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Chronic Prostatitis / Chronic Pelvic Pain Syndrome: Histological Evidences of treatment with an Immunotherapeutic Agent in a Murine Model

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Abstract: Chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) has a strong autoimmune component and is the most important type of prostatitis in worldwide. Here,

using a modification of the murine model of experimental autoimmune prostatitis (EAP), we showed that besides an infiltrate of inflammatory cells in the prostatic tissue, animals developed benign prostatic hyperplasia. Different doses of immune regulatory agent were administrated to experimental animals, observing that the dialyzable leukocyte extract (DLE) decreased the infiltrate of inflammatory and mast cells.

Key words: *autoimmune, prostatitis, Chronic Prostatitis, inflammation, Dialyzable leucocyte Extract*

INTRODUCTION

Prostatitis indicates an inflammation state of the prostate gland and comprises several syndromes[1,] Chronic inflammatory prostatic disease of non- bacterial prostatitis (CP/CPPS) is difficult to diagnose and treat and it is the most common type of prostatitis. Epidemiological data suggest that its incidence exceeds the bacterial prostatitis, however, its etiology is unknown although an autoimmune origin is suggested. Moreover, the disease have been also associated in some cases with benign prostatic hyperplasia (BPH). Other evidences indicate that chronic prostatic inflammation could be related with prostatic cancer, which is the second cause of cancer-related deaths in males[2,] Many reports indicated that inflammation may play a critical role in the proliferation and transformation of the luminal epithelium[3] and many authors have emphasized the relevance of specific studies to determine the immunological mechanisms of prostate gland inflammation. Experiments done in animal models for experimental autoimmune prostatitis (EAP), date the possible molecular and cellular mechanisms involved[4]. Rivero and coworkers[5], reported infiltrating mononuclear cells (MNC) approximately 7 days after priming, growing up rapidly 28 days later. Infiltrating MNC were located in the stroma of the prostate gland, surrounding the acini and very close to blood vessels. The presence of mast cells, hemorrhage, disorganization of tissue architecture and fibrosis were also typical features. Infiltrates consisted mainly of CD4+ and CD8+T cells. Many treatments for chronic prostatitis such as the receptor agonist BXL-628 (elocalcitol) of vitamin D, have been evaluated in different EAP models. Elocalcitol studies showed that 2 weeks of administration, significantly inhibits the intraprostatic inflammation, leading to an important reduction in the number of CD-4 and CD8-T cells, B cells, macrophages and dendritic cells[6]. In addition, BXL-628 inhibited the production of proinflammatory cytokines and chemokines[7].

The objective of this work was to modify the EAP model developed for Motrich *et al.*[1] to induce a higher inflammatory process besides the induction of a prostatic hyperplasia to evaluate the putative effects of an immune modulatory agent in the chronic autoimmune prostatitis.

METHODS

Animals: Normal and immunized male Wistar rats of 3 months old, were used in this study. Another normal rats were used for the preparation of prostatic antigen for immunization.

Antigens preparation: Male sex accessories glands (RAG) excised from Wistar rats were homogeneized in 0.01M PBS at pH 7.2 using protease inhibitors. The homogenate was centrifuged at 10000 g for 30 min and the supernatant was used them as RAG homogenate. For the immunization procedure, the RAG homogenate was chemically modified (MRAG) by coupling the RAG saline extract to diazonium derivate of sulphanilic and arsalinilic acids.

Immunization and treatment: Rats (n=33) were intradermal injected at day 0, then animals were intraperitoneal injected at day 15 and finally rats were again intradermal injected at day 30. The immunization was doing with 5 mg of MRAG emulsified with 0.5ml of Freund's complete adjuvant (FCA). After second immunization we administrated dexamethasone (Dex 0.15mg/kg/day), DLE (1u) or placebo. A control group with the pathology without treatment and a normal group (without immunization) were included. The rats were sacrificed after 34 days and prostatic tissue was obtained.

