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Effect of Oral and Parenteral Sensitization to Bovine β-Lactoglobulin on Intestinal Structure in Rats

Nabila Mehedi, Hanane Kaddouri, Djamel Saidi and Omar Kheroua *

ABSTRACT

Wistar rats were sensitized by intradermal administration of β-Lactoglobulin (β-Lg). The sensitization was boosted once again after two weeks. The oral sensitization was monitored by drinking fresh cow milk for 21 d. Both types of sensitization led to high IgG titers against β-Lg**,** especially in parenterally immunized animals. Histological examination of intestinal tissue revealed a marked decrease in villus length in sensitized rats by the parenteral route. An increase in the number of intraepithelial lymphocytes and goblet cells was observed in both groups of sensitized rats. The results indicate that chronic exposure to cow's milk proteins leads to intestinal injury.

Keywords: Cow's milk allergy, Wistar rat, Intraepithelial lymphocytes, Villus length.

Abbreviations used:

CMA : Cow's Milk Allergy
IELs : Intraepithelial Lymphocytes
β-Lg : β-Lactoglobulin
CMP : Cow Milk Proteins

1. INTRODUCTION

Cow's Milk Allergy (CMA) is one of the major causes of food hypersensitivity in children. Approximately, 2.5% of infants exhibit cow's milk hypersensitivity in their first year of life (Host, 1997; Paupe, 1997). CMA is associated with a broad spectrum of IgE-mediated and non-IgE-mediated hypersensitivity disorders circulating immune complexes and effector cells (Olives and Breton, 1998; Moneret-Vautrin, 1999; Sanz Ortega et al., 2001; Shek et al., 2005). The mechanisms involved in this pathology are difficult to elucidate in children. Several experimental models of allergy (guinea pig, rat, mouse, rabbit) have shown that oral or parenteral administration of Cow's Milk Proteins (CMP) generates IgG and IgE antibodies (Ju et al., 1995; Kitagawa et al., 1995; Li et al., 1999; Knippels et al., 2000; Adel-Patient et al., 2003) that are of a similar specificity to those produced by humans (Varaala et al., 1995; Sampson, 1997; Jenmalm and Bjorkstén, 1998). Findings have demonstrated that mediators released by mast cells and other immune cells in allergy affect epithelial function. These mediators increase transcellular and paracellular antigen uptake (Scudamore et al., 1995; Berin et al., 1997; Marano et al., 1998; Sicherer et al., 1998) and chloride secretion (Barrett, 1991; Perdue and MacKay, 1994; Oprins et al., 2000). Most facets of enterocyte function can be regulated by cytokines, including ion transport, permeability and restitution and proliferation (Chang et al., 1990; Dignass and Podolsky, 1993). Few data demonstrated the CMP effect on the intestinal epithelium. The aim of the present study was to compare the effect of being exposed to β-Lactoglobulin in CMP by oral and parenteral route on intestinal morphology and intraepithelial lymphocyte counts in a rat model of CMA.

2. MATERIALS AND METHODS

Rats

Six week-old female Wistar rats were obtained from

* Laboratoire de Physiologie de la Nutrition et de Sécurité Alimentaire, Département de Biologie, Faculté des Sciences, Université d'Oran Es-Sénia, Algier. Received on 16/8/2005 and Accepted for Publication on 30/7/2006.

IFA Credo (Dijon, France), housed individually and allowed their diets (commercial pellets: ONAB, Algeria) and water ad libitum. The animal room was kept at 20-24 C° with a 12 h light-dark cycle.

Experimental Protocol

Oral Immunization

Six animals were sensitized to cow's milk protein by giving them fresh milk to drink for 21 d. They were returned to drinking water 3-4 d before the experiments. Control rats received only water. The rats were killed on day 25.

Parenteral Immunization

Six rats were sensitized on day 0 with a subcutaneous injection in the back. Each rat was injected with 100 $\mu g.ml^{-1}$ of β-Lg (Sigma Chemicals Co., St. Louis, MO. USA) emulsified with 250 µL complete Freund's adjuvant (Sigma Chemicals Co., St. Louis, MO. USA) and 50 µL of saline. On day 14, each rat was boosted with the same dose of protein, incomplete Freund's adjuvant and saline.

Antibody Titer Measurements

IgG anti β-Lg were assayed in serum samples by an Enzyme-Linked Immunosorbent Assay (ELISA). The protocol was similar to that described by Heyman et al. (1990).

