

# B cells are involved in the modulation of pathogenic gut immune response in food-allergic enteropathy

C. R. Cardoso,\* P. R. Provinciatto,\*  
D. F. Godoi,<sup>†</sup> T. S. Vieira,\*  
B. R. Ferreira,\* G. Teixeira,<sup>‡</sup>  
M. A. Rossi,<sup>§</sup> F. Q. Cunha<sup>¶</sup> and  
J. S. Silva\*

\*Department of Biochemistry and Immunology,

<sup>†</sup>Internal Medicine, <sup>§</sup>Pathology and

<sup>¶</sup>Pharmacology, School of Medicine of Ribeirão

Preto, University of São Paulo, Ribeirão Preto, SP,

and <sup>‡</sup>Biology Institute, Fluminense Federal

University, Niterói, RJ, Brazil

Accepted for publication 11 July 2008

Correspondence: J. S. Silva, School of Medicine  
of Ribeirão Preto – USP – Department of Bio-  
chemistry and Immunology, Av. Bandeirantes  
3900 – 14049-900 Ribeirão Preto, SP, Brazil.

E-mail: jsdsilva@fmrp.usp.br

## Summary

Food enteropathies involve uncontrolled or hypersensitivity reactions to ingested nutrients and may result in IgE and T-helper type 2 (Th2) responses as in food allergy. However, the precise role of B cells in the development of food enteropathies remains uncertain. In this work, we used B cell-deficient mice (B KO) and a model of peanut sensitization to examine the involvement of B lymphocytes in the pathogenesis of food allergy. Results showed that priming of wild-type (WT) mice with peanut proteins induced specific IgG1 and IgE responses in serum, with edema, tissue destruction, epithelial exulceration and inflammatory infiltrate in the gut of sensitized and challenged (*S + Peanut*) WT animals. In contrast, there was no sera immunoglobulin detection and absence of tissue destruction in the gut of B KO mice, which presented moderate inflammatory infiltrate and villous enlargement after peanut challenge. These animals presented marked decrease in IL-4 and TNF- $\alpha$  and high levels of IL-10, TGF- $\beta$ , IL-12p40 and IFN- $\gamma$  mRNA in the gut. Moreover, the expression of CCL5, CCL11 and CXCL1 was reduced in the gut of B KO mice, in contrast to elevated messages of CCL2 or similar detection of Th1-related chemokines in *S + Peanut* WT mice. Finally, we provided evidence that B cells are necessary to the development of food-related enteropathies and induction of gut inflammation during allergic reactions to food.

**Keywords:** B cell-deficient, enteropathy, food allergy, gut inflammation, immune response

## Introduction

Food enteropathies involve a state of uncontrolled or hypersensitivity responses to ingested nutrients. The mechanism underlying the initiation and development of allergic responses is not completely clear but it is known that the physiological penetration of dietary macromolecules into the organism through the gut mucosa has several recognized immunological consequences like food allergy, which is defined as an adverse immunological reaction to food. Allergy to dietary nutrients may result in a large number of clinical presentations in the skin, circulatory system, and the gastrointestinal tract, and can lead to severe, potentially life-threatening anaphylaxis [1–3]. In addition to other atopic diseases, the incidence and prevalence of food allergy are increasing [4,5] and fatal anaphylactic reactions induced by peanut seeds, one of the most severe food allergens, are frequently observed [6,7].

Responses generated to orally administered antigens have local and systemic features that distinguish them from other immunological reactions [8] and breakdown of mucosal tolerance to fed antigens can lead to the development of enteropathies [9] that require interactions between non-degraded proteins and gut-associated lymphoid tissue. The resulting active immunity against such non-pathogenic matters can lead to hypersensitivity responses and inflammatory disorders with subsequent allergic reaction [10,11], participation of IgE-producing B cells in response to activated T-helper type 2 (Th2) effector lymphocytes and cytokines like IL-4 or IL-13 [9].

B cells far outnumber all other lymphoid cells in the Peyer's patches [12]. They might therefore serve as a specialized set of antigen-presenting cells (APCs) able to induce tolerance or to drive particular immune responses. Although it is largely known that food allergy may involve systemic immunoglobulin responses in humans and

animals, the precise role of B cell producing antibodies in the pathogenesis of the allergic reaction to fed antigens is still uncertain. Most importantly, the B cell dependent mechanisms by which the mucosal immune system responds to food allergens in the gut remain unclear.

Accordingly, in this work, we used B cell-deficient mice and a murine model of peanut sensitization described previously by our group [9] to examine the role played by B lymphocytes in the development of food allergy. Lastly, this study provided evidence that B cells are necessary for the induction of gut mucosal alterations and inflammatory response in the onset of allergic enteropathy.

## Materials and methods

### Mice sensitization and induction of food enteropathy

For the induction of food enteropathy, C57BL/6 wild-type (WT) and B KO mice (Igh-6<sup>tm1Cgn</sup>), obtained from Jackson Laboratories (Bar Harbor, ME, USA), were age (6–8 weeks old) and gender matched ( $n = 5$  animals/group) and sensitized twice, 3 weeks apart, with 100 µg of peanut proteins extract (PPE) [9] in the presence (1st sensitization) or absence (2nd sensitization) of 1 mg of aluminum hydroxide by subcutaneous injection. One week after the last sensitization, mice were fed peanut seeds *ad libitum* for 30 days uninterruptedly, in the absence of the conventional chow (S + Peanut group). Control mice were divided as follows: the NS (non-sensitized) group, which received the first sensitization with PBS plus aluminum hydroxide, followed by a second sensitization with PBS only and was not submitted to the diet containing peanut seeds; the Peanut group, which was submitted to the same protocol of sham-sensitization as the NS group, but the animals were fed peanut seeds for 30 days, beginning a week after the last sensitization; and the S group (sensitized), which was sensitized twice with PBS containing PPE but was not challenged with the peanut diet. Water was available during all of the experiment. All mice were anesthetized, bled and sacrificed on day 30 after introduction of the peanut diet. The experimental procedures followed the guidelines of the Brazilian Council for Use of Animals in Research.

