

Adoptive Transfer of Mast Cells Abolishes the Inflammatory Refractoriness to Allergen in Diabetic Rats

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Key Words

Mast cells · Diabetes · Allergy · Inflammation

Abstract

Mast cells are pivotal secretory cells primarily implicated in allergen-evoked inflammatory responses and are downregulated following experimental chemical diabetes. Here we tested the hypothesis that a decrease in the number and reactivity of mast cells would account for the hyporesponsiveness of diabetic rats to allergen-induced inflammation. We found that the anaphylactic release of histamine from sensitized ileum, trachea and skin tissues was clearly reduced in rats turned diabetic via intravenous administration of alloxan. Likewise, actively and passively sensitized diabetic rats mounted a weaker allergen-evoked pleural mast cell degranulation and protein extravasation, as compared to sensitized nondiabetic animals, which paralleled a marked reduction in the mast cell population in the pleural cavity. The number of mast cells enumerated in the mesentery from diabetic rats was also clearly reduced. The allergen-evoked plasma leakage in diabetic rats was restored by the transfer of mast cells from sensitized nondiabetic

rats. Moreover, the adoptive transfer of sensitized mast cells from diabetics to naive animals led to a lower allergen-induced exudation as compared to the response noted after the transfer of sensitized naive mast cells. Purified mast cells from diabetic rats were hyporesponsive to antigen and compound 48/80 stimulation in vitro as attested by histamine release. Thus, our results show that the phenomenon of refractoriness of diabetic animals to allergen challenge appears to be accounted for by a reduction in the number and reactivity of mast cells.

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Introduction

The immediate hypersensitivity reaction is the hallmark of allergic diseases including rhinitis, atopic dermatitis, asthma and anaphylaxis, and the central involvement of mast cells in the pathogenesis of such disorders has long been considered [1]. In general, mast cells are found around blood vessels, predominantly in tissues forming an interface between the host and the external environment such as skin, airways and gastrointestinal

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tract [2], their number being dependent on both the rate of production of mast cell precursors and the survival of mature mast cells within the tissue [3]. Microenvironmental factors such as the stem cell factor play an important role in regulating mast cell numbers in the periphery [4]. They are also located in mucosal and serosal surfaces, a fact that has been used for experimental purposes in order to collect free mast cells. In response to antigen, mast cells can release cytoplasmic granule-associated mediators such as the vasoactive amines and have also been shown to produce lipid mediators such as cysteinyl-leukotrienes and PAF [5]. Mast cells can also secrete a wide range of cytokines, which contribute to the initiation and perpetuation of IgE-dependent allergic inflammation in different sites [6, 7]. In studies using the adoptive transfer system, in genetically mast cell-deficient mice, it was demonstrated that mast cells are essential for the leukocyte recruitment in the skin after challenge with IgE and specific antigen [8].

There is a high incidence of allergic dysfunctions in the population of both developed and developing countries [9], although the occurrence of such diseases in patients with diabetes mellitus is smaller than predicted [10, 11]. The mechanism implicated in this phenomenon is not yet fully understood. We and others reported that diabetic rats exhibit a decrease of the allergen-evoked late eosinophil infiltration in the pleural [12] and bronchoalveolar spaces [13], a phenomenon which was temporally correlated with a selective reduction in the local mast cell population [14]. Since mast cells play a critical role not only in the eosinophil recruitment but also in other key features of the allergic inflammatory response, this study was undertaken in order to evaluate how a diabetes-associated mast cell disturbance could effect the suppression of allergen-mediated inflammation under diabetic conditions. To address this point, we used the model of adoptive transfer of mast cells and the quantification of allergen-induced tissue histamine release and pleural protein leakage as inflammatory markers.

Material and Methods

Animals and Diabetes Induction

Isogenic and nonisogenic male Wistar rats (180–200 g) were obtained from the Oswaldo Cruz Foundation Breeding (Rio de Janeiro, Brazil) and used in accordance with the guidelines of the Committee on Use of Laboratory Animals of the Oswaldo Cruz Foundation (licence 0085-02). Diabetes mellitus was induced 21 days before challenge with a single intravenous injection of 40 mg/kg alloxan monohydrate, diluted with saline (0.9% NaCl) into 12-hour-fasted rats supplied with water ad libitum. Animals injected with vehicle and

submitted to similar experimental conditions were used as negative controls. Rats with blood glucose levels below 11 mM were excluded from further experiments.

