Journal of Advancement in Medicine
Volume 3, Number 2, Summer 1990

Abnormal Fecal Microflora and Malabsorption Phenomena in Atopic Eczema Patients

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ABSTRACT: Fecal microflora and biological markers of the contaminated small bowel syndrome (CSBS) were quantitatively estimated in 58 atopic eczema (AE) patients and in 21 apparently healthy controls.

The dramatic reduction of lactobacilli (82.7 %), bifidobacteria (77.6 %) and/or enterococci (62 %) noted in fecal samples from AE patients was generally associated with increased counts of pathogenic strains, including Candida, atypical coliforms, Cl. perfringens, Cl. innocuum, Proteus, Staph. aureus, Klebsiella and/or aerobic spore formers.

In addition, significant alterations of specific CSBS markers, including serum hypoproteinemia ($p < 0.01$), lactose malabsorption ($p < 0.0001$), raised indicanuria ($p < 0.005$) and fecal fat excretion ($p < 0.05$) demonstrate the occurrence of malabsorption phenomena linked to a disturbed intestinal flora in these patients. Although no direct correlations could be found between individual strain counts and the levels of CSBS markers, however, several combinations of microorganisms appeared to be related to the above-mentioned changes.

The relationship between abnormal fecal microflora, hypogammaglobulinemia (in 53.4 % of the patients, $p < 0.005$) and malabsorption phenomena, as well as their significance in the AE pathogenesis are also analysed.

Introduction

The gastrointestinal tract proximal to the distal ileum is usually sparsely populated with bacteria in healthy individuals. The fasting concentration of microorganisms in the stomach, duodenum and mid jejunum is generally less than 10^4 cfu/ml (colony-forming-units/ml), the predominant forms being aerobes as Lactobacillus, Streptococcus,

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Staphylococcus and various fungi. Aerobic coliforms are occasionally present in small numbers but strict anaerobes are notably absent (1).

In the distal ileum gram-negative bacteria begin to outnumber gram-positive organisms, reversing the trend seen in the proximal bowel. Coliforms are consistently present and anaerobic bacteria such as Bifidobacterium, Bacteroides and Clostridium are found in substantial concentrations (10^3-10^8 cfu/ml) (1, 2).

Distal to the ileocaecal valve the bacterial populations increase dramatically to 10^9-10^{11} cfu/gram of faeces whereby the anaerobic bacteria outnumber aerobes by a factor of 10^2 to 10^4. The predominant isolates are Bifidobacterium, Bacteroides, Eubacterium, Clostridium, enterococci, anaerobic lactobacilli, and various species of Enterobacteriaceae (2, 3). The vast numbers of microorganisms in the colon contrast with the relative sterility of the small intestine and especially of the upper jejunum. When the mechanisms regulating the million-or-more-fold difference between these bacterial populations fail, microbial contamination of the gut occurs and leads to the so called "contaminated small bowel syndrome" (CSBS) (4). This may have serious clinical consequences including diarrhea and nutrient malabsorption associated with weight loss, malnutrition and growth failure in infancy (4, 5, 6).

As young atopic eczema (AE) patients often exhibit a similar clinical picture and obviously increased mucocutaneous infectious susceptibility, we have asked if this might be associated with abnormal intestinal flora too. Moreover, in a previous study, we have not been able to correlate the increased intestinal permeability values for larger molecules (4 ooo S) in AE patients with their histamine and/or IgE-containing circulating immune-complex levels. We therefore suggest that additional factors, possibly an early CSBS setting, may account for mucosal injury and defective intestinal permeability in these patients (7).

The most reliable method in the diagnosis of CSBS—although expensive and unpleasant, remains the intestinal intubation followed by appropriate bacterial culture of the aspirate (3, 5, 7). However, besides clinical symptoms indicating small bowel disturbances, the use of non-invasive tests for detecting the presence of bacterial overgrowth becomes increasingly important in the long-term management of these patients in a wide range of clinical situations (5).

The aim of this study is to demonstrate that microbiological and chemical investigation of stool samples from AE patients and healthy controls, together with determinations of serum proteins, urinary in-

dican and lactose loading tests, provide consistent evidence for a small bowel bacterial overgrowth in a significant number of AE patients. The interrelations between abnormal fecal microflora, malabsorption phenomena and immunological deficiencies in the pathogenesis of atopic eczema are also analysed.

Materials and Methods

Patients and Controls

Fifty-eight patients (38 female and 20 male, aged 10 to 34 yrs.) with clinically proved AE (8) and twenty-one apparently healthy volunteers (11 female, 10 male, aged 16 to 45 yrs.) with no atopic history, gave their consent to take part in this study.

All patients avoided any steroid or antihistaminic treatment for at least 10 days before admission and none received antibiotics within the previous year.

Thirty-eight of them showed severe AE with widespread deep excoriation and weeping or bleeding of lesions over the face, limbs and trunk; all of these were carriers of Staph. aureus skin infections and 34 of them had additional mucocutaneous foci with Candida sp., Streptococcus pyogenes, enterococci and occasionally with Enterobacteriaceae, Corynebacterium and/or herpes simplex virus.