### Detection of PPE-specific immunoglobulin production

The PPE-specific IgG, IgG1, IgG2a and IgE serum levels were measured by ELISA on 96-well polystyrene plates (Corning, New York Costar, Acton, MA, USA) coated with PPE (20 µg/well). The reactions were performed with anti-IgG, anti-IgG1, anti-IgG2a or anti-IgE biotinylated antibodies (Southern Biotechnology Associates, Inc., Birmingham, AL, USA), followed by incubation with streptavidin-HRP-specific conjugates (Vector Laboratories, Inc., Burlingame, CA) and 3,3',5,5'-tetramethylbenzidine (TMB; KPL, Gaithersburg, MD, USA). Antibody levels in optimal sera dilution

(previously established, 1:100) were expressed as ELISA index (EI) [13], according to the formula:  $EI = \text{mean OD of the sample} / \text{cut off}$ , where the cut off is calculated as the OD mean values of negative controls sera plus three standard deviations. EI values > 1.0 were considered positive. All samples were analysed for a minimum of two to three times.

### Histopathological analysis

Small gut segments (jejunum) were collected, fixed in formalin solution and embedded in paraffin. Sections were stained with hematoxylin and eosin and inspected for the presence of mucosal inflammation.

### Transmission electron microscopy

For transmission electron microscopy (TEM) assays, jejunum segments were collected and immediately fixed in 4% sodium cacodylate buffered glutaraldehyde, pH 7.4 at 4°C for 24 h. The segments were post-fixed in 1% osmium tetroxide at 4°C for 1 h. Tissues were dehydrated in ascending concentrations of acetone and embedded in Araldite® 502 resin (Polysciences Inc., Warrington, PA, USA). Ultrathin sections were stained with uranyl acetate, lead citrate and examined in a Zeiss EM 109 electron microscope at an 80 kV accelerating voltage (Carl Zeiss, Oberkochen, Germany).

### Analysis of gene expression in the gut by real-time PCR

Total RNA from jejunum segments was extracted using the TRIZOL® reagent (Invitrogen™, Carlsbad, CA, USA) and Promega RNA extraction kit (Promega, Madison, WI, USA), according to the manufacturer's instructions. Complementary DNA (cDNA) was synthesized using 1 µg of RNA through a reverse transcription reaction (M-MLV reverse transcriptase, Promega). Real-time PCR analyses were performed on the ABI Prism 7000 Sequence Detection System using the SYBR-green fluorescence quantification system (Applied Biosystems, Warrington, UK). The standard PCR conditions were 95°C for 10 min, 40 cycles for 1 min at 94°C, 56°C (1 min), and 72°C (2 min), followed by the standard denaturation curve. The sequences of murine primers were designed using the Primer Express software (Applied Biosystems) using nucleotide sequences present in the GenBank data base and are depicted in Table 1. SYBR Green PCR Master Mix (Applied Biosystems), 0.1–0.2 µg/µl specific primers, and 2.5 ng of cDNA were used in each reaction. Threshold for positivity of real-time PCR was determined based on negative controls. The results were demonstrated as mRNA expression of the NS, Peanut, S and S + Peanut animals, relative to naïve non-sensitized, non-exposed to alumen and non-challenged mice, for both WT and B KO groups. Calculations to determine the relative level of gene expression were made according to the instructions from

**Table 1.** Primers sequences used in real-time PCR assays.

| Primers        |           | Sequences                     |
|----------------|-----------|-------------------------------|
| $\beta$ -actin | Sense     | AGC TGC GTT TTA CAC CCT TT    |
|                | Antisense | AAG CCA TGC CAA TGT TGT CT    |
| TGF- $\beta$   | Sense     | GCT GAA CCA AGG AGA CGG AAT   |
|                | Antisense | GCT GAT CCC GTT GAT TTC CA    |
| IL-10          | Sense     | TGG ACA ACA TAC TGC TAA CC    |
|                | Antisense | GGA TCA TTT CCG ATA AGG CT    |
| IL-4           | Sense     | CTG ACG GCA CAG AGC TAT TGA   |
|                | Antisense | TAT GCG AAG CAC CTT GGA AGC   |
| IL-12 p40      | Sense     | AGC ACC AGC TTC TTC ATC AGG   |
|                | Antisense | GCG CTG GAT TCG AAC AAA G     |
| IFN- $\gamma$  | Sense     | GCA TCT TGG CTT TGC AGC T     |
|                | Antisense | CCT TTT TCG CCT TGC TGT TG    |
| TNF- $\alpha$  | Sense     | TGT GCT CAG AGC TTT CAA CAA   |
|                | Antisense | CTT GAT GGT GGT GCA TGA GA    |
| CCL5           | Sense     | TTC CCT GTC ATC GCT TGC TCT   |
|                | Antisense | CGG ATG GAG ATG CCG ATT TT    |
| CCL11          | Sense     | ATA TCA GCA CCA GTC GCC CA    |
|                | Antisense | TCA TGC CCC CCT TTT AAG ATG   |
| CXCL1          | Sense     | CTT CCC TTG GAC ATT TTG TGT C |
|                | Antisense | TTT GAA CGT CTC TGT CCC GAG   |
| CCL2           | Sense     | AGA AGG AAT GGG TCC AGA CA    |
|                | Antisense | TCA TTT GGT TCC GAT CCA G     |
| CCL3           | Sense     | TTC TGC TGA CAA GCT CAC CCT   |
|                | Antisense | ATG GCG CTG AGA AGA CTT GGT   |
| CXCL10         | Sense     | AGC GTT TAG CCA AAA AAG GTC   |
|                | Antisense | TGG CTT CAC TCC AGT TAA GGA   |

Applied Biosystems User's Bulletin #2 (P/N 4303859), by reference to the  $\beta$ -actin in each sample, using the cycle threshold method. Negative controls without RNA and without reverse transcriptase were also performed. Results show one experiment representative of three.

