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Distribution characteristics of RIG-I receptors of innate immunity in experimental diabetes mellitus and administration of nonspecific blockers of TNF-α

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Abstract

Background: We study type 1 diabetes as examples in which interactions between host and viruses have been implicated in autoimmune pathology. The RIG-I-like receptors (RLRs) as a sensors of RNA virus infection which can initiate and modulate antiviral immunity have been studied. The aim of the study was to determine the features of expression of retinoic acid-inducible gene-I (RIG-I) receptors in GALT in experimental diabetes mellitus (EDM) and after administration of pentoxifylline.

Materials and Methods: To determine structure of population of RIG +-cells we used the analysis of serial histological sections using the method of indirect immunofluorescense with monoclonal antibodies to RIG-I of rat (Santa Cruz Biotechnology, USA).

Results: It has been established that diabetes development was accompanied by an increase in total density RIG+ cells, population density of RIG+ macrophages and increase the concentration of the RIG protein in these cells in the lymphoid structures of ileum at 2nd week. But this data showed a dynamics to decrease to control values by the 4th week of disease. Pentoxifylline (PTX) administration of diabetic animals resulted in a decrease of the total density RIG+ cells, population density RIG+ dendritic cells and RIG+ lymphocytes on the at 2nd week of pathology, and on the 4th week of the disease this data showed dynamics to an increase.

Conclusions: All of these show that expression of RIG-I in ileum immunopositive cells can influence the differentiation of immunopositive cells and their ability to produce pro-inflammatory cytokines, thus acting as one of triggers of diabetes development and progression.

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Introduction

Currently there is no doubt the fact that changes in the intensity of expression of pattern-recognition receptors (PRR) of innate immune system by the structures gut-associated lymphoid tissue (GALT) may play a role of trigger the start of development and progression of type 1 diabetes mellitus (1, 2). One of these classes are the PPR family of cytoplasmic RIG-like receptors, which includes three members - retinoic acid-inducible gene-I (RIG-I), melanoma differentiation associated gene 5 (MDA5) and laboratory of genetics and physiology 2 (LGP2) (3). RLR actively expressed by lymphocytes and APC of intestine except plasmacytoid pre-DCs, which makes them universal sensors of RNA-genomic viruses (4). RIG-I is a typical member of the RLR family, which have a repressor C-terminal domain (RD) that interacts with RNA. Usually, in uninfected cells RIG-I is an inactive monomer, but virus infection and RNA binding is the trigger of changes in conformation that lead to self-organization and interaction with molecules of the subsequent stages (5, 6). After the RNA recognition, RIG-I and MDA5 transmitted signal through the adapter interferon-β promoter stimulator 1 (IPS-1, also known as MAVS, Cardif and VISA) to a number of signaling proteins that leads to the transcription of interferon genes (7, 8). RLR activity is an ATP-dependent, which is probably due to the need unfolding of double-stranded and duplex RNA structures by interaction with helicase domain of RLR. As in the case of TLR / NLR, the intracellular signaling pathway of RLR activated by homophilic interactions: CARD-domain of RLR binds with adapter IPS-1 which associated with the outer membrane of mitochondria, that then interacts with a ubiquitin ligase TRAF3, is also necessary for TLR-induced production of INF I type. TRAF3 activates the kinase TVK1 and IKK-ε, which in turn phosphorylate and activate transcription factors IRF3 and IRF7 (9). Thus since TRAF3 from RLR-signaling pathway is similar to the signaling pathway of TLR3. RLR can also activate NF-κβ and induce the expression of proinflammatory genes (10). In experiments on mice deficient in RIG-I or MDA5 have been shown that these two helicases are critical for adequate antiviral response and recognition of various viruses. RIG-I is required for an adequate response to the RNA of influenza viruses, parainfluenza and Japanese encephalitis, at the same time MDA5 is needed for picornavirus detection (11).

