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A Monocyte-Specific Peptide from Herpes Simplex Virus Type 2 Glycoprotein G Activates the NADPH-Oxidase but Not Chemotaxis through a G-Protein-Coupled Receptor Distinct from the Members of the Formyl Peptide Receptor Family

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We have recently identified a peptide derived from the secreted portion of the HSV-2 glycoprotein G, gG-2p20, to be proinflammatory. Based on its ability to activate neutrophils and monocytes via the formyl peptide receptor (FPR) to produce reactive oxygen species (ROS) that down-regulate NK cell function, we suggested it to be of importance in HSV-2 pathogenesis. We now describe the effects of an overlapping peptide, gG-2p19, derived from the same HSV-2 protein. Also, this peptide activated the ROS-generating NADPH-oxidase, however, only in monocytes and not in neutrophils. Surprisingly, gG-2p19 did not induce a chemotactic response in the affected monocytes despite using a pertussis toxin-sensitive, supposedly G-protein-coupled receptor. The specificity for monocytes suggested that FPR and its homologue FPRL1 like-1 (FPRL1) did not function as receptors for gG-2p19, and this was also experimentally confirmed. Surprisingly, the monocyte-specific FPR homologue FPRL2 was not involved either, and the responsible receptor thus remains unknown so far. However, the receptor shares some basic signaling properties with FPRL1 in that the gG-2p19-induced response was inhibited by PBP10, a peptide homologue FPRL2 was not involved either, and the responsible receptor thus remains unknown so far. However, the receptor shares some basic signaling properties with FPRL1 in that the gG-2p19-induced response was inhibited by PBP10, a peptide that has earlier been shown to selectively inhibit FPRL1-triggered responses. We conclude that secretion and subsequent degradation of the HSV-2 glycoprotein G can generate several peptides that activate phagocytes through different receptors, and with different cellular specificities, to generate ROS with immunomodulatory properties. The Journal of Immunology, 2007, 179: 6080–6087.

Herpes simplex virus 2 is a sexually transmitted pathogen that infects the genital tract mucosa and is the most common causative agent of genital ulcer disease in humans. Once HSV-2 infects the epithelium and replicates, it can be transmitted to the sacral ganglia via nerve axons and establish a latent infection, which in association with factors such as stress, fever, UV-irradiation, and immunosuppression can reactivate and cause recurrent disease (1, 2). Professional phagocytes (neutrophil granulocytes and monocytes/macrophages) constitute an important first line of defense against microbial intruders, including many viruses. Their potential role in HSV infection is suggested by studies showing that reduced numbers of granulocytes, or functional defects in these cells, may lead to severe and recurrent infections due to a deficiency in the control of HSV replication (3, 4). Functional restoration of the impaired cells is also associated with a recovery of the infected host (4).

We recently identified an HSV-2 peptide, named gG-2p20, which possesses proinflammatory properties in being an activator of professional phagocytes (5). Based on our results, we introduced a new concept for the role of inflammatory cells in HSV-2 pathogenesis; the fact that gG-2p20-activated phagocytes could impair NK-cell function suggested that this HSV-2-derived proinflammatory peptide may contribute to the survival of virus-infected cells and thereby be of immunomodulatory importance in HSV-2 pathogenesis (5). We were also able to identify both the receptor through which the HSV-2 peptide triggered its proinflammatory activity and the mechanism by which the NK cell function was impaired. The triggering receptor was identified as one of the classical pertussis toxin sensitive G-protein coupled chemoattractant receptors (GPCR) known as the formyl peptide receptor (FPR) (6). This is a high-affinity pattern recognition receptor with the ability to track not only formylated peptides released from bacteria, but also virus-derived proteins, e.g., peptides from the envelope proteins of HIV-1 (7) or the earlier mentioned peptide from HSV-2 (5), as well as a number of different endogenous agonists (8). In addition to FPR, there are two other members of the formyl peptide receptor

5 Abbreviations used in this paper: GPCR, G-protein coupled chemoattractant receptors; CL, chemiluminiscence; FPR, formyl peptide receptor; FPRL1, FPR like-1; FPRL2, FPR like-2; KRG, Krebs-Ringer phosphate buffer; N-t-Boc-MLF, N-t-butoxycarbonyl-methylonyl-leucyl-phenylalanine; ROS, reactive oxygen species; sgG-2, secreted portion of HSV-2 glycoprotein G.

