

Intestinal B cell-activating factor: an indicator of non-IgE-mediated hypersensitivity reactions to food?

G. Arslan Lied^{*,†}, K. Lillestøl^{*,†}, J. Valeur^{*,†} & A. Berstad^{*,†}

^{*}Institute of Medicine, University of Bergen.

[†]Division of Gastroenterology, Department of Medicine, Haukeland University Hospital, Bergen, Norway.

Correspondence to:

Dr G. Arslan Lied, Division of Gastroenterology, Department of Medicine, Haukeland University Hospital, N-5021 Bergen, Norway.
E-mail: Gulen.Arslan@med.uib.no

Publication data

Submitted 21 December 2009
First decision 14 January 2010
Resubmitted 8 February 2010
Accepted 23 March 2010
Epub Accepted Article 26 March 2010

SUMMARY

Background

Medically confirmed hypersensitivity reactions to food are usually IgE-mediated. Non-IgE-mediated reactions are not only seldom recognized but also more difficult to diagnose.

Aim

To examine B cell-activating factor (BAFF) in serum and gut lavage fluid of patients with self-reported food hypersensitivity, and to study its relationship to atopic disease.

Methods

Gut lavage fluid was obtained from 60 and serum from another 17 patients with self-reported food hypersensitivity. Twenty healthy volunteers served as controls, gut lavage fluid was obtained in all, serum from 11 of 20. The patients were divided into atopic and non-atopic subgroups. BAFF was measured by ELISA in both serum and gut lavage fluid.

Results

B cell-activating factor levels in serum and gut lavage fluid were significantly higher in patients than in controls ($P < 0.03$ and $P < 0.002$ respectively). Non-atopic patients had significantly higher levels of BAFF in serum than both atopic patients ($P < 0.05$) and controls ($P < 0.05$). There was no significant correlation between serum levels of BAFF and IgE.

Conclusions

The results suggest that BAFF might be a new mediating mechanism in food hypersensitivity reactions. Significantly higher levels in non-atopic compared with atopic patients, and no correlation between BAFF and IgE, suggest that BAFF might be involved particularly in non-IgE-mediated reactions.

Aliment Pharmacol Ther 2010; 32: 66–73

INTRODUCTION

Food hypersensitivity is an 'umbrella term' which refers to all types of adverse reactions to food, both allergic and non-allergic (food intolerance) reactions. While food allergy involves immunological mechanisms, non-allergic food hypersensitivity does not.^{1, 2} Food allergy can be IgE-mediated (type I) or non-IgE-mediated (type III or IV). Type I hypersensitivity is characterized by an immediate reaction shortly after contact with the allergen (within minutes – up to 2 h). Here, high-affinity IgE receptor (Fc ϵ RI)-bearing mast cells and basophils are key effector cells. After cross-linking Fc ϵ RI receptors by antigen-specific IgE molecules, mast cells and basophils release various mediators such as histamine, heparin, leucotrienes, prostaglandins and cytokines. Delayed-type hypersensitivity reactions occur 2–24 h after allergen exposure without involvement of IgE and can be both immune and non-immune-mediated.^{3, 4}

B cell-activating factor (BAFF) is a member of the tumour necrosis factor (TNF) superfamily (TNFSF13B) and an important regulator of peripheral B cell survival, maturation and immunoglobulin class-switch recombination (CSR).⁵ CSR is a biological mechanism by which activated B cells (plasma cells) change their antibody production from one isotype to another, for example, from IgM to IgG. Naive mature B cells produce both IgM and IgD, which contain the first two heavy chain segments of the immunoglobulins. To make a new isotype of antibody, CSR requires two signals. The first signal normally comes through T cell cytokines (IL-4, IL-10, IL-13 and TGF- β), while the second is delivered by engagement of CD40 on B cells.⁶ In addition, BAFF impact is involved in this process by one of its specific receptors, called TACI. Despite the fact that CSR is generally restricted to lymphoid organs, previous studies show that local immunoglobulin CSR and IgE production also occur in the nasal and airway mucosa of patients with allergic asthma and allergic rhinitis, as well as in the oesophageal mucosa of patients with eosinophilic oesophagitis.^{7, 8}

B cell-activating factor is mainly produced and secreted not only by myeloid cells (macrophages, monocytes and dendritic cells) but also by non lymphoid cell types (salivary gland epithelial cells, astrocytes and fibroblast-like synoviocytes) and epithelial cells including bronchial and nasal epithelial cells. The biological role of BAFF is mediated through the specific receptors; two high-affinity receptors, BAFF receptor (BAFF-R) and transmembrane activator-calcium modulator and cyclophilin ligand interactor (TACI), and a low-affinity

receptor, B cell maturation antigen (BCMA). BAFF-R present on the surface of effector T cells is a potent regulator of mature B-cell survival and IgE production by BAFF, whereas TACI on surfaces of B cells is critical for CSR.^{9–11}

