PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

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CLINICAL RESEARCH

Colorectal mucosal histamine release by mucosa oxygenation in comparison with other established clinical tests in patients with gastrointestinally mediated allergy

M Raithel, M Weidenhiller, R Abel, HW Baenkler, EG Hahn

M Raithel, R Abel, EG Hahn, Functional Tissue Diagnostics, Gastroenterology, Department of Medicine I, University Erlangen-Nuremberg, Germany
HW Baenkler, Clinical Immunology and Allergology, Department of Medicine III, University Erlangen-Nuremberg, Germany
M Weidenhiller, Medicine Clinics III, Klinikum Augsburg, Germany
Correspondence to: Martin Raithel, MD, Functional Tissue Diagnostics, Gastroenterology, Department of Medicine I, University Erlangen-Nuremberg, Ulmenweg 18, Erlangen 91054, Germany. martin.raithel@med1.imed.uni-erlangen.de
Telephone: +49-9131-8535151 Fax: +49-9131-8535152
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Abstract

AIM: This study evaluated colorectal mucosal histamine release in response to blinded food challenge-positive and -negative food antigens as a new diagnostic procedure.

METHODS: 19 patients suffering from gastrointestinally mediated allergy confirmed by blinded oral provocation were investigated on grounds of their case history, skin prick tests, serum IgE detection and colorectal mucosal histamine release by *ex vivo* mucosa oxygenation. Intact tissue particles were incubated/stimulated in an oxygenated culture with different food antigens for 30 min. Specimens challenged with anti-human immunoglobulin E and without any stimulus served as positive and negative controls, respectively. Mucosal histamine release (% of total biopsy histamine content) was considered successful (positive), when the rate of histamine release from biopsies in response to antigens reached more than twice that of the spontaneous release. Histamine measurement was performed by radioimmunoassay.

RESULTS: The median (range) of spontaneous histamine release from colorectal mucosa was found to be 3.2 (0.1%-25.8%) of the total biopsy histamine content. Food antigens tolerated by oral provocation did not elicit mast cell degranulation 3.4 (0.4%-20.7%, P = 0.4), while anti-IgE and causative food allergens induced a significant histamine release of 5.4 (1.1%-25.6%, P = 0.04) and 8.1 (1.5%-57.9%, P = 0.008), respectively. 12 of 19 patients (63.1%) showed positive colorectal mucosal histamine release in accordance with the blinded oral challenge responding to the same antigen (s), while the specificity of the functional histamine release to accurately recognise tolerated foodstuffs was found to be 78.6%. In comparison with the outcome of blinded food challenge tests, sensitivity and specificity of history (30.8% and 57.1%), skin tests (47.4% and 78.6%) or antigen-specific serum IgE determinations (57.9% and 50%) were found to be of lower diagnostic accuracy in gastrointestinally mediated allergy.

CONCLUSION: Functional testing of the reactivity of colorectal mucosa upon antigenic stimulation in patients with gastrointestinally mediated allergy is of higher diagnostic efficacy.

Key words: Gut; Histamine release; Mucosa oxygenation; Food allergy diagnostics; Gastrointestinally mediated allergy

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INTRODUCTION

Gastrointestinal complaints after the ingestion of certain foodstuffs often pose diagnostic problems in various clinical situations such as food hypersensitivity, enzyme deficiencies, irritable bowel syndrome, inflammatory bowel disease, dyspepsia, eosinophilic gastroenteritis and several others. However, identification of immunologically mediated food hypersensitivity at the gastrointestinal level remains problematic, since skin tests and allergen specific serum IgE detection (e.g. RAST) may fail to show clear signs of food-specific sensitisation[1-5] and do not necessarily indicate symptomatic food allergy. This is also valid for functional tests using blood cells (basophil histamine, or leucotriene release), lymphocyte transformation tests or measurement of mediators in blood or serum[6-8]. Oral provocation, referred to as the 'gold standard' for food allergy diagnosis, is both time consuming and cost intensive, may put the patient at a

more or less severe risk, allows only one food to be tested per day, is often difficult to evaluate and is without doubt an unpopular and irritating procedure for the patient[1,5,8].