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family, formyl peptide receptor like-1 and -2 (FPRL1, FPRL2). The FPR family members are differently expressed in phagocytes, with neutrophils expressing FPR and FPRL1, and monocytes/macrophages expressing all three. A number of agonists that trigger cells through FPRL1 have been described whereas the monocyte-specific family member FPRL2 was until very recently considered an orphan receptor (9).

Ligation of the FPR family receptors with specific agonists (including gG-2p20 for FPR) results in oxygen radical generation by activation of the NADPH-oxidase, an electron transport system assembled in the plasma membrane during cell activation in both neutrophils and monocytes/macrophages (10). The primary products of the assembled oxidase are superoxide anions and hydrogen peroxide, and these reactive oxygen species (ROS) can function as regulators of other immune reactive cells, providing a molecular explanation behind the functional changes seen as apoptotic/necrotic processes in NK cells exposed to activated phagocytes (11–14).

The HSV-2 glycoprotein G, from which the proinflammatory peptide gG-2p20 was derived, is the only HSV-2 envelope protein to be cleaved post-translationally, generating two proteins of which one has the unique property of being secreted from the infected cells (sgG-2) (15–18). Previous to our study of the sgG-2-derived peptide, indicating proinflammatory activity, the secreted protein had not been ascribed a specific function. In this study, we have continued our investigation on the immunomodulatory properties of peptides derived from the sgG-2 amino acid sequence. In addition to the earlier identified neutrophil/monocyte activating peptide, we found one peptide, gG-2p19, which induced NADPH-oxidase activation in monocytes, but not neutrophils. Although the activity induced by gG-2p19 was monocyte specific and mediated through a pertussis toxin-sensitive GPCR, it was not triggered by FPRL2 (nor by FPR or FPRL1), which is the only member of the FPR family that is expressed solely in monocytes. Intriguingly, gG-2p19 was not a monocyte chemoattractant, suggesting that the receptor involved has a more restricted functional spectrum compared with other GPCRs.

Materials and Methods

Peptides and reagents

Three 15-mer synthetic peptides (Fig. 1A) from the secreted portion of the HSV-2 glycoprotein G (sgG-2) of strain HG52 (19) were synthesized as described earlier using F-moc synthesis chemistry (20) and were from two different sources, Kjos Ross-Petersen (used in Fig. 1) and GenScript (used in Figs. 2–8). The purity of the peptides was found to be ≥95% by reverse phase HPLC. The HSV-2 sgG-2 peptides were dissolved in either water, sodium bicarbonate (NaHCO3), 0.02 M in H2O), or DMSO to 6 mM and stored at 70°C until use.

The hexapeptides WKYMVM and WKYMVm, which were synthesized and HPLC purified by Kjos Ross-Petersen, and the formylated tripeptide formyl-methionyl-leucyl-phenylalanine (MLF), which was purchased from Sigma-Aldrich, were dissolved in DMSO (0.01 M in H2O), or DMSO to 6 mM and stored at 70°C until use. Subsequent dilutions of all peptides were made in Krebs-Ringer phosphate buffer (KRG (pH 7.3); 120 mM NaCl, 5 mM KCl, 1.7 mM KH2PO4, 8.3 mM NaH2PO4, and 10 mM glucose) supplemented with Ca2+ (1 mM) and Mg2+ (1.5 mM).

Cyclosporin H was provided by Novartis Pharma. Ficol-Paque was obtained from Amersham Biosciences N-t-butoxycarbonyl-methionyl-leucyl-phenylalanne (N-t-Boc-MLF), isologomin, and PMA were obtained from Sigma-Aldrich, and HRP was obtained from Boehringer Mannheim. The WRW4 was from GenScript. The gelosin-derived peptide FBPI0 (residues 160–169, as sequence ORLFOVKGR) was prepared by solid phase peptide synthesis and coupled to rhodamine as described earlier (21).