High levels of BAFF have been reported in patients with allergic disease (asthma) and autoimmune diseases such as systemic lupus erythematosus, Sjögren's syndrome, rheumatoid arthritis, systemic sclerosis, mixed cryoglobulinaemia, myasthenia gravis and coeliac disease.^{12–16} Overexpression of BAFF in animal models leads to B-cell hyperplasia, lymphoproliferation, hypergammaglobulinaemia and symptoms of autoimmunity. Conversely, BAFF-deficient animals exhibit defects in peripheral B-cell maturation and decreased levels of immunoglobulins.^{9, 10} Recently, BAFF has emerged also as an important regulator of T-cell function, particularly during T-cell-mediated reactions.¹⁶

Patients with self-reported food hypersensitivity complain of a wide range of unexplained somatic symptoms related to the intake of food. Often, the patients feel quite ill with considerably impaired quality of life.¹⁷ The condition is common. In the general population, approximately 1 of 5 (20%) adults reports food-related hypersensitivity reactions. Food allergy is confirmed in only 3–5% and the confirmed reactions are usually IgE-mediated.¹ Non-IgE-mediated reactions are seldom recognized. Thus, in most cases, the cause of self-reported food hypersensitivity remains elusive. A possible role of BAFF has not yet been investigated in these patients. In the present explorative study, we therefore wanted to investigate whether BAFF could be determined in blood and gut lavage fluid in patients with self-reported food hypersensitivity. Retrospectively, we investigated the relationship between BAFF and atopic disease in the patients.

MATERIALS AND METHODS

Patients

Eighty consecutive patients referred to Haukeland University Hospital for abdominal complaints self-attributed to food hypersensitivity were eligible for the study. Patients with organic diseases, such as inflammatory bowel disease ($n = 1$), patients reporting severe anaphylactic reactions ($n = 2$), and pregnant or lactating women were excluded. The remaining 77 patients went through an extensive clinical examination programme including allergological as well as gastroenterological examinations as reported previously.¹⁸ BAFF was examined in gut

lavage fluid obtained from 60 of the patients and in serum from the other 17 patients.

Briefly, the allergological examination consisted of a full medical history including atopy, family history, suspected food items, serum levels of total and food-specific IgE and skin prick tests using a panel of inhalant and food antigens. Open provocation test and double-blind placebo-controlled provocation test (DBPCFC) were performed by a dietician in some patients when indicated according to medical history and results of skin prick test and serum food-specific IgE levels. The gastroenterological examination included a screening questionnaire based on the Rome II criteria for the diagnosis of irritable bowel syndrome (IBS)¹⁹ and gastroscopy with gastric and duodenal biopsies to diagnose *Helicobacter pylori* infection and coeliac disease respectively. Stool tests were performed to exclude gastrointestinal infections. Inflammatory bowel disease was ruled out by measuring intestinal permeability and levels of calprotectin in gut lavage fluid.²⁰ The method for intestinal lavage is described in detail below.

The study was approved by the Western Norway Regional Committee for Medical Research Ethics.

Controls

The control group consisted of 20 volunteers who claimed to be healthy. Gut lavage fluid was obtained from all 20, while serum was obtained from only 11 of them. They filled in questionnaires regarding abdominal complaints (IBS-SSS)²¹ and allergic diseases including a history of atopy, but they were not examined otherwise.

Intestinal lavage procedure

Intestinal lavage was performed primarily to measure mucosal permeability, but the permeability results are not reported here. The method has been described previously.²⁰ Briefly, after an overnight fast, a thin nasoduodenal feeding tube (Freka Feeding Tube, Fresenius Kabi, GmbH, Germany) was positioned with its tip in the distal part of the duodenum by a gastroscop. Two litres of isotonic polyethylene glycol solution (PEG) (MW 3350 daltons, Laxabon, Tika, Sweden) was administered through the nasoduodenal feeding tube over a period of 40 min using a peristaltic pump (Watson Marlow 505S/RL, Falmouth, UK). Approximately 50 μ Ci of ⁵¹Cr-labelled ethylenediaminetetraacetic acid (⁵¹CrEDTA, Amersham international, Amersham, UK) was added to the solution. Depending on intestinal permeability, trace amounts of this substance is absorbed and excreted in the urine. Urine was collected for 5 h (i.e. 4 h and

40 min. after finishing the infusion) and analysed for radioactivity using a gamma counter (Wallac, 1282 Com-pugamma Counter, Norway). When the PEG solution reached the distal colon, bowel movements started. The first clear fluid passed per rectum was collected and passed through gaze to remove solid particles. Aliquots (4.5 mL) were added to tubes containing 0.5 mL of an antiseptic and antiproteolytic solution. A stock solution of the latter was prepared by adding 1 mL of 10% sodium azide (NaN₃) to 50 mL of soybean trypsin inhibitor (Sigma, Steinheim, Germany). The samples were stored frozen at -80 °C until assayed. Repeated freeze-thaw cycles were avoided.

