Intestinal involvement in atopic disease

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INTRODUCTION

The incidence of atopic disorders is on the increase in industrialized countries. Atopic eczema frequently represents the first manifestation of such disorders. The development of new strategies for the prevention and treatment of atopic disease calls for a better understanding of the processes which initiate sensitization and regulate the inflammatory response in patients with atopic eczema. The underlying denominators in atopic predisposition may be outlined as genetic susceptibility, aberrant barrier functions of skin epithelium and gut mucosa, and dysregulation of immune response to ubiquitous environmental antigens[1-3].

Hereditary predisposition is one important denominator in atopy. Elevated concentrations of serum IgE to common environmental antigens may indicate dysregulation of B-cell responses in atopic eczema[4]. IgE responses are under the control of cytokines produced by competing signals from T helper 1- and T helper 2-like cells[5]. Interleukin (IL)-4 is essential for the development of T helper 2 cells, which induce enhanced IgE production and eosinophilia. Consequently, aberrant cytokine production may play a central role in the pathogenesis of atopic eczema[6]. The fact that allergic reactions to foods frequently comprise the first manifestation of allergic symptomatology in infants with atopic eczema may indicate impaired gut barrier function in atopic disease[7,8].

ATOPIC DERMATITIS: THE FIRST MANIFESTATION OF ATOPIC DISEASE

Atopic dermatitis is one of the clinical manifestations of atopic disease. It usually begins in infancy and early childhood, while allergic rhinitis and asthma generally have their onset at school age. The phenomenon of atopic infants frequently outgrowing their eczema as they develop allergic respiratory disease is called the atopic march or cascade. Hence, atopic dermatitis may be considered an important initial link in the allergic disease family.

Atopic eczema is a chronically relapsing skin disease. The rash is characterized by pruritic, erythematous, papulovesicular lesion progressing to a scaly lichenified state[9]. In infancy, atopic eczema involves the face, scalp and extensor surfaces of extremities. In childhood, it involves flexor surfaces, and in adolescents, the flexor surfaces, hands and feet are affected.

The immunological abnormalities encountered in patients with atopic dermatitis involve both humoral and cell-mediated immune response. Atopic dermatitis is immunologically characterized by increased systemic production of allergen-specific IgE, elevated levels of which can be demonstrated by positive skin prick test reactivity to multiple allergens. T cell response is also altered in atopic patients, involving impaired suppressor T cell function with concomitant activation of T helper 2 cells releasing cytokines relevant to the allergic inflammation, among them IL-4, IL-5 and IL-13, but little interferon-γ. IL-4 regulates IgE synthesis and is critical for the development of T helper 2 cells[6]. IL-5 promotes eosinophil differentiation, proliferation and function. The typical eczematous skin lesions are infiltrated by activated T cells, and keratinocytes show evidence of cytokine-induced activation[10]. The lack of interferon-γ production and the concomitant activation of IL-4 and IL-5, is thought to play a critical role in the pathophysiology of atopic dermatitis.

Atopic eczema patients do not constitute a homogeneous group. The majority of infants with atopic eczema have elevated antigen-specific IgE concentrations, as opposed to a subgroup with no detectable IgE antibodies[8]. Some of them are polysensitized, some monosensitized[8,11]. These patients have also been classified according to their reaction type and reaction onset time to oral food challenge[12,13]. Double-blind placebo-controlled food challenges have demonstrated acute-onset clinical reactions consisting of urticaria, pruritus and erythema in a subset of patients with atopic dermatitis, while others have developed delayed-onset eczematous reactions[8]. Skin prick and patch tests have been found to yield markedly discrepant results in these subgroups. Prick tests were frequently positive in the cases with acute-onset reactions to milk challenge, whereas patch tests tended to be negative, and patch tests were positive in most of those with delayed-onset reactions, though prick tests were frequently negative.

Data are accumulating to suggest that environmental factors contribute to the development of atopic eczema[14]. Infant feeds containing foreign dietary antigens, domestic and industrial air pollutants and exposure to tobacco smoke have been considered as risk factors for atopic sensitization. Recent epidemiological evidence suggests that early

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infection by household contact may exert a protective effect on atopic sensitization[15]. This could be explained by the activation of mainly T helper 1-like cells by viral infections, which may prevent the generation of a T helper 2-like cell population. Susceptibility to atopic sensitization during infancy has been associated with the maturation process of the immune defence mechanisms[16]. Maturation of the mucosal immune system is brought about by microbial stimulation from the natural environment, including the normal microbial flora of the gastrointestinal tract[17–20].