Histopathology analyses: Prostates from immunized and control rats were studied by light microscopy. At necropsy, the prostate was separated from the adjacent tissues and weighted. The specimens were immediately placed in 10% formalin for at least 24h, dehydrated in alcohol, cleared in xilol, and embedded in paraffin. The glands were sectioned at 5 µm. After cutting, the sections were stained with hematoxylin-eosin. For identification of mast cells, sections were stained in toluidine blue at pH 3.5. The slides were examined and scored by 2 experienced pathologists independently. The inflammatory cells were quantified as density per unit area by randomly counting on 10 spots under high- power fields (HPF, 400x magnification). Every slide had 2 values, and the mean of the 2 values was the final score for the slide. The mean of the 4 slides scores in given prostate was the final score for each prostate.

RESULTS AND DISCUSSION

The use of two different pathways for immunization produced two different kind of signs together: we found signs of Benign Prostatic Hyperplasia (BPH), like the increase of glandular and fibro muscular cells; and also a very important infiltration of inflammatory cells, which have not been reported previously in similar models of EAP (**Figure 1. Panel A**). This results could be due the pro-inflammatory micro-environment generated, inducing stromal hyper proliferation and tissue remodeling with a local hypoxia induced by increased oxygen demands of proliferating cells. This support the chronic inflammation as a source of oxidative stress, leading to tissue injury in infiltrating area[8, 9]. Moreover, the constant prostatic inflammation also has been associated with prostate intraepithelial neoplasia and prostate cancer[1, 9]. Analysis of prostate needle biopsies from patients suspected of prostate cancer revealed an important association between chronic inflammation and prostate epithelial malignant changes[1]. Taking in consideration these studies, the animals with the pathology but without treatment could be developed other pathologies like BPH. Studies in other murine models suggest that these conditions could evolve in other more serious diseases like prostatic cancer[1, 8].

In hematoxylin-eosin staining, we observed in the group without treatment a considerable amount of inflammatory cells (**Figure 1. Panel A and B)** like in other murine models of EAP, where is reported that immunization of rats or mice with prostate gland extracts produce infiltration of inflammatory cells [10,11]. It is known that the infiltrate of inflammatory cells present in this model of EAP are mainly T-cells CD-4 and CD-8. Some studies shown that T-cell in EAP could produce IFN-γ, IL-6, IL-17 and other pro-inflammatory cytokines that have an essential role in disease induction, perpetuation and evolution of the prostatitis[9],[12]. The cytokine production may contribute to local growth factor production and angiogenesis in the prostatic tissue[13].

In general, after treatment of animals with the different doses schemes, we observed a significantly decrease in the inflammatory infiltrates in the groups treated with DLE (1.3 cell per HPF) compared with the pathologic group (3 cell for HPF) and placebo group (5.6 cell for HPF) (Figure 1, panel B). In concordance with the studies that shown beneficial effects of DLE in some autoimmune diseases like thrombocytopenic

purple[14] and allergies; the DLE also show beneficial effects in different kinds of cancer, for example breast[15] and prostatic cancer[16]. In this work non-treated animal groups showed significantly more inflammatory infiltrate, compared to that of groups treated with dexamethasone (1.9 cell for HPF) and DLE (Figure 1 and 2). *t* Student analysis showed significant differences between experimental and control groups (p=0.05).