Sensitization Procedure and Allergen Challenge

Active sensitization was performed by means of a subcutaneous injection of a mixture containing 50 µg ovalbumin (OVA) and 5 mg of Al(OH)₃. After 14 days, animals submitted to light ether anesthesia received an intrapleural (i.pl.) injection of OVA (12 µg/cavity) dissolved in sterile saline. IgE passive sensitization was carried out by the i.pl. injection of murine anti-dinitrophenylated (DNP) IgE mAb (1 µg/cavity) [15] followed by challenge with DNP-bovine serum albumin (BSA; 1 µg/cavity) 24 h later. In both experimental conditions, rats were killed by terminal CO₂ atmosphere followed by cervical dislocation, 30 min post-antigen challenge. The pleural cavity was opened, rinsed with 3 ml of heparinized (10 U/ml) Hanks' balanced salt solution (HBSS), and the volume was measured in a graduated syringe. Negative control groups were represented by sensitized rats in which allergen was replaced by saline as a challenge.

Pleural Fluid Analysis

The fluid recovered from the pleural cavity was centrifuged at 1,500 g for 10 min and the total protein content spectrophotometrically quantified (540 nm) in the supernatant by means of the Biuret technique. Mast cells were counted in a Neubauer chamber after dilution of pleural fluid samples (90 µl) with toluidine blue dye solution (10 µl) and evaluated as total and intact cells ($\times 10^3$ cells/cavity).

Mast Cell Enumeration in the Mesentery

After having been killed, the animals were injected intraperitoneally with 5 ml of paraformaldehyde (4%) diluted with sucrose solution (4%). After 15 min, the peritoneal cavity was opened, the mesentery was recovered and placed on glass slides to dry at room temperature. The mast cell population was identified in the mesenteric membrane after staining with Giemsa as well as Alcian blue (1%) and safranin (0.5%) dyes [16]. The number of mast cells was counted by means of an optical microscope (Olympus BX50), which was coupled to a video camera (Optronics Engineering, DEI-750). The camera output was processed and analyzed by image analyzer software (Image-Pro Plus 4). The number of mast cells was expressed per square millimeter, and at least 16 fields (1 mm²/field) per mesentery were examined.

Adoptive Transfer of Mast Cells

Mast cells were recovered from the peritoneal cavity of naive or diabetic isogenic Wistar rats and purified by means of continuous Percoll gradient according to Inagaki et al. [17] with some modifications. After peritoneal washing with heparinized (10 U/ml) calcium and magnesium-free HBSS (HBSS⁻), the cells were centrifuged at 150 g for 10 min, the supernatant was discarded and the pellet resuspended in HBSS⁻ containing 0.1% BSA. The cell suspension was mixed with Percoll (72%) and overlaid with 1 ml of HBSS⁻ to be further centrifuged at 150 g for 25 min. Pelleted mast cells were washed twice with HBSS⁻ and purity was over 96% as attested by toluidine blue staining. Viability tested by means of trypan blue exclusion was higher than 95%. 4×10^5 mast cells diluted with 0.1 ml of sterile saline were injected into the pleural cavity of the recipient animals. One hour after the cell transfer, the animals were i.pl. challenged with OVA (12 µg/cavity) and 30 min later they were killed. The pleural fluid

analyses were made as indicated above. A similar volume of the vehicle was used in the negative control group.

Mast Cell Enumeration in Intestine Segments

Animals were killed with CO₂ followed by cervical dislocation, the peritoneal cavity was opened and the small intestine removed and washed with HBSS. The material was fixed in a solution containing formalin 40% (pH 7.2), for 1 h at 4°C, and then washed with buffer. Thin sections were obtained in a Reichert Jung Ultramicrotome OmU3 and observed stained with Giemsa dye by means of an optical microscope (Olympus BX50), which was coupled to a video camera (Optronics Engineering, DEI-750). The camera output was processed and analyzed by an image analyzer software (Image-Pro Plus 4). The number of mast cells was expressed per square millimeter, and at least 16 fields (1 mm²/field) per sample were examined.

