APOPTOTIC RESPONSES TO ACUTE AND CHRONIC INFLAMMATION IN THE TISSUES OF YOUNG PIGS

JOANNA ZUBEL-ŁOJEK*, KRYSTyna PIERZCHAŁA-KOZIEC, EWA OCOŁOŃ AND ANNA LĄTACZ

Department of Animal Physiology and Endocrinology, University of Agriculture,
Al. Mickiewicza 24/28, 30-059 Cracow, Poland

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Apoptosis is a physiological, programmed process for the elimination of damaged cells from a living organism. Abnormalities in cell death regulation can be a significant component of diseases. The present study was undertaken to examine the influence of acute and chronic inflammation on the apoptosis process in the piglet spleen and liver. The experiment was carried out on young pigs (10-week-old, n=24). The animals (females) were divided into 4 experimental groups: I– control, II– chronic inflammation (overweight), III– acute inflammation, and IV– overweight with acute inflammation. Piglets from groups II and IV received a high-calorie diet in order to develop overweight. In order to induce acute inflammation the animals received a single i.p. injection of streptozotocin. Twenty-four hours after the injection the spleen and liver were quickly removed. Tissue fragments were placed in liquid nitrogen and then they were used to detect apoptotic cells with the TUNEL technique and In Situ Cell Death Detection Kit. The analysis of TUNEL positive cells showed their presence both in the spleen and in the liver but their numbers were different. The obtained results showed that different types of inflammation (chronic or acute) induced different responses in the tested tissues.

Key words: inflammation, apoptosis, spleen, liver, young pigs

INTRODUCTION

Obesity is considered as a low-degree chronic inflammatory state of the adipose tissue, caused by the immune system activation that generates obesity-related metabolic disorders, mainly insulin resistance (HOTTAMISLIGIL, 2006). The adipose tissue is an endocrine organ that produces adipokines with various biological activities (FRUH-BECK and SALVADOR, 2004; JENSEN, 2006). Some of them, such as interleukin 6 (IL-6) and interleukin 18 (IL-18) or tumor necrosis factor (TNF), are of a proinflammatory character and modulate metabolism and activity of insulin (SENN et al., 2002). One of the main effects of IL-6 is the induction of hepatic acute phase protein (CRP) synthesis, which is known to be a major risk marker of cardiovascular complications.

*e-mail: jzubel@ar.krakow.pl
The spleen is the largest lymphoid organ in the body and plays an important role in the regulation of the immune function and blood filtration via the removal and destruction of aged or damaged erythrocytes and other blood cells (DAMESHEK, 1955). The gene expression of pro-inflammatory cytokines, such as TNF-α or IL-6 and IL-10 (potent anti-inflammatory cytokine), was observed in the splenic tissue (LAMAS et al., 2004). It is suggested that the altered functioning of the spleen may modulate inflammatory processes in the organism. The spleen plays an important role in the modulation of the immune system and in the maintenance of peripheral tolerance via the clearance of circulating apoptotic cells, differentiation and activation of T and B cells, and production of antibodies (THACKER and JANSSEN, 2012).

On the other hand, obesity, mainly of the visceral type, and insulin resistance are associated with liver inflammation. Increased adiposity with the consequence of chronic low-grade inflammation and insulin resistance or type 2 diabetes have been linked to the development of nonalcoholic fatty liver disease (NAFLD). The most common form of this inflammation is triglyceride accumulation in hepatocytes (steatohepatitis). Recent data indicate that an alteration in the regulation of hepatocyte apoptosis could play an important role in hepatic damage and steatohepatitis progression (FELDSTEIN et al., 2003). What is more, metabolic and endocrine functions of the spleen were emphasized in obesity and nonalcoholic fatty liver disease. In conclusion, both the organs are involved in the regulation of metabolic diseases and inflammation.

Programmed cell death (mainly apoptosis) is involved in all fundamental processes of the immune system. Apoptosis is a physiological process for the elimination of cells from a living organism. In the physiological state, apoptosis plays an important role in normal development as well as in tissue proliferation (ZIMMERMANN and GREEN, 2001). Abnormalities in cell death regulation can be a significant component of various diseases, such as cancer, autoimmune lymphoproliferative syndrome, AIDS or metabolic diseases.

The present study was undertaken to examine the influence of acute (single streptozotocin injection) and chronic (high-calorie diet) inflammation on the apoptosis process in the piglet spleen and liver.

MATERIALS AND METHODS

All the procedures were approved by the First Local Animal Ethics Commission in Cracow, Poland (No. 14/2009).

The experiment was carried out on young pigs (Polish Landrance, 10-week-old, n=24). The animals (females) were kept under standard conditions and divided into 4 experimental groups: I- control (C), II- chronic inflammation – overweight (O), III- acute inflammation (STZ), and IV- overweight with acute inflammation (O+STZ). Piglets from groups I and III were fed a commercial diet, whereas those from groups II and IV received high-calorie diet in order to developed overweight. In order to develop acute inflammation, the animals received a single i.p. injection of streptozotocin (STZ, 100 mg/kg b.w.). The piglets were weighed prior to injection, and STZ was freshly dissolved in dilution buffer (0.1 M sodium citrate, pH 4.5, with HCl, stored at 4° C). Twenty-four hours after the injection the spleen and the liver were quickly removed. Tissue fragments were placed in liquid nitrogen and then they were used to prepare microscopic sections with Leica cryostat. Apoptotic cells were detected in the tissues using the TUNEL technique and In Situ Cell Death Detection Kit (Roche, Germany), according to the manufacturer’s protocols. The number of labelled cells per unit area (mm²) was calculated with MultiScan v.14.02.

The results were expressed as mean±SEM and their statistical comparison was made by analysis of variance followed by Duncan’s test. Differences were considered significant at the level of 0.05.