### Statistical analysis

Results were submitted to the one-way analysis of variance, followed by Tukey's post-test. Values of  $P < 0.05$  were considered statistically significant.

## Results

### Mice sensitization induces PPE-specific immunoglobulin production in WT but not in B KO animals

One of the main hallmarks of experimental allergy is the B cell production of antigen-specific antibodies throughout the course of the disease. Accordingly, both sensitized (S) and the sensitized and challenged WT mice (S + *Peanut*) presented higher production of PPE-specific IgG antibodies than the controls *NS* and *Peanut* (Fig. 1a), demonstrating that sensitized mice responded to PPE by means of immunoglobulin production. The sensitization also induced antibody class switch towards a Th2 allergic profile, as seen in

Fig. 1(b–d). As expected, B KO mice presented undetectable levels of specific IgG, IgG2a, IgG1 or IgE antibodies in sera (Fig. 1a–d), thus confirming the absence of immunoglobulin response in B KO animals.

### B cells contribute to intestinal inflammation in food allergy

To determine if the presence of B cells and allergic antibodies could be related to any intestinal damage due to pathological Th2 responses in WT animals, jejunum segments from WT and B KO mice were collected and processed for microscopic analysis. As demonstrated in Fig. 2, WT mice sensitized and challenged with peanut seeds developed intestinal inflammation, characterized mainly by marked edema in the lamina propria accompanied by epithelial exulceration, disruption of small gut villous and leukocyte infiltrate. In addition, a great influx of plasma cells with abundant and notably dilated cisterns of the rough endoplasmic reticulum was found in the interior of the lamina propria. On the other hand, no marked changes were found in the intestinal mucosa of WT or B KO control mice (*NS*, *Peanut* and *S*). Animals from B cell-deficient group, sensitized and challenged with peanut seeds, showed an increase in the inflammatory infiltrate in the lamina propria with a moderate villous enlargement when compared with *NS*, *Peanut* and *S* B KO mice. Moreover, all groups from B KO mice showed mild signs of congestion in the interior of the lamina propria despite the sensitization protocol to which they were submitted. However, these animals did not present with the marked edema, epithelial exulceration or disruption of small gut architecture observed in *S* + *Peanut* WT group. These results, together with high serum-specific antibody production in *S* + *Peanut* WT mice, argued for a role for B lymphocytes or their products in the pathogenesis of the intestinal alterations observed in food allergy, thus indicating that B cells might be responsible for the overt pathology in WT allergic animals.

### Altered cytokine expression in the gut of S + *Peanut* mice in the absence of B cells

To verify if the presence of B cells contributed to the damage in the WT gut by mechanisms dependent on cytokine production, we further characterized the modulation of cytokine expression in the jejunum of B cell-deficient mice after peanut proteins exposure, and compared the results with WT ones. Along with elevated TGF- $\beta$  mRNA in the gut of B KO sensitized and challenged mice (Fig. 3a,  $P < 0.05$ ), a huge increase in IL-10 expression was overall observed in B KO animals when compared with WT ones, especially when *S* + *Peanut* groups were compared (Fig. 3b,  $P < 0.05$ ). Moreover, B cell-deficient mice presented elevated levels of IL-12p40 mRNA when fed peanut seeds (Fig. 3d, *Peanut* WT versus *Peanut* KO and *S* + *Peanut* WT versus *S* + *Peanut* KO,

