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Interferon-gamma (IFN-γ) regulates production of IL-10 and IL-12 in human herpesvirus-6 (HHV-6)-infected monocyte/macrophage lineage

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SUMMARY

To determine whether HHV-6 infection induces expression and production of IL-10 and IL-12 in monocytes/macrophages, and to explore the influence of IFN-γ on cytokine production in HHV-6-infected cells, expression and production of IL-10 and IL-12 were evaluated through reverse transcription-polymerase chain reaction (RT-PCR) and sandwich ELISA. HHV-6 infection induced the expression and the production of IL-10 and IL-12 in monocytes and THP-1 cells. Kinetic study showed that the expression of IL-12 mRNA decreased with accumulation of IL-10 mRNA. Expression and production of IL-12 were markedly increased when anti-human IL-10 MoAbs were added to the cultures, implying that endogenous IL-10 induced by HHV-6 inhibited IL-12 production. Addition of increasing concentrations of IFN-γ to the cultures of HHV-6-infected cells enhanced the expression of IL-12 gene, while the accumulation of IL-10 mRNA was down-regulated. Determination of protein levels of IL-10 and IL-12 by ELISA also showed that IFN-γ increased IL-12 and decreased IL-10 production. These results suggest that IFN-γ regulates the production of IL-10 and IL-12 at transcriptional level mainly through inhibiting endogenous IL-10 production in HHV-6-infected monocyte/macrophage lineage.

Keywords human herpesvirus-6 interferon-gamma IL-10 IL-12

INTRODUCTION

HHV-6 is a new identified herpesvirus originally isolated from patients with AIDS and lymphoproliferative disorder [1]. The established clinical manifestation of primary HHV-6 infection is exanthem subitum in young children [2]. Other potential disease associations have been inferred from higher HHV-6 seroprevalence and antibody titres in patients with a variety of diseases, such as systemic lupus erythematosus (SLE), Hodgkin's disease, AIDS, chronic fatigue syndrome (CFS) and Kawasaki disease [1–3]. The tropism and infectivity of HHV-6 for the monocyte/macrophage lineage have been demonstrated [4], suggesting that HHV-6 infection may have a direct or indirect effect on the immune response. IL-12 and IL-10 produced by monocytes/macrophages play an important role in the regulation of the Th1/Th2 response [5,6]. This study shows that HHV-6 induced IL-12 and IL-10 production, and endogenous IL-10 inhibited IL-12 mRNA expression and protein production in HHV-6-infected THP-1 cells and monocytes. Our results also show that IFN-γ could regulate IL-12 and IL-10 production at transcriptional level in the HHV-6-infected monocyte/macrophage lineage.

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MATERIALS AND METHODS

Cell lines and monocytes

The human monocyte cell line THP-1 and the human T cell line HSB-2 (obtained from the American Type Culture Collection, Rockville, MD) were grown (37°C, 5% CO_2 in air) in RPMI 1640 supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin. Cells were passed serially every 4 days by suspending at a concentration of 2×10^5 cells/ml in fresh media. Human peripheral blood mononuclear cells (PBMC) were separated on Ficoll–Hypaque density gradients. Monocytes were obtained after adherence of PBMC (1×10^7/ml) to Petri dishes (1 h, 37°C). These preparations contained > 95% $CD14^+$ cells.

Virus and cell infection

The GS strain of HHV-6 (gift of Dr D. Carrigan, Medical College of Wisconsin, Milwaukee, WI) was propagated in HSB-2 cells. Infected cells were collected at the peak of cytopathic effect (day 8–10 post-infection), and 10^7 cells were suspended in 1 ml of medium and frozen and thawed twice. Cell debris was removed by centrifugation and supernatant fluid was used as virus stock. Infectivity titres were measured as previously described [7]. The virus preparation had a titre of 10^4 50% tissue culture infective

Table 1. Primer sequences and polymerase chain reaction (PCR) condition

Cytokine			Denaturation/time	Annealing/time	Extension/time	Cycles
IL-12 (p40)	sense	5′ CCACATTCCTACTTCTC 3′				
	antisense	5′ GTCTATTCCGTTGTGTC 3′	94°C/45 s	55°C/45 s	72°C/45 s	35
IL-10	sense	5′ ATGCCCCAAGCTGAGAACCAAGACCCA 3′				
	antisense	5′ TCTCAAGGGGCTGGGTCAGCTACCCA 3′	94°C/1 min	60°C/1 min	72°C/1·5 min	35
β-actin	sense	5′ TGACGGGGTCACCCACACTGTGCCCATCTA 3′				
	antisense	5′ CTAGAAGCATTTGCGGTGGACGATGGAGGG 3′	94°C/1 min	65°C/1 min	72°C/1·5 min	35

