Biochimica et Biophysica Acta 1793 (2009) 1768–1775

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamcr

Regulation and function of the cytosolic viral RNA sensor RIG-I in pancreatic beta cells

Mónica García [a], Zeynep Dogusan [a], Fabrice Moore [a], Shintaro Sato [b], Gunther Hartmann [c], Decio L. Eizirik [a], Joanne Rasschaert [a,*]

[a] Laboratory of Experimental Medicine, Université Libre de Bruxelles, Brussels, Belgium
[b] Division of Mucosal Immunology, Department of Microbiology and Immunology, The Institute of Medical Science, University of Tokyo, Tokyo, Japan
[c] Institute of Clinical Chemistry and Pharmacology, University Hospital Bonn, Bonn, Germany

ARTICLE INFO

Article history:
Received 2 July 2009
Received in revised form 26 August 2009
Accepted 3 September 2009
Available online 10 September 2009

Keywords:
Diabetes
RNA helicases
Toll-like receptor 3
Apoptosis

ABSTRACT

Enteroviral infections are associated with type I diabetes. The mechanisms by which viruses or viral products such as double-stranded RNA (dsRNA) affect pancreatic beta cell function and survival remain unclear. We have shown that extracellular dsRNA induces beta cell death via Toll-like receptor-3 (TLR3) signaling whereas cytosolic dsRNA triggers the production of type I interferons and apoptosis via a TLR3-independent process. We presently examined expression of the intracellular viral RNA sensors, the RNA helicases RIG-I and MDA5, and documented the functionality of RIG-I in pancreatic beta cells.

FACS-purified rat beta cells and islet cells from wild-type or TLR3$^{-/-}$ mice were cultured with or without the RIG-I-specific ligand 5′-triphosphate single-stranded RNA (5′triP-ssRNA), the synthetic dsRNA polyI:C (PIC) or 5′OH-ssRNA (negative control); the RNA compounds were added in the medium or transfected in the cells using lipofectamine. RIG-I and MDA5 expression were determined by real-time RT-PCR. NF-κB and IFN-β promoter activation were studied in the presence or absence of a dominant-negative form of RIG-I (DN-RIG-I). Both extracellular (PICex) and intracellular (PICin) PIC increased expression of RIG-I and MDA5 in pancreatic beta cells. TLR3 deletion abolished PICex-induced up-regulation of the helicases in beta cells but not in dendritic cells. PICin-induced NF-κB and IFN-β promoter activation were prevented by the DN-RIG-I. The RIG-I-specific ligand 5′triP-ssRNA induced IFN-β promoter activation and beta cell apoptosis. Our results suggest that the RIG-I pathway is present and active in beta cells and could contribute to the induction of insulitis by viral RNA intermediates.

1. Introduction

Type 1 diabetes (T1D) is a severe chronic disease resulting from a progressive and selective loss of functional insulin-producing beta cells through an autoimmune-mediated process [1]. The incidence of T1D shows seasonal and geographic-dependent variations. This, together with the increased occurrence of the disease in the last decades, suggests that both genetic and environmental factors contribute for its pathogenesis. Various putative environmental agents have been implicated in the etiology of T1D, and there is now increased evidence suggesting that viral infections may play a role in the process [2,3]. This hypothesis is supported by studies showing the presence of enterovirus or enteroviral RNA in the serum and in pancreatic islets of newly diagnosed T1D children and adults [4–7]. Moreover, high levels of antibodies against enteroviruses were detected [8,9] and found associated with the presence of beta cell autoantibodies at early onset of the disease [10,11]. The molecular mechanisms involved in virus-induced beta cell damage and apoptosis remain, however, to be clarified.

Recognition of viral pathogen-associated molecular patterns (PAMPs) by the host cells is essential to initiate innate immune responses and overcome infections [12]. Cells possess a variety of innate immune pattern recognition receptors to detect viral PAMPs. These include the Toll-like receptors (TLRs), the double-stranded RNA (dsRNA)-dependent protein kinase R (PKR) and the newly identified retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs) which include RIG-I, MDA5 and Lgp-2. TLR3 and RLRs detect viruses by binding dsRNA [13–15], a common intermediary product of the viral life cycle [16]. In the course of viral infection, dsRNA accumulates both in the cytosol of infected cells and in the extracellular compartment and activates antiviral responses, contributing to trigger the subsequent adaptive immune response [17,18]. TLR3 detects mostly extracellular dsRNA phagocytosed in endosomes, while RLRs sense cytosolic viral dsRNA [14,19].

Poly (I:C) (PIC), a dsRNA synthetic analogue, mimics the effects of viral dsRNA in vivo and in vitro and triggers cellular dysfunction and apoptosis in pancreatic beta cells [20–23]. Exposure of purified rat beta cells to extracellular PIC or infection of human pancreatic islets

* Corresponding author. Laboratory of Biochemistry and Nutrition, Université Libre de Bruxelles, Lennikstreet, 808, CP 611, B-1070 Brussels, Belgium. Tel.: +32 2 555 6307; fax: +32 2 555 6230.
E-mail address: jrasscha@ulb.ac.be (J. Rasschaert).

0167-4889/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamcr.2009.09.005

with the coxsackievirus B5, a diabetogenic strain, induces TLR3 and RIG-I mRNAs expression [23–25]. Of note, PIC is recognized by both TLR3 and RLRs [14,15,26,27]. In a previous study, we demonstrated that extracellular PIC-mediated beta cell apoptosis and expression of pro-inflammatory genes is abolished in islet cells from TLR3 knockout ($TLR3^{-/-}$) mice, indicating that the TLR3 pathway is required for extracellular dsRNA-mediated beta cell death and inflammatory signaling [28]. The $TLR3^{-/-}$ islet cells are, however, only partially protected against internal dsRNA-triggered modifications in mRNA expression and subsequent apoptosis, suggesting a role for other signaling pathways that remain to be determined [28].

RIG-I and melanoma differentiation-associated gene 5 (MDA5) are homologous cytosolic proteins members of the RLRs family [29]. They contain a C-terminal helicase domain that binds dsRNA and two NH_2-terminal caspase activation and recruitment domains (CARDs) acting as signaling domains and implicated in homotypic interactions related to inflammatory or cell death pathways [30]. Besides recognizing dsRNA, RIG-I also recognizes uncapped 5′-triphosphate single-stranded RNA (5′triP-ssRNA), a well-defined molecular structure of viral nucleic acids [31,32]. The binding of dsRNA or 5′triP-ssRNA to RIG-I or MDA5 promotes their interaction with the adaptor molecule VISA [33]. VISA further transmits downstream signals that result in the activation of the transcription factors NF-κB and IRF-3 and the subsequent synthesis and secretion of type I interferons (IFNs) [34,35].

We have recently shown that intracellular PIC activates IRF-3 and NF-κB in pancreatic beta cells, inducing a massive production of type I IFNs which contributes to cytosolic dsRNA-induced apoptosis in a TLR3-independent process [28]. To clarify the nature of these TLR3-independent mechanisms, which may play a relevant role for dsRNA-induced beta cell death and insulitis, we presently examined the expression of the RNA helicases RIG-I and MDA5 in purified primary rat beta cells and in islet cells isolated from $TLR3^{-/-}$ or wild-type mice. The functionality of RIG-I was then evaluated using a RIG-I-specific ligand and a dominant-negative form of the protein. The results obtained suggest that the RIG-I pathway is present and active in pancreatic beta cells and might contribute to the deleterious effects of viral RNA intermediates on beta cell function and to the induction of insulitis.

2. Materials and methods

2.1. Animals

Adult male Wistar rats (Charles River Laboratories, Brussels, Belgium), C57/BL6 (Harlan CBP, Zeist, The Netherlands) and TLR3 knockout ($TLR3^{-/-}$) mice [13] were housed and used according to the guidelines of the Belgian Regulations for Animal Care. $TLR3^{-/-}$ mice were kept under specific pathogen-free (SPF) conditions at the Katholieke Universiteit van Leuven (KUL). All experiments were conducted with the approval of the Animal Ethics Committees of the Université Libre de Bruxelles and KUL.