The remaining 20 patients were designated as having mild AE with erythema, xerosis, lichenification and superficial flexural excoriations; they were also carriers of Staph. aureus infections and 9 of them further exhibited skin contamination with enterococci and/or corynebacteria.

Physical examination revealed weight loss in 23 cases and growth retardation in 11 children of school age, all belonging to the severe eczema subset. Intermittent diarrhea, constipation, flatulence, instestinal rushes and abdominal discomfort (particularly after meals) were registered in 28 AE cases. Twelve patients with diagnosed food allergy were also aware of severe symptoms after food challenge including nausea, bronchospasm, diarrhea, vomiting and skin itching. Pallor, chronic fatigue and cold extremities were usual clinical hallmarks in the atopic group. Neither microbial skin foci nor clinical signs of intestinal distress (excepting flatulence after meals in 4 cases) were recorded in the control group. No medication was administered the first 48 hrs. after admission and both patients and control

subjects received for 2 days the same standard meals (western diet), including 200 g protein and 100 g fat per 24 hrs. in exactly weighed usual foods.

Blood and Urinary Tests

Routine laboratory investigations, including serum total proteins, albumin, hemoglobin and serum iron were estimated using commercially available kits (Boehringer Mannheim, FRG). Laser nephelometry techniques (Behring, FRG) were used to evaluate serum IgG, IgM and IgA. Total serum IgE and specific IgE's against three microbial antigens (Sacch. cerevisiae, Candida albicans and Staph. aureus) were measured with standard enzyme-immunoassays (Padezym PRIST and Padezym RAST / German Pharmacia, FRG), whereby levels above 100 IU/ml for total IgE and RAST classes 2-4 were considered pathological.

Lactose Malabsorption Test. Thirty three AE patients aged 12-34 years and 21 controls took part in this study. After an overnight fast the patients emptied their bladders and were given 150 mg of ethanol/kg body weight and 50 g lactose dissolved in 400 ml of water, orally, at the same time (9).

A fingertip blood sample and an urinary sample were taken 40 min. later. Blood and urinary galactose concentrations were measured with a galactosedehydrogenase kit (Galactose UV-Test, Boehringer, Mannheim, FRG) according to the instructions of the manufacturer. Subjects with blood galactose less than 0.2 mmol/l and with urine galactose less than 4 mmol/l were classified as lactose malabsorbers and those with levels above 0.2 mmol/l and 4 mmol/l respectively, as lactose absorbers; herewith the presence or absence of symptoms was used as an additional criterion in setting the cut-off point for lactose malabsorption. Statistical analysis of the data was performed using the Student t-test.

Urinary Indican Excretion. Bacterial tryptophan deamination in the bowel with indole formation and urinary excretion of indican was estimated with a standard method in a 24 hour alkalinised urine (10). The red-violet coloured complex resulting from the reaction between indican (potassium indoxyl sulfate) and 4—hexylresorcin in the presence of Obermeyer's reagent was measured photometrically. Results from 58 AE patients were compared statistically with those from 21 control subjects (Student t-test).

Investigation of Fecal Microflora

All stool specimens were collected in anaerobic vials with caps containing a CO_2 generating and O_2 absorbing mixture after water addition (silicium dioxide, natrium carbonicum, iron powder and citric acid / Merck Anaerocult Powder, Darmstadt, FRG) and processed as follows.

One gram of stool specimen was placed in a scintillation tube containing about 3-4g of glass beads (2-3 mm in diameter). After adding 9 ml of dilution medium (DM), the contents of the tube were thoroughly mixed on a Vortexer (Thermolyne Maxi Mix) to get a homogeneous suspension. Starting with this first dilution, further 10-fold dilutions were performed using DM without glass beads.

Immediately after dilution, 1 µl (pipetted by an Automatic Precision Pipette, Nichiryo, Japan) each of 10^{-1} to 10^{-6} dilution was spread on the respective nutrient or selective agars to give final dilutions ranging from 10^{-4} to 10^{-9}.

Media. Dilution medium (DM): 15 g standard nutrient broth II (Merck # 7884) was dissolved in 1 000 ml H_2O and autoclaved at 121 ° C for 15 min.

Blood-agar (BA), containing 7.5 % defibrinated sheep blood, Columbia-blood-colistin nalidixic acid-agar (CNA) (11), modified reinforced clostridial agar pH 5.9 (RCA) (12) and Columbia-blood-neomycin-agar (CBN) containing 100 µg neomycin/ml (13), were obtained as "ready to use" media from Heipha, Heidelberg, FRG. After inoculation, an antibiotic-test-ring "Mastring SM ID 8 " (Mast Diagnostica Hamburg, FRG) was put on the surface of the CBN agar plate for differentiation of clostridia and Bacteroides (19). Dehydrated MacConkey-agar (14) from Becton and Dickinson, Heidelberg, FRG, was prepared according to instructions (# 11387).