Separation of human monocytes

Human PBMC were separated from buffy coats from healthy blood donors. After Ficol-Paque centrifugation, mononuclear cells were separated into lymphocytes and monocytes using counter current centrifugal elutriation as described in detail elsewhere (22). This procedure yielded a fraction with >90% monocytes at a flow rate of 20–22 ml/min. The cells were washed and resuspended in KRG and stored on melting ice until use.

Separation of human neutrophils

Human peripheral blood neutrophils were isolated from buffy coats from healthy blood donors using dextran sedimentation and Ficol-Paque gradient centrifugation as described (23). The cells were washed and resuspended in KRG and stored on melting ice until use.

Stable expression of FPR, FPRL1, and FPRL2 in undifferentiated HL-60 cells

The stable expression of FPR, FPRL1, and FPRL2 in undifferentiated HL-60 cells has been described previously (24, 25). Transfected cells were cultured in RPMI 1640 (PAA Laboratories GmbH) containing FCS (10%) (PAA Laboratories GmbH), penicillin/streptomycin (1%) (PAA Laboratories GmbH), and G418 (1 mg/ml). The maximal density was maintained (PAA Laboratories GmbH), and G418 (1 mg/ml). The maximal density was maintained by guest on October 30, 2017 http://www.jimmunol.org/ Downloaded from

Measurement of superoxide release

The NADPH-oxidase activity was determined using isoluminol-ECL chemiluminescence (CL) (26). The CL activity was measured in a six-channel Biolumat LB 9505 (Berthold), using disposable 4-ml polypyrlylene tubes with a 400-μl reaction mixture containing 4 × 105 cells. The tubes were equilibrated in the Biolumat for 5 min at 37°C, after which the
stimulus (20 μl) was added. The light emission was recorded continuously, and the light intensity was measured 15 times per minute. The relation between the amount of superoxide produced and the amount of detected light is dependent on the luminometer, but it can easily be determined by a direct comparison of the superoxide dismutase-inhibitable reduction of cytochrome c with the integrated value of the superoxide dismutase-inhibitable chemiluminescence (27, 28). With the equipment used, 7.2 × 10^7 counts were found to correspond to the production of 1 nmol superoxide (using a millimolar extinction coefficient for cytochrome c of 21.1).

When experiments were performed with antagonists and inhibitors such as N-t-Boc-MLF, cyclosporin H, WRW4, or PBP10, these substances were included in the reaction mixture during the 5 min equilibration period. In desensitization experiments, the primary stimulus was added to the reagent tubes on ice or at 37°C, followed by incubation in the Biolumat for 10 min before addition of the second stimulus.

Monocyte chemotaxis

Monocyte chemotaxis was determined using ChemoTX multiwell chambers (Neuro Probe) with a membrane pore diameter of 5 μm, according to instructions provided by the manufacturer. Cells were allowed to migrate through the membranes toward stimulus added to the lower chambers during a 90-min incubation at 37°C, after which the cells in the lower chamber were lysed with 0.2% cetyltrimethylammonium bromide in PBS with 0.2% BSA. Twenty microliters of each lysate were transferred into a 96-well ELISA plate (MaxiSorp, Nunc) and quantified based on myeloperoxidase activity by the addition of 45 μl peroxidase substrate solution containing 0.1 mg/ml tetramethylbenzidine and 0.04% H₂O₂ in 0.2 M phosphate-citrate buffer (pH 5). The reaction was stopped after 2 h by the addition of 25 μl 1 M H₂SO₄ and the absorbance (OD) was measured at 450 nm.