2.2. Cell culture

Pancreatic islets were isolated, digested, hand-picked under a stereomicroscope and dissociated into single cells in the presence of dispase (0.5 mg/ml). Beta cells were purified by autofluorescence-activated cell sorting (FACS, FACStar; Becton-Dickinson, Sunnyvale, CA) [23]. Purified beta cells (>90% pure) were then cultured in HAM's F-10 medium as previously described [23].

Mouse spleen cells were digested with collagenase P (3.5 U/ml), further dissociated in calcium-free medium and separated into low- and high-density fraction on a Nycodenz gradient (Nycomed). Low-density cells were cultured for 2 h in RPMI 1640 containing 5% heat-inactivated fetal bovine serum (FBS); non-adherent cells were then eliminated by vigorous pipetting and adherent cells were cultured overnight at 37 °C. After overnight culture, non-adherent cells contained $87 \pm 2\%$ of CD11c-positive dendritic cells ($n = 7$) as assessed by CD11c staining (data not shown).

2.3. Real-time RT-PCR

FACS-purified rat beta cells, mouse dispersed islet cells, CD11c or INS-1E cells were cultured in Falcon 24-well plates (10^5 cells per well); for beta cells and dispersed islet cells culture, the plates were pre-coated with poly-L-lysine. Cells were maintained in control condition or were exposed to either external dsRNA (tested in the form of polyinosinic–polycytidilic acid; PICex; 100 μg/ml; Sigma) or to internal dsRNA (PICin). Introduction of foreign dsRNA into the cells was achieved by treating them for 4 h with a mixture of lipofectamine and PIC (1 μg/ml for rat beta cells and 10 μg/ml for mouse dispersed islet cells). As control condition for the internal PIC treatment, cells were treated with lipofectamine alone (LF). Cells were harvested 2, 6 or 24 h after treatment, and mRNA ($poly(A)^+$ RNA) was extracted and reverse transcribed as previously described [36]. Real-time quantitative PCR for RIG-I, MDA5 and GAPDH was done using SYBR Green fluorescence on a Roche Lightcycler instrument in a 20-μl reaction containing 3 mmol/l $MgCl_2$, 0.5 μmol/l forward and reverse primers, 2 μl FastStart SYBR Green mix (Roche) and 2 μl template cDNA. The primer sequences and their respective fragment lengths are described in Table 1 (qPCR). The method used for quantification was the standard curve approach [37]. To obtain the specific standard curves, the primers sequence and their respective PCR fragment sizes are described in Table 1 (standard). The target cDNAs present in each sample were corrected for their respective GAPDH values. Expression

Table 1
Primer sequences for RIG-I, MDA5 and GAPDH and their respective fragment length.

Gene		Primer sequence	Size (bp)
RIG-I	qPCR	Sense 5′-AAAGCCAGAGACCAAGACCA-3′ Antisense 5′-TATCTCCGCTGGCTCTGAAT-3′	200
	Standard	Sense 5′-CACAAAGCGTGCTCAGTGTT-3′ Antisense 5′-GTATGCGGTGAACCGTCTTT-3′	802
MDA5	qPCR	Sense 5′-TGTCTTGGQCQCTTGCTTCG-3′ Antisense 5′-TGCTGAGAAGGATTGTGCAG-3′	121
	Standard	Sense 5′-TGACGAGTGTCTCCACTTGC-3′ Antisense 5′-TCCATTTGGTAAGGCCTGAG-3′	612
GAPDH (rat)	qPCR	Sense 5′-AGTTCAACGGCACAGTCAAG-3′ Antisense 5′-TACTCAGCACCAGCATCACC-3′	118
	Standard	Sense 5′-TCCCTCAAGATTGTCAGCAAA-3′ Antisense 5′-AATGTATCCGTTGTGGATCT-3′	308
GAPDH (mouse)	qPCR	Sense 5′-AACTTTGGCATTGTGGAAGG-3′ Antisense 5′-GGATGCAGGGATGATGTTCT-3′	138
	Standard	Sense 5′-TAACATCAAATGGGGTGAGG-3′ Antisense 5′-TGTTGCTGTAGCCGTATTCA-3′	719

of the "housekeeping" gene GAPDH is not affected by exposure to PIC [22], a finding confirmed in the present experiments (data not shown).

2.4. Assessment of cell viability

Rat beta cells (10^4 cells per well) were cultured in Falcon 96-well microtiter plates pre-coated with poly-L-lysine. ssRNA (polyC; 1 μg/ml; Sigma), dsRNA (polyI:C; 1 μg/ml; Sigma), in vitro-transcribed uncapped 5′-triphospate RNA, namely 5′triP-ssRNA (5′-pppGGGGCUGACCCUGAAGUUCAUCUU-3′; 1.33 μg/ml; prepared as described [32]) or a chemically synthesized 5′hydroxyl-ssRNA used as negative control (5′-OH-GGGGCUGACCCUGAAGUUCAUCUU-3′; 1.33 μg/ml; Eurogentec, Belgium) were introduced into the cells by a 4-h treatment with a mixture containing lipofectamine and each of the compounds. Cells treated with lipofectamine alone (LF) were used as control. Dose–response studies were performed to select the most adequate intra- and extracellular dsRNA concentrations for the present experiments (data not shown). The transfected cells were then maintained in culture for up to 5 days. The percentage of viable, apoptotic or necrotic cells was assessed by incubation for 15 min with propidium iodide (PI, 10 μg/ml) and Hoechst (HO) 342 (10 μg/ml) [38]. This fluorescence assay for single cells is quantitative and has been validated by comparison against electron microscopy observations [39], determination of DNA strand breaks [40] and caspase-3 activation [41,42]. The method has been successfully used to evaluate apoptosis/necrosis in rat [38,39], mouse [43,44] and human [45] beta cells. Counting of viable, apoptotic and necrotic cells was done by two independent observers, with one of them unaware of the tested conditions. At least 500 cells were counted per experimental condition, and the agreement between the observers was always >90%.

2.5. Reporter gene assay

Purified beta cells were transfected using lipofectamine with plasmid constructs containing the firefly luciferase gene under the control of either multiples copies of the NF-κB consensus sequence (BD Biosciences Clontech) or the mouse IFN-β promoter [23,46]; a pRL-CMV plasmid was co-transfected as internal control for transfection efficiency. In some experiments, cells were transfected with the plasmids described above and with an expression plasmid encoding for a dominant-negative form of RIG-I [14] or its empty vector pFLAG. After 24 h, the cells were transfected either with dsRNA (PICin; 1 μg/ml), ssRNA (1 μg/ml; used as negative control), 5′triP-ssRNA (1.33 μg/ml) or 5′OH-ssRNA (1.33 μg/ml) in the presence of lipofectamine (see above); cells treated with lipofectamine alone (LF) were used as controls. Luciferase activities were assayed 16 h later with the Dual Luciferase Reporter Assay System (Promega, Madison, WI) [23]. Test values were corrected for the luciferase activity value of the internal control plasmid, pRL-CMV.

2.6. Statistical analysis

Data are presented as means ± SEM of at least four independent experiments. Statistical differences between groups were determined either by paired Student's *t*-test or by ANOVA followed by *t*-test with the Bonferroni correction, as indicated. A *p* value of less than 0.05 was considered as statistically significant.

3. Results

3.1. DsRNA induces RIG-I and MDA5 mRNA expression in rat pancreatic beta cells

To examine expression of RIG-I and MDA5 mRNAs, primary rat beta cells were cultured in control condition or exposed to extracellular dsRNA (PICex) or intracellular dsRNA (PICin) for 2, 6 or 24 h. Addition of PICex for 6 h led to a 23- and 11-fold induction of RIG-I (Fig. 1A) and MDA5 (Fig. 1B) expression, respectively. This induction was maintained up to 24 h in the case of RIG-I (Fig. 1A), while MDA5 mRNA returned to basal value at this time point (Fig. 1B). When PIC was introduced in the cells by lipofection, the up-regulation of both RIG-I and MDA5 appeared earlier (starting already after 2 h), was more pronounced (around 50-fold induction) and was maintained up to 24 h (Fig. 1). Of note, expression of the adaptor molecule VISA was also detected in rat beta cells but was not affected by PICex or PICin (data not shown).

