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Kilham rat virus-induced type 1 diabetes involves beta cell infection and intra-islet JAK–STAT activation prior to insulitis

Aimon K. Alkanani [1], Naoko Hara [1], Roberto Gianani, Danny Zipris *

Barbara Davis Center for Childhood Diabetes, University of Colorado Denver, 1775 Aurora Ct., Mail Stop B-140, Aurora, CO 80045, United States

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ABSTRACT

We used the LEW1.WR1 rat model of Kilham Rat Virus (KRV)-induced type 1 diabetes (T1D) to test the hypothesis that disease mechanisms are linked with beta cell infection and intra-islet immune activation prior to insulitis. KRV induces genes involved in type I and type II interferon pathways in islet cell lines in vitro and in islets from day-5-infected animals in vivo via mechanisms that do not involve insulitis, beta cell apoptosis, or impaired insulin expression. Immunohistochemistry studies indicated that KRV protein is expressed in beta cells 5 days following infection. KRV induces the phosphorylation of Janus Kinase 1/2 (JAK1/2) and signal transducer and activator of transcription 1 (STAT-1) in islet cells via a mechanism that could involve TLR9 and NF-κB pathways. These data demonstrate for the first time that KRV-induced islet destruction is associated with beta cell infection and intra-islet innate immune upregulation early in the disease process.

Introduction

It is hypothesized that viruses play a key role in triggering type 1 diabetes (T1D) in genetically susceptible individuals (Fujinami et al., 2006; Jun and Yoon, 2001, 2003; Laitinen et al., 2014), but how they lead to T1D is not yet clear. Recent studies support the hypothesis that the process of islet destruction involves beta cell infection and intra-islet innate immune upregulation; however the data supporting this hypothesis were obtained from in vitro studies (Schulte et al., 2012; Sarkar et al., 2012), or pancreata from humans with established islet autoimmunity (Dotta et al., 2007, 2010; Roep et al., 2010).

We used the LEW1.WR1 rat model of Kilham Rat Virus (KRV)-induced T1D to identify in vivo disease mechanisms in the preinsulitis stage. The LEW1.WR1 rat has normal T lymphocyte levels and function (Mordes et al., 2005). Infection of this rat with KRV leads to islet infiltration and destruction detectable beginning at 2 weeks following virus inoculation (Zipris et al., 2003). KRV is a single stranded DNA parvovirus (Jacoby et al., 1996), encoding three overlapping structural proteins, VP1, VP2, and VP3, and two overlapping nonstructural proteins, NS1 and NS2 (Jacoby et al., 1996).

STAT pathways have recently been implicated in the mechanism of T1D (Kim et al., 2007; Eizirik et al., 2009). They play a central role in mediating the delivery of IFN-α/β and IFN-γ signaling (for reviews, see Refs. Platanias (2005); Shuai and Liu (2003)). The binding of IFN-α/β and IFN-γ to their receptors results in the rapid auto-phosphorylation and activation of the receptor-associated Janus activated kinases (JAKs) and tyrosine kinase 2 (TYK2). Activation of the JAKs leads to tyrosine phosphorylation of STAT-1 and STAT-2 and to the formation of homodimeric (STAT-1–STAT-1), heterodimeric (STAT-1–STAT-2), and heterotrimeric (STAT-1–STAT-2–IFN-regulatory factor 9) complexes. These complexes translocate to the nucleus and bind IFN-stimulated response elements in DNA to initiate the transcription of genes involved in host defense.

It is not yet clear whether and how pancreatic islets are linked with early mechanisms of KRV-induced diabetes prior to insulitis. Previous in situ hybridization studies suggested that KRV-induced islet destruction does not involve beta cell infection (Brown et al., 1993). In this study, we revisit this issue using newly developed KRV-specific antibodies and further examine the hypothesis that early disease mechanisms involve the upregulation of intra-islet immune mechanisms. We demonstrate that inoculation of LEW1.WR1 rats with KRV leads to the expression of virus transcripts and protein in islet cells and to activation of the JAK–STAT signaling

* Correspondence to: Barbara Davis Center for Childhood Diabetes, University of Colorado Denver, 1775 Aurora Ct., Mail Stop B-140, Aurora, CO 80045, United states. Tel.: +1 303 724 0108; fax: +1 303 724 6839.
E-mail address: danny.zipris@ucdenver.edu (D. Zipris).
[1] Authors equally contributed to this work.