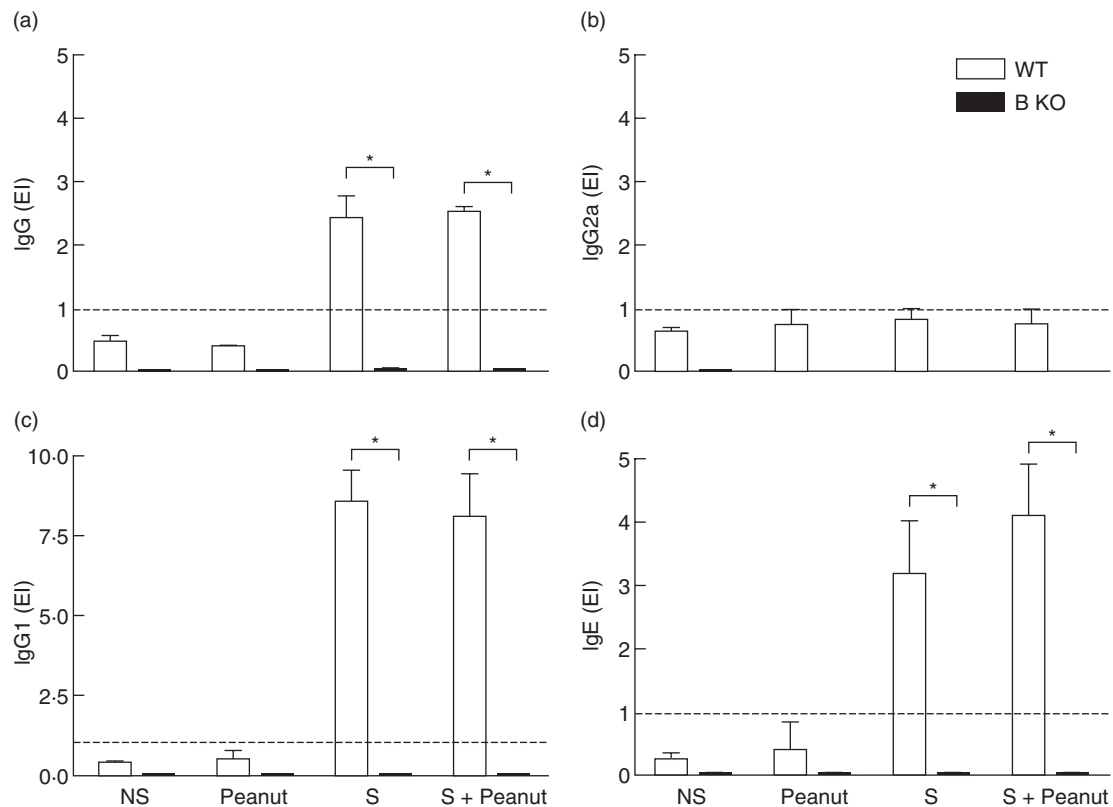


**Fig. 1.** Induction of immunoglobulin production specific to PPE in WT but not B KO animals. Mice (*S + Peanut*) were sensitized and challenged with peanut seeds for 30 days, when sera were collected for antibody detection, as described in 'Methods'. Results were expressed as ELISA index (EI), where values of EI > 1.0 (horizontal dashed line) were considered positive. (a) IgG, (b) IgG2a, (c) IgG1 and (d) IgE. Data (mean + s.e.m.) represent values from one experiment representative of three. NS, Peanut and S; control groups. \* $P < 0.05$ .

$P < 0.05$ ) as well as increased Th1 cytokine expression (IFN- $\gamma$ ) in sensitized and challenged animals in comparison with allergic WT mice (Fig. 3e,  $P < 0.05$ ). On the other hand, B cells were involved in the induction of IL-4 and TNF- $\alpha$  messages in WT food allergy, because there was a significant decrease in the expression of these cytokines in the gut of B KO mice sensitized and challenged with peanut seeds (Fig. 3c and 3f, respectively, *S + Peanut* WT versus *S + Peanut* KO,  $P < 0.05$ ). These results demonstrated the relevant role of B lymphocytes in the modulation of cytokine expression and in the intestinal alterations observed in this model of food allergy.

#### Gut damage is accompanied by differential chemokine expression in the gut of allergic animals

To elucidate if the disease could be mediated by specific leukocyte recruitment to the damaged tissues, we investigated the differential induction of chemokine expression in the gut of allergic WT groups compared with B KO mice. Results showed that *S + Peanut* WT mice presented increased expression of the chemokines CCL5, CCL11 and CXCL1, associated with neutrophil, eosinophil and mast cell recruitment, when compared with B KO mice sensitized and chal-

lenged with peanut seeds (Fig. 4a, b and c, respectively). On the contrary, in the absence of B cells, these mice presented higher expression of CCL2 (Fig. 4d, *S + Peanut* WT versus *S + Peanut* KO,  $P < 0.05$ ) and similar mRNA levels for CCL3 and CXCL10 chemokines, as demonstrated in Fig. 4 (e and f, respectively). These results suggest that the local altered morphology may be mediated by differential chemokine expression that recruits specific leukocytes to the intestinal damaged tissue.

#### Discussion

There are several ways by which the intestinal environment might influence an immune response resulting in tolerance or active immunity in the gut. In the present study, we demonstrated the participation of B lymphocytes in the breakdown of mucosal tolerance in a model of food-associated enteropathy. Differently from current models of food allergy that reflect a variety of causes and evaluate acute clinical reactions, our study focused on revealing the detailed modulation of gut immunity responsible for the breakdown of mucosal tolerance to food antigens.

B cells play an important role in the allergic response by producing allergen-specific immunoglobulins like IgE.