Relative migration (%) was obtained by dividing the sample OD₄₅₀ with the OD₄₅₀ of cells added directly to the lower compartment of the multiwell chamber (representing 100% migration). Spontaneous movement, i.e., OD₄₅₀ values obtained from samples with no added stimulus, was used as negative control. As positive control, migration toward fMLF (10⁻⁸ M) and gG-2p20 (varying concentrations) were used. The OD₄₅₀ values used are means of duplicates.

Cytosolic calcium mobilization

Monocytes or undifferentiated HL-60 cells (2 × 10⁶/ml) stably expressing FPR, FPRL1 (26), or FPRL2 (29) were incubated with fura-2AM (2 μM)
in calcium free KRG supplemented with BSA (0.1%) at room temperature for 30 min in the dark. Cells were then diluted to twice the original volume with RPMI 1640 culture medium without phenol red (PAA Laboratories GmbH) and centrifuged. The cells were washed once and resuspended to a density of 2 × 10^7/ml in KRG containing 1.0 mM Ca^{2+}. Cells were equilibrated for 5 min at 37°C, after which gG-2p19 or positive control peptides (fMLF for FPR and WKYMVM for FPRL1 and FPRL2) were added. The fura-2 fluorescence was measured by a luminescence spectrometer (LS50B; PerkinElmer) using excitation wavelengths of 340 and 380 nm and an emission wavelength of 505 nm. Maximal and minimal fluorescence were determined in the presence of Triton X-100 and EGTA/Tris-HCl, respectively. The intracellular calcium levels are shown as Ex340/Ex380 ratios.

Results

An HSV-2 glycoprotein G-derived peptide triggers the monocyte NADPH-oxidase to produce superoxide anions

Through screening of peptides spanning the whole amino acid sequence of the secreted portion of HSV-2 glycoprotein G, we have...
previously identified a peptide, gG-2p20, that exerted downregulatory functions on human NK cells by activating phagocytes to produce ROS (5). In this study, we analyze two neighboring peptides derived from the same protein, overlapping the gG-2p20 sequence with 5 aa in either end of the peptide, and extended with 10 aa to the N-terminal side (gG-2p19) or the C-terminal side (gG-2p21) (Fig. 1A). When exposing neutrophils or monocytes to these peptides we found that whereas gG-2p20 activated a response in both monocytes and neutrophils (Fig. 1B), gG-2p19 activated a respiratory burst response in monocytes but was without effect on neutrophils (Fig. 1C). The gG-2p19-induced oxidase activation in monocytes was concentration dependent (Fig. 1C, inset). No respiratory burst activity was triggered by gG-2p21 in either of the cells (data not shown).

The NADPH-oxidase activity triggered in monocytes by gG-2p19 is pertussis toxin sensitive

The gG-2p19-induced production of ROS in monocytes (Fig. 1 and 2) with a time course resembling that induced by the earlier described FPR agonists gG-2p20 (Fig. 1 and (5)) and fMLF (Fig. 2; inset). The ROS production induced by gG-2p19 was inhibited by preincubation of the monocytes with pertussis toxin, known to specifically block signaling induced by Gi GPCRs (30), suggesting that also gG-2p19 induces activation through a GPCR.

The gG-2p19 is not chemotactic for monocytes

The signals generated by GPCRs in phagocytes usually mediate a chemotactic response. No such response was however triggered by the gG-2p19 peptide, irrespective of the concentration used to trigger the response (Fig. 3). The earlier-described FPR ligands fMLF and gG-2p20 were used as controls, both inducing a potent chemotactic response in monocytes (Fig. 3).

The gG-2p19 does not interact with FPR or FPRL1

The gG-2p19-induced NADPH-oxidase response closely resembled responses induced by other peptides (bacterial and synthetic) acting through different GPCRs, including those of the FPR family. This, together with the fact that the earlier-described HSV-2 peptide gG-2p20 is an FPR agonist, gave us cause to investigate the involvement of FPR family members in the gG-2p19-induced monocyte response. The basis for the first part of this investigation was desensitization protocols using gG-2p19, the FPR agonist fMLF, and the FPRL1/FPRL2 agonist WKYMVM. In these experiments we exploit the fact that agonist binding to a particular receptor induces receptor-specific desensitization and subsequent inability of the cells to respond to the same or a second agonist that ligates the same receptor (29).