Anaphylactic Histamine Release from Rat Tissues in vitro

Small segments of the ileum, tracheal and dorsal hypoderm were removed from actively sensitized diabetic or nondiabetic rats, washed with Tyrode solution and placed in 24-well plates containing HBSS. The tissues were challenged with OVA (400 µg/ml) and 15 min later the plates were centrifuged at 150 g for 10 min, the samples were collected and added to 0.8 N perchloric acid (1:2 dilution). After centrifugation at 170 g for 10 min, the supernatant was collected and stored at -20°C for histamine quantification according to Shore et al. [18]. Briefly, this method consists of sample dilution in 0.1 N HCl followed by 0.8 N of NaOH, and further addition of the substrate *o*-phthalaldehyde. After 4 min incubation, the reaction was stopped with 3 N HCl and the fluorescence measured in a Shimadzu RF1501 spectrofluorophotometer (Japan; excitation at 360 nm; emission at 450 nm). The tissue fragments were dried in incubators at 40°C for 15 min and the results expressed as the amount of histamine release (ng/mg) of tissue.

Mast Cell Purification and Reactivity to Degranulating Agents in vitro

Peritoneal mast cells were purified as indicated above. In the case of antigen stimulation, isolated mast cells (1×10^5 /ml) were cultured in 24-well plates with IgE anti-DNP antibody (0.5 µg/ml) at 37°C for 12 h. Following sensitization, the cells were incubated with antigen (DNP-BSA, 0.05 µg/ml) for 1 h at 37°C in 5% CO₂ in air. After centrifugation at 150 g, the supernatant was collected and stored at -20°C. Mast cells were also stimulated with compound 48/80 (0.5 µg/ml). In both cases, histamine release (%) was assayed fluorimetrically [18].

Drugs

OVA (grade V) was purchased from Biochemica Fluka (Switzerland), DNP-BSA from Calbiochem (San Diego, Calif., USA), sodium heparin from Roche (São Paulo, Brazil) and the murine anti-DNP IgE mAb from Calbiochem (San Diego, Calif., USA). BSA, *o*-phthalaldehyde, HBSS, compound 48/80 and Percoll were obtained from Sigma Chemical Co. (St. Louis, Mo., USA). All solutions were freshly prepared immediately before use.

Statistic Analysis

The data were reported as mean $\pm$ standard error of the mean (SEM) and statistically analyzed by Student's test or ANOVA followed by the Newman-Keuls-Student t test. Probability values (*p*) of 0.05 or less were considered significant.

Results

Effect of Alloxan-Induced Diabetes on the Anaphylactic Histamine Release from Rat Tissues in vitro

Stimulation with antigen (OVA, 400 µg/ml) in vitro caused a marked increase in the amount of histamine released from ileum, trachea and skin fragments from sensitized rats (fig. 1A–C, respectively). Under conditions of 21-day alloxan-induced diabetes, a drastic reduction in the anaphylactic secretion of histamine was observed in all tissues tested (fig. 1). No difference in the basal histamine release from ileum, trachea and skin was noted under diabetic conditions as compared to controls (fig. 1). Moreover, we detected, by means of histopathological analysis, that intestine fragments from diabetic animals exhibit a lower number of mast cells in comparison with that noted in controls. Values were 301.8 ± 1.9 and 135.2 ± 12.9 mast cells/mm² (mean $\pm$ SEM, *n* = 5; *p* < 0.01) in naive and diabetic rats, respectively.

Influence of Diabetic State on Mast Cell Reactivity in vitro

Purified peritoneal mast cells, previously passively sensitized with IgE and then activated with antigen (DNP-BSA, 0.05 µg/ml), exhibited a marked increase in the levels of histamine released, a phenomenon clearly suppressed in mast cells recovered from diabetic rats (table 1). Similar refractoriness was noted when compound 48/80 was used as a stimulus (table 1).

Effect of Alloxan-Induced Diabetes on the Antigen-Induced Pleural Protein Leakage in Actively and IgE-Passively Sensitized Rats

As illustrated in figure 2A, the i.pl. injection of OVA (12 µg/cavity) into 14-day actively sensitized rats caused a marked increase in the protein content of the pleural cavity. A similar exudative response was noted after the i.pl. injection of DNP-BSA (1 µg/cavity) into naive rats passively sensitized by prior injection of anti-DNP IgE mAb (fig. 2B). In the case of alloxan-diabetic rats, the antigen-induced protein leakage was clearly suppressed either in actively or in IgE-passively sensitized rats (fig. 2A and B, respectively). In addition, a significant drop in the number of pleural mast cells in diabetic versus nondiabetic rats was noted. Values were $651.75 \pm 53.7 \times 10^3$ and $477.4 \pm 23.7 \times 10^3$ cells/cavity (mean $\pm$ SEM, *p* < 0.014; *n* = 6) in naive or diabetic rats, respectively. It is of interest to note that alloxan-induced diabetes did not modify the amount of mast cell-stored histamine, which is a sign