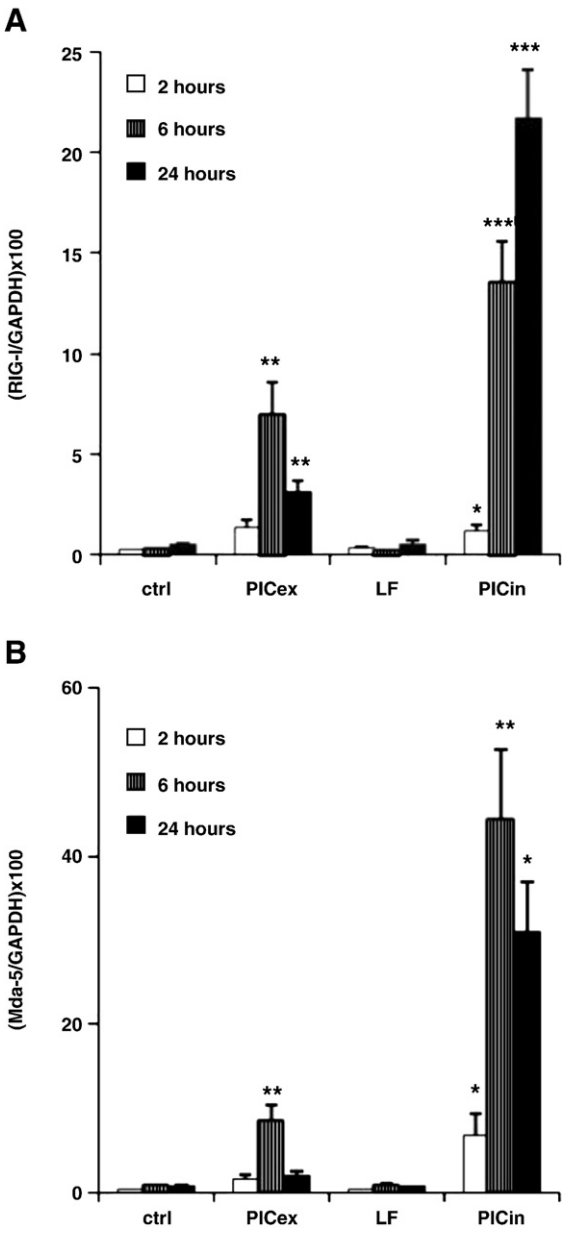

Fig. 1. Expression of RIG-I and MDA5 is regulated by PICin and PICex in rat beta cells. Purified rat beta cells were cultured in the absence (Ctrl or LF) or presence of PICex (100 μg/ml) or PICin (1 μg/ml) for 2–24 h. RIG-I (A) and MDA5 (B) mRNA expression were analyzed by real-time RT-PCR and the data were normalized for the housekeeping gene GAPDH. The results are means ± SEM of four to six experiments. *$p<0.05$; **$p<0.01$; and ***$p<0.005$ vs. respective control; Student's paired *t*-test.

3.2. TLR3 signaling controls RIG-I and MDA5 expression in primary beta cells

In a previous study, we described that islet cells isolated from TLR3$^{-/-}$ mice produce 4-fold less IFN-β than islet cells from wild-

type (WT) mice in response to PICin [28]. We therefore investigated whether the TLR3 pathway regulates expression of MDA5 and/or RIG-I in pancreatic beta cells. Dispersed islet cells were isolated from WT or TLR3$^{-/-}$ mice and exposed to either PICex or PICin. As observed in rat beta cells, exposure of WT mouse islet cells to PICex or PICin increased both RIG-I (Fig. 2A) and MDA5 (Fig. 2B) expression. Disruption of the TLR3 signaling pathway prevented PICex-triggered MDA5 and RIG-I up-regulation (Fig. 2). In contrast,

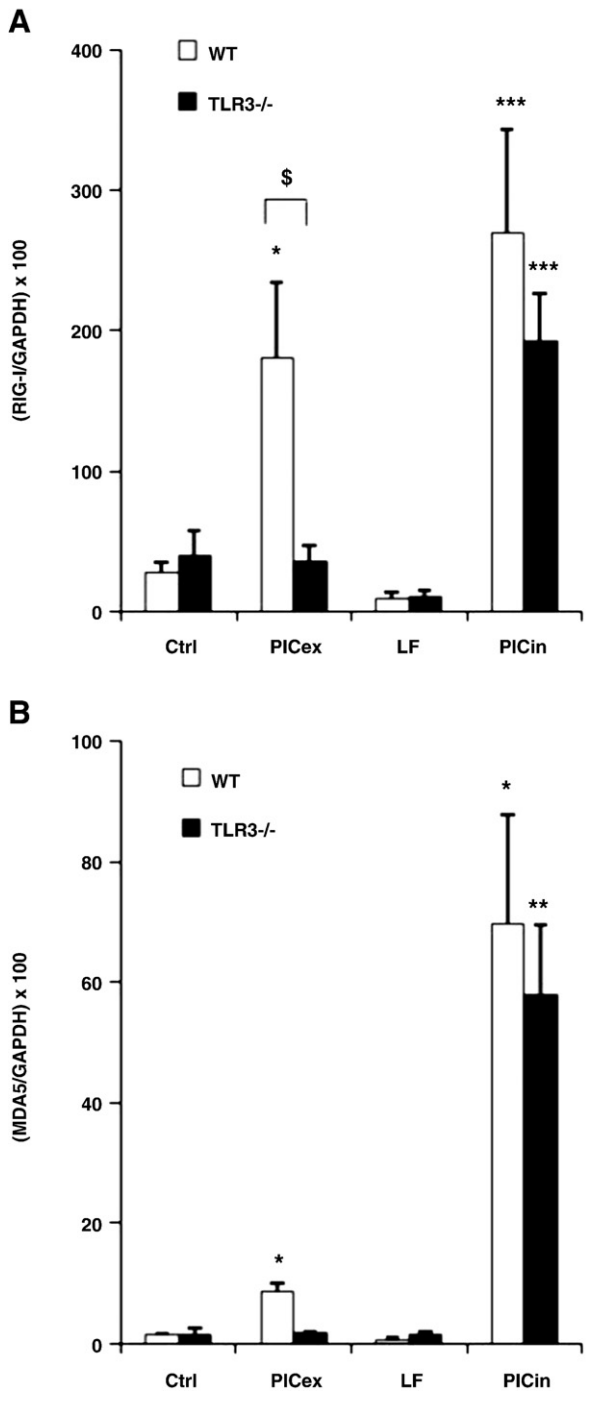

Fig. 2. The TLR3 pathway regulates PICex-induced RIG-I and MDA5 expression in mouse pancreatic islet cells. Dispersed islet cells from wild-type (WT) and TLR3$^{-/-}$ mice were cultured for 6 h in the absence (Ctrl or LF) or presence of PICex (100 μg/ml) or PICin (10 μg/ml). RIG-I (A) and MDA5 (B) mRNA expression were analyzed by real-time RT-PCR and the data were normalized for the housekeeping gene GAPDH. The results are means ± SEM of six to nine experiments. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. respective control; and $^{\$}p < 0.05$ vs. WT; ANOVA.