Blood samples

Blood samples were taken for analysis of serum IgE and BAFF while the patients were on their customary diet. Levels of serum total and food-specific IgE were analysed by ImmunoCap-System (Phadia, Uppsala, Sweden) at the Laboratory of Clinical Biochemistry (LKB), Haukeland University Hospital at the same day. Remaining blood samples were centrifuged for 10 min at 3000 rpm at 4 °C and serum was stored frozen at -80 °C until the assay of BAFF was performed.

Analysis of BAFF

The gut lavage fluid specimens were thawed and centrifuged for 10 min at 3000 rpm at 4 °C and the supernatants were used to analyse BAFF levels.

The BAFF levels in serum and gut lavage fluid were measured by enzyme-linked immunosorbent assay (ELISA) (R&D System, Abingdon, England). The Quantikine Human BAFF Immunoassay is designed to measure human BAFF in cell culture supernates, serum and plasma. It contains *E. coli*-expressed recombinant human BAFF and has been shown to quantify the recombinant factor accurately. The analysis of BAFF was performed according to the manufacturer's manual. Briefly, a monoclonal antibody specific for BAFF was pre-coated onto a microplate. Standards and samples were pipetted into the wells and any BAFF present was bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for BAFF was added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells and colour developed in proportion to the amount of BAFF bound in the initial step. The colour development was stopped and the intensity of the colour (absorbance) was measured at 450 nm for 96-well plates. The results of the test samples

were calculated from the standard curves and expressed as picogram per mL (pg/mL) fluid or serum.

Statistical analysis

Data were analysed using the GraphPad Prism 5 (GraphPad Software Inc., San Diego, CA, USA) statistical software package. The values were reported as individual data and medians with interquartile ranges (IQR). Differences between groups were evaluated by Mann–Whitney *U*-test and one-way ANOVA test. Spearman's correlation coefficient was used to evaluate correlation between BAFF levels and serum total IgE levels. A *P*-value of less than 0.05 was considered statistically significant.

RESULTS

Subject characteristics

Gut lavage fluid was collected from 60 patients (lavage group: 43 women, 17 men, age range: 20–73 years, mean age 41) and 20 controls (13 women, 7 men, age range: 24–44 years, mean age 32), while blood samples were drawn from another group of 17 patients (serum group: 13 women, 4 men, age range: 20–62 years, mean age 40) and from 11 of the 20 controls (7 women, 4 men, age range: 24–42 years, mean age 34). The mean of total IgE levels in serum were 465 kU/L (range: 2–9600 kU/L) in the lavage group ($n = 60$), 351 kU/L (range: 5–3106 kU/L) in the serum group ($n = 17$) and 102 kU/L (range: 2–555 kU/L) in controls.

The patients were divided into atopic and non-atopic subgroups, according to history, serum-specific IgE and skin prick tests: atopic individuals were defined as having at least one positive skin prick test and/or elevated levels of serum specific IgE to at least one allergen, as well as personal history of at least one of the following atopic symptoms: dermatitis, asthma or rhinoconjunctivitis.²² Thirty-two of 60 patients in the lavage group and 8 of 17 patients in the serum group were classified as having atopic disease (altogether 40 individuals).

In the patients, cow's milk was the most commonly suspected food item (71%), followed by wheat flour (61%), and diarrhoea was the most common gastrointestinal symptom (78%), followed by abdominal pain (70%). All 40 patients with atopic disease were sensitized to at least one inhalant allergen, as verified by skin prick test and/or serum specific IgE, whereas 34 of the atopic patients were sensitized to at least one food allergen. Birch (75%) and timothy grass (70%) were the most common inhalant allergen, whereas wheat flour (45%) and peanuts (30%) were the most common food allergen.

Thirty-three of the atopic patients (83%) reported atopic diseases (rhinoconjunctivitis 78%, atopic dermatitis 40% and asthma 25%). DBPCFC was performed in seven patients and came out positive in three persons (4% of the whole study population), all having non-IgE-mediated allergy or non-allergic food hypersensitivity. Hence, none of the patients had IgE-mediated food allergy as confirmed by both skin prick test, food-specific IgE levels in serum and DBPCFC with the same food item. Majority of patients (96%) had gastrointestinal symptoms consistent with a functional gastrointestinal disorder, predominantly IBS, according to the Rome II criteria.

None of the controls had IBS, but one had borderline score for IBS on the IBS-SSS questionnaire. Three controls had seasonal pollen allergy.