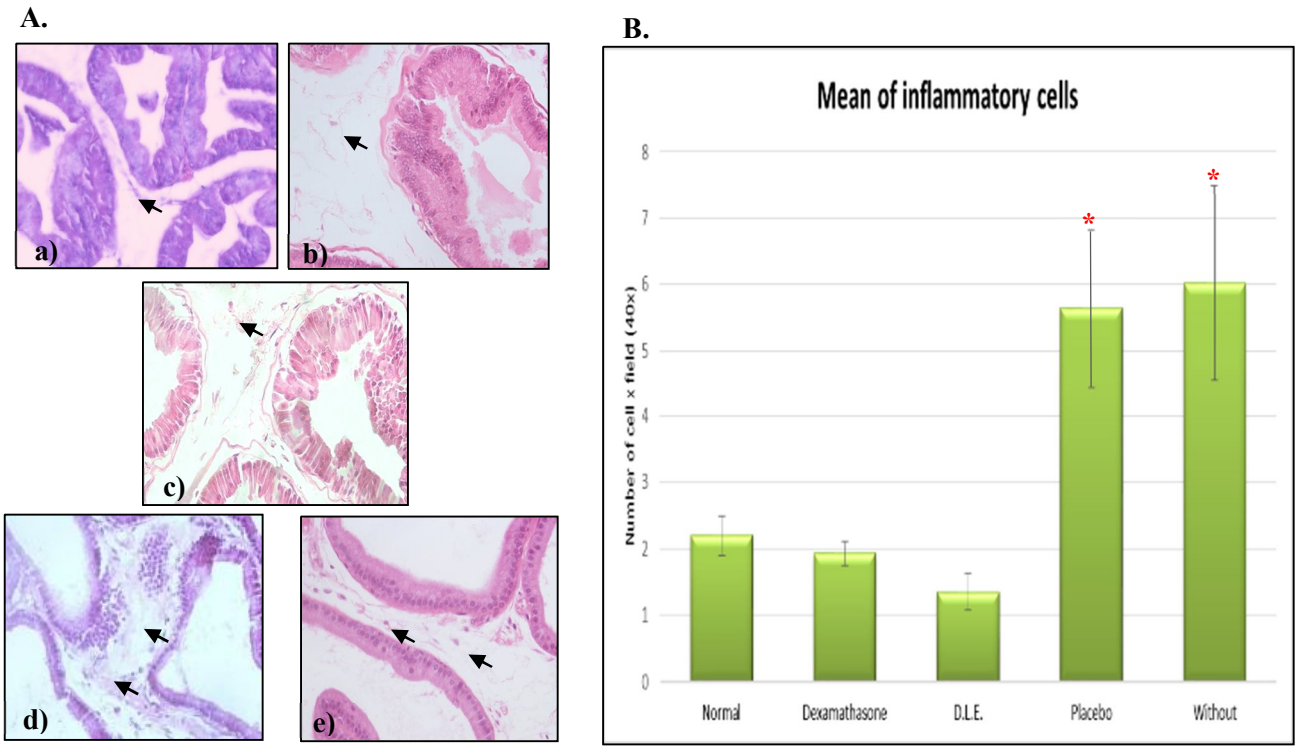

Figure 1: A. Hematoxiline-eosine staining. The arrows indicate inflammatory cells. a) Normal group, b) Dexamethasone group, c) DLE group, d) Placebo group and e) non-treated group, B. Graphic of the mean of inflammatory cell for high- power field in each treated group. * Staditically significantly differences (p=0.05).

On the other hand, we also observed an increment of mast cell in the non-treated group (2.9 cells for HPF). This is consistent with findings of other authors like Rivero *et al*.[5] in 1997 **(Figure 2. Panel A)** The mast cell is considered an integrator or amplifier of autoimmune responses, it has been involved in the breakdown of immune tolerance and/or autoimmune activation and recognition of different tissue, like prostatic tissue[17]. Evidences indicate that mast cells are activated via the complement pathway but are known to be activated also directly by T cells[18]. Activate mast cell secrete pro-inflammatory and nociceptive mediators that include histamine, cytokines and proteolytic enzymes, promoting the perpetuation of the inflammatory microenvironment and pelvic pain[19]. For example, in CPPS is observed that the synthesis of Neural Grown Factor (NGF) correlated with the presence of mast cell in prostatic nerves, linking mast cells to chronic pain[6, 18, 20.] Moreover the amount of mast cell in non- treated group was similar to 2.9 mast cells for HPF observed by Papadoukakis *et al.* [21], who found benign prostatic hyperplasia, reinforcing our observation that prostatic rat tissue showed inflammation and prostatic hyperplasia[21]. Mast cells have also been linked to prostate cancer; evidences indicate that those cells could regulate angiogenesis, tissue remodeling, and immunomodulation in human and murine cancer; however mores investigations are necessary[17].

In the DLE group the amount of mast cells (1.93 cell for HPF) decrease significantly compared with the amount of cells presented in non- treated group (2.9 cell for HPF) and in the group treated with placebo (4.4 cell for HPF) (Figure 2. Panel B). Furthermore DLE group did not show statistically significant differences with dexamethasone group (1.8 cell for HPF) (Figure 2. Panel B). Besides DLE and dexamethasone groups did not show statistically significant differences with the normal group **(Figure 2. Panel B).** In contrast, in the placebo group was observed more inflammatory cells that the others groups. The effect of DLE could modify the perpetuation of inflammatory process in prostatic tissue, suggesting that it could induce the return to the autoimmune tolerance or the reduction of autoimmune activation.

