

Allergy and intestinal dysmotility – evidence of genuine causal linkage?

Simon Murch

Purpose of review

The relationship between allergy and motility has been controversial. There is, however, accumulating evidence demonstrating that mucosal allergic responses may disrupt gut motility, and may also potentially alter nociceptive pathways to cause visceral hyperalgesia.

Recent findings

Experimental studies implicate T helper 2 cells and the cytokines interleukin-4 and -13 in antigen-induced dysmotility, and interleukin-5 in the pathogenesis of mucosal eosinophilia. Both mast cells and eosinophils play obligatory roles in different forms of experimental antigen-induced dysmotility. Overall clinical findings appear to implicate eosinophil infiltration in proximal and distal dysmotility syndromes (oesophageal, gastric and colorectal), and induced mast cell degranulation in mid-gut dysmotility. There is also evidence that mucosal allergic responses may induce long-term changes in visceral perception, including alteration of limbic response, leading to sustained abnormality in visceral sensation.

Summary

Clinical evidence implicating mucosal allergic responses in dysmotility has been extended to include disorders considered previously entirely functional, such as in some cases of irritable bowel syndrome. Only a proportion of cases are, however, caused by food allergy and a future challenge is to differentiate patients with similar symptoms, but induced by different mechanisms.

Keywords

allergy, eosinophils, irritable bowel syndrome, intestinal motility, mast cells, T helper 2 cytokines

Introduction

Food allergy has been linked frequently with intestinal dysmotility, although hard evidence for genuine causal association is relatively recent. Diverse conditions, ranging from infant colic to irritable bowel syndrome (IBS) and constipation, have been reported to improve with exclusion diets; however, many patients manifest apparently identical symptoms induced by nonimmunological or unknown mechanisms [1]. Thus, clear evidence of causal linkage for specific diseases may await better clinical stratification of cases of functional bowel disorders, to identify those subgroups where allergic responses are unequivocally responsible for symptoms.

The major mechanisms potentially linking allergic responses and dysmotility are through T helper (T_h) 2 cytokine production, and recruitment and activation of either mast cells or eosinophils.

Mechanisms of allergic dysmotility

The strongest evidence that intestinal allergic responses can modulate enteric motility has come from a series of studies in animal models.

A role for T helper 2 cells and cytokines

Much understanding of the role of T_h2 cytokines in acquired motility disturbance has come from experimental studies of helminth expulsion from the gut and it has become apparent that these cytokines influence function of both smooth muscle and mast cells. Effective expulsion requires a T_h2 polarised response from CD4⁺ T cells and, thus, induction of a contrary T_h1 polarisation by the cytokine interleukin (IL)-12 impairs parasite expulsion [2]. In study of *Trichinella*-induced hypercontractility, Akiho *et al.* [3] demonstrated that local release of the T_h2 cytokines IL-4 and -13 induced an exaggerated contractile response to carbachol within intestinal smooth muscle. These cytokines altered motility by upregulation of transforming growth factor- β , leading in turn to increased cyclooxygenase-2 and prostaglandin E2 expression within smooth muscle. In follow-up of these animals, it was found that increased transforming growth factor- β expression persisted after T_h2 cytokine production returned to normal levels [3].

Evidence is increasing for the importance of IL-13 in regulating intestinal motility. It shares some actions with IL-4, possibly due to a common signalling

Curr Opin Gastroenterol 22:664–668. © 2006 Lippincott Williams & Wilkins.

Warwick Medical School, Clinical Sciences Research Institute, Coventry, UK

Correspondence to Simon Murch, PhD, FRCP, FRCPCH, Professor of Paediatrics and Child Health, Warwick Medical School, Clinical Sciences Research Institute, Clifford Bridge Road, Coventry CV2 2DX, UK
Tel: +44 0 2476 968693; e-mail: s.murch@warwick.ac.uk

Current Opinion in Gastroenterology 2006, 22:664–668

Abbreviations

ACC	anterior cingulate cortex
DBPCFC	double-blind placebo-controlled food challenge
IBS	irritable bowel syndrome
IL	interleukin
T _h	T helper