Histology

Rats were anesthetized by intraperitoneal injection of 10 % chloral, and a 15 cm segment of jejunum was excised (beginning 5 cm distal to ligament of Treiz). One of the paired pieces of jejunum from each animal was challenged with β-Lg (final concentration: 100 $\mu g.ml^{-1}$) added to the bathing Ringer solution (140 $mmol.L^{-1}$ Na^{+}, 120 $mmol.L^{-1}$ Cl^{-}, 5.2 $mmol.L^{-1}$ K^{+}, 1.2 $mmol.L^{-1}$ Ca^{++}, 1.2 $mmol.L^{-1}$ Mg^{++}, 25 $mmol.L^{-1}$ HCO_3^{-}, 2.4 $mmol.L^{-1}$ HPO_4^{-}, 0.4 $mmol.L^{-1}$ $H_2PO_4^{-}$) at 37c°. Fifteen minutes later, the tissues were fixed for histological examination. As a control, companion jejunal segments from the same animal were also incubated in Ringer for the same time and not challenged with antigen before fixation. All pieces of jejunum were fixed in formalin at 10% for 24-48 h. Sections were stained with haematoxylin and eosin.

Length of Villi

The measurements of length of villi were made using a micrometer eyepiece. Villus length was expressed in µm.

Intraepithelial Lymphocyte Counts

A differential cell count was made under oil immersion (x100 magnification) of the cells within the epithelium covering the villi, and intraepithelial lymphocytes counts were expressed as the number of intraepithelial lymphocytes/100 epithelial cells.

Statistical Analysis

The results were analyzed by Student t test and are presented as mean values ± SE. A p value <0.05 was considered significant.

3. RESULTS

IgG Anti β-Lg Titer

Immunization by β-Lg was evaluated by measuring the titer of the IgG antibodies to β-Lg in sensitized rats and controls. As shown in Figure (1), antibodies were higher in the sensitized animals than in the control. However, parenterally sensitized rats had significantly higher titer than orally sensitized animals ($p< 0.01$).

Sensibilisation Effect on Intestinal Structure

General Effect

Intestinal morphology was examined by light microscopy. The morphological appearance of sections from control animals was normal. Jejunal villus appeared long and fine with an unistratified epithelium. The villus epithelium was predominantly populated by columnar enterocytes and a smaller number of goblet cells that showed no evidence of secretion. The aspect of lamina propria was fibrous and appeared polymorphous containing several immune cells (Fig.2-E). We could distinguish in the immunized rats a shorter villus and an epithelium pseudostratification. Histologic examination showed separation of the epithelium cell layer from the lamina propria in some immunized rats. All experimental groups had high IEL counts and goblet cells (Fig.2).

Effect on Villus Length

The mean value of villus length determined from sections of jejunum from control and immunized rats are shown in Figure (3). Length of villi measured in control rats was 64.49 ± 1.6 µm. It was significantly reduced in the rats immunized to β-Lg by parenteral route (47.36 ± 1.51 µm, $p<0.0001$) and in the rats immunized to cow's milk proteins by oral route (53.99 ± 2.6 µm, $p<0.05$).

Effect on IELs Count

The mean value of IELs from control rats was 16.67 ± 1.63 lymphocytes/100 epithelial cells. The number of IELs has significantly increased in rats immunized to cow's milk proteins by oral route (29.52 ± 1.42 lymphocytes/100 epithelial cells (p<0.01)) and those immunized to β-Lg by parenteral route (37.43 ± 1.86 lymphocytes/100 epithelial cells (p<0.01) (Fig.4). However, no difference in the number of IELs between the two immunization route was found (p=0.22).

Challenge Effect of β-Lg on IELs Number

The IELs number of jejunal fragments incubated with β-Lg for 15 min was 32.52 ± 1.78 lymphocytes/100 epithelial cells compared to 29.52 ± 1.42 lymphocytes/100 epithelial cells of jejunal fragments not challenged with β-Lg (p=0.07). The same results were obtained in rats immunized to β-Lg by parenteral route. The IELs number of jejunal fragments incubated with β-Lg for 15 min was 34.22 ± 1.32 lymphocytes/100 epithelial cells compared to 37.43 ± 1.86 lymphocytes/100 epithelial cells of jejunal fragments not challenged with β-Lg (p=0.08). IELs number remained unchanged in all β-Lg challenged fragments (Fig.5).

