2[d]
		1.3×10^5			61.4	19.4
III + II	None	5.0×10^5	CyA	5.0×10^5	16.0	< 1.0
		2.5×10^5			23.9	< 1.0
		1.3×10^5			20.1	< 1.0
III + IV	CyA	5.0×10^5	CyA	5.0×10^5	6.2[d]	< 1.0
		2.5×10^5			14.3	< 1.0
		1.3×10^5			23.5	< 1.0

[a] BALB/c mice received four daily i.p. injections of CyA (1 mg/injection) or were left untreated. Animals were inoculated i.p. with 10^5 p.f.u. of HSV-2 on the 3rd day of treatment. Plastic adherent and non-adherent PEC were assayed 2 days after infection.

[b] 5.0×10^5 non-separated PEC lysed 70.8% of YAC and 20.8% of Neuro targets when derived from non-treated mice. The respective data for CyA-treated mice were 11.3% and less than 1.0%.

[c] 10^4 target cells per well.

[d] Suppression was significant at $P \leq 0.05$ according to Students t-test.

therefore also prevent the induction of NK responses. Table 7 clearly demonstrates that this is not a mode of action of CyA. In order to elicit detectable interferon levels in vivo we had to use HSV type 1 instead of type 2. It has, however, similar properties in the animal model used with respect to pathogenesis and generation of resistance functions [14] (reviewed in ref. 11). We compared the highly resistant C3H/HeJ with the less resistant BALB/c mice. Both were similarly affected by CyA treatment resulting in increased susceptibility to HSV infections (data for the former not published). It is obvious from the results that interferon levels in mice assayed 5 or 18 h after HSV-1 infection

TABLE 7

Influence of i.p. cyclosporin A treatment on the in vivo induction of interferon by HSV-1 in normal and vaccinated mice

Experiment	Mouse strain	Vaccination[c]	Cyclosporin A treatment[a]	Challenge (h before interferon harvest)[b]	Interferon titers (units/ml)
I	C3H/HeJ	Attenuated HSV-2	None	18	< 20
			4 X		< 20
			2 X		< 20
		None	None		90
			4 X		110
			2 X		40
	C3H/HeJ	None	None	18	240
			4 X		240
			2 X		320
II		None	None	5	35
			4 X		160
			2 X		200
	BALB/c	None	None	5	60
			4 X		320
			2 X		960

[a] 1 mg/day/mouse starting 4 or 2 days before challenge. Control mice received oil. Five mice per group.

[b] 10^6 p.f.u. 8 days before challenge, i.p.

[c] 10^7 p.f.u. HSV-1, i.p.

were not diminished by CyA treatment but rather enhanced. Enhancement was most pronounced after two doses of CyA given on day 2 and day 1 before infection. It was also interesting to note that presensitization of mice with attenuated HSV-2 8 days before challenge completely prevented interferon induction in all mouse groups.

DISCUSSION

I.p. infections of adult mice with HSV provide a useful system for studying the genetic and cellular basis of natural resistance. Cumulative evidence from our laboratory [2, 4] and those of others [9, 11, 12, 14–16, 18] has identified a variety of genetically determined potential resistance functions. Among these are those sustained by macrophages [2, 16, 18], natural killer cells [4], interferon producing cells [11, 12] and bone marrow-associated cell populations [5]. A major part of the evidence for the role of these cell activities was derived from two lines of experimentation. The first attempted to correlate

the presence or absence of certain cell functions with resistance in genetically defined mouse strains [2, 4, 11, 12, 14, 16]. The second approach used suppressive treatments in the hope of selectively inactivating particular subpopulations of cells in order to elucidate their respective importance in resistance (reviewed in ref. 11). Our studies on the effects of CyA on inborn and acquired resistance to HSV-2 infection as presented here corroborate these earlier findings.

In the preceding paper [5] we demonstrated a dramatic increase in the susceptibility of mice to i.p. HSV-2 infection after local CyA treatment. Here we show that at least two cell populations are severely affected by the drug: macrophages and natural killer cells. The former exhibited enhanced susceptibility to virus replication; the latter was drastically suppressed during the treatment course and for several weeks thereafter. It was most interesting to note that these cell functions were not impaired by the drug if the mice had been presensitized with attenuated HSV. This vaccination procedure was also shown by us [5] to ensure resistance against HSV infection during CyA treatment. The reported effects of CyA were locally restricted since oral application of the compound did not interfere with resistance against i.p. infection and the putative resistance functions. Thus, with regard to the clinical situation where CyA is applied by the oral route [8], the danger of aggravating potential infections is probably low. Furthermore, one could expect adults to have developed an immunological memory for the more hazardous of infections. Immunological memory is apparently not affected by CyA [1].

Resistance of macrophages to virus infection and activation of NK cells have both been reported as being mediated by interferon [9] (reviewed in ref. 20). It was therefore surprising that interferon induction was not diminished but rather enhanced by CyA in our system. Although we had to use HSV-1 for significant stimulation of interferon production, it is unlikely that the drug would suppress mediator generation for one virus and not for the other. Hence, failure to induce NK cell responses in the presence of significant levels of interferon could mean that these cells are inhibited either directly by the drug or via an external suppressive mechanism induced by CyA. Since intrinsic macrophage functions were affected by the fungal metabolite, the possibility existed that other properties of this cell population were also changed. We have demonstrated suppression of the virus/oil-induced stimulation of macrophage phagocytosis late post-infection, and the presence of adherent PEC which were inhibitory for NK cell activity. The first effect may not account for the decrease in natural resistance, since it represents a late function and is antibody-dependent. Inhibition of NK activity was not very dramatic when effector cells from untreated mice were tested. The fact that residual NK activity in CyA-treated mice was more prone to suppression by macrophages suggests a functional defect in the NK cells themselves. This defect may have been caused by suppressor macrophages or other cells during, or before, activation of the NK cells. We favour the idea of a more direct effect of CyA on this cell class in a manner analogous to the inhibitory activity of the drug on specific cytotoxic T cells [1]. Thus it is possible that CyA blocks interferon binding similarly to the blocking of the receptor for HLA-DR as reported by Palacios and Möller [19]. The latter hypothesis, however, is in contrast to the findings

of Introna et al. [10], who reported the inhibition of spontaneous human NK activity by CyA and the restoration of NK function by fibroblast interferon in vitro. Hence one could speculate that the spontaneous killers already possess interferon receptors, whereas the induced NK cells would have to produce binding sites for interferon. Thus it could be that CyA prevents generation of interferon receptors perhaps in a manner analogous to that described for the interleukin 2 receptor on killer T cells by Larsson [13].

Finally, the finding that in presensitized CyA-treated mice, even when the sensitization took place during CyA treatment, natural resistance functions were preserved in the absence of measurable interferon suggests that alternative mechanisms exist for the maintenance and induction of these functions.

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