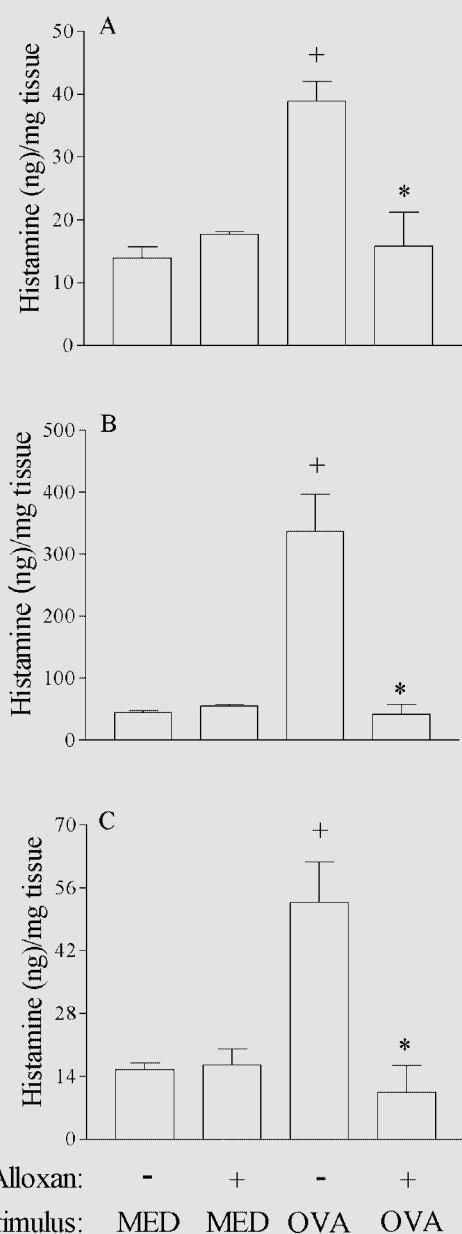


Fig. 1. Influence of alloxan-induced diabetes on the histamine release from fragments of ileum (A), trachea (B) and skin (C) obtained from 14-day actively sensitized rats. Alloxan (40 mg/kg, i.v.) was injected 21 days before in vitro challenge with OVA (400 µg/ml) or medium (MED). Data are expressed as mean $\pm$ SEM from at least 5 animals. + = Statistically significant compared to medium-stimulated tissues from nondiabetic sensitized rats; * = statistically significant compared to OVA-stimulated tissues from nondiabetic sensitized rats.

Table 1. Effect of alloxan diabetes on the mast cell degranulation triggered by antigen and compound 48/80 in vitro

Condition	Sensitization	Stimulus	Dose µg/ml	Histamine release, %	n
Naive	+	Medium	–	5.59 $\pm$ 0.62	15
	+	Antigen	0.05	24.79 $\pm$ 1.60 ⁺	22
	–	Medium	–	4.54 $\pm$ 0.47	6
	–	C48/80	0.50	34.98 $\pm$ 3.86*	6
Diabetic	+	Medium	–	3.99 $\pm$ 0.47	16
	+	Antigen	0.05	17.75 $\pm$ 1.00 ⁺	22
	–	Medium	–	4.26 $\pm$ 0.58	5
	–	C48/80	0.50	20.60 $\pm$ 1.47*	6

Peritoneal mast cells were collected from normal or diabetic rats and sensitized with IgE (0.5 µg/ml) for 12 h followed by stimulation with antigen (DNP-BSA) for 60 min. Mast cells from normal and diabetic rats were also stimulated with compound 48/80 for 60 min. Data are expressed as mean $\pm$ SEM.

⁺ Statistically significant compared to medium-stimulated cells.

* Statistically significant compared to antigen- or C 48/80-stimulated naive mast cells.

of an absence of cell damage. Histamine values for mast cells from naive and 21-day-diabetic rats were 11.66 ± 1.56 pg/cell (mean $\pm$ SEM; n = 6) and 14.04 ± 1.04 pg/cell (n = 10), respectively.

