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Induction of Interferon-α by Glycoprotein D of Herpes Simplex Virus: A Possible Role of Chemokine Receptors

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The induction of type I interferons by most RNA viruses is initiated by virus-derived double-stranded (ds)RNA. However, retro- and DNA-viruses, which do not synthesize dsRNA, must rely on different mechanisms of induction. For human immunodeficiency virus type 1 (HIV-1), recombinant glycoproteins 120 or 160 suffice to induce interferon (IFN)-α in blood-derived lymphocytes [H. Ankel, M. R. Capobianchi, C. Castilletti, and F. Dianzani (1994). *Virology* 205, 34–43]. Here we show that for herpes simplex virus type 1 (HSV-1) recombinant glycoprotein, gD is the major inducer, whereas gB, gC, gE, gG, gI, and the complex of gH and gL are poor inducers. The recombinant extramembrane fragment of gD was sufficient to induce IFN-α levels comparable to that of intact virus. Like with HIV-1, induction was inhibited by a monoclonal antibody that recognizes cerebrosides and sulfatides. Furthermore, monoclonal antibodies specific for the chemokine receptors CCR3 and CXCR4 also blocked induction. We conclude that HSV-1 induces IFN-α by interaction of its glycoprotein gD with appropriate receptors on IFN-producing cells. Based on the known receptor roles of galactosyl cerebrosides and chemokine receptors in HIV infection, such structures on IFN-producing cells could also participate in the induction of IFN-α by HSV-1.

INTRODUCTION

Interferons (IFNs) are important mediators of the early host defense against many viral infections, and most viruses induce IFNs in infected individuals and in cells in culture. IFN-producing cells are contained in freshly isolated PBMCs from health donors (Fitzgerald-Bocarsly, 1993). When challenged with viruses such as herpes simplex virus (HSV) or human immunodeficiency virus (HIV), these cells produce IFNs of the α type (IFN-α), representing a family of homologous polypeptides (Ferbas *et al.,* 1994; Fitzgerald-Bocarsly, 1993; Svensson *et al.,* 1996). Analogous results are seen using UV-inactivated virus as inducer, indicating that replication is not required for IFN-α induction. In fact, virus-infected cells that express viral envelope components on their surface are excellent inducers, even when cross-linked by glutaraldehyde, which renders endogenous virus noninfectious (Ankel *et al.,* 1996; Capobianchi, 1996; Lebon, 1985). Previous studies have shown that antibodies against the major envelope glycoprotein of HIV, gp120, blocked IFN-α induction in PBMCs not only by the virus but also by cross-linked HIV-1-infected cells. Furthermore, the pretreatment of PBMCs with antibodies to the CD4 receptor of HIV-1 suppressed induction of IFN-α by the virus as well as by HIV-1-infected cells (Francis and Meltzer, 1993). A direct role of gp120 in induction was shown by the fact that a recombinant preparation of the glycoprotein induces IFN-α in the absence of any other viral component (Ankel *et al.,* 1994; Capobianchi *et al.,* 1992). Thus for the retrovirus HIV-1, these data provide evidence for an induction mechanism that is initiated by interaction of its viral envelope glycoprotein with the cell surface antigen CD4. Furthermore, antagonistic effects of exogenous sulfatides or cerebroside/sulfatide specific antibodies suggest an additional role for these glycolipids during induction (Ankel *et al.,* 1996). The involvement of other peptide sequences of gp120 in addition to its CD4 binding site is suggested by inhibitory effects of monoclonal antibodies to overlapping epitopes on its V3 loop (Ankel *et al.,* 1994). Indeed, this domain is implicated in interaction of HIV-1 with glycolipids and with chemokine receptors, recently discovered coreceptors of HIV infection (Alizon, 1997; Baggiolini, 1998; Cook *et al.,* 1994; D'Souza and Harden, 1996; Wu *et al.,* 1997). An interferonogenic glycoprotein has also been described for transmissible gastroenteritis virus (TGEV) (Laude *et al.,* 1992).

In this communication, we investigated whether the enveloped DNA virus HSV-1 relies on a similar mechanism of IFN-α induction. HSV-1 expresses $\geq$11 glycoproteins, of which 8 are known constituents of the viral envelope. Of the latter group, glycoproteins gB, gC, and

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gD and a heterodimeric complex of gH and gL are directly involved in virus attachment and/or entry into susceptible cells (Cai *et al.,* 1988; Forrester *et al.,* 1992; Herold *et al*., 1991; Hutchinson *et al.,* 1992; Johnson and Ligas, 1988; Peng *et al.,* 1998; Spear, 1993). Although glycoproteins gE, gG, and gI are also constituents of the viral envelope, their role in HSV-1 infection remains undefined (Spear, 1993). However, they appear to have other important functions in that they have been maintained throughout evolution and structural homologs are present on most other members of the alphaherpesvirus family. Glycoproteins gB and gC are known to bind to heparan sulfate side chains on membrane proteoglycans, and both are important in the initial phase of virus attachment (Kuhn *et al.,* 1990; Trybala *et al.,* 1993). In contrast, limited numbers of specific cell membrane receptors exist for gD, as judged from binding studies using radiolabeled recombinant gD constructs (Johnson *et al.,* 1990). Recently a human cell membrane component related to the TNF receptor family was cloned, which specifically bound recombinant gD. When HSV-1-resistant Chinese hamster ovary cells were transfected to express this component, they became sensitive to infection, suggesting that it can serve as a receptor in HSV-1 infection by attaching to gD (Montgomery *et al.,* 1996; Whitbeck *et al.,* 1997).

