Thymus involution in alloxan diabetes: analysis of mast cells

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We previously reported that alloxan-induced diabetes results in reduction in the number and reactivity of mast cells at different body sites. In this study, the influence of diabetes on thymic mast cells was investigated. Thymuses from diabetic rats showed marked alterations including shrinkage, thymocyte depletion, and increase in the extracellular matrix network, as compared to those profiles seen in normal animals. Nevertheless, we noted that the number and reactivity of mast cells remained unchanged. These findings indicate that although diabetes leads to critical alterations in the thymus, the local mast cell population is refractory to its effect. This suggests that thymic mast cells are under a different regulation as compared to those located in other tissues.

Key words: diabetes - mast cells - thymus - extracellular matrix

It has been demonstrated that diabetic patients present many functional abnormalities which can be partially responsible for their failure in mounting an appropriate inflammatory response (Garcia-Leme 1989). In line with this concept, some authors reported that the occurrence of allergic disorders concomitantly with type 1 diabetes is markedly reduced when compared with the incidence of each disease alone (Olesen et al. 2001, Rosenbauer et al. 2003). It is thus possible that the resistance to allergic provocation results from an imbalance in the T-helper 1/ T-helper 2 (Th1/Th2) response. Accordingly, since autoimmune type 1 diabetes is Th1-dependent and allergy is Th2-dependent, the susceptibility to one disease might lead a state of refractoriness to the other (Huang 1999).

We previously described that alloxan-diabetic rats showed reduction of the allergen-induced acute plasma leakage and late eosinophil accumulation in the pleural cavity, phenomena which simultaneously occurred in parallel with decrease in the number of mast cells recovered from the pleural space (Diaz et al. 1996). Antigen-evoked plasma leakage in diabetic rats was restored by adoptive transfer of mast cells from sensitized non-diabetic rats (de Oliveira Barreto et al. 2003), supporting the idea that refractoriness of diabetic animals to allergen stimulation seems to be at least partially accounted for by depletion in the mast cell population. Mast cells act as central effector and regulatory cells in many inflammatory disorders, such as autoimmunity, allergy and parasite infections (Galli et al. 1999). They were shown to be widely distributed in many tissues including skin, airways, gastrointestinal tract (Wasserman 1990), and also in the thymus (Huntley et al. 1990). Based on the above observations, we investigated herein the interference of alloxan-diabetes on thymic mast cell population.

MATERIALS AND METHODS

Male Wistar rats from the Oswaldo Cruz Foundation breeding were used. All procedures involving care and use of laboratory animals in this study were examined and approved by the Animal Ethics Committee of the Oswaldo Cruz Foundation (License 0085-02). Diabetes was induced by a single injection of alloxan monohydrate (Sigma St. Louis, US) (40 mg/kg, i.v.) into 12 h fasted rats. Control animals were injected with the vehicle alone. Blood glycaemia was determined by means of a glucose monitor in samples obtained from the tail vein and only rats with glucose levels above 200 mg/dl were considered for further experiments. To evaluate thymus atrophy, the organ was removed from diabetic and control animals, dried for 24 h at 40ºC and weighed. For cell analyses, the thymus was passed through a mesh stainless steel grid and the supernatant centrifuged at 150 x*g* for 10 min. Cells were harvested, washed three times with phosphate buffer solution (PBS) and counted in a Neubauer chamber, after dilution of samples in Türk solution. For histological analysis, thymus fragments were immediately frozen in liquid nitrogen after removal and then kept until use. Five µm thick frozen sections were fixed in acetone, washed in PBS and stained with haematoxylin-eosin, alcian blue (1 %, pH = 2.0) and safranin (0.5%). For immunhistochemistry, frozen sections were washed with PBS and then subjected to a given primary antibody for 24 h, extensively washed in PBS and then exposed to the streptavidin-peroxidase-labelled secondary antibody for 1 h. After washing with PBS, the reaction were developed with aminoethylcarbazole and 3% hydrogen peroxide. The slides were mounted and analyzed under an optical microscope (Olympus BX50), which was coupled to a videocamera (Optronics Engineering, DEI-750). The camera output was processed and analyzed by an image analyzer software (Image-Pro Plus 4). For mast cell responsiveness, thymus fragments were submitted to passive sensi-

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tization after incubation with anti-DNP IgE mAb (0.5 µg/ml) at 37°C for 12 h, followed by challenge with antigen DNP-BSA (0.05 µg/ml) during 1 h. After 150 x*g* centrifugation, supernatants were collected and stored at –20°C until histamine quantification by means of fluorimetric assay (Shore et al. 1959). The data were statistically analyzed by ANOVA followed by the Newman-Keuls-Student's t test and values ≤ 0.05 were considered significant.