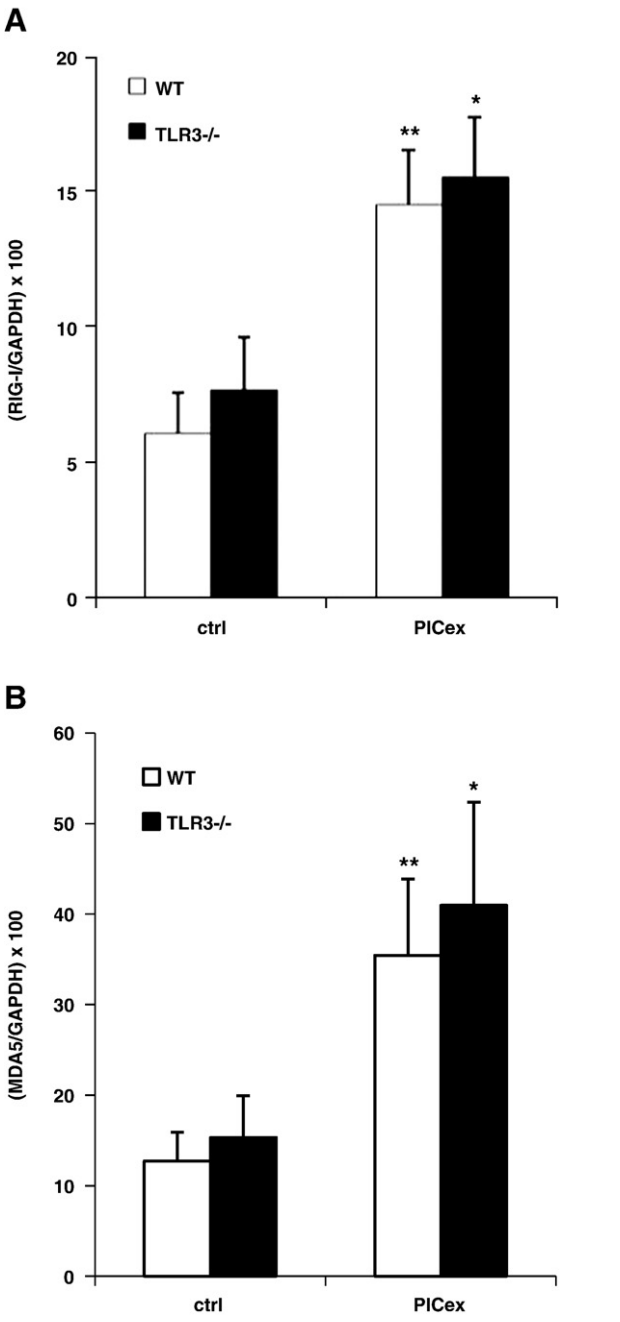

Fig. 3. Expression of RIG-I and MDA5 in dendritic cells from wild-type and TLR3$^{-/-}$ mice. Splenic dendritic cells (CD11c) isolated from wild-type (WT) and TLR3$^{-/-}$ mice were cultured for 6 h in the absence (ctrl) or presence PICex (100 μg/ml). RIG-I and MDA5 mRNA expression were analyzed by real-time RT-PCR and the data were normalized for the housekeeping gene GAPDH. The results are means ± SEM of six to nine experiments. *$p < 0.05$; **$p < 0.01$ vs. control; Student's paired *t*-test.

intracellular dsRNA-induced up-regulation of RIG-I (Fig. 2A) and MDA5 (Fig. 2B) was not affected by TLR3 disruption.

These results demonstrate that the TLR3 signaling pathway controls RIG-I and MDA5 expression in response to extracellular, but no intracellular, dsRNA in pancreatic beta cells.

To establish whether such a role for the TLR3 pathway is also observed in other cell types, we studied expression of the helicases in immune cells, namely splenic dendritic cells (CD11c). CD11c cells were isolated from WT or TLR3$^{-/-}$ mice and exposed to PICex. As observed in rat beta cells, exposure of WT CD11c cells to PICex increased expression of RIG-I (Fig. 3A) and MDA5 (Fig. 3B) mRNAs. In

the dendritic cells, however, deletion of the TLR3 pathway did not prevent MDA5 and RIG-I mRNA up-regulation by PICex (Fig. 3) as it was the case for beta cells (Fig. 2).

These results show that the key role for the TLR3 pathway in PICex-triggered MDA5 and RIG-I expression is present in beta cells but not in splenic dendritic cells.

3.3. RIG-I regulates IFN-β promoter and NF-κB activation in primary beta cells

To investigate the functionality of RIG-I in pancreatic beta cells, we examined whether PICin-induced IFN-β promoter and NF-κB activation are affected by the expression of a dominant-negative form of RIG-I [14]. For this purpose, beta cells were co-transfected with an IFN-β promoter or an NF-κB reporter plasmid and with a plasmid encoding for a dominant-negative form of RIG-I (DN-RIG-I) or its empty vector pFLAG. As shown in Fig. 4, internal dsRNA, but not ssRNA, increased by 7-fold the IFN-β promoter activity. PICin-induced IFN-β promoter activation was prevented by the DN-RIG-I (Fig. 4). Likewise, PICin-induced NF-κB activation was inhibited by the DN-RIG-I (Fig. 5). Of note, NF-κB activation by IL-1β (8.0 ± 1.9-fold activation; $n = 4$), used as positive control, was not affected by the presence of the DN-RIG-I (9.6 ± 2.2-fold activation; $n = 4$).

3.4. RIG-I stimulation by its specific ligand triggers pancreatic beta cell apoptosis

It was recently described that 5′-triphosphate ssRNA (5′triP-ssRNA) is a specific ligand for RIG-I [32]. We therefore evaluated the effect of this compound on pancreatic beta cell viability. Introduction of 5′triP-ssRNA in the cytosol by lipofection increased beta cell apoptosis but not necrosis (Fig. 6). Importantly, the non-phosphorylated form of the ssRNA fragment (5′OH-ssRNA), which is not recognized by RIG-I, did not affect beta cell viability (Fig. 6).

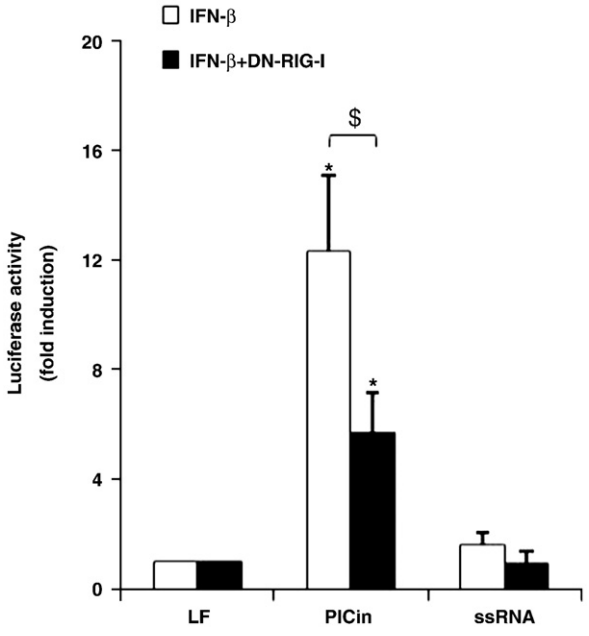

Fig. 4. RIG-I mediates internal dsRNA-induced IFN-β promoter activation in rat beta cells. Purified beta cells were co-transfected with an IFN-β promoter reporter plasmid, a pRL-CMV plasmid (used as internal control) and, as required, an expression plasmid encoding for the dominant-negative form of RIG-I or its empty vector pFLAG. After overnight culture, the cells were transfected with PIC (1 μg/ml) or ssRNA (1 μg/ml), used as negative control. Luciferase activities were measured 16 h later and corrected for the values obtained with the pRL-CMV plasmid in the same sample. The results are means ± SEM of 8–14 experiments, $^{*}p < 0.01$ vs. LF; $^{\$}p < 0.05$ vs. PICin without DN-RIG-I; ANOVA.