Dehydrated Biggy-agar (15) and Rogosa-agar(16) were obtained from Oxoid, Wesel, FRG, and prepared according to instructions (# CM 589 and # CM 627, respectively). Rifampicin-blood-agar (RBA) (17) was prepared as follows: 37g Columbia-agar-base (Oxoid, # CM 331) and 1g glucose (Merck) were dissolved in 900 ml H_2O and autoclaved for 20 min. at 121 ° C. After cooling to 55 ° C, 50 ml sterile, defibrinated sheep blood (Biologische Arbeitsgemeinschaft GmbH, Lich, FRG) and 50 ml rifampicin stock solution (see below) were added; agar plates were poured immediately after mixing.

Rifampicin stock solution: 100 mg rifampicin (Sigma # R-3501) was dissolved in 20 ml 96% ethanol (Merck); H_2O was added to give a

final volume of 100 ml. Clostridia tubes: Clostridium perfringens selective agar (18) (Oxoid # CM 587) was prepared according to instructions and distributed in 5 ml portions into 10 ml screw-cap tubes. Prior to use, the agar was melted again by boiling in a water bath; after cooling to 50 ° C, 20 µl of an aqueous solution of 1 mg polymyxin B-sulfate / ml (Sigma, # P-1004) was added to each tube. The tubes were immediately inoculated with 1 µl to the respective fecal dilutions and mixed by gentle rotation.

Incubation of Media. After inoculation, the media were incubated as follows:

BA and MacConkey-agar:	37 ° C; 24 h;	aerobically.
Biggy-agar and CNA:	37 ° C; 48 h;	aerobically.
Clostridia tubes:	37 ° C; 48 h;	anaerobically.
Rogosa-agar:	37 ° C; 48 h;	in an anaerobic jar (Oxoid) under an atmosphere of 80 % CO (Minimax, Munich, FRG) and 20 % air.
RCA, RBA and CBN:	37 ° C; 48 h;	anaerobically, using anaerobic jars, (Merck) Merck Anaerocult A (# 13829).

Colony Counts and Identification of Microorganisms. Organisms were isolated and identified as described elsewhere (19). In brief, gram staining, cell morphology and specific biochemical reactions (oxidase, catalase, computer aided API 20E System, API O/F test, API 20 Strep-System, API Staphyslide-test / API Bio Merieux, Nürtingen, FRG) were used in identification of the isolates. Gas chromatographic analysis of volatile and non-volatile fermentation products (20) (GC Perkin Elmer Sigma 3 B, TCD) API 20A System, and the sulfite reduction test (21) enabled further differentiation of anaerobes. Species of B. praeacutus, B. oralis and Cl. butyricum, Cl. spiroforme, Cl. bifermentans, Cl. barati and other unidentifiable strains, respectively, were recorded as "other Bacteroides" or "other Clostridial strains" (Table 1). Aerobic spore formers were represented by Bacillus ssp.

Since many of the organisms grew on more than one media, counts of particular organisms were recorded from the medium giving the highest yield, using a Cook Electronic Colony Counter. The mean count values for each isolate in different subsets of the AE and con-

trol groups are recorded in Table 1 and express cfu/g of wet weight stool. Data were analysed not only in terms of mean counts but also in terms of absence or presence of organisms and high versus low counts; the normal range of the most prevalent species is given in Table 1 according to the statistics of our laboratory for a healthy German population (19). Microbial strains showing colony numbers less than lx 10^4/g feces for bacteria and less than lx 10^3/g feces for fungi (Candida sp.) were not detectable with this method.

Fecal Fat. Total fatty acids (esterified and free) were estimated in stool samples from 29 AE patients and 14 controls after saponification with potassium hydroxide and extraction with petroleum benzine in the presence of hydrochloric acid. The fatty acids were then titrated with O,l N NaOH in the residue of the organic phase after concentration under vacuum (22). Values exceeding 6 g / 24 hrs. feces were considered pathological and the difference between the two groups was statistically analysed by Student t-test.

Macroscopic and microscopic investigations of feces including appearance, consistency, pH, as well as stool smears for detection of muscle fibres, lipids, starch and blood were also performed with standard methods.

Results

Blood and Urinary Tests

Serum total protein levels ranged between 5.6 and 7.5 g/dl in the AE group, in 28 cases (48,3 %) values below 6.7 g/dl were registered. Statistically there was a significant difference between the AE group and controls ($p < 0.01$; Fig. 1).

Albumin concentrations below 4 g/dl (in spite of normal liver function and urine tests) were recorded in 13 AE patients (22.4 %) and although less than those observed in control subjects, there was no statistical difference between the two groups.