Previous studies of the mechanism of IFN-α induction in human PBMCs by HSV-1-infected Vero cells indicated a possible role for glycoprotein gD. When the inducer cells were pretreated with monoclonal antibodies against gB, gC, or gD, only the gD-specific one resulted in pronounced inhibition of induction, suggesting a direct function of this glycoprotein in the induction process (Lebon, 1985). Progress in cloning and expression of individual HSV glycoproteins has provided new tools to more clearly define the role of individual envelope glycoproteins in the induction of IFN-α (Ghiasi *et al.,* 1991, 1992a, 1992b, 1992c, 1992d, 1992e, 1994; Westra *et al.,* 1997). Specifically it allows one to ask whether induction is triggered by one or several envelope glycoproteins, or whether other viral components are also necessary. As described in this communication, we used *Spodoptera frugiperda* insect cells (Sf9) expressing individual HSV-1 glycoproteins on their surface, as potential IFN-α inducers in human PBMCs (Ghiasi *et al.,* 1994). In comparing such glycoprotein vehicles carrying six of the known surface membrane glycoproteins individually, we show that among them gD is the most potent IFN-α inducer. Glycoproteins gB, gC, gE, gG, and gI, when used under identical conditions, showed little induction. A possible exclusive role of gD in induction was confirmed by using a recombinant-soluble version of the extramembranous portion of the polypeptide. This preparation induced IFN-α to levels comparable to those observed with the intact virus or with gD-expressing Sf9 cells. A soluble recombinant gH–gL complex, on the other hand, was inactive, supporting the conclusion that gD is the major IFN-α-inducing component of the viral envelope. Surface components of target cells involved in IFN-α induction were studied by the use of monoclonal antibodies to defined cell membrane antigens. Based on these studies, we propose a scenario of IFN-α induction that is similar for both HSV and HIV and involves galactosyl cerebrosides/sulfatides and the chemokine receptors CCR3 and CXCR4.

RESULTS

IFN-α induction by HSV-derived glycoproteins expressed by insect Sf9 cells

Like intact or UV-inactivated virus, HSV-infected mammalian cells, cross-linked with glutaraldehyde, effectively induce IFN-α in PBMCs (Lebon, 1985). To investigate whether IFN-α induction by HSV-1 is dependent on a specific glycoprotein of the virus, we used insect Sf9 cells that presented individual HSV-1 membrane glycoproteins gB, gC, gD, gE, gG, and gI on their surface. Sf9 cells infected with baculovirus expressing HSV-2-derived glycoprotein D (Sf9-gD2) were also included for comparison. As indicated in Table 1 the various Sf9 cell preparations showed strong fluorescence with their cognate monoclonal antibodies (mAbs) but were negative for the others. They also fluoresced with human and rabbit anti-HSV-1 antisera, as did gI-expressing cells for which no specific antibody was available to us. Surface expression of specific glycoproteins on Sf9 cells was confirmed by immunofluorescence of nonpermeabilized cells and corresponded to previously published results (Ghiasi *et al.,* 1994). As expected, uninfected or wild-type virus-infected control cells lacked immunofluorescence in response to the HSV-specific antibodies.

Freshly isolated human PBMCs were incubated overnight with glutaraldehyde-fixed Sf9 cells presenting individual HSV-derived glycoproteins, at an Sf9/PBMC cellular ratio of 1:30.

Levels of human IFN-α were then determined in the incubation supernatants. Table 2 compares yields of IFN-α that were obtained in repeated experiments using PBMCs from different donors. As seen in the Table, IFN-α induction was strongest with Sf9 cells expressing glycoprotein gD. Sf9 cells containing gD from the KOS strain of HSV-1 yielded the same amounts of IFN-α as the McIntyre strain-derived construct (not shown). Also, Sf9-gD2 cells containing HSV-2-derived glycoprotein were equally potent IFN-α inducers. Clearly, Sf9 cells expressing gB, gC, gE, gG, or gI induced only small amounts of antiviral activity. Even less was seen with wild-type baculovirus-infected cells. The Table gives mean values and standard deviations of IFN-α levels, which demonstrate the variability of the IFN response in different donors. Thus using mixtures of Sf9-gD cells with PBMC preparations from 16 different donors, levels var-

TABLE 1

Immunofluorescence with Indicated Antibodies

SF9 Cells expressing	% Fluorescent cells[a]							
	HSV-1[b]		mAB to baculo gp67	mABs to HSV-1 glycoproteins				
	H	R		gB	gC	gD	gE	gG
gB (1[c])	3+	2+	3+	4+	—	—	—	—
gC (1)	3+	3+	3+	—	4+	—	—	—
gD1 (5)	3+	3+	3+	—	—	4+	—	—
gD2 (3)	3+	3+	2+	—	—	3+	—	—
gE (1)	2+	3+	4+	—	—	—	3+	—
gG (4)	2+	2+	3+	—	—	—	—	3+
gI (2)	2+	3+	3+	—	—	—	—	—
WT[d] (3)	—	—	2+	—	—	—	—	—
Uninfected	—	—	—	—	—	—	—	—

[a] t4+: 75–100%; 3+: 50–75%; 2+: 25–50%; —: background fluorescence.
[b] H, human antiserum; R, rabbit antiserum.
[c] Number of SF9-cell isolates for which complete antibody panel was done.
[d] SF9 cells infected with wild-type baculovirus.

ied beween 400 and 3200 IU/ml. In the associated column, yields of IFN-α obtained with different glycoprotein constructs are expressed as percent of those obtained with Sf9-gD cells in parallel experiments using the same PBMC preparations and represent ~≤5%.

Figure 1 compares representative experiments of the dose response of IFN-α induction by HSV-1 (top) with that by Sf9-gD (bottom). Half-maximal induction occurred at an Sf9-gD/PBMC ratio of ~1:100 and at a virus/PBMC ratio of ~1:2000, indicating greater inducing efficiency of the viral particles. That the antiviral activity produced in

TABLE 2

IFN-α Induction by SF9 Cells Expressing Individual HSV Glycoproteins

Glycoproteins expressed	Number of donors	IFN-α	
		IU/ml[a]	% of gD induced[b]
gB	6	84 ± 41	6 6
gC	3	53 ± 49	5 ± 1
gD1	16	1625 ± 934	100
gD2	4	1879 ± 1033	91 ± 44
gE	4	47 ± 49	5 ± 2
gG	3	24 ± 20	2 ± 1
gI	3	52 ± 7	3 ± 3
Control Wt[c]			
Infected cells	11	6 ± 19	1 ± 2
Control			
Uninfected cells	4	<1	<1

[a] Mean ± standard deviation of IFN-α induced.
[b] Mean ± standard deviation relative to SF9-gD induced amount in the same PBMC isolate.
[c] Wild-type baculovirus.