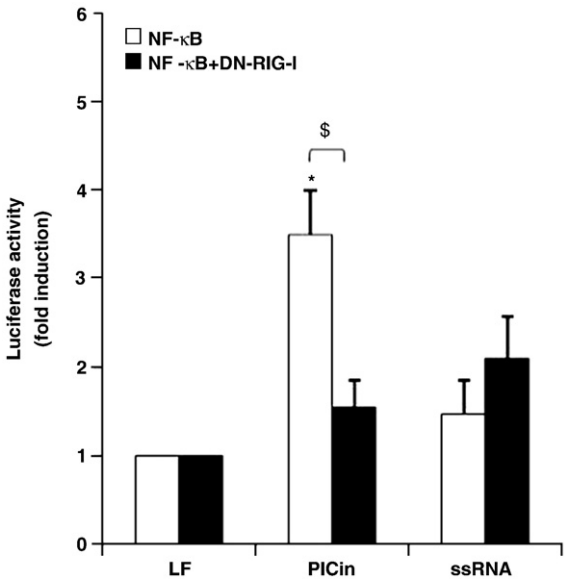

Fig. 5. RIG-I mediates internal dsRNA-induced NF-κB activation in rat beta cells. Purified rat beta cells were co-transfected with an NF-κB reporter plasmid and the internal control plasmid pRL-CMV, in the presence of either an expression plasmid encoding for the dominant-negative form of RIG-I or pFLAG, its empty vector. 24 h later, the cells were transfected with PIC (1 μg/ml) or ssRNA (1 μg/ml). Luciferase activities were measured 16 h later and corrected for those obtained for the pRL-CMV plasmid in the same sample. The results are means ± SEM of 7–11 experiments, $^{*}p < 0.01$ vs. LF; $^{\$}p < 0.05$ vs. PICin without DN-RIG-I; ANOVA.

We further determined whether intracellular 5′triP-ssRNA induces NF-κB and IFN-β promoter activation. As shown in Fig. 7, both NF-κB (Fig. 7A) and the IFN-β promoter (Fig. 7B) were activated by internal 5′triP-ssRNA, but not by 5′OH-ssRNA (used as negative control). Co-transfection with the DN-RIG-I reduced 5′triP-ssRNA-induced IFN-β promoter activation but did not affect NF-κB activation.

4. Discussion

Enteroviral infections, acting in conjunction with a predisposing genetic background, may trigger insulitis and eventually lead to the development of T1DM. The molecular mechanisms by which viruses or viral RNA intermediates induce insulitis and pancreatic beta cell death are still unclear. It has been proposed that during viral infection the local production of cytokines and chemokines by both invading immune cells and the pancreatic beta cells themselves may interact to initiate and/or accelerate the transition from innate immune response to a chronic autoimmune assault [28,47,48].

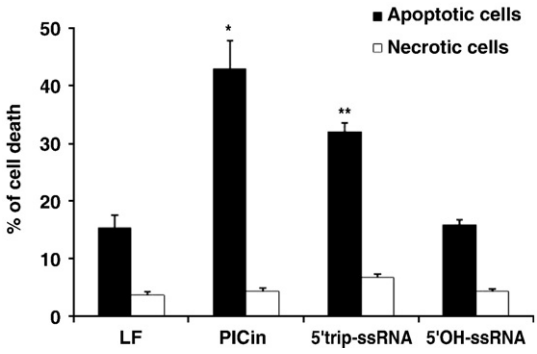

Fig. 6. RIG-I stimulation by its specific ligand 5′triP-ssRNA induces beta cell apoptosis. FACS-purified rat beta cells were transfected with PIC, 5′triP-ssRNA or 5′OH-ssRNA (1 μg/ml); cells treated with lipofectamine alone were used as control (LF). Cell viability was determined with the DNA-binding dyes HO 342 and PI. The results are means ± SEM of four individual experiments. $^{*}p < 0.05$ vs. LF; Student's paired t-test.

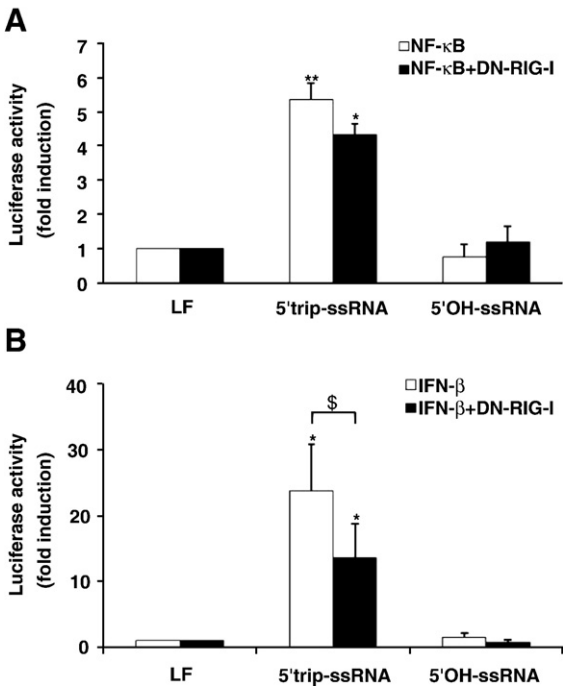

Fig. 7. 5′triP-ssRNA activates NF-κB and the IFN-β promoter in beta cells. Purified beta cells were co-transfected with an NF-κB (A) or an IFN-β promoter (B) reporter plasmid and the pRL-CMV plasmid (internal control), in the presence of either the DN-RIG-I or pFLAG. After overnight culture, the cells were transfected with 5′triP-ssRNA or 5′OH-ssRNA (1 μg/ml); cells treated with lipofectamine alone were used as control (LF). Luciferase activities were corrected for those obtained for the pRL-CMV plasmid in the same sample. The results are means ± SEM of four to six experiments; $^{*}p < 0.05$, $^{**}p < 0.01$ vs. LF, $^{\$}p < 0.05$ vs. PICin without DN-RIG-I; ANOVA.

The host response to viral infection triggers the production of type I IFNs through activation of the transcription factors NF-κB and IRF-3. The subsequent transcription of IFN-stimulated genes such as pro-inflammatory cytokines and chemokines will then allow prevention of viral replication and spreading [49].

We have recently shown that pancreatic beta cells express functional TLR3 and that this immune pattern recognition receptor plays a key role in the modulation of beta cell gene expression and subsequent apoptosis triggered by extracellular dsRNA [23,28]. We further demonstrated that introduction of dsRNA in the cytosol by lipofection induces beta cell apoptosis through activation of IRF-3 and production of type I IFN [28]. Deletion of the TLR3 pathway, however, neither suppressed internal dsRNA-triggered NF-κB and IRF-3 activation nor prevented beta cell apoptosis [28]. These observations suggest that in pancreatic beta cells TLR3-independent pathway(s) contribute to type I IFNs production, de novo gene expression and apoptosis in response to intracellular dsRNA. The present work was therefore initiated to clarify the nature of these TLR3-independent mechanisms, which may play a relevant role for intracellular dsRNA-induced beta cell death and insulitis, especially by inducing NF-κB activation and a massive production of type I interferons, both phenomenon having a pro-apoptotic role in pancreatic beta cells. For this purpose, we performed the experiments in primary and pure beta cell preparations (>90% beta cells), a model which enables us to understand beta cell responses to viral components without the confounding signals generated by other endocrine- and non-endocrine islet cells.

On the other hand, use of purified beta cells isolated from their native environment poses limitations to the extrapolation of the findings obtained to the *in vivo* diabetes situation. Future studies, using mouse models of virus-induced insulitis and diabetes, will be required to further clarify the role for RNA helicases and TLRs in diabetes.

The helicases RIG-I and MDA5 were recently identified as important cytosolic viral RNA sensors [29], triggering immune responses such as type I IFNs production through activation of NF-κB and IRF-3 [50]. Against this background, we have presently evaluated whether beta cells express RIG-I and MDA5 and the putative role of these cytosolic proteins for beta cell responses to intracellular dsRNA and ssRNA. The results obtained indicate that the dsRNA synthetic analogue PIC, when added to the culture medium, induces a mild up-regulation of both MDA5 and RIG-I mRNA expression, whereas introduction of PIC in the cytosol triggers an early and more marked increase in their mRNA levels. RIG-I and MDA-5 up-regulation depends on type I IFNs production and we have previously shown that PICin, but not PICex, induces a massive and sustained production of type I IFNs in pancreatic beta cells [28].