All PCR reactions listed above were extended 10 min at 72°C following 35 cycles.

doses ($TCID_{50}$)/ml. Mock-infected HSB-2 cells were prepared and handled identically to the virus stock preparation and were used as cell control. All virus preparations and cells were found to be negative for mycoplasm (Gen-Probe, San Diego, CA) and endotoxins (limulus amebocyte assay; Sigma, St Louis, MO).

THP-1 cells and monocytes (1×10^6/ml) were infected by HHV-6 at a multiplicity of infection of 5×10^3 $TCID_{50}/10^6$ cells. Cells were harvested for isolating total RNA at 6 h, 12 h, 24 h following the infection and supernatants were collected for measuring of cytokines at 48 h after HHV-6 infection. In some experiments, cells were infected by HHV-6 in the presence of recombinant human IL-10 (rhIL-10; R&D Systems, Minneapolis, MN), rhIFN-γ (Sigma) and neutralizing anti-human IL-10 MoAb (Clone BS; Biosource International, Camarillo, CA).

IL-10 and IL-12 determination

A sandwich ELISA was used to determine the levels of IL-10 and IL-12 in the HHV-6-infected cell supernatants according to the manufacturer's technical guidelines. ELISA kits used were purchased from Genzyme (Boston, MA) and R&D Systems.

Reverse transcription-polymerase chain reaction

Total RNA was isolated by RNAzol B solution (Biotex Labs, Houston, TX) according to the manufacturer's instructions. For the reverse transcription (RT) reaction, 1 μg of total RNA was incubated at 65°C for 5 min and then reverse transcribed in a total volume of 20 μl, using 4 μl of 5×RT buffer (GIBCO/BRL, Grand Island, NY), 1 μl of $Oligo(dT)_{15}$ (100 pM/μl; Boehringer Mannheim, Indianapolis, IN), 10 mM of each dNTP (Boehringer Mannheim), 20 U of rRNasin (Promega, Madison, WI), 200 U of MMLV reverse transcriptase (GIBCO/BRL), 0·5 μl of 0·1 M dithioltreitol, at 37°C for 60 min, followed by 95°C for 5 min to stop cDNA synthesis.

Two microlitres of the reverse transcript reaction were used for each amplification, and the conditions for 50 μl polymerase chain reaction (PCR) were as follows: 5 μl of 10×PCR buffer (500 mM KCl, 100 mM Tris–HCl pH 8·3, 15 mM MgCl and 0·1% gelatin), 8 μl of 1·25 mM dNTP, and 25 pM of each primer, 1·5 U of Taq polymerase (Perkin Elmer Cetus, Foster City, CA) and 32 μl of DEPC-treated water. Trace amounts (5 μCi) of α-^{32}P-dATP were added. Primers used for the amplification of cytokine and β-actin genes were synthesized on an applied Biosystems DNA synthesizer according to published sources (Table 1) [8,9]. Since the p35 subunit of IL-12 was found to be constitutively expressed in THP-1 cells and monocytes in our prior study and some reports [5,10], we only examined expression of IL-12 p40 subunit mRNA. Amplification was carried out in a Perkin Elmer thermocycler and PCR conditions were empirically selected so that the reaction products of individual samples could be compared in a semiquantitative manner (Table 1). The PCR products were analysed by electrophoresis on 5% polyacrylamide gel and autoradiographed using autoradiography film. For quantitative PCR, the amount of each band was determined by densitometry (BioRad, Hercules, CA), and cytokine PCR products were normalized according to the amount of β-actin detected in the same mRNA. The data are presented as the ratio of cytokine:β-actin mRNA.

Statistical analysis

Student's *t*-test for paired data was used and a P value $<0{\cdot}05$ was considered significant.

RESULTS

HHV-6 infection induced IL-10 and IL-12 expression and production

Figure 1 shows the time course for the accumulation of the IL-10 and IL-12 mRNA in HHV-6-infected THP-1 and monocytes for various periods of times. The induction of IL-12 mRNA by HHV-6 was apparent at 6 h and then rapidly decreased. IL-10 mRNA expression was first seen weakly at 6 h and reached a peak at 12 h following infection. HHV-6 appeared to induce more accumulation of IL-10 mRNA than that of IL-12. IL-10 and IL-12 proteins could be detected by ELISA in the cultured supernatants of HHV-6-infected cells, compared with very low levels of cytokines in HSB-2 inocula infected cell control (Table 2), indicating that IL-10 and IL-12 are products induced by HHV-6, and not non-specific products induced by HSB-2 cell metabolites.

