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Multiple mechanisms for HSV-1 induction of interferon α production by peripheral blood mononuclear cells

Q. Rong[1], **T. S. Alexander**[2], **G. K. Koski**[3], and **K. S. Rosenthal**[1]

[1]Northeastern Ohio Universities College of Medicine, Rootstown, Ohio, U.S.A.
[2]Summa Health System, Akron, Ohio, U.S.A.
[3]University of Pennsylvania Medical Center, Philadelphia, Pennsylvania, U.S.A.

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Summary. UV-inactivated, infectious, and other forms of herpes simplex virus 1 (HSV-1) induced interferon (IFN) production by different routes in myeloid origin mononuclear cells (MOMC) (consisting predominantly of monocytes). GM-CSF activated the MOMC (G-MOMC) to produce greater amounts of interferon while differentiation to DC, by the addition of granulocyte macrophage colony stimulating factor (GM-CSF) and calcium ionophore (GA-MOMC), reduced the levels of interferon production upon challenge with some HSV strains. UV-inactivated virus induced more interferon than infectious virus. L-fucose, an antagonist of the mannose receptor, inhibited the induction of IFN-α by UV-inactivated virus and gB^- virus (defective in penetration) in MOMC and GA-MOMC but not G-MOMC. L-fucose had little effect on interferon induction by infectious HSV-1. The insensitivity of the G-MOMC to fucose inhibition distinguishes these interferon producing cells from the pDC2 cells previously described as natural interferon producing cells. The mannose receptor appears to be involved in the response to non-infectious forms of HSV but infectious virus appears to use a different pathway. These studies suggest that non-infectious virions and HSV infected cell debris effectively stimulate monocytes and predendritic cells to produce IFN-α to initiate host protection against HSV infection.

Introduction

Interferon alpha (IFN α) production is one of the earliest responses to virus infection, acting locally and systemically [6] to inhibit virus replication and induce protective immune responses. IFN α can inhibit infection and the spread of HSV from neurons to epidermal cells [37] *in vitro*, and application of plasmid DNA

expressing IFN α is sufficient to prevent disease upon vaginal [27] or corneal [42] infection in animal models.

The most potent inducer of IFN-α is double stranded RNA (dsRNA), formed as the replicative intermediate of RNA viruses or as a result of complementary RNAs of a DNA virus [30]. Complementary transcripts, which may provide an inducer, have been detected in vaccinia virus [40], adenovirus [45], herpes simplex virus (HSV) [31], and SV40 [1]. IFN-α production can also occur upon inhibition of protein synthesis which can block the synthesis of repressors of IFN-α mRNA synthesis [52].

Certain enveloped viruses, including Sendai virus [44], human immunodeficiency virus (HIV) [2, 18, 23, 25], and HSV [11, 12, 16, 19–22, 24, 25, 34, 35, 38, 44, 46, 47, 50, 57, 58] promote the production of large amounts of IFN-α upon interaction with a population(s) of cells in freshly isolated peripheral blood. Human monocytes produce IFN-α in response to Sendai virus or HIV [25, 49] and may also produce interferon in response to HSV. HSV induces high levels of IFN α in a minor cell component which have been termed natural interferon producing cells (NIPC). The IFN α producing activity of these cells is lost upon overnight *in vitro* culture of the cell population [58]. A candidate for the NIPC has been identified as the pDC2 (dendritic cell 2 precursor) [53]. These cells lack myeloid or monocytic markers including CD33 (myeloid origin marker), CD13, CD11b, CD15 (markers expressed when precursor cells differentiate into myeloid lineage mononuclear cells), CD11b and CD15 (strongly expressed on granulocytes), CD14 (monocyte marker) antigens, and also lack B-cell and T-cell antigens. Other types of HSV responsive interferon-producing cells may also be present in peripheral blood or the peripheral tissue. For example, in the mouse, the marginal metallophilic macrophages and marginal zone macrophages of the spleen are the major interferon producers in response to IV challenge with HSV and murine DC lines can produce interferon in response to bacteria and viruses, including HSV [16].

Several forms of HSV can induce the IFN α response including infectious and UV inactivated virus [21, 57] indicating that replication of the virus is not required. Internalization of the virus is also not required since HSV fixed to glutaraldehyde cross-linked cells [12, 47] and genetically engineered HSV-1 glycoprotein D (gD) obtained from mosquito cells are sufficient for induction of IFN-alpha [3].