4. DISCUSSION

The effect of chronic dietary antigen challenge on the intestine was examined in sensitized animals. Parenteral administration of cow's milk was followed rapidly by extensive intestinal changes compared to oral administration. These results concomit with IgG anti-β-Lg production. These findings were similar to data from experimental CMA (Ju et al., 1995; Li et al., 1999; Knippels et al., 2000). Patients with IgE-mediated CMA have an elevated poly-isotypic response to cow milk proteins (Shek et al., 2005). A partial atrophy was shown in the group of rats immunized by parenteral route. The injury appeared to begin with epithelial separation from the core of the lamina propria. A marked vascular congestion and edema of lamina propria were observed in both groups of immunized rats.These abnormalities were not seen in control rats. The partial atrophy was noticed in non-IgE-mediated hypersensitivity in children presenting a cow's milk enteropathy (Olives and Breton, 1998; Moneret-Vautrin, 1999). The intestinal mucosa reproduced the same marks found in coeliac disease but they were less severe (Lake et al., 1991; Navarro et al., 1993). We have found an important increase in IELs and lymphocytes counts in lamina propria from rats immunized to cow's milk proteins by oral route and from those immunized to β-Lg by parenteral route. These cells give a stratified aspect of intestinal epithelium. An increased IELs was shown in mice immunized to ovalbumin (Mowat and Ferguson, 1981), in cow's milk enteropathy (Hankard et al., 1997) and in some gastrointestinal diseases (Jarry et al., 1990). During delayed hypersensitivity, the IELs and mucosal T lymphocytes contribute to cell mediated immune response (Olives and Breton, 1998; Mauneret-Vautrin, 1999). IELs are the first immune cells exposed to luminal antigens and they participate in inflammatory reactions (Tlaskalova-Hogenova et al., 1995). Studies on coeliac disease have shown that T cells activation led to villus atrophy (Marsh and Crowe, 1995). IELs participated closely in the preservation of intestinal integrity (Cerf-Bensussan and Guy Grand, 1991). These different experimental models indicated that T cells activation can be responsible for histologic injury in food allergy. We have noted a strong increase of goblet cells in rats immunized by oral and parenteral route to cow's milk proteins. Mucine production by goblet cells has been found to increase in intestinal anaphylaxis (Lake et al., 1991) and in food IgE-dependant in rats (Sakamoto et al., 1998) and in mice (Adel-Patient et al., 2003). The role of the rapidly secreted mucus during intestinal anaphylaxis is possibly to play as a barrier limiting antigen access from the lumen to sensitized mast cells, which is likely to occur with dietary antigens. Histamine and cytokines released in inflammatory reaction lead to an increase in mucus production. The interaction of mucosal lymphocytes and intestinal epithelial cells is thought to be important in regulating immune response in the intestinal mucosa.

5. CONCLUSION

We demonstrated that sensitization of rats to cow's milk proteins by different routes can lead to epithelial injury in the intestine associated with an increase in IELs and goblet cells. The morphological changes were most important in the parenteral route. These data may help to evaluate the mucosal immunopathogenic mechanisms involved in CMA.

Acknowledgements

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Fig.1. Antibodies to β-Lg detected by ELISA in rats sensitized to cow's milk proteins by oral route (OR) and to β-Lg by parenteral route (PR) and control rat (C). Sera from different groups of rats (n=6) as indicated were obtained at day animals sacrifice. Values represent mean ± SE. $^{**}p<0.01$ in parenterally sensitized animals compared with orally sensitized animals.

Fig.2. Histologic examination of jejunum from immunized and control rats stained with HE. **A** Jejunal fragment of rats sensitized to cow's milk proteins by oral route, edema appeared, an increase in lymphocyte infiltration and goblet cells (x40). **B** An increase of LIEs and goblet cells were observed in jejunal fragment of rats sensitized to β-Lg by parenteral route and (x25). **C** Jejunal fragment of rats sensitized to cow's milk proteins by oral route and challenged with β-Lg (x40). **D** Jejunal fragment of rats sensitized to β-Lg by parenteral route and challenged with β-Lg (x40), a partial atrophy was observed and no significant changes in IELs number was noticed 15 min after β-Lg challenged tissues. **E** Villi and epithelium appear normal in control rats (x40).