FOOD ALLERGY: A LINK BETWEEN GUT AND SKIN IN ATOPIC DERMATITIS

Food allergy is defined as an immunologically-mediated adverse reaction against dietary antigens. The disorder has been convincingly linked to the development of atopic dermatitis[21]. Cow's milk allergy frequently comprises the first major allergy in infants, since cow's milk is the first important source of antigenic protein consumed in large quantities. Allergy to cow's milk is most prevalent at the age of 1 year, and it coincides with the peak prevalence of atopic dermatitis. Cow's milk allergy has been reported in 2.5% of the general child population[22]. In infants with atopic dermatitis, studies with double-blind, placebo-controlled oral challenge showed the incidence of cow's milk allergy to be 50–60%[8].

Further evidence that food allergy is implicated in atopic eczema is afforded by the clinical benefit from exclusion diets in infants suffering from atopic eczema. Dietary elimination of the antigen responsible is associated with alleviation of clinical symptoms of eczema. In like manner, recent studies have brought evidence that the use of synthetic amino acid-based formulae with further reduced allergenicity compared to the extensive hydrolysed formulae may restore normal growth in infants with atopic dermatitis and multiple food allergies[23,24]. These patients are at risk of nutritional insult due to persistent inflammation in the skin or in the gut, leading to increased utilization or loss of nutrients, and to inadequate intake during a highly restricted dietary regimen[25]. Appropriate antigen elimination based on formal elimination-challenge procedure also reverses some disturbances of the humoral and cell-mediated immune responses to the antigen[23,26].

THE GUT MUCOSAL BARRIER

The mucosal surface forms the largest area of the body in contact with the external environment. The intestinal mucosa is an important organ of host defence, providing a barrier against antigens encountered by the enteric route[17]. Continuous exposure of antigens such as food and microorganisms places high demands on the defence barrier. Protection against such potentially harmful agents is ensured by many non-immunological factors, including saliva, gastric acid, peristalsis, mucus, intestinal proteolysis and intestinal flora. Epithelial cell membranes and intercellular junctional complexes further promote the intestine's barrier function. An immunological barrier is maintained by the unique mucosal immune system, which constitutes an important element in the total immunological capacity of the host. The increased propensity of young infants to food-allergic reactions is believed to be the result of immaturity of the intestine's barrier functions.

The barrier functions are incompletely developed in early infancy. The binding of antigens to immature gut microvillus membrane is increased compared to the mature mucosa, and this has been shown to correlate with the increased uptake of antigens[27]. Intestinal permeability can be secondarily increased as a result of inflammation in the intestinal mucosa induced by viruses, bacteria or dietary antigens. A greater amount of antigens may thus traverse the mucosal barrier and the routes of transport may be altered. During mucosal dysfunction caused by immaturity, infection or hypersensitivity reaction, the normal pattern of antigen handling is impaired[17,19], which may evoke aberrant immune responses and lead to sensitization[28].

ANTIGEN TRANSFER ACROSS THE GUT MUCOSAL BARRIER

Apart from the barrier function, the intestinal mucosa is efficient in assimilating antigens, for which purpose there are specialized transport mechanisms in the villous epithelium[17,19]. Antigens are absorbed across the epithelial layer by transcytosis along two functional pathways[29]. The main degradative pathway includes lysosomal processing of the antigen to smaller fragments, which reduces the immunogenicity of the antigen load. A minor pathway allows the transport of intact unprocessed antigens, which results in antigen-specific immune responses. In particular, this type of antigen transport across Peyer's patches is an important element in mucosal and systemic immune defence. Peyer's patches are areas where specific immune responses are generated. They are the source of IgA-committed plasma cells.

Intestinal antigen handling determines the subsequent immune response to the antigen. An inflammatory reaction is an adaptive response to intraluminal pathogens. By contrast, the mucosal immune system contains mechanisms for selective downregulation of an immunoinflammatory response to ubiquitous antigens such as food.

