Lymphocytes from Peyer's Patches and Mesenteric Lymph Nodes Proliferation in a Model of Oral and Systemic Sensitization with Ovalbumin

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Abstract: *Background*: In previous works we demonstrated that subcutaneous sensitization with Ovalbumin (OVA) induce generation of specific IgE antibodies and modifications of immune cells populations in different mucosal sites in rabbit. The aim of the study was the evaluation of OVA specific lymphoproliferation in mesenteric lymph nodes (MLNs), spleen and Peyer's patch from OVA orally and subcutaneous sensitized and challenged rabbits.

Methods: New Zealand white rabbits were divided into six groups: G1 (n=8): orally sensitized and challenged with OVA; G2 (n=10): subcutaneous sensitized with OVA and oral challenged (OVA); G3 (n=10): subcutaneous sensitized and oral challenged with PBS (phosphate buffer saline). G4-G5 and G6 (n=9 each) controls. Four hours after challenge animals were sacrificed and obtained samples were processed for lymphoproliferation studies: isolated cells from MLNs, spleen and Peyer's patch from the different groups were suspended in culture media containing OVA or Concanavaline A and were cultured for 48, 72 and 120 hours. Proliferation was measured as incorporation of radioactive element as counts per minute (CPM).

Results: Spleen derived lymphocytes showed important proliferation at subcutaneous sensitized groups when incubated with OVA. Meanwhile, proliferation was significantly higher in lymphocytes originated in MLNs from subcutaneous OVA sensitized and oral challenged rabbits at 48, 72 and 120 hours of incubation. No significant proliferation was observed in Peyer's Patch derived lymphocytes.

Conclusions: We conclude that proliferation of OVA-specific spleen originated lymphocytes was successful after systemic sensitization but after oral challenge with the antigen, only OVA incubated MLNs-originated lymphocytes showed proliferation as compared with Peyer's patch. This fact evidence a main participation of MLNs in this model of food allergy.

Keywords: Rabbit, Mesenteric Lymph Nodes, Peyer's Patch, Ovalbumin, Sensitization.

INTRODUCTION

The mucosal system in association with Gut Associated Lymphoid Tissue (GALT), is the first line of defense against large quantity of different pathogens [1, 2]. It is continuously exposed to dietary and microbial antigens, and thus the host must maintain a homeostatic environment between commensal microbiota and pathogenic infections as well as the exclusion of potentially antigenic proteins from food [3, 4].

The GALT is mainly formed by peripheral lymphoid tissues, such as lymph nodes and Peyer's patches (PPs) that are organs required to develop a highly efficient immune responses to different kind of antigens (Ag). The compartmentalization of immune system supports the function of these tissues [5, 6].

Peyer's patches are located along the wall of the small intestine and their particular morphology is essential for the generation of the immune response [7]. Considered as an inductive site of GALT, we observed the dome with follicle-associated epithelium (FAE) with M cells, and dendritic cells (DC) beneath the subepithelial dome (SED), able to sample antigens from the intestine lumen [8, 9].

Lymph node is morphologically divided in superficial (outer) cortex, and deep (inner) cortex, medular zone (cordonal) and marginal, cortical and medullary lymphatic sinusis. The outer cortex is mainly a B cell area (lymph follicle and germinal center) meanwhile deep cortex is a T cell zone with abundant DCs and high endothelial venules (HEVs) [9].

The present data suggest that lymph nodes receive antigen from class II positive dendritic cells or drained from lymph vessels in a free form [10]. Besides, spleen monitors blood meanwhile GALT is strategically associated to mucosal surfaces [11].

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Rabbit is a useful model for allergy experimentation [12]. Lagomorphs have an important genetic preservation in humans and generate high levels of IgE after sensitization. Ovalbumin is soluble antigen that induces specific IgE sensitization after subcutaneous injection [13]. Oral challenge with this antigen in sensitized individuals induces an allergic anaphylactic response in digestive tract [14].

The aim of the study was the evaluation of OVA specific lymphoproliferation in Peripheral lymphoid tissues associated with GALT, mesenteric lymph nodes, spleen and Peyer's patch from orally OVA sensitized versus subcutaneous OVA sensitized and challenged rabbits.

MATERIAL AND METHODS

New Zealand white rabbits were divided into six groups: G1 (n=8): orally sensitized and challenged with OVA; G2 (n=10): subcutaneous sensitized with OVA and oral challenged (OVA); G3 (n=10): subcutaneous sensitized and oral challenged with PBS (phosphate buffer saline). G4-G5 and G6 (n=9 each) controls. G4: orally sensitized with OVA and PBS Challenged: G5: Non sensitized and OVA challenged and G6 normal non sensitized / non challenged rabbits [15].