Consequently, different methodical approaches have emerged for the improvement and acceleration of the cumbersome diagnostic way to identify patients with food allergy primarily involving the gastrointestinal tract. Already in 1942, 1984 and 1997, direct endoscopic observation has repeatedly been claimed to be of diagnostic value for recognition of food allergy when antigenic solutions were applied to the intestinal mucosa[9-11]. However, direct endoscopic evaluation of allergen application and endoscopic or fluoroscopic balloon perfusion techniques harbour similar disadvantages to those experienced in oral provocation tests. All of these tests require special conditions with strict medical supervision because of the risk of allergic reactions *in vivo* during endoscopy or allergen perfusion[10-12]. Only a few allergens can be tested during one examination and, in case of an endoscopic allergen injection, the endoscopic procedure is prolonged by at least 20 min, providing a higher risk of procedure-related complications, bacterial translocation and discomfort to the patient[10-14]. Finally, the outcome of these endoscopic methods and the results obtained from double-blind, placebo-controlled food challenge procedures have never been directly compared on a scientific basis. Nevertheless, this is absolutely necessary for an appropriate evaluation in routine practice with respect to their diagnostic effectiveness[1,5,8].

Another possibility to identify food allergy at the gastrointestinal tract was described in 1989. Baenkler *et al* used endoscopically taken samples from the duodenum to show antigen-induced histamine release *ex vivo*[15,16]. Since many patients with suspected gastrointestinally mediated allergy (GMA) have to undergo endoscopic procedures for differential diagnostic reasons, this approach has the advantage that the principle demonstration of a food-induced mediator release can be performed outside of the patient, thus avoiding the risk of allergic reactions *in vivo*. In addition, several food antigens can be tested simultaneously without further burden to the patient[15-17]. However, performance of functional histamine release experiments requires certain laboratory equipment and, initially, should be compared with the 'gold standard' for food allergy diagnostics before introducing this approach into clinical practice.

Similar to Baenkler's approach using duodenal mucosa, functional histamine release should also be expected from mucosa of other regions of the intestine[15-17], provided that these tissue samples contain large enough numbers of (mucosal) mast cells. However, *ex vivo* histamine release from viable tissue samples of the lower gastrointestinal tract in response to nutritive antigens has not yet been studied together with the standardised *in vivo* oral challenge tests in order to provide a direct comparison of the two diagnostic methods. For this reason, this study investigated the rate of histamine release from colorectal mucosal samples in a group of patients with proven food allergy and compared the results of *ex vivo* mucosa oxygenation with the outcome of standardised blinded oral provocation tests.

Table 1 Patient data, allergens used for oral provocation, colorectal mucosal histamine release, skin prick test, serum IgE detection (RAST test), and patient's history

Pat. No.	Sex	Age	Allergen	Oral provocation	Colorectal HR	Skin test	RAST IgE	H
1WT	F	44	Cheese	+	+	-	+	?
			Rye bran	-	-	+	+	?
2DF	F	49	House dust	+	+	+	+	?
			Rye pollen	-	+	-	+	?
3OL	M	41	Soya flour	+	+	+	+	+
			Rye flour	-	-	-	+	?
4CG	M	23	Rye flour	+	+	-	-	-
5NA	F	42	Wheat flour	+	-	+	+	?
			Soya flour	-	-	-	+	?
6IL	F	43	Wheat bran	+	+	-	+	?
			Barley flour	-	-	+	-	?
7GG	F	19	Soya flour	+	+	+	+	?
			Wheat flour	-	+	-	+	?
8FJ	F	25	Soya lecithin	+	-	+	+	+
			Milk	-	-	-	+	+
9MR	F		Milk	+	-	-	-	-
			Potato	-	-	-	-	-
10MB	F	23	Wheat/rye	+	-	+	+	-
			Egg	-	-	-	-	+
11FB	F	51	Wheat flour	+	+	-	+	+
12RA	M	38	Spices	+	+	-	-	+
			Milk	-	-	-	-	+
13KF	M	32	Apple	+	+	+	+	-
			Potato	-	-	-	-	-
14AC	F	40	Nuts	+	+	-	-	-
			Maize	-	-	-	+	-
15KF	M	38	Nuts	+	-	+	+	-
16TM	F	28	Nuts	+	-	-	-	-
17SJ	F	37	Nuts	+	-	-	-	-
18LS	M	44	Nuts	+	+	-	-	-
			Rice	-	-	-	-	-
19SL	M	29	Wheat flour	+	+	+	-	?
			Potato	-	+	+	-	?