BAFF in gut lavage fluid

The concentration of BAFF in gut lavage fluid in the patients ($n = 60$; median, 34 pg/mL; IQR, 24.3–44.8 pg/mL) was significantly higher than in the controls ($n = 20$; median, 0 pg/mL; IQR, 0–26.8 pg/mL; $P < 0.002$) (Figure 1). There were no significant differences in BAFF concentration between atopic and non-atopic patients. The highest BAFF value in gut lavage fluid was seen in the control person who had the borderline scoring for IBS (see above). To eliminate the possibility that high concentrations of BAFF merely reflects vascular leakage (7), we normalized the BAFF values to the concentration of albumin by dividing median BAFF levels to the respective median albumin level. The thus calculated BAFF-to-albumin ratio in lavage fluid (median

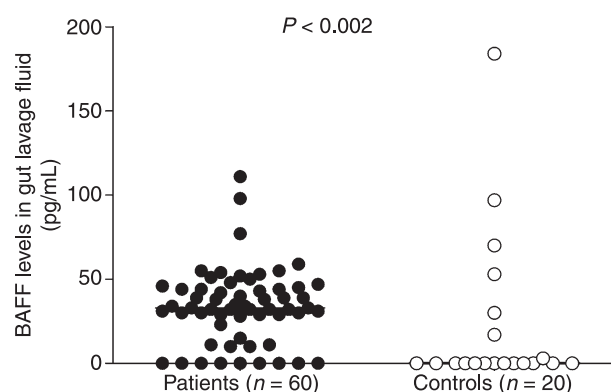


Figure 1 | B cell-activating factor concentrations in gut lavage fluid in patients with self-reported food hypersensitivity ($n = 60$) and in healthy controls ($n = 20$). Individual results are reported with median values ($P < 0.002$).

albumin 1.7×10^{-4} g/mL) was approximately 180 times higher than that in blood (median albumin 40 g/L), suggesting that BAFF in gut lavage is not derived from blood.

BAFF in serum

The concentration of BAFF in serum was significantly higher in the patients ($n = 17$; median, 1249 pg/mL; IQR, 959–1507 pg/mL) than in the controls ($n = 11$; median, 914 pg/mL; IQR, 780–1106 pg/mL; $P < 0.03$) (Figure 2). Non-atopic patients ($n = 9$; median, 1435 pg/mL; IQR, 1036–1828 pg/mL) had significantly higher concentrations of BAFF in serum than both atopic patients ($n = 8$; median, 1012 pg/mL; IQR, 917.8–1278 pg/mL; $P < 0.05$) and controls ($n = 11$; median, 914 pg/mL; IQR, 780–1106 pg/mL; $P < 0.05$) (Figure 3). Serum total IgE levels were generally lower in non-atopic patients (median, 39 kU/L; IQR, 18–265 kU/L) than in both atopic patients (median, 221 kU/L; IQR, 79–441 kU/L) and controls (median, 43 kU/L; IQR, 10–101 kU/L), but the differences were not significant.

Relationship between BAFF concentration and total IgE levels

There was no significant correlation either between serum BAFF concentration and serum total IgE levels ($r = 0.07$, $P = 0.79$) or between BAFF concentration in gut lavage fluids and serum total IgE levels ($r = -0.003$, $P = 0.98$) (Figures 4 and 5).

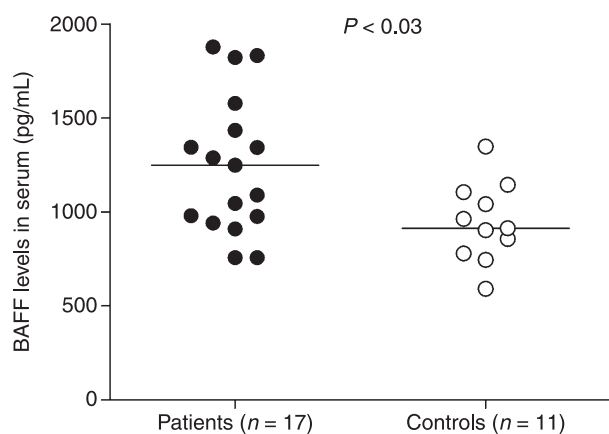


Figure 2 | B cell-activating factor concentrations in serum in patients with self-reported food hypersensitivity ($n = 17$) and in healthy controls ($n = 11$). Individual results are reported with median values ($P < 0.03$).

