

Increased levels of hyaluronan and albumin after intestinal challenge in adult patients with cow's milk intolerance

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Summary

Background The mechanisms for adverse reactions to foods in the gastrointestinal tract are poorly understood. Presently, only limited possibilities are available for identification of adverse immunological reactions to different foods.

Objective The intestinal inflammatory reactions in adult patients with a history of milk-related gastrointestinal symptoms were studied after intestinal challenges by a jejunal perfusion technique and compared with the reactions in a control group.

Methods Five skin-prick test and radioallergosorbent test negative and lactose tolerant patients with a history of milk-related gastrointestinal symptoms, verified by double-blind placebo-controlled challenge, and eight healthy controls were investigated. Perfusions were performed allowing analyses of a well-defined 'closed' jejunal segment. Milk perfusions were performed in patients and controls after an overnight fast. Ten millilitres of milk were administered to the segment at 3 mL/min. The jejunal fluid levels of hyaluronan (hyaluronic acid) and albumin were measured.

Results In the five patients the milk challenges induced as a mean fivefold increased levels of hyaluronan compared with prestimulation values, whereas no such increases were seen in the control subjects. Albumin, as a marker of plasma leakage, was also increased in the patients but not in the control subjects.

Conclusion The underlying mechanisms for locally increased levels of hyaluronan and also albumin in the intestinal lumen may be secretion of lymph rich in hyaluronan and reflect the mucosal oedema. This capacity of the intestinal mucosa to react with lymph leakage towards a locally infused allergen may be a possible way to delineate gastrointestinal reactions in food-related disorders.

Keywords: gastrointestinal, food allergy, food intolerance, hyaluronan, albumin, challenge

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Introduction

The mechanisms for adverse reactions to foods in the gastrointestinal tract are poorly understood. Presently,

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only limited possibilities are available for identification of adverse immunological reactions to different foods. Moreover, the results of these methods do not always relate to the history of adverse reactions despite severe symptoms. Mucosal oedema is frequently seen in allergy-like reactions and in food intolerance oedema of the ileum has been reported [1]. In the lung, oedema formation was recently found to be related to the levels of hyaluronan [2]. Hyaluronan is a polysaccharide found

extracellularly in all tissues with the highest concentration in soft connective tissues. It has also been shown [3] that local inflammatory processes in lung, skin, middle ear and intestine are accompanied by increased levels of hyaluronan.

The aim of the present study was to elucidate whether or not hyaluronan was present in the jejunal perfusate in patients with a history of milk-induced gastrointestinal symptoms and could be used as an inflammatory marker. Thus, after local food challenge of a perfused jejunal segment, repeated measurements of hyaluronan and albumin, as markers for intestinal secretion of substances from lymph fluid and plasma, were performed.

Materials and methods

Patients

In a pilot study 14 allergic individuals and 24 healthy control subjects were investigated to establish the basal levels of albumin and hyaluronan.

Five women with a history of milk-related diarrhoea and eight healthy volunteers (five men, three women), with mean ages of 54 years and 28 years (ranges 50–59 and 22–36, respectively), were studied (Tables 1 and 2). The patients were diagnosed by double-blind placebo-controlled milk challenges. Skin-prick test and radioallergosorbent test (RAST) for milk were negative and all patients were tested for lactose intolerance. This was achieved by an oral dose of 100 g lactose, which had to produce a blood-glucose of 1.2 mmol or more to be considered as normal. A blood glucose less than 1.2 mmol indicated lactose intolerance.

Most of the patients had a history of migrating arthralgia, joint swelling and mucose in stools and they also had a history of abdominal distention after milk challenge. Abdominal distention was measured by

determining their abdominal circumference at the umbilicus, before and after open milk challenge.

When we planned the actual study we tried to enrol control subjects of the same age as the patients. However, patients have a disease in need of treatment and are easier to enrol in invasive investigations than healthy control subjects. That was why the latter were younger.

Patients and healthy controls all had to answer a standard questionnaire regarding earlier allergic reactions and the study was approved by the Ethics Committee of the Medical Faculty, Uppsala University.