In this study, we evaluated an alternative source of cells to study the nature of the interferon response to HSV-1. Large numbers of non-lymphocytic mononuclear cells were obtained by leukophoresis and countercurrent centrifugal elutriation [15]. These cells are predominantly of myeloid origin (MOMC) with a small percentage of immature dendritic cells. Following treatment with granulocyte macrophage colony stimulating factor (GM-CSF) and A23187 (calcium ionophore, ionomycin, 'A') in serum-free cell culture, the monocytes and immature dendritic cells (iDC) undergo a rapid and consistent change to become activated dendritic cells (DC) [17, 36]. The differentiation to DC includes down-regulation of CD14 expression, acquisition of dendritic cell morphological properties, up-regulation of MHC class II and co-stimulatory molecule expression, and enhanced

capacity for T cell sensitization [15]. We demonstrate that the extent of interferon induction is different upon challenge with different strains and forms of HSV and that monocytes, GM-CSF treated monocytes, and the mature dendritic cell populations respond differently to these challenges. The response of the MOMC to some strains of HSV-1 is enhanced by GM-CSF to levels similar to that reported for NIPC. Comparison of the activities of different strains of infectious HSV, UV-inactivated HSV, and a mutant HSV incapable of penetration (KΔT) [9] and results of treatment with a mannose receptor antagonist indicate that there are more than one mechanism for HSV induction of interferon in the MOMC origin cell populations.

Materials and methods

Preparation of MOMC and cell culture

Mononuclear myeloid cells (MOMC) isolated from different healthy donors on different occasions by leukopheresis and countercurrent centrifugal elutriation [15] were frozen and thawed for each experiment. The MOMC preparations consist predominately of CD33+ cells (>98%) (a myeloid cell surface marker) with approximately 90% monocytes ($CD14^+CD33^+$) and 1–10% iDC ($CD14^-CD33^+$) [15]. After thawing, MOMC were washed once in macrophage-SFM medium (Gibco) containing 1% penicillin-streptomycin (Cellgro) and grown in 24-well (3×10^6/well) cluster plates (Costar) in 2 ml serum free macrophage-SFM medium (Gibco) with 1% penicillin-streptomycin in an atmosphere of 5% CO_2 at 37 °C. Recombinant human granulocyte-monocyte colony stimulation factor (GM-CSF, 20 ng/ml) (Immunex) was added to specific wells of the MOMC (GM-CSF treated MOMC, G-MOMC) directly after the plating. Calcium ionophore (A) (A23187, ionomycin; Sigma Chemical Co, 225 ng/ml) was added to a set of GM-CSF treated MOMC at 20 h after thawing to induce differentiation of the cells (GM-CSF plus A23187 treated MOMC, GA-MOMC) [15, 17, 36].

Cells for each experiment were analyzed by flow cytometry to determine their immunophenotype [15]. Cells were incubated with 1 mg/ml human IgG (Sigma Chemical Co.) for 15 min to block Fc receptors and double stained with fluorescein isothiocyanate (FITC)-conjugated mouse anti-human CD14 and phycoerythrin (PE)-conjugated mouse anti-human CD80 (B7.1) or CD86 (B7.2) (PharMingen) for 30 min at 4 °C.

Virus

Virus stocks were prepared by freeze-thaw and sonication of infected Vero cell lysates and culture media. Vero (African Green Monkey Kidney) cells were grown in Medium 199 (Gibco BRL) supplemented with 5% fetal calf serum (Hyclone) and 1% penicillin-streptomycin (Cellgro) at 37 °C. Virus stock was quantitated in a standard plaque assay on Vero cell monolayers.

KOS321, a lab attenuated virus, was kindly provided by Tom Holland, Wayne St. U. School of Medicine, Detroit, MI. SP7 and SLP10 were generated for other studies in our lab [7]. SP7 is a clinical strain obtained post mortem from the brain of a 10-day-old infant with disseminated HSV-1 disease. SLP10 was generated by serial passage (10 times) of plaque purified SP7 virus in Vero cells at low MOI (<0.01). ANG and ANG-PATH virus [39], two related syncytial virus strains, were kindly provided by Bradley M. Mitchell (Baylor College of Medicine, Houston, TX). ANG-PATH was derived from ANG by passage in mice. Stocks of virus were prepared and quantitated as mentioned above.

UV-inactivated virus was prepared by exposure of virus to UV light for 15 min and then tested by plaque assay for residual infectivity. The quantity of UV-virus is referred to as pfu or MOI-equivalents, which are based on the amount of infectious virus prior to UV inactivation.

KΔT is a mutant of HSV-1 KOS with a gene deletion in the transmembrane region of the glycoprotein B (gB) to prevent implantation into membranes and the virion envelope [9]. KΔT was kindly provided by John Docherty (Northeastern Ohio Universities College of Medicine, Rootstown, OH). Stocks of KΔT containing gB (*KΔT-gB*) were made by infecting D6 cells, a Vero cell line expressing gB, with KΔT-gB. Quantitation of KΔT-gB was done in a standard plaque assay on D6 cells. Plaque assay was done on Vero cells to detect potential revertants to the parental KOS virus.