A surprisingly large subgroup of 31 AE patients (53.4 %) showed lowered gammaglobulin levels (range 0.31-0.89g/dl) and in most cases correlated with decreased total serum proteins. The difference was highly significant when compared to the control group ($p < 0.005$) and was mainly due to an obvious drop in the IgE and IgM values (Fig. 1). Six patients, including 4 of 12 with severe clinical symptoms

TABLE 1

Estimation of Fecal Microflora in 58 Atopic Dermatitis

	Lactobacillus sp.	Bifidobacteria	Bacteroides fragilis	other Bacteroides strains	Eubacteria	Clostridium perfringens	Clostridium innocuum	other Clostridial strains
Normal range	5×10^6-10^8	$\geq3\times9^{10}$	$\geq3\times10^9$	$>10^7$	$<10^6$	$<10^5$	$<10^5$	$<10^5$
AD PATIENTS (n = 58)	0<10^4	0<10^4			↑↑↑ 2.1×10^7			↑↑↑↑ 1.8×10^9
	33(56.9%)	4(6.9%)			5(8.6%)			21(36.2%)
	↓↓↓ 3.7×10^5 15(25.8%)	↓↓↓ 6.6×10^6 19(32.7%)	↓↓ 2.2×10^8 3(5.2%)		↑↑ 4.7×10^6 13(22.4%)	↑↑ 8.6×10^6 6(10.3%)	↑↑↑ 3.1×10^7 9(15.5%)	↑↑↑ 8.7×10^7 18(31%)
	↓↓ 2.1×10^6 6(10.3%)	↓↓ 3.5×10^8 22(37.9%)	↓ 1.1×10^9 26(44.8%)	↓↓ 4.3×10^7 12(20.7%)	↑ 1.6×10^6 11(19%)	↑ 2.2×10^5 9(15.5%)	↑↑ 5.5×10^5 4(6.9%)	↑↑ 7.6×10^6 12(20.7%)
	N3.9×10^7 4(6.9%)	N8.7×10^9 13(22.4%)	N7.2×10^9 29(50%)	N2×10^5 46(79.3%)	N4.3×10^5 29(50%)	N<10^5 43(74.1%)	N<10^5 45(77.6%)	N5.3×10^4 7(12%)
CONTROLS (n = 21)	0<10^4							
	2(9.5%)							
	↓↓↓ 5.1×10^5 4(19%)	↓↓↓ 5.9×10^6 3(14.3%)						↑↑↑ 9×10^7 3(14.3%)
	↓↓ 2.4×10^6 8(38%)	↓ 1.3×10^9 11(52.3%)	↓ 8.6×10^8 13(62%)	↑ 2.1×10^7 9(42.8%)	↑ 2.5×10^6 5(23.8%)			↑↑ 8.1×10^6 9(42.8%)
	N4.5×10^7 7(33.3%)	N5.1×10^9 7(33.3%)	N6.3×10^9 8(38%)	N1.4×10^5 12(57.1%)	N3.9×10^5 16(76.2%)	N<10^5 21(100%)	N<10^5 21(100%)	N4×10^4 9(42.8%)

Legend 0 = not detectable; ↓↓↓ = very low; ↓↓ = significantly reduced; ↓ = lightly diminished; N = in normal range; ↑↑↑↑ = extremely high; ↑↑↑ = very high; ↑↑ = significantly raised; ↑ = ligthly increased

after food challenge, demonstrated depressed serum levels of both albumin and gammaglobulins; all belonged to the severe eczema subset.

Although no direct correlation could be established between serum protein values and counts of individual bacterial strains, the concentration of urinary indican, or stool fat content, 23 of 28 patients with decreased total serum proteins and gammaglobulins consistently

Patients and in 21 Apparently Healthy Controls

Escherichia coli	Enterococcus sp.	Enterobacter sp.	Aerobic spore formers	Proteus sp.	Klebsiella sp.	Staphylococcus aureus	Candida sp.
10^7-10^8	5×10^6-5×10^7	$<10^5$	$0<10^4$	$0<10^5$	$0<10^5$	$0<10^4$	$0<10^3$
Atypical strains (H_1,H_4,F_3) 20(34.5%)	0 6(10.3%)		↑↑↑ 1.4×10^6 3(5.2%)				↑↑↑ 8.8×10^5 5(8.6%)
↑↑ to ↑↑↑ 4.4×10^8 21(36.2%)	↓↓↓ to ↓ 1.1×10^5 30(51.7%)	↑↑↑ 4.2×10^6 2(3.5%)	↑↑ 5×10^5 11(19%)	↑↑↑ 5.8×10^6 3(5.2%)		↑↑↑ 6.7×10^6 4(6.9%)	↑↑ 1.9×1 7(12%)
↑↑ to ↑ 5.2×10^6 18(31%)	↑ to ↑↑ 6.4×10^7 10(17.2%)	↑ 2.9×10^5 6(10.3%)	↑ 2.2×10^5 4(6.9%)	↑↑ 7.5×10^5 5(8.6%)	↑ 2.6×10^5 4(6.9%)	↑↑ 4.4×10^5 6(10.3%)	↑ 2.5×10^4 11(19.1%)
$N3.7\times10^7$ 19(32.7%)	$N7.1\times10^6$ 12(20.7%)	$N0<10^5$ 50(86.2%)	$N0<10^4$ 40(69%)	$N0<10^5$ 50(86.2%)	$N0<10^5$ 54(93.1%)	$N0<10^4$ 8(82.8%)	$N0<10^3$ 35(60.3%)
Atypical strains H_2,H_3,F_3) 2(9.5%)							
↑↑ 3.1×10^6 4(19%)	↓↓↓ 4.3×10^5 1(4.8%)						
↓ 5×10^5 6(28.6%)	↓↓ 1.8×10^6 3(14.3%)						↑ 3.1×10^4 2(9.5%)
$N4.3\times10^7$ 11(52.4%)	$N4.2\times10^7$ 17(89.9%)	$N0<10^5$ 21(100%)	$N0<10^4$ 21(100%)	$N0<10^5$ 21(100%)	$N0<10^5$ 21(100%)	$N0<10^4$ 21(100%)	$N0<10^3$ 19(91.5%)

