Dengue Virus Induces Expression of CXC Chemokine Ligand 10/IFN-γ-Inducible Protein 10, Which Competitively Inhibits Viral Binding to Cell Surface Heparan Sulfate

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Dengue virus is an arthropod-borne flavivirus that causes a mild febrile illness, dengue fever, or a potentially fatal syndrome, dengue hemorrhagic fever/dengue shock syndrome. Chemokines primarily orchestrate leukocyte recruitment to the areas of viral infection, which makes them critical mediators of innate and acquired immune responses. In the present study, we investigated the induction and function of chemokines in mice early after infection with dengue virus in vivo. We found that CXCL10/IP-10 expression was rapidly and transiently induced in liver following infection. The expressed CXCL10/IP-10 likely mediates the recruitment of activated NK cells, given that anti-CXCL10/IP-10-treated mice showed diminished NK cell infiltration and reduced hepatic expression of effector molecules in activated NK cells after dengue virus infection. Of particular interest, we found that CXCL10/IP-10 also was able to inhibit viral binding to target cells in vitro. Further investigation revealed that various CXCL10/IP-10 mutants, in which the residues that mediate the interaction between the chemokine and heparan sulfate were substituted, failed to exert the inhibitory effect on dengue binding, which suggests that CXCL10/IP-10 competes with dengue virus for binding to heparan sulfate on the cell surface. Moreover, subsequent plaque assays showed that this inhibition of dengue binding blocked viral uptake and replication. The inhibitory effect of CXCL10/IP-10 on the binding of dengue virus to cells may represent a novel contribution of this chemokine to the host defense against viral infection.

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The mosquito-borne human pathogen dengue virus (DV) is a single-stranded, positive RNA flavivirus prevalent in tropical and subtropical areas of the world. Infection of 50–100 million individuals each year, DV infection has a high mortality rate among children (1–3). Individuals with a primary infection usually develop dengue fever, an acute febrile illness accompanied by debilitating headache and myalgia; however, a life-threatening syndrome, dengue hemorrhagic fever/dengue shock syndrome, frequently occurs after a second DV infection due to a virus-specific serotype-cross-reactive immune response that elicits Ab-mediated enhancement (4–6) and inappropriate T cell activation (7). Symptoms of dengue hemorrhagic fever/dengue shock syndrome include high fever, plasma leakage, and multifac-
hepatic expression and function of chemokines early after infection. We found that among the chemokines studied, CXCL10/IFN-γ-inducible protein 10 (IP-10) was the most prominent and that it was rapidly and transiently induced following DV infection. The possible function of CXCL10/IP-10 under these circumstances was investigated both in vitro and in vivo.

Materials and Methods

Mice

Specific pathogen-free C57BL/6 mice originally from The Jackson Laboratory were purchased from the National Laboratory Animal Center (Taipei, Taiwan). All mice used for the experiments were 4–6 weeks old and were housed under specific pathogen-free conditions at the Institute of Biomedical Sciences, Academia Sinica (Taipei, Taiwan). Mouse handling and all experimental procedures were in accordance with the guidelines of the Institutional Animal Care and Utilization Committee at Academia Sinica.

Cell culture

Hepa-1 cells, a mouse hepatoma cell line, were obtained from the American Type Culture Collection (ATCC) and cultured in DMEM supplemented with 10% FBS (HyClone) and 2 mM glutamine. Both cell types were maintained at 37°C in a 5% CO2 incubator. The C6/36 mosquito cell line was obtained from Aedes albopictus cells. C6/36 mosquito cells were cultured in RPMI 1640 supplemented with 10% FBS at 28°C in the presence of 5% CO2.