Double-blind, placebo-controlled food challenge (DBPCFC)

Patients were accepted in the study group only after a significant reaction in two repeated double-blind challenges using the suspected antigen, with absence of reaction in two repeated double-blind placebo-controlled challenges in the same patient. The amount of food that elicited symptoms in the blinded tests was the same as in previously performed open studies. To accept a blind challenge as positive the same symptoms, as were produced with the open challenge, had to be repeated. The onset of time and the strength and duration of symptoms should be similar to the open challenge in at least one of two active challenges to be considered as positive.

The challenges were performed at the outpatient clinic. In the open challenges foods were used in their natural form. In the blind challenges the same foods were used, freeze-dried and pulverized if necessary. Dextrose was used as placebo. Foods as well as placebo were placed in opaque gelatin capsules, size 1, and tinted with titanium oxide.

The patients had prior to the investigation been on elimination diet, deprived of all different foods suspected

Table 1. Clinical atopy-related features in five patients with a history of milk-related gastrointestinal symptoms

Patient	Atopic family history	Skin-prick test		Asthma	Rhinitis/conjunctivitis	Eczema	Urticaria
		Inhalants	Foods (not milk)				
1	–	–	–	+	–	–	–
2	+	+	+	+	–	–	–
3	–	+	–	+	+	–	–
4	+	–	–	+	+	–	–
5	–	–	–	+	+	–	–

Patient 2. Skin-prick test positive to soy protein, wheat flour, rye flour and barley meal. These positive reactions were regarded without clinical relevance to the patients cow's milk intolerance.

Table 2. Clinical non-atopy-related features in five patients with a history of milk-induced gastrointestinal symptoms

Patient	Arthralgia	Joint swelling	Mucose in stools
1	++	+	++
2	++	—	+
3	++	+	++
4	+	+	+
5	+	+	++

++. Every day. + Intermittently.

of causing symptoms. Thus, symptoms elicited by the challenge could easily be distinguished. Before and after challenge the patients fasted for 2 h. After each challenge the patients stayed at the clinic for a 2 h observation period and they were also asked to complete a 48-h diary recording food, drugs and symptoms. The time between oral challenges and the perfusion studies always exceeded 3 weeks and the time between the different perfusion studies in the same patient or subject always exceeded 4 weeks. Anti-histamines and drugs containing acetylsalicylic acid were withdrawn during the diary period and for 48 h before the challenges.

Skin-prick test (SPT), allergen extracts for SPT and RAST

The SPT were performed and the reactions were graded according to the recommendations of the Standardization Committee of Northern Society of Allergology [4]. The allergen extracts were prepared and standardized in the laboratory of the Asthma and Allergy Centre at Sahlgrens Hospital, Gothenburg, Sweden. For SPT one panel of alimentary allergens (meat, fish, shellfish, cow's milk, soy protein, egg yolk, egg white, wheat flour, rye flour, oat meal, barley meal, hazelnut, chocolate and vegetables) and another panel of aeroallergens (mite, mould, dander, grass, herb and tree) were used as in an earlier study [5]. All patients and control subjects were tested by Phadebas RAST[®] (Pharmacia Diagnostics AB, Uppsala, Sweden) for milk as described in the test kit instructions from the manufacturer.

Segmental jejunal perfusion

A tube made of polyvinyl chloride and with an outer diameter of 16 French (5.3 mm) was used (LOC-I-GUT, Pharmacia AB, Uppsala, Sweden). The precise use of the technique is explained elsewhere [6]. In short, the tube included six channels and was provided distally with two

