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Short communication

Chemokine scavenging by the human cytomegalovirus chemokine decoy receptor US28 does not inhibit monocyte adherence to activated endothelium

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Abstract

The human cytomegalovirus has found smart ways to exploit the chemokine network in order to subvert immune attack. Chemokines trigger the arrest and firm adhesion of inflammatory cells to the vascular wall. Scavenging of chemokines by viral decoy receptors, such as US28, might prevent arrest of leukocytes to the vascular wall and impair an antiviral immune response. We determined the effect of chemokine scavenging by endothelium-expressed signaling mute US28 (US28R129A) on static monocyte adhesion. Despite the chemokine scavenging capacity of US28R129A, expression of this construct by endothelial cells was insufficient to disrupt leukocyte adhesion to cytokine-activated monolayers. Our results suggest that the concentrations of chemokines that trigger firm leukocyte adhesion are too high to be efficiently scavenged by viral chemokine decoy receptors like US28. From the results of this experimental model a role for US28 in viral immune evasion by chemokine scavenging would appear therefore unlikely.

Keywords: Immune evasion; Human cytomegalovirus; Leukocyte adhesion; Chemokine receptor; Chemokine

Chemokines are important mediators of immunity against viral infections. They activate cytotoxic T lymphocytes and enhance the recruitment of inflammatory cells (Mahalingam et al., 2003; Salazar-Mather and Hokeness, 2003). Scavenging of chemokines by virus-encoded decoy receptors is a strategy to impair leukocyte recruitment and subsequent antiviral activity. The human cytomegalovirus gene US28 encodes a chemokine receptor homolog that internalizes β-chemokines with high efficiency in vitro (Billstrom et al., 1999; Bodaghi et al., 1998; Randolph-Habecker et al., 2002). Immobilized chemokines on the apical surface of activated endothelial cells trigger leukocytes to become adherent by inducing rapid integrin avidity and affinity changes (Alon and Feigelson, 2002; Johnston and Butcher, 2002). In monocytes, β-chemokines, such as MCP-1/CCL2, MIP-1α/CCL3 and RANTES/CCL5, increase the expression of β2 (CD18) integrins and facilitate adherence (Vaddi and Newton, 1994). A chemokine gradient directs extravasated inflammatory cells towards the site of infection. Although chemokine scavenging was sufficient to reduce monocyte chemotaxis in vitro (Randolph-Habecker et al., 2002), the effect of chemokine scavenging by HCMV-infected endothelial cells on leukocyte arrest is not known.

In addition to chemokine scavenging, US28 also constitutively activates pro-inflammatory transcription factors such as NF-κB and CREB (Casarosa et al., 2001; Waldhoer et al., 2002), which might upregulate the expression of adhesion molecules, cytokines and chemokines that enable leukocyte adhesion. Expression of US28 in vascular smooth muscle cells results in a chemotactic response towards RANTES/CCL5 and MCP-1/CCL2 gradients (Streblow et al., 1999). The role of US28 in the viral life cycle remains unclear. More likely, chemokine scavenging and signaling are two different activities of US28 that occur simultaneously. The significance of signaling and scavenging by US28 may differ between different phases of the viral life cycle. Evasion of antiviral immune responses enables the virus to persist, whereas

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for virus replication and dissemination immune activation is indispensable.

We aimed to investigate the effect of chemokine scavenging by US28 on leukocyte arrest. To exclude the influence of US28 signaling, we constructed a mutant receptor US28R129A that did not activate NF-κB (data not shown). Chemokine internalization was measured in confluent monolayers of CHO-k1 cells that were grown in 24 well plates. At 24 h after transfection, 44 nCi of ^{125}I-labeled RANTES/CCL5 was added to each well and incubated for 30, 60, 90 and 120 min at 37 °C. To remove cell surface-bound chemokines, cells were washed with 0.01 M phosphate buffer followed by incubation with 200 mM acetic acid/50 mM NaCl, pH 2.5, for 5 min. Subsequently cells were lysed by addition of 0.5 M NaOH. Total activity in the cell lysate was quantified in an LKB multichannel counter. We found for both constructs that RANTES/CCL5 is rapidly sequestered within the intracellular fraction (Fig. 1A). The US28R129A appears to internalize RANTES/CCL5 more efficiently than the wild type US28. The uptake of RANTES/CCL5 is temperature-sensitive as no uptake was found after incubation for 90 min at 4 °C. This indicates that chemokine uptake depends on active transport mechanism, and is not due to spontaneous diffusion of the protein (Fig. 1B). The observation that US28 rapidly internalizes chemokines and that mutation of the DRAI motif blocks G protein-mediated signaling is in concordance with published data (Michelson et al., 1997; Waldhoer et al., 2003). The US28R129A construct appeared to be slightly more efficient in chemokine internalization than the wild type receptor. This might reflect an increased recycling rate of the signaling mute receptor (Waldhoer et al., 2003) as transfection efficiencies were comparable for both constructs (∼10%, data not shown).