KΔT virus was quantitated by two methods which gave comparable results. KΔT viral DNA concentration was compared with that of a KOS virus stock for which the titer was already known. Viral DNA was purified for both viruses using Wizard Genomic DNA purification kit (Promega, Madison, WI). The DNA concentration was quantitated by absorbence at 260 nm and KΔT PFU-equivalents were calculated by comparison with the DNA concentration of KOS stock. In addition, KΔT strain was quantitated using a plaque assay in which polyethylene glycol (PEG) was used to promote viral penetration and plaque formation (adapted from Sarmiento et al., 1979 [51]). After a 1 h adsorption period, the monolayer was washed once with PBS and the cells were exposed to a solution (1 ml per well) that contained 40 g of melted PEG 6000 (Sigma, St. Louis, MO) and 36.4 ml of M199 without serum. The PEG solution was removed by washing with solutions of 1:3 PEG: serum-free M199, 1:7 PEG: serum-free M199, and three washes with M199 containing 5% fetal calf serum. The cultures were incubated with M199 containing 5% fetal calf serum for 24 h at 37 °C to allow the cells to recover. The medium was then removed and M199 containing 0.5% methylcellulose (Kodak, Rochester, NY) and 5% fetal calf serum was added. The cultures were incubated for two or three days at 37 °C until plaques formed.

Induction and measurement of IFN-α

Cells treated with UV inactivated viruses were incubated for 24 h at 37 °C. For infectious virus, cells were incubated with HSV-1 for 1 h at 37 °C, the medium was replaced with SFM medium and incubated for 24 h at 37 °C. To evaluate the effect of fucose on interferon induction, different concentrations of fucose were added 15 min before the addition of virus. After the 24 h incubation, 1 ml aliquots were removed and frozen at −20 °C. The cells and remaining medium were frozen at −70 °C and thawed for quantitation of virus production. IFN-α concentration was determined by ELISA (Biosource, Camarillo, CA). The antibodies in the ELISA kit can recognize the most common subtypes of IFN-α.

Results

Myeloid origin cell populations

Myeloid origin mononuclear cell populations (*MOMC*) obtained by leukopheresis and countercurrent elutriation were used as an alternative source of non-lymphocytic mononuclear cells to study HSV induction of IFN α. MOMC are predominantly (>90%) monocytes, contain a minor population of immature dendritic cells (iDC) (1–10%) and have minimal contamination by lymphocytes and neutrophils [15]. MOMC treated with GM-CSF (*G-MOMC*) appeared similar to the untreated MOMC but maintained their viability to a greater extent over

the 4-day-course of the experiment. Both the MOMC and G-MOMC expressed high levels of CD14, the LPS receptor, although G-MOMC expressed lower levels than MOMC with low or absent CD80 (B7.1) or CD86 (B7.2) but a sub-population expressed higher levels of CD86 expression. G-MOMC maintained in serum free medium and treated with the calcium ionophore A23187 (*GA-MOMC*) readily and reproducibly converted to mature DC [17, 36]. The GA-MOMC cells could be distinguished from the MOMC and G-MOMC by the lack of CD14 expression and upregulation of both CD80 and CD86 expression, characteristics of mature dendritic cells [15]. The loss of CD14 expression and up-regulation of CD80 and CD86 was seen in each experiment. Maintenance of these cells in serum free medium and separation from lymphocytes and granulocytes prevents the effects of bovine serum factors from affecting their development. The conversion was reproducible in cells from different donors.

Virus production by myeloid origin cell populations

Herpes simplex virus production by MOMC, G-MOMC and GA-MOMC was evaluated by quantitating the amount of virus released to the media by plaque assay on Vero cells. All three cell populations were poor virus producing cells with an average yield of one virus per cell (data not shown). This was less than the input (MOI = 2) infectious virus. The low permissivity of myeloid cells for HSV replication has been reported by others [56]. Infection with higher multiplicities of infection (MOI = 10) caused considerable cytopathological effect and cell death.

HSV induction of IFN-α production

Initial studies were performed to determine whether MOMC, G-MOMC and GA-MOMC have the ability to produce IFN α in response to UV-inactivated HSV-1. The cells were challenged with different MOI equivalents of UV-inactivated KOS, a highly passaged, attenuated HSV-1 strain and extracellular medium was obtained after 24 h and IFN α analyzed by ELISA.