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pathway in beta cells on day 5 post-infection. We hypothesize that the mechanism of virus-induced islet destruction involves beta cell infection and innate immune upregulation early in the disease process.

Results

KRV induces proinflammatory gene expression in islet cells in vitro and in vivo

Recent in vitro studies suggested that human T1D involves the induction of proinflammatory responses in pancreatic islets (Sarkar et al., 2012; Schulte et al., 2012). We tested the hypothesis that KRV-induced T1D in the LEW1.WR1 rat involves proinflammatory gene expression in islet cells. To do so, we employed two experimental systems. First, we measured the level of transcripts for proinflammatory genes in the INS-1 and RIN-14B islet cell lines which produce insulin and somatostatin, respectively (Moore et al., 2011), following incubation in the presence or absence of KRV. We also analyzed proinflammatory gene expression in islets isolated from naïve uninfected ($n=3$ to 7) versus day-5-infected rats ($n=5$ to 7). Data presented in Fig. 1A indicate that KRV induces the expression of transcripts for IRF-7, a transcription factor involved in type I interferon signaling (Honda et al., 2005), in both INS-1 and RIN-14B cell lines ($p<0.007$ and $p<0.002$, respectively). KRV also induced the expression of transcripts for CXCL-10 in RIN-14B ($p<0.002$) but not in INS-1 cells. Consistent with these observations, data presented in Fig. 1B further demonstrate that there was a significant increase in the level of transcripts for IFN-γ ($p=0.04$), CXCL-10 ($p=0.02$), CXCL-11 ($p=0.004$), IRF-7 ($p=0.001$), IFN-α ($p=0.02$), STAT-1 ($p=0.04$), and JAK-2 ($p=0.003$) in islets 5 days following infection compared to uninfected rats. Fig. 1C further indicates that islets from days 3, 5, and 8 post-infection are insulitis-free. Taken together, these data imply that infection with KRV results in proinflammatory gene activation in islets on day 5 post-infection via a mechanism that is not associated with insulitis.

KRV infects islet cell lines in vitro and beta cells in vivo

Previous data implied that virus-induced T1D does not involve beta cell infection (Brown et al., 1993); however, the observation that KRV upregulates proinflammatory gene expression in islets in vitro and in vivo prompted us to revisit the issue of KRV infectivity in islet cells. To that end, we tested the ability of KRV to infect islet cells in vitro and in vivo. To assess in vitro infectivity, the INS-1 and RIN-14B cell lines were cultured in the presence or absence of KRV and the expression of virus transcripts was assessed with quantitative RT-PCR. The data presented in Fig. 2A indicate that transcripts for KRV are detectable in both the INS-1 and RIN-14B cell lines following a 4 h incubation in the presence of KRV but not medium only ($n=3$–7, $p<0.001$). Fig. 2A further shows that transcripts for KRV VP2 are readily detectable in pancreatic islets from 5-day-infected animals but not naive uninfected rats ($p=0.001$ versus uninfected).

We next assessed the expression of KRV protein in islet cells from day-5-infected ($n=5$ to 7) versus naïve uninfected ($n=3$ to 7) rats. We used spleens from infected and uninfected rats as positive and negative controls, respectively. A polyclonal rabbit antibody against the C terminus of KRV VP1/VP2 was used to detect KRV expression in beta cells. Because immunofluorescence was not