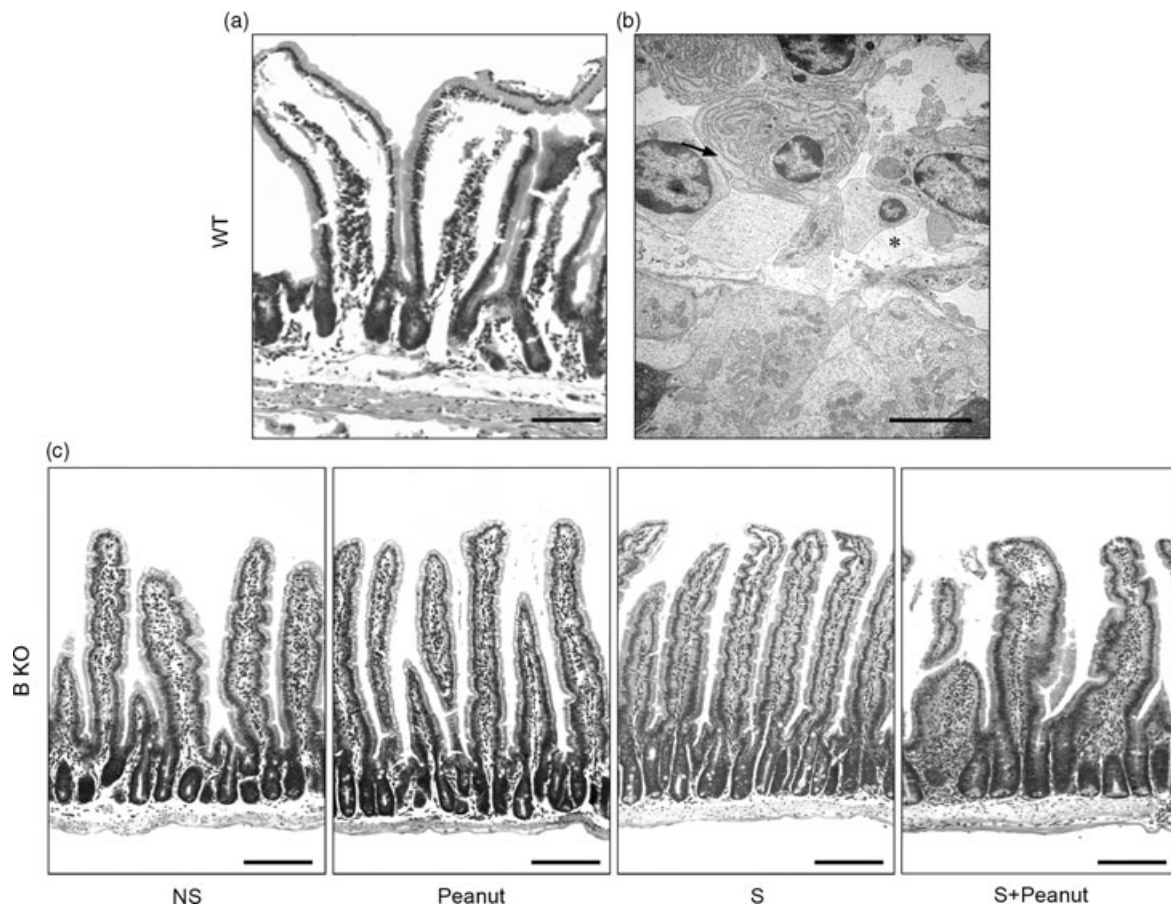


**Fig. 2.** Sensitization and challenge with peanut seeds do not induce tissue destruction in the gut of B KO mice. Mice (S + Peanut) were sensitized and challenged with peanut seeds for 30 days, when jejunum segments were collected and processed for histopathology and TEM analysis. In (a) note oedema, tissue destruction, epithelial exulceration and inflammatory infiltrate in S + Peanut WT mice, by conventional light microscopy. In (b) TEM shows influx of plasma cells with dilated cisterns of rough endoplasmic reticulum (arrow) and edema (\*) in S + Peanut WT animals. In (c), mild congestion, inflammatory infiltrate in the lamina propria, moderate villous enlargement but absence of tissue destruction in S + Peanut B KO animals. NS, Peanut and S; controls. Scale bars = 10  $\mu$ m (light microscopy) and 250  $\mu$ m (TEM). Results are representative of 3–5 independent experiments.

These antibodies mediate hypersensitivity reactions and play a central role in food allergic diseases. In this work, the high production of allergenic antibodies concomitant with abundant plasma cells in the gut of WT mice sensitized and challenged with peanut proteins highlighted the hypothesis of an antibody-mediated enteropathy in these animals. Therefore, the availability of genetically manipulated B cell-deficient mice allowed us to directly evaluate the influence of B cells and thus antigen-specific immunoglobulins in the development of the allergic intestinal inflammation observed previously by our group [9].

In our model, allergen sensitization and challenge resulted in differential antigen-driven responses in WT and B KO animals. Although WT mice lost weight when fed peanut seeds [9], we did not find any evidence of differential weight loss or altered healthy status in the B KO animals exposed to peanut proteins. Obviously, as expected, with the lack of B cells, there were no detectable levels of immunoglobulin pro-

duction and consequently no contribution of antibodies to the pathogenesis of the disease. The B cell deficiency also contributed significantly to the diminished manifestations of food allergy in the gut. Even though the lack of protective IgA in the intestinal mucosa could have contributed to the tissue alterations in B KO mice, the detectable morphological changes in these animals were mainly restricted to a moderate villous enlargement, probably due to the increase in the inflammatory infiltrate in the lamina propria, in contrast to the pathological findings in WT allergic mice, which did not lack IgA but instead presented high levels of serum PPE-specific allergenic antibodies. Although IgA deficiency is correlated with diminished accumulation of B lymphocytes with consequent abrogation of the development of allergic lung inflammation [14], in another murine model of allergen-induced disease, no role for B cells or antigen-specific antibodies was required, as B cell-deficient mice developed unabated eosinophilic esophagitis [15].