Virus preparation

Mouse-adapted, neurovirulent DV type 2 (strain New Guinea C-N) was kindly provided by Dr. Ching-Juh Lai (National Institutes of Health, Bethesda, MD) and was propagated in C6/36 cells. Virus type 2 strain 16681 (35) was used for in vivo neutralization experiments and also was propagated in C6/36 cells. The cells were collected, resuspended in medium containing 2% FBS in a centrifuge tube, and infected at a multiplicity of infection (MOI) of 0.1 at 28°C. After exposure to the virus for 2 h, the cells were centrifuged, and the virus-containing supernatant was removed. The cells were then resuspended in medium containing 5% FBS and cultured at 28°C. Culture supernatants containing the virus were harvested every 2–3 days, and fresh medium was added until the infected cells fully expressed the cytopathic effect. Then after removing cell debris by centrifugation, the virus-containing supernatants were frozen and stored at −80°C until used. Virus titers were determined using plaque assays with BHK-21 cells.

DV infection

For in vivo studies, mice were infected i.v. with DV at a dose of 3–10 x 103 PFU in a volume of 0.5 mL. For in vivo neutralization experiments, mice were infected i.p. with 250 µg of anti-CXCL10/IP10 Ab (mouse IgG1) provided by Dr. Thomas Lane or control Ab on the day before infection; mice were then infected with 104 PFU of DV-2 strain 16681. For in vitro experiments, Hepal-1 cells were initially grown to 30% confluence and maintained, and resolved on 5% sequencing gels, which were dried and subjected to autoradiography.

Real-time PCR analysis

Total RNA was isolated from liver using TRIzol (Invitrogen Life Technologies), after which a 5-µg sample was reverse transcribed using an oligo(dT)20 primer and SuperScript II reverse transcriptase (Invitrogen). For real-time PCR analysis, the 2ΔΔCt method was used to quantify the relative changes in gene expression (36). Real-time PCR for IFN-γ, perforin, granzyme A and B, and GAPDH was conducted using Assays-on-Demand Gene Expression products (Applied Biosystems), which consisted of a 20× mix of unlabeled PCR primers and TaqMan MGB probe (FAM dye-labeled). The PCR cycling protocol entailed 1 cycle at 50°C for 2 min and then 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 60 s. The resultant PCR products were measured and elaborated using an ABI Prism 7700 Sequence Detection System (Applied Biosystems). All samples were run in duplicate. All quantifications were normalized to the level of GAPDH gene expression. Analysis of the relative expression levels required calculations based on the threshold cycle (CT), the fractional cycle number at which the amount of amplified target reaches a fixed threshold as follows: ΔCT was calculated as the difference between the mean CT values of samples evaluated using specific primers and the mean CT values of the same samples evaluated with GAPDH-specific primers; ΔΔCT was calculated as the difference between the ΔCT values of the samples and that of the calibrator sample; 2−ΔΔCT was the relative change in mRNA levels represented by the fold induction over control. To verify that the efficiencies of the primers for the target and reference were approximately equal, we serially diluted the template and conducted real-time PCR for each target gene. We then plotted log input template vs ΔCT and calculated the slope of the resultant linear function. We found that the slope for each gene was <−0.1 (~−0.0887 for perforin; 0.009 for granzyme A; −0.094 for granzyme B), which indicates that the efficiencies of the primers targeting the genes and the reference were approximately equal.

ELISA

Livers were weighed and homogenized on ice in protein extraction buffer (Biochain Institute) using a tapered tissue grinder (Wheaton Scientific Products). The resultant homogenates were centrifuged at 15,000 x g for 20 min at 4°C, after which the supernatants were collected and assayed for CXCL10/IP-10 using an ELISA. CXCL10/IP-10 levels in serum, liver homogenate and culture supernatant were determined using a purified unconjugated capture mAb (clone 134013) and a biotinylated detection Ab (biotin-conjugated goat anti-mouse CRG-2 polyclonal Ab). The Ab pairs for CXCL10/IP-10 were purchased from R&D systems.

Isolation of intrahepatic monoclonal cells

Liver was perfused with ice-cold PBS, homogenized in RPMI 1640 containing 5% FBS, and centrifuged. The pellet was resuspended in 10 ml of buffer containing collagenase and DNase I in RPMI 1640 and incubated for 30 min at 37°C. After digestion, the pellet was centrifuged and resuspended in 4 ml of HBSS. The cell suspension was overlaid onto 3 ml of Ficoll-Paque (Amersham Biosciences) and centrifuged at 900 x g for 10 min at room temperature. After centrifugation, the mononuclear cells in the interface were collected and washed three times with HBSS. The cells were then stained with anti-mouse DX5-FITC (clone DX5; BD Biosciences) and anti-CD3-PE (clone 145-2C11; BD Biosciences) followed by FACS (FACSCalibur; BD Biosciences).