For many years, scientists have been made to establish a link between infectious disease and the induction of the autoimmune diseases, such as type 1 diabetes. They were based on the fact that infectious and autoimmune pathologies accompanied by activation of the adaptive immune system components and the subsequent development of inflammation, but such a causal relationship has not been established. At the same time, recent findings demonstrate the link between genes that involved in the induction of type 1 diabetes, and the genes responsible for the interaction in the system of virus-host (12). Therefore, the aim of our study was to determine the features of expression of RIG-I receptors in GALT in experimental diabetes mellitus (EDM) and after administration of pentoxifylline (PTX).

Material and methods

Animals and tissue isolation

A total of 80 adult male Wistar rats were used. Animals were obtained from the nursery of Veterinary Medicine Association Ltd. "Biomodelservis" (Kiev). We divided test animals into 5 experimental groups: control rats, which were administered once intraperitoneal with 0.5 ml of 0.1 M citrate buffer (pH = 4.5) (group 1); rats with 14-day EDM (group 2); rats with 28 days EDM (group 3); rats with 14-day (group 4) and 28-day EDM (group 5), which were respectively treated by pentoxifylline during 2 and 4 weeks at a dose of 9 mg / kg from 1st day of diabetes induction.

Induction of diabetes

Streptozotocin (STZ) (SIGMA Chemical, USA) was injected intraperitoneally at a dose of 50 mg / kg dissolved in 0.5 ml of 0.1 M citrate buffer (pH 4.5) prior to the moment of administration. The time elapsed since the date of introduction of the drug in the future presentation of the material has been interpreted as the duration of diabetes. For further studies were selected animals with fasting glucose level > 8.0 mmol/l. Determination of glucose concentration was performed after 6 hours from the last meal on 3rd day after injection of streptozotocin. For this needs were used blood collected from the tail vein. Glucose concentration were determined by the glucose-oxidase method using the instrument "BIONIME RightestTM GM 110" (Switzerland) a 12 hours and 1, 2, 3, 5, 7, 10, 14 and 28 days after injection STZ.

Immunohistochemical staining

Population structure of RIG-I-cells was studied on the basis of morphometric and densitometric characteristics of serial histological sections (5 micron serial sections of ileum). For their preparing is a rotary microtome MICROM HR -360 (Microm, Germany) were used. These sections were deparaffinized in xylene, rehydrated in a descending carried concentrations of ethanol (100%, 96%, 70%) and washed in 0.1M phosphate buffer (pH = 7.4). Then they were stained with a rabbit monoclonal primary antibodies (MAbs) to the RIG-I receptor of rats (Santa Cruz Biotechnology, USA) during 18 hours in a humid chamber at t = 4°C. The excess of primary antibody were washed in a 0.1 M phosphate buffer. After that sections were incubated for 60 minutes (t = 37°C) with a secondary antibody molecule to the total rabbit IgG (Santa Cruz Biotechnolog, USA), conjugated with FITC. Then sections were washed with 0.1 M phosphate buffer and embedded in a mixture of glycerol and a phosphate buffer (9:1) and studied by fluorescence microscope PrimoStar (ZEISS, Germany) in the ultraviolet spectrum of excitation 390 nm (FITC) with using a highly sensitive camera AxioCam 5c (ZEISS, Germany) and the software package for a work with images AxioVision 4.7.2 (ZEISS, Germany). Then images were immediately entered into the computer and analiesed with using software ImageJ (NIH, USA). In the automatic mode, identified areas with the statistically significant fluorescence characteristics of cells that express RIG-I, their morphometric and densitometric characteristics. When painting the MAbs RIG-I-cells in the lamina propria of the villi (LP) and subepithelial zone isolated lymphoid follicles (ILF) were examined, which are, respectively, an effector and inductive zones of immune response in GALT.