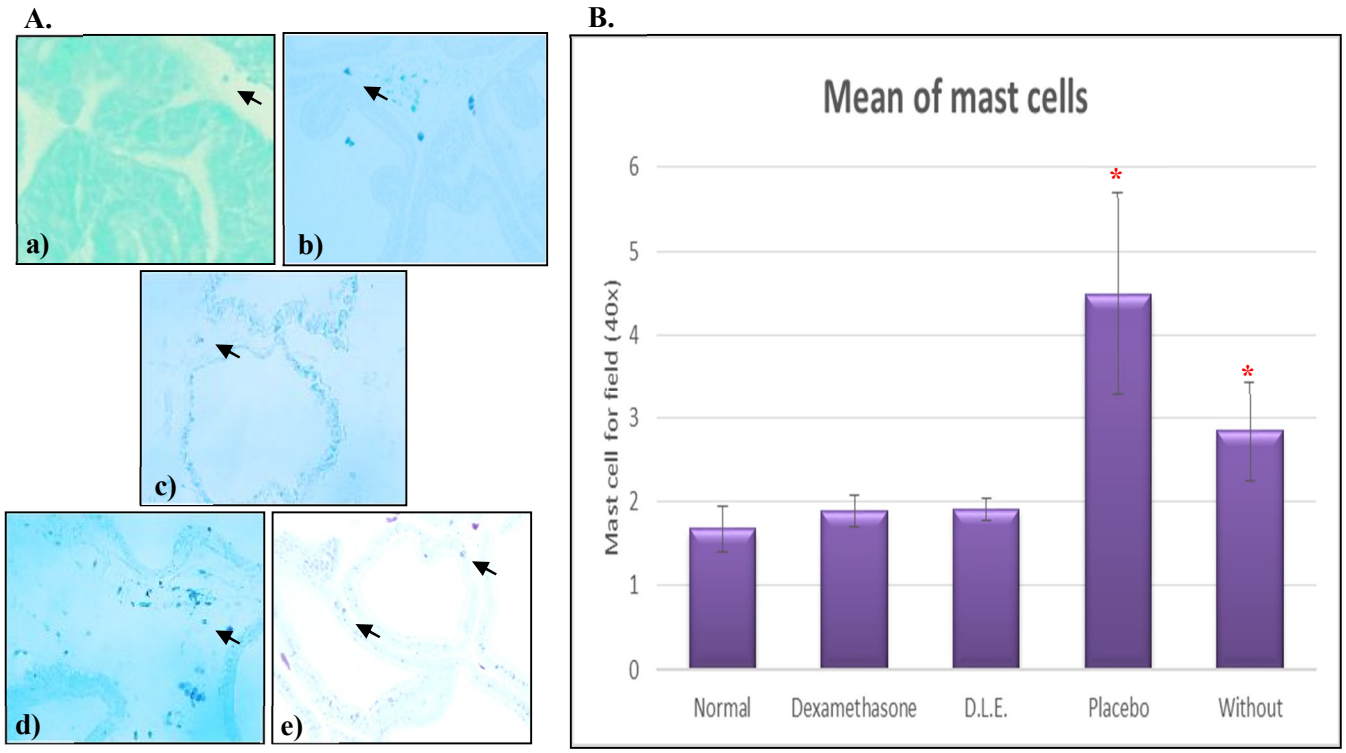

Figure 2: A. Toloudine blue stainning. The arrows indicate inflammatory cells. a) Normal group, b) Dexamethasone group, c) DLE group, d) Placebo group and e) non-treated group, B. Graphic of the mean of mast cell for high- power field in each treated group. * Staditically significantly differences (p=0.05).

CONCLUSIONS

The method of EAP developed in this work presented signs of prostatic inflammation and moreover BPH.

It presents a notorious infiltrate of inflammatory cells, particularly mast cell. It was observed that DLE could decrease the signs of inflammation, it reduce the infiltrate of inflammatory cells, particularly of mast cells and diminish the hyperplasia process.

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