IFN-α production, by each of the cell populations, increased upon challenge with increasing MOI equivalents of UV-inactivated HSV-1 KOS (Fig. 1). The G-MOMC cell population produced the greatest amount, GA-MOMC produced an intermediate amount and the MOMC produced the least amount of IFN-α in response to UV inactivated KOS. There was some variation in the amounts of interferon produced by cells obtained from different donors and for different dates of donation but the G-MOMC treated cells always produced the most IFN α. Cells that were mock infected or treated with uninfected Vero cell extract produced no IFN α (data not shown). The response to UV inactivated HSV confirms other studies [3, 47] that show that complete virus replication is not necessary for IFN α induction. Also, GM-CSF activates the cells to produce more interferon upon HSV induction.

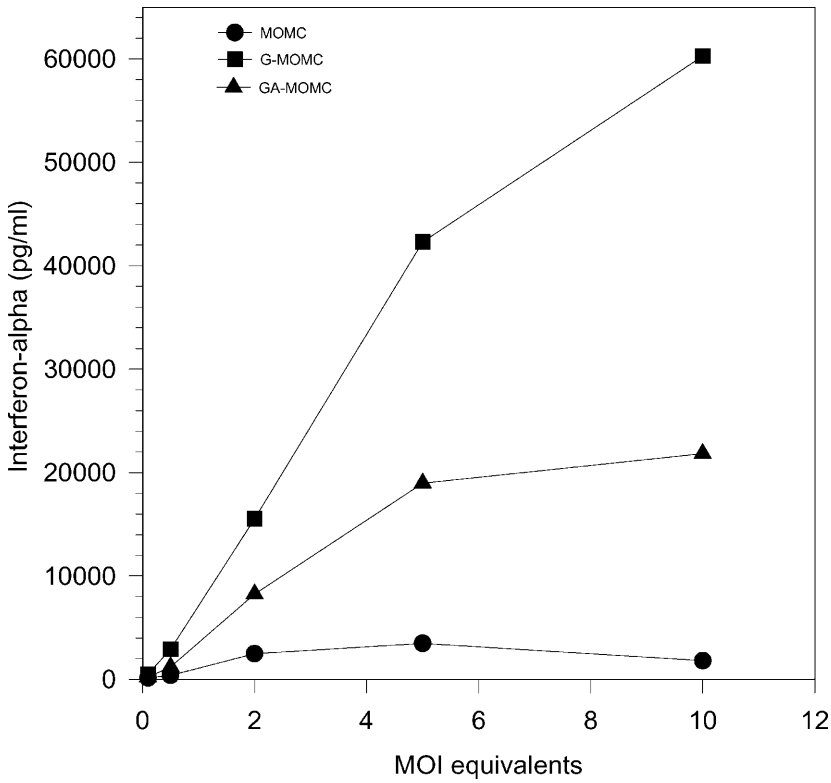

Fig. 1. IFN-α production induced by UV inactivated HSV-1. Different MOI equivalents of (MOI = 0.1, 0.5, 2, 5, 10) UV inactivated HSV-1 KOS were added to the three different cell types on the fourth day after thawing. Supernatants of the cell culture media were removed after an additional 24 h incubation and stored at −20 °C for ELISA analysis of IFN-α (pg/ml)

Virus strain dependent differences in induction of IFN-α

The magnitude, range and trends for IFN α responses were different for UV inactivated and infectious virus and for different strains of HSV-1 (Fig. 2). The difference in response to infectious and UV-inactivated virus is clearly shown for HSV-1 KOS and UV-inactivated KOS. The strain dependent difference in response was evaluated for HSV-1 strains that differ in their passage history, tissue culture behavior and their ability to cause lethal neuroinvasive disease in the mouse footpad and other models of HSV infection [7, 26, 39]. A MOI of 2 or the equivalent amount of UV-inactivated virus was chosen for the virus challenge because the cytopathological effect of infectious HSV on the different types of MOMC cells was minimal at this dose. Cells from different individuals were used for some of the experiments (different panels of Fig. 2).

The first set of viruses to be tested included SP7, a low passage neuroinvasive virus and SLP10, an attenuated virus derived from SP7 by passage in Vero cells (Fig. 2A, 2B). The response to UV-SP7 was greater than for UV-SLP10 or UV-KOS (data not shown). The response to infectious virus was much less than for