RESULTS

We first noticed a significant decrease in thymus/body weight ratio under conditions of 21 day-alloxan diabetes. The thymus is a primary lymphoid organ which exhibits lobules comprising an extraparenchymal compartment, which includes capsule, septae and the perivascular spaces. As regards the histology of the thymic parenchyme, each lobule consists of an inner pale medulla and a peripheral, darkly staining lymphocyte-rich cortex. We observed that, as compared to control animals, thymuses from alloxan-diabetic rats presented a very advanced atrophy, characterized by lobules with loss of a clear lobular definition and disappearance of cortical-medullary distinction (Fig. 1A, B). These alterations directly correlated with a marked drop in thymic cell numbers, from 9.16 ± 0.17 x 10^8 cells/thymus in normal rats to 2.44 ± 0.27 10^8 cells/thymus in diabetic rats.

Mast cells in the rat thymuses are selectively located in the septae of the organ (Fig. 2A), exhibiting a connective tissue phenotype (Fig. 2C). Different from what was observed in the case of total thymic cell types, mast cells remained unchanged under conditions of alloxan-diabetes (Fig. 2B, C). In order to investigate the responsiveness of thymic mast cells, tissue fragments were passively sensitized with IgE anti-DNP and then activated with an-

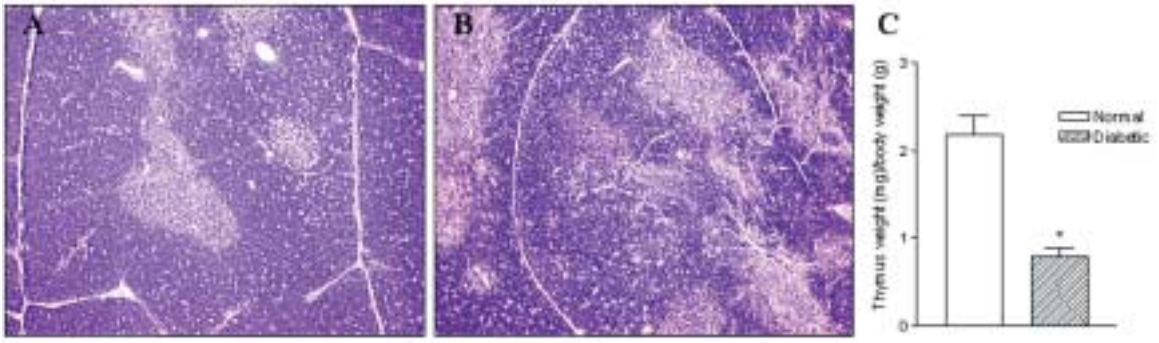

Fig. 1: general histological features of thymus from normal (A) and diabetic rats (B) (haematoxylin-eosin staining, × 100). Reduction in thymus/body weight ratio in diabetic animals as compared to normals (C). Values are the mean ± SEM for at least eight animals. *P< 0.05 significantly different from normal animals.