Interestingly, we observed that expression of RIG-I and MDA5 is modulated by the TLR3 pathway in pancreatic beta cells but not in splenic dendritic cells suggesting a cell-type-specific role for the innate immune pattern recognition receptor, as documented in epithelial cells [51] and plasmacytoid dendritic cells [27].

5′-Triphosphate ssRNA is an important ligand for RIG-I [31,32], but recent evidence indicates that RIG-I also interacts with dsRNA [52,53]. In the present study, we demonstrated that internal dsRNA-induced NF-κB and IFN-β promoter activations are decreased by the presence of a dominant-negative form of RIG-I, suggesting that RIG-I recognizes dsRNA in pancreatic beta cells and thus contributes to trigger the antiviral response. The presence of the dominant-negative form of RIG-I reduced but did not suppress IFN-β promoter activation by dsRNA, suggesting that other intracellular RNA sensor such as MDA-5 could also play a role in IFN-promoter activation in beta cells. The RIG-I pathway was shown to enhance PIC-induced apoptosis in macrophages [54] and activation of the transcription factor NF-κB has a pro-apoptotic role in pancreatic beta cells [55]; activation of RIG-I could therefore contribute to internal dsRNA-induced beta cell apoptosis. In line with this possibility, the RIG-I ligand 5′triP-ssRNA triggers beta cell apoptosis and activates NF-κB and the IFN-β promoter. The role for the RNA helicases RIG-I and MDA-5 in dsRNA-induced beta cell death requires additional investigation, since a decrease (>85%) in RIG-I or MDA-5 mRNA expression mediated by specific siRNAs failed to prevent dsRNA-induced beta cell death [56; Colli M.L. et al., manuscript in preparation].

A dominant-negative form of RIG-I partially prevented activation of the IFN-β promoter but did not affect activation of the transcription factor NF-κB by 5′triP-ssRNA. This last observation might be explained by the recent demonstration that PKR, a well-known activator of NF-κB in response to intracellular dsRNA, is also activated by 5′triP RNA, independently of RIG-I [57].

The cell-type-specific responses to viral infections may reflect the different capacity of infected cells to recognize virally produced molecules such as dsRNA or uncapped 5′-triphosphate ssRNA, leading to a preferential induction of TLR3-, RIG-I- or MDA5-dependent responses [58–61]. Expression of several antiviral receptors and pathways was up to now considered to be restricted to epithelial cells [62], in which stimulation of these receptors activate antiviral but not pro-inflammatory responses [63]. The present and previous data from our group [23,28] and others [25] demonstrate that pancreatic beta cells are remarkably well equipped to respond to viral RNA intermediates, expressing several functional innate immune pattern recognition receptors involved in the production of cytokines, chemokines and type I IFNs [64]. Of interest, Chehadeh et al. [65] demonstrated that infection of human islets by coxsackievirus leads to viral replication in both alpha and beta islet cells while it triggers the synthesis of IFN-α in beta cells only, suggesting that the beta cell-specific IFN-α production may play a role in the initiation and/or the maintenance of chronic CVB infection in human islets. High levels of type I IFNs have been detected in the pancreas or islets of type 1 diabetic patients [66,67] and these cytokines are implicated in the pathogenesis of

viral-induced type 1 diabetes [68,69]. Our results suggest that the presence of viral compounds in the cytosol of beta cells triggers a massive production of type I IFNs, partially through activation of RIG-I, leading to the initiation of antiviral responses in neighboring cells. Since TLR3 is highly expressed in pancreatic beta cells [70], the viral dsRNA released by dying cells will also activate the TLR3 pathway in surrounding non-infected cells. Stimulation of the TLR3 pathway, acting in conjunction with the IFNs produced by both the beta cells and the invading immune cells [23,28], may therefore improperly initiate the apoptotic machinery in non-infected beta cells, amplifying beta cell death and accelerating the transition from the innate immune response to a chronic autoimmune assault in genetically predisposed individuals.

Acknowledgements

This study was supported by grants from the EFSD/Lilly European Diabetes Research Programme, the "Fonds David et Alice Van Buuren", the "Fonds de la Recherche Scientifique Médicale" (convention no. 3.4514.03/avenant 3.4554.05), the Communauté Française de Belgique-Actions de Recherche Concertées (ARC) and the European Union (STREP Savebeta, contract no. 036903, in the Framework Programme 6 of the European Community). We are grateful to Prof. Lena Alexopoulou (CNRS-INSERM-Université de la Mediterranée, Marseille, France) for providing the TLR3$^{-/-}$ mice and to Prof. Chantal Mathieu and her group (Katholieke Universiteit van Leuven, Leuven, Belgium) for the maintenance of the TLR3$^{-/-}$ colony in Belgium. We thank Dr. Hendrik Poeck for providing the 5′triP-ssRNA compounds under the guidance of Prof. G. Hartmann.

We are very grateful to J. Schoonheydt, M. Urbain and G. Vandenbroeck for expert technical assistance.