© 2006 Lippincott Williams & Wilkins
0267-1379

pathway through one of two IL-4 receptors (which comprises IL-4R α 1 and -13R α 1 chains) [4]. IL-13 may also bind to another receptor (IL-13R α 2), which appears to have inhibitory or decoy function as it does not induce intracellular signalling. Study of the distribution of IL-13 and its receptors within mouse intestine demonstrated high expression of its inhibitory receptor IL-13R α 2 within smooth muscle, while its activating receptor IL-13R α 1 was more widely expressed within epithelium, smooth muscle and myenteric neurones [4]. Mice deficient in the IL-13R α 2 receptor showed exaggerated spontaneous contractility of smooth muscle in small bowel and colon. If applicable to humans, these data imply that regulation of IL-13 responses in allergy might be important in antigen-driven dysmotility.

One long-standing problem in understanding the physiological implications of intestinal food allergy has been the difficulty in establishing animal models of luminal sensitisation. This may have been overcome in a murine model of food allergic enteropathy, induced by feeding egg white to ovalbumin 23-3 T cell receptor transgenic animals [5 $\bullet$]. Chronic egg white feeding induced loose stools and poor weight gain, due to small intestinal enteropathy. Mucosal and mesenteric lymph node T cell responses were skewed towards T_H2 and there was tissue infiltration of IgE⁺ degranulating mast cells. While motility was not directly studied in this initial report, future assessment should allow dissection of events underlying allergic dysmotility.

A role for mast cells

Abnormal increase in mucosal mast cells is not only seen in response to intestinal helminth infection, but has also been recognised for many years in human dysmotility disorders such as IBS. Their proximity to enteric nerves and their triggering by sensitising antigen makes them likely candidates for a genuine role in allergic dysmotility [6]. In a model of food allergy, in which rats were sensitised to ovalbumin by either parenteral or enteral administration, Saavedra and Vergara [7 $\bullet$] have studied mucosal mast cell infiltration and release of mast cell protease, as well as measuring intestinal motility by strain gauge. This study was notable for achieving sensitisation in Sprague-Dawley rats, rather than the high IgE-producing Brown Norway rats usually studied. Unsensitised animals showed regular duodenal contractions at intervals of 1.25–5 min, while sensitised rats showed disturbed motility, with intense clusters of contraction followed by inactivity. These changes were most marked in the duodenum, and independent of systemic IgE response. The changes induced by food sensitisation were chronic, related to mast cell status and persisting after the antigen was withdrawn. It was also noted that *N* ω -nitro-L-arginine did not additionally augment motor

activity, implying that decreased inhibitory tone may mediate hypermotility after sensitisation [7 $\bullet$].

Specific links between mast cell degranulation and altered neural function are likely to centre on released products such as histamine, which may act to stimulate presynaptic inhibitory receptors [8]. Histamine receptors have been characterised within human intestine, with the surprising finding that, although H₁, H₂ and H₄ receptors are widely expressed, H₃ was absent [9]. This is unexpected, as there is physiological evidence for the importance of H₃ signalling in regulation of motility in other species [8]. Ganglion cells expressed H₁ and H₂R, while enterocytes expressed H₄R in addition. H₁R and H₂R were upregulated within the mucosa in food allergy and IBS [9].

A role for eosinophils

Allergy-induced recruitment of eosinophils within the mucosa has substantial clinical and experimental support as an important mechanism of dysmotility [10 $\bullet$]. Evidence that eosinophil recruitment could induce experimental allergic gastric dysmotility was provided in a study [11] of the gastric clearance of ovalbumin-coated beads in sensitised mice. Allergen-induced dysmotility was clearly demonstrated and shown to be abrogated in the absence of the eosinophil chemokine eotaxin-1. Eosinophil mural gastritis has also been reported in man [12], associated with infiltration of eosinophils in the deep layers of the gastric wall, causing severe gastric dysmotility. For reasons currently unclear, the oesophagus is particularly likely to recruit eosinophils in response to both ingested and inhaled antigens, and indeed to systemic T_H2 responses, with the cytokine IL-5 and the chemokine eotaxin-1 critical mediators [10 $\bullet$]. Intriguingly, IL-5 and eotaxin-1 contribute only in part to the mucosal eosinophilia induced by helminths, and do not affect worm expulsion [13]. Thus, an evolutionary explanation for this important oesophageal recruitment pathway has yet to emerge.