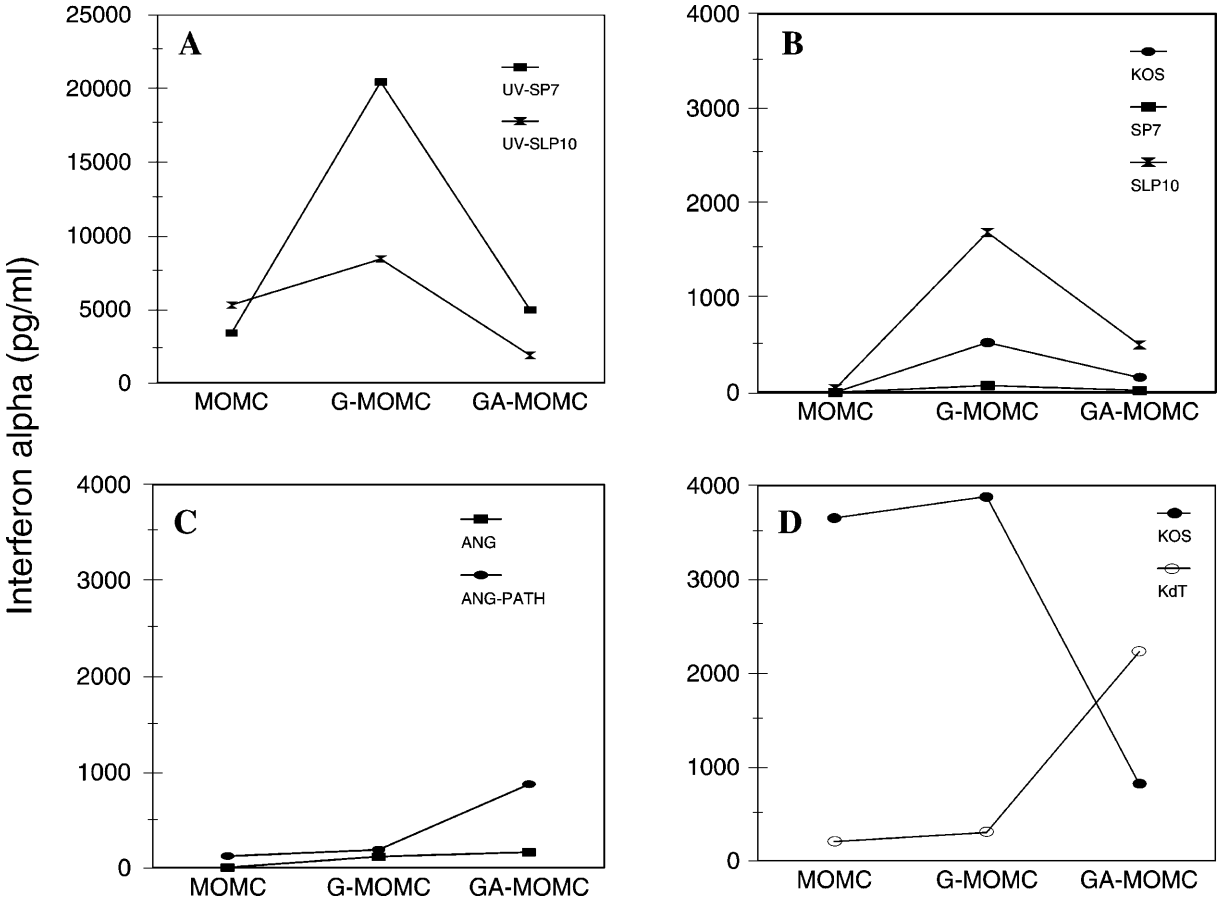

Fig. 2. IFN-α production induced by different strains of HSV-1. Cells were incubated with UV-inactivated or live virus on the fourth day after thawing. Cells were obtained from two different donors. Data from individual experiments are shown in each set of boxes (**A–D**). The MOI, or MOI-equivalents for all experiments was 2

UV-inactivated virus. The G-MOMC response was greater than for MOMC or GA-MOMC but SP7 was the poorest inducer of interferon in all three cell populations. These trends were observed for different individuals and on different occasions.

A different trend in cellular response was observed for the ANG and ANG-path set of viruses. ANG-path was derived from ANG, a clinical virus, by passage in mouse brains to select for a more neuroinvasive virus [39]. Both ANG and ANG-path cause syncytia formation in all three cell populations. ANG-PATH induced interferon, but unlike the previously described viruses, the GA-MOMC produced more interferon upon induction by ANG-PATH than did G-MOMC (Fig. 2C). ANG was a poor inducer of interferon but induced a small, but measurably greater amount of IFN α in GA-MOMC. This strain dependent difference in the trend may indicate a different type of interaction of ANG-path and ANG with the MOMC-origin cells.

IFN α response to a gB⁻ mutant virus

In order to address the question of whether virus entry is required for the induction of IFN-α in MOMC origin cell populations, an HSV mutant defective in penetration was evaluated for its abilty to induce interferon production. The KΔT mutant was developed from KOS by deletion of a 969-base-pair *Bst*EII fragment in the gB-coding region, corresponding to 323 amino acids of the transmembrane region. This virus yields normal virions lacking the glycoprotein B. The KΔT mutants bind efficiently, but cannot enter cells [9]. Stocks of infectious virus (*KΔT-gB*) were prepared by growth in a gB expressing Vero cell line (D6 cells) and stocks of virus lacking gB (*KΔT*) were obtained upon infection of the non-complementing Vero cells. An equivalent titer (with respect to KOS) of KΔT virus was quantitated by two methods. Aliquots of KΔT virus were allowed to bind to D6 cells and fusion of the cell-bound virus was promoted with PEG treatment [51]. This allowed plaque formation to occur in the complementing D6 cells. In addition, the DNA concentration of aliquots of KΔT was compared to similar aliquots of KOS, for which the titer was known. These assays indicated that the equivalent-titer of the KΔT virus stock was approximately 6×10^7/ml.