HR: histamine release; no.: number; pat.: patient; H: patients' history with regard to causative allergens and tolerated foodstuff.

MATERIALS AND METHODS

Patients

A total of 19 patients (7 male, 12 female; median age 38.0, range 19-51 years) were included in this study (Tables 1 and 2). All patients gave their informed consent and the study protocol was approved by the local ethics committee (No. 331). All patients (100%) reported abdominal symptoms, nausea, pain, vomiting and/or diarrhoea (98%) after certain meals, while postprandial extraintestinal signs of allergy such as skin reactions, asthma bronchiale and allergic rhinoconjunctivitis occurred only in a small percentage of patients (32%). Every patient was assessed on grounds of their history and detailed skin prick tests of environmental allergens (moulds, fibres, bacteria, pollen, dust) and food allergens (fish, fruit, vegetables, grains and different types of wholemeal, flour, bran, tea, coffee, eggs, milk, and cheese). Serum IgE detection of the putative allergens was performed according to the patients' history or skin tests (Tables 1 and 2). In the case of uncertainties about non-tolerated foods, tests were conducted for basic foodstuff. Case history, skin test reactions and RAST results were then compared with the outcome of oral

Table 2 Clinical symptoms induced by blinded, placebo-controlled food challenge, atopy status and predominant type of allergy according to Coombs and Gell

Pat. No.	Main symptoms	Atopy status	Type of allergy
1	Diarrhoea, flush, pruritus	-	Type I (systemic IgE)
2	Abdominal pain, loose stools bloating	+	Type I (systemic IgE)
3	Vomiting, diarrhoea	+	Type I (systemic IgE)
4	Abdominal pain, urticaria	-	Type I (local IgE)
5	Diarrhoea, abdominal pain dyspepsia, vomiting	+	Type I (systemic IgE)
6	Diarrhoea	-	Type I (systemic IgE)
7	Vomiting, loose stools right lower quadrant pain	+	Type I (systemic IgE)
8	Profuse watery diarrhoea	-	Type I (systemic IgE)
9	Diarrhoea, bloating, tachy-cardia	+	Type III (immune com-plexes present) or IV (?)
10	Pruritus, Rhinitis, tachycardia bloating, diarrhoea	-	Type I (systemic IgE)
11	Colitis, diarrhoea, arthragia rhinitis	-	Type I (systemic IgE)
12	Bloody diarrhoea, hypotension, abdominal pain, bloating	-	Type I (local IgE) and /or type III (immune complexes present)
13	Fever, diarrhoea, hypotension	+	Type I (systemic IgE)
14	Diarrhoea, vomiting, abdominal pain	-	Type IV (cellular hyper-sensitivity ?)
15	Bloating, diarrhoea, eosinophilia	+	Type I (systemic IgE)
16	Atopic eczema, diarrhoea, colitis, abdominal pain	+	Type I (local IgE) and /or type IV (cellular hyper-sensitivity ?)
17	Rhinitis, vomiting, diarrhoea	+	Type I (local IgE)
18	Diarrhoea, bloating	+	Type I (local IgE)
19	Eosinophilia, bloating, diarrhoea	+	Type I (systemic IgE)

Atopy status was defined as positive, when history or clinical manifestation of the patient gave evidence for milk crust, atopic eczema, asthma bronchiale and/or allergic rhino-conjunctivitis. For definition of the allergy type, the most dominant immunological signs were chosen to classify the ongoing allergic mechanisms in this population of patients with manifest gastrointestinally mediated allergy. However, some patients displayed symptoms that suggested more than one definitive type of allergy (see for example patient No. 9, 12, 16): Type I allergy (systemic IgE) was recognised when positive skin or antigen specific IgE levels were present in serum, type I allergy (local IgE) was diagnosed when intestinal lavage fluid contained elevated levels of IgE[20]. Type Ⅲ allergy was found in patients no. 9 and 12 who showed formation of either IgA, IgM and/or IgE immune complexes during or after allergen application by blinded food challenge. Additionally, type IV allergy was suspected in patients with heightened serum TNF levels during food challenge, and additionally in patient no. 14 who had a positive antigen-specific lymphocyte proliferation test.

challenge tests.