Rabbits from group 2 and 3 were twice subcutaneously sensitized with 70 µg OVA in 30 mg ALUM/ml (aluminium hydroxide). An interval of 15 days among sensitizations were developed. After 15 days post sensitization, rabbits were 24 hours fasten and G1 and G2 animals were oral challenged with 50 mg OVA in 5 ml of phosphate buffer saline (PBS) [15].

Four hours after challenge animals were sacrificed, according to considerations of Ethical Committee of Rosario School of Medicine and obtained samples were processed for lymphoproliferation studies: isolated cells from MLNs, spleen and Peyer's patch from the different groups were suspended in culture media containing OVA or Concanavaline A and were cultured for 48, 72 and 120 hours. Proliferation was measured as incorporation of radioactive element as counts per minute (CPM). Specific anti-OVA IgE titres were evaluated by passive cutaneous anaphylaxis (PCA) [16].

RESULTS

Passive cutaneous anaphylaxis showed specific anti OVA IgE in sensitized groups (G2 and G3) at 1/160 dilution. Histopathology showed patchy distribution of mucosal oedema, lymphangiectasy and eosinophils infiltration in sensitized and challenged groups (G3) (Figure **1**). Eosinophils infiltrate mucosa near surface epithelium. No changes could be detected in control group (G1, G4, G5 and G6) and sensitized but not challenged group (G2).

Results are shown in graphics. Spleen derived lymphocytes showed important proliferation at subcutaneous sensitized groups when incubated with OVA (Figure **2**). Meanwhile, proliferation was significantly higher in lymphocytes originated in MLNs from subcutaneous OVA sensitized and oral challenged rabbits at 48, 72 and 120 hours of incubation (Figure **3**). No significant proliferation was observed in Peyer's Patch derived lymphocytes.

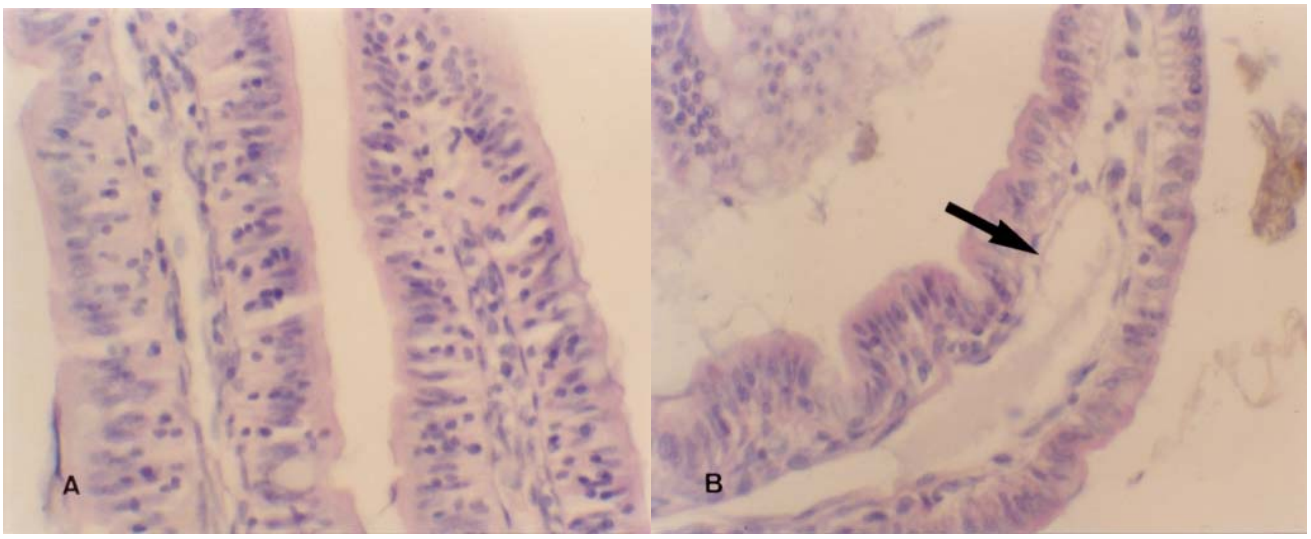

Figure 1: A. Normal intestinal villi. **B**. Oedema and lymphangiectasy after oral OVA challenge (G2).