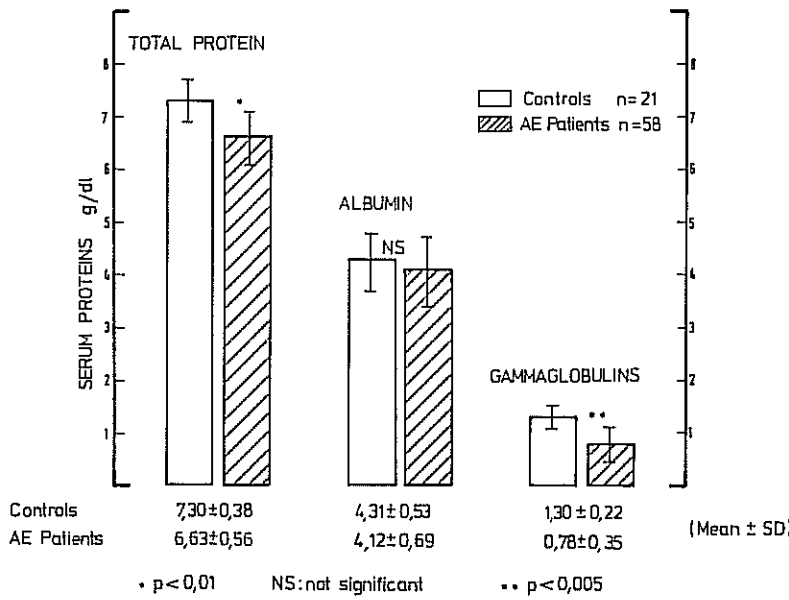
The numbers right to the arrows represent mean values of colony numbers recorded in the respective subset of patients or controls (cfu/g of wet faeces); numbers belows the arrows indicate patients or controls and their frequency (brackets).

showed raised counts of Candida, E. coli (atypical strains), Clostridium, Proteus and/or Staph. aureus in stools, associated with dramatically reduced counts of lactobacilli, bifidobacteria and/or enterococci.

Increased total IgE levels and frequent eosinophilia were observed in the AE patients. Thirty three of them had positive mucocutaneous Staph. aureus cultures and raised concentrations of specific IgE's against staphylococcal antigen (56.9 %). Twenty five of 34 demon-

FIGURE 1

Total protein, albumin and gammaglobulins in serum of atopic eczema patients and healthy controls.



strated mucocutaneous or intestinal candidiasis and had positive RAST results with Candida antigen (73.5 %). Since specific anti-candida and anti-staphylococcal IgE's are known to aggravate the clinical picture in atopic patients (23), we assume that this may also be the case for the frequent anti-Sacch. cerevisiae IgE's detected in 67.2 % of the AE cases.

In contrast, the controls, with the exception of two subjects who had moderately decreased gammaglobulins, showed neither hypoproteinemia, increased IgE levels, nor positive RAST results with the investigated microbial allergens.

Moderate microcytic anemia was found in only 10 AE patients (17.2 %) despite a relatively frequent finding of serum iron deficiency in 32.7 % (19 patients). Serum iron concentrations ranged from 18-67 µg/dl. Persistently positive blood tests in stools from 25 patients (43.1 %) were also detected. The latter finding seems to be an important

indicator of mucosal injury, since mild gastrointestinal blood loss has been reported in animals with experimental overgrowth / blind loop syndrome who developed ulcerations of the small bowel mucosa (24, 25). This could be further related to the frequent intestinal contamination with haemolytic strains of Staph. aureus and E. coli, as noticed in 17 to 34.5 % of the atopic fecal samples (Table 1).

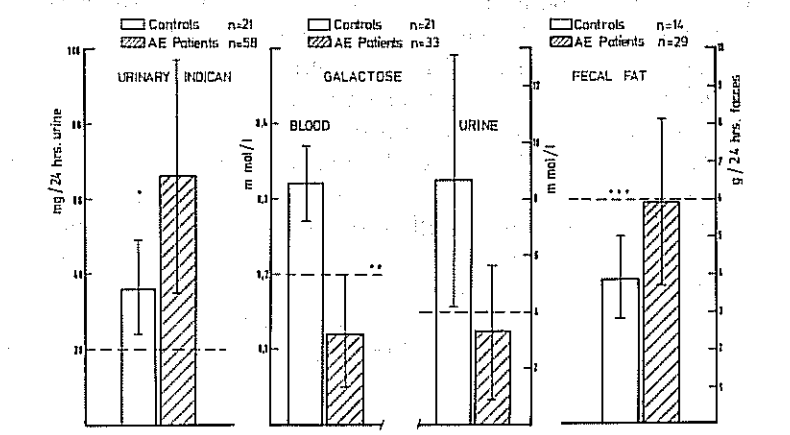
Of 21 control subjects, only one had a positive blood test in stool. Two others exhibited a moderate iron deficiency in serum, but no sign of anemia was evident.

