Proteasome Activator and Antigen-Processing Aminopeptidases Are Regulated by Virus-Induced Type I Interferon in the Hepatitis C Virus-Infected Liver

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ABSTRACT

Many components of the class I antigen-processing pathway are thought to be regulated solely by interferon-γ (IFN-γ). Herein, we report type I IFN-mediated induction of proteasome activator (PA28) subunits α and β, endoplasmic reticulum aminopeptidase 1 (ERAP1), ERAP2, and leucine aminopeptidase (LAP). This mechanism was initiated by either synthetic RNA (poly(I-C)) or by hepatitis C virus (HCV) RNA-mediated induction of type I IFN and abrogated by blocking of type I IFN. In serial liver biopsies of chimpanzees with acute HCV infection, increases in PA28 subunit and aminopeptidase mRNA levels correlated with intrahepatic type I IFN responses and preceded intrahepatic IFN-γ responses by several weeks. Thus, viral RNA-induced type I IFN regulates the antigen-processing machinery early during viral infection and prior to IFN-γ response. This mechanism may contribute to the high effectiveness of type I IFN-based therapies if administered early during acute HCV infection.

ENDOGENOUSLY SYNTHESIZED MOLECULES, such as viral and self proteins, are processed by intracellular enzymes and presented on major histocompatibility complex (MHC) class I molecules to CD8+ T cells. Specifically, the cytoplasmic proteasome complex generates small peptides,1,2 which are then translocated into the endoplasmic reticulum lumen,3 trimmed to optimal length by aminopeptidases, loaded on MHC class I molecules, and transported to the cell surface.2,4

Expression and activity of the molecules involved in this MHC class I antigen-processing pathway are regulated by cytokines.5 Interferon-γ (IFN-γ), a major cytokine in many viral infections, is known to induce the immunoproteasome subunits β1i (low-molecular mass polypeptide 2 [LMP2]), β2i (multicatalytic endopeptidase complex-like-1 [MECL-1]), and β5i (LMP7), and the proteasome activator 28 (PA28).1,5,6 The resulting immunoproteasome/PA28 complex digests proteins more efficiently than its constitutive counterpart and alters the panel of generated CD8 T cell epitopes.1,6 Because immunoproteasomes and PA28 had been reported to be induced solely in an IFN-γ-dependent manner in a murine model of lymphocytic choriomeningitis virus (LCMV) infection7 and because IFN-γ also induces the expression of transporters associated with antigen processing (TAP),8 aminopeptidases,9,10 and MHC class I,11 it is regarded as the main cytokine to facilitate the processing and presentation of viral antigens for recognition by CD8+ T cells.

We challenged this dogma and reported that induction of immunoproteasomes in the liver in response to hepatitis C virus (HCV) infection occurs several weeks prior to the IFN-γ response and, instead, is mediated by type I IFN.12 In the present study, we extend this analysis to additional components of the MHC class I antigen-processing machinery. Specifically, we investigate the role of type I IFN in the induction of the PA28 subunits α and β, endoplasmic reticulum aminopeptidase 1 (ERAP1), ERAP2, and leucine aminopeptidase (LAP) in vitro and in vivo in the virus-infected target organ.

Human hepatoma Huh-7 cells were treated with either consensus sequence IFN-α (IFN-α-con1), IFN-β, or as a positive control, IFN-γ. All cytokines induced the expression of PA28α, PA28β, ERAP1, ERAP2, and LAP at the mRNA (Fig. 1A) and...

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FIG. 1. Type I IFN induces PA28 subunits and ER aminopeptidases in vitro. Huh-7 cells were treated with 3 ng/mL IFN-α-con1 (kindly provided by Dr. L. Blatt, InterMune, Brisbane, CA), 3 ng/mL IFN-β1b (Betaseron, Chiron, Emeryville, CA) or 10 ng/mL IFN-γ (Pepro Tech, Rocky Hill, NJ) for 24 h or 48 h, respectively, and expression of PA28α, PA28β, ERAP1, ERAP2, LAP, and PREP was assessed by (A) TaqMan real-time PCR and (B, C) Western blot. Bar graphs indicate the respective Western blot band density normalized to that of the α4 subunit of constitutive proteasome (B) or GAPDH (C), respectively. (D) Two sets of primary human hepatocytes, obtained through the Liver Tissue Procurement and Distribution System (Pittsburgh, PA; funded by NIH contract N01-DK-9-2310) and grown in hepatocyte maintenance medium (Cambrex, Walkersville, MD), were treated with 1.8 ng/mL (500 U/mL) IFN-α2a (Research Diagnostics, Inc., Flanders, NJ) or 3 ng/mL IFN-α-con1 or 10 ng/mL IFN-γ for 24 h, and expression of PA28α, PA28β, ERAP1, ERAP2, LAP, and PREP was assessed by TaqMan real-time PCR. Mean values from two sets of hepatocytes are presented. In TaqMan real-time PCR analysis, the amount of mRNA was normalized to that of β-actin as an endogenous control and expressed as relative mRNA level over the corresponding mRNA level in untreated cells.
The induction of these components of the antigen-processing machinery by type I IFN was confirmed in primary human hepatocytes (Fig. 1D). In contrast, the expression levels of the controls, prolyl endopeptidase (PREP) (Fig. 1A,D) and proteasome α4 subunit (Fig. 1B), remained stable.