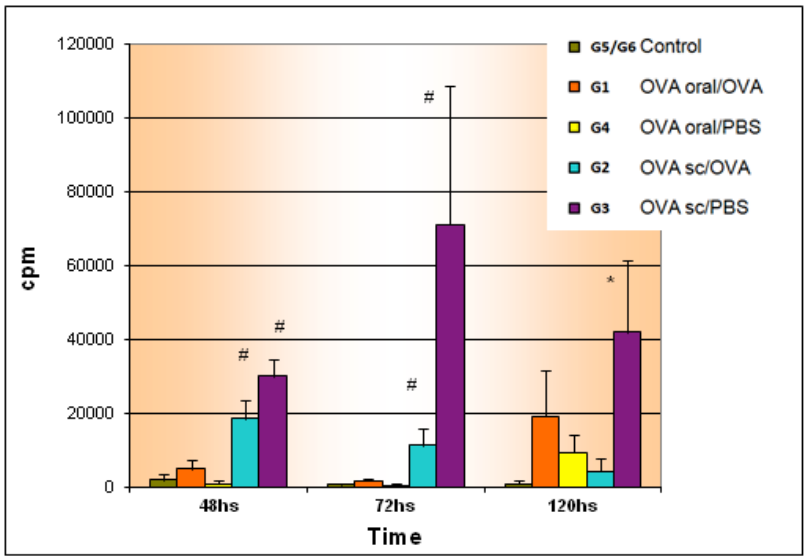

Figure 2: Blastogenesis of splenic cells with OVA. Mean SE. cpm (count per minute). # p<0,03, *p<0,05.

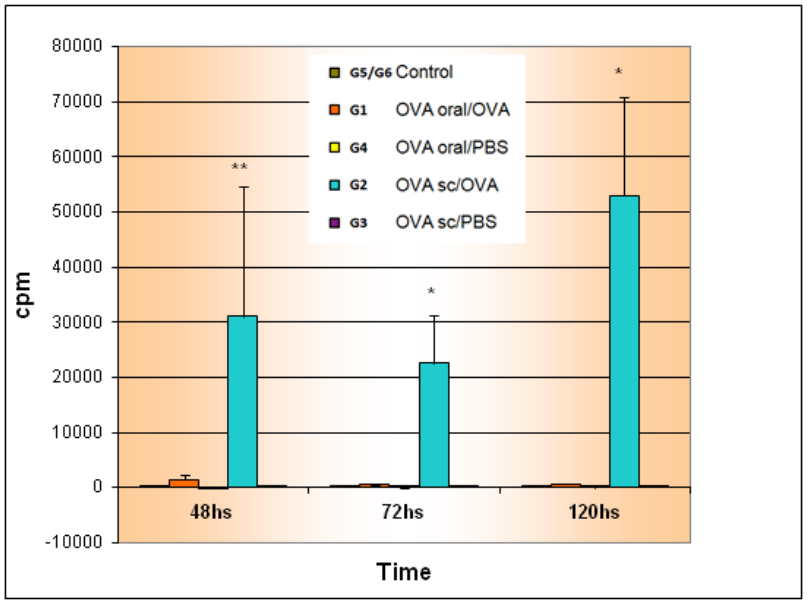

Figure 3: Blastogenesis of MLN's cells with OVA. Mean SE. cpm (count per minute). ** p<0,01, * p<0,05.

DISCUSSION

Ovalbumin is a soluble antigen which, when subcutaneously administered, induces sensitization that elicits specific IgE antibodies. Challenge with OVA in sensitized individuals produces an anaphylactic allergic response in different mucosal sites. In previous works we found modifications in number of lymphatic cells, mast cells and eosinophils in gastrointestinal tract from OVA-sensitized and challenged rabbit. These changes associated to high levels of specific anti-OVA IgE, indicated an immediate hypersensitivity reaction [17].

Maximal histopathological expression in gut mucosa developed 4 hours after challenge. Vasoactive and pro-inflammatory mediators induced mucosal oedema, lynphangiectasy and eosinophil infiltration. This finding is similar to that found by other authors and our group in previous works in different regions of digestive tract from rabbit [15, 16, 18].

One possible explanation for the absence of response at PP in the experimental model could be due to the soluble property of ovalbumin as an antigen. Many authors describes that PP mainly deals with particulate antigens as compared with soluble type such as OVA [19].

We conclude that proliferation of OVA-specific spleen originated lymphocytes was successful after systemic sensitization but after oral challenge with the antigen, only OVA incubated MLNs-originated lymphocytes showed proliferation as compared with Peyer's patch. This phenomena evidence a potential migration of the antigen from intestinal lamina propria to mesenteric lymph nodes. All data suggest a main participation of MLNs in this model of food allergy.

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