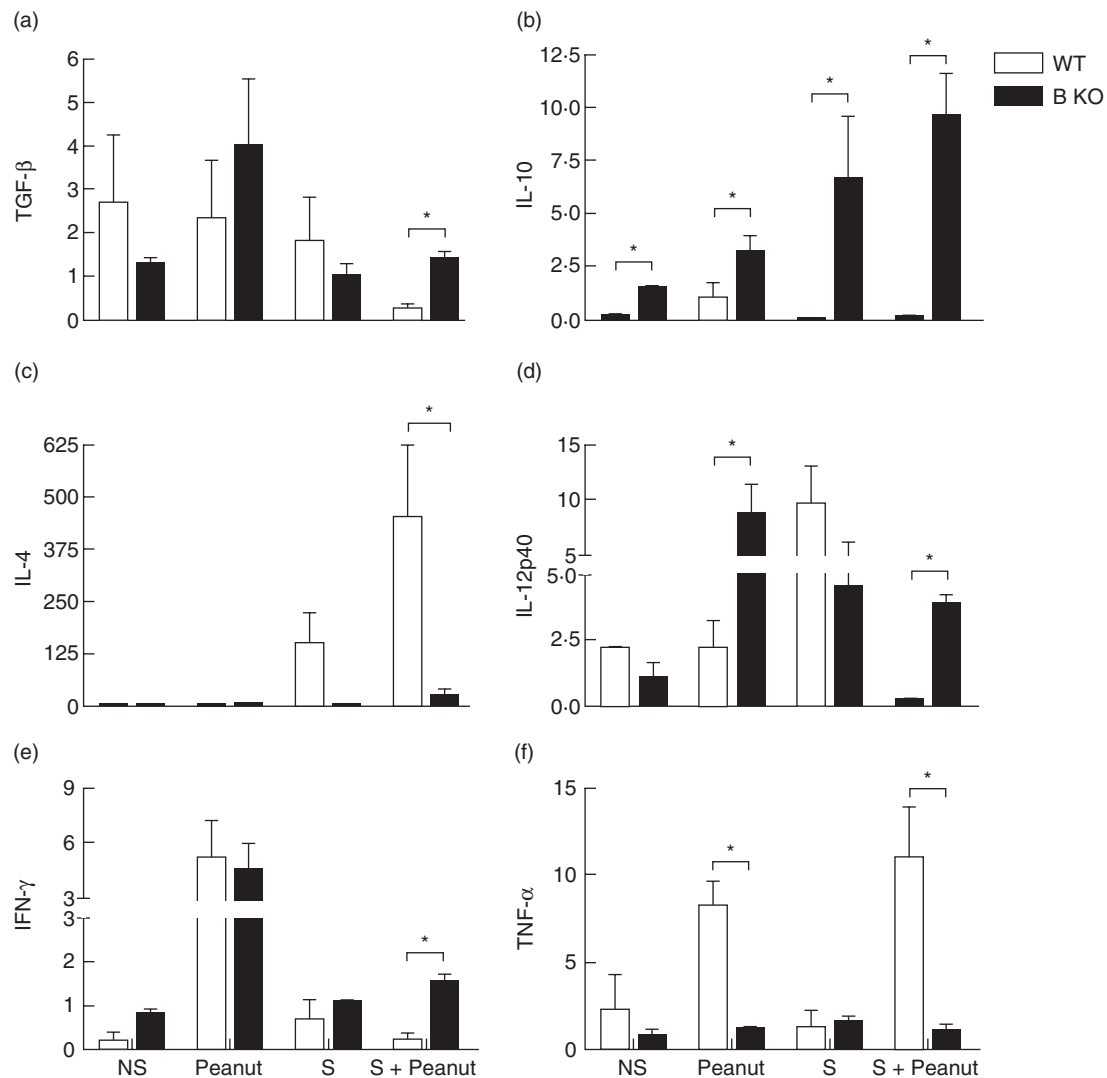


**Fig. 3.** Altered cytokine expression in the gut of B cell-deficient mice sensitized and challenged with peanut seeds. TGF- $\beta$  (a), IL-10 (b), IL-4 (c), IL-12p40 (d), IFN- $\gamma$  (e) and TNF- $\alpha$  (f) mRNA was quantified by real-time PCR, as described in 'Methods'. Results are demonstrated as mRNA expression of NS, Peanut, S and S + Peanut animals, relative to naïve non-sensitized, non-exposed to alum and non-challenged WT or B KO animals. The relative levels of gene expression were calculated by reference to the  $\beta$ -actin in each sample, using the cycle threshold method. Data (mean + s.e.m.) represent values from one experiment representative of three. NS, Peanut and S; controls. \* $P < 0.05$ .

Aside from being the precursors of the antibody-secreting cells, B lymphocytes are engaged in other immune functions. Though these cells are semiprofessional [16], rather than professional APCs [17], they may play a relevant role in antigen presentation, especially for soluble protein antigens [18–22]. Therefore, besides dendritic cells [23], the putative role of B lymphocytes as potent APCs and their absence resulting in diminished immunopathology should be considered in the present model of food allergy.

In our study, S + Peanut B KO mice also showed significantly impaired cytokine responses in the gut. The marked decrease in IL-4 and TNF- $\alpha$  mRNA suggested the ability of B cells to induce and/or produce cytokines in response to allergenic proteins as part of the pathogenic process of the enter-

opathy studied. Indeed, B cells may preferentially favor the development of IL-4-producing Th2 cells [24] and also down-regulate IL-12 released by dendritic cells, thereby inhibiting the development of IFN- $\gamma$ -producing Th1 cells [25]. Alternatively, the lack of B cells allowed an increase in the messages for the regulatory cytokines TGF- $\beta$  and IL-10, critical for gut homeostasis [26]. A recent study indicated that IL-10 AA, the genotype with lower IL-10 production in atopic children, is associated with higher IgE levels in the serum [27], demonstrating the importance of IL-10 in the regulation of allergic responses. Together with induction of regulatory responses, in the absence of B lymphocytes the gut immune system responded to the allergenic antigens by inducing a shift towards a Th1 profile that also certainly