Interferon induction by KΔT was compared to KOS at MOI equivalents of 2 (data not shown) and 10. The results are corrected for levels of interferon produced by equivalent numbers of wild-type virus to the infectious virus that may be present in KΔT due to genetic reversion to the parental KOS (approximately 2.5 KOS virus per 10,000 KΔT virus) or the small amounts of KΔT viruses which would acquire or retain gB on their envelope (KΔT-gB) (2 KΔT-gB virus per 1,000 KΔT virus).

Figure 4D shows that KΔT induced lower levels of IFN-α production than the parental KOS virus in MOMC and G-MOMC cells. The GA-MOMC produced a larger amount of IFN α than MOMC or G-MOMC in response to KΔT and this response was greater than for KOS.

The effect of fucose on HSV activation of interferon production

Studies by other investigators implicated the mannose receptor as an important mediator of HSV-induced IFN-α production and fucose as an effective inhibitor of this interaction [38]. Initial studies demonstrated a concentration dependent inhibition of UV-inactivated HSV-1 KOS induction of interferon in MOMC, G-MOMC and GA-MOMC. Cells pretreated with fucose for 15 min were incubated with UV-KOS (MOI = 10) for 24 h in the presence of fucose and then aliquots were removed and tested for IFN-α production. IFN-α production in the absence of fucose treatment was set as the 100% control. Fucose treatment caused a biphasic concentration dependent inhibition of IFN α production by MOMC and GA-MOMC in response to UV-KOS (Fig. 3). Although the 50% inhibitory dose was 25 mM, the extent of inhibition (slope) was less at higher concentrations of fucose (50–200 mM). Interestingly, the effect of fucose treatment of G-MOMC was different from that of MOMC and GA-MOMC. IFN α induction in G-MOMC

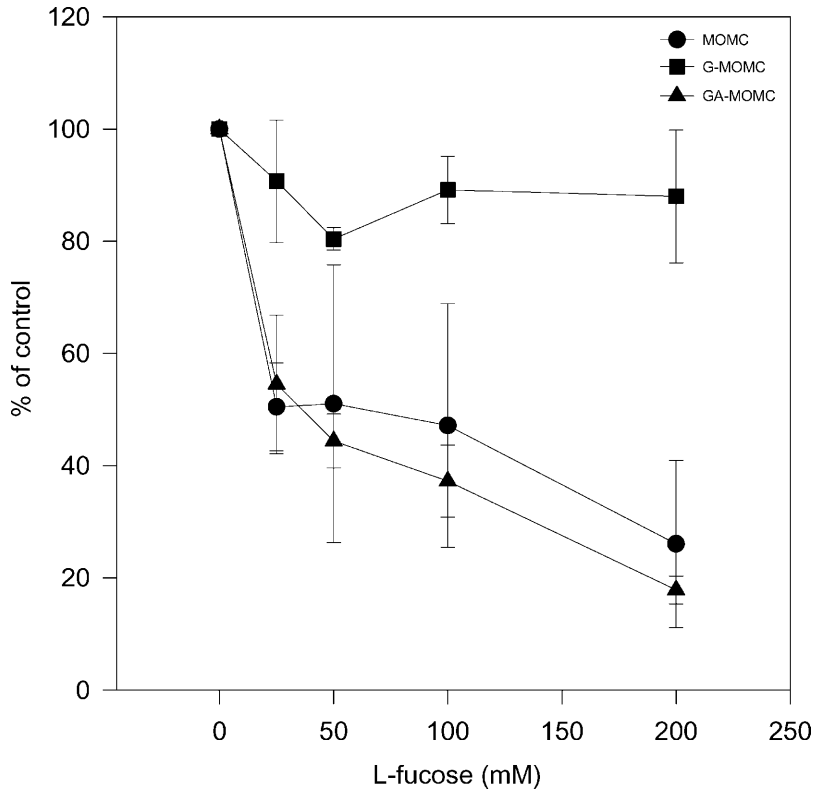

Fig. 3. Fucose inhibition of IFN-alpha production in response to UV-inactivated KOS. MOMC origin cells were pretreated with different concentrations of fucose and then 15 min later, UV-inactivated KOS (MOI equivalents equivalent to 10) was added. The results are presented as a percentage of the IFN-alpha production of UV-inactivated KOS in the absence of fucose

was inhibited by only 20% at 25 mM fucose and this was the maximum level of inhibition (Fig. 3). These results indicate that there are different mechanisms for IFN α induction between these different cell populations with fucose sensitive, fucose less sensitive and fucose insensitive routes of interferon induction. In addition, the primary route of interferon induction for UV-HSV in G-MOMC cells is a fucose insensitive route.