RESULTS

The obtained data showed that different types of inflammation (chronic or acute) induced different responses in the tested organs.

The number of TUNEL-positive cells in the spleen (Fig.1.) of the control group was 12.45±0.89 cells/mm². The number of apoptotic cells after 3 weeks of a high-calorie diet increased to 14.91±0.95 cells/mm² (P<0.05), while no changes were observed after a single injection of streptozotocin (13.27±0.73). Interestingly, in piglets
from group IV (with both types of inflammation) the number of cells was 60% higher (19.86±1.38) compared with the control group (12.45±0.89, P<0.05, Fig. 2).

The analysis of TUNEL-positive cells confirmed their presence also in the liver of 10-week-old piglets (Fig. 3.), although they were less numerous. The numbers of liver apoptotic cells in the control and overweight with acute inflammation groups were similar (2.75±0.08 vs 2.85±0.11). The high-calorie diet (chronic inflammation) decreased the number of apoptotic cells in the liver tissue to 2.21±0.08, whereas the STZ injection (causing acute inflammation) increased the number of TUNEL-positive cells by 25% to 3.45±0.17 (P<0.05, compared with the control group, Fig. 4.).

DISCUSSION

The presented data indicate that different types of inflammation induced various apoptotic responses in the piglet spleen and liver. Programmed cell death, apoptosis, is an important mechanism regulating the development, maturation, and activation of lymphocytes (Van Parijs and Abbas, 1998). Apoptosis present in parasitic infections (overreaction of the immune system) may be involved in down-regulating inflammatory T-helper cell–1 responses (Fallon et al., 1998). Other factors involved in apoptosis may include tumor necrosis factor, nitric oxide, or reactive oxygen (Villewal et al., 1990), all of which are present at high concentrations in the mouse spleen during acute infection. In our study spleen cells showed
Apoptosis During Inflammation in Young Pigs

Apoptotic changes in overweight animals as well as in piglets with overweight and those injected with streptozotocin. Streptozotocin (STZ, 2-deoxy-2,3-(methyl-3-nitrosoureido)-D-glucopyranose) isolated from Streptomyces achromogenes is used to induce both insulin-dependent and non-insulin-dependent diabetes mellitus by damaging the pancreas cells. It is known that STZ action is mediated by activation of the immune mechanism (Ito et al., 1999). In our experiment we used a single injection of streptozotocin which induced acute inflammation. We observed increased levels of proinflammatory cytokines (TNF, IL-6, CRP) in the blood twenty-four hours after STZ administration, which confirmed the inflammatory state (unpublished data).

We observed that acute inflammation, twenty-four hours after STZ administration, had no effect on the apoptotic process in the porcine spleen. Unexpectedly, our results did not prove the pro-apoptotic effect of STZ on the spleen cells. It may suggest that during the inflammation state the main action of the spleen is directed toward synthesis and release of proinflammatory cytokines. This suggestion is in agreement with the results obtained earlier by Cockfield et al., (1989) who showed that small but cumulative doses of STZ significantly increased the plasma level of IFN-γ, the major indicator of an inflammatory state. On the other hand, streptozotocin has been shown to damage not only islets but also many other tissues including lymphoid organs (Koib, 1987). A decreased state of immune reactivity has been reported after high or multiple low doses of streptozotocin (Schwartz and Eardley, 1985). In chronic inflammation (overweight animals), as well as in group IV, we observed an increase in the number of apoptotic cells, which suggested the likely impact of the altered immuno-metabolic status on the splenic tissue. We have also observed a correlation between the number of apoptotic cells and the level of cytokines (TNF, IL-6) in the piglet spleen (unpublished data). The results may indicate that an increase in proinflammatory adipokine secretion plays an important role in the spleen damage.

The onset of diabetes is accompanied by the development of major biochemical and functional abnormalities in the liver, including alterations in carbohydrate, lipid and protein metabolism, as well as changes in the antioxidant status (Chatila and West, 1996; Harrison et al. 2006). It was found that hyperglycemia increases the production of mitochondrial reactive oxygen species.

Fig. 4. Apoptotic changes in the piglet liver (A – control group, B – overweight group, C – acute inflammation group, → apoptotic cells). Bar 20 μm.
(ROS), which could represent a key mechanism in the development of diabetic complications (Nishikawa et al., 2000; Kiritoshi et al., 2003). The initial cellular response to high glucose challenge is the generation of ROS, which rapidly induces apoptotic cell death (Park et al., 2001). Reactive oxygen species are involved in the induction of apoptosis by different stimuli as well as pathological cell death that occurs in many diseases (Oh et al., 2004). The prevalence of liver diseases is increased in patients with either type 1 or type 2 diabetes (Saxena et al., 1993; Bell and Allbright, 2007). Clinically, changes in the size of the liver are seen in both juvenile and adult diabetic patients, as a result of alterations in cell numbers, cell growth, and/or cell death and apoptosis (Chatila and West, 1996).

In our study a single streptozotocin injection resulted in a higher number of apoptotic cells in the liver, which may indicate a direct effect of acute inflammation on hepatic cells, probably related to the removal of toxins. We observed that a 3-week high-calorie diet effectuated a significant decrease in the number of apoptotic cells; however no effects of STZ on the prolonged inflammation were found.

The present study has clearly shown that different types of inflammation (chronic or acute) induce different apoptotic responses in the testes tissues. In overweight animals we observed a direct, potent effect of a high-calorie diet on the apoptotic process in the spleen, whereas in the liver, a direct effect of streptozotocin (acute inflammation) was noted. The knowledge of the spleen and liver functions could be helpful in developing appropriate prevention strategies and managing the pandemic of obesity.

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