Statistical analysis

All the experimental data were processed on a personal computer with using application package statistical programs EXCEL of the MS Office 2010 (Microsoft Corp., USA), Statistica 6.0 (Stat-Soft, 2001). In all cases were calculated the arithmetic mean value of the sample (m), its variance and the standard error of the mean (SEM). To identify the significance of differences in the results of studies of experimental and control groups of animals was determined by Student's coefficient (t), after which it was determined by the possibility of difference (p) and the average confidence interval. Critical significance level when testing statistical hypotheses assumed to be equal to 0.05.

Results

The development of diabetes was accompanied by a significant increase in the total density of RIG-I+-cells in LP by 46% ($p<0,05$), and in ILF on 52% ($p<0,05$) at 14th day of the experimental diabetes as compared to control group, but it demonstrated the trend to decrease by the 28th day of pathology (Fig 1A). The distribution of individual classes of RIG-I+-cells in LP in these groups of experimental animals showed an increase in population density (PD) of RIG-I+-macrophages (2,5-fold, $p<0,05$ at 2nd and 84%, $p <0.05$ on 4th week of EDM) and their percentage was increased by 81% ($p<0,05$) at day 14 and 75% ($p<0,05$) on day 28 of diabetes. In ILF also observed an increase in the PD and percentage of RIG-I+-macrophages by 14th days of the pathological process in a 2,6-fold ($p<0,05$) and 67% ($p<0,05$), respectively. At the same time there is a decrease of percentage RIG-I+-lymphocytes by 27% ($p<0,05$) compared with control.

Pentoxifylline administration to diabetic animals was associated with significant reduction of total density RIG-I+-cells in LP on the 14th day of development EDM by 24% ($p<0,05$) (Fig 1B) and increase that index by 38% ($p<0,05$) on the 28th day. (Fig 1C) In the study of the distribution of individual classes RIG-I+-cells has been showed a significant decrease in PD RIG-I+-dendritic cells by 25% ($p<0,05$) and RIG-I+-lymphocytes by 37% ($p<0,05$), and increase the proportion of RIG-I+-macrophages on 36 % ($p<0,05$) at the 2nd week of pathology compared with the groups of diabetic animals. On the 4th week of EDM observed the following changes in the distribution of RIG-I+-cells: increased PB of RIG-I+-macrophages and RIG-I+-dendritic cells by 46% and 57% ($p<0,05$), respectively, a decrease of PD RIG-I+-lymphocytes by 33% ($p<0,05$). With administration of pentoxifylline observed changes in the subepithelial zone ILF. The total density RIG-I+-cells on the 2nd week of pathology development were significantly reduced by 30% ($p<0,05$) compared to diabetic animals (Fig 1B), to whom the preparation was not administered. Regarding the distribution of individual classes RIG-I+-cells revealed the following changes: on 2nd week of EDM development reduction PD RIG-I+-dendritic cells in 2,3 times and RIG-I+-lymphocytes by 32% ($p<0,05$) were observed, on the 4th week increase PD and the percentage of RIG-I+-macrophages in 2,3, and 2,1-fold ($p<0,05$) respectively, and reducing the PD and percentage of RIG-I+-lymphocytes by 37% and 39% ($p<0,05$) respectively over corresponding groups of diabetic animals which pentoxifylline was not administered.