Influence of Alloxan-Induced Diabetes on the Mast Cell Population of the Mesentery

To address the potential effect of diabetes on the mast cell population of the mesenteric region, tissue fragments were fixed and stained with Giemsa and Alcian blue and safranin dyes, and the number of total mast cells was enumerated as cells per mm². We noted that, compared with nondiabetic rats, 21-day-diabetic animals presented a significant reduction in the number of mast cells (fig. 3A and B, respectively). For Giemsa staining, values were 84.4 ± 10.1 and 37.9 ± 9.1 cells/mm² (mean $\pm$ SEM, n = 18) in naive and diabetic rats, respectively. No degranulated mast cells were observed in the mesenteric tissue from diabetic rats (data not shown).

Influence of Diabetes-Induced Mast Cell Depletion on the Downregulation of Allergic Pleurisy in Sensitized Rats

In order to investigate the hypothesis that there is a potential relationship between the reduction in the mast cell population in diabetic rats and their refractoriness to

antigen provocation, we used the system of cell adoptive transfer. Freshly isolated peritoneal mast cells (400×10^3 cells) from sensitized donors were injected into the pleural cavity of diabetic sensitized recipient rats. We noted that mast cell counts and the protein content in the pleural fluid of mast cell-reconstituted diabetic rats were not different from those of naive rats. Values of pleural mast cells were $783.2 \pm 14.0 \times 10^3$ and $657.1 \pm 74.1 \times 10^3$ cells/cavity (mean $\pm$ SEM, $n = 6$) and values of total protein were 4.7 ± 1.2 and 4.8 ± 0.9 mg/cavity (mean $\pm$ SEM, $n = 6$) in naive and mast cell-reconstituted diabetic animals, respectively. As illustrated in figure 4, i.pl. OVA challenge of diabetic sensitized rats which had undergone selective reconstitution of their mast cell population evoked a marked increase in the protein leakage, at levels comparable with those previously noted in nondiabetic rats, indicating that the hyporesponsiveness of diabetic rats to antigen can be corrected by means of mast cell reconstitution.

Next, to address the mast cell reactivity under diabetic conditions, we attempted to transfer isolated mast cells from sensitized diabetic or nondiabetic donors to naive recipient rats. The data in figure 5 show that the pleural protein extravasation noted after OVA stimulation was significantly smaller in rats transferred with mast cells from diabetic rats as compared with those from nondiabetic animals, suggesting a complementary possibility that a deficiency in the mast cell reactivity might also contribute to refractoriness of diabetic animals to antigen challenge.

Discussion

It has been reported that the susceptibility to type 1 diabetes dysfunction might induce resistance to allergic inflammatory processes [10, 19, 20]. We previously demonstrated that the population of free mast cells was down-regulated in the celomic cavities of alloxan-diabetic animals, a phenomenon which could be responsible for at least part of their refractoriness to antigen challenge [12, 14]. In this study, we found a marked reduction in the antigen-evoked histamine release from tissue and pleural protein extravasation in diabetic rats following either active or passive sensitization. The reconstitution of the pleural mast cell population in diabetic animals, by means of cell adoptive transfer, restored the antigen-induced plasma leakage. We showed that the transfer of diabetic sensitized mast cells to normal animals caused a lower pleural exudative response compared to the response pro-