40 mm long, elongated latex balloons, each separately connected to one of the smaller channels. The two wider channels in the centre of the tube were for infusion and aspiration of perfusate from the segment. The two remaining peripheral smaller channels were used for administration of marker substances or for drainage. This permitted isolation and perfusion of a segment of 10 cm. A teflon-coated guide wire was used during insertion of the tubes. To further facilitate passage, an 8 cm long weight made from short tungsten plates was attached to the distal end of the tube. The tube was introduced orally and after positioning of the tube in the desired part of the small intestine, as checked fluoroscopically, the guide wire was withdrawn. Continuous gastric drainage was achieved by a separate Salem-sump tube (12 French). The subjects were free to choose a comfortable position sitting or lying down. Air was inflated in the balloons and the test segment was rinsed with 154 mm NaCl, at 3 mL/min for 30 min, using a syringe pump. After the rinsing period, the segment was perfused at 3 mL/min with a solution containing 10 mm glucose, 5.4 mm KCl, 120 mm NaCl, 2 mm Na₂HPO₄, 1 g/L polyethylene glycol (PEG) MW 4000 and 35 mM mannitol. The osmolality of the perfusion fluid was 290 mosm/L. ¹⁴C-labelled polyethylene glycol (¹⁴C PEG, molecular weight 4000 Da, 2.5 µCi/L, Amersham Lab., Buckinghamshire, UK) was added to the perfusate as a volume marker [7]. Ten millilitres of aprotinin (10 000 kIU/mL Trasylol, Bayer AG, Leverkusen, Germany) were added to every litre of perfusion solution in order to inhibit a possible proteolytic activity from proteases that might have contaminated the effluent. Phenol red solution was infused into the stomach through the sump line of the Salem-sump tube at a rate of 1 mL/min and was analysed in the effluent from the intestinal segment. This was made to rule out leakage, i.e. contamination from intestinal fluid by-passing the proximal balloon into the segment. All solutions were infused at 37 °C, collected from the stomach by suction (Ameda Suction Pump Type 23, Ameda Ag, Zug, Switzerland) and from the segment by gravity drainage. Samples of perfusate were collected on ice at 20 min intervals and frozen at -70 °C in plastic vials. Perfusions were performed during 120 min.

Allergens for jejunal challenges

The milk allergen was commercial pasteurized cow's milk and was given as 10 mL (3 mL/min) of undiluted milk after a 40-min baseline period. Whey and casein were obtained from Sigma Chemical Co., St Louis, MO, USA. These allergens were given in the same way as milk allergen.

Analytical measurements

Hyaluronan was analysed by a radioimmunoassay, originally described by Laurent [8], and used according to the manufacturer's instructions (Kabi Pharmacia Diagnostics, Uppsala, Sweden). The test is based on the use of specific hyaluronan binding proteins (HABP) isolated from bovine cartilage. Albumin in the perfusion fluid (50 μ L sample) was measured by double antibody radioimmunoassays (Kabi Pharmacia Diagnostics AB). The coefficient of variation of this method was below 13%. ^{14} C-PEG was determined by liquid scintillation and phenol red was measured spectrophotometrically [9,10]. Since some of the radioimmunoassays could be influenced by the presence of even small amounts of proteases in the effluent, all samples were carefully thawed on ice with the addition of 2 mm (10 μ L of a 0.2 m stock solution) of Phenylmethylsulphonyl fluoride (PMSF; Sigma Chemical Co., St Louis, MO, USA), dissolved in absolute alcohol before all radioimmunoassay (RIA) analyses. PMSF is an irreversible serine protease inhibitor. The samples of effluent were analysed in duplicate for hyaluronan and albumin.

Calculations and statistical analysis

Absolute secretory rates of the analytes were calculated after correction for the minimal ^{14} C losses using the formula: concentration in perfusion fluid 3 mL/min 60 min/10 cm and presented as secretion per cm of bowel and hour. Results are presented as mean $\pm$ SEM. Each patient served as his own control and all results were analysed individually. Statistical significances of differences were performed using Mann-Whitney *U*-test for unpaired and Wilcoxon's test for paired variates. Kendall's rank correlation test was used for correlation of net increases. Differences were considered significant at a *P*-value < 0.05 .

Results

Clinical findings

All five patients had a history of bronchial asthma and three of five patients had a history of rhinitis and/or conjunctivitis. Two of the five patients had positive skin-prick test for inhalant allergens and one of the five patients had a positive skin-prick test for alimentary allergens other than milk (Table 1). Skin-prick test and RAST were negative for milk in all five patients.