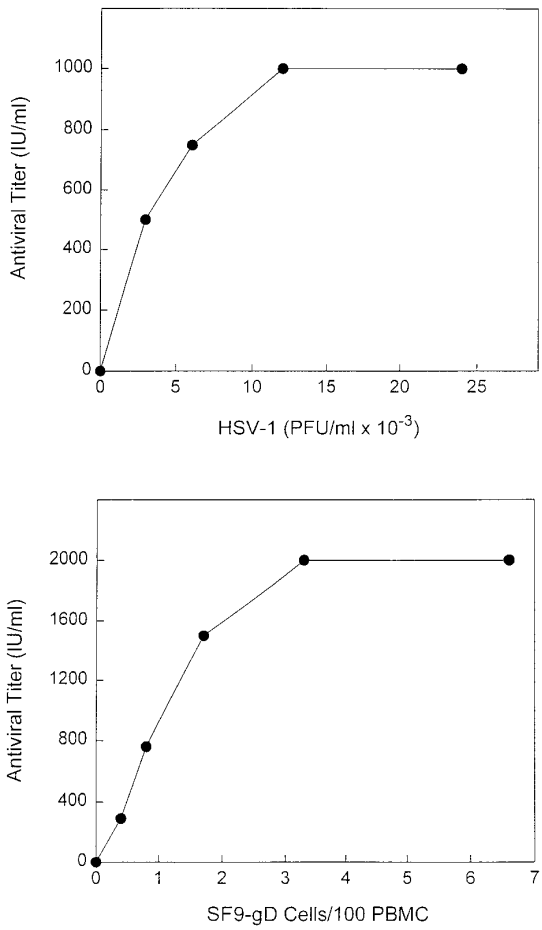

FIG. 1 Induction of IFN-α by HSV-1 or Sf9 cells expressing gD (Sf9-gD). PBMCs were induced with indicated amounts of HSV-1 (top) or Sf9-gD (bottom), and antiviral titers in culture supernatants were determined as described under Materials and Methods. Representative examples from a total of 5 (top) and 13 (bottom) experiments are shown.

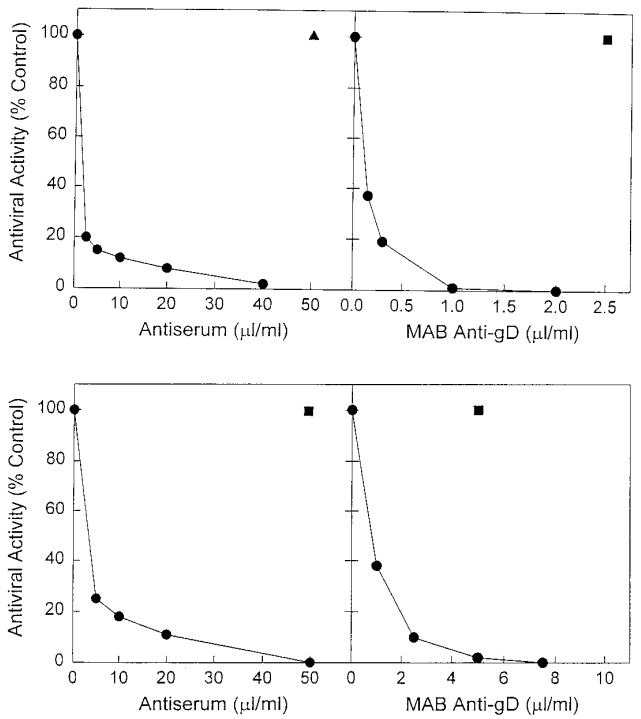

FIG. 2. Inhibition of HSV-1 or Sf9-gD dependent IFN-α induction by anti-HSV-1 human serum or mAb anti-gD. HSV-1 (top) or Sf9-gD (bottom) was preincubated either with indicated amounts of human HSV-1-positive serum (left) or gD-specific mAb LP2 (right). HSV-1 concentrations were 10^4 PFU/ml (left) and 10^5 PFU/ml (right). Sf9-gD concentrations were 2×10^4 cells/ml (left) and 5×10^4 cells/ml (right). PBMCs were then added, and incubations continued for 18 h, followed by determinations of antiviral titers as described under Materials and Methods. Control titers were 500 IU/ml (top left), 4000 IU/ml (top right), 100 IU/ml (bottom left), and 4000 IU/ml (bottom right). Squares represent control experiments using VSV (top) or Sendai virus (bottom) as inducers in the presence of indicated amounts of antibody. The triangle shows lack of inhibition by an HSV-1-negative control serum. Representative examples of a total of 7 (top left), 10 (top right), 4 (bottom left), and 3 (bottom right) experiments are shown.

response to Sf9-gD is of the α type was concluded from its antiviral effect on MDBK cells, which respond poorly to human IFNs β or γ (Gresser *et al*., 1974). This was confirmed by complete (>99%) neutralization by IFN-α-specific antiserum. The same neutralization was seen with Sf9-gD2-and HSV-1-induced antiviral activities.