Reproducibility and stability of the BAFF assay

Gut lavage fluid from 10 patients was re-analysed to determine reproducibility and stability of BAFF in gut lavage fluid. The correlation between the first (test) measurement and the second (retest) measurement ($r = 0.77$, $P = 0.009$) indicated good inter-assay precision. Stability was analysed by preparing two samples (sample 1 and sample 2) from each of 10 patients. Exogenous BAFF (250 pg/mL) 0.075 mL was added to gut lavage fluid

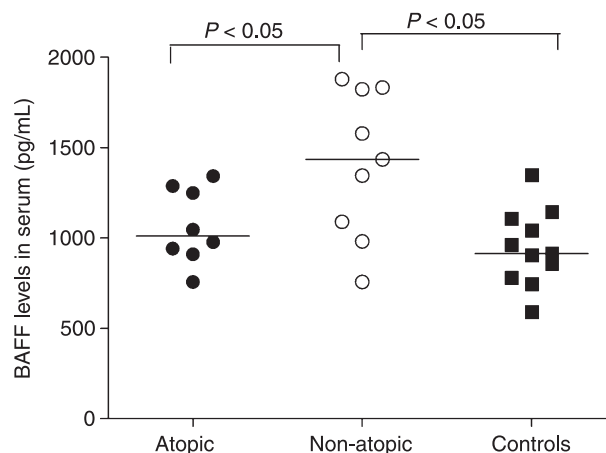


Figure 3 | Serum BAFF concentrations in atopic patients ($n = 8$), non-atopic patients ($n = 9$) and in controls ($n = 11$). Individual results are reported with median values (atopic vs. non-atopic $P < 0.05$, non-atopic vs. controls $P < 0.05$).

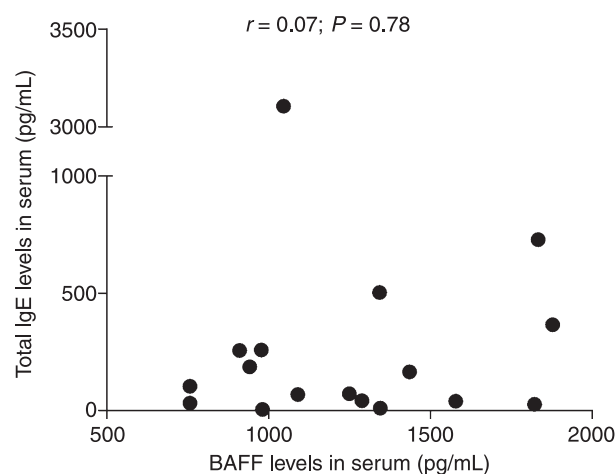


Figure 4 | Correlation between serum BAFF concentration and serum total IgE levels ($n = 17$; $P =$ not significant).

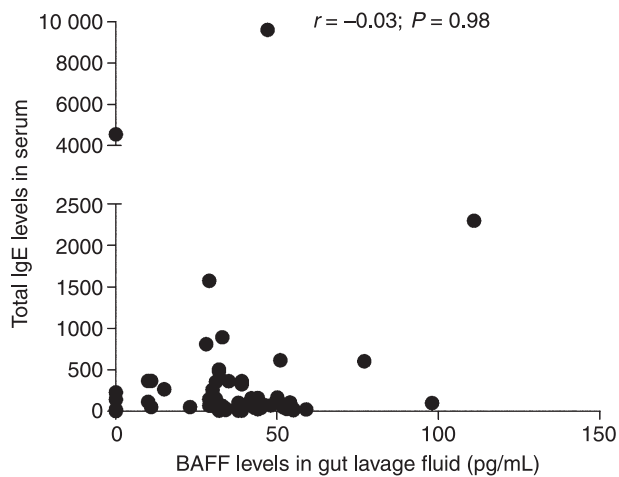


Figure 5 | Correlation between BAFF concentrations in gut lavage fluid and serum total IgE levels ($n = 60$; $P =$ not significant).

0.150 mL (sample 1) and left on the bench at room temperature for 2 h. Then, sample 2 was prepared similarly and analysed immediately together with sample 1 on one ELISA-plate. Mean BAFF concentrations in sample 1 and 2 were equal (84.3 pg/mL and 84.2 pg/mL respectively), indicating good stability of BAFF in gut lavage fluid over 2 h at room temperature and good intra-assay precision (coefficient of variation 2.7%).

DISCUSSION

A relationship between food intolerance and IBS is well recognized.²³ Up to 70% of the IBS patients report adverse reactions to one or more food items.^{24, 25} The prevalence of self-reported food hypersensitivity differs greatly, however, from that of medically confirmed food allergy. A reason for the discrepancy could be that current tests of food allergy are inadequate. While the diagnosis of IgE-mediated allergy is based primarily on history, blood concentration of specific IgE and skin prick tests, non-IgE-mediated or delayed-type hypersensitivity is much more difficult to diagnose²⁶ and, maybe as a consequence, seldom recognized in clinical practice. Patients with self-reported food hypersensitivity usually present with IBS-like symptoms in response to food ingestion. In addition, systemic, non gastrointestinal symptoms like joint pain and chronic fatigue are often reported.¹⁸ This widespread somatic symptomatology is often associated with anxiety and depression, and much attention has been paid to the possibility that somatisation could be a major explanatory mechanism.²⁷ However, in a recent study, only around 10% of the total

variance in somatic symptoms could be explained by psychological factors. Ninety percent of the variance remained unexplained.²⁸ Hence, neither classical IgE-mediated allergy nor psychological factors seem to be the major cause of self-reported food hypersensitivity.