Food allergy was confirmed in each patient by blinded, placebo-controlled food challenge tests (BPCFC) adding the putative allergen to a basic diet containing rice, potato, oligopeptides (Survimed OPD, Fresenius, Germany) and tea. For provocation of flours (wheat, rye, soya, barley), commercially available allergen solutions for skin tests were used (Maser, Bochum, Germany). These were applied orally, while all other allergens were freshly prepared and given to the patients via a nasogastric tube[5,18].

BPCFC was performed in a standardised fashion, while patients were hospitalised. Food antigen was administered in three different doses. Initially, a 1/20 dilution of the native allergen solution was administered, followed by 1/10 of the dose 3 h later and finally, a dose of 5 mL of the full strength native allergen solution was provided[18,19]. One single food antigen was tested per day. Placebo consisted of an oligopeptide-diet (protein source: hydrolysed soybean, Survimed OPD, Germany), which was also used for base-line nutrition (minimum: 1800 kcal/d), in conjunction with a rice-potato diet in order to prevent a catabolic state[18,19]. A single blind challenge was performed in 42% (patients unaware of provocation protocol), while a double-blind challenge was carried out in 58% (patients and physicians unaware of the provocation protocol)[18,19]. Blinding of the food antigens was managed by nutritionists, who were responsible for the preparation and addition of the allergens to usually tolerated foodstuff or to the oligopeptide solution, respectively.

Physicians selected the type of food to be tested either on the basis of the patients' history, previous results of skin prick tests and RAST tests or from a list of basic foodstuff. During the provocation procedure, the patients were provided with a peripheral venous line, and all medical staff involved was trained for medical intervention in case of an anaphylactic reaction. For the definition of food allergic reactions, a modified scoring system for symptoms was applied[18] and main symptoms of patients evoked by the food challenge are listed in Table 2.

Food hypersensitivity was diagnosed only when food-specific immune events were detected through positive skin tests (mean wheal diameter of 3mm or greater than negative control[1,9,18,19]), serum RAST-IgE (≥ class Ⅱ[8,19]) or through proof of intestinal IgE by endoscopically guided segmental lavage[18,19] in conjunction with a reproducible clinical adverse reaction to the food antigen(s) applied[1,5,8,18,19]. During BPCFC, at least one reproduction of an allergen induced clinical reaction and one or two placebo challenges were included for every patient. Whenever possible, both antigens causing clinical symptoms as well as tolerated antigens were applied to the patient, or else investigated on grounds of case history, skin tests, RAST and mucosal histamine release. In this way, a provocation allergen and a control allergen (Table 1) was determined for most patients (14 of 19 patients 74%), which enabled the direct comparison of the mucosal histamine release results with those of the BPCFC.

Before the execution of food challenge tests, additional examinations including endoscopy and histology of the upper and lower gastrointestinal tract were conducted[5,18-20]. Patients with macroscopic alterations of the mucosa or with histological signs of acute inflammation (Crohn's Disease, ulcerative colitis *etc*) were excluded from the study as well as those suffering from other digestive diseases (e.g. celiac disease, autoimmunopathy, mastocytosis, eosinophilic gastroenteritis *etc*).

At least two weeks in advance of colonoscopy and BPCFC, any antiallergenic, immunosuppressive or steroid treatments had been discontinued for all patients. Patients were prepared for colonoscopy using a commercial polyethylenglycol solution. To facilitate colonoscopy, benzodiazepins (midazolam, diazepam) and meperidine were used at a dose of 2.5-10 mg (midazolam, diazepam) and 25-150 mg (meperidine), respectively[20,21].

Colorectal mucosal histamine release

For colorectal mucosal histamine release by mucosa

oxygenation, 138 samples from the left-sided colon were obtained from all 19 patients. In 14 of 19 patients (74%), 10-12 mucosal samples were taken during colonoscopic examination. Whenever possible, 8 biopsies (4 repeats) were used for mucosa oxygenation and 2-4 for histological examination. The biopsies were immediately placed into a portable mucosa oxygenator (Intestino-Diagnostics, Erlangen, Germany) containing tubes filled with 2000 μL of oxygenated Hank's solution (pO_2 85-95 mmHg, pH 7.0, 37℃)[16,17,20]. Each incubation medium was bubbled with a steady flow of room air to ensure sufficient oxygen pressure inside the biopsy, to facilitate allergen distribution into the tissue or mediator release from the tissue and to avoid ischemic damage of the tissue[16,17,20,21].