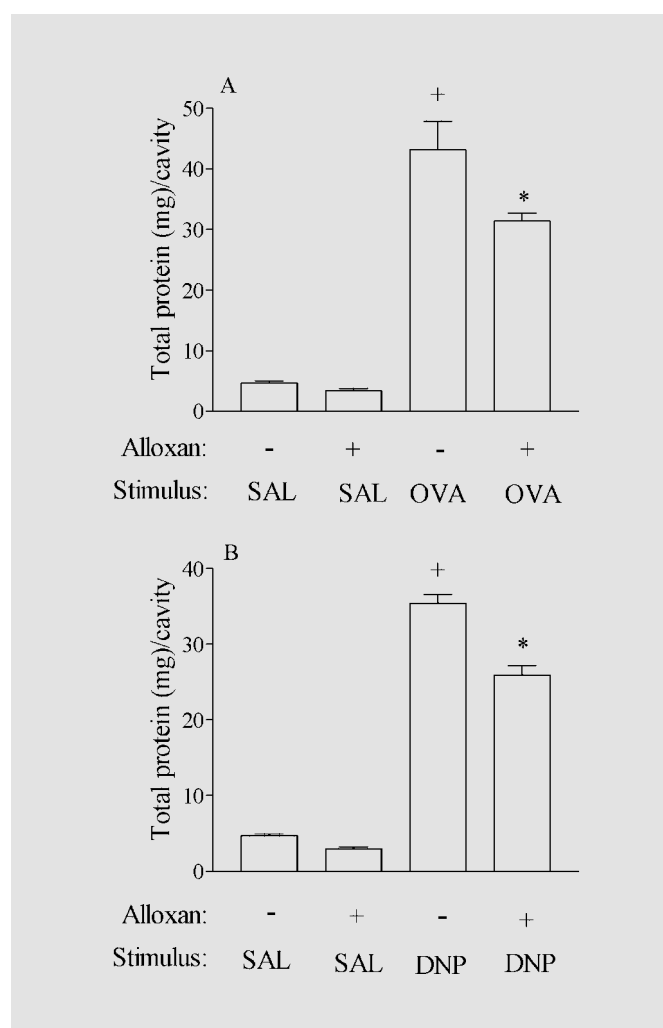


Fig. 2. Influence of alloxan-induced diabetes on the protein extravasation induced by antigen in actively (**A**) and in IgE-passively (**B**) sensitized rats. Alloxan (40 mg/kg, i.v.) was injected 21 days before i.pl. challenge with OVA (12 μ g/cavity) or DNP-BSA (1 μ g/cavity) into actively or IgE-passively sensitized rats, respectively. Control values for total protein extravasation were obtained from sensitized rats injected with saline (SAL). The pleural fluid analysis was made 1 h postchallenge. Data are reported as mean $\pm$ SEM from at least 5 animals. ⁺ = Statistically significant compared to nondiabetic sensitized rats injected with saline; ^{*} = statistically significant compared to nondiabetic sensitized rats challenged with antigen.

duced by nondiabetic mast cells. Purified mast cells from diabetic rats were also hyporesponsive to antigen stimulation *in vitro*. Taken together, our findings indicate that the refractoriness of diabetic animals to allergen challenge is associated with the reduction in the number and reactivity of mast cells.