Subsequent studies evaluated the ability of fucose (200 mM) to block interferon induction by UV-inactivated ANG, infectious ANG, infectious KOS, and KΔT. As shown in Fig. 4, fucose inhibited IFN α induction by UV inactivated KOS and UV-inactivated ANG in MOMC and GA-MOMC but not the G-MOMC cell population, consistent with the results shown in Fig. 3. Interestingly, fucose did not inhibit IFN-α production in response to infectious KOS or ANG virus in any of the three cell types. Unexpectedly, fucose treatment appeared to enhance the IFN-alpha production in the G-MOMC cells. Interferon induction by KΔT virus was very sensitive to fucose treatment. IFN α production was reduced by 90% in MOMC and GA-MOMC, and by 80% in G-MOMC. The difference in

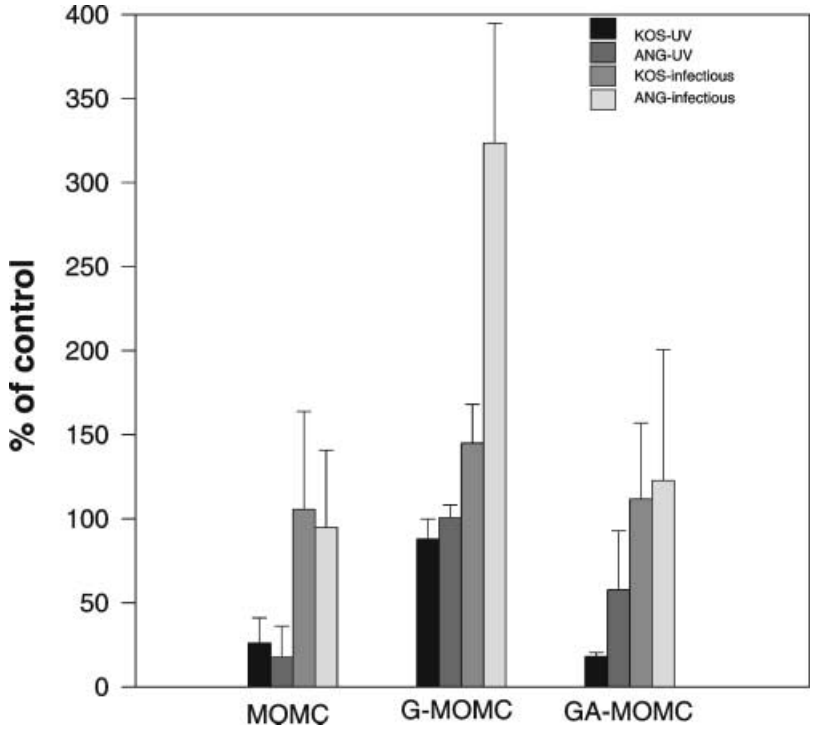

Fig. 4. Fucose inhibition of IFN-α production in response to infectious and UV inactivated virus. Different strains of virus, UV inactivated KOS (MOI equivalent equal to 10), UV inactivated ANG (MOI equivalent equal to 2), live KOS and live ANG (MOI = 2) were used to induce IFN-α production in the presence or absence of fucose (200 mM). The 100% value represents IFN-α production of the cells in the absence of fucose

sensitivity to fucose inhibition suggests that infectious virus induces IFN α by mechanisms different from UV-inactivated virus, and KΔT. These results confirm that the mechanism by which HSV induces interferon in G-MOMC is different from MOMC and GA-MOMC.

Discussion

Different forms of HSV, including infectious virus, UV-inactivated-non-infectious virus, fixed HSV coated onto glutaraldehyde fixed cells [12] and purified glycoprotein D [3, 34] can induce an IFN α response. The nature of the responses to these different forms of virus has been assumed to be similar and the descriptions of the responses have been used interchangeably in many studies. The results from our study indicate that various forms and different strains of HSV induce IFN α to different extents and likely, by divergent pathways. In addition, cell types other than the pDC2 cell are likely to produce IFN α in response to HSV and that response is different for different forms or strains of virus. Also, GM-CSF potentiates the interferon response to some forms and strains of virus.