The patients were openly challenged with cow's milk with the resulting symptoms according to Table 3. Beside abdominal discomfort and diarrhoea the most

noteworthy finding was that four of five patients increased the abdominal circumference within 60 min after challenge. During and after the jejunal challenge procedures the patients had only diffuse abdominal symptoms.

Presence of allergic mediators

There were no differences in basal appearance rates of the different substances studied between patients and control subjects regardless of age. In fact, when there were repeated studies in the same subjects the mean basal appearance rate of albumin among the patients ($n = 14$ different experiments) was 527 ± 166 and in control subjects ($n = 24$) 661 ± 105 μ g/cm/h, respectively. An even closer relationship was observed regarding basal appearance of hyaluronan, where in patients (same number of experiments) the rate was 628 ± 195 and in control subjects 689 ± 127 ng/cm/h, respectively.

The intestinal challenge with regular cow's milk induced an increased permeation of albumin in all five patients and of hyaluronan in four of five patients in the first 20 min period following luminal administration (Figs 1 a and b). This increase subsided during the following 20 min period. The intestinal challenge induced a more than twofold (albumin; Fig. 1c) up to fivefold (hyaluronan; Fig. 1d) increase, shown as mean values, in the luminal appearance rate of the allergic mediators in the patient group not followed by the control subjects. This significant increase was present during the first 20 min period after challenge only. The highest individual leakage of hyaluronan and albumin was observed in the same patient. The correlation between the increased appearance rate of hyaluronan and albumin after challenge showed a close relationship ($P < 0.001$), indicating that the mechanism underlying the antigen-reaction concomitantly acted on the permeation of these two macromolecules.

There was a close relationship between milk induced increase of abdominal circumference (after open challenge) and the appearance rate of hyaluronan 20 min after jejunal milk challenges ($P < 0.01$) (Fig. 2). No such relationship was seen between the appearance rate of albumin and increase of abdominal circumference.

Challenge tests with casein and whey showed similar results with increases of hyaluronan and albumin among the patients, but not among the control subjects. These data did not reach significant difference, however (data not shown).

Discussion

The data presented in this study revealed that luminal

Table 3. Symptoms related to milk challenges in five patients with a history of milk-induced gastrointestinal symptoms

Patient	Open milk challenges	DBPCFC	Increase of abdominal circumference after open milk challenge (cm)
1	D, OMB	D	6
2	D, AP, AD	D, AP, AD	14
3	D, N	D	2
4	D, AP, N	D, AP, N	6
5	AP, AD, H, N, OMB	AP, AD, H	0

AD, Abdominal distention. AP, Abdominal pain. D, Diarrhoea. H, Headache. N, Nausea. OMB, Oral Mucosal Blebs.