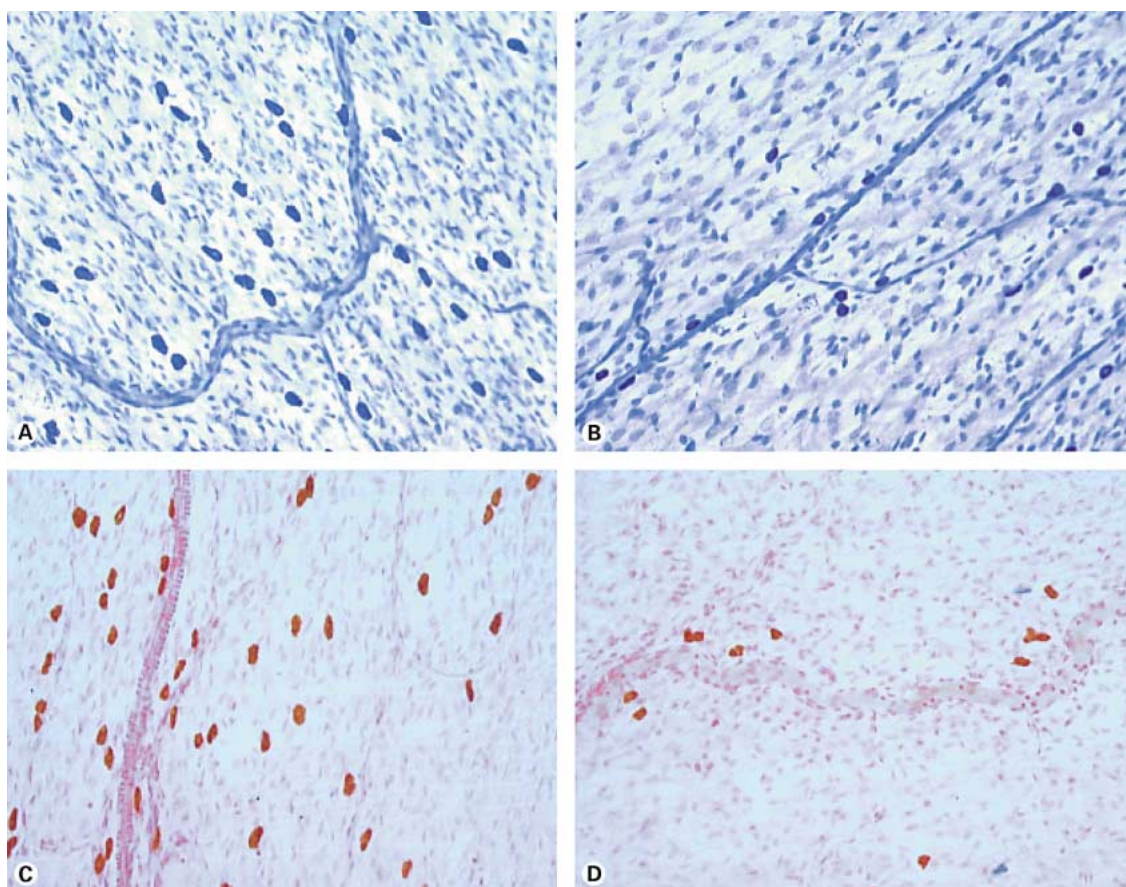


Fig. 3. Comparative analysis of mast cell population present in the mesenteric region of normal (**A, C**) and 21-day-diabetic rats (**B, D**). Tissue fragments were fixed and stained with Giemsa (**A, B**) and Alcian blue/safranin dyes (**C, D**). The analysis was made under an optical microscope with a 40 $\times$ objective.

Several works state that some tissues exhibit the capacity to respond rapidly to the release of inflammatory mediators after antigen stimulation and that mast cells are the main cells of such responses [21]. In the current study we showed that isolated fragments of ileum, trachea and skin from actively sensitized rats were quite sensitive to *in vitro* antigen provocation, as attested by the amount of secreted histamine. In contrast, in the case of diabetic animals, not only did trachea and skin but intestine as well show refractoriness to the antigen challenge. These observations provide evidence that similar to what was noted in case of free mast cells [12, 22], those located in tissues seem to be negatively regulated under diabetic conditions. In line with this proposition, we also noted that the number of mast cells present in the mesenteric tissue of diabetic animals was clearly reduced as compared to that of normal animals. Since mast cells exhibit a morphological and functional heterogeneity in different

sites in the body [21], our findings provided additional evidence that both connective tissue and mucosal mast cell subtypes are affected under diabetic conditions.

We further evaluated the interference of the diabetic state with allergen-evoked protein extravasation and noted that diabetic sensitized rats presented a less intense pleural vascular permeability response 1 h postchallenge. Similarly, allergen-evoked exudation also appeared significantly attenuated in alloxan-diabetic rats passively sensitized with IgE. These findings indicate that pleural protein leakage caused by allergen provocation is downregulated under diabetic conditions following either active or passive sensitization. This phenomenon could be associated with functional changes in the behavior of microvessels [23] and/or decreased responsiveness of endothelial cells to vasoactive agents [24]. In fact, a number of studies reported that diabetic animals present a decreased response to intradermally administered bradykinin and va-

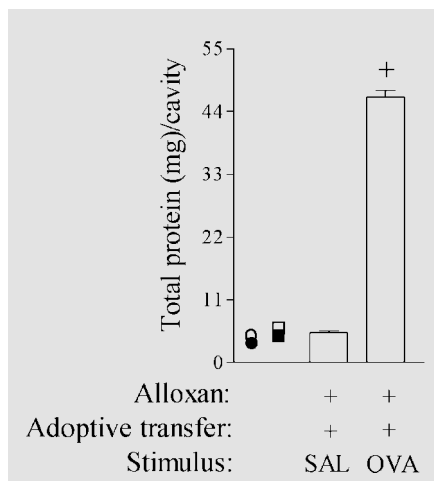


Fig. 4. Effect of the mast cell adoptive transfer on the suppression of pleural protein extravasation noted in 21-day-diabetic rats. Isolated mast cells recovered from sensitized nondiabetic donors were injected i.pl. into diabetic sensitized recipient animals 1 h before the antigen challenge (OVA, 12 µg/cavity). Control values were obtained from sensitized rats injected with saline (SAL). The pleural fluid analysis was made 1 h postchallenge. Values of basal pleural protein content from nondiabetic (○) and diabetic (●) rats and from mast cell-transferred nondiabetic (□) and diabetic rats (■) are shown. Data are reported as mean ± SEM from at least 5 animals. + = Statistically significant compared to saline-injected rats.

