Short Communication

Mechanism of Interferon Induction and Pteridine Production in HIV-Infection

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Introduction

Elevated neopterin levels are found in body fluids of HIV-infected individuals (1), whereas biopterin is in the normal range. Neopterin elevation is assumed to be due to endogenous interferon (IFN) production induced by HIV. Raised IFN- α and IFN- γ levels are observed *in vivo* in sera of HIV-infected patients (2, 3), but *in vitro* IFN production of HIV-infected cells is deficient. HIV replicates, among other CD4-receptor positive cells, in monocytes/macrophages (M/M), which are considered a reservoir for persistent viral infection. M/M release neopterin when activated by IFNs. Upon infection with conventional viruses M/M produce IFN. However, HIV-infected M/M do not produce IFNs (4); retroviruses are poor IFN inducers. *In vitro* spontaneous as well as IFN-triggered neopterin release is not altered by infection with HIV (5). These observations imply that GTP-cyclohydrolase is neither activated directly by HIV-infection of M/M nor indirectly via IFN induction, since no IFN activity is produced by HIV-infected M/M. GTP-cyclohydrolase can be activated by IFNs in HIV-infected as in uninfected M/M.

To explain the mechanism of neopterin induction by HIV-infection, a model system is required which demonstrates induction of IFNs, leading to activation of GTP-cyclohydrolase, and subsequently increased neopterin release. Cytokine production can be initiated by M/M presenting antigen to T-lymphocytes. Therefore we investigated, whether HIV-infected M/M can induce IFN production and neopterin release upon interaction with lymphocytes.

Material and Methods

M/M from buffy coat of healthy blood donors were infected with HIV-1 strain HTLV-III_B. Viral replication was assessed by determination of the HIV-1 core protein p24 in supernatants (DuPont). 8 to 10 days post infection freshly isolated peripheral blood lymphocytes (PBL) of healthy donors were added to HIV-infected M/M at a ratio of 5:1 to 10:1. The time course of IFN activity in the supernatants was determined by a biosassay, using WISH-cells and Vesicular Stomatitis Virus. This assay determines the antiviral activity of IFNs; IFN- α as well as IFN- γ are active. Neopterin and biopterin were determined by radioimmunoassay.

Results and Discussion

IFN was induced upon coculturing of HIV-infected M/M with PBL (Table 1); IFN activity appeared early, with a maximum at 24 h. Control cultures of uninfected M/M, either alone or cocultivated with PBL, or HIV-infected M/M alone were consistently negative for IFN activity. Cell-cell contact between HIV-infected M/M and PBL was required for IFN induction. To characterize the IFNs, supernatants were incubated with antisera against IFN- α and IFN- γ , respectively, and then assayed for residual antiviral activity. Between 70 to 90% of IFN activity was abolished by these antisera, showing that IFN- α as well as IFN- γ are induced in this *in vitro* model system (5). The results indicate a strong synergistic effect of both IFNs.

Considerable amounts of neopterin were found in the supernatants upon coculturing of HIV-infected M/M with PBL (Table 1, 2). The time course of neopterin release is different from the time course of IFN activity, which is produced early during cocultivation. Neopterin, induced by IFN, is high on day 4, when IFN activity is already back to undetectable levels. In contrast to IFNs, cocultivation of PBL and uninfected M/M lead to neopterin release; however, neopterin is significantly higher upon coculturing of PBL with HIV-infected M/M (Table 2). Differences of biopterin levels were not so pronounced in cocultures of PBL with either uninfected or HIV-infected M/M; the high background level of biopterin is due to the biopterin in the fetal calf serum used in the culture medium.

IFN induction by HIV-infected M/M through cell-cell interaction with PBL offer an explanation for detectable levels of IFNs found in sera of HIV-infected patients. IFNs activate GTP-cyclohydrolase leading to neopterin release, whereas biopterin is only slightly elevated.

References

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Table 1. Time course of neopterin release (N; pmol/ml) and interferon activity (IFN; U/ml) induced by cell-cell interaction of HIV-infected M/M with PBL. Viral replication is given as p24 (ng/ml), determined immediately before cocultivation of M/M ($\cdot 10^5$ /ml) with PBL ($1 \cdot 10^6$ /ml). 90% of the medium was exchanged after 48 h.

Experiment	1		2		3	
	N	IFN	N	IFN	N	IFN
24 h	6	174	27	437	13	937
48 h	30	103	52	312	130	812
72 h	13	32	36	26	69	65
96 h	81	—	240	—	190	—
p24 (ng/ml)	2.1		4.0		3.4	
M/M ($\cdot 10^5$ /ml)	2.0		1.4		1.4	

Table 2. Neopterin and biopterin (N, B; pmol/ml) release after cocultivation of uninfected or HIV-infected M/M with lymphocytes (PBL). Uninfected and HIV-infected M/M were from the same donor in each experiment.

Experiment	1		2		3	
	N	B	N	B	N	B
Uninfected M/M						
24 h	5	25	5	25	4	27
48 h	16	28	21	29	7	26
72 h	21	30	31	31	15	26
96 h	30	33	50	35	32	29
HIV-infected M/M						
24 h	7	28	7	25	5	26
48 h	32	29	29	32	18	28
72 h	45	35	39	33	36	30
96 h	86	51	75	38	94	39