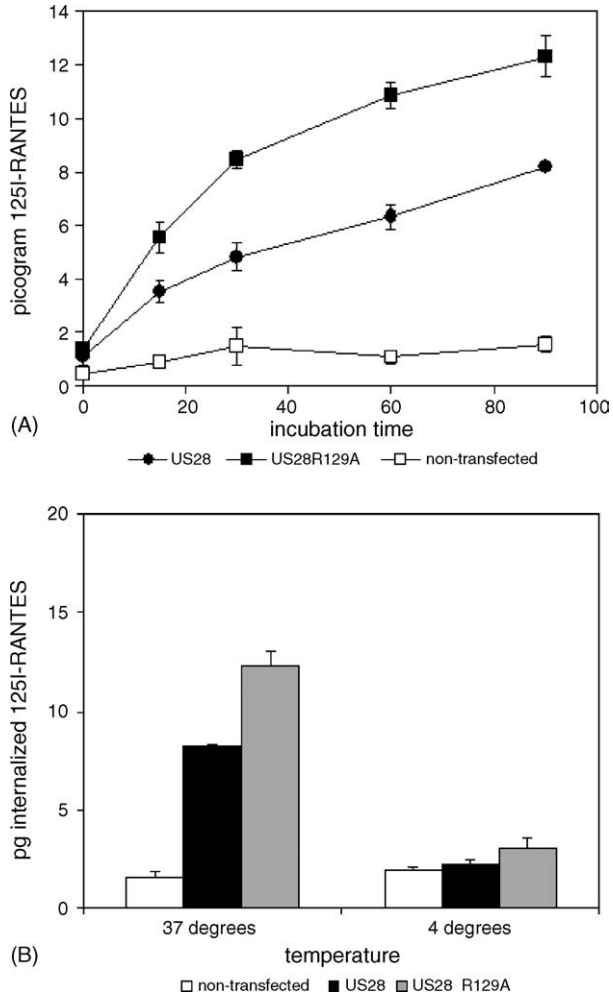

Fig. 1. RANTES binding and internalization by transfected CHO-k1 cells. Per 12 wells approximately 100 pg of ^{125}I-RANTES was added. Activity of the internalized fraction was measured over time. Cells were incubated at 37 °C for 90 min (A). Furthermore, the ligand uptake was measured at 37 °C and 4 °C for 90 min (B). Indicated are the average and S.E.M. of three independent triplicate measurements.

For leukocyte adhesion studies, we selected the human pre-monocytic cell line MonoMac-6 as this cell line responds to β-chemokines (Cross et al., 1997), such as RANTES/CCL5. MonoMac-6 is also well characterized in a static adhesion assay (Erl et al., 1995). Furthermore, MonoMac-6 cells express the integrin Mac-1 that interacts with ICAM on endothelial cells that is required for sustained adherence (Erl et al., 1995). As a model for endothelial cells, we used primary cell cultures of human umbilical vein endothelial cells (HUVEC) in which we expressed the US28 gene through retroviral transduction. For the construction of retroviruses we used the bicistronic LZRS–linker–IRES–GFP vector in which the gene of interest was linked to a downstream internal ribosomal entry site (IRES) and the green fluorescent protein (GFP) marker gene that allowed independent translation of the products of both genes in the transduced target cells (Heemskerk et al., 1997). Recombinant retroviruses were produced after transfection of the 293T-based ΦNX-A amphotropic packaging cell line (kindly provided by Dr. G. Nolan, Stanford University, Palo Alto, CA) with the LZRS–US28R129Amyc–IRES–GFP. HUVECs were grown to subconfluency and incubated for 18 h with undiluted retrovirus-containing supernatant containing 5 μg/ml polybrene (Sigma–Aldrich). Efficiency of transduction was determined by measuring the number of GFP-positive cells by flow cytometry. Previously, it was shown that expression of US28 in HUVEC resulted in efficient chemokine internalization (Billstrom et al., 1999). The static adhesion assay was performed according to a protocol adapted from Molema et al. (1998). At 7 days after transduction, subconfluent monolayers of HUVECs were grown in 24 well plates (Corning, Cambridge, MA) and stimulated with 20 ng/ml TNFα (Boehringer Ingelheim GmbH, Basel, Swiss) overnight. For fluorescent staining, MonoMac-6 cells were loaded with 0.8 μg/ml of 1,1′-dioctadecyl3,3,3′,3′-tetramethylindocarbocyanide perchlorate (DiI) (Molecular Probes, OR) at 37 °C for 1 h in 5% CO_2 atmosphere and intensively washed before use. To each well 0.25×10^6 MonoMac-6 cells were added. After incubation at 37 °C for 30 min, adherent cells were washed three times using pre-warmed PBS. HUVECs and adherent MonoMac-6 cells were harvested by adding trypsin/EDTA solution (0.5/0.2 mg/ml; Sigma–Aldrich); and short incubation at 37 °C. After adding 25 μl of Flow Count Fluorospheres (Coulter Corporation, Miami) samples were analyzed by flow cytometry (Coulter EPICS Elite Flow Cytometer, Coulter Corporation). Based on their fluorescence, the number of