The phenomenon of oral tolerance, a state of systemic hyporesponsiveness induced by prior oral administration of dietary antigens, has been characterized in experimental animal models[30]. Oral tolerance has been taken to be a concomitant effect of immune exclusion and suppression of

systemic immune response. To test this hypothesis, the ELISPOT or ELISA plaque assay has been used as indirect evidence of gut local immune response[31]. The total number of immunoglobulin-secreting cells (ISC) as an index of antigen-nonspecific immune response and the number of specific antibody-secreting cells (sASC) as an index of antigen-specific immune response have been measured during a cow's milk challenge[13]. The progress of patients with challenge-proven cow's milk allergy was then followed for one year on elimination diet, whereafter they were subjected to rechallenge[16]. Correspondingly, the development of humoral immune response to cow's milk antigens has been evaluated by the ELISPOT method in healthy infants during their first year of life[32,33]. In healthy formula-fed infants IgA sASC to cow's milk antigens were measurable while the serum IgA antibody level was still low. In another study, moreover, during oral milk challenge healthy infants mounted a focused sASC response to cow's milk antigens while antigen-nonspecific ISC responses were not seen. The pattern of the immune response was different in patients with cow's milk allergy. These evinced a high, antigen-nonspecific ISC response to the oral milk challenge. Notwithstanding the distinct increase in the total number of ISC, the sASC response specifically directed against β-lactoglobulin and casein was small and inconsistent. By the time these infants acquired cow's milk tolerance, the total numbers of ISC did not increase during provocation, but they had sASC of IgA isotype. These results indicate that in cow's milk allergy, immune elimination of cow's milk antigens is ineffective. They further suggest that the ability to mount a local immune response against cow's milk antigens, particularly in the IgA class, is related to the suppression of clinical sensitivity.

There is evidence that during the process of absorption across the intestinal mucosa, dietary antigens are altered into tolerogenic form (Figure 1). This implies that notwithstanding the massive antigen load in the normal diet, a well-functioning gut mucosal barrier protects the host against hypersensitivity reactions. The gut microflora is an important constituent in the intestine's defence barrier, particularly against gastrointestinal hypersensitivity disorders[34]. It has been demonstrated that in its absence antigen transport is increased[35]. Moreover, the induction of oral tolerance may be abrogated[18].

GUT DEFENCE IN PATIENTS WITH ATOPIC DERMATITIS AND FOOD ALLERGY

Intestinal inflammation

Antigen-specific IgE antibodies have been found in faeces and intestinal washings of patients with food allergy[36,37] as evidence of local inflammation. Moreover, a significant correlation has been shown between intestinal mast cell
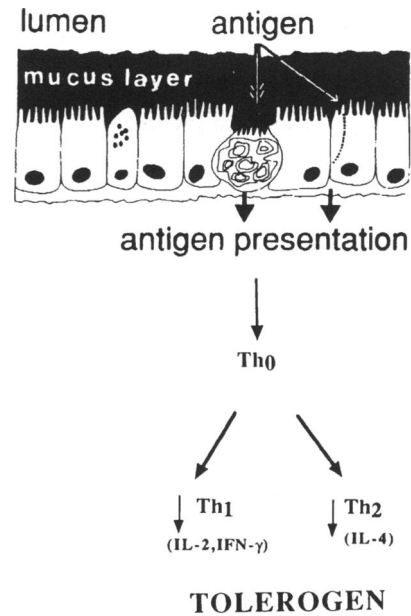

Figure 1 **Hyporesponsiveness to dietary antigens is a hallmark of the intestinal immune system. Oral administration of dietary antigens crossing the intestinal epithelium elicit selective downregulation of immune response. The underlying denominators in selection have been outlined as type and dose of the antigen, age of the host and timing of the encounter**

degranulation and positive clinical response to blinded intragastric challenge[38]. To assess the mucosal responses induced by double-blind, placebo-controlled cow's milk challenge in infants with atopic dermatitis, the concentrations of tumour necrosis factor-α (TNF-α), eosinophil cationic protein (ECP) and α-1 antitrypsin in faeces as indicators of intestinal inflammation have been determined[25]. An increased α-1 antitrypsin concentration was detected in 43% of the infants positive as compared to 11% of those negative to challenge, $P=0.02$. In the cases positive to the oral challenge, an elevated concentration of ECP in faeces was associated with immediate-type reactions to the cow's milk challenge, while TNF-α in faeces was associated with delayed-type reactions to the challenge.