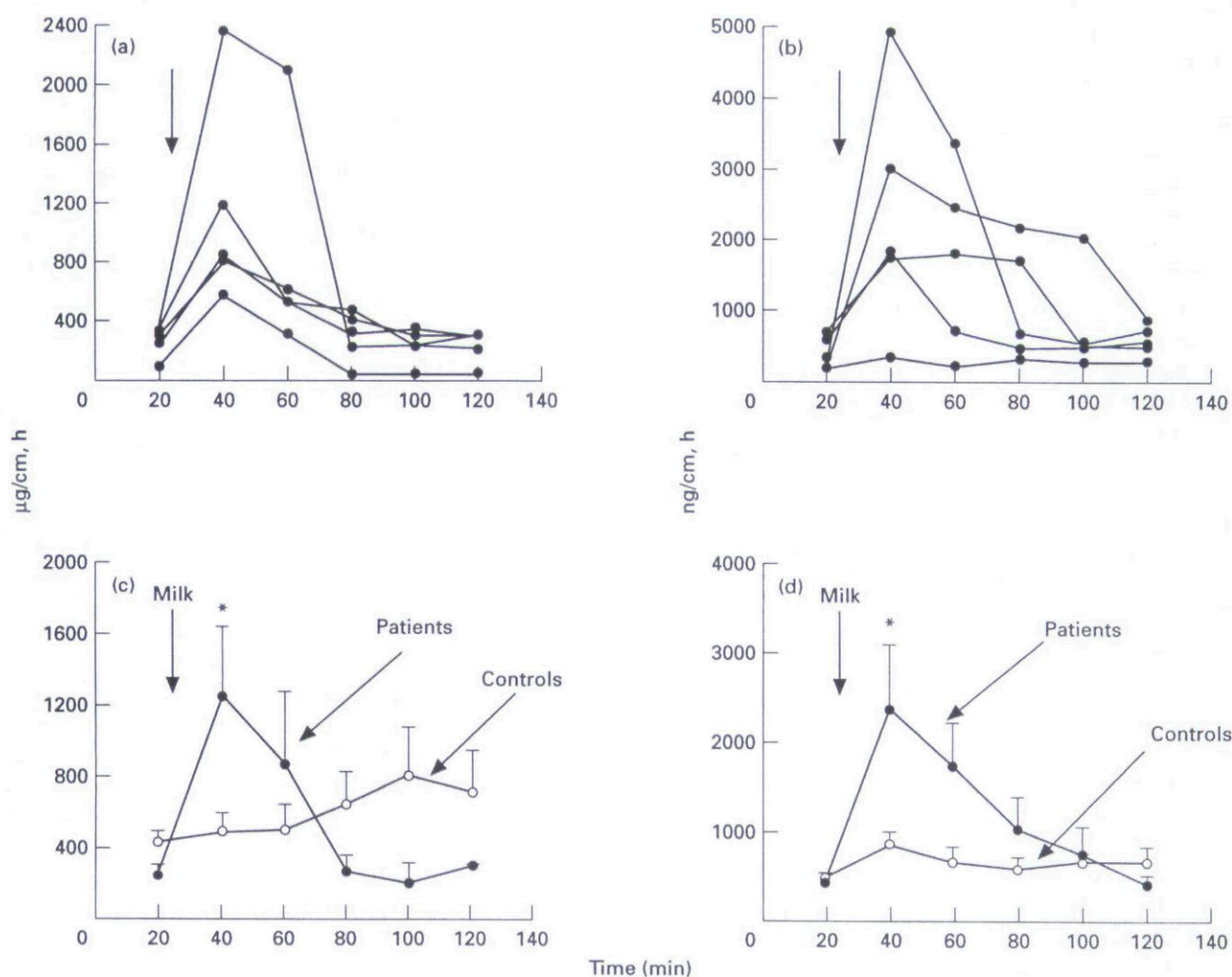


Fig. 1. Intestinal provocation with regular cow's milk (arrow) induced an increased permeation of albumin (a) and of hyaluronan (b) in the patient group. The highest individual leakage of albumin and hyaluronan was observed in the same patient. Data given as mean $\pm$ SEM show a significant increase in the luminal appearance rate of albumin (c) among the same patients ($*P < 0.05$ vs basal values) not followed by a control group. A concomitant fivefold increase in the appearance rate of hyaluronan (d) was consequently noted ($*P < 0.05$) vs basal values) not followed by the control group.

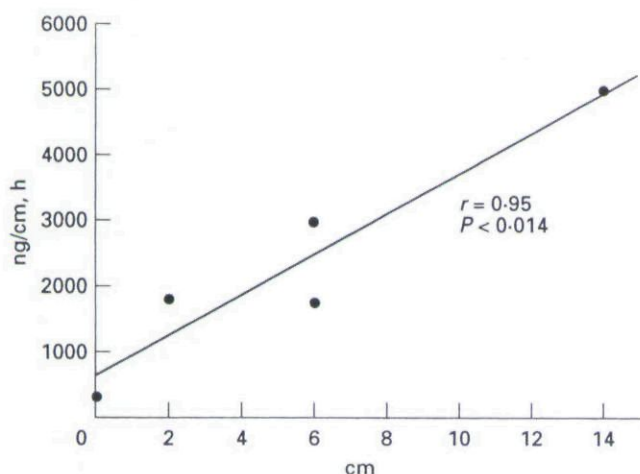


Fig. 2. Correlation between milk induced increase of abdominal circumference (after open challenge) and the appearance rate of hyaluronan 60 mins after jejunal milk challenges ($P < 0.01$).