Histamine release into the culture medium was measured at 0, 7.5, 15, and 30 min by removal of 200 μL of the supernatant at each sampling time[17,20,21]. To obtain as accurate histamine measurements as possible, each 200 μL sample was immediately denatured by heating to 95℃ for 5 min in order to destroy all histamine metabolising enzymes that may have been contained within the drawn supernatant[16,17,20,21].

Allergen induced histamine release was achieved by addition of 200 μL Hank's solution containing 5 μL of native allergen solution to the culture medium at the sampling time of 0 min, thus providing a final concentration of 0.01 μg allergen/mL. The same procedure was applied for positive control using anti-human immunoglobulin E except that 20 μL of pure anti-IgE solution (Behringwerke, Marburg, Germany) were used for dilution. The final concentration of anti-human-IgE was 0.01 μg/mL, which has previously been found to be the optimal stimulation concentration[16,17]. For negative control (spontaneous mucosal histamine release), only 200 μL Hank's solution were added to the culture medium. The stimulation procedure of the 8 samples of each patient was arranged as follows: two samples were studied for spontaneous mucosal histamine release (negative control), two for anti-IgE induced histamine release (positive control), two for a BPCFC-positive allergen (provocation allergen) and two for provocation negative, i.e. tolerated antigens (control allergen).

After a stimulation period of 30 min, the rest of the volume of 1400 μL containing the biopsy (1200 μL Hank's + 200 μL stimulus) was also heated to 95℃ for five minutes in order to determine the remaining tissue histamine content and to denature tissue histamine catabolising enzymes[17,20,21].

Histamine measurement

Histamine was measured using a sensitive and specific radioimmunoassay (Histamine RIA, Beckman-Coulter, Krefeld, Germany)[17,21,22]. The actual rate of histamine release was expressed as the percentage of the total tissue histamine content of the biopsy. This was calculated from the discharged histamine into the supernatant and the remaining histamine content in the tissue at the sampling time of 30 min[17,20-22]. Intra-assay and inter-assay coefficients of variation for the histamine radioimmunoassay ($n > 150$ samples) were 6.2% and 8.8% for supernatants, and 13.7% and 18.2% for detection of the remaining tissue histamine content, respectively. The individual rates of histamine release were found to vary by up to 24.0% within the same person.

Histamine content was also measured in native allergen solutions to exclude histamine contamination.

Table 3 Results from colorectal mucosal histamine release (% of total tissue histamine content) following *ex vivo* mucosa oxygenation in patients with GMA

Pat. No.	Spontaneous HR	Control Anti-IgE	Control Allergen	Provocation allergen
1	1.7	5.4	3.1	8.7
2	2.6	12.6	11.5	17.3
3	3.4	5.2	4.5	39.1
4	3.2	6.2	-	8.8
5	3.3	6.0	3.1	3.0
6	0.5	3.7	0.9	5.1
7	4.1	1.6	8.9	27.6
8	7.5	2.9	8.3	3.2
9	7.0	6.6	4.9	8.1
10	2.5	3.6	3.0	4.8
11	0.3	3.6	-	1.5
12	1.4	7.9	2.4	11.3
13	5.3	-	3.7	11.2
14	22.4	25.6	20.7	57.9
15	25.8	23.0	-	28.7
16	1.5	-	-	1.8
17	4.8	9.3	-	6.7
18	2.9	3.8	2.8	5.8
19	0.1	1.1	0.4	2.4
Median:	3.20	5.40[b]	3.40	8.10[a]
(range)	0.1-25.8	1.1-25.6	0.4-20.7	1.5-57.9

Significance levels: [a]$P = 0.008$ *vs* spontaneous histamine release; [b]$P = 0.04$ *vs* spontaneous histamine release. HR: histamine release (average value from two measurements); no.: number; pat.: patient; anti-IgE: anti-immunoglobulin E. Control and provocation allergen are allergens, which were either tolerated or induced clinical symptoms during blinded oral food challenge procedures. Rates of histamine release are expressed as percentage (%) of total tissue histamine content. Each release value represents the average value from two repeat experiments (two separate biopsies).