Next, we asked if endogenously induced type I IFN had the same effect as exogenously added type I IFN on PA28 and
aminopeptidase expression. Endogenous induction of type I IFN in response to a viral infection occurs by sensing of dsRNA by retinoic acid-inducible gene I (RIG-I), which causes phosphorylation and activation of IFN response factor-3 (IRF-3) and subsequent production of IFN-α/β.13 Secreted IFN-α/β binds to the IFN-α/β receptor in an autocrine and paracrine manner, causing activation of the Jak-Stat signaling pathway, and ultimately transcription of IFN-stimulated genes (ISG) and production of various IFN-α subtypes.13 To mimic viral infection, Huh-7 cells were first transfected with the synthetic dsRNA poly(I-C), which resulted in an increase in PA28α, PA28β, ERAP1, ERAP2, and LAP mRNA levels but not PREP mRNA levels (Fig. 2A). Poly(I-C) also induced the expression of IFN-α/β and downstream 2,5-oligoadenylate synthetase (2,5-OAS-1), suggesting that the induction of the components of the antigen-processing machinery was mediated by type I IFN (Fig. 2A). To prove this hypothesis, we performed the same experiment in the presence of the vaccinia virus-encoded B18 receptor protein (VV B18R), which competes with the IFN-α/β receptor for IFN binding.14 As shown in Figure 2B, the addition of VV B18R abrogated the induction of PA28α, PA28β, ERAP1, ERAP2, and LAP along with abrogation of 2,5-OAS-1 induc-
tion. Thus, induction of PA28 subunits and ERAPs in response to intracellular dsRNA was indeed mediated by endogenously produced type I IFN.

Furthermore, transfection of Huh-7 cells with HCV RNA, which was in vitro transcribed from pHCV-H77 (kindly provided by Dr. J. Bukh, NIAID), increased mRNA levels of PA28α, PA28β, ERAP1, ERAP2, and LAP (but not that of the control PREP) and induced IFN-β production and the downstream response of 2,5-OAS-1 (Fig. 2C). The induction was abrogated by the addition of VV B18R (Fig. 2D). These data demonstrate that PA28 and ERAPs were induced not only by synthetic dsRNA but also by HCV RNA itself in a type I IFN-dependent manner. The poly(I-C) and HCV RNA transfection experiments were validated by studying other ISGs, such as Stat1 and IP-10. These ISGs were also induced upon either poly(I-C) or HCV RNA transfection, and the induction was abrogated by the addition of VV B18R (data not shown).

To clarify PA28, ERAP1, ERAP2, and LAP induction in the HCV-infected liver in relation to intrahepatic type I IFN and IFN-γ, we prospectively studied five chimpanzees with acute HCV infection (Fig. 3). The study was approved by the Animal Care and Use Committee and the Public Health Service Interagency Model Committee. Intrahepatic PA28/H9251, PA28/H9252, ERAP1, ERAP2, and LAP mRNA levels started to increase as early as 2 weeks after infection and peaked 5–8 weeks after infection (Fig. 3B,C,D; p < 0.05 comparing baseline and peak mRNA levels for PA28α, PA28β, ERAP2, and LAP, respectively), whereas PREP mRNA levels remained constant (Fig. 3D). Surprisingly, intrahepatic IFN-γ mRNA levels started to increase only weeks after the peak of PA28 and ERAP mRNA levels was reached (as indicated by the dashed vertical lines in Fig. 3E). The lack of an early IFN-γ response was confirmed by the absence of induction of monokine induced by gamma-interferon (Mig) mRNA, which is strictly IFN-γ dependent.

In contrast, an increase in the 2,5-OAS-1 mRNA level was detectable as early as 2 weeks after infection, and its expression kinetics correlated closely to those of PA28 subunits and ERAPs (Fig. 3F). In parallel to the 2,5-OAS-1 response, an increase in IFN-α and IFN-β mRNA was detectable, as previously described. Collectively, these results indicate an early role for type I IFN in the induction of PA28 subunits and ERAPs during acute HCV infection.

The impact of PA28 subunit and ERAP induction on recognition of virus-infected hepatocytes by HCV-specific CD8+ T cells is difficult to investigate at present because aminopeptidase-dependent T cell epitopes have not been studied and identified in the HCV. Overall, the time point of PA28 subunit and ERAP induction in the liver was comparable for all chimpanzees, indicating that it was not a major factor in the outcome of infection.

The duration of type I IFN-dependent induction of PA28 subunits and ERAPs during natural infection was transient. It is, therefore, conceivable that prolonged induction with high doses of exogenous IFN, the current standard therapy of acute hepatitis C, may contribute to viral clearance. Extended duration of type I IFN-dependent expression of PA28 subunits and ERAPs may reduce the gap in peak time between increased expression of antigen-processing enzymes in the liver (peak at 2–8 weeks after infection) and infiltration of HCV-specific CD8+ T cells (peak at 12–16 weeks after infection). Because an effective response of HCV-specific CD8+ T cells against HCV-infected hepatocytes is an important determinant for the outcome of HCV infection, prolonged expression of antigen-processing enzymes may contribute to the high (95%) effectiveness of type I IFN-based therapies in acute HCV infection.

In this respect, it is also of interest that melanoma cells have been reported to be unresponsive to type I IFN-mediated induction of antigen-processing enzymes, a possible mechanism of tumor escape from CD8+ T cell responses. Thus, type I IFN plays a previously unknown role in the induction of components of the antigen-processing machinery in vitro and in vivo.

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