Immunological modulation of nociception

While there is increasing evidence for disruption of motility by allergens, there are also emerging data suggesting that mucosal allergic responses may affect visceral sensation, so that induced dysmotility may cause increased symptoms though modulation of nociceptory pathways. The evolving field of psychoneuroimmunology is likely to prove important in understanding the complex interplay between cognitive function, genetic predisposition, immune sensitisation and enteric neural function that may underpin allergic dysmotility syndromes [14]. An extensive consensus review of the field of neurogastroenterology [15 $\bullet$] has highlighted potential links between allergic responses and modulation of visceral afferent neural input. Cytokines and other inflammatory mediators may potentially modulate ion channel activity or postreceptor signalling conduction pathways in

the short term, or induce longer-term alteration in expression and function of receptor molecules or other functional components of the signalling pathway. Functional brain-imaging studies may be able to demonstrate whether peripheral sensitisation due to mucosal immune responses, aberrant central processing or a combination of these factors may be found in allergic dysmotility syndromes characterised by abdominal pain.

Study of central nervous system responses to mucosal allergy has been based on rodent models of 'intestinal anaphylaxis', in which initial sensitisation is followed by later mucosal challenge. Such a protocol [16] was shown to induce brainstem activation within the tractus solitarius, mediated by histamine and 5-hydroxytryptamine signalling through histamine H1 and 5HT3 receptors. By contrast, prostaglandin secretion was not implicated in the central nervous system response in this model.

An important study [17^{••}] identifies for the first time a potentially direct link between mucosal allergic responses and altered nociception within the central nervous system. Visceral hypersensitisation was induced in rats by intraperitoneal injection of ovalbumin in adjuvant. A control group who had not been sensitised was also studied. Limbic function within the anterior cingulate cortex (ACC) was specifically examined by examining single ACC neuronal responses. Three days after initial sensitisation, the animals were given daily rectal infusions of ovalbumin, with subsequent induction of rectal distension after a further 30 min. Controls received distension with rectal saline infusions at similar volumes. The findings were of an abnormal ACC neural response to visceral distension, but only in sensitised animals exposed to ovalbumin. Sensitisation affected the visceral afferent input only and there was a contrasting failure to increase ACC responses to cutaneous noxious heat stimulation [17^{••}]. This study provided evidence that mucosal allergy may induce a visceral nociceptive input that chronically sensitises the ACC neurones which respond specifically to colorectal distension. The effect of such limbic sensitisation is likely to increase pain-related unpleasant sensation and may thus be very relevant to human IBS, as these chronically altered central responses led to a decreased distension threshold and increased neural response when distension occurred.

The presence and function of T cells may be an important modulator of nociception. Early studies had identified β -endorphin production by T cells, acting to reduce peripheral inflammation-induced pain [18]. Evidence from T cell-deficient severe combined immunodeficient mice suggests that mucosal T cells might play an important constitutive role in preventing visceral nociception. In

studies [19^{••}] of colonic distension, comparing unreconstituted severe combined immunodeficient mice with those who had received reconstitution with lymphocytes, it was found that CD4⁺ T cells, but not B cells, were able to restore normal visceral nociception. Severe combined immunodeficient mice without T cells showed abnormal visceral nociception and a reduced pain threshold upon colonic distension. Importantly, the effects of CD4⁺ cells in normalising nociception was abolished by use of the opioid antagonist naloxone methiodide. The authors [19^{••}] demonstrated both production of β -endorphin by activated T cells and increase of β -endorphin within the enteric nervous system upon reconstitution with T cells.