Northern analysis

Twenty micrograms of total RNA extracted from Hepal-1 cells were separated on denaturing formaldehyde gels and transferred to nitrocellulose membranes. Mouse CXCL10/IP-10 gene cloned into pBlueScript KS (37), kindly provided by Dr. Joshua Farber (National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD), was used as the probe for hybridization. The probe was generated using the random primer method (Stratagene), and the hybridization was conducted as described previously (38). Hybridizations with an oligonucleotide probe for 18S rRNA were also as described (39).

Analysis of virus binding by flow cytometry

Hepal-1 cells (105) were suspended in 50 µl of buffer containing HBSS supplemented with 1% FBS and 10 mM HEPES (pH 7.4) and incubated with DV (MOI 1) on ice for 2 h in the presence of various concentrations of anti-CXCL10/IP-10 mAb. Cells were then fixed in 2% paraformaldehyde (PFA) for 10 min, washed in PBS, and resuspended in 50 µl of phosphate buffered saline (PBS) containing 1% FBS, 1% BSA, and 0.1% sodium azide. One of the following Abs was used for each experiment: anti-CXCL10/IP-10 mAb (clone 134013) and a biotinylated detection Ab (biotin-conjugated goat anti-mouse CRG-2 polyclonal Ab). This was followed by fixing the cells in 4% paraformaldehyde (PFA) for 15 min, followed by washing the cell in 2% PFA-PBS. After 30 min, the cells were incubated with Alexa Fluor 647-conjugated capture mAb (clone 134013) and a biotinylated detection Ab (biotin-conjugated goat anti-mouse CRG-2 polyclonal Ab). The Ab pairs for CXCL10/IP-10 were purchased from R&D systems.

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Dr. Andrew Luster (Harvard Medical School, Boston, MA). After the incubation, the cells were washed three times to remove unbound virus and labeled for 1 h at 4°C with a rabbit Ab (1/2000 dilution) against DV envelope protein (kindly provided by Dr. Wen Chang, Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan) or an isotype Ab. Then after three additional washes, the cells were incubated for 1 h at 4°C with PE-conjugated goat anti-rabbit Ab (1/2000 dilution), washed, and fixed in buffer containing 0.8% paraformaldehyde. DV binding to Hepa1-6 cells was then analyzed using a FACS Caliber flow cytometer (BD Biosciences). Specific DV binding in the absence of chemokine was set to 100%.

**Plaque assays**

Hepa1-6 cells were plated in 96-well plates to a density of 10^4 cells/well and then incubated in DMEM supplemented with 10% FCS. After the cells were washed with binding buffer (HBSS, 1% FBS, 10mM HEPES), they were incubated with DV (MOI 5) for 2 h at 4°C, with or without heparin (Sigma-Aldrich) or CXCL10/IP-10 (PeproTech), and then washed three times with RPMI 1640. The cells were then scraped from the plates, resuspended in serum-free medium, and ruptured by trituration using a syringe with a 26-gauge needle. After removal of the cell debris, the remaining lysates were placed onto BHK-21 monolayers in 6-well plates and allowed to incubate for 2 h at 37°C in a 5% CO2 incubator. The supernatants were then removed. 5 ml of 1% SeaPlaque Agarose (Cambres) in RPMI 1640 containing 2% FBS were overlaid on the BHK-21 monolayer, and the cultures were then incubated for 5 days to develop the plaques. The top agarose was then removed, and the infected BHK-21 cells were stained with 0.125% crystal violet in 145 mM NaCl plus 50% ethanol to visualize the plaque for counting.

**Statistical analysis**

The Mann-Whitney U test was used to assess the significance of the observed difference between two groups. p < 0.05 was considered significant.