Attempts to challenge gG-2p19-pretreated monocytes with the same agonist a second time failed, meaning that the HSV-2 peptide acts through a receptor that can be homologously desensitized (Fig. 4A). Cells pretreated with the FPR agonist fMLF were however still able to mount an oxidative response when stimulated with gG-2p19 (Fig. 4B), i.e., fMLF did not heterologously desensitize the cells to the HSV-2 peptide. Furthermore, preincubation of the cells with the FPR receptor antagonists N-t-Boc-MLF or...
cyclosporin H, both of which completely abrogate fMLF-induced responses (Fig. 5A), did not prevent the cells from responding to gG-2p19 (Fig. 5C), even though both N-t-Boc-MLF and cyclosporin H pretreatment did affect the magnitude of the gG-2p19-induced responses (Fig. 5C). Taken together, we conclude that FPR is not involved in the response to gG-2p19.

No substantial desensitization of the gG-2p19-induced activation could be seen in monocytes first treated with the FPRL1/FPRL2 agonist WKYMVM (Fig. 4C), suggesting that the peptide uses neither FPRL1 nor FPRL2. For FPRL1, this is supported by inhibition experiments using the FPRL1-specific antagonist WRW4 (Fig. 5B), which did not prevent a gG-2p19-induced response (Fig. 5C).

The gG-2p19 does not trigger any response in transfected cells expressing FPRL2

Because gG-2p19 was a potent activator of the NADPH-oxidase in monocytes (expressing FPR, FPRL1, and FPRL2) but not in neutrophils (which express FPR and FPRL1), FPRL2 was more plausible as a receptor for gG-2p19 than FPR and FPRL1. To determine whether gG-2p19 could activate cells specifically through FPRL2, we investigated the triggering potential of the peptide in HL-60 cells stably transfected to express one or the other of the three FPR family members. In accordance with our desensitization data, gG-2p19 could not trigger any transient Ca\(^{2+}\) response in cells expressing either FPR (Fig. 6A) or FPRL1 (Fig. 6B). The same was true also for FPRL2, i.e., gG-2p19 could not activate the FPRL2-expressing cells (Fig. 6C). Undifferentiated HL-60 cells transfected with an irrelevant receptor, CXC\(\text{R}2\), do not show a Ca\(^{2+}\) response to FMLF or WKYMVM (25). In contrast, gG-2p19 did induce a Ca\(^{2+}\) response in purified monocytes (Fig. 6D). All taken together, our data indicate that gG-2p19 interacts with a GPCR that is not a member of the formyl peptide receptor family.

The gG-2p19 activation of monocytes is inhibited by PBP10

We have recently shown that the signaling cascades induced in neutrophils by agonists binding to FPR and FPRL1 differ in their sensitivity to the gelsolin-derived, PIP2-binding peptide PBP10 (31). This membrane-permeable gelsolin-derived peptide specifically inhibits signaling through FPRL1. In an attempt to determine whether the signaling route induced by the gG-2p19 receptor was in any way similar to that induced by FPRL1, we investigated the inhibitory effect of PBP10 on the gG-2p19-induced response in monocytes. The inhibitory profile of PBP10 with respect to FPR and FPRL1 was the same as in monocytes as in neutrophils, i.e., the response induced in monocytes by WKYMVM (FPRL1/FPRL2 agonist) was completely inhibited in presence of PBP10 (Fig. 7A), whereas the response induced by fMLF (FPR agonist) was unaffected (Fig. 7B). Interestingly, the monocyte NADPH-oxidase response induced by gG-2p19 was substantially inhibited in the presence of PBP10 (Fig. 7C), indicating that the gG-2p19-binding GPCR shares the PBP10 sensitive signaling route with FPRL1.