Indirect mechanisms linking food allergy and immune responses

An important indirect mechanism potentially linking allergy and dysmotility is through abnormalities within the gut flora, which may disrupt motility through their effects upon the migrating motor complex [20,21]. Dysbiosis is not only associated with allergic sensitisation, but also may be induced as a consequence of carbohydrate malabsorption in food-sensitive enteropathy. Use of the probiotic *Lactobacillus paracasei* reduced the visceral hypersensitivity developing following antibiotics in mice maintained in a strict pathogen-free colony [22]. The development of dysbiosis-induced hyperalgesia was matched by an induced substance P expression within the myenteric and submucous plexus, which may have been mediated by concomitant low-grade inflammation.

Sensitisation to dietary antigen may also be promoted by psychological stress, in addition to the recognised effects of stress on gastrointestinal motility and visceral pain perception. Chronic stress in rats promoted increased uptake of antigen by follicle associated epithelium [23] and also promoted allergic sensitisation to ingested horseradish peroxidase [24[•]]. The induced T_H2 skewing in this latter model, characterised by increased mucosal IL-4 and decreased interferon- γ , was abrogated by corticotrophin-releasing hormone antagonist [24[•]]. Thus, exposure to oral antigen during periods of chronic psychological stress may promote a tendency to sensitisation and induce a milieu in which induced dysmotility becomes more likely.

Clinical disorders of motility

Recent findings of localised mucosal upregulation of IL-4 and ϵ germline transcripts in food allergy, demonstrating in-situ induction of IgE without systemic IgE responses [25^{••}], suggests that study of circulating immunoglobulins may give misleading insights into genuine mucosal allergic responses. Further uncertainty is added by findings [26[•]] on abdominal ultrasound assessment, following

duodenal allergen challenge, which suggest the unreliability of double-blind placebo-controlled food challenge (DBPCFC) in identifying genuine intestinal allergic responses. Abnormal findings of duodenal wall swelling and fluid exudation were seen not only in patients with positive DBPCFC, but also in several symptomatic patients characterised clinically as showing no response on DBPCFC. It is thus possible that organic symptoms due to genuine mast cell-mediated mucosal allergic responses may be misdiagnosed as psychologically mediated in at least some patients.

Irritable bowel syndrome

Symptoms of apparent food hypersensitivity are commonly reported in IBS, but improvement of symptoms on exclusion diet has often been attributed to food intolerance rather than allergy [1]. Reports of low-grade inflammation with increased mucosal mast cells have been published intermittently for over 40 years [27], although more specific recent data suggest also direct T cell–neural interaction and increased circulating gut-homing lymphocytes expressing β_7 integrin [28].

The evidence for immunological involvement is increasing, and a high tumour necrosis factor-producer/low IL-10-producer phenotype appears significantly more common in IBS patients [29]. Abdominal pain has been found to correlate with activated mast cells adjacent to colonic enteric nerves [30]. This raises the question whether mast cells might be triggered by specific dietary antigen, thus inducing symptoms by the direct effects of mast cell products on enteric nerves. A study of attempted dietary elimination in IBS found reduction in symptom severity in patients excluding foods based on the results of IgG antibody testing, significantly greater than that seen in response to diets based on negative testing antigens [31]. The status of IgG testing in diagnosis of food allergy remains controversial and the implications of this study, and another which identified raised IgG4 titres to wheat, beef, pork and lamb in IBS patients, without increase in specific IgE [32], are as yet uncertain.

Gastro-oesophageal reflux and eosinophilic oesophagitis

There has been long-standing recognition that a proportion of infants with colic or gastro-oesophageal reflux may improve on cow's milk exclusion [33]. While infant colic is unlikely to be a unitary entity, a proportion of infants suffer milk-induced gastro-oesophageal reflux [33]. It remains uncertain whether this disorder is associated, like allergic constipation, with mucosal eosinophilia, and a recent comparative study [34] was unable to differentiate mucosal eosinophil counts between milk allergic and nonallergic infants with reflux. The disorder of milk-induced reflux may thus be distinct from the emerging clinical disease of eosinophilic oesophagitis, in which

oesophageal dysmotility leading to dysphagia is associated with infiltration of eosinophils [35]. A population-based study [36] in the USA suggests that the incidence of eosinophilic oesophagitis may be as high as Crohn's disease, if appropriate investigative strategies are used. In one study [37] of adult eosinophilic oesophagitis, 84% had a history of allergic disease and specific IgE to food allergens or inhaled aeroallergens were identified in 80% of cases. Amongst paediatric cases, up to 75% have been reported to show symptomatic and histological improvement upon dietary antigen elimination [38].