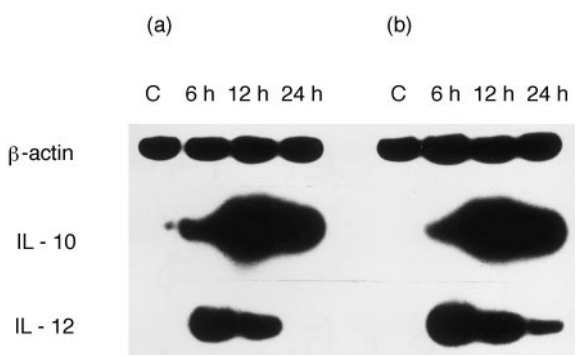

Fig. 1. The kinetics of IL-10 and IL-12 mRNA expression in HHV-6-infected THP-1 cells (a) and monocytes (b). C, HSB-2 cell controls, in which THP-1 cells and monocytes were incubated with HSB-2 inocula for 12 h.

Table 2. Effects of endogenous IL-10, IFN-γ on cytokine production in HHV-6-infected THP-1 cells and monocytes (pg/ml, ± s.d.)

Cytokine	HSB-2 cell control	HHV-6	HHV-6 + anti-IL-10 MoAb	HHV-6 + IFN-γ
THP-1 cells				
IL-10	168 ± 64	4156 ± 477	–	1970 ± 254**
IL-12	< 10	332 ± 53	908 ± 114*	564 ± 87**
Monocytes				
IL-10	184 ± 42	4968 ± 605	–	2066 ± 396*
IL-12	< 10	490 ± 131	1648 ± 359*	1028 ± 149*

Cells were infected by HHV-6 for 48 h and production of cytokines was determined in the supernatants by sandwich ELISA. Compared with HHV-6 infection alone, production of IL-10 in the presence of IFN-γ (100 U/ml) was significantly decreased (*$P < 0{\cdot}001$) and production of IL-12 in the presence of anti-IL-10 MoAb (10 μg/ml) of IFN-γ was markedly enhanced (*$P < 0{\cdot}001$ or **$P < 0{\cdot}005$). THP-1 cells: data represent the average from five independent experiments; monocytes: number of donors = 5.

Endogenous IL-10 induced by HHV-6 inhibited IL-12 expression and production

It has been reported that IL-10 inhibits IL-12 production in PBMC activated with *Staphylococcus aureus* Cowan strain 1 (SAC) or lipopolysaccharide (LPS) [10]. We further extended the experiment to determine whether endogenous IL-10 inhibited IL-12 expression and production in HHV-6-infected monocyte/macrophage lineage. The interference of exogenous IL-10 with accumulation of IL-12 mRNA was first observed. When HHV-6 infection was performed in the presence of rhIL-10 (20 ng/ml), expression of IL-12 mRNA was significantly inhibited and transcripts of IL-12 were almost abrogated (Fig. 2). THP-1 cells and monocytes were infected by the virus in the presence or absence of neutralizing anti-human IL-10 MoAb (10 μg/ml) for 12 h and mRNA expression was analysed by RT-PCR. As shown in Fig. 2, expression of IL-12 mRNA (IL-12:β-actin) was enhanced from $0{\cdot}44 \pm 0{\cdot}12$, $0{\cdot}84 \pm 0{\cdot}25$ to $1{\cdot}36 + 0{\cdot}28$ ($P < 0{\cdot}001$), $3{\cdot}21 + 0{\cdot}74$ ($P < 0{\cdot}001$) in THP-1 cells and monocytes, respectively. Determination of cytokines also showed that levels of IL-12 protein were significantly increased in the presence of anti-human IL-10 MoAb (Table 2).