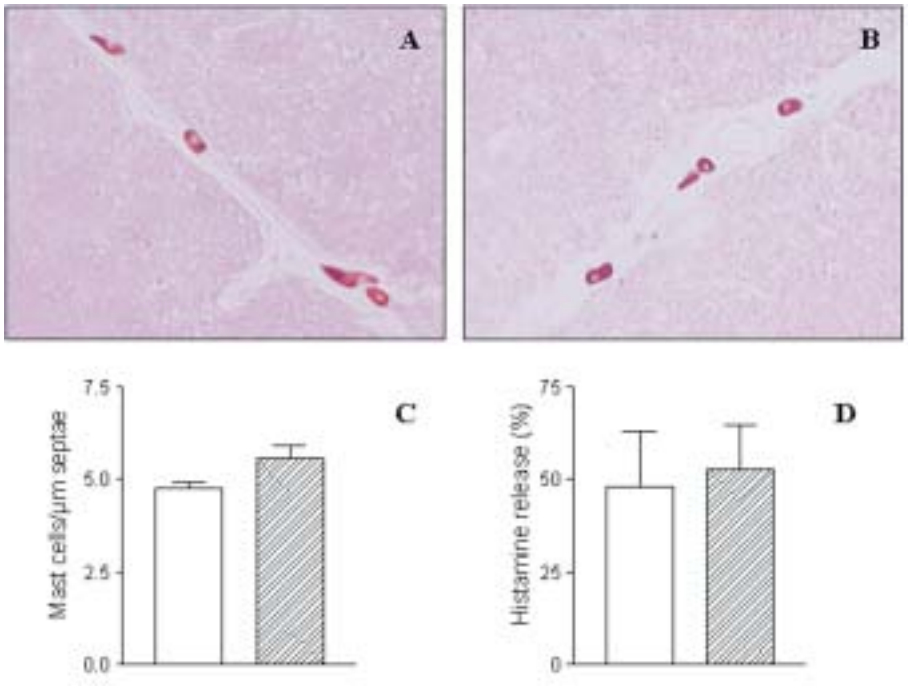

Fig. 2: light micrographs showing mast cell localization in the thymus septae from normal (A) and diabetic rats (B). Alcian blue/safranin staining was used (× 400). Number of mast cells/septa and percentage of histamine from normal (white columns) and diabetic rat thymuses (hatched columns) (C and D, respectively). Values are the mean ± SEM for at least five animals.

tigen (DNP-BSA, 0.05 µg/ml). Under such conditions, mast cells from diabetic rats released similar amount of histamine as compared to that from normal animals. Lastly, we evaluated the extracellular matrix (ECM) containing network in both control and diabetic rats. As illustrated in Fig. 3, in normal thymus laminin appeared in the interstitium of the thymic parenchyma and around the vessels (Fig. 3A). Fibronectin appeared more frequently as fibers or large patches in medulla region of normal thymus (Fig. 3C). In parallel to thymic atrophy in diabetic rats, we observed that laminin appeared widely distributed, also including cortex, medulla and septae (Fig. 3B) and fibronectin more expressed in the cortex and septae regions (Fig. 3D).

DISCUSSION

Under alloxan diabetic conditions, reduction in numbers and reactivity of mast cells were in different body sites such as celomatic cavities, mesentery and intestine (Carvalho et al. 2003), and this was directly correlated with the refractoriness to allergen provocation seen in diabetic rats (de Oliveira Barreto et al. 2003). We investigated herein the effect of diabetic state on thymic mast cell population. Alloxan-induced diabetes led to thymus atrophy, as ascertained by a marked a decreased in the thymus relative weigh and depletion in cell numbers. In the same vein, we showed that diabetes induced loss of cortico-medullary definition. Our data are in line with previous observations which described that spontaneous non-obese diabetic (NOD) mice exhibit disorganization of thymus microenvironment (Savino et al. 1991, 1993, Atlan-Gepner et al. 1999). Since the intrathymic T cell differentiation is a very complex sequence of biological events, alterations in different steps including cell proliferation, differential membrane protein expression, programmed cell death and cell migration could contribute to the depletion of thymocyte under diabetic conditions.