**Results**

**Infection with DV i.v. predominantly induces CXCL10/IP-10 expression**

To determine which chemokines are important in DV infection, we infected mice i.v. with the virus and then analyzed chemokine expression using RPAs. DV infection led predominantly to induction of CXCL10/IP-10 mRNA expression in liver, which peaked 12 h postinfection (Fig. 1A). This finding led us to test whether there were corresponding increases in the expression of the encoded protein and, consistent with the time-dependent increase in transcription, levels of CXCL10/IP-10 protein were correspondingly increased in both liver (Fig. 1B) and serum (Fig. 1C) following DV infection. In addition, CCL1/TCA-3, CCL5/RANTES, CCL3/MIP-1α, CCL4/MIP-1β, and CCL5/RANTES mRNAs were also induced by DV infection, but to a much lesser degree than CXCL10/IP-10 mRNA.

**DV infection induces expression of effector molecules in activated NK cells**

CXCL10/IP-10 serves as a chemoattractant mainly for activated T and NK cells (41–43), the latter of which play a key role in innate immunity to viral infection (44). Therefore, given our observation that CXCL10/IP-10 is rapidly and transiently induced in the liver following DV infection (Fig. 1), we hypothesized that this chemokine may be responsible for recruiting the activated NK cells that produce the effector molecules needed for clearance of the virus. To test that idea, we measured hepatic levels of IFN-γ, perforin, granzyme A, and granzyme B, all of which are expressed by activated NK cells. Using real-time PCR, we found that expression of all of these molecules was up-regulated 12 h postinfection and that their levels peaked 24 h postinfection (Fig. 2), which suggested that CXCL10/IP-10 might play a role in the recruitment of activated NK cells to the sites of DV infection.

**CXCL10/IP-10 inhibits the binding of DV to cells by competing for heparan sulfate on the cell surface**

CXCL10/IP-10 is a basic protein that interacts with both heparin and heparan sulfate (40, 45), the latter of which is the putative receptor for DV (46–49). We therefore hypothesized that CXCL10/IP-10 may competes with the virus for binding to heparan sulfate on the cell surface, thereby inhibiting viral entry and replication. To test this idea, we initially verified that DV infection induces CXCL10/IP-10 transcription in Hepa1-6 cells and noted that CCL5/RANTES mRNA was also induced, but to a lesser extent (Fig. 4A). Using an ELISA, we then found that secretion of CXCL10/IP-10 protein from Hepa1-6 cells was significantly increased following DV infection (Fig. 4B). This induction of CXCL10/IP-10 required replication-competent virus, as heat- or

**Depletion of CXCL10/IP-10 reduces expression of effector molecules by activated NK cells**

To assess the extent to which CXCL10/IP-10 actually mediates recruitment of activated NK cells, mice were treated with neutralizing anti-CXCL10/IP-10 Ab for 24 h before i.v. infection with DV; numbers of intrahepatic NK cells and levels their effector molecules were then assessed 24 h postinfection. Treatment mice with anti-CXCL10/IP-10 significantly reduced the numbers of intrahepatic NK cells (p = 0.22) (Fig. 3, left) and the expression of perforin, granzyme A, and granzyme B (p = 0.034, p = 0.058, and p = 0.037, respectively) (Fig. 3, right). These results indicate that CXCL10/IP-10 is critical for recruiting activated NK cells to sites of DV infection, where they produce effector molecules needed for viral clearance.
UV-irradiated virus failed to induce CXCL10/IP-10 expression (Fig. 4C).