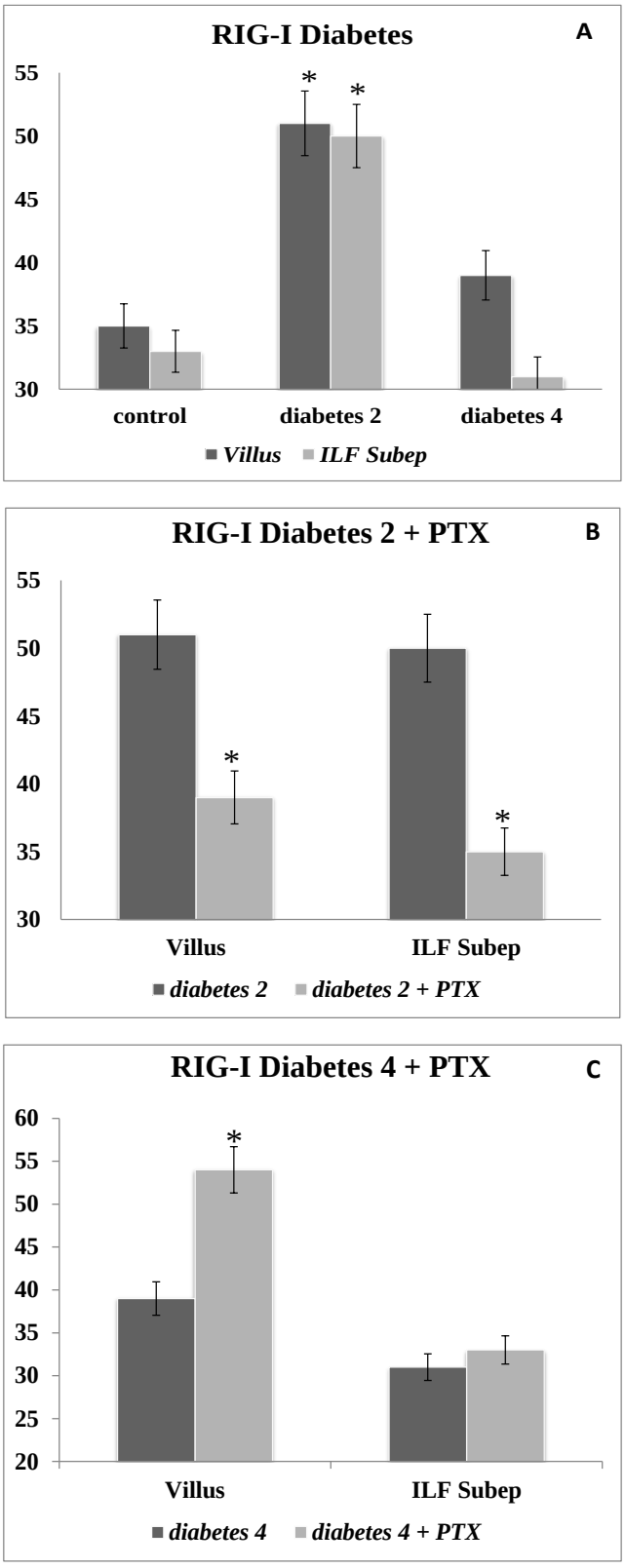

Figure 1. The number (on 1 mm^2) of RIG-I+ cells in lamina propria of villus mucous layer (Villus) and in subepithelial zone of isolated lymphoid follicles (ILF Subep). The development of STZ diabetes (A). The administration of PTX (B, C) to experimental animals. Note: * P < 0. 05.

