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Journal of Autoimmunity 24 (2005) 269–279

journal of autoimmunity

www.elsevier.com/locate/issn/08968411

Expression of the NK-1 receptor on islet cells and invading immune cells in the non-obese diabetic mouse

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Received 16 August 2004; revised 28 February 2005; accepted 28 February 2005

Abstract

The underlying mechanistic causes of immune cell infiltration in the islets of Langerhans and beta cell failure in the non-obese diabetic (NOD) mouse is still to be completely revealed. Substance P (SP) is a substance known to have pro-inflammatory, endocrine, neuromodulatory and trophic effects, and its preferred receptor, the neurokinin receptor 1 (NK-1 R), is reported to be involved in extravasation of granulocytes and in inflammation and tissue derangement. Therefore, we have investigated the expression of NK-1 R during development of insulitis in the NOD mouse. We show that the magnitude of immunoreactivity scoring NK-1 R expression in the islets was increased in the 12-week-old NOD mouse. Expression of NK-1 R co-localized with expression of glucagon. In line with this expression pattern, we did not detect any effect of SP on glucose-induced insulin release. NK-1 R expression was particularly observed in islet cells in association with the clusters of immune cells. Expression of NK-1 R was also demonstrated in a fraction of the infiltrating B and T lymphocytes, as well as on infiltrating macrophages and dendritic cells. The observations show that the level of NK-1 R expression is increased in 12-week-old NOD mice, being correlated with the occurrence of islet mononuclear infiltration. Our data suggest that SP may act as a chemoattractant, contributing to the pathogenic mononuclear infiltration process in the NOD mouse. On the whole, the observations suggest that SP and the NK-1 R to certain extents are involved in the changes that occur during the development of insulitis in the NOD mouse.

Keywords: Substance P; Neuropeptides; Islets; NOD mouse; Inflammation

1. Introduction

In the normal mouse, the endocrine pancreas is densely innervated by neurons of extrinsic and intrinsic origin that take part in the regulation of hormone release [1–3]. In addition to cholinergic and adrenergic nerve fibers, there is also a population of sensory nerve fibers containing substance P (SP) innervating the islets [2], SP being one member of the tachykinin family of neuropeptides. In the mouse, SP containing nerve fibers are regularly observed within or close to islets [1,4]. Substance P shows high affinity binding to the neurokinin-1 receptor (NK-1 R) belonging to the class of seven transmembrane domain receptors that are G-protein-coupled [5]. Generally, stimulation of NK-1 R

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doi:10.1016/j.jaut.2005.02.003

induces metabolism of phosphoinositol, leading to synthesis of inositol-1,4,5-trisphosphate and diacylglycerol which results in release of Ca^{2+} from the endoplasmic reticulum and activation of protein kinase C [6]. There have been reports on the effects of SP on hormone release, but these reports have diverged why the role of SP in the islets is not clear (cf. refs. [2,7–9]). No extensive analysis on the occurrence of NK-1 R in the endocrine pancreas has been reported.

Neuropeptides not only have a neurotransmitter function, they are also important in the regulation of inflammation and are associated with the "neuroimmune-axis". In this feed-back system, there is a communication between immunological cells and neurons with their content of neuropeptides [10]. Substance P has apart from neuromodulatory and trophic actions, pro-inflammatory effects, and there is an increasing amount of data that imply a role for NK-1 R in regulating immune function (e.g. see ref. [10]). There is e.g. for an overall upregulation of NK-1 R expression in the colon in inflammatory bowel disease [11]. Expression of NK-1 R has been demonstrated for inflammatory cells in the gut (e.g. see refs. [12,13]) and it has recently been suggested that SP modulates inflammation in the bowel partly via SP-receptors on intestinal T lymphocytes [14]. Expression of NK-1 R has also been shown for inflammatory cells in the pancreas [15]. In addition, it has been suggested that NK-1 R is associated with pro-inflammatory reactions, development of inflammatory edema and pain in pancreatitis [15–18].

The non-obese diabetic (NOD) mouse is a model for human type 1 diabetes (e.g. see ref. [19]). In the NOD mouse, the development of insulitis is characterized by an initial infiltration of antigen presenting cells (APC) like dendritic cells and macrophages in the peripheral parts of the islets, followed by infiltration of inflammatory CD4+ T cells, CD8+ T cells and B lymphocytes. Several factors have been demonstrated to contribute to the infiltration process, including cell adhesion molecules as well as a battery of chemokines and their receptors [20,21]. However, the role of NK-1 R and its ligand SP has not been examined. Therefore, in this study, the presence of NK-1 R was analyzed in islets from normal mice and NOD mice. We demonstrate here that there is an increased NK-1 R expression on islet-derived cells in NOD mice, and that a substantial fraction of the islet infiltrating mononuclear cells expresses NK-1 R, suggesting that SP may contribute to the infiltration process and insulitis in the autoimmune lesion.

2. Materials and methods

2.1. Animals

Animal experiments followed the principles set forth in the "Guide for the Care and Use of Laboratory Animals" (NIH publication no. 86-23, revised 1985). In accordance with Swedish national law, the experiments were approved by the Local Ethical Committee on Animal Experiments in Northern Sweden, No A 38-03. Female NOD, C57BL/6 and Umeå-+/? mice were taken from local colonies and were used at 1-, 3- and 12-weeks of age as indicated. Umeå-+/? mice were used as controls if not otherwise stated. The mice were given free access to pelleted food and tap water.

2.2. Immunohistochemistry

2.2.1. Fixation and sectioning

Five to six NOD and Umeå-+/? mice were used in each age group (1-, 3- and 12-week-old) for the immunohistochemical analyses. The mice were killed by decapitation and the pancreas was dissected and fixed at 4 °C for 24 h in 4% formaldehyde in 0.1 M phosphate buffer (pH 7.0). After that the specimens were rinsed in Tyrode's solution (pH 7.2), containing 10% (w/v) sucrose, for 24 h, and were then mounted on cardboard with OCT embedding medium (Miles Laboratories, Naperville, IL, USA) and frozen in propane chilled by liquid N_2. The specimens were cut in a cryostat at a thickness of 8 μm.

2.2.2. Immunohistochemical processing and analysis of the sections

Staining for NK-1 R, glucagon, somatostatin: the sections were dried in room temperature and subsequently incubated in 1% (v/v) Triton X-100 for 20 min. After that the sections were rinsed in phosphate-buffered saline (PBS) three times (5 min each). Endogenous peroxidase activity was blocked by incubation in 1% H_2O_2 for 30 min. After rinsing in PBS the sections were incubated with normal swine serum for 15 min. Incubation with primary antibodies (cf. below) was performed for 1 h at 37 °C. Thereafter the sections were rinsed in PBS, incubated with normal serum and then incubated with secondary antibody (swine anti-rabbit, 1:100, code Z196; Dakopatts, Glostrup, Denmark). Finally, the sections were rinsed in PBS, developed in diamino-benzidine (DAB), dehydrated and mounted in DPX. The sectioned pancreases were analyzed in a Zeiss Axioscop. When staining for glucagon and somatostatin was performed, an initial pre-treatment with acid potassium permanganate (cf. ref. [22]) was applied.

Staining for insulin: when staining for insulin was performed, TRITC staining protocol was applied (cf. ref. [4]). It included the use of an initial treatment with acid potassium permanganate (cf. ref. [22]).

Double staining: the sections were first incubated with the NK-1 R antibody and after that incubated with the secondary antibody that was conjugated to TRITC. After that the free epitopes were blocked with normal