Fig.3. Effect of sensitization on villus length. Jejunal fragments from different groups of rats (n=6) as indicated were obtained from sensitized and control rats. Oral no β-Lg challenged (ONC), Parenteral no β-Lg challenged (PNC) and control no β-Lg challenged (CNC).
Data are based on examination of 6 villus units per slide and are expressed as mean ± SE. $^{*}p<0.05$ and $^{***}p<0.0001$ compared with control.

Fig.4. Effect of sensitization on LIEs numbers. Jejunal fragments from different groups of rats (n=6) as indicated were obtained from sensitized and control rats. Oral no β-Lg challenged (ONC), Parenteral no β-Lg challenged (PNC) and control no β-Lg challenged (CNC).
Values represent mean ± SE derived from examination of 4 segments of each animal.** $p<0.01$ and * $p<0.01$ compared with control.

Fig.5. Effect of β-Lg challenged tissues on IELs numbers. Jejunal fragments from different groups of rats (n=6) as indicated were obtained from sensitized and control rats. All experiments were made with paired preparations, one of these were exposed to β-Lg during 15 minutes. Cell counts were performed on 4 segments of each animal. Oral no β-Lg challenged (ONC), Oral β-Lg challenged (OC), Parenteral no β-Lg challenged (PNC), Parenteral β-Lg challenged (PC), control no β-Lg challenged (CNC) and control β-Lg challenged (CC).
Values are expressed as means ± SE.