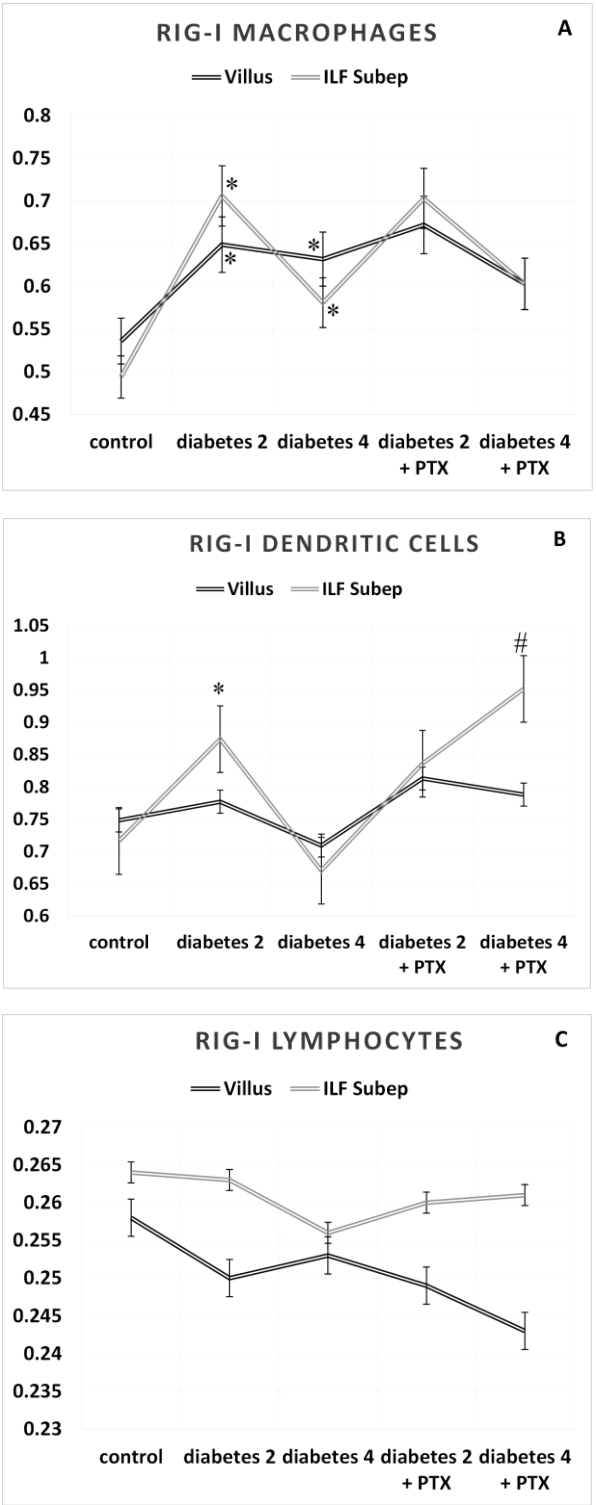

Figure 2. Concentration of RIG-I (fluorescence intensity in arbitrary units, AU) in RIG-I+-macrophages (A), RIG-I+-dendritic cells (B) and RIG-I+-lymphocytes (C). Note: * $P < 0,05$ relative to the control, $ - $P < 0,05$ relative to the diabetes 2, # $P < 0,05$ relative to diabetes 4.

Measurement of fluorescence intensity RIG-I+-cells displaying the concentration of intracellular RIG-receptors in immunopositive cells has been showed a significant increase in this parameter during the development of diabetes in RIG-I+-macrophages in LP on 21% ($p<0,05$) and 18% ($p<0,05$), respectively, on the 2nd and 4th week of EDM (Fig 2A). In subepithelial zone of ILF on the 2nd week of EDM development as compared with control fluorescence intensity increased in RIG-I+-macrophages and RIG-I+-dendritic cells on 43% ($p<0,05$) and 22% ($p<0,05$) (Fig 2A, 2B), respectively, and on the 4th week this parameter significantly increased in RIG-I+-macrophages by 18% ($p<0,05$) compared with control (Fig 2A).
Pentoxifilline administration of diabetic animals was reflected in an increase in fluorescence intensity RIG-I+-dendritic cells on 42% ($p <0,05$) in ILF at the 4th week of pathology development compared to intact diabetic animals (Fig 2B).