Effects of antibodies to HSV glycoproteins

We further confirmed the role of gD in the induction of IFN-α by comparing the effects of antisera and monoclonal antibodies on inducing activities of intact HSV-1 and Sf9-gD cells. As shown in Fig. 2 (left), decomplemented serum from an HSV-1-positive patient progressively suppressed induction by intact HSV-1, whereas serum from an HSV-1 negative control did not. The patient serum also blocked induction by Sf9-gD. Similar results were seen with two additional HSV-1 positive sera (not shown). Furthermore, the virus-neutralizing gD-specific mAb LP2 blocked IFN-α induction of both the virus and the gD-expressing Sf9 cells in a similar manner (Fig. 2, right). LP2, which neutralizes both HSV-1 and HSV-2, also blocked IFN-α induction by gD2-expressing Sf9 cells (not shown). Another neutralizing gD-specific mAb (4S) that blocked induction by HSV-1-infected Vero cells (Lebon, 1985) was also inhibitory to induction by HSV-1 and by Sf9-gD (not shown). On the other hand, the non-neutralizing mAbs H2B4B1, B1C1B4, E5G3G9, and B1E6A5 recognizing gB, gC, gD, and gE, respectively, had no effects on IFN-α induction by HSV-1. All antisera and mAbs tested in these experiments had no effects on IFN-α induction by vesicular stomatitis virus (VSV) or Sendai virus when used under comparable conditions.

Effects of antibodies to cell membrane constituents

In previous work, HIV-1-dependent IFN-α induction was found to be blocked by preincubation of PBMCs with an mAb that recognizes the galactosyl-sphingosine moieties of galactosyl cerebrosides and sulfatides (mAb GalC; Ankel *et al.,* 1996). We tested the effects of the same antibody on IFN induction by HSV-1. As can be seen in Fig. 3, similar inhibitory effects on IFN-α induction occurred with either intact HSV-1 (left) or Sf9-gD cells (right) as inducers. IFN-α induction by intact VSV was not influenced by the presence of mAb GalC, indicating that the results are not due to nonspecific and/or toxic effects. The same lack of inhibition by mAb GalC was seen when a poliovirus–immunoglobulin complex was used as inducer. In this case, induction occurs via interaction with the CD32 receptor on the responder cells (P. Lebon, unpublished observation).

Chemokines represent an extended family of che-

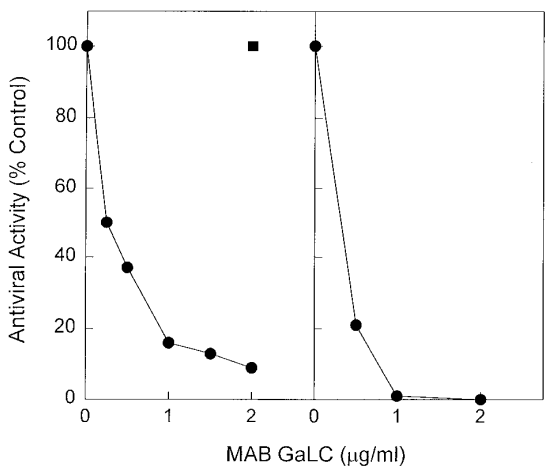

FIG. 3. Inhibition of HSV-1- or Sf9-gD-dependent IFN-α induction by galactosyl cerebroside/sulfatide-specific mAb GalC. PBMCs were preincubated with indicated amounts of the mAb, followed by the addition of HSV-1 (10^4 PFU/ml; left) or Sf9-gD (10^5 cells/ml; right). Control IFN-α levels were 100 IU/ml (left) and 1500 IU/ml (right). Lack of inhibition of VSV-dependent induction is indicated by the square. Representative examples of four (left) and three (right) experiments are shown.

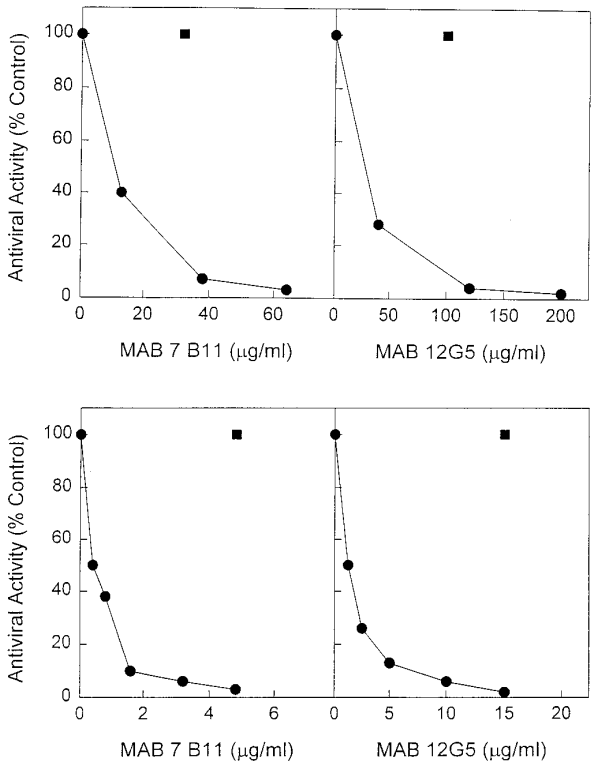

FIG. 4. Effects of mAbs specific for chemokine receptors CCR3 or CXCR4 on IFN-α induction by HSV-1 or Sf9-gD. PBMC were preincubated with indicated amounts of mAbs 7B11 (CCR3 specific) or 12G5 (CXCR4 specific) before the addition of the inducers HSV-1 (10^4 PFU/ml; top) or Sf9-gD (2×10^5 cells/ml; bottom). Control IFN-α titers were 600 IU/ml (top) and 2700 IU/ml (bottom). Squares indicate lack of inhibition when VSV was used as the inducer. Representative examples of two (top left), five (top right), two (bottom left), and five (bottom right) experiments are shown.