challenge with cow's milk significantly increased permeation of macromolecules into the proximal jejunum in a group of patients intolerant to this food constituent. Hyaluronan, as a marker for interstitial leakage and oedema and albumin, as a marker of plasma leakage, increased two to five times compared with pre-stimulation values. This increased permeation was not observed in a control group. Luminal challenge with the different milk antigens casein and whey, however, did not induce any significant changes in luminal transport in these patients.

The conclusions stated in the report are based on differences after allergic intestinal provocation, where the patients and healthy control subjects are acting as their own controls and not due to comparisons between the groups. We have in our current studies of inflammatory markers in different diseases not seen any differences that could be correlated to gender whatsoever.

Mucosal inflammation both in the lung and intestine often results in increased permeation into the lumen of plasma proteins as well as proteins synthesized in the target organ itself [11]. In inflammatory bowel disease the increased appearance rate of luminal inflammatory mediators and proteins correlates well with increased disease activity [12]. Both infections [13] and local anaphylactic reactions induce an increased permeation of macromolecules [14]. Accumulated data from studies using bronchial lavage, have demonstrated that hyaluronan is a marker of potential value for the evaluation of disease progress and activity in interstitial lung disease as well as in bronchial asthma [15–18]. Furthermore, in the intestine the luminal appearance rate of hyaluronan is increased in patients with inflammatory bowel disease

[19] and luminal challenges with gliadin in patients with coeliac disease induce pronounced release of hyaluronan [20].

The patients in the present study were SPT and RAST test negative. However, the appearance of hyaluronan in intestinal secretions within 20 min after milk challenge may suggest a local intolerance in the intestine. A mechanism similar to that was also demonstrated in a recent report comparing the mucosal response in allergic and non-allergic patients [21]. Locally appearing IgE antibodies may mediate this intolerance and the reaction can take place in the wall of the intestine. This has been indicated in studies in patients with nasal allergy [22,23], in children with IgE antibodies to foods in faeces [24] and in a recent study with food-related diarrhoea with IgE-staining mast cells [5]. In the present study the content of IgE-antibodies in the jejunal perfusate was not evaluated.

Hyaluronan plays an important role in water balance in several tissues. The overall shape of the molecule is spherical, but only 0.1% of the content is polysaccharide, while the rest is water. Thus, the hyaluronan molecule possesses a pronounced water-binding capacity [25]. Its influence on pulmonary physiology by contribution to interstitial alveolar oedema has earlier been reported [15–18] and excessive accumulation of hyaluronan, as a basis for transplantation oedema, has been demonstrated in rejecting kidneys [26] and transplanted small intestine [27]. Intestinal oedema as a manifestation of food allergy has been reported and might reflect intestinal leakage of lymph/interstitial fluid as a consequence of the inflammatory reaction induced by the allergen [1]. Enalapril, an angiotensin converting-enzyme inhibitor, has also been known to induce mucosal and submucosal oedema, indicating an enhanced response from the intestinal connective tissue [28]. Abdominal distention is a common complaint in patients with food-related symptoms [29] as it was in the present study. This was objectively verified 30 min after intestinal challenge in one patient, in whom the abdominal circumference increased 14 cm during the study. This reaction was interpreted as intestinal swelling and oedema resembling other reactions of intolerance.

The close relationship in time between the increased jejunal appearance rate of hyaluronan and albumin may suggest mutual pathways for activation and release. Both substances are probably secreted into the intestinal lumen mainly as a contribution from lymph/interstitial fluid (hyaluronan) and to a lesser extent from plasma (albumin). Allergic reactions are mediated through histamine release from activated mast cells [30] and also through the release of several cytotoxic constituents from eosinophils mediating cell damage [31]. Morphometric

studies of the gut have revealed increased numbers of intraepithelial eosinophils in cow's milk allergy with circulating IgE antibodies [32]. Such basic means of allergic activation have also been reported regarding local challenge in patients with rhinitis [33]. It can be argued that increasing age in the study group would influence the outcome of the present study and enhance intestinal reactivity. Increasing age *per se* has not been found to be positively correlated with allergic reaction, in fact both prevalence for positive skin tests and weal index for histamine are reported to decrease with age [34,35].