This study provides the first demonstration of increased concentration of BAFF in serum and gut lavage fluid of patients with self-reported food hypersensitivity. The fact that the increase in BAFF was seen particularly in patients without indications of IgE-mediated atopic disease is interesting. BAFF is an important regulator of B-cell activation, proliferation, immunoglobulin CSR and immunoglobulin production.⁵ Increased BAFF and B-cell activation are typically seen in patients with rheumatic diseases like rheumatoid arthritis and systemic lupus erythematosus, i.e. diseases with widespread symptomatology and often sparse findings, at least initially, a situation that, to some extent, is reminiscent of the condition of the present patient population. An exciting question is whether BAFF-mediated immunological reactions can explain the systemic symptoms in patients with self-reported food hypersensitivity.

In vitro experiments indicated good test-retest performance and good stability of BAFF in gut lavage fluid at room temperature for 2 h. A much higher ratio of BAFF to albumin in gut lavage fluid than in blood suggests that BAFF is produced locally in the intestines. Local BAFF may thus contribute to intestinal accumulation of B-lymphocytes and local production of immunoglobulins in patients with self-reported food hypersensitivity. As BAFF is able to induce CSR, the locally produced immunoglobulins may well be of another isotype than IgE.

Recently, Kato *et al.* reported that BAFF is up-regulated in the airways of allergic subjects after allergen exposure.⁷ They collected bronchoalveolar lavage fluids from 12 patients with allergic asthma and four patients with allergic rhinitis after challenge with an allergen. The concentration of BAFF was significantly increased in the bronchoalveolar lavage fluid 20–24 h after challenge, suggesting that BAFF production occurs locally within the airways. BAFF can also be measured in synovial fluids of patients with rheumatoid arthritis.²⁹ The results thus support the notion that BAFF is produced locally in different compartments of the body like synovium, airway and gut during inflammation and allergic reactions.

Previous results in patients with asthma resemble to some extent the present results. Asthma might be both IgE- and non-IgE-mediated, and high serum levels of BAFF were shown in patients with non-IgE-mediated disease, possibly explaining the airway hyper-responsive-

ness in these patients.¹² Anti-asthmatic therapy for 3–6 months reduced the serum BAFF levels significantly, suggesting that BAFF could be a novel parameter to monitor the severity of asthma. Likewise, in patients with coeliac disease, serum BAFF levels correlated with anti-transglutaminase antibody levels, and significant reduction in BAFF was observed after a gluten-free diet.¹⁶ Thus, changes in BAFF levels may be valuable for the follow-up of patients with coeliac disease while on gluten-free diet. In autoimmune diseases, BAFF was correlated with the number of swollen joints in patients with rheumatoid arthritis and treatment with methotrexate reduced the serum levels of BAFF in parallel with improvements in clinical activity and fall in autoantibody titres (IgM rheumatoid factor and anti-cyclic citrullinated peptide).³⁰ BAFF-targeting therapy by BAFF antagonists are therefore promising new therapeutic agents, currently being tried in B-cell-related autoimmune diseases, especially rheumatoid arthritis and systemic lupus erythematosus.³¹ In a previous study, 12% of the patients with self-reported food hypersensitivity also reported joint pain,¹⁸ but whether there exists an association between joint pain and BAFF in these patients remains to be investigated.

Establishment of tolerance is important for preventing the development of food hypersensitivity. In children with cow's milk allergy and in mice models of oral tolerance, IgG subgroups (IgG₄ and IgG_{2a} respectively) appear to play a role in the induction of tolerance.^{32–34} Specific IgG is able to block the binding of IgE to allergen, leading to inhibition of mast cell degranulation and attenuation of IgE-facilitated allergen presentation to specific T cells. Increased specific IgG production as a result of BAFF-induced immunoglobulin CSR may thus be partly responsible for the development of oral tolerance.