Constipation

Constipation is another disorder in which allergic children with treatment-resistant disease may respond to antigen exclusion. A study of 52 children, treated by exclusion of milk alone, or by more extensive oligoallergenic diet if unresponsive, identified 30 who achieved remission of symptoms on exclusion diet, of whom six were allergic to multiple antigens [39]. Findings upon rectal biopsy in the children with antigen-responsive constipation included epithelial erosions, increased intraepithelial lymphocytes and lamina propria eosinophils. In particular, rectal mucus production was reduced during relapse and restored on exclusion diet.

The same authors have identified adult patients with food allergy associated constipation, and four such patients who were resistant to treatment were placed on an oligoallergenic diet and then underwent successive DBPFC [40]. All normalised bowel function on exclusion diet, relapsing after DBPFC challenge triggered the reappearance of constipation. In comparison with intractably constipated patients unresponsive to exclusion diet, the patients had a higher incidence of self-reported food intolerance and modest decrease in body mass index. These patients also showed lymphocyte and eosinophil infiltration in duodenal and rectal mucosa.

Conclusion

There is increasing clinical evidence, supported by experimental findings, to suggest a true causal link between allergy and intestinal dysmotility. This may be mediated by mast cells, eosinophils and/or T_H2 cytokines such as IL-13. The consequences may include reprogramming of central and peripheral neural function, inducing a state of hyperalgesia in which nociceptive mechanisms remain enhanced even after the antigen is withdrawn. Such mechanisms may be clinically relevant for a currently unknown proportion of patients with functional abdominal pain and other dysmotility syndromes. The challenge is to identify these patients amongst the many presenting with functional bowel disorders. The lack of detectable systemic IgE responses or measurable biomarkers makes this a challenging area of clinical practice.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 692–693).