Intestinal permeability

In patients with atopic dermatitis, disturbances in intestinal permeability compared to controls have been reported[39,40]. There is a tendency for younger children to have the highest lactulose/rhamnose ratios. However, some studies have failed to show any permeability disorder in atopic patients[41].

It has been shown that during a cow's milk elimination diet the lactulose/mannitol recovery ratios in urine after an

oral load are comparable in patients with cow's milk allergy and their age-matched controls, while a rise in the lactulose/mannitol excretion ratio follows a positive oral cow's milk challenge[42]. Intestinal permeability changes have been found to be identical in patients who manifested cow's milk allergy cutaneously and gastrointestinally. In like manner, enhanced absorption of macromolecules across the jejunal mucosa has been detected with only minor morphological abnormalities in the small intestinal mucosa in patients with cow's milk allergy, most of whom had predominantly gastrointestinal symptoms[43]. The barrier function was normalized when the antigen responsible was eliminated from the patients' diet. In keeping with this, in adult cow's milk allergic patients it has been shown that cow's milk challenge results in albumin secretion in the gut[44]. These results indicate that intestinal permeability changes are not gross histopathological alterations in the gut mucosa. They further suggest that increased intestinal permeability is caused by a local hypersensitivity reaction.

To explore the intestine's barrier function and antigen transfer across the intestinal mucosa in patients with atopic dermatitis, the absorption and degradation of horseradish peroxidase (HRP) have been studied *in vitro* in Ussing chambers[3]. HRP was chosen as a tracer macromolecule because its structure and transport pattern are comparable to those of dietary antigens. 18 biopsy samples of upper small intestinal mucosa from 14 patients (aged 0.5–8 years) with atopic dermatitis and 18 samples from 15 age-matched controls were examined. The histology of the samples was normal. The mean (95% confidence interval) absorption of intact HRP was significantly higher in children with atopic dermatitis than in controls: 242 (81–404) pmol/ h.cm^2 as against 23 (12–33); $P=0.007$. The absorption of degraded HRP was 972 (732–1213) pmol/h.cm^2 in patients with atopic dermatitis and 672 (532–811) in controls; $P=0.03$. The finding suggests that altered macromolecular absorption in the gut may proceed even when the patient is on elimination diet. The result further supports the notion that enhanced macromolecular absorption may occur even when the mucosal histology is normal.

Reduced secretion of interferon-γ, a constant finding in patients with atopic dermatitis and those with cow's milk allergy[45-47], may explain the aberrant antigen transport observed in these patients. It has been shown that at early stages of gut maturation, interferon-γ can promote the uptake of antigens in Peyer's patches, which play an important immunoregulatory role in the mucosal immune system implying suppression of T cell responses and induction of mucosal IgA responses. The Peyer's patch-targeted effect of interferon-γ may be important in eliciting mucosal immune responses against dietary antigens early in life and aiding immune exclusion of foreign antigens in the normal diet.
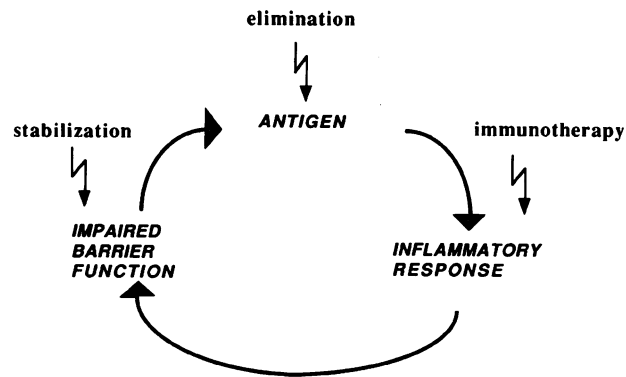

Figure 2 **Food allergy: targets of the treatment. Dietary antigens induce an immunoinflammatory response that impairs the intestine's barrier function in a sensitized subject. Mucosal dysfunction may lead to aberrant absorption of intraluminal antigens. In addition to antigen elimination, therapy exploiting endogenous mechanisms that terminate inflammatory response may offer novel ways of treating hypersensitivity disorders and of preventing the progression of sensitization to debilitating disease**