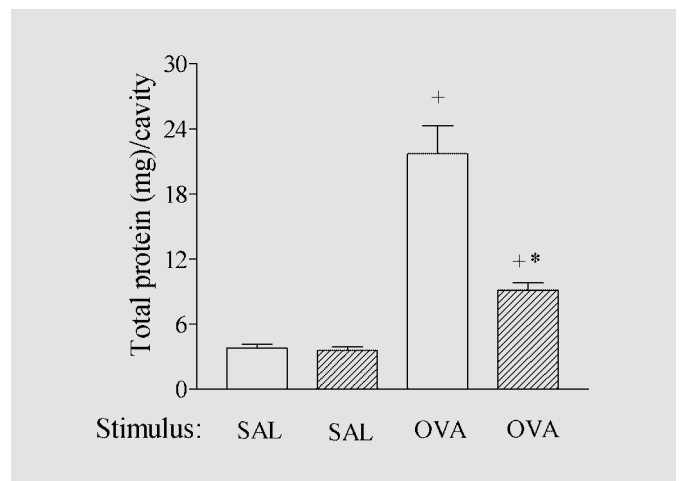


Fig. 5. Analysis of the cell reactivity by means of the adoptive transfer of isolated mast cells from diabetic (■) or nondiabetic sensitized (□) rats to the pleural cavity of normal rats. The transfer was done 1 h before OVA challenge (12 µg/cavity) and the protein extravasation analyzed 1 h postchallenge. Control animals were injected with saline (SAL). Data are reported as mean ± SEM from at least 5 animals. + = Statistically significant compared to matching controls; * = statistically significant compared to rats transferred with sensitized mast cells from nondiabetic rats.

soactive amines [25] as well as swelling induced by carrageenan, cellulose sulfate and antigen stimulation [26]. Alternatively, the refractoriness of diabetic sensitized rats to allergen challenge could be accounted for by a defective response of the IgE-mast cell system. Malaviya and Abraham [27] described that mast cell-derived leukotrienes play a role in the host defense by mediating neutrophil influx and consequently bacterial clearance at infection sites. Considering the higher susceptibility of diabetic patients to bacterial infection, it is plausible to speculate that such a phenomenon could be partially dependent on the depletion of the mast cell population. It is important to bear in mind that the variety of mediators released from mast cells in response to many different signals, adding allergen provocation, make these cells important enough to participate in a wide spectrum of adaptive, innate and several other pathological immune responses, potentially including diabetes.

For better evaluation of the contribution of the diabetes-induced mast cell disturbance to the refractoriness to allergen provocation, we studied the exudative response in alloxan-diabetic animals which had undergone a selective and local repair of their mast cell deficiency.

Freshly isolated mast cells from normal donors were intrathoracically injected into diabetic sensitized rats, which were then challenged with OVA. The increase in pleural protein leakage in mast cell-reconstituted diabetic recipient rats was significantly higher than the increase noted in mast cell-deficient animals, clearly showing that the hyporeactivity of diabetic rats to allergen-evoked protein extravasation was indeed dependent on mast cells. Finally, we thoroughly investigated the mast cell responsiveness to antigen challenge. The transfer of sensitized mast cells from diabetic donors to naive animals caused a lower exudative response to allergen provocation as compared to the response noted after the transfer of sensitized mast cells from nondiabetic rats. We found that purified mast cells recovered from diabetic rats exhibited a decreased reactivity to stimulating agents such as antigen and compound 48/80 in vitro, showing that the hyporeactivity associated with diabetes is not restricted to antigen challenge. Altogether, our findings indicate that not only might the number but also the reactivity of mast cells seem to be modified under diabetic conditions. Early studies demonstrated that glucocorticoids induce depletion of mast cells at different body sites including lung,

intestine and skin by a mechanism related to inhibition of local production of factors involved in the control of mast cell recruitment, development and survival [28, 29]. These include stem cell factor [30] and the cytokines IL-3 and IL-4 [31]. We previously reported that following alloxan diabetes, rats exhibited a significant increase in the adrenal gland/body weight rate in parallel to an elevation in serum corticosterone levels [32]. Hyperactivity of hypothalamopituitary-adrenal axis with consequent hypercortisolism has been described for uncontrolled streptozotocin-induced diabetes [33, 34] and is frequently observed in patients with type 1 and type 2 diabetes [35, 36]. Thus, we can propose that, as previously shown for mast cell numbers under diabetic conditions [32], inhibition by glucocorticoids of cytokine production and/or a direct effect of these hormones on mast cells may be a plausible explanation for the reduction in their reactivity. Experiments are underway to clarify this point.

In conclusion, our findings indicate that the alloxan-induced decrease in the number and reactivity of mast cells seems to actually contribute to the refractoriness to allergen challenge detected in different tissues of diabetic animals. They also provide evidence of the potential relevant role of depletion in the mast cell population in some of the pathological-related processes associated with diabetic dysfunctions.

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