These studies were made possible by the use of myeloid origin mononuclear cells (MOMC), a large population of cells which are predominantly monocytes, with small numbers of dendritic cell precursors and minimal contamination from lymphocytes and neutrophils [15]. Unlike cells used in other studies, the MOMC can be frozen and thawed and maintained in tissue culture for greater than 24 h. Use of serum free medium for these cells minimizes the interference from unknown cytokines and the serum free conditions may be more representative of the extra-vascular environments where pDC or monocytes may encounter an HSV infection [32].

GM-CSF appeared to prime or activate the interferon response to UV-inactivated and infectious forms of KOS and SP7 viruses but not for all the viruses. The amount of IFN-α produced by G-MOMC in response to UV inactivated HSV was in the same range as suggested in the literature for the NIPC [21, 53], although direct comparisons are difficult due to differences in the means of analysis (ELISA vs bioassay), the virus strain, and individual donor variation. The GM-CSF also enhanced interferon production by peripheral blood mononuclear cells following stimulation by HSV bound to glutaraldehyde fixed WISH cells [12]. In vivo, GM-CSF is an early response to infection and is produced by activated T cells, macrophages, endothelial cells or fibroblasts [24]. Treatment with recombinant GM-CSF [59] is sufficient to elicit protection against HSV-1 encephalitis in a rat model. Our studies would suggest that an important component of the GM-CSF induced protection is the potentiation of the interferon response to HSV-1.

Differentiation of the G-MOMC into mature DC-like cells by treatment with A23187 (GA-MOMC) was accompanied by a reduction in the production of IFN α in response to KOS, SP7 and SLP10 strains of HSV. Decreased response upon differentiation is consistent with the loss (reduction) of interferon induction observed by others upon overnight incubation of peripheral blood mononuclear cells under normal cell culture conditions [53].

The GA-MOMC dendritic cells were more responsive to challenge with ANG-PATH and KΔT viruses than G-MOMC or MOMC. The difference in interferon response may reflect differences in the interaction of the virus with the interferon producing cells since ANG and ANG-PATH cause syncytia formation, KΔT binds, but is incapable of entering the cell, and all three viruses have mutations in or lack the glycoprotein B. Other studies support our findings that DC can make IFN α in response to HSV and also HIV [18, 24].

The different fucose inhibition patterns for the varied forms and strains of virus and for the different cell types suggests that there are different routes of HSV induction of interferon. The fucose sensitive cell surface route probably uses the mannose receptor and is activated by UV-KOS, UV-ANG and KΔT in both MOMC and GA-MOMC cell populations. This may also be the route used by UV-inactivated-non-infectious virus, fixed HSV coated onto glutaraldehyde fixed cells [12], and purified glycoprotein D [3, 34]. A route that is less sensitive to fucose, as distinguished at high concentrations of fucose, may also be used by these activators. The mannose receptor does not seem to be extensively involved

in induction of IFN α by infectious virus or by any of the forms of virus in G-MOMC. The insensitivity of the G-MOMC to fucose inhibition distinguishes the interferon producing cells in this population from the pDC2 cells that have been called NIPC, which are sensitive to fucose inhibition [38].

The large enhancement of interferon induction by UV-inactivation of infectious HSV observed herein and by Linnavuori and Hovi [35] suggests that infectious virus may have the ability to limit IFN α production in MOMC related cells. HSV strains appear to differ in their ability to utilize this mechanism to evade host protection as indicated by comparison of the trend for interferon induction for the UV-inactivated viruses (SP7 > SLP10) and infectious viruses (SLP10 ≫ SP7). For the limited numbers of virus strains tested herein, the virus strains with a history of more extensive passage in non-human hosts (SLP10, KOS, ANG-PATH) appeared to induce more IFN-α production than the low-passage viruses (SP7, ANG). This suggests that the suppression of interferon production may be selected during human infection as a means to escape host defenses but this property may be lost upon infection of cells or animals of other species. Other human-specific HSV mechanisms for escaping host protective responses include the HSV-1 UL47 protein block of MHCI expression by blocking the TAP [28] and glycoprotein E binding to the Fc portion of IgG [41].

The results of this study open up the possibility that myeloid origin monocytes and pre-dendritic cells are an important source of IFN α for host protection against HSV infection. The mechanism of induction for these cells may be different from the pDC2 cells (based on the fucose blocking studies) described by others [38]. The greater response to UV-inactivated virus suggests that non-infectious virions and possibly HSV infected cell debris are the more potent activators of IFN α production and that replicating virus can limit the induction of interferon production as a means of escaping host protection. Locally produced GM-CSF would activate monocytes or pDC to enhance production of IFN α and protective responses.

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Author's address: Ken S. Rosenthal, Ph.D., Northeastern Ohio Universities College of Medicine, 4209 SR44, Box 95, Rootstown, OH 44272, U.S.A.; e-mail: ksr@neoucom.edu