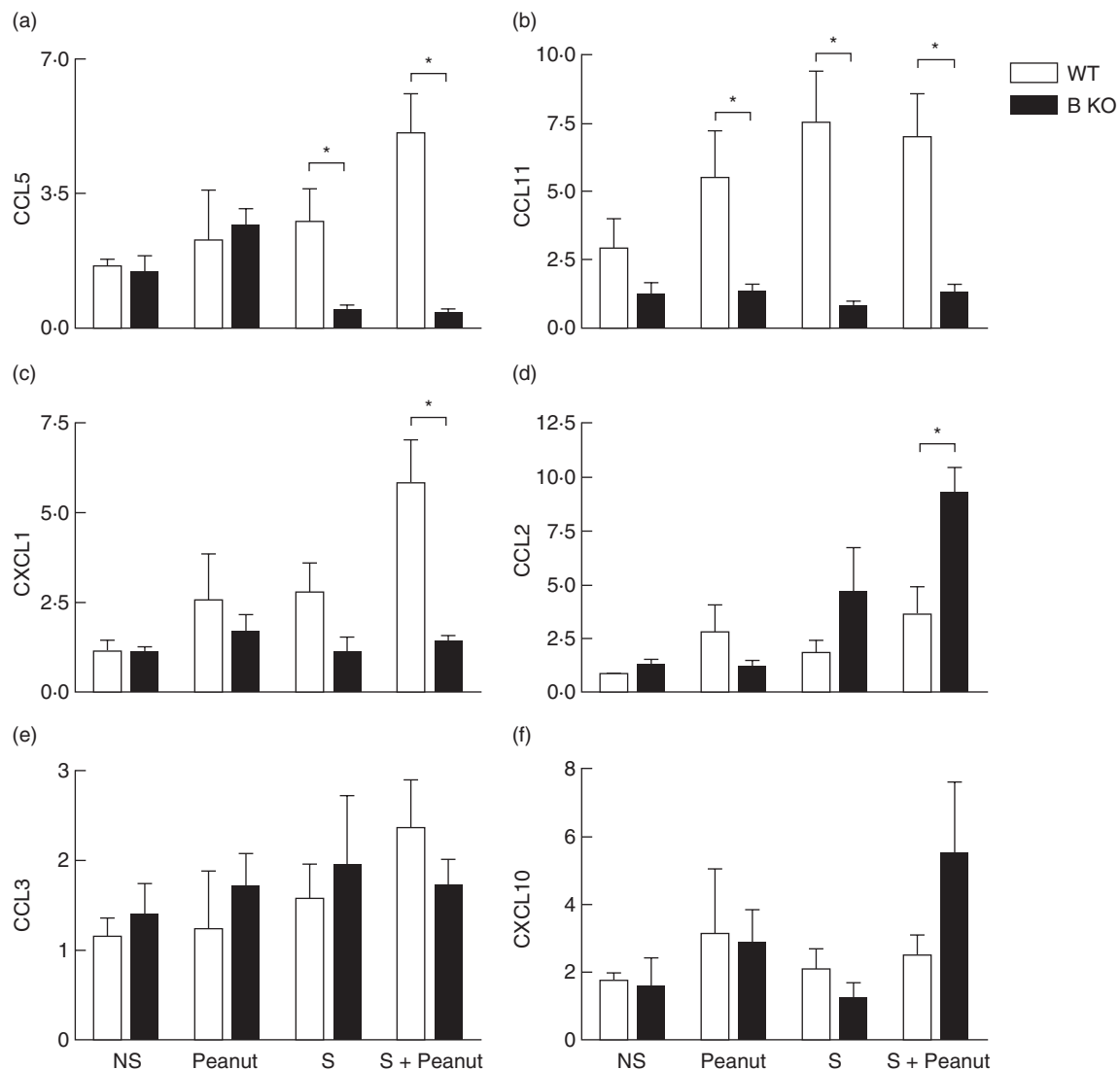


**Fig. 4.** Differential chemokine expression in the gut of allergic animals. CCL5 (a), CCL11 (b), CXCL1 (c), CCL2 (d), CCL3 (e) and CXCL10 (f) mRNA was quantified by real-time PCR, as described in 'Methods'. Results are demonstrated as mRNA expression of NS, Peanut, S and S + Peanut animals, relative to naïve non-sensitized, non-exposed to alumen and non-challenged WT or B KO animals. The relative levels of gene expression were calculated by reference to the  $\beta$ -actin in each sample, using the cycle threshold method. Data (mean + s.e.m.) represent values from one experiment representative of three. NS, Peanut and S; controls. \* $P < 0.05$ .

contributed to the remission of the pathological intestinal alterations visualized in WT mice. This aspect of regulation of the immune response and, in particular, modulation of the B-cell response is extremely important and should be considered when devising strategies to effectively block antigen-driven IgE responses, as in food allergy.

Immunological reactions to dietary antigens involve specific types of local and systemic responses and a unique scenario in the intestine with specialized antigen-handling M cells and Peyer's patches [11]. The B cell-deficient animals used in this study are the  $\mu$ MT mice, which contain no B cells due to a targeted mutation in the transmembrane region of the IgM heavy chain [28] and no M cells or Peyer's patches because of the lack of B lympho-

cytes [29]. Although some authors postulated that despite these deficiencies, the  $\mu$ MT mice make perfectly normal systemic T-cell responses to oral antigens [30], the singular scenario demonstrated here pointed to relevant differences in the induction and control of local immune response when mucosal tolerance is broken by allergenic food antigens in B KO mice. The lack of both M cells and Peyer's patches enabled us to speculate whether the allergic inflammatory cytokines, chemokines expression and tissue immunopathology observed in WT but not in  $\mu$ MT mice could also be an effect of these issues on intestinal responses, thus providing additional evidence that mice lacking B cells have a relevant defect in mounting allergen-specific responses in the intestinal mucosa.

Besides the deficiency in the formation of gut-associated lymphoid tissue, *S + Peanut* B KO mice presented an increased inflammatory infiltrate in the lamina propria in response to the antigen challenge, although not resulting in destructive enteropathy. Additionally, the data presented in this paper corroborate our previous study [9] in which the intestinal manifestations of food allergy were supposed to be a consequence of eosinophils and mast cells recruitment to WT intestine. In accordance, *S + Peanut* B KO animals showed reduced expression of chemokines related to the migration of these cells (e.g. CCL11) [31–33] and elevated messages or similar detection for chemokines that recruit preferentially macrophages or cells from the Th1 response when compared with *S + Peanut* WT mice [34,35].

In conclusion, the systemic exposure and oral challenge with peanut allergenic proteins promoted inflammation in the WT gut by pathways leading to systemic and local induction of B-cell responses, providing evidence for the involvement and the critical role of B cells in the development of food-related enteropathies such as food allergy.

## Acknowledgements

The authors thank L. Santoro, M. Abreu and M. E. Riul for technical assistance in TEM and histology procedures. This study was supported by grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP).