Urinary Indican Excretion

As Fig. 2 shows, there was a significant increase of urinary indican excretion in the AE group ($p < 0.005$) although both patients and controls ingested similar protein quantities for 2 days before the trial. In 12 atopics we measured indican levels above 100 mg/24 hrs., strongly suggesting the presence of CSBS (26, 27). Moreover, oral antibiotic therapy reduced the increased indican values in 10 of the 12 cases into the normal range, further supporting the assumption of CSBS (Ionescu G: unpublished observations). No direct correlation was ob-

FIGURE 2

Urinary indican, lactose malabsorption test and fecal fat excretion in atopic eczema patients and healthy controls.



served between the concentration of urinary indican, total serum protein levels, or in vitro counts of individual tryptophan deaminating bacteria. However, in 17 of 19 patients with indicanuria above 80 mg/24 hrs., there were consistently high stool counts of E. coli, Proteus and/or Klebsiella, together with sharply diminished numbers of lactobacilli and enterococci.

The mean value of urinary indican in the control group (36.38 mg/24 hrs.), was 16 mg higher than the upper limit of normal indicated in our method (10). This may reflect occasional disturbances of intestinal microflora in people consuming an average western diet with moderate animal protein intake (Table 1).

Lactose Malabsorption Test

Lactose malabsorption based on the inherited delayed onset of lactase deficiency is common in adults, worldwide (28, 29). It was thus not surprising that most of our control subjects showed blood and urinary galactose levels in the lower normal range (Fig. 2). In 4 patients moderate abdominal distension, flatulence, borborygmi or mild diarrhea occurred following lactose administration. The mean values recorded in the atopic group were 2.5-fold lower than in controls (31 of 33 patients had blood levels below 0.2 mmol/l and 25 of 33 showed less than 4 mmol/l galactose in urine). This was usually associated with severe abdominal distension, colicky pain and diarrhea after challenge. Only 2 of 33 tested AE patients remained asymptomatic after lactose ingestion (6 %) compared with 9 of 21 controls (42.9 %).

Although both blood and urinary galactose investigations provided a highly significant difference between the two groups ($p < 0.0001$), the decreased blood values in the AE group correlated better with the severity of the lactose intolerance symptoms.

No correlation was observed between the extent of lactose malabsorption and urinary indican excretion of serum albumin levels. However, 16 of 26 AE patients with blood galactose levels below 0.15 mmol/l showed reduced gammaglobulins in serum and 8 of them demonstrated an elevated fecal fat excretion. Finally, 20 of the 26 hypogalactosemic patients were consistently found to have increased fecal counts of Candida, atypical coliforms, Clostridium and/or Bacillus spp., together with drastically reduced numbers of lactobacilli and bifidobacteria.

Fecal Fat Excretion

The investigation of total fatty acids in fresh stool samples from 29 AE and 14 control subjects revealed a significantly elevated fat excre-

tion in the atopic group ($p < 0.05$; Fig. 2). Eight of the 29 AE patients (27.6%) showed steatorrhea with values between 6.6 and 15.8 g fat/24 hrs. In contrast, only one of 14 control subjects (7.1 %) revealed a fecal fat excretion (6.9 g fat/24 hrs.) above the normal limit (5.05 g fat/24 hrs.).

A direct relation between fecal fat excretion, total serum protein, serum albumin or indicanuria was not observed but 6 of 8 patients with steatorrhea showed hypogammaglobulinemia and blood galactose levels less than 0.15 mmol/l after lactose challenge. All 6 exhibited indican values over 100 mg/24 hrs. urine.

Moreover, a strikingly high incidence of elevated counts of Clostridium, aerobic spore formers (Bacillus ssp.) and Bacteroides associated with sharply reduced numbers of lactobacilli and enterococci was seen in all 8 cases with steatorrhea. Oral antibiotic treatment administered to the 6 AE patients with steatorrhea and high indicanuria lowered the fecal fatty acids in 5 cases to the normal range, strongly suggesting the involvement of an intestinal dysbacteriosis (CSBS) in the setting of an increased fecal fat excretion (30, 31).

The microscopic examination of fecal smears for lipids further confirmed a high incidence of fatty acid crystals and neutral fat droplets in 51.7% of the atopic samples. Macroscopically, a greasy, clay-like consistency of stools was also frequently noted in the AE group.

Bacterial Counts

In spite of individual variations in fecal microflora several trends were evident in the AE group in respect to the investigated strains (Table 1).

The most consistent finding in the AE patient samples was the absence or dramatic reduction of lactobacilli counts (particularly Lb. acidophilus) in 82.7% of the tested fecal specimens. Only 4 of 58 patients (6.9%) showed normal lactobacilli counts when compared with 7 of 21 control subjects (33.3%). Since the stool moisture content was within normal limits in both patients and controls, we may assume that lactobacilli from small bowel compartments were also lacking. Interestingly, 42 of the 58 patients (72.4%) with absent or sharply reduced lactobacilli counts had been formula rather than breastfed after birth, a practice with very serious consequences for the intestinal flora of neonates (32).