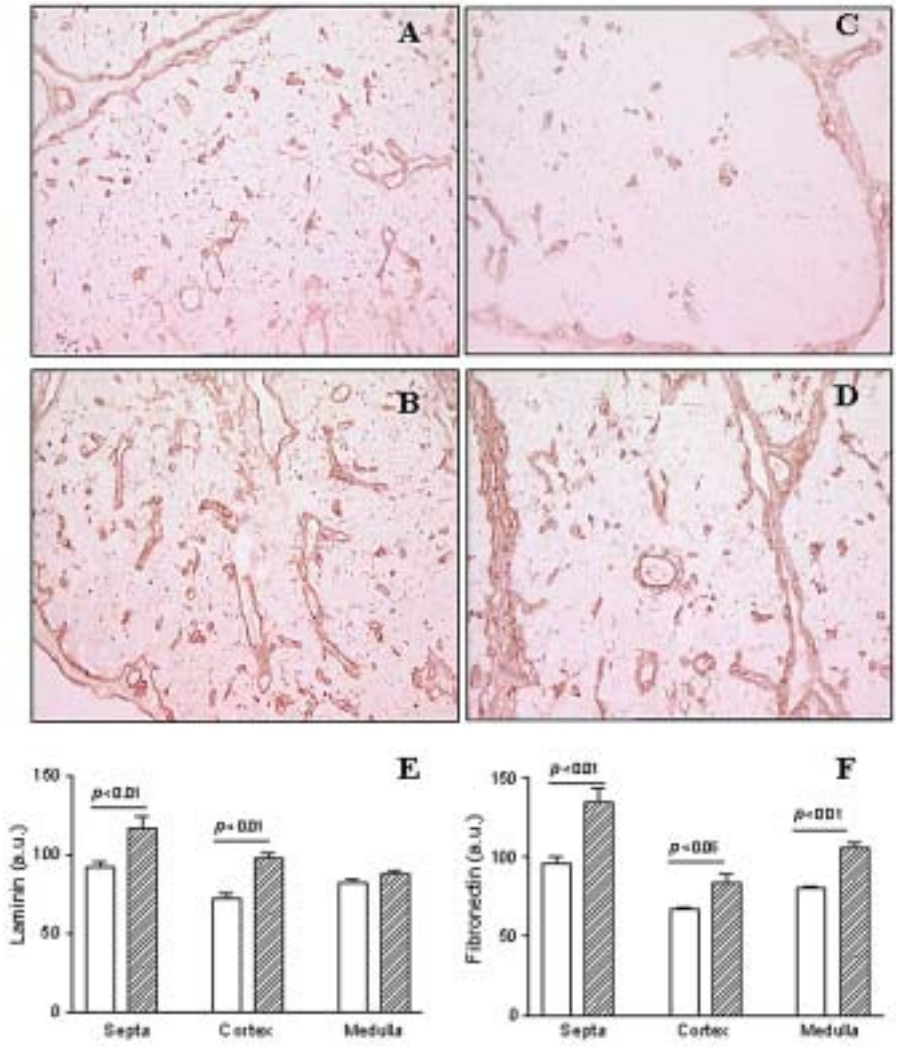

Fig. 3: immunohistochemical analysis of laminin in the thymus of normal (A) and diabetic (B) rats. Similar analysis was done for fibronectin (C and D, normal and diabetic animals, respectively). Number of mast cells/septa and percentage of histamine release from normal (white columns) and diabetic rat thymuses (hatched columns) are shown in C and D, respectively. Arbitrary unit (a.u.) values of laminin (E) and fibronectin (F) expression in septa, cortex, and medulla in thymuses from normal (white columns) and diabetic (hatched columns) rats. Values are the mean ± SEM for at least five animals.

Thymic mast cells are selectively localized in the septal region of the lobules and exhibited a connective tissue phenotype. Surprisingly, this population was not modified by alloxan-induced diabetes, when considering both cell numbers and reactivity to allergen stimulation. There results suggest that thymic mast cell population have a distinct behavior as compared to those present in other tissue sites. We previously demonstrated that mast cell depletion in alloxan diabetic rats well correlated with an increase in the levels of serum glucocorticoids (Diaz et al. 2001). Surgical adrenalectomy and treatment with steroid receptor antagonist RU 486 completely reversed mast cell depletion under diabetic conditions (Diaz et al. 2001). Thus, since steroids are known to induce thymus atrophy (Durant et al. 1984), it is possible that the reduction in the number and/or sensitivity of glucocorticoid receptors (GR) accounts for the refractoriness of thymic mast cells seen in diabetic animals. Further experiments are now in progress to approach this issue.

We also studied the distribution of selected ECM components in the thymus of diabetic rats. An augmentation in the deposition of both laminin and fibronectin were detected in the case of diabetes. Interestingly, an increase in ECM proteins have already been reported in the NOD mouse thymus (Savino et al. 1991, 1993). Since mast cells are under the direct influence of microenvironment factors, it is conceivable that an intrathymic augmentation ECM also contributes (even if partially) to impair the down-regulatory effect of diabetes on thymic mast cells.

In conclusion, our findings indicate that though alloxan-induced diabetes leads to a clearcut thymus atrophy due to lymphocyte depletion, mast cells are kept unaltered regarding their number and reactivity. From a conceptual point of view, these data indicate that thymic mast cells are under a different regulation, as compared to their counterparts located in other body sites.

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