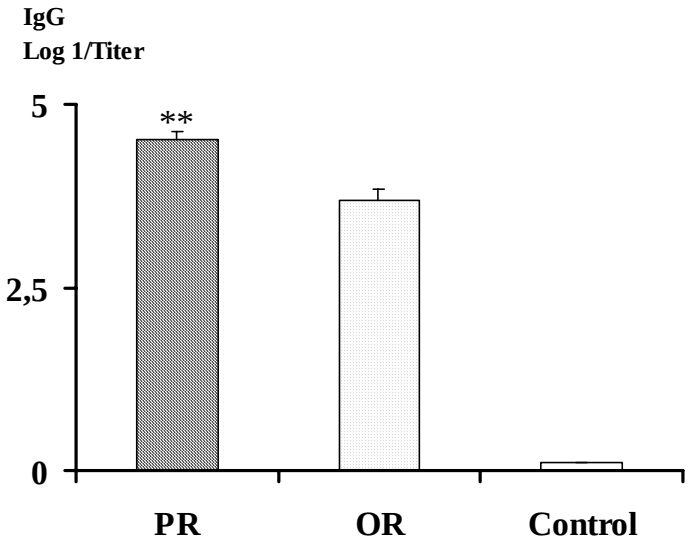

Fig. 1

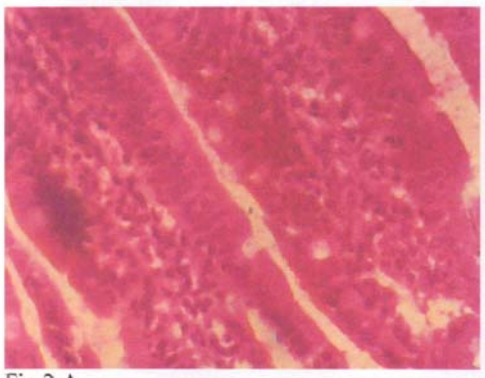

Fig.2 A

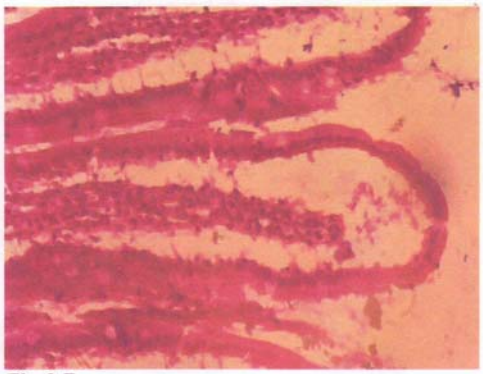

Fig.2 B

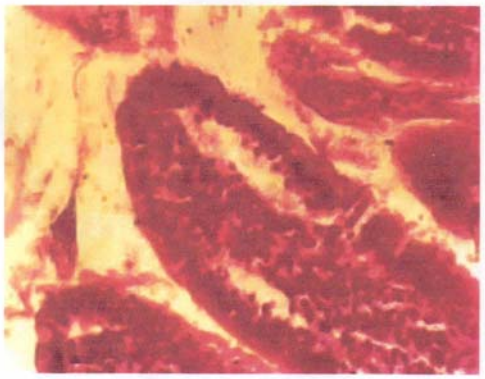

Fig.2 C

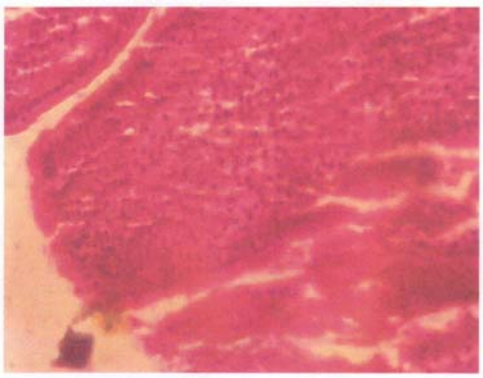

Fig.2 D

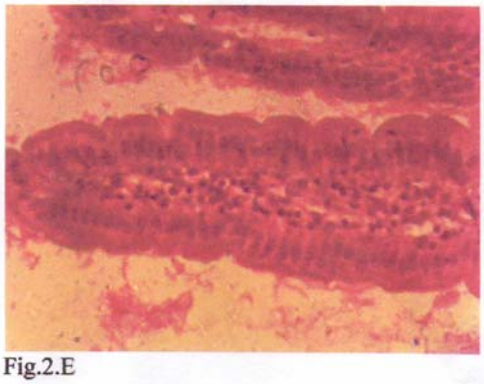

Fig.2.E

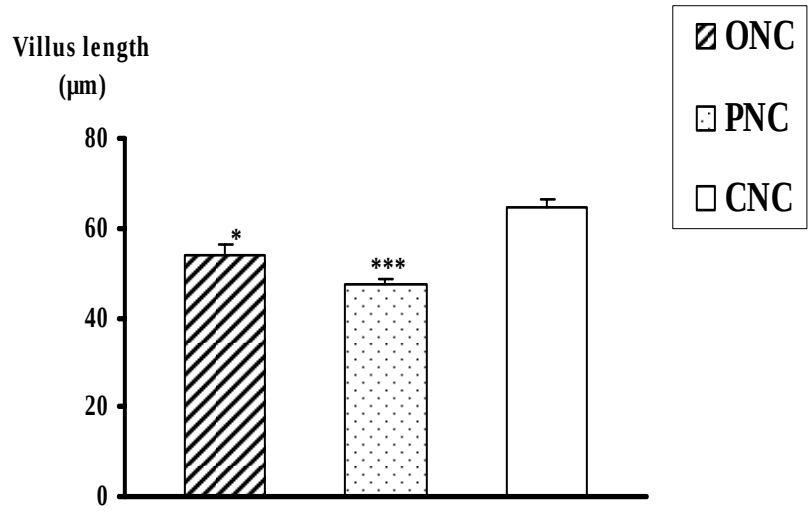
Villus length
(µm)
80
60
40
20
0
*
***
ONC
PNC
CNC

Fig. 3

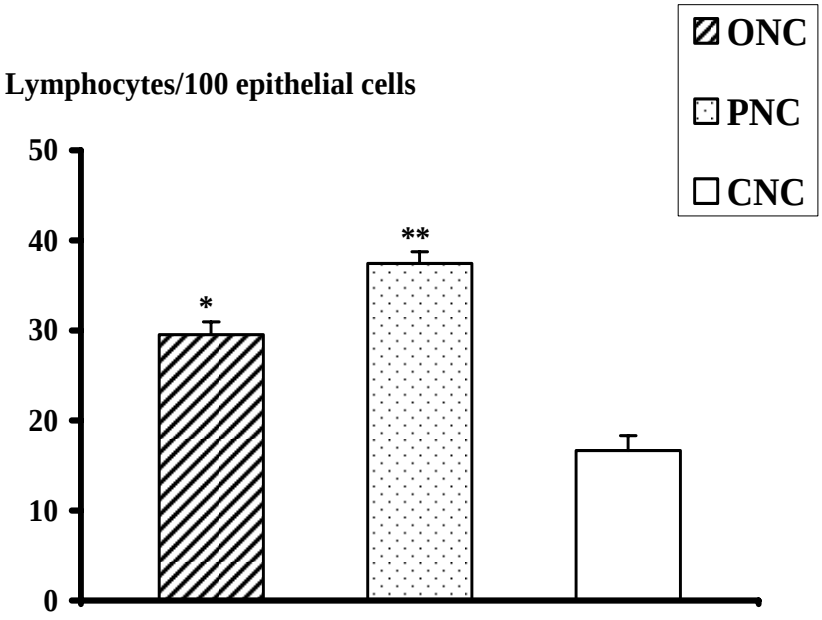
Lymphocytes/100 epithelial cells
50
40
30
20
10
0
*
**
ONC
PNC
CNC