Statistical analysis

From each patient, two rates of histamine release were obtained for each of the parameters spontaneous histamine release, anti-IgE, provocation and control allergen. The average value for each pair of release data was calculated and used for final statistics as listed in Table 3.

For descriptive statistics of the whole group, the median and range were chosen. Statistical comparisons were made using the *U*-test (Wilcoxon, Mann & Whitney) and significance levels are given in brackets. For comparison of the clinical tests, *ex vivo* biopsy stimulation by mucosa oxygenation was considered successful (positive) when the antigen containing solution caused an increase of the histamine release up to more than twice that of the spontaneous release.

RESULTS

Histamine release from colorectal tissue

Spontaneous histamine release from viable colorectal mucosal fragments amounted to only 3.2% of the total tissue histamine content, indicating that mast cells are able

Table 4 Colorectal mucosal histamine release in comparison with the outcome of oral provocation tests in patients with GMA

	Positive HR	Negative HR	Line sum
Positive BPCFC	12	7	19
Negative BPCFC	3	11	14
Column sum	15	18	

HR: histamine release, BPCFC: (double- or single) blinded, placebo-controlled oral food challenge. Colorectal mucosal histamine release: Sensitivity 63.1%, specificity 78.6%.

Table 5 Patients' history in comparison with the outcome of oral provocation tests in patients with GMA

	Positive H	Negative H	Line sum
Positive BPCFC	4	9	13
Negative BPCFC	3	4	7
Column sum	7	13	

H: patients' history, BPCFC: (double- or single) blinded, placebo-controlled oral food challenge. Note: Only 13 of 19 patients (68%) felt confident to answer questions concerning suspected provocation antigens, while the remaining were uncertain or had no experience of adverse reactions to the particular antigen. For evaluation of the specificity of patients' history, data from only 7 patients (37%) were available. Patients' history: Sensitivity 30.8%, specificity 57.1%.

Table 6 Skin prick tests in comparison with the outcome of oral provocation tests in patients with GMA

	Positive prick test	Negative prick test	Line sum
Positive BPCFC	9	10	19
Negative BPCFC	3	11	14
Column sum	12	21	

BPCFC: (double- or single) blinded, placebo-controlled oral food challenge Skin prick test: Sensitivity 47.4%; specificity 78.6%.

Table 7 Allergen-specific serum IgE determinations (RAST test) in comparison with the outcome of oral provocation tests in patients with GMA

	Positive RAST	Negative RAST	Line sum
Positive BPCFC	11	8	19
Negative BPCFC	7	7	14
Column sum	18	15	

BPCFC: (double- or single) blinded, placebo-controlled oral food challenge Allergen-specific IgE detection in serum: Sensitivity 57.9%; specificity 50.0%.

to maintain their normal metabolism and their mediators within granules during mucosa oxygenation (Table 3). Application of anti-human-IgE induced a clearly enhanced rate of histamine release of 5.4% ($P = 0.04$) within 30 min, confirming the functional reactivity of mucosal immune effector-and intestinal mucosal mast cells towards IgE-receptor cross-linking[15-17,21,23]. Four of 17 patients (23.5%) were found to be unresponsive to the anti-IgE concentrations used. Interestingly, these patients had the highest rates of spontaneous histamine release, possibly indicating that mast cells had already been degranulated or that a high rate of spontaneous histamine secretion may exert some negative feed-back mechanisms on mast cell triggering by anti-IgE (Table 3).

Incubation of intact colorectal tissue with BPCFC-negative food antigens did not induce a significant increase in histamine release (median increase 1.2 fold; range 0.6-4.4 fold of spontaneous mediator release) in patients with GMA. Histamine release with control antigens amounted to 3.4% and was not statistically different from spontaneous histamine release (Table 3).

In contrast, provocation allergens that evoked clinically significant reactions in allergic individuals (BPCFC-positive food antigens) already induced a 2.6 fold increased rate of histamine release compared to the spontaneous release (range 0.9-24 fold) during 30 min of mucosa oxygenation. The percentage of histamine release in response to provocation allergens was 8.1% and significantly different from spontaneous histamine release ($P = 0.008$).