We then tested whether CXCL10/IP-10 is able to inhibit DV binding to Hepa1-6 cells. After exposure to DV for 2 h in the presence of various concentrations of CXCL10/IP-10, the cells were incubated first with an Ab against the DV envelope protein and then with a PE-conjugated secondary Ab and subjected to FACS analysis. The results showed that CXCL10/IP-10 dose-dependently inhibited DV binding to Hepa-6 cells with an IC50 of 5.6 μg/ml (Fig. 5A). Campanella et al. (40) characterized the amino acid residues of CXCL10/IP-10 that mediate the binding of the chemokine to CXCR3 and/or heparin. They found that Arg22 has the highest specificity for binding to heparin, whereas basic residues along the C-terminal helix (Lys71, Arg72, Lys74, and Arg75) also bind to heparin, mainly through an electrostatic effect (40). Bearing that in mind, we tested whether the inhibitory effect of CXCL10/IP-10 on DV binding to cells reflected competition for heparan sulfate on cell surface by examining the effect of a set of CXCL10/IP-10 mutants. We found that substituting Arg22 with either Ala or Glu (R22A and R22E) abolished the ability of CXCL10/IP-10 to inhibit DV binding (Fig. 5A, b and c). Similarly, mutation of the aforementioned four basic C-terminal residues (K71E/R72Q-K74Q/R75E; C-t mut) also abolished the ability of CXCL10/IP-10 to inhibit DV binding to cells (Fig. 5A, d), as did substitution of all five residues (C-tR22A; Fig. 5Ae). By contrast, R8A substitution, which abolishes binding to CXCR3, but not heparin (40), had no effect on the ability of CXCL10/IP-10 to inhibit DV binding (Fig. 5Af). CXCL10/IP-10 thus appears to inhibit DV binding by competing with the virus for heparan sulfate on the cell surface.
The inhibition of DV binding to heparan sulfate by CXCL10/IP-10 was further confirmed using plaque assays, in which heparin served as a positive control. Hepa1-6 cells were incubated with DV (MOI 1) for 2 h in the presence of various concentrations of CXCL10/IP-10 (a) or one of its mutant forms (b–f). The cells were then washed three times and incubated with anti-DV envelope Ab followed by a PE-conjugated secondary Ab. After three washes, the virus bound to cells was analyzed by flow cytometry. Shown are the relative levels of specific DV binding; specific binding in the absence of chemokine was set to 100%. Data are representative of four independent experiments and are presented as means ± SEM of duplicate assays. B, Inhibition of DV binding by CXCL10/IP-10 reduces viral replication. Hepa1-6 cells were incubated with DV (MOI 5) for 2 h, with or without heparin (10 μg/ml) or CXCL10/IP-10 (10 μg/ml). The cells were then washed and ruptured, and the resultant cell lysates were subjected to plaque assay as described in Materials and Methods; the percent of viral replication = (number of plaques produced with DV + heparin or CXCL10/IP-10/ number of plaques produced with DV only). Data are means ± SEM from four independent experiments. *, p < 0.05 compared with the cells infected with DV only.

CXCL9/Mig and CXCL10/IP-10, but not CXCL11/I-TAC, inhibit DV binding to cells

CXCL9/Mig, CXCL10/IP-10, and CXCL11/I-TAC are all IFN-γ-inducible CXC chemokines and share the same receptor, CXCR3. Given that CXCR3 ligands have been shown to have similar biological functions in vitro, we tested whether the ability of CXCL10/IP-10 to inhibit DV binding to Hepa-6 cells was shared by other CXCR3 ligands. Using binding assays, we found that CXCL9/Mig also exerted an inhibitory effect on virus binding to Hepa-6 cells and was slightly more potent than CXCL10/IP-10 (IC₅₀ = 4.3 μg/ml for CXCL9/Mig vs IC₅₀ = 5.9 μg/ml for CXCL10/IP-10; Fig. 6). By contrast, CXCL11/I-TAC had no effect on DV binding to Hepa-6 cells (Fig. 6). Because infecting Hepa-6 cells with DV also induced expression of CCL5/RANTES, although to a lesser degree than CXCL10/IP-10 (Fig. 4A), we tested the ability of CCL5/RANTES to inhibit DV binding to cells. We found that although CCL5/RANTES was reported to interact with glycosaminoglycan (GAG; Refs. 50 and 51), CCL5/RANTES failed to inhibit DV binding (data not shown), suggesting that the inhibitory effect of CXCL10/IP-10 and CXCL9/Mig on DV binding to Hepa-6 cells is specific to those chemokines.