In conclusion, the intestinal mucosa in milk intolerant patients without circulating IgE antibodies, but not control subjects, reacted to a locally infused allergen with increased transport of macromolecules. The appearance rate of albumin and hyaluronan reflected an antigen-induced lymphoedema and can provide an approach to comprehend cellular and mucosal events in food-related disorders.

References

- Robertson D. Oedema of the ileum: a possible manifestation of food allergy. *BMJ* 1987; 294:350.
- Nettelblad O, Bergh J, Schenholm M, Tengblad A, Hällgren R. Accumulation of hyaluronic acid in the alveolar interstitial tissue in bleomycin-induced alveolitis. *Am Rev Resp Dis* 1989; 139:759–62.
- Engström-Laurent A, Laurent TC. Hyaluronan as a clinical marker. In: Lindh E, Thorell J, eds. *Clinical impact of bone and connective tissue markers*. London: Academic Press, 1989; 235–52.
- Aas K, Backman A, Belin L, Weeke B. Standardization of allergen extract with appropriate methods. The combined use of skin prick testing and radioallergosorbent tests. *Allergy* 1978; 33:130–1.
- Bengtsson U, Rognum TP, Brantzaeg P, Kilander A, Hansson G, Ahlstedt S, Hanson LÅ. IgE-positive duodenal mast cells in patients with food-related diarrhea. *Int Arch Allergy Appl Immunol* 1991; 95:86–91.
- Knutson L, Odlin B, Hällgren R. A new technique for segmental jejunal perfusion in man. *Am J Gastroenterol* 1989; 84:1278–84.
- Hydén S. The recovery of polyethylene glycol after passage through the digestive tract. *Ann Roy Agr Coll* 1956; 22:411–24.
- Laurent UBG, Tengblad A. Determination of hyaluronate in biological samples by a specific radioimmunoassay. *Ann Biochem* 1980; 109:394–6.
- George JD. New clinical method for measuring the rate of gastric emptying: the double sampling test meal. *Gut* 1968; 9:237–42.
- Hohman A, Labrooy J, Davidson GP, Shearman DJC. Measurement of specific antibodies in human intestinal aspirate: Effect of the protease inhibitor Phenylmethylsulphonyl Fluoride. *J Immunol Methods* 1983; 64:199–204.
- Persson CGA. Role of plasma exudation in asthmatic airways. *Lancet* 1986; ii:1126–9.
- Ahrenstedt Ö, Knutson L, Nilsson-Ekdahl K, Krogh M, Odlin B, Hällgren R. Evidence of enhanced local production of the complement components C4, C3 and factor B in the small intestine in Crohn's disease. *N Eng J Med* 1990; 322:1345–9.
- Murray M, Jarret WFH, Jennings FW. Mast cells and macromolecular leak in intestinal immunological reactions. The influence of sex of rats infected with *Nippostrongylus brasiliensis*. *Immunology* 1971; 21:17–31.
- Gustavsson B, Persson CGA. Allergen-induced mucosal exudation of plasma into rat ileum and its inhibition by budesonide. *Scand J Gastroenterol* 1992; 27:587–93.
- Bjerner L, Engström-Laurent A, Thunell M, Hällgren R. Mastocytosis in bronchoalveolar lavage fluid from patients with sarcoidosis is related to increased lavage levels of hyaluronic acid, a potential marker of fibroblast activation. *Thorax* 1987; 42:933–8.
- Bousquet J, Chanez P, Lacoste JY et al. Indirect evidence of bronchial inflammation assessed by titration of inflammatory mediators in BAL fluid of patients with asthma. *J Allergy Clin Immunol* 1991; 88:649–60.
- Hällgren R, Eklund A, Engström-Laurent A, Schmekel B. Hyaluronate in bronchoalveolar lavage fluid: a new marker in sarcoidosis reflecting pulmonary disease. *Br Med J* 1985; 290:1778–81.
- Larsson K, Eklund A, Malmberg P, Bjerner L, Lundgren R, Belin L. Hyaluronic acid (Hyaluronan) in BAL fluid distinguishes farmers with allergic alveolitis from farmers with asymptomatic alveolitis. *Chest* 1992; 101:109–14.
- Ahrenstedt Ö, Knutson L, Hällgren R, Gerdin B. Increased luminal release of hyaluronan in uninvolved jejunum in active Crohn's disease but not in inactive disease or in relatives. *Digestion* 1992; 52:6–12.
- Lavö B, Knutson L, Löf L, Odlin B and Hällgren R. Signs of increased leakage over jejunal mucosa during gliadin challenge of patients with coeliac disease. *Gut* 1990; 31:153–7.
- Knutson TW, Bengtsson U, Dannæus A, Ahlstedt S, Stålenheim G, Hällgren R, Knutson L. Intestinal reactivity in allergic and non-allergic patients. An approach to determine the complexity of the mucosal reaction. *J All Clin Immunol* 1993; 91:553–9.
- Huggins KG, Brostoff J. Local production of specific IgE antibodies in allergic-rhinitis patients with negative skin tests. *Lancet* 1975; ii:148–50.
- Small P, Barrett D, Frenkiel S, Rochon L, Cohen C, Black M. Measurement of antigen-specific IgE in nasal secretions in patients with perennial rhinitis. *Ann Allergy* 1985; 55:68–71.
- Kolmannskog S, Haneberg B. Immunoglobulin E in feces from children with allergy. Evidence of local production of IgE in the gut. *Int Arch Allergy Appl Immunol* 1985; 76:133–7.