IFN-γ-regulated IL-10 and IL-12 production

THP-1 cells and monocytes were infected by HHV-6 for 12 h in the absence or presence of different concentrations of IFN-γ (1, 10, 100, 1000 U/ml). The transcription of IL-12 and IL-10 was measured by quantitative RT-PCR. The results show that IL-12 mRNA expression was enhanced with increasing concentrations of IFN-γ, while accumulation of IL-10 mRNA was down-regulated by IFN-γ (Fig. 3). When IFN-γ (100 U/ml) was added to the cultures of HHV-6 infection, IL-12 and IL-10 in the supernatants were remarkably increased and decreased, respectively (Table 2). In order to exclude the possibility that increased IL-12 mRNA was induced by IFN-γ, THP-1 cells and monocytes were mock-infected and cultured with the same concentration of IFN-γ used in the previous experiment for 6 h and 12 h. No expression and production of IL-12 were induced by IFN-γ alone (data not shown).

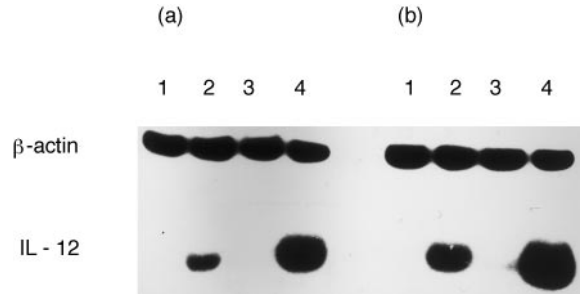

Fig. 2. The effects of endogenously produced IL-10 and exogenous IL-10 on expression of IL-12 mRNA in HHV-6-infected THP-1 cells (a) and monocytes (b). Cells were infected by HHV-6 for 12 h in absence (2) or presence of rhIL-10 (3), and anti-human IL-10 MoAb (4). 1, HSB-2 cell controls.

DISCUSSION

IL-10 and IL-12 are pleiotropic cytokines produced by monocytes/macrophages, T lymphocytes and B lymphocytes. Numerous studies have shown that IL-10 and IL-12 can be induced by bacteria, bacterial products and intracellular parasites [5,6], but little is known as to whether IL-10 and IL-12 could be induced by virus. It has recently been reported that IL-10 is induced by HIV infection in the human monocyte/macrophage lineage [11], and IL-12 mRNA expression was barely detected early in respiratory syncytial virus-infected neonatal macrophages [12]. The present study shows that HHV-6 infection *in vitro* induced IL-12 (p40) and IL-10 mRNA expression and protein production. Kinetic studies showed that IL-12 mRNA occurred relatively early and rapidly decreased with increasing IL-10 mRNA accumulation, suggesting that IL-12 gene expression in HHV-6 infection might be interfered with by endogenous IL-10. Through the use of anti-human IL-10 MoAb to block endogenous IL-10, levels of IL-12 mRNA expression and protein production were significantly up-regulated. Thus, one of the main conclusions from the present study is that endogenous IL-10 can down-regulate IL-12 production in HHV-6-infected cells. There seems to exist a complex regulatory network in HHV-6-infected monocytes/macrophages. In one aspect, the virus triggers a host cell-mediated immune response against HHV-6 by induction of IL-12 secretion, while on the other hand the virus suppresses the response through stimulation of IL-10 production. However, the description presented above might be over-simplified. In this study, cytokine profiles following virus infection were evaluated through determination of cytokine