moattractants that direct blood leukocytes to sites of infection and inflammation. They also act as regulatory molecules of lymphocyte migration and adhesion and have other regulatory functions related to the development of leukocytes and lymphoid tissue (Baggliolini, 1998). Recent work has established that several of the known chemokine receptors, including CCR3, CCR5, and CXCR4, can also serve as coreceptors for infection by HIV-1 and related retroviruses (Alizon, 1997; D'Souza and Harden, 1996). To investigate the role of these receptors in HSV-1-dependent IFN-α induction, we studied the effects of chemokine-specific mAbs that block infection by HIV-1. As shown in Fig. 4, pretreatment of PBMC with either mAb 7B11 (CCR3 specific) or mAb 12G5 (CXCR4 specific) suppressed IFN-α induction by either the intact virus (top) or Sf9-gD cells (bottom). However, with both antibodies, much higher concentrations were required for inhibition of the viral inducer compared with the Sf9-gD cell-associated one. This could be due to tighter binding of the virus to both receptors compared with gD-expressing cells, requiring higher antibody concentrations to compete off the virus. Both antibodies were without effects on IFN induction by VSV, suggesting different modes of induction and excluding cytotoxic effects of the antibody preparations.

IFN-α induction by soluble recombinant gD1

To further substantiate that gD is, indeed, capable of inducing IFN-α, it was desirable to show induction by a soluble version of the glycoprotein in the absence of other viral components. We used a recombinant truncated form of gD representing its extramembraneous portion, which was isolated from the culture supernatant of *Spodoptera frugiperda* cell cultures after infection with a baculovirus construct expressing the N-terminal 315 residues of the mature glycoprotein (gDt). As shown in Fig. 5, IFN-α was dose-dependently induced, and half-maximal induction was estimated to occur at a glycoprotein concentration of ~0.3 μM. Maximal amounts of antiviral activity were similar to those obtained with intact virus or Sf9-gD cells and occurred at inducer concentrations from ~0.8 μM on. Because the heterodimer complex of gH and gL is necessary for cell penetration of HSV-1, we also investigated the IFN-α-inducing potential of a recombinant soluble version of this complex that was truncated at the amino end of the transmembrane sequence of gH (gHt/gL; Westra *et al.,* 1997). As indicated in Fig. 5, gHt/gL did not induce an antiviral response at doses close to those for half-maximal induction by gDt. To further corroborate this result, we used a gH/gL-specific rabbit antiserum (5R127) that had been previously found to block virus entry at a 1000-fold dilution (Peng *et al.,* 1998). In experiments essentially identical to those described for Fig. 2, we observed no inhibition of HSV-1-dependent induction up to a 1:50 dilution

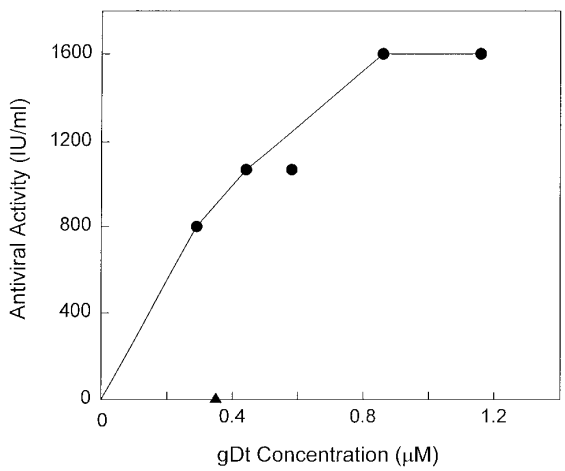

FIG. 5. IFN-α induction by soluble recombinant glycoprotein gDt. PBMCs were incubated with indicated amounts of gDt, and antiviral titers were determined essentially as described under Materials and Methods. The triangle shows lack of IFN-α induction by a soluble recombinant gHt/gL heterodimer at the indicated micromolar concentration. A representative example of five experiments is shown.

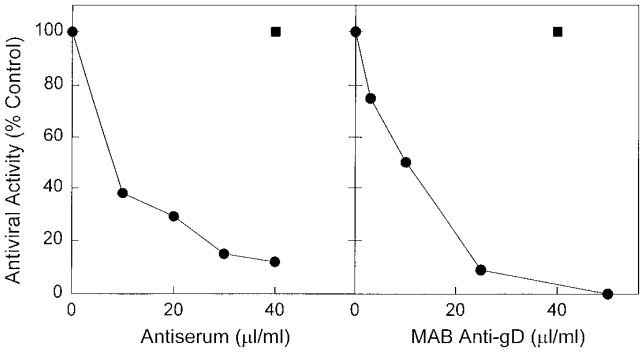

FIG. 6. Inhibition of gDt-dependent IFN-α induction by anti-HSV-1 serum or mAb anti-gD. Glycoprotein (0.3 μM) was preincubated with indicated amounts of human HSV-1-positive serum (left) or gD-specific mAb LP2 (right). PBMCs were then added, and antiviral titers were determined after an additional 18 h of incubation. Control titers of IFN-α were 250 IU/ml (left) and 100 IU/ml (right). The squares represent control experiments using VSV as inducer. In each case, a representative example of a total of four experiments is shown.

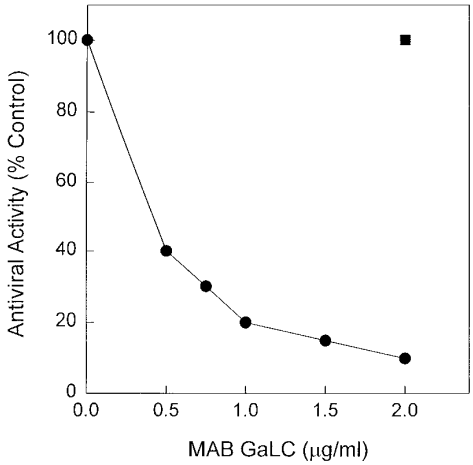

FIG. 7. Effects of the galactosyl cerebroside/sulfatide-specific mAb GalC on IFN-α induction by gDt. The experiment was carried out essentially as described under Materials and Methods, using 0.3 μM gDt as inducer. Control titer of IFN-α was 300 IU/ml. The square shows lack of inhibition with VSV as the inducer. A representative example of a total of three experiments is shown.

of the antiserum (not shown). This result also suggests that the gH–gL complex plays no role in IFN-α induction.