- 1 Cummings JH, Antoine JM, Azpiroz F, *et al.* PASSCLAIM – gut health and immunity. *Eur J Nutr* 2004; 43 (Suppl 2):II/118–II/173.
- 2 Khan WJ, Blennerhassett PA, Deng Y, *et al.* IL-12 gene transfer alters gut physiology and host immunity in nematode-infected mice. *Am J Physiol Gastrointest Liver Physiol* 2001; 281:G102–G110.
- 3 Akiho H, Deng Y, Blennerhassett P, *et al.* Mechanisms underlying the maintenance of muscle hypercontractility in a model of postinfective gut dysfunction. *Gastroenterology* 2005; 129:131–141.
- 4 Morimoto M, Morimoto M, Zhao A, *et al.* Functional importance of regional differences in localized gene expression of receptors for IL-13 in murine gut. *J Immunol* 2006; 176:491–495.
- 5 Nakajima-Adachi H, Ebihara A, Kikuchi A, *et al.* Food antigen causes T_H2-dependent enteropathy followed by tissue repair in T-cell receptor transgenic mice. *J Allergy Clin Immunol* 2006; 117:1125–1132.
- A transgenic mouse model which is the first to replicate important clinical features of food-sensitive enteropathy.
- 6 Siddiqui AA, Miner PB Jr. The role of mast cells in common gastrointestinal diseases. *Curr Allergy Asthma Rep* 2004; 4:47–54.
- 7 Saavedra Y, Vergara P. Hypersensitivity to ovalbumin induces chronic intestinal dysmotility and increases the number of intestinal mast cells. *Neurogastroenterol Motil* 2005; 17:112–122.
- A study which underlines the importance of mast cell reactivity in antigen-induced dysmotility. Induced changes in motility persisted for a notably long time after antigen withdrawal.
- 8 Wood JD. Histamine, mast cells, and the enteric nervous system in the irritable bowel syndrome, enteritis, and food allergies. *Gut* 2006; 55:445–447.
- 9 Sander LE, Lorentz A, Sellge G, *et al.* Selective expression of histamine receptors H1R, H2R, and H4R, but not H3R, in the human intestinal tract. *Gut* 2006; 55:498–504.
- 10 Rothenberg ME, Hogan SP. The eosinophil. *Annu Rev Immunol* 2006; 24:147–174.
- An outstanding and comprehensive review of eosinophil biology and its relevance to allergic disease.
- 11 Hogan SP, Mishra A, Brandt EB, *et al.* A pathological function for eotaxin and eosinophils in eosinophilic gastrointestinal inflammation. *Nat Immunol* 2001; 2:353–360.
- 12 Martin ST, Collins CG, Fitzgibbon J, *et al.* Gastric motor dysfunction: is eosinophilic mural gastritis a causative factor? *Eur J Gastroenterol Hepatol* 2005; 17:983–986.
- 13 Dixon H, Blanchard C, Deschoolmeester ML, *et al.* The role of Th2 cytokines, chemokines and parasite products in eosinophil recruitment to the gastrointestinal mucosa during helminth infection. *Eur J Immunol* 2006; 36:1753–1763.
- 14 Berstad A, Arslan G, Lind R, Florvaag E. Food hypersensitivity – immunologic (peripheral) or cognitive (central) sensitisation? *Psychoneuroendocrinology* 2005; 30:983–989.
- 15 Grundy D, Al-Chaer ED, Aziz Q, *et al.* Fundamentals of neurogastroenterology: basic science. *Gastroenterology* 2006; 130:1391–1411.
- An important and comprehensive consensus statement on the current state and future challenges of neurogastroenterology research.
- 16 Kreis ME, Muller M, Zittel TT, *et al.* Mediators of neuronal activation in the rat brainstem following intestinal anaphylaxis. *Neurosci Lett* 2000; 289:45–48.
- 17 Gao J, Wu X, Owyang C, Li Y. Enhanced responses of the anterior cingulate cortex neurones to colonic distension in visceroally hypersensitive rats. *J Physiol* 2006; 570:169–183.
- The first study to demonstrate that mucosal allergy may induce a chronic visceral afferent nociceptive input leading to altered limbic function. Neurones within the anterior cingulate cortex showed increased spontaneous activity and showed increased response to rectal distension with lowered threshold of activation.
- 18 Cabot PJ, Carter L, Gaiddon C, *et al.* Immune cell-derived β -endorphin. *J Clin Invest* 1997; 100:142–148.
- 19 Verma-Gandhu M, Bercik P, Motomura Y, *et al.* CD4⁺ T-cell modulation of visceral nociception in mice. *Gastroenterology* 2006; 130:1721–1728.
- T cell function may be important in prevention of intestinal hyperalgesia. Production of β -endorphin by CD4 T cells affects neural response to colonic dilatation. Further study may elucidate whether this property is restricted to particular T cell subtypes and whether β -endorphin production is modulated in allergic diseases.
