Synthetic Double-Stranded RNA Stimulates the Expression of Interferon-Inducible Protein 10 in Human Mesothelial Cells

Monika Merkle,1 Matthias Sauter,2 Andrea Ribeiro,2 Thomas Mussack,3 Roland Ladurner,3 Thomas Sitter,2 and Markus Wörnle2*

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Interferon-inducible protein 10 (IP-10) is a chemokine playing an important role in the restriction of viral spread. A time- and dose-dependent increase in IP-10 is found upon activation of viral receptors expressed on mesothelial cells, which provides novel evidence for a link between viral infections and inflammation of serous membranes.

Interferon-inducible protein 10 (IP-10) is a chemokine of the CXC family. By binding to the receptor CXCR3, it is a potent chemoattractant for activated T cells and NK cells and thus plays an important role in the restriction of viral spread as well as in early viral elimination (6). IP-10 is induced by gamma interferon (IFN-γ) in several human cell types (28). Other cytokines typically elevated during infection or inflammation, such as type I interferons and tumor necrosis factor alpha (TNF-α) as well as double-stranded RNA (dsRNA), lipopolysaccharide (LPS), and certain viruses, can upregulate IP-10 expression too (8). Mesothelial cells (MC) line the inner surfaces of body cavities, including the pleura, pericardium, and peritoneal cavity. In the peritoneum, MC are the main source of chemokines (22), which play a pivotal role in attracting neutrophils and macrophages to the sites of inflammation in cases of peritonitis (1, 7, 18). Cytokines such as interleukin 1β (IL-1β), TNF-α, and IFN-γ activate peritoneal MC, thereby inducing an increased synthesis of several chemokines (12, 19) involved in the induction and progression of peritonitis and peritoneal fibrosis. A mesothelial expression of IP-10 has been found during these inflammatory processes (22), but so far no functional link between viral receptors and IP-10 expression in mesothelial cells has been described. However, many viral infections cause serositis, such as viral peritonitis in patients undergoing continuous ambulatory peritoneal dialysis (CAPD) (11). Viral infections of pleural MC are more frequent, especially in immunosuppressed patients (4). Furthermore, viral mesothelial infections are responsible for transformation to malignant mesothelioma (5, 20). Viral infections of the heart can lead to dilated cardiomyopathy and congestive heart failure (10). Toll-like receptors (TLRs) are an essential part of the innate immune system. TLRs recognize conserved pathogen-associated molecular patterns (PAMPs) associated with microbial pathogens and induce an immune response, with each TLR binding specifically distinct components of an infectious agent (2, 16). In particular, TLR3 recognizes dsRNA of viral origin, as exemplified by poly(I:C) RNA, a synthetic analogue of viral dsRNA (3, 24) that is well known for mimicking viral infection in experimental settings. The helicases retinoic acid-inducible gene I (RIG-I) and melanoma differentiation-associated gene 5 (MDA5) may also act as sensors of viral infections through recognition of viral dsRNA (15, 23, 27). We have previously shown that human MC express TLR3, RIG-I, and MDA5 and that activation of these viral receptors upregulates several cytokines and chemokines (25, 26).

In the present study, we analyzed the effects of viral infection of MC, prepared for in vitro experiments by stimulation with poly(I:C) RNA, on IP-10. MC were isolated from the omental tissues of consenting patients and grown as described previously (7, 21), real-time reverse transcription (RT)-PCR analysis was done as described previously (13), and enzyme-linked immunosorbent assay (ELISA) was performed on cell culture supernatants using commercial assay kits (Quantikine; R&D Systems). A time- and dose-dependent increase in the expression and synthesis of IP-10 upon activation of viral receptors is shown [Fig. 1A and B, which show stimulation with poly(I:C) RNA at 5 μg/ml, and Fig. 1C and D, which show stimulation for 24 h]; significant differences are indicated for P values of <0.05 and 0.01. Furthermore, mesothelial IP-10 is inducible by each of the interferons IFN-α, IFN-β, and IFN-γ, with IFN-β having the most pronounced effect (Fig. 1E, for which the stimulation time was 24 h). Disparate activations of interferon-regulatory factors by IFN-α and IFN-β might account for the differences in effects on chemokine regulation despite their use of a common receptor. We have previously shown and now confirm a poly(I:C)-dependent increase in mesothelial IFN-α and IFN-β (26; data not shown); whether the induction of IP-10 in MC by poly(I : C) is a direct effect or requires the upregulation of IFN-α and IFN-β needs further investigation. However, type I interferons are known to be induced under a variety of conditions, and they mediate the influx of monocytes, ultimately contributing to chronic inflammatory processes. Being specifically interested in early and potentially reversible stages of virally induced serositis, we focused primarily on IP-10 as a mediator known to be more specifically involved in viral clearance.
To elucidate signaling pathways responsible for IP-10 upregulation by ligand binding to viral receptors, knockdown experiments with predesigned small interfering RNAs (siRNAs) specific for TLR3, RIG-I, and MDA5 (Ambion, Japan) were performed; transfection of siRNA into the cells was done using the siPORT-NeoFX transfection agent as described previously (15), and scrambled siRNA served as a nonspecific negative control of siRNA (Ambion). We have shown previously the efficiency of receptor knockdowns with these siRNAs, which result in a significant downregulation of the basal expression of TLR3 (86%), RIG-I (89%), and MDA5 (90%), whose TLR3 and RIG-I synthesis was confirmed at the protein level (26). In the actual experiments, transfection of cells with siRNA specific for TLR3 and RIG-I significantly blocked the poly(I:C) RNA-induced increase in IP-10 expression and synthesis (Fig. 2A and B, for which experiments the stimulation with poly(I:C) RNA was at 5 μg/ml for 24 h). Neither siRNA specific for MDA5 nor negative controls with unspecific RNA had an effect. Therefore, knockdown exper-
ments argue in favor of a predominant role for TLR3 and RIG-I in mediating the induction of mesothelial IP-10. Further studies will be necessary to define whether the TLR3 and RIG-I pathways in mesothelial cells are activated independently of each other.

So far, the role of IP-10 has been well defined only for bacterial peritonitis (9). The novel finding of mesothelial IP-10 expression and synthesis being differentially regulated processes in viral infections may also be relevant not only for viral peritonitis but also for the initiation of the inflammatory response in viral infections of pleural or pericardial MC. As previously discussed, with regard to processes of matrix generation and the regulation of virally induced inflammation and fibrosis (17), we suppose viral receptors to be of crucial importance for balancing a variety of inflammatory pathways, including viral clearance, tissue regeneration, and scarring. The observation that single-stranded RNA also activates TLR3 (14), implying that RNA generated in the course of replication of DNA viruses could also act as a ligand for TLR3, adds to the potential impact of the present results. Finally, one can speculate about a potential role for the binding of nucleic acid fragments to TLR3 in autoimmune diseases.

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