Dysregulation of Th1 and Th2 responses may contribute to the pathogenesis of inflammation, autoimmune diseases and asthma. BAFF over-expression in transgenic mice enhances Th1-driven delayed-type hypersensitivity

and inhibits Th2 cell-mediated allergy, resulting in increased secretion of Th1 cytokines like INF γ and less secretion of Th2 cytokines like IL-4 and IL-5.³⁵ Previous studies have shown that inflammatory Th1 cytokines trigger BAFF secretion in affected tissues and that BAFF in turn stimulates B cell and T cell responses locally as well as in neighbouring lymph nodes. In chronic inflammation, BAFF levels may gradually increase in the blood to levels that affect B cell maturation and immune tolerance.³⁶ Some patients with IBS have indications of low-grade chronic inflammation in the intestine.³⁷ Although BAFF signalling may contribute to intestinal inflammation, the mucosal inflammation in patients with IBS is usually subtle³⁸ and the inflammation may not itself be a direct cause of the symptoms, which seem rather related to colonic malfermentation.³⁹ Hence, the possible role of BAFF in symptom generation needs further studies.

In conclusion, increased levels of BAFF in blood and gut lavage fluid suggest that BAFF might be a new mediating mechanism of hypersensitivity reactions to food. Significantly higher levels in non-atopic compared with atopic patients and no correlation between BAFF and IgE levels suggest that BAFF might be particularly involved in non-IgE-mediated reactions. Locally and/or systemically distributed BAFF affects Th1 and Th2 responses and might have an impact on both local (gastrointestinal) and systemic (extraintestinal) symptoms in patients with self-reported food hypersensitivity.

ACKNOWLEDGEMENT

Declaration of personal interests: None. We thank Marianne Eidsheim (The Gade Institute) for skilful and diligent assistance during analysis. We also acknowledge the other members of the MAI (Food Allergy and Intolerance) investigation team at Haukeland University Hospital, for important contributions in the collection of data and for many enriching discussions. *Declaration of funding interests:* None.

REFERENCES

- O'Leary PF, Shanahan F. Food allergies. *Curr Gastroenterol Rep* 2002; **4**: 373–82.
- Sampson HA. Food allergy. Part 1: immunopathogenesis and clinical disorders. *J Allergy Clin Immunol* 1999; **103**: 717–28.
- Kuefner MA, Janson C, Raithel M, Uder M, Bautz W, Schwab SA. A retrospective study of enteroclysis in patients with manifest food allergy. *Clin Imaging* 2009; **33**: 204–8.
- Sicherer SH, Munoz-Furlong A, Murphy R, et al. Symposium: pediatric food allergy. *Pediatrics* 2004; **111**: 1591–4.
- Mackay F, Browning JL. BAFF: a fundamental survival factor for B cells. *Immunology* 2002; **2**: 465–75.
- Stavnezer J, Amemiya CT. Evolution of isotype switching. *Semin Immunol* 2004; **16**: 257–75.
- Kato A, Xiao H, Chustz RT, Liu MC, Schleimer R. Local release of B-cell activating factor of the TNF family after segmental allergen challenge of allergic subjects. *J Allergy Clin Immunol* 2009; **123**: 369–75.