The gDt-induced antiviral activity is of the α type based on its activity in MDBK cells and on its inhibition with IFN-α-specific antiserum. As shown in Fig. 6, induction by gDt was inhibited by a human anti-HSV-1 antiserum and by the gD-specific mAb LP2, excluding that induction is due to non-HSV-1 or non-gD contaminants present in the preparation. Higher mAb concentrations were necessary to block gDt-dependent induction compared with induction by intact virus. This is expected in that actual amounts of gD that cause induction are much higher for soluble gD than for the virus-bound version.

We also assessed the effects of the cell membrane-specific mAbs that blocked induction by HSV-1- or cell-bound inducer on induction by soluble gDt. The results in Fig. 7 show the inhibitory effects of mAb GalC for the soluble inducer, which are similar to the effects seen for intact HSV-1 and Sf9-gD (see Fig. 3). Thus the same glycolipid involvement in IFN-α induction is suggested for all three inducers. In addition, the same CCR3- and CXCR4-specific mAbs that blocked induction by HSV-1 and Sf9-gD also blocked induction by gDt (Fig. 8). As seen in the Figure, much lower concentrations of both antibodies were required to block gDt-dependent induction compared with induction by the intact virus, comparable to the results seen with gD-expressing Sf9 cells (see Fig. 4). As suggested above, this could be due to tighter binding of the virus to each of both receptors relative to the soluble form of gD.

DISCUSSION

We studied a possible role of HSV-derived envelope glycoproteins in the induction of IFN-α in human PBMCs. In one approach, we incubated PBMCs with insect Sf9 cells that individually expressed six of these glycoproteins in the absence of any other herpes virus- or mammalian cell-derived antigens (Ghiasi *et al.,* 1994). In a second approach, we tested soluble recombinant glycoproteins lacking transmembrane and intracellular domains. Because the infection-promoting form of gH and gL is a heterodimeric complex of both glycoproteins, we used a recombinant version in which full-length gL is complexed with truncated gH component (gHt–gL). Such a preparation, when injected into rabbits, produced antibodies that blocked HSV-1 infection, thus appearing to have a conformation close to its functional form (Peng *et al.,* 1998). However, we did not observe induction of IFN-α with the heterodimer construct that we used. In

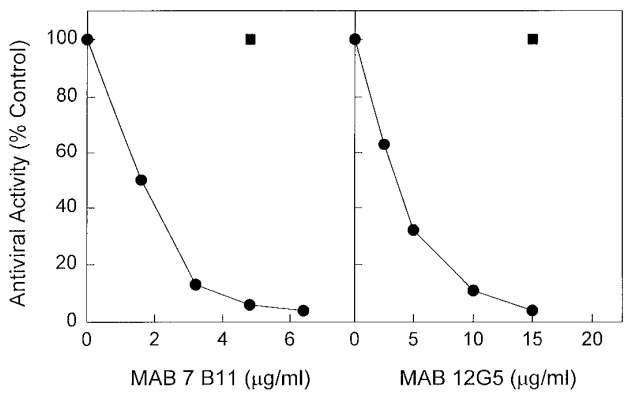

FIG. 8. Effects of chemokine receptor-specific mAbs on IFN-α induction by gDt. Experiments were carried out essentially as described under Materials and Methods, using 0.3 μM gDt as inducer. Control levels of IFN-α were 800 IU/ml (left) and 500 IU/ml (right). Squares represent control experiments using VSV as inducer. Representative examples of four (left) and five (right) experiments are shown.

addition, the truncated construct of gD was used to confirm the role of this glycoprotein in IFN-α induction in the absence of other viral components. A similar gD construct was previously shown to block HSV infection and to specifically bind to a limited number of cell receptors, also suggesting that it retained its natural conformation (Johnson *et al.,* 1990). Based on our results, we conclude that glycoprotein gD is the main membrane component that is responsible for IFN-α induction by HSV-1. Furthermore, the results with Sf9 cells expressing HSV-2-derived gD (gD2) suggest a similar role for this glycoprotein in IFN induction by HSV-2.

One half of maximal induction by gDt occurred at an inducer concentration of ~0.3 μM. This value is close to a dissociation constant of 0.26 μM for the soluble gDt–cell receptor complex (Johnson *et al.,* 1990). This result suggests that 50% of maximal induction occurs close to a receptor occupancy of 50% and thus favors a receptor-mediated phenomenon involving intact gDt without prior processing. However, reactive concentrations of gDt are much higher than those of the virus-bound inducer; thus different induction mechanisms for soluble and particle-bound gD cannot be excluded. In fact, prior attachment of intact virus to responder cells via gB or gC, or both, might allow for tighter interaction of virus-bound gD with responder cell receptors, despite their relatively low affinity for soluble gD. Similar observations were made in studies of IFN-α induction in PBMCs by HIV-1-derived soluble gp120. The gp120 concentration yielding half-maximal IFN-α induction was also close to the dissociation constant of the soluble glycoprotein cell–receptor complex and thus compatible with half-saturation of receptor occupancy. Again it was much higher than that of virus-bound glycoprotein that yielded the same response (Ankel *et al.,* 1994).

Similarities between induction by HIV-1 and HSV-1 extend further, as in both cases IFN-α induction is inhibited by an mAb that specifically recognizes galactosyl-sphingosinyl moieties of galactosyl cerebrosides and sulfatides. These glycolipids are prominent membrane components and might be necessary participants in membrane fusion of both viruses via their respective glycoproteins. Alternatively they might be active participants in transmembrane signaling via their lipid components ceramide or sphingosine, as discussed previously (Ankel *et al.,* 1996). In fact, a regulatory role of ceramides in the function of dendritic cells has been proposed (Sallusto *et al.,* 1996).

