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Research paper

Feline pancreatic islet-like clusters and insulin producing cells express functional Toll-like receptors (TLRs)

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ABSTRACT

Toll-like receptors (TLRs) are cellular receptors that recognize molecules derived from pathogens, endogenous molecules generated after cellular stress, and free fatty acids. TLR activation leads to a proinflammatory reaction that is fundamental in the initiation of an innate immune response and subsequent adaptive responses but also can damage tissues. TLRs are not only expressed within the immune system, but also in most other organ systems including the pancreas. TLR4 is expressed in pancreatic β-cells of rodents and humans and its stimulation affects insulin secretion in response to glucose. A low-grade inflammation is often associated with disturbed performance of β-cells and insulin resistance, the cardinal metabolic event of type-2 diabetes. Feline diabetes mellitus shares many similarities with type-2 diabetes in humans. Our objective was to elucidate the role of TLRs in feline pancreatic islets and islet-like clusters (ILC) that consist of islets with their adjacent tissue. We tested whether TLRs are triggered by their agonists and lead to the expression of inflammatory cytokines. We confirmed the expression of all known feline TLRs in pancreas and ILC. Furthermore, stimulation with TLR agonists increased IL-6 mRNA and protein content and the expression of other proinflammatory cytokines indicating a clear proinflammatory response. The reactivity to TLR ligands was stronger in β-cell enriched populations obtained after sorting by FACS indicating that inflammatory stimuli can also be generated within β-cells. We conclude that the microenvironment of feline β-cells harbor the potential for inflammatory reactions, that can be initiated by molecules released from bacteria or viruses or other molecules recognized by TLRs. Therefore infections associated with bacteriemia and viremia can induce inflammation in islets and damage the endocrine pancreatic tissue.

1. Introduction

Toll-like receptors recognize specific molecular structures of pathogens and initiate innate and adaptive immune response mechanisms (Takeda et al., 2003). They also detect endogenous molecules like heat shock proteins (Tsan and Gao, 2004), mRNA (Kariko et al., 2004) or free fatty acids (Shi et al., 2006) and regulate cellular homeostasis in several tissues (Blander and Medzhitov, 2004; Rakoff-Nahoum et al., 2004; Zarember and Godowski, 2002). TLR-triggering results in nuclear translocation of NFκB and subsequent expression of proinflammatory proteins including TNFα, IL-1β, IL-6, iNOS, as well as chemokines that contribute to cellular infiltration, tissue damage and apoptosis. Activation of TLRs may disturb glucose homeostasis and lead to type-2 diabetes melli-

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tus (T2DM). In a viral mediated model of diabetes in rats, administration of TLR ligands increased the percentage of animals developing diabetes up to 100% (Zipris et al., 2005). Inflammation may favor T2DM at two different levels: first generalized inflammatory processes influence glucose uptake within the target organs. Thus, in human patients with bacterial endotoxin sepsis, insulin resistance is observed (White et al., 1987). Experimental administration of LPS (the best investigated TLR4 agonist) in humans initially causes an increase in glucose utilization that is followed by insulin resistance. LPS treatment in cats causes increase in blood glucose concentration (Declue et al., 2009) and decreased GLUT4 mRNA in omental fat and reduced insulin receptor substrate-1 mRNA in skeletal muscle suggesting localised insulin resistance (Osto et al., 2009). Second, inflammatory processes influence the metabolic performance of islets. Bacterial endotoxins have been considered a factor that may favor the development of diabetes. Human β-cells express the three main components of the LPS recognition complex: CD14, MD-2 and TLR4, and thus possess the full potential to react to bacterial endotoxin. In a β-cell line, LPS induced mRNA specific for proinflammatory molecules, enhanced insulin release at low glucose concentrations and inhibited insulin release at higher glucose concentrations (Vives-Pi et al., 2003). LPS administration in mice decreased insulin specific mRNA in β-cells (Saitoh et al., 2004). TLR4 can also be activated by nutritional fatty acids, thus providing a link between obesity related to T2DM, insulin resistance and inflammation (Shi et al., 2006). β-cells also express TLR2 that binds components of Gram positive bacteria such as, Lipoteichoic acid (Vives-Pi et al., 2003). Primary human β-cells express TLR3 (Rasschaert et al., 2005) that recognizes double stranded RNA of viral origin and endogenous mRNA (Kariko et al., 2004). Apart from generalized inflammatory events that seem to influence the homeostasis of pancreatic islets, local inflammation in the exocrine part of the pancreas have effects on its endocrine function. Indeed, human patients with chronic pancreatitis (CP) show inadequate production of insulin (Peters et al., 1966). CP has been statistically associated to T2DM and there are some indications that it could be the primary event in developing this disease (Hardt et al., 2000, 2002; Meisterfeld et al., 2008). Feline diabetes is very similar to human T2DM in many aspects. It affects middle-aged cats and is associated with obesity, development of insulin resistance, loss of β-cells and amyloid formation (Johnson et al., 1986, 1989a,b; O'Brien et al., 1986). Therefore, similar interactions between inflammatory processes, function of β-cells, and peripheral events associated with insulin sensitivity may occur in cats. Islets like clusters (ILC) can be isolated from cat pancreas, they consist of islets and part of the adjacent acinar tissue, and can be used for functional studies and as a source of β-cells (Maeno et al., 2006).