Fig. 4

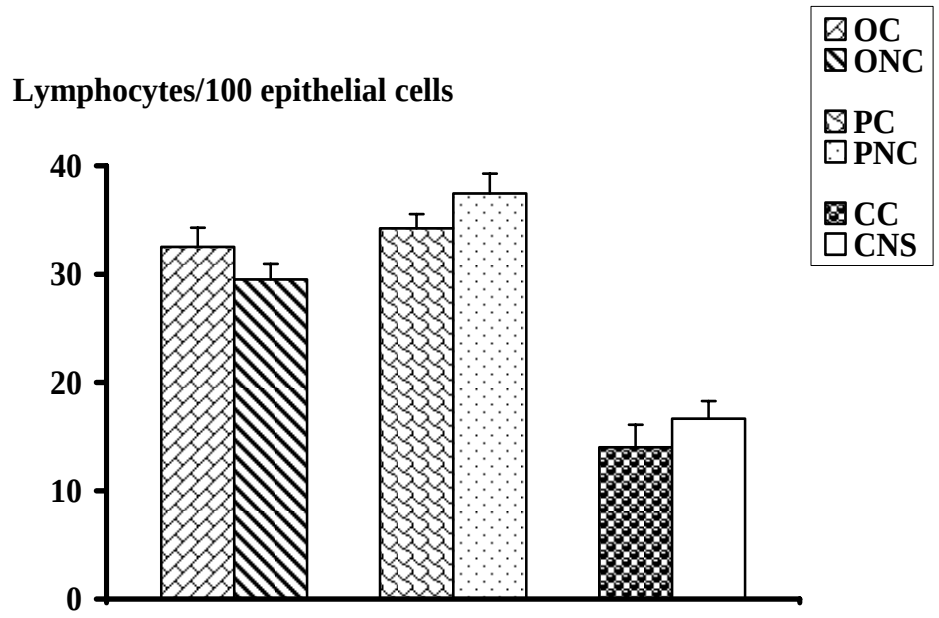

Fig. 5

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تأثير حساسية البيتاجلوبلين بروتين حليب البقر على شكل مخاطية أمعاء الجرذان

نبيلة مهيدي، حنان قدوري، جمال سعيدي، وعمر خروع *

ملخـص

تم حقن جرذان من نوع Wistar عن طريق الجلد بواسطة بروتين حليب β-Lg (β-Lactoglobulin)، وأعيدت العملية مرة بعد أسبوعين، وتم تحسيس مجموعة أخرى من الجرذان منعت عن طريق شرب حليب البقر الطازج لمدة 21 يوماً.أظهرت نتائج تقدير الأجسام المضادة المصلية النوعية IgG anti β-Lg أن هناك تماثلا بين المجموعتين في الاستجابة المناعية، وتم تقييم تأثير الحساسية على هيئة الخلايا المخاطية المعوية وتركيبها، وتبين أن هناك نقصا في أطوال الخملات المعوية عند الحيوانات المحسسة بـ β-Lg عن طريق الجلد وزيادة في أعداد الخلايا المناعية من نوع الخلايا اللمفاوية بين الطلائية (Intraepithelial lymphocytes) والخلايا الكاسية (Goblet cells) عند الحيوانات المحسسة.

وخلصت النتائج إلى أن تعرض الجرذان إلى بروتينات حليب البقر يؤدي إلى خلل في الطبقات الطلائية للأمعاء.

الكلمات الدالة : الحساسية لحليب البقر، جرذان، خلايا لمفاوية بين طلائية، اطوال الخملات.

* قسم البيولوجيا، كلية العلوم، جامعة وهران، الجزائر. تاريخ استلام البحث 2005/8/16، وتاريخ قبوله 2006/7/30.