Drastically lowered counts of gram positive bifidobacteria and enterococci were also registered, respectively in 77.6 % and 62 % of the AE patients, further suggesting a severe intestinal dysbiosis, possibly

associated with lowered acetic/lactic acid production (the stool pH oscillated in most AE cases between 6.2-7.4 when compared to 5.5-6.4 in the control group). By contrast, only 14.3% and 19.1% of the control subjects showed significantly reduced numbers of bifidobacteria and/or enterococci, respectively (Table 1).

A marked difference between the two groups was further demonstrated by the dramatic increase of clostridia, atypical E. coli (i.e. strains showing hemolytic activity, reduced β-galactosidase activity and/or strongly mucoid forms) Eubacteria and atypical Bacteroides counts noted in 67.2%, 34.5%, 31% and 20.7% of the atopic fecal specimens, respectively (Table 1). Of special interest is the high incidence of Cl. perfringens (25.8%), and Cl. innocuum (22.4%) strains in the AE samples but none of the control group. Finally, another hallmark of the AE group appeared to be the relatively high frequence of microflora usually occurring only in very low numbers, including Candida (39.7%), aerobic spore formers (31.1%), Staph. aureus (17.2%), Proteus (13.8%) and Klebsiella (6.9%), again suggesting the pattern of a marked dysbiosis.

In contrast, only two of the control fecal specimens showed contamination with such organisms (Candida), Table 1.

No direct relationship was evident between single strain counts and the investigated biologic markers in the AE group, but several combinations of microorganisms appeared to be related to the increased indican and fat excretion or to the obviously diminished lactase activity (see above). Moreover, 27 patients showing a drop in gram positive lactic acid-producing organisms associated with a sharp rise in fungal or atypic gram negative bacterial counts belonged to the severe eczema subset exhibiting multiple skin infections too. In 20 of them we also noticed gammaglobulin levels below 0.8 g/dl, suggesting a close link between the defective humoral immune response and the high incidence of chronic recurrent infections in these cases.

Discussion

A. Although it was impossible to find a unique pattern of microorganisms in fecal samples from AE patients, the quantitative investigation of the most prevalent strains showed that the drop in gram positive, lactic acid-producing bacteria, was generally associated with an obvious rise in clostridia, fungi and/or pathogenic gram negative forms (Table 1).

Since, despite normal moisture content of the stool specimens, the lactobacilli or the enterococci were not detectable in these samples, we may assume that they were also seriously diminished or lacking in the small bowel. Conversely, increased counts of Proteus, Staph. aureus, Candida, aerobic spore formers, Klebsiella or atypical E. coli in the same samples may indicate an abnormal bacterial population in the upper intestinal compartments also.

The significance of microbial interactions in the context of a normal or a disturbed intestinal flora is extremely complex. Several reports demonstrate the eminent role of lactobacilli and bifidobacteria in maintaining a low pH in the intestinal lumen via generation of lactic and acetic acid with inhibition of colonic putride microflora (32,33). By contrast, the atopic fecal samples in our study demonstrated an obvious pH increase when compared with the control specimens. Lactobacilli have further been reported to produce hydrogen peroxide (33) and antibiotics (34,35), with definite antagonistic action to undesirable intestinal flora including Proteus, Cl. perfringens, Salmonella, E. coli, Staph, aureus and Pseudomonas. Hence, the absence or dramatic reduction of lactobacilli and bifidobacteria in the intestine and feces following formula feeding in infancy (32,36), or inappropriate antibiotic/drug treatment, may destroy the natural barriers against food contaminants or colonic flora with the setting of an overgrowth syndrome in the ileal and jejunal compartments. In this context, our finding that 42 out of 58 AE patients with absent or sharply lowered lactobacilli counts had not been breast-fed after birth is consistent with previous studies of R. Chandra (37) and Narayanan et al. (38), which clearly demonstrate the negative effects of formula feeding on the incidence of infection and allergy in neonates, particularly when they have atopic siblings (37).

Other mechanisms, including impaired small bowel motility, lowered gastric acid secretion or anatomical defects may also favor the setting of an intestinal dysbiosis (4,5), but specific investigations are lacking in this study.

On the other hand, the relatively high incidence of gastrointestinal symptoms in the AE group (especially after meals), as well as significant changes in biologic markers related to the overgrowth syndrome provide additional support to the CSBS concept in more than one third of our patients.

B. Disturbances of protein metabolism involving an increased bacterial degradation of amino acids in the small bowel and serum hypoproteinemia have previously been related to an elevated indicanuria (26,27) and to the CSBS setting (39,40).