## References

- 1 Fischer R, McGhee JR, Vu HL *et al.* Oral and nasal sensitization promote distinct immune responses and lung reactivity in a mouse model of peanut allergy. *Am J Pathol* 2005; **167**:1621–30.
- 2 Metcalfe DD. Food allergy. *Curr Opin Immunol* 1991; **3**:881–6.
- 3 Sicherer SH, Leung DY. Advances in allergic skin disease, anaphylaxis, and hypersensitivity reactions to foods, drugs, and insects. *J Allergy Clin Immunol* 2005; **116**:153–63.
- 4 Sampson HA. Clinical practice. Peanut allergy. *N Engl J Med* 2002; **346**:1294–9.
- 5 Sampson HA. Update on food allergy. *J Allergy Clin Immunol* 2004; **113**:805–19; quiz 20.
- 6 Bock SA, Munoz-Furlong A, Sampson HA. Fatalities due to anaphylactic reactions to foods. *J Allergy Clin Immunol* 2001; **107**:191–3.
- 7 Pons L, Palmer K, Burks W. Towards immunotherapy for peanut allergy. *Curr Opin Allergy Clin Immunol* 2005; **5**:558–62.
- 8 Husband AJ. Novel vaccination strategies for the control of mucosal infection. *Vaccine* 1993; **11**:107–12.
- 9 Cardoso CR, Teixeira G, Provinciatto PR *et al.* Modulation of mucosal immunity in a murine model of food-induced intestinal inflammation. *Clin Exp Allergy* 2008; **38**:338–49.
- 10 Bischoff S, Crowe SE. Gastrointestinal food allergy: new insights into pathophysiology and clinical perspectives. *Gastroenterology* 2005; **128**:1089–113.
- 11 Mowat AM. Anatomical basis of tolerance and immunity to intestinal antigens. *Nat Rev Immunol* 2003; **3**:331–41.
- 12 Veldkamp J, De Reuver MJ, Willers JM. Distribution of different cell types in the lymphoid organs of the mouse, as determined with sera against thymus and Peyer's patches. *Immunology* 1974; **26**:359–66.
- 13 Ferro EA, Silva DA, Bevilacqua E, Mineo JR. Effect of *Toxoplasma gondii* infection kinetics on trophoblast cell population in *Calomys callosus*, a model of congenital toxoplasmosis. *Infect Immun* 2002; **70**:7089–94.
- 14 Arnaboldi PM, Behr MJ, Metzger DW. Mucosal B cell deficiency in IgA-/- mice abrogates the development of allergic lung inflammation. *J Immunol* 2005; **175**:1276–85.
- 15 Mishra A, Schlotman J, Wang M, Rothenberg ME. Critical role for adaptive T cell immunity in experimental eosinophilic esophagitis in mice. *J Leukoc Biol* 2007; **81**:916–24.
- 16 Stockinger B, Zal T, Zal A, Gray D. B cells solicit their own help from T cells. *J Exp Med* 1996; **183**:891–9.
- 17 Lassila O, Vainio O, Matzinger P. Can B cells turn on virgin T cells? *Nature* 1988; **334**:253–5.
- 18 Byersdorfer CA, Dipaolo RJ, Petzold SJ, Unanue ER. Following immunization antigen becomes concentrated in a limited number of APCs including B cells. *J Immunol* 2004; **173**:6627–34.
- 19 Constant S, Schweitzer N, West J, Ranney P, Bottomly K. B lymphocytes can be competent antigen-presenting cells for priming CD4+ T cells to protein antigens in vivo. *J Immunol* 1995; **155**:3734–41.
- 20 Kurt-Jones EA, Liano D, HayGlass KA, Benacerraf B, Sy MS, Abbas AK. The role of antigen-presenting B cells in T cell priming in vivo. *Studies of B cell-deficient mice. J Immunol* 1988; **140**:3773–8.
- 21 Linton PJ, Harbertson J, Bradley LM. A critical role for B cells in the development of memory CD4 cells. *J Immunol* 2000; **165**:5558–65.
- 22 Rivera A, Chen CC, Ron N, Dougherty JP, Ron Y. Role of B cells as antigen-presenting cells in vivo revisited: antigen-specific B cells are essential for T cell expansion in lymph nodes and for systemic T cell responses to low antigen concentrations. *Int Immunol* 2001; **13**:1583–93.
- 23 Kelsall BL. Innate and adaptive mechanisms to control of pathological intestinal inflammation. *J Pathol* 2008; **214**:242–59.
- 24 Liu Q, Liu Z, Rozo CT *et al.* The role of B cells in the development of CD4 effector T cells during a polarized Th2 immune response. *J Immunol* 2007; **179**:3821–30.
- 25 Moulin V, Andris F, Thielemans K, Maliszewski C, Urbain J, Moser M. B lymphocytes regulate dendritic cell (DC) function in vivo: increased interleukin 12 production by DCs from B cell-deficient mice results in T helper cell type 1 deviation. *J Exp Med* 2000; **192**:475–82.
- 26 Ruiz PA, Shkoda A, Kim SC, Sartor RB, Haller D. IL-10 gene-deficient mice lack TGF-beta/Smad-mediated TLR2 degradation and fail to inhibit proinflammatory gene expression in intestinal epithelial cells under conditions of chronic inflammation. *Ann NY Acad Sci* 2006; **1072**:389–94.
- 27 Negoro T, Orihara K, Irahara T *et al.* Influence of SNPs in cytokine-related genes on the severity of food allergy and atopic eczema in children. *Pediatr Allergy Immunol* 2006; **17**:583–90.
- 28 Kitamura D, Roes J, Kuhn R, Rajewsky K. A B cell-deficient mouse by targeted disruption of the membrane exon of the immunoglobulin mu chain gene. *Nature* 1991; **350**:423–6.

- 29 Golovkina TV, Shlomchik M, Hannum L, Chervonsky A. Organogenic role of B lymphocytes in mucosal immunity. *Science* 1999; **286**:1965–8.
- 30 Alban O, Rudomen G, Matzinger P. The role of dendritic cells, B cells, and M cells in gut-oriented immune responses. *J Immunol* 2001; **166**:4843–52.
- 31 Fulkerson PC, Fischetti CA, McBride ML, Hassman LM, Hogan SP, Rothenberg ME. A central regulatory role for eosinophils and the eotaxin/CCR3 axis in chronic experimental allergic airway inflammation. *Proc Natl Acad Sci USA* 2006; **103**:16418–23.
- 32 Oyamada H, Kamada Y, Saito N *et al.* RANTES production from mononuclear cells in response to the specific allergen in asthma patients. *Allergol Int* 2006; **55**:253–9.
- 33 Salib RJ, Kumar S, Wilson SJ, Howarth PH. Nasal mucosal immunorepression of the mast cell chemoattractants TGF-beta, eotaxin, and stem cell factor and their receptors in allergic rhinitis. *J Allergy Clin Immunol* 2004; **114**:799–806.
- 34 Hori M, Nobe H, Horiguchi K, Ozaki H. MCP-1 targeting inhibits muscularis macrophage recruitment and intestinal smooth muscle dysfunction in colonic inflammation. *Am J Physiol Cell Physiol* 2008; **294**:C391–401.
- 35 Ito R, Shin-Ya M, Kishida T *et al.* Interferon-gamma is causatively involved in experimental inflammatory bowel disease in mice. *Clin Exp Immunol* 2006; **146**:330–8.