The antagonistic effects of anti-chemokine receptor antibodies to IFN-α induction by HSV-1 and its glycoprotein gD were unexpected. A possible functon of chemokine receptors in IFN-α induction could be based on a role of chemoattraction of IFN-α-producing cells and antigen-presenting cells (Baggliolini, 1998; Bancherau and Steinman, 1998). In fact, using immunocytochemistry to visualize intracellular IFN-α, clusters of IFN-producing cells with nonproducing cells have been observed (Feldman and Fitzgerald-Bocarsly, 1990; Fitzgerald-Bocarsly, 1993).

On the other hand, chemokine receptors play an important coreceptor role in HIV infection, although such a role for HSV-1 has not previously been described (Alizon, 1997; D'Souza and Harden, 1996). The mAbs that we found to be inhibitory to IFN-α induction block either the CXCR4 or the CCR3 receptors. They are known antagonists of infection by certain laboratory-adapted or primary isolates of HIV-1 (Endres *et al.,* 1996; Heath *et al.,* 1997). Our results suggest that the same receptors on IFN-producing cells might also interact with the gD component of HSV. In fact, enriched IFN-producing cells resemble dendritic cells, which are known to express both CCR3 and CXCR4 receptors (Banchereau and Steinman, 1998; Dittmar *et al.,* 1997; Granelli-Piperno, 1996). Chemokine receptors are membrane components that meander in and out of the cellular membrane. They contain seven membrane-spanning domains and display four peptide sequences on the exterior side of the cell. Theoretically an infecting virus such as HIV could attach to any one of the exterior segments of such a structure. The CXCR4-specific mAb 12G5 attaches to the third extracellular domain of the receptor (Alizon, 1997; Brelot *et al.,* 1997). Apparently this loop is also involved in the interaction with gD because gD-dependent IFN-α induction is blocked by 12G5. The epitope that is recognized by the CCR-3-specific antibody 7B11 has not as yet been characterized. However, because 7B11 also blocks induction, the latter epitope likewise appears to interact with gD.

Our findings provide another example for the phenomenon that a virus-encoded glycoprotein that is essential for infection can also trigger the induction of an antiviral response that can limit its spread. The same phenomenon was described earlier for HIV-1, which is unrelated to the DNA virus studied here but codes for the same type of bifunctional glycoprotein (Ankel *et al.,* 1994, 1996; Capobianchi *et al.,* 1992). Like gD of HSV, gp120 of HIV-1 is essential for infection, yet it also induces antiviral activity via IFN-α. Probably such dual activity has evolved to allow for the coexistence of both the infected host and the infectious agent. In addition, our results suggest that interaction of HSV-1 with immune cells other than those producing IFN could cause agonistic or antagonistic actions on chemokine function, which could play a role in the pathobiology in the virus. Thus consequences for the infected host beyond cytopathic action of the virus, such as stimulatory or inhibitory effects of gD on the immune system via cytokine or chemokine dysfunction, should be considered.

MATERIALS AND METHODS

Cells and virus

Sf9 cells (CRL 1711) were from ECACC (Salisbury, UK). They were grown in TC-100 medium (Aldrich, France)

containing 10% fetal bovine serum (FBS) at 28°C. Wild-type *Autographa californica* nuclear polyhydrosis virus (baculovirus) was obtained from Novagen (Abingdon, UK). All HSV-derived glycoproteins referred to in the text are those of HSV-1 except gD2, which is the HSV-2-derived species. Baculovirus constructs expressing individual HSV-1(KOS) glycoproteins gB, gC, gD, gE, gG, and gI were generously supplied by Dr. Homayon Ghiasi (Los Angeles, CA) (Ghiasi *et al.,* 1991, 1992a, 1992b, 1992c, 1992d, 1992e, 1994). Baculovirus constructs expressing HSV-1 (McIntyre)-derived gD1 and HSV-2 (strain 333)-derived gD2 were produced essentially as described for the other glycoproteins (Damhof, 1995; Ghiasi *et al.,* 1994). Titers of baculovirus stocks ranged from 5×10^7 to 2×10^8/ml. Monolayers of Sf9 cells were infected with baculovirus suspensions at an m.o.i. of ~10–20 and incubated for 48–72 h at 28°C, when most of the cells no longer adhered to the culture flasks. Cells were harvested and washed several times with PBS and then suspended in PBS containing 0.1% glutaraldehyde for 1 h at 4°C. Cells were washed again with PBS and suspended in 0.3% glycine containing PBS to inactivate all traces of glutaraldehyde. After overnight storage at 0–4°C, cells were washed again with PBS and then suspended to 10^6/ml in RPMI–10% FBS and stored at 0°C. Expression of individual glycoproteins was monitored by immunofluorescence using antisera or individual mAbs (see below). Of the transfected Sf9 cells that were used in the IFN-α induction assays, routinely 50–90% expressed the HSV-derived glycoprotein under study.

Stocks of HSV-1 (Shealy) were prepared from supernatants of infected Vero cells cultured in RPMI–2% FBS 72 h p.i. at an m.o.i. of 0.1 and had a titer of 10^6 to 10^7 PFU/ml. Stocks of VSV were likewise obtained from RPMI-cultured Vero cell supernatants 48–72 h p.i. at an m.o.i. of ~0.01. Their titer was 10^7 PFU/ml. Stocks of Sendai virus (E 72) were obtained after infection of embryonated chicken eggs and had a titer of 10^8 infectious doses/ml. In control induction mixtures, both viruses were used at dilutions of 10^{-3}. Inactivated poliovirus vaccine was obtained from Pasteur (Merieux, Paris). One volume of a 1:1 vaccine dilution in RPMI–10% FBS was mixed with 1 volume of a 5% γ-globulin solution (LFB, Paris) and used as controls in some of the IFN-α induction assays (P. Lebon, unpublished observations). Peripheral blood mononuclear cells from healthy donors negative for HIV, HTLV-1, hepatitis B virus, and hepatitis C virus were prepared from fresh buffy coats obtained from Etablissement de Transfusion Sanguine (Paris). Centrifugation on Ficoll-Hypaque gradients was routinely used to isolate PBMCs. Freshly isolated cells were suspended in RPMI medium containing 10% heat-inactivated FBS and used in experiments on the same day.