References

[1] K.M. Gillespie, Type 1 diabetes: pathogenesis and prevention, CMAJ 175 (2006) 165–170.
[2] M. Honeyman, How robust is the evidence for viruses in the induction of type 1 diabetes? Curr. Opin. Immunol. 17 (2005) 616–623.
[3] K.M. Drescher, S.M. Tracy, The CVB and etiology of type 1 diabetes, Curr. Top. Microbiol. Immunol. 323 (2008) 259–274.
[4] C. Nairn, D.N. Galbraith, K.W. Taylor, G.B. Clements, Enterovirus variants in the serum of children at the onset of type 1 diabetes mellitus, Diabet. Med. 16 (1999) 509–513.
[5] W. Chehadeh, J. Weill, M.C. Vantyghem, G. Alm, J. Lefebvre, P. Wattre, D. Hober, Increased level of interferon-alpha in blood of patients with insulin-dependent diabetes mellitus: relationship with coxsackievirus B infection, J. Infect. Dis. 181 (2000) 1929–1939.
[6] F. Dotta, S. Censini, A.G. van Halteren, L. Marselli, M. Masini, S. Dionisi, F. Mosca, U. Boggi, A.O. Muda, S.D. Prato, J.F. Elliott, A. Covacci, R. Rappuoli, B.O. Roep, P. Marchetti, Coxsackie B4 virus infection of beta cells and natural killer cell insulitis in recent-onset type 1 diabetic patients, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 5115–5120.
[7] S.J. Richardson, A. Willcox, A.J. Bone, A.K. Foulis, N.G. Morgan, The prevalence of enteroviral capsid protein vp1 immunostaining in pancreatic islets in human type 1 diabetes, Diabetologia 52 (2009) 1143–1151.
[8] P. Muir, N.B. Singh, J.E. Banatvala, Enterovirus-specific serum IgA antibody responses in patients with acute infections, chronic cardiac disease, and recently diagnosed insulin-dependent diabetes mellitus, J. Med. Virol. 32 (1990) 236–242.
[9] A. Elfaitouri, A.K. Berg, G. Frisk, H. Yin, T. Tuvemo, J. Blomberg, Recent enterovirus infection in type 1 diabetes: evidence with a novel IgM method, J. Med. Virol. 79 (2007) 1861–1867.
[10] L. Andreoletti, D. Hober, C. Hober-Vandenberghe, I. Fajardy, S. Belaich, V. Lambert, M.C. Vantyghem, J. Lefebvre, P. Wattre, B. Coxsackie, Virus infection and beta cell autoantibodies in newly diagnosed IDDM adult patients, Clin. Diagn. Virol. 9 (1998) 125–133.
[11] G. Frisk, T. Tuvemo, Enterovirus infections with beta-cell tropic strains are frequent in siblings of children diagnosed with type 1 diabetes children and in association with elevated levels of GAD65 antibodies, J. Med. Virol. 73 (2004) 450–459.
[12] E. Meylan, J. Tschopp, M. Karin, Intracellular pattern recognition receptors in the host response, Nature 442 (2006) 39–44.
[13] L. Alexopoulou, A.C. Holt, R. Medzhitov, R.A. Flavell, Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3, Nature 413 (2001) 732–738.
[14] M. Yoneyama, M. Kikuchi, T. Natsukawa, N. Shinobu, T. Imaizumi, M. Miyagishi, K. Taira, S. Akira, T. Fujita, The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses, Nat, Immunol. 5 (2004) 730–737.
[15] H. Kato, O. Takeuchi, S. Sato, M. Yoneyama, M. Yamamoto, K. Matsui, S. Uematsu, A. Jung, T. Kawai, K.J. Ishii, O. Yamaguchi, K. Otsu, T. Tsujimura, C.S. Koh, C. Reis e Sousa, Y. Matsuura, T. Fujita, S. Akira, Differential roles of MDA5 and RIG-I helicases in the recognition of RNA viruses, Nature 441 (2006) 101–105.
[16] P. Ahlquist, Parallels among positive-strand RNA viruses, reverse-transcribing viruses and double-stranded RNA viruses, Nat. Rev. Microbiol. 4 (2006) 371–382.
[17] B.L. Jacobs, J.O. Langland, When two strands are better than one: the mediators and modulators of the cellular responses to double-stranded RNA, Virology 219 (1996) 339–349.
[18] J.A. Majde, N. Guha-Thakurta, Z. Chen, S. Bredow, J.M. Krueger, Spontaneous release of stable viral double-stranded RNA into the extracellular medium by influenza virus-infected MDCK epithelial cells: implications for the viral acute phase response, Arch. Virol. 143 (1998) 2371–2380.
[19] K. Takeda, S. Akira, Toll-like receptors in innate immunity, Int. Immunol. 17 (2005) 1–14.
[20] M.R. Heitmeier, A.L. Scarim, J.A. Corbett, Double-stranded RNA inhibits beta-cell function and induces islet damage by stimulating beta-cell production of nitric oxide, J. Biol. Chem. 274 (1999) 12531–12536.
[21] D.O. Sobel, J. Newsome, C.H. Ewel, J.A. Bellanti, V. Abbassi, K. Creswell, O. Blair, Poly I:C induces development of diabetes mellitus in BB rat, Diabetes 41 (1992) 515–520.
[22] D. Liu, A.K. Cardozo, M.I. Darville, D.L. Eizirik, Double-stranded RNA cooperates with interferon-gamma and IL-1 beta to induce both chemokine expression and nuclear factor-kappa B-dependent apoptosis in pancreatic beta-cells: potential mechanisms for viral-induced insulitis and beta-cell death in type 1 diabetes mellitus, Endocrinology 143 (2002) 1225–1234.
[23] J. Rasschaert, L. Ladriere, M. Urbain, Z. Dogusan, B. Katabua, S. Sato, S. Akira, C. Gysemans, C. Mathieu, D.L. Eizirik, Toll-like receptor 3 and STAT-1 contribute to double-stranded RNA+ interferon-gamma-induced apoptosis in primary pancreatic beta-cells, J. Biol. Chem. 280 (2005) 33984–33991.
[24] P. Ylipaasto, B. Kutlu, S. Rasilainen, J. Rasschaert, K. Salmela, H. Teerijoki, O. Korsgren, R. Lahesmaa, T. Hovi, D.L. Eizirik, T. Otonkoski, M. Roivainen, Global profiling of coxsackievirus- and cytokine-induced gene expression in human pancreatic islets, Diabetologia 48 (2005) 1510–1522.
[25] M. Hultcrantz, M.H. Huhn, M. Wolf, A. Olsson, S. Jacobson, B.R. Williams, O. Korsgren, M. Flodstrom-Tullberg, Interferons induce an antiviral state in human pancreatic islet cells, Virology 367 (2007) 92–101.
[26] G.C. Sen, S.N. Sarkar, Transcriptional signaling by double-stranded RNA: role of TLR3, Cytokine Growth Factor Rev. 16 (2005) 1–14.
[27] H. Kato, S. Sato, M. Yoneyama, M. Yamamoto, S. Uematsu, K. Matsui, T. Tsujimura, K. Takeda, T. Fujita, O. Takeuchi, S. Akira, Cell type-specific involvement of RIG-I in antiviral response, Immunity 23 (2005) 19–28.
[28] Z. Dogusan, M. Garcia, D. Flamez, L. Alexopoulou, M. Goldman, C. Gysemans, C. Mathieu, C. Libert, D.L. Eizirik, J. Rasschaert, Double-stranded RNA induces pancreatic beta-cell apoptosis by activation of the toll-like receptor 3 and interferon regulatory factor 3 pathways, Diabetes 57 (2008) 1236–1245.
[29] E.M. Creagh, L.A. O'Neill, TLRs, NLRs and RLRs: a trinity of pathogen sensors that co-operate in innate immunity, Trends Immunol. 27 (2006) 352–357.
[30] M. Yoneyama, T. Fujita, RIG-I family RNA helicases: cytoplasmic sensor for antiviral innate immunity, Cytokine Growth Factor Rev. 18 (2007) 545–551.
[31] A. Pichlmair, O. Schulz, C.P. Tan, T.I. Naslund, P. Liljestrom, F. Weber, C. Reis e Sousa, RIG-I-mediated antiviral responses to single-stranded RNA bearing 5′-phosphates, Science 314 (2006) 997–1001.
[32] V. Hornung, J. Ellegast, S. Kim, K. Brzozka, A. Jung, H. Kato, H. Poeck, S. Akira, K.K. Conzelmann, M. Schlee, S. Endres, G. Hartmann, 5′-Triphosphate RNA is the ligand for RIG-I, Science 314 (2006) 994–997.
[33] L.G. Xu, Y.Y. Wang, K.J. Han, L.Y. Li, Z. Zhai, H.B. Shu, VISA is an adapter protein required for virus-triggered IFN-beta signalling, Mol. Cell 19 (2005) 727–740.
[34] M. Yoneyama, M. Kikuchi, K. Matsumoto, T. Imaizumi, M. Miyagishi, K. Taira, E. Foy, Y.M. Loo, M. Gale Jr, S. Akira, S. Yonehara, A. Kato, T. Fujita, Shared and unique functions of the DExD/H-box helicases RIG-I, MDA5, and LGP2 in antiviral innate immunity, J. Immunol. 175 (2005) 2851–2858.
[35] C.L. Johnson, M. Gale Jr, CARD games between virus and host get a new player, Trends Immunol. 27 (2006) 1–4.
[36] A.K. Cardozo, M. Kruhoffer, R. Leeman, T. Orntoft, D.L. Eizirik, Identification of novel cytokine-induced genes in pancreatic beta-cells by high-density oligonucleotide arrays, Diabetes 50 (2001) 909–920.
[37] A. Giulietti, L. Overbergh, D. Valckx, B. Decallonne, R. Bouillon, C. Mathieu, An overview of real-time quantitative PCR: applications to quantify cytokine gene expression, Methods 25 (2001) 386–401.
[38] D. Liu, M. Darville, D.L. Eizirik, Double-stranded ribonucleic acid (RNA) induces beta-cell Fas messenger RNA expression and increases cytokine-induced beta-cell apoptosis, Endocrinology 142 (2001) 2593–2599.
[39] A. Hoorens, M. Van de Casteele, G. Kloppel, D. Pipeleers, Glucose promotes survival of rat pancreatic beta cells by activating synthesis of proteins which suppress a constitutive apoptotic program, J. Clin. Invest. 98 (1996) 1568–1574.
[40] I. Kharroubi, L. Ladriere, A.K. Cardozo, Z. Dogusan, M. Cnop, D.L. Eizirik, Free fatty acids and cytokines induce pancreatic beta-cell apoptosis by different mechanisms: role of nuclear factor-kappaB and endoplasmic reticulum stress, Endocrinology 145 (2004) 5087–5096.
[41] Z. Ling, M. Van de Casteele, D.L. Eizirik, D.G. Pipeleers, Interleukin-1beta-induced alteration in a beta-cell phenotype can reduce cellular sensitivity to conditions that cause necrosis but not to cytokine-induced apoptosis, Diabetes 49 (2000) 340–345.