- 20 Husebye E, Hellstrom PM, Sundler F, *et al.* Influence of microbial species on small intestinal myoelectric activity and transit in germ-free rats. *Am J Physiol Gastrointest Liver Physiol* 2001; 280:G368–G380.
- 21 Barbara G, Stanghellini V, Brandi G, *et al.* Interactions between commensal bacteria and gut sensorimotor function in health and disease. *Am J Gastroenterol* 2005; 100:2560–2568.
- 22 Verdu EF, Bercik P, Verma-Gandhu M, *et al.* Specific probiotic therapy attenuates antibiotic induced visceral hypersensitivity in mice. *Gut* 2006; 55:182–190.
- 23 Velin AK, Ericson AC, Braaf Y, *et al.* Increased antigen and bacterial uptake in follicle associated epithelium induced by chronic psychological stress in rats. *Gut* 2004; 53:494–500.
- 24 Yang PC, Jury J, Soderholm JD, *et al.* Chronic psychological stress in rats induces intestinal sensitization to luminal antigens. *Am J Pathol* 2006; 168:104–114.
- A study of potential clinical relevance, suggesting that psychological stress may play a role in promoting food allergic sensitisation.
- 25 Coeffier M, Lorentz A, Manns MP, Bischoff SC. Epsilon germ-line and IL-4 transcripts are expressed in human intestinal mucosa and enhanced in patients with food allergy. *Allergy* 2005; 60:822–827.
- Compartmentalisation of food allergic responses. Mucosal IgE production is enhanced in food allergy, but without matched systemic IgE responses.
- 26 Arslan G, Gilja OH, Lind R, *et al.* Response to intestinal provocation monitored by transabdominal ultrasound in patients with food hypersensitivity. *Scand J Gastroenterol* 2005; 40:386–394.
- Is the DBPCFC really the gold standard? Similar intestinal secretory responses to antigen challenge seen by ultrasound in patients deemed to have positive or negative challenges.
- 27 Tornblom H, Abrahamsson H, Barbara G, *et al.*, The Swedish Motility Group. Inflammation as a cause of functional bowel disorders. *Scand J Gastroenterol* 2005; 40:1140–1148.
- 28 Öhman L, Isaksson S, Lundgren A, *et al.* A controlled study of colonic immune activity and β_7^+ blood T lymphocytes in patients with irritable bowel syndrome. *Clin Gastroenterol Hepatol* 2005; 3:980–986.
- 29 Van der Veek PP, Van den Berg M, De Kroon YE, *et al.* Role of tumor necrosis factor- α and interleukin-10 gene polymorphisms in irritable bowel syndrome. *Am J Gastroenterol* 2005; 100:2510–2516.
- 30 Barbara G, Stanghellini V, De Giorgio R, *et al.* Activated mast cells in proximity to colonic nerves correlate with abdominal pain in irritable bowel syndrome. *Gastroenterology* 2004; 126:693–702.
- 31 Atkinson W, Sheldon TA, Shaath N, Whorwell PJ. Food elimination based on IgG antibodies in irritable bowel syndrome: a randomised controlled trial. *Gut* 2004; 53:1459–1464.
- 32 Zar S, Benson MJ, Kumar D. Food-specific serum IgG4 and IgE titers to common food antigens in irritable bowel syndrome. *Am J Gastroenterol* 2005; 100:1550–1557.
- 33 Heine RG. Gastroesophageal reflux disease, colic and constipation in infants with food allergy. *Curr Opin Allergy Clin Immunol* 2006; 6:220–225.
- 34 Nielsen RG, Fenger C, Bindslev-Jensen C, Husby S. Eosinophilia in the upper gastrointestinal tract is not a characteristic feature in cow's milk sensitive gastro-oesophageal reflux disease. Measurement by two methodologies. *J Clin Pathol* 2006; 59:89–94.
- 35 Furuta GT. Eosinophilic esophagitis: an emerging clinicopathologic entity. *Curr Allergy Asthma Rep* 2002; 2:67–72.
- 36 Noel RJ, Putnam PE, Rothenberg ME. Eosinophilic esophagitis. *New Engl J Med* 2004; 351:940–941.
- 37 Simon D, Marti H, Heer P, *et al.* Eosinophilic esophagitis is frequently associated with IgE-mediated allergic airway diseases. *J Allergy Clin Immunol* 2005; 115:1090–1092.
- 38 Spergel JM, Andrews T, Brown-Whitehorn TF, *et al.* Treatment of eosinophilic esophagitis with specific food elimination diet directed by a combination of skin prick and patch tests. *Ann Allergy Asthma Immunol* 2005; 95:336–343.
- 39 Carroccio A, Scalici C, Maresi E, *et al.* Chronic constipation and food intolerance: a model of proctitis causing constipation. *Scand J Gastroenterol* 2005; 40:33–42.
- 40 Carroccio A, Di Prima L, Iacono G, *et al.* Multiple food hypersensitivity as a cause of refractory chronic constipation in adults. *Scand J Gastroenterol* 2006; 41:498–504.