Receptor cross-talk and hierarchy

There is a hierarchical receptor cross-talk within the phagocyte GPCR family. Cells desensitized to FPR or FPRL1 agonists are down-regulated not only in the response to their respective receptor-specific agonists but also to a second stimulation with the GPCR agonists IL-8 or platelet-activating factor (32, 33). The fact that desensitization of FPR or FPRL1 does not affect the gG-2p19-induced response suggests not only that monocyte activation by gG-2p19 does not involve the FPR family receptors, but also that the gG-2p19 receptor is not subordinated FPR or FPRL1. However, desensitization experiments, in which the order of the agonists was changed, revealed receptor cross-talk between the gG-2p19 receptor and FPR. We found that the FPR-triggered response was reduced when monocytes were desensitized with gG-2p19 before stimulation with fMLF (Fig. 8A). No such cross-talk or inhibition was seen when fMLF was replaced by the FPRL1 agonist WKYMVM (Fig. 8B). Our interpretation of these results is that activation of the gG-2p19 receptor cross-desensitizes the FPR, i.e., that the gG-2p19 receptor is hierarchically above the FPR. The fact that FPRL1 differs from FPR by being unaffected by desensitization of the gG-2p19 receptor gives support to the notion that these two receptors, although closely related, differ in their intracellular signaling.

Discussion

A peptide derived from the secreted portion of HSV-2 glycoprotein G has been identified that selectively activates monocytes through a pertussis toxin-sensitive receptor. The peptide, denoted gG-2p19, triggered activation of the superoxide-generating NADPH-oxidase, but was not able to induce monocyte chemotaxis. The main receptor candidate, FPRL2, the monocyte-specific member of the formyl peptide receptor family, was found not to be involved in cell activation. The peptide used a receptor distinct from the formyl peptide receptor family, but the receptor appeared to share at least one crucial step in its signaling pathway with one of the family members, FPRL1, based on the inhibition of the response to gG-2p19 by the PIP2-binding, gelsolin-derived peptide PBP10, known to block FPRL1 signaling (31).

The strategy used to identify gG-2p19 as a monocyte-activating peptide has been used earlier in the search for neutrophil-activating peptides from the HSV-2 glycoprotein G (5). We then identified gG-2p20 as a chemotactic peptide for both neutrophils and monocytes and we were also able to disclose the identity of the gG-2p20 binding receptor, which was the FPR. We originally used thirty-three 15-mer, partly overlapping peptides covering the entire secreted portion of the HSV-2 glycoprotein G. The fact that the only two proinflammatory peptides, gG-2p19 and gG-2p20, originate from the same part of the protein and contain an identical sequence of five amino acids, suggested to us that they should bind to two closely related receptors.

Because gG-2p19 did not activate neutrophils, the receptor could be differently expressed in neutrophils and monocytes, which made FPRL2 the most obvious candidate. The FPRL2 was originally cloned from a promyelocyte cDNA library using low-stringency hybridization with the FPR cDNA as a probe (34, 35), and it was for a long time regarded as an orphan receptor. In contrast to the other two members of the receptor family (FPR and FPRL1), the expression of FPRL2 is restricted to cells in the monocyte/macrophage lineage.

From our data we conclude, however, that the activating signal in monocytes induced by gG-2p19 is not transduced through FPRL2. This conclusion is based on the facts that no signal is generated by gG-2p19 in HL-60 cells transfected to express FPRL2 and that the FPRL2 agonist WKYMWM did not desensitize monocytes to the gG-2p19 peptide. The fact that no activity is induced in FPRL2-expressing HL-60 cells by gG-2p19 strongly indicates that another monocyte-specific receptor is involved, but desensitization or binding inhibition experiments with an FPRL2-specific ligand would of course add strength to the conclusion. Such an agonist has been described (9). We have tried to use the FL2 peptide (with the sequence MLGMIKNSLFGSVETWPWQVL), however, in our experimental systems with FPRL2-expressing cells or monocytes this peptide was without effect (our unpublished data). We can at present only speculate on the reason for this but it has been shown that FL2 induces a strong response.
in around 50% of the blood cell donors suggesting that we might have obtained all our samples from nonresponders. More importantly, however, is that these cells still responded to gG-p2–19. We cannot at present explain why FL2 fail to trigger a response in FPRL2 expressed in HL-60 cells, but the receptor might look a bit different when expressed in another cell line such as cho-K1 cells. Even though the identity of the gG-2p19 receptor remains to be disclosed, our results suggest that it is a receptor distinct from FPRL2.