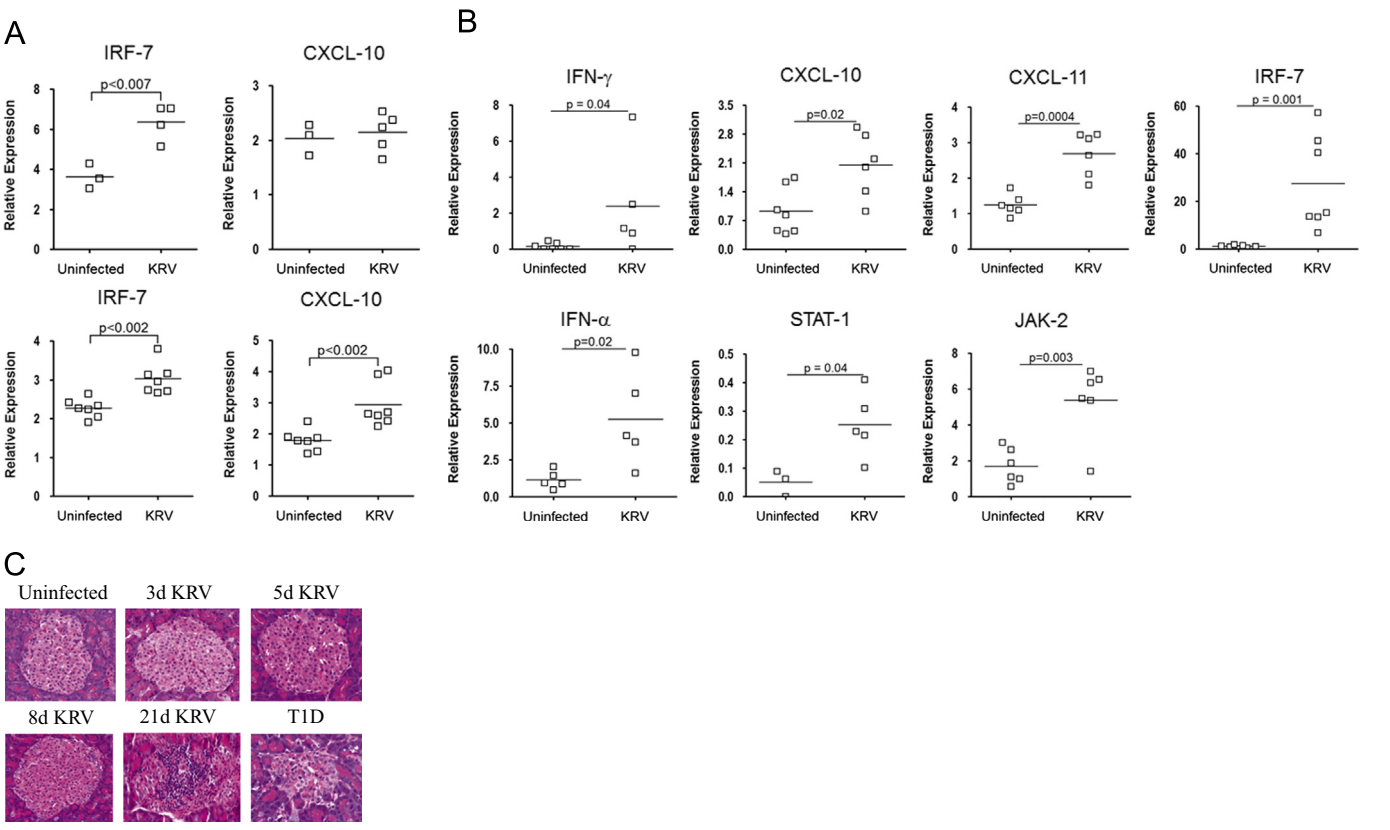

Fig. 1. Proinflammatory gene expression in islet cell lines and primary islets from virus-infected rats. RNA was extracted from INS-1 (upper panel) and RIN-14B (lower panel) islet cell lines cultured in the presence or absence of KRV for 4 h (A) or pancreatic islets purified from 5-day-infected or naïve rats (B). The expression level of transcripts for the indicated genes was assessed using quantitative RT-PCR. The results are expressed as the mRNA expression of the gene of interest relative to the expression of β-actin. Statistical analyses were performed using the Mann–Whitney *U* test. Paraffin sections of pancreata from infected and uninfected animals at different time points following infection as indicated in the figure were stained for insulitis with hematoxylin and eosin (C). The images shown are representative of at least three independent experiments.