Taking these results together, impaired barrier function and defective handling of antigens in the epithelial cells may be an important pathogenic mechanism in atopic dermatitis. A hypothesis for the interrelationship between gut and skin in atopic disease is presented in Figure 2. Dietary antigens may evoke a local hypersensitivity reaction which may or may not induce local symptoms. The immunoinflammatory reaction further impairs the intestine's barrier function. Early sensitization to dietary antigens may put the atopic infant in a vicious circle and thereby at risk for developing multiple food allergies.

MODULATION OF THE GUT DEFENCE MECHANISMS IN INFANTS WITH ATOPIC DERMATITIS

Promotion of endogenous barrier mechanisms by probiotic bacteria

Here the relevant study[34] included 14 infants with atopic dermatitis fed an extensively hydrolysed whey formula (group Wh) and 13 given an extensively hydrolysed whey formula supplemented with an intestinal bacterial strain *Lactobacillus* GG (ATCC 53103, group Wh-GG). To evaluate the effect of *Lactobacillus* GG on the clinical course of atopic dermatitis, the severity of eczema was assessed by clinical scoring, SCORAD[9], before and after dietary intervention. To assess the effect of bacteriotherapy on intestinal inflammation, the concentrations of α-1 antitrypsin, TNF-α and ECP in faeces were determined. There was a significant improvement in SCORAD score at one month in group Wh-GG but not in group Wh. In parallel, the concentrations of faecal α-1 antitrypsin and TNF-α decreased significantly in Wh-GG but not in Wh during the one-month management. The concentration of

faecal ECP remained unaltered during dietary management in both groups alike. The result could be explained by stabilization of the intestine's mucosal barrier (Figure 2). Mechanisms beyond this effect could be related to data demonstrating a rise in the production of interferon-γ by probiotic bacteria[48]. Increased interferon-γ secretion might prevent mucosal dysfunction induced by proinflammatory cytokines and restore normal antigen transport mechanisms in the epithelial cells.

Antigen processing in the gut: an immunotherapeutic approach

The capability of intestinal bacteria to modify immunoactivity to dietary antigens may introduce an immunotherapeutic model for food allergy in patients with atopic dermatitis. In a study designed to test this hypothesis, lymphocyte transformation tests were carried out in healthy adults to determine the mitogen-induced proliferative responses of peripheral blood mononuclear cells to caseins with and without in vitro degradation by Lactobacillus GG[49]. In experiments for caseins hydrolysed with pepsin and trypsin, β- and α_{S1}-caseins significantly suppressed proliferation when compared to corresponding control cultures without these hydrolysates. In contrast, κ-casein significantly stimulated proliferation. The hydrolysis of caseins with Lactobacillus GG-derived proteases reversed this stimulation and enhanced the suppression by β- and α_{S1}-caseins. Next, study was made to investigate whether caseins degraded by Lactobacillus GG-derived enzymes could modulate the cytokine production by anti-CD3 antibody-induced peripheral blood mononuclear cells from 14 atopic patients, aged 5–29 months[50]. Without hydrolysation, casein increased the production of IL-4 in cultures of patients with atopic dermatitis, while Lactobacillus GG-hydrolysed casein reduced the production of IL-4.

The results of these studies indicate that degradation of food antigens with intestinal bacteria-derived enzymes modifies their immunomodulatory activity. They further suggest that intestinal bacteria can be a beneficial tool to downregulate the hypersensitivity reactions in the gut.

CONCLUSIONS

Intestinal inflammation, a common phenomenon in food allergy, is an important risk factor in atopic sensitization. As a result of inflammation, a greater amount of antigens may traverse the mucosal barrier, favouring the sensitization process. The approach seeking to control allergic inflammation by antigen elimination only has not proved satisfactory, particularly in patients with multiple food allergies. The results of the studies reviewed here indicate that the gut not only constitutes a target of allergic inflammation but may also act as a route for tolerance

induction. The capability of probiotic bacteria to modify immunoactivity to food antigens and to promote endogenous barrier mechanisms may introduce novel tools for the management of atopic dermatitis (Figure 2).

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