8. Vicario M, Blanchard C, Stringer KF. Local B cells and IgE production in the oesophageal mucosa in eosinophilic oesophagitis. *Gut* 2010; **59**: 12–20.
9. Mackay F, Leung H. The role of the BAFF/APRIL system on T cell function. *Semin Immunol* 2006; **18**: 284–9.
10. Moisini I, Davidson A. BAFF: a local and systemic target in autoimmune diseases. *Clin Exp Immunol* 2009; **158**: 155–63.
11. Ng LG, Mackay CR, Mackay F. The BAFF/APRIL system: life beyond B lymphocytes. *Molecular Immunol* 2005; **42**: 763–72.
12. Kang JS, Yoon YD, Ahn JH, *et al.* B cell activating factor is a novel diagnosis parameter for asthma. *Int Arch Allergy Immunol* 2006; **141**: 181–8.
13. Stohl W, Metyas S, Tan SM, *et al.* B lymphocyte stimulator overexpression in patients with systemic lupus erythematosus: longitudinal observations. *Arthritis Rheum* 2003; **48**: 3475–86.
14. Jonsson MV, Szodoray P, Jellestad S, *et al.* Association between circulating levels of the novel TNF family members APRIL and BAFF and lymphoid organization in primary Sjögren's syndrome. *J Clin Immunol* 2005; **25**: 189–201.
15. Ragheb S, Lisak R, Lewis R, *et al.* A potential role for B-cell activating factor in the pathogenesis of autoimmune myasthenia gravis. *Arch Neurol* 2008; **65**: 1358–62.
16. Fabris M, Visentini D, De ReV, *et al.* Elevated B cell-activating factor of the tumor necrosis factor family in celiac disease. *Scand J Gastroenterol* 2007; **42**: 1434–9.
17. Arslan G, Lind R, Olafsson S, Florvaag E, Berstad A. Quality of life in patients with subjective food hypersensitivity: applicability of the 10-item short form of the Nepean Dyspepsia Index. *Dig Dis Sci* 2004; **49**: 680–7.
18. Arslan G, Kahrs GE, Lind R, Froyland L, Florvaag E, Berstad E. Patients with subjective food hypersensitivity: the value of analysing intestinal permeability and inflammation markers in gut lavage fluid. *Digestion* 2004; **70**: 26–35.
19. Thompson WG, Longstreth GF, Drossman DA, *et al.* Functional bowel disorders and functional abdominal pain. *Gut* 1999; **45**(Suppl 2): II43–7.
20. Berstad A, Arslan G, Folvik G. Relationship between intestinal permeability and calprotectin concentration in gut lavage fluid. *Scand J Gastroenterol* 2000; **35**: 64–9.
21. Francis CY, Morris J, Whorwell PJ. The irritable bowel severity scoring system: a simple method of monitoring irritable bowel syndrome and its progress. *Aliment Pharmacol Ther* 1997; **11**: 395–402.
22. Johansson SGO, Bieber T, Dahl R, *et al.* Revised nomenclature for allergy for global use: report of the nomenclature review committee of the world allergy organization, October 2003. *J Allergy Clin Immunol* 2004; **113**: 832–6.
23. Jones VA, McLaughlan P, Shorthouse M, Workman E, Hunter JO. Food intolerance: a major factor in the pathogenesis of irritable bowel syndrome. *Lancet* 1982; **ii**: 1115–7.
24. Simren M, Mansson A, Langkilde AM, *et al.* Food-related gastrointestinal symptoms in the irritable bowel syndrome. *Digestion* 2001; **63**: 108–15.
25. Monsbakken KW, Vandvik PO, Farup PG. Perceived food intolerance in subjects with irritable bowel syndrome: etiology, prevalence and consequences. *Eur J Clin Nutr* 2006; **60**: 667–72.
26. Lillestøl K, Helgeland L, Arslan Lied G, *et al.* Indications of “atopic bowel” in patients with self-reported food hypersensitivity. *Aliment Pharmacol Ther* 2010; **31**: 1112–22.
27. Lillestøl K, Berstad A, Lind R, Florvaag E, Lied GA, Tangen T. Anxiety and depression in patients with self-reported food hypersensitivity. *Gen Hosp Psychiatry* 2010; **32**: 42–8.
28. Lind R, Lied GA, Lillestøl K, Valeur J, Berstad A. Do psychological factors predict symptom severity in patients with subjective food hypersensitivity? *Scand J Gastroenterol* 2010 [Epub ahead of print].
29. Tan SM, Xu D, Roschke V, *et al.* Local production of B lymphocyte stimulator protein and APRIL in arthritic joints of patients with inflammatory arthritis. *Arthritis Rheum* 2003; **48**: 982–92.
30. Bosello S, Youinou P, Daridon C, *et al.* Concentrations of BAFF correlate with autoantibody levels, clinical disease activity, and response to treatment in early rheumatoid arthritis. *J Rheumatol* 2008; **37**: 1256–64.
31. Sun J, Lin Z, Feng J, Li Y, Shen B. BAFF-targeting therapy, a promising strategy for treating autoimmune diseases. *Euro J Pharma* 2008; **597**: 1–5.
32. Ruiter B, Knol EF, Van Neerven RJ, *et al.* Maintenance of tolerance to cow's milk in atopic individuals is characterized by high levels of specific immunoglobulin G4. *Clin Exp Allergy* 2007; **37**: 1103–10.
33. Li X, Huang CK, Schofield BH, *et al.* Strain-dependent induction of allergic sensitization caused by peanut allergen DNA immunization in mice. *J Immunol* 1999; **162**: 3045–52.
34. Burks AW, Laubach S, Jones SM. Oral tolerance, food allergy and immunotherapy: implications for future treatment. *J Allergy Clin Immunol* 2008; **121**: 1344–50.
35. Sutherland APR, Ng LG, Fletcher CA, *et al.* BAFF augments certain Th1-associated inflammatory responses. *J Immunol* 2005; **174**: 5537–44.
36. Groom J, Kalled SL, Cutler AH, *et al.* Association of BAFF/BLyS overexpression and altered B cell differentiation with Sjögren's syndrome. *J Clin Invest* 2002; **109**: 59–68.
37. Collins SM. Is the irritable gut or inflamed gut? *Scand J Gastroenterol* 1992; **27**: 102–5.
38. Barbara G, De Giorgio R, Stanghellini V, Cremon C, Corinaldesi R. A role for inflammation in irritable bowel syndrome? *Gut* 2002; **51**: i41–4.
39. Madden JA, Hunter JO. A review of the role of the gut microflora in irritable bowel syndrome and the effects of probiotics. *Br J Nutr* 2002; **88**: 67–72.