In our study we searched for TLRs in feline pancreas and ILC and tested, whether or not their stimulation with specific agonists would lead to inflammatory responses. In addition we investigated potential inflammatory reactions taking place in β-cells, which might influence their insulin expression. From our data we conclude that inflammation can be induced upon activation of TLRs in islets and/or their immediate vicinity without affecting insulin expression.

2. Material and methods

2.1. Source of pancreases

Twenty-nine cats were used for the study, including 14 client-owned cats that were euthanized because of a variety of end-stage disorders (i.e. metastatic tumors, chronic liver and renal failure, congestive heart failure) and 15 healthy cats that were euthanized in animal experiments (Veterinary Office of Zürich, Switzerland, study permission nr. 51/2007, 116/2007).

2.2. Isolation of ILC

The procedure for isolation of ILC was published elsewhere (Zini et al., 2009). Briefly, cats were euthanized, the pancreas was excised under sterile conditions and immersed in cold Hank's buffered saline solution (HBSS). Within 15 min the pancreas was infiltrated with 40 ml of an endotoxin free collagenase solution prepared as follows: 50 PZ U NB8 collagenase (Serva Electrophoresis GmbH, Heidelberg, Germany) dissolved in 40 ml HBSS, with addition of 350 μl 1 M $CaCl_2$ and 1 ml 1 M HEPES at pH 7.4. A first infiltration was made with 20 ml solution using a 10 ml syringe and a 0.4 mm needle (27G). The pancreas was incubated in this solution in a 50 ml tube in a water bath at 37 °C for at least 45 min. The incubation was prolonged up to 1 h until a substantial number of ILC appeared in the solution. Then, the organ was filtered using a 2 mm mesh steel sieve. The semidigested pancreas was reincubated with 20 ml of the collagenase solution for additional 20 min. The first filtrate was filled in a 50 ml tube and ice cold HBSS with 10% FCS. The tube was kept on ice until ILC settled on the bottom (about 10 min). The supernatant was aspirated and the clusters were washed with ice cooled HBSS without FCS until the supernatant appeared clear. The same procedure was repeated for the second digest and finally ILC were pooled. The pellet with ILC was resuspended in either HBSS or cell culture medium depending on the assay performed (see below).

2.3. ILC stimulations with TLR agonists

ILC were resuspended in DMEM containing 1 g/l glucose, 10% FCS and antibiotics. FCS was certified for very low endotoxin content (not detectable) and for absence of spontaneous induction of IL6 and TNFα in cat PBMC. ILC were resuspended at a density of 100/ml and seeded in a 24 well cell culture plate in a volume of 250 μl. After 2 h the TLR agonists were added in a volume of 50 μl in triplicate wells. The following concentrations were used: LPS 1 μg/ml, poly (I:C) 10 μg/ml, LTA 1 mg/ml, Pam3Cys 1 mg/ml, Staurosporin 200 nM. The concentrations of LPS and poly (I:C) were chosen for maximal effect. Preliminary dose-effect assay showed significant increases in IL-6 mRNA for LPS at 1 ng/ml and for poly (I:C) at 100 ng/ml (not shown).