Discussion

In addition to the ability to induce IFN expression and production of ISG products, viral infection in signal which transmit through RLRs can also stimulates the expression of IFN-λ, belonging to the IL-10-dependent type III interferons (13, 14) and various proinflammatory cytokines (15). In some experiments have been showed that signaling via RIG-I, IPS-1, TBK1 and IRF-3 are required for induction of synthesis of IFN-λ in infectious pathologies caused by paramyxoviruses, and during the analysis of the promoter regions of IFN-λ gene were revealed numerous elements responsible for interaction with IRFs and NF-κB (3) Thus we can say that the answer through RLRs occurs by two mechanisms. The first of these involves the IPS-1-CARD9-Bcl-10-dependent transcription of proinflammatory genes and a lot of them are NF-κB-dependent (15). The second includes RIG-I association with ASC-protein that stimulates caspase-1 inflammasome activation according to type and processing of proinflammatory cytokines such as IL-1 β and IL-18 into their mature forms. There is no doubt that INF play a major role in the modulation of adaptive immune responses, because INF promotes clonal differentiation of T-cells after antigen stimulation. The main inducers of Th1-response are IL-12p70 and IFN type I, for their optimal production must be combined activation of MyD88- and TRIF-dependent signaling pathways (16). Furthermore, interferon is able to induce cytolytic activity of NK-cells and cytotoxic T-cells (17), and also plays a role in the stimulation of B cell differentiation and antibody production (18). In mice which are deficient in IPS-1 upon infection with West Nile virus has been identified increasing production INF, proinflammatory chemokines and cytokines, but also it has been noted disregulation of T-and B-cells activation, decrease concentrations of neutralizing antibodies against the increase in production of total antibody (19). It was also shown that the expansion of Treg was disrupted in mice deficient for the IPS-1 during acute viral infection due to the inability signalling through RLRs. Furthermore Anz et al. (2010) found RIG-I and MDA5 expressed by effector and regulatory T cells, and RLR-signaling is required for the regulation of Treg-cells functioning within picornavirus-induced infection (20). However, despite this role RLR which are critical for the production of INF in the regulation of adaptive

responses is not entirely clear and may depend on the type of virus inducing corresponding RLR.

Recently has been accumulated evidence that a disturbed induction of interferon synthesis and signaling through PRRs are closely connected with the development and progression of autoimmune pathology (21). In the course of genetic research has been installed multiple polymorphism in IFIH1 (gene MDA5), which is associated with resistance to the induction of type 1 diabetes, including T946A, E627*, I923V, R843H, IVS8+1, and IVS14+1 (22, 23), which was associated with decreased levels of transcription IFIH1 (24), violation of splicing, leading to the formation of defective variants of MDA5 (23) and/or expression of mutated MDA5, and therefore a violation of binding to RNA synthesis and subsequent induction of INF (25). Additional confirmation is the fact of the presence and accumulation of picornavirus RNA and antigens in pancreatic islet cells in patients with type 1 diabetes compared with healthy people, as well as patients with type 2 diabetes (26, 27). Recently, it was also found that the RIG-I is involved in the detection of bacteria located intracellularly. RNA of Salmonella enterica serovar Typhimurium activates INFβ products by RIG-I-dependent pathway in nonphagocitic cells. However, the successful detection of this pathogen depends on the colocalization of sensor and PAMPs, as well as a potential countermeasures of pathogen during infection (28). Kocic G, Pavlovic R. et al (2011) indicate that a significantly lower level of expression of RIG-I is observed in PBMCs cultured with circulating RNA isolated from children with type 1 diabetes, compared with intact PBMCs (29). It was also established that expression of RIG-I receptor in epithelial cells of intestinal mucosa decreased in autoimmune pathologies such as Crohn's disease, and in RIG-I-deficient mice spontaneously develop autoimmune colitis which accompanied by inflammation and "leaky" mucous membranes, and the reduction of the size and number Peyer's patches and suppression of expression of Gαi2 (30). All this points to the fact with regard to violations of the expression of RIG-I and induction of autoimmune disorders involving GALT (1).

Conclusion

1. Diabetes development has been associated with an increased amount of RIG-I+-cells in the intestine on 45-50% on 14th day of pathology, but by the 28th day their number returned to control values. Induction of diabetes led to increased concentrations of RIG-I on 20-40% in macrophages and dendritic cells, but remained virtually unchanged in lymphocytes.

2. TNFα-nonspecific blocker pentoxifylline administration in diabetic animals resulted in a decrease of the total density of RIG-I+-cells on the 2nd week of development EDM on 24 (LP) -30% (ILF), but on the 4th week this index was restored to control values in ILF and exceeded them by 38% in LP. The concentration of protein RIG-I demonstrated the dynamics of an increase in the range of 40% in dendritic cells only in ILF on the 28th day of pathology.

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