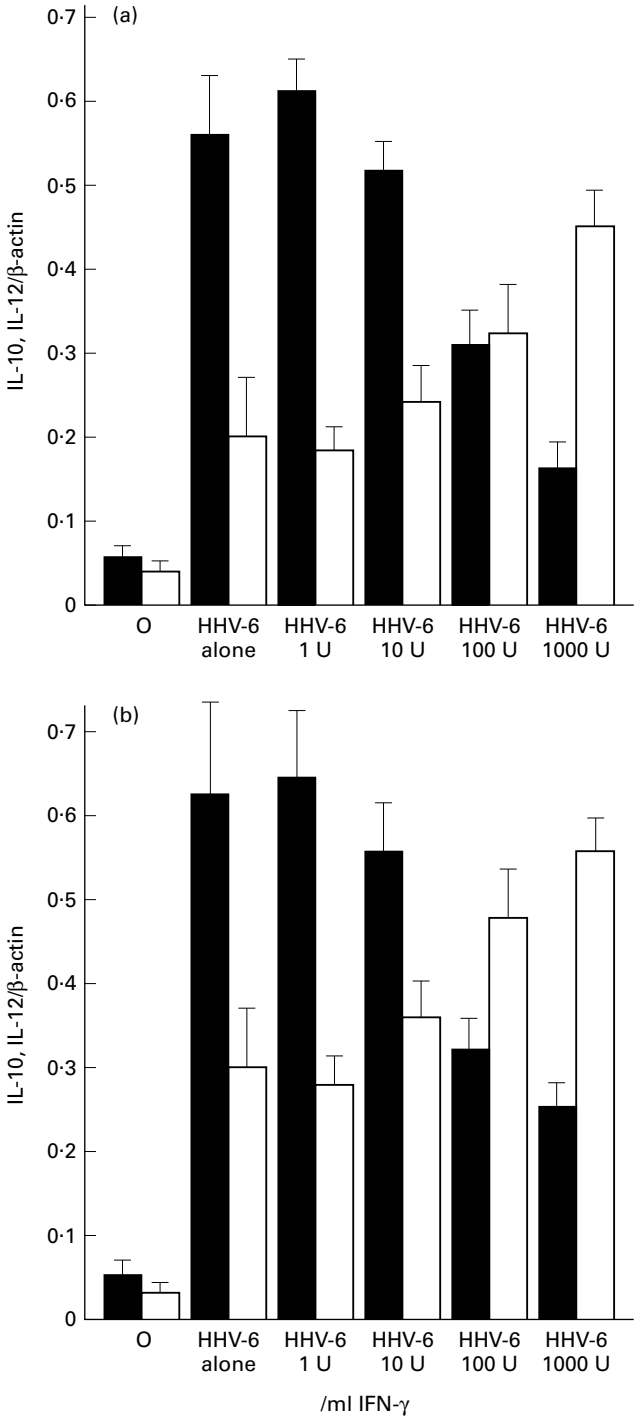

Fig. 3. The influence of IFN-γ on expression of IL-10 (■) and IL-12 (□) mRNA in HHV-6-infected THP-1 cells (a) and monocytes (b). Cells were infected by HHV-6 for 12 h in the presence of 1, 10, 100, 1000 U/ml of IFN-γ. ○, HSB-2 controls. Cytokine mRNA levels are expressed as the ratio of cytokine mRNA/β-actin mRNA in the same sample (± s.d.; THP-1 cells: data represent the average of five independent experiments; monocytes: number of donors = 5).

mRNA expression and relevant proteins, but not by testing for biologically active substances. More detailed studies will be necessary to define completely the mechanism of HHV-6-mediated cytokine production.

IL-12 is a critical cytokine for the generation of the Th1-dominant immune response, due to its capacity to induce IFN-γ [5]. IL-10 inhibits IL-12 synthesis in accessory cells and down-regulates the Th1 response [6,10]. Thus, levels of IL-12 and IL-10 produced by monocytes/macrophages in the initial response to potential pathogens can ultimately determine the nature of the subsequent immune response. Regulation of IL-12 production has been the subject of intensive research. It has been reported that IFN-γ inhibits IL-10 production by monocytes [13]. In this study, an experiment was done to determine whether IFN-γ modulates IL-12 production by inhibition of endogenous IL-10 production in HHV-6-infected THP-1 cells and monocytes. The results show that IL-12 and IL-10 mRNA expression was up-regulated and down-regulated, respectively, with increasing concentrations of IFN-γ. Increase and decrease between them were proportional. Determination of cytokine levels also showed that IFN-γ suppressed IL-10 production. Thus, the data described above indicate that IFN-γ enhances IL-12 production mainly through inhibition of endogenous IL-10 at transcriptional level in HHV-6-infected monocytes/macrophages. In addition, it has been recently reported that IFN-γ can enhance, though not induce, LPS-induced IL-12 expression and production [14,15]. Our experimental system could not completely exclude the possibility that IFN-γ might exert partial effects in facilitating IL-12 production. No matter what mechanism might be involved, the ultimate effect of IFN-γ regulation is to control the relative balance of IL-10 and IL-12 production and to maintain normal Th1/Th2 responses in HHV-6 infection. Abnormal Th1/Th2 responses are involved in the pathogenesis of immunological disorders, and predominant Th2 activation has been assumed to be associated with atopic disorders, SLE and progression to AIDS in HIV infection [16]. The potential disease association of HHV-6 infection with SLE, Hodgkin's disease, Kawasaki disease, CFS and AIDS has been inferred, although the mechanisms involved are not very clear. This study provides a preliminary theoretical basis for the hypothesis. Further investigation is required to elucidate the complex network of cytokine production and to understand the effects of such immunoregulators as IFN-γ on cytokine production, which might be helpful for seeking a therapeutic clue to the diseases associated with HHV-6 infection.

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