[42] D.L. Eizirik, F. Moore, D. Flamez, F. Ortis, Use of a systems biology approach to understand pancreatic beta-cell death in type 1 diabetes, Biochem. Soc. Trans. 36 (2008) 321–327.
[43] D. Pavlovic, N.A. Andersen, T. Mandrup-Poulsen, D.L. Eizirik, Activation of extracellular signal-regulated kinase (ERK)1/2 contributes to cytokine-induced apoptosis in purified rat pancreatic beta-cells, Eur. Cytokine Netw. 11 (2000) 267–274.
[44] D. Liu, D. Pavlovic, M.C. Chen, M. Flodstrom, S. Sandler, D.L. Eizirik, Cytokines induce apoptosis in beta-cells isolated from mice lacking the inducible isoform of nitric oxide synthase (iNOS−/−), Diabetes 49 (2000) 1116–1122.
[45] C.A. Delaney, D. Pavlovic, A. Hoorens, D.G. Pipeleers, D.L. Eizirik, Cytokines induce deoxyribonucleic acid strand breaks and apoptosis in human pancreatic islet cells, Endocrinology 138 (1997) 2610–2614.
[46] M. Yamamoto, S. Sato, H. Hemmi, K. Hoshino, T. Kaisho, H. Sanjo, O. Takeuchi, M. Sugiyama, M. Okabe, K. Takeda, S. Akira, Role of adaptor TRIF in the MyD88-independent toll-like receptor signaling pathway, Science 301 (2003) 640–643.
[47] H.S. Jun, J.W. Yoon, The role of viruses in type I diabetes: two distinct cellular and molecular pathogenic mechanisms of virus-induced diabetes in animals, Diabetologia 44 (2001) 271–285.
[48] D.L. Eizirik, M.L. Colli, F. Ortis, The role of inflammation in insulitis and beta-cell loss in type 1 diabetes, Nat. Rev. Endocrinol. 5 (2009) 219–226.
[49] T. Taniguchi, A. Takaoka, The interferon-alpha/beta system in antiviral responses: a multimodal machinery of gene regulation by the IRF family of transcription factors, Curr. Opin. Immunol. 14 (2002) 111–116.
[50] Y.M. Loo, J. Fornek, N. Crochet, G. Bajwa, O. Perwitasari, L. Martinez-Sobrido, S. Akira, M.A. Gill, A. Garcia-Sastre, M.G. Katze, M. Gale Jr, Distinct RIG-I and MDA5 signaling by RNA viruses in innate immunity, J. Virol 82 (2008) 335–345.
[51] P. Liu, M. Jamaluddin, K. Li, R.P. Garofalo, A. Casola, A.R. Brasier, Retinoic acid-inducible gene I mediates early antiviral response and Toll-like receptor 3 expression in respiratory syncytial virus-infected airway epithelial cells, J. Virol. 81 (2007) 1401–1411.
[52] P. Gee, P.K. Chua, J. Gevorkyan, K. Klumpp, I. Najera, D.C. Swinney, J. Deval, Essential role of the N-terminal domain in the regulation of RIG-I ATPase activity, J. Biol. Chem. 283 (2008) 9488–9496.
[53] M. Yoneyama, K. Onomoto, T. Fujita, Cytoplasmic recognition of RNA, Adv. Drug Deliv. Rev. 60 (2008) 841–846.
[54] J. Rintahaka, D. Wiik, P.E. Kovanen, H. Alenius, S. Matikainen, Cytosolic antiviral RNA recognition pathway activates caspases 1 and 3, J. Immunol. 180 (2008) 1749–1757.
[55] H. Heimberg, Y. Heremans, C. Jobin, R. Leemans, A.K. Cardozo, M. Darville, D.L. Eizirik, Inhibition of cytokine-induced NF-kappaB activation by adenovirus-mediated expression of a NF-kappaB super-repressor prevents beta-cell apoptosis, Diabetes 50 (2001) 2219–2224.
[56] M.L. Colli, F. Moore, E.N. Gurzov, F. Ortis, D.L. Eizirik, PTPN2 and MDA5, two candidate genes for type 1 diabetes, modify beta cell responses to double-stranded RNA, Diabetologia 52 (Suppl.1) (2009) S 153.
[57] S.R. Nallagatla, J. Hwang, R. Toroney, X. Zheng, C.E. Cameron, P.C. Bevilacqua, 5′-triphosphate-dependent activation of PKR by RNAs with short stem-loops, Science 318 (2007) 1455–1458.
[58] K. Li, Z. Chen, N. Kato, M. Gale Jr, S.M. Lemon, Distinct poly(I-C) and virus-activated signaling pathways leading to interferon-beta production in hepatocytes, J. Biol. Chem. 280 (2005) 16739–16747.
[59] J.M. Andersen, D. Al-Khairy, R.R. Ingalls, Innate immunity at the mucosal surface: role of toll-like receptor 3 and toll-like receptor 9 in cervical epithelial cell responses to microbial pathogens, Biol. Reprod. 74 (2006) 824–831.
[60] T. Kawai, S. Akira, Antiviral signaling through pattern recognition receptors, J. Biochem. (Tokyo) 141 (2007) 137–145.
[61] L. Malmgaard, Induction and regulation of IFNs during viral infections, J. Interferon Cytokine Res. 24 (2004) 439–454.
[62] B.N. Kalali, G. Kollisch, J. Mages, T. Muller, S. Bauer, H. Wagner, J. Ring, R. Lang, M. Mempel, M. Ollert, Double-stranded RNA induces an antiviral defense status in epidermal keratinocytes through TLR3-, PKR-, and MDA5/RIG-I-mediated differential signalling, J. Immunol. 181 (2008) 2694–2704.
[63] A. Uehara, Y. Fujimoto, K. Fukase, H. Takada, Various human epithelial cells express functional Toll-like receptors, NOD1 and NOD2 to produce anti-microbial peptides, but not proinflammatory cytokines, Mol. Immunol. 44 (2007) 3100–3111.
[64] J. Rasschaert, D. Liu, B. Kutlu, A.K. Cardozo, M. Kruhoffer, T.F. ORntoft, D.L. Eizirik, Global profiling of double stranded RNA- and IFN-gamma-induced genes in rat pancreatic beta cells, Diabetologia 46 (2003) 1641–1657.
[65] W. Chehadeh, J. Kerr-Conte, F. Pattou, G. Alm, J. Lefebvre, P. Wattré, D. Hober, Persistent infection of human pancreatic islets by coxsackievirus B is associated with alpha interferon synthesis in beta cells, J. Virol. 74 (2000) 10153–10164.
[66] A.K. Foulis, M.A. Farquharson, A. Meager, Immunoreactive alpha-interferon in insulin-secreting beta cells in type 1 diabetes mellitus, Lancet 2 (1987) 1423–1427.
[67] X. Huang, J. Yuang, A. Goddard, A. Foulis, R.F. James, A. Lernmark, R. Pujol-Borrell, A. Rabinovitch, N. Somoza, T.A. Stewart, Interferon expression in the pancreases of patients with type I diabetes, Diabetes 44 (1995) 658–664.
[68] D. Devendra, G.S. Eisenbarth, Interferon alpha—a potential link in the pathogenesis of viral-induced type 1 diabetes and autoimmunity, Clin. Immunol. 111 (2004) 225–233.
[69] K.S. Lang, M. Recher, T. Junt, A.A. Navarini, N.L. Harris, S. Freigang, B. Odermatt, C. Conrad, L.M. Ittner, S. Bauer, S.A. Luther, S. Uematsu, S. Akira, H. Hengartner, R.M. Zinkernagel, Toll-like receptor engagement converts T-cell autoreactivity into overt autoimmune disease, Nat. Med. 11 (2005) 138–145.
[70] L. Wen, J. Peng, Z. Li, F.S. Wong, The effect of innate immunity on autoimmune diabetes and the expression of Toll-like receptors on pancreatic islets, J. Immunol. 172 (2004) 3173–3180.