Colorectal mucosal histamine release in comparison with established clinical tests

Colorectal mucosal histamine release was positive in 12 of 19 patients (sensitivity 63.1%, Table 4), who experienced a reproducible clinical reaction in response to the same provocation antigen. In contrast, 3 of 14 patients (21.4% false positive) discharged significant histamine amounts although oral provocation was negative. Control antigens, tolerated by the patient during BPCFC, were also found to be negative with regard to histamine release in 11 of 14 patients (specificity 78.6%).

When comparing established clinical parameters for food allergy diagnostics with the outcome from blinded provocation tests (gold standard), a lower diagnostic accuracy was obtained through reference to patients' history (Table 5), skin test results (Table 6) and allergen-specific IgE detection in serum (Table 7) than with histamine release experiments.

The comparison between patients' history and BPCFC (Table 5) was somewhat impeded, since only 13 of 19 patients (68.4%) knew their allergen inducing clinical symptoms, 7 of 14 patients (50%) with recurrent gastrointestinal complaints tried to give exact answers on questions about their well tolerated foods, while all other individuals had significant uncertainties about adverse reactions to or tolerance of the food antigens tested.

DISCUSSION

Diagnosis and existence of gastrointestinal food allergy are still a matter of debate[1,4,5,18]. To date, no exact diagnostic and practical relevant means are readily available for the gastroenterologist or endoscopist to examine the gastrointestinal mucosa for signs of food hypersensitivity, when patients with recurrent episodes of variable gastrointestinal complaints are referred for further diagnostics[5,8,10]. One innovative approach for the diagnosis of food hypersensitivity by endoscopy may be the use of a mucosa oxygenation system, which allows culturing of small viable endoscopic samples outside of

the patient for immunological release experiments[15-17,20,23]. Since histamine is one important and very early secreted mediator of different types of IgE and non-IgE mediated allergic reactions[1,3,17,24], this study was designed to evaluate colorectal mucosal histamine release from patients with gastrointestinal food hypersensitivity. In contrast to allergic individuals with typical extraintestinal signs of type I food hypersensitivity, diagnostic problems have been repeatedly reported in this patient population primarily involving the gastrointestinal tract with symptoms of allergic diarrhoea, vomiting, nausea, *etc*. In addition, the frequency of local gastrointestinal allergy in several important clinical conditions is also still unclear[1,4,5,8-11,25-27].

This study demonstrates that a significant histamine release can be induced from colorectal mucosa upon IgE receptor cross-linking or antigenic stimulation *ex vivo* by mucosa oxygenation using endoscopically taken samples[15-17,23]. This confirms that functional antigen-specific tests using histamine as the primary test parameter are equally feasible with mucosa from the lower gastrointestinal tract as from the upper gastrointestinal tract (duodenum[15]) or more long-lived mediators like mast cell tryptase or eosinophilic cationic protein[20,23]. However, in contrast to previously published work featuring tryptase or eosinophilic cationic protein, histamine release tests using bioptic tissue bear the distinct practical advantage that they can be performed within a short period of time of only 30 min, providing the appropriate technique to rapidly destroy all histamine catabolising enzymes in the drawn culture supernatants is applied[16,17,20,21]. The quick performance of mucosa oxygenation using histamine as a diagnostic parameter may qualify this test for its use in clinical practice, possibly as a refinement or complement of existing endoscopic-diagnostic procedures when patients with suspected gastrointestinal food allergy are being referred for diagnostic work-up.

Compared to other human tissues or isolated mast cells, histamine release from colorectal mucosa was found to be of a smaller magnitude than expected. This could be a result of the short cultivation period and of the fact that colorectal tissue harbours large quantities of histamine metabolising enzymes, which are still active within the viable cultured tissue at a physiological rate during mucosa oxygenation[6,11,17,20,21,28]. However, compared with the spontaneous rate of histamine release, functional mast cell stimulation by anti-IgE or BPCFC-positive antigens achieved a significantly higher rate of histamine release, while control antigens showed a similar degree of histamine release as the spontaneous secretion. This study proved unambiguously the reactivity of histologically normal gut mucosa in allergic patients upon specific challenge. In view of that, gastroenterologists will find a valuable addition to their diagnostic methods through the establishment of a functional test using biopsies of the involved and reacting allergic shock organ[15,21,23]. Depending on the number of biopsies taken during endoscopy, this functional test allows the simultaneous examination of different food antigens during mucosa oxygenation[15-17,21]. In this way, any contact between the antigen and the patient's immune system is avoided and the patient is not put at risk of any allergic symptoms or reactions.