Discussion

CXCL10/IP-10, a non-ELR CXC chemokine and mouse homologue originally named Crg-2 (37), is a potent chemoattractant for activated T and NK cells (41, 42), and induction of its expression is seen in a variety of viral infections (25, 26). Studies with CXCL10/IP-10-deficient mice or Ab neutralization revealed that
CXCL10/IP-10 is involved in effector T cell recruitment into inflammatory sites in mice with viral infections (52–56). In the present study, we found that, in vivo, the induction of CXCL10/IP-10 occurred very early after DV infection and was transient: levels peaked ~12 h postinfection, returning to basal levels within <48 h. We also found that expression of several molecules (perforin, granzymes A and B, IFN-γ) known to be produced by activated NK cells was induced. Taken together, these findings suggest that the early induction of CXCL10/IP-10 during DV infection plays a key role in innate immunity through the recruitment and activation of NK cells. Consistent with this idea, one recent study showed that by coordinating innate immune responses, CXCL10/IP-10 mediates the defense following corona-virus infection of the CNS; i.e., decreased disease development and increased survival was associated with a significant increase in the recruitment of activated NK cells by CXCL10/IP-10 within the CNS (57).

That CXCL10/IP-10 expression was induced rather early (within 6 h) and its induction required live virus leads us to postulate that induction of CXCL10/IP-10 is not a secondary effect mediated, for example, via induction of TNF-α or IFNs. It is therefore likely that DV RNA and/or DV protein(s) are directly involved in the induction of CXCL10/IP-10. For instance, one recent study showed that the DV nonstructural protein NS5 induces CXCL8/IL-8 transcription and secretion (58). Other virus-encoded proteins have also been shown to regulate chemokine expression; the core and NS5A proteins of hepatitis C virus are able to activate the promoter activities of CCL2/MCP-1 and CCL5/RANTES (59); hepatitis C viruses NS5, NS4A, and NS4B induce CXCL8/IL-8 expression (60, 61); HIV gp120 induces CXCL10/IP-10 expression (62); and HIV tat regulates CCL2/MCP-1 expression (63). The mechanism by which CXCL10/IP-10 is induced by DV is currently under investigation.

In addition to its ability to recruit NK cells, our in vitro studies suggest that CXCL10/IP-10 also has a novel function following DV infection; i.e., it inhibits the binding of the virus to the cell surface, thereby blocking its entry and replication. It is known that the interaction of chemokines with GAGs such as heparan sulfate is physiologically important for sequestrating the molecules on the cell surface to create a gradient for the recruitment of leukocytes to inflamed tissues (50, 51). And given that heparan sulfate is a putative receptor for initiating DV infection (46–49), our contention is that CXCL10/IP-10 inhibits DV binding by competing for heparan sulfate on host cells. Campanella et al. conducted extensive mutational studies on murine CXCL10/IP-10. Their results indicate that the N-terminal Arg residue is critical for CXCR3 signaling but not for heparin binding; Arg23 is critical for specific binding to heparin; and the four C-terminal basic amino acids (Lys71, Arg72, Lys74, and Arg75) also contribute to heparin binding, mainly via an electrostatic effect (40). Our results show that substitution of Arg23 and/or the four C-terminal amino acids, but not Arg3, abolishes the ability of CXCL10/IP-10 to inhibit DV binding, indicating that the amino acid residues responsible for heparin binding are critical for inhibiting DV binding to cells. That DV reportedly interacts with cells mainly through the interaction of the external loop region of domain III with heparan sulfate on the host cells (46, 49) suggests CXCL10/IP-10 likely competes with the external loop region of domain III for binding to heparan sulfate.

Virtually all cells have heparan sulfate on their surface, which raises a question concerning the specificity of the interaction between DV and cell surface GAGs. DV patients frequently show liver abnormalities, indicating its tropism for DV infection (15, 64, 65). One would therefore expect that DV has a higher affinity for GAGs on hepatocytes than on other cell types, which would facilitate infection of the liver. In that regard, soluble high sulfate forms of heparan sulfate appear to inhibit the infectivity of the virus more efficiently than low sulfate forms (46); thus, it would be expected that the heparan sulfate in the liver should have a high degree of sulfation to facilitate the infection. Indeed, comparison of heparan sulfate chains isolated from different tissues has shown that heparan sulfate isolated from liver has the highest degree of sulfation among tissues (66), which is consistent with the notion that liver is a tropic tissue for DV infection. Therefore, our in vitro study showing that CXCL10/IP-10 is able to compete with DV for binding to heparan sulfate on Hepa-1-6 cells may imply that, in vivo, the high levels of CXCL10/IP-10 expressed by DV-infected hepatocytes create locally high concentrations of the chemokine that protect uninfected hepatocytes from DV infection by competing for heparan sulfate binding sites. In this way, CXCL10/IP-10 may limit the spread of infection and contribute to host defense early during DV infection.