In the AE group, the urinary indican excretion was significantly higher than in controls and this appeared to be related to increased counts of tryptophan deaminating bacteria (E. coli, Proteus, Klebsiella) in feces. The high incidence of positive guaiac tests noted in the atopic fecal samples may further suggest ulcerations of intestinal mucosa with plasma protein loss in the lumen, as earlier described in the experimental blind loop syndrome (24,25) and in patients with allergic gastroenteropathy and eczema (41). Raised local concentrations of bacterial metabolites (indol, phenol, cresol, volatile fatty acids, endotoxins, deconjugated bile acids, etc.) or histamine releases after food challenge, may account for this phenomenon.

The markedly diminished gammaglobulins (especially IgG and IgM) in over 50 % of the AE sera also contribute to the drop of total proteins; this may be due either to an inherited defect or to an acquired deficiency following an increased immunoglobulin turn-over and/or consumption in circulating immune complexes with food or microbial antigens (7). Most AE patients with low gammaglobulin levels also showed marked intestinal contamination with atypical strains and belonged to the severe eczema subset. Similar results have recently been reported in children with food allergy and eczema (42), and these findings are often associated with a defective cellular immune function in superinfected atopic patients (57). It therefore seems likely that a deficient immune status may be related to an increased cutaneous and / or intestinal germ contamination (44), causing skin/mucosal damage and abnormal entry of adjuvantized antigen, with resulting sensitization (45). As neonatal experience may be critical in becoming sensitized, this could be avoided either by elimination of possibly damaging allergens or by establishing a beneficient intestinal flora via breast-feeding (32, 37, 46).

C. Abnormalities of carbohydrate metabolism involving disaccharidase deficiency (47), monosaccharide malabsorption (48) and an increased sugar degradation by small bowel bacteria (5,6) have also been described in humans and experimental animals with overgrowth syndrome. More specifically, the absorptive disturbances have been associated with the intestinal brush border injury (48) and disaccharidase depletion by protease-containing bacterial extracts (49).

The clear-cut reduction of blood and urinary galactose concentrations of AE patients in our study appears to be related to increased fecal counts of Candida, clostridia, atypical coliforms and/or aerobic spore formers, as well as markedly reduced gammaglobulin levels. Our results suggest, therefore, that depression of brush border lactase

by enteric bacteria, or by Candida, as already demonstrated in vitro (50) and in vivo (51), along with the absence of lactase-producing lactobacilli in the small bowel, could be responsible for the dramatically lowered galactose levels and for the exacerbated lactose intolerance symptoms in these patients.

The observed abnormal microflora and low gammaglobulin levels in most of our hypogalactosemic AE subjects are in agreement with earlier findings in children with disaccharidase deficiency and immunologic deficits (51), as well as in patients with disturbed intestinal flora and hypogammaglobulinemia (42, 44), respectively.

In our experience disaccharidase deficiency in AE is a significant marker of the disease, explaining various carbohydrate intolerance reactions in these patients, including diarrhea, flatulence, intestinal rushes, tiredness, nausea and headache.

Specific anti-fungal therapy, associated with carbohydrate-low diet, is an important measure in controlling this condition.

D. Malabsorption of fat is known to be a prominent feature of clinical and experimental CSBS (4, 5, 6, 31). In this context the abnormal fatty acid concentrations found in 27.6% of the fecal samples in AE patients could be due either to bacterially induced alterations in bile salt metabolism (6, 31), or to an increased microbial carbohydrate and peptide degradation with fatty acid production (52, 53), or to both mechanisms. On the other hand, the elevated fecal fat content appeared to correlate with increased fecal counts of Clostridium. Bacteroides and Bacillus spp., strains particularly capable of deconjugating bile salts, with resulting steatorrhea (31, 54, 55). It is conceivable that a diminished small bowel carbohydrate absorption due to disaccharidase deficiency may result in additional sugar degradation by the overgrowth microflora, with production of large amounts of short chain fatty acids and negative effects for the host metabolism (52, 56) and/or with yeast-mediated sugar fermentation and production of increased alcohol levels measurable in blood (43), on the other side. The disturbances of sugar and fat absorption in patients with CSBS may therefore be related.

The above data are in good agreement with parallel investigations of the duodenal aspirates in AE patients demonstrating a significant colonization of the upper intestine with pathogenic strains (57).

Conclusions

Although no direct intestinal intubation technique was used in this study, the above fecal, urinary and blood investigations associated with the successful antibiotic treatment of the malabsorption phenomena strongly suggest the setting of the overgrowth syndrome in an important subset of AE patients. This may happen as a result of small bowel contamination in early life following contamination at birth, inappropriate feeding and/or immune deficiencies.

Injury of the upper gut mucosa may occur in these subjects as a consequence of increased levels of deconjugated bile salts (4, 5), bacterial proteases (49), short chain fatty acids, alcohol and bacterial toxins (48), which lead to malabsorption of nutrients and permeability changes (7). This, in turn, will allow increased entry of food antigens and adjuvantising microbial metabolites, followed by sensitization (45) and high levels of circulating immune complexes, with detrimental side effects for the host (7).

Correction of skin and intestinal dysbiosis, accompanied by long-term hypoallergic diet and immune stimulation therapy are, according to our investigations and clinical experience the most important measures in the management of atopic eczema.

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J Adv Med: in press.