- 25 Laurent TC. Biochemistry of Hyaluronan. *Acta Otolaryngol* 1987; Suppl. 442:7–24.
- 26 Hällgren R, Gerdin B, Tufveson G. Hyaluronic acid accumulation and redistribution in rejecting rat kidney graft. Relationship to the transplantation edema. *J Exp Med* 1990; 171:2063–76.
- 27 Knutson L, Meurling S, Wahlberg J, Hällgren R, Tufveson G. Functional studies on the human transplanted small intestine. *J Clin Transplant* 1993; 7:151–7.
- 28 Farraye FA, Pappercorn MA, Steer ML, Joffe N, Rees M. Acute small-bowel mucosal edema following enalapril use. *JAMA* 1988; 259:3131.
- 29 Nanda R, James R, Smith H, Dudley CRK, Jewell DP. Food intolerance and irritable bowel syndrome. *Gut* 1989; 30:1099–104.
- 30 Perdue MH, Chung M, Gall DG. The effect of intestinal anaphylaxis on gut function in the rat. *Gastroenterology* 1984; 86:391–7.
- 31 Parillo JE, Fauci AS. Human eosinophils. Purification and cytotoxic capability of eosinophils from patients with the hypereosinophilic syndrome. *Blood* 1978; 51:457–73.
- 32 Maluenda C, Philips AD, Briddon A, Walker-Smith JA. Quantitative analyses of small intestinal mucosa in cows milk sensitive enteropathy. *J Pediatr Gastroenterol Nutr* 1984; 3:349–56.
- 33 Svensson C, Andersson M, Persson CGA, Venge P, Alkner U, Pipkorn U. Albumin, bradykinins and eosinophil cationic protein on the nasal mucosal surface in patients with hayfever during natural allergen exposure. *J Allergy Clin Immunol* 1990; 85:829–33.
- 34 Mesinga TT, Schouten JP, Rijcken B, Weiss ST, Van Der Lende R. Host factors and environmental determinants associated with skin test reactivity and eosinophilia in a community-based population study. *Ann Epidemiol* 1994; 4:382–92.
- 35 Niemeijer NR, De Monchy JG. Age-dependency of sensitization to aero-allergens in asthmatics. *Allergy* 1992; 47:431–5.

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