However, we have clues to some characteristics of the gG-2p19 receptor. Firstly, the receptor is a pertussis toxin-sensitive receptor. Pertussis toxin inhibits the G_i group of GPCRs in phagocytes, and receptor. Firstly, the receptor is a pertussis toxin-sensitive receptor. Secondly, the unknown receptor shares signaling properties with FPRL1 in that it is sensitive to the inhibitory activity of BPB10, a membrane-permeable polypeptide-binding peptide derived from the cytoskeletal protein gelsolin (21). We have previously shown that BPB10 blocks FPRL1-induced ROS secretion in neutrophils, while having no effect on the neutrophil response to agonists for FPR, C5aR, PAFR, or CXCR3 (31). The precise mechanism by which BPB10 selectively interferes with signaling by some receptors but not by others remains to be determined in detail, but it is reasonable to believe that the signaling intracellular parts of the two sensitive receptors, FPRL1 and the gG-2p19 receptor, contain structural similarities.

Thirdly, the gG-2p19 receptor cross-talks with other phagocyte GPCRs. The process known as desensitization (homologous as well as heterologous) is most probably important for the limitation or termination of the cellular response to high concentrations of a chemoattractant, avoiding prolonged activation and by that limiting the inflammation-associated tissue damage at an inflammatory site (8). With respect to cross-talk hierarchy, it is obvious that some activators are strong whereas others are weak. The fact that ligation of the gG-2p19 receptor desensitized the hierarchically strong FPR, indicates that the novel receptor is even stronger. Further desensitization studies have to be performed to more precisely place this receptor in the GPCR hierarchy. It should also be kept in mind that yet other gG-2p19 receptor(s) may be present that is responsible for inhibitory signal(s). We have shown earlier this to be the case for a peptide derived from the N terminus of annexin I, a peptide that triggers NADPH-oxidase activation through one receptor, comconitantly with eliciting inhibitory signals through another receptor (38).

We can only speculate on whether gG-2p19 could exert any effects in vivo. However, there should be high levels of sGg-2 at sites with massive local viral replication, i.e., in herpetic lesions, where >10^{10} viral particles are found to be present (5). In such an environment, gG-2p19-induced ROS has the potential to eliminate viral particles, but could also lead to local tissue destruction, enhancing the damage induced by the virus itself. However, ROS secreted from neutrophils and monocytes also have a strong negative impact on cell-mediated immunity. Numerous studies have shown that both the cytotoxic capacity and the survival of NK cells and CTL are impaired in the presence of activated phagocytes, in a process that is reversed by ROS scavengers (11–14). Thus, sGg-2 released from HSV-2-infected cells could, through its interaction with a GPCR (as suggested in this study and in (5)) and subsequent activation of the NADPH-oxidase, contribute to the documented down-regulation of the cellular immune response seen during HSV infection (39–41).

In summary, we have identified a peptide derived from HSV-2 glycoprotein G that induces release of ROS from monocytes through binding to a GPCR. In vitro experiments showed that the gG-2p19 peptide did not induce a chemoattractive response, indicating that the receptor involved has a more restricted functional spectrum than other GPCRs. Furthermore, we were able to show that the gG-2p19-interacting GPCR shares at least one common signaling pathway with FPRL1 because both these receptors, as opposed to, for instance, FPR, are sensitive to the PI_3K-specific gelsolin-derived peptide PBP10. This is of considerable interest for further elucidation of chemoattractant signal transduction, because FPRL1 is a chemoattractant receptor whereas the gG-2p19-binding GPCR is not.

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Disclosures
The authors have no financial conflict of interest.

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