Antisera, monoclonal antibodies, and recombinant glycoproteins

Patient-derived neutralizing anti-HSV-1 sera had anti-HSV-1 titers of 3000–4000 IU/ml by ELISA (assay kit supplied by Behringwerke, Marburg, Germany). These sera were decomplemented before use. A rabbit anti-HSV-1 antiserum and the non-neutralizing mouse mAbs reactive with HSV-1- and -2-derived glycoproteins gB, gC, gD, and gE were kindly furnished by Dr. Stig Jeansson (Oslo, Norway) (H2G4B1, B1C1B4, E5G3G9, and B1E6A5; Bergstroem *et al.,* 1992; Trybala *et al.,* 1993). Neutralizing mouse mAbs recognizing HSV-1- and -2-derived gD were donated by Dr. Tony Minson (Cambridge, UK) (LP2; Minson *et al.,* 1986) and Dr. Martin Zweig (Frederick, MD) (4S; Showalter *et al.,* 1981). The polyclonal rabbit anti-gH/gL serum (R 137) recognizing the native gH–gL complex was a kind gift of Drs. Gary Cohen and Roselyn Eisenberg (Philadelphia, PA) (Peng *et al.,* 1998). A gG-specific mouse mAb was obtained from Advanced Biotechnologies (Columbia, MD). An IFN-α-specific neutralizing sheep polyclonal antibody was prepared at Unité 43, INSERM (Paris, France). The mouse mAb GalC was from Boehringer-Mannheim (Mannheim, Germany). This antibody binds galactocerebrosides and psychosine equally well and cross-reacts with sulfatides with a 16-fold lower titer (Rantscht *et al.,* 1982). The mAbs to the chemokine receptors CXCR4 (12G5) and CCR3 (7B11) were provided by the NIH Aids Research and Reference Reagent Program (Rockville, MD) and by the MRC Aids Reagent Project (Potters Bar, UK). Anti-CXCR4 receptor had been made available by Dr. James Hoxie (Philadelphia, PA), and anti-CCR3 receptor had been made available by LeukoSite (Cambridge, MA) (Endres *et al.,* 1996; Heath *et al.,* 1997; Wu *et al*., 1997). The 12G5 hybridoma line was made available by Dr. Marc Alizon (Paris). A mouse hybridoma line expressing mAb ACN5 to the baculovirus glycoprotein gp67 was a gift from Dr. Peter Faulkner (Kingston, Ontario, Canada) (Whitford *et al.,* 1989). Hybridoma culture supernatants were used directly for immunofluorescence studies of baculovirus-infected Sf9 cells. The soluble recombinant gHt–gL complex contains the extramembranous domain of gH (residues 1–719, including the signal sequence) elongated by a hexahistidinyl tail at the carboxyl end and full-length mature gL [gH-1t(His)6/gL-1]. It was purified by metal chelate affinity chromatography from culture supernatants of Sf9 cell cultures infected with a baculovirus construct expressing both glycoproteins (Westra *et al.,* 1997; Westra and Welling-Wester, in preparation). The recombinant extramembranous portion of glycoprotein gD was isolated from culture supernatants of Sf9 cells infected with a baculovirus construct expressing the N-terminal 315 amino acids of the complete mature polypeptide of HSV-1 (gD-1t; Damhof, 1995; Westra and Welling-Wester, unpublished results).

IFN induction and assay

Freshly isolated PBMCs (6×10^6/ml) were incubated at 37°C for ~18 h in 0.1 ml of RPMI–10% FBS with added HSV-1 or control virus, inducer Sf9 cells, or glycoprotein at indicated concentrations. At the end of the reaction, 5 μl of 1 N HCl was added to inactivate live virus where applicable. Supernatants were removed and serially diluted 2-fold in duplicate. MDBK cells in MEM–10% FBS were added, and mixtures were incubated for an additional 24 h at 37°C, followed by infection with VSV. Cytopathic effects were scored under the microscope 24 h later. Titration end points represent dilutions that gave destruction of 50% of the cells. A laboratory reference of human IFN-α, which had been standardized with the NIH Ga 023–902-530 reference, was included with each titration. Duplicates usually varied by no more than one dilution. One international unit of IFN activity (IU) represents the reciprocal of the dilution that results in 50% of cell destruction, corrected for the value obtained with the IFN-α standard. Although MDBK cells are of bovine origin, they are as sensitive to human IFN-α as human fibroblasts. They do not respond to human IFN-γ and poorly to human IFN-β, and thus define human cell-derived antiviral activity as IFN-α (Gresser *et al.,* 1974). In addition, antiviral activies induced by Sf9-gD and recombinant glycoprotein gDt were further characterized by neutralization with IFN-α-specific antiserum.

Immunofluorescence and inhibition studies

For immunofluorescence, PBS-washed Sf9 cells expressing HSV glycoproteins were fixed onto slides with acetone, and then primary antibodies were added for 30 min at 37°C. After washing with PBS, slides were developed with appropriate fluorescein-conjugated anti-IgG second antibody. To visualize surface-expressed viral glycoproteins, washed cells were suspended in PBS containing sera or mAbs at 4°C for 1 h, washed again, and further processed with the appropriate fluoresceine-labeled anti-IgG second antibody. In the inhibition studies using antisera or mAbs against gD, both inducer and mAb were mixed and preincubated for 15 min at 37°C before PBMCs were added, and incubation was continued overnight. In the studies with antisera or mAbs that recognize cell surface components, PBMC and antibodies were preincubated for 15 min at 37°C before inducers were added.

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