CXCL10/IP-10, CXCL9/Mig, and CXCL11/T-A are known as IFN-γ-inducible CXC chemokines and share the same receptor, CXCR3. Both CXCL9/Mig and CXCL10/IP-10 inhibited the binding of DV to cells, though CXCL11/T-A did not. Despite earlier reports showing functional redundancy of IFN-γ-inducible CXC chemokines in vitro, our results suggest that the functions of these molecules do not overlap entirely. In addition, although CCL5/RANTES reportedly interacts with GAGs (50, 51), it did not inhibit DV binding to Hepa-1-6 cells, which implies that different chemokines induced by viral infection may have different tissue-specific affinities for specific GAGs. This could explain the in vivo specificity of the functions of chemokines in host defense, despite their apparent functional redundancy in vitro.

Studying DV infection in vivo has been difficult because mice are not natural hosts for the virus and are not easily infected, and even when infected they seem to efficiently clear the virus. In our study, we infected mice i.v. and found that CXCL10/IP-10 was always significantly induced, and though viral RNA was only occasionally detected, peak viral RNA levels were consistently associated with peak CXCL10/IP-10 levels. It may be that we only occasionally detected viral RNA because mice are not the natural host for DV and thus cannot be efficiently infected. On the other hand, it is also plausible that the consistently strong induction of CXCL10/IP-10 by DV may efficiently prevent the virus from binding to and entering cells, thereby reducing/eliminating infection.
When patients previously infected by DV are infected a second time with a different serotype of DV, they usually develop severe dengue hemorrhagic fever or dengue shock syndrome. One possible explanation for this phenomenon is Ab-dependent enhancement (ADE) (67). It is hypothesized that a nonneutralizing antibody (Ab) bound during the first infection will bind to DV during the second infection, thereby enhancing virus entry into target cells via the FcR. In addition, Mahalingam and Lidbury (68) demonstrated that Ab-dependent enhancement of macrophage infection by Ross River virus suppressed CXCL10/IP-10 expression. Given that macrophages also are target cells for DV, it seems plausible that production of CXCL10/IP-10 may be reduced in macrophages infected by DV via the ADE mechanism. Additionally, infection of macrophages by DV induces expression of IL-1β (27), which reportedly stimulates synthesis of sulfated GAGs (69). Thus, DV infection via the ADE mechanism may reduce CXCL10/IP-10 production and increase production of sulfated GAGs, thereby facilitating DV entry and replication in FcR-bearing cells. That would explain why a second DV infection always produces a more serious disease state.

In summary, we have shown that infecting mice i.v. with DV results in prominent induction of CXCL10/IP-10, which plays an important role in the recruitment of activated NK cells early after DV infection. Additionally, our in vitro results showing the inhibitory effect of CXCL10/IP-10 on dengue virus binding to cells during the second infection, thereby enhancing virus entry into target cells via the FcR. In addition, Mahalingam and Lidbury (68) demonstrated that Ab-dependent enhancement of macrophage infection by Ross River virus suppressed CXCL10/IP-10 expression. Given that macrophages also are target cells for DV, it seems plausible that production of CXCL10/IP-10 may be reduced in macrophages infected by DV via the ADE mechanism. Additionally, infection of macrophages by DV induces expression of IL-1β (27), which reportedly stimulates synthesis of sulfated GAGs (69). Thus, DV infection via the ADE mechanism may reduce CXCL10/IP-10 production and increase production of sulfated GAGs, thereby facilitating DV entry and replication in FcR-bearing cells. That would explain why a second DV infection always produces a more serious disease state.

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DENGUE VIRUS INFECTION INDUCES CXCL10/IP-10 EXPRESSION