Although only a small group of patients was investigated in this study, colorectal mucosal histamine release was found to yield a diagnostic sensitivity and specificity of 63.1% and 78.6%, while skin tests and serum IgE detection reached only a sensitivity and specificity of 47.3% and 78.6% or 57.5% and 50%, respectively. Similar low rates of diagnostic accuracy of the skin prick tests and RAST tests have also been reported by other investigators in GMA[1,2,4,5,12,25-27]. This may be explained by the fact that sensitisation in the cutaneous and serological compartment may not always accurately reflect the immunological response of the mucosal microenvironment at the large surface area of the gastrointestinal tract[1,18,25,27]. The presence of different (immunological) compartments with separate mechanisms for local IgE production in food hypersensitivity may also account for the known multitude of allergic manifestations in food allergy[1,8,29]. This is illustrated by the fact that several individuals in this study experienced significant clinical symptoms during BPCFC despite negative skin or serum IgE tests, confirming the need for further diagnostic means directly targeting the involved shock organ[1,5,25-27,29].

Mucosa oxygenation with histamine or more long-lived mediators bears the potential to predict the outcome of double-blind, placebo-controlled food challenges[17,21,23]. Hence, a future cost-effective approach of the gastroenterologist to diagnose gastrointestinal food allergy could possibly be based on the additional use of gut mucosal histamine release (Table 4) to avoid time-consuming, cost-intensive and sometimes risky challenge procedures. However, the real value of such a diagnostic procedure has yet to be established within greater patient populations[21,23]. The rationale of this proposal for future diagnostics is given by the fact that gut mucosal histamine release was proven to be of superior diagnostic value compared to that of the commonly used allergological means such as case history, skin prick tests or RAST tests, as these are not always concise or do not directly examine the involved organ in patients with GMA, respectively[1,2,25,27]. The data presented here suggest that patients with a food antigen-induced mucosal histamine release exceeding more than twice the amount of the spontaneous one be could directly subjected to a specific elimination diet.

As part of appropriately provided health care, prospective long-term analysis of the clinical course of these patients is always necessary. Diminishing financial resources, however, dictate the need for more economical investigations, which may be sufficed by mucosa oxygenation. Although this approach is more invasive than blinded food challenges, it needs only one endoscopic examination with testing of several antigens, while DBPCFC needs at least one (in-patient) test day for each individual allergen application. The data presented here suggest that mucosa oxygenation could perhaps eliminate the need to perform DBPCFC in a significant number of patients suspected of having GMA[16,17,21].

However, the fact that colorectal mucosal histamine release by mucosa oxygenation did not identify all BPCFC-positive allergens is more likely to be related to the

complex pathophysiology and compartmentalisation of gastrointestinal food allergy rather than to inadequately applied methods. Patients with positive provocation but negative colorectal histamine release, which was the case for 7 of 19 patients (36.8%), may develop their allergic reactions in more proximal parts of the gastrointestinal tract (e.g. stomach, duodenum). Or else, these patients may react only in blood or at extraintestinal sites, respond to the tested antigen mainly with other mediators rather than with histamine (e.g. arachidonic acid products, eosinophilic proteins *etc*) or may have produced the antigenic epitope after passage through the liver[1,5,8,25,29]. Conversely, control allergens, which elicited a significant histamine release in 3 of 14 patients (21.4%), may fail to provoke a clinically significant reaction unless histamine catabolism is sufficiently active[1,28]. In addition, the demonstration of food-specific histamine release from colorectal mucosa may also explain why some patients experience postprandial extraintestinal symptoms (urticaria, hypotension, asthma bronchiale *etc*) despite negative skin tests. In such cases, intestinally produced and released histamine may reach peripheral extraintestinal organs and activate their histamine receptors inducing classical extraintestinal allergic symptoms without the presence of food-specific IgE in the periphery. These different pathophysiological parameters in combination with several yet unknown or ill-defined factors (e.g. neurovegetative impulses, gut flora *etc*) contribute to or induce the great variability of clinical manifestations in GMA[1,3-5,10,18,23,29].

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