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Intestinal Permeability and Antigen Absorption in Rheumatoid Arthritis

Effects of Acetylsalicylic Acid and Sodium Chromoglycate

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Abstract. Intestinal permeability was measured using cow's milk beta-lactoglobulin absorption (BLG) as a permeability marker in 14 patients with active and inactive rheumatoid arthritis (RA) under three different conditions: after a washout period, after treatment with acetylsalicylic acid (ASA) associated with disodium chromoglycate (DSCG), and with ASA only. No intolerance to cow's milk was present and serum IgE levels were in the normal range in 12 of 14 patients. IgG anti-IgE were present in 7 of 13 patients tested. When off treatment the intestinal permeability to BLG in RA patients was not increased as compared to controls, but we found a significative difference between active and inactive RA. ASA administration strongly increased BLG absorption, not prevented by DSCG pretreatment. In normal controls treated with a single dose of ASA we obtained similar results. Our results suggest that prolonged treatment with nonsteroidal anti-inflammatory drugs induces an increase of food antigen absorption, apparently not related to anaphylaxis mediator release, with possible clinical effects.

Introduction

Recent interest has developed on the possible relationship of rheumatic diseases, especially rheumatoid arthritis (RA), to foods or food-related products. It has been postulated that some rheumatic disorders may arise from food allergy and/or intolerance [1, 2] or alternatively that food-derived antigens may perpetuate inflammation so that dietary manipulation would affect clinical manifestations [3–5]. Prevalence of atopy in RA has been found to be similar to that observed in the general population, with levels of IgE within the normal range [6]. Since most authors reported beneficial effects from diets and/or fasting [4, 7, 8], it has been proposed that excess food antigen absorption may contribute to chronic joint manifestations in RA.

We studied the intestinal permeability to a macromolecular food antigen (cow's milk B-lactoglobulin – BLG) in RA patients in relationship to administration of acetylsalicylic acid (ASA) with/out disodium chromoglycate (DSCG), a mast cell stabilizer which is known to be effective in reducing gut absorption in food-allergic patients [9].

Materials and Methods

Patients

We selected 14 patients with classical RA, diagnosed according to ARA criteria [10], 2 males and 12 females, with mean age 51.6 ± 8.9 years (range 33–68). Mean duration of symptoms was over 3 years, and at the time of the study 9 had active disease (according to symptoms and laboratory findings). Eleven had been previously treated with nonsteroidal anti-inflammatory drugs, 6 with anti-malaria drugs, 3 with steroids and 2 with gold salts. They had been off all treatment for at least 7–30 days prior to this study.

Controls

Ten normal subjects selected among Hospital staff for absence of atopic or joint disease, or any other present inflammatory or microbial disease, acted as controls. None were receiving therapy or self-medication. Four were males and 6 females, mean age was 34.6 ± 9.2 years (range 25–50).

Oral Challenges

Patients and controls were subjected to oral challenge with fresh cow's milk (10 ml/kg BW) after an average 14 days washout and overnight fast. Blood samples were collected before and 2 and 4 h after drinking the milk. Sera were then separated and stored in aliquots at –20 °C until tested. After this baseline value for the assessment of intestinal permeability, 9 patients (1 male, 8 females, age range 38–63 years, 6 with active RA) were treated with DSCG (10 mg/kg daily) and ASA (1 g daily) for 2 weeks and then chal-

Table 1. Levels of IgE, and presence of rheumatoid factor (RF) and IgG anti-IgE antibodies in RA patients with active (A) or inactive (I) disease

Subjects	Sex	Age	A/I	RF	IgE IU/ml	IgG anti-IgE
1 M.S.	F	54	A	–	51	n.t.
2 T.B.	F	33	I	+	9	+
3 T.V.	M	68	A	–	12	++
4 G.A.	F	63	A	+	426	–
5 T.P.	F	53	I	+	1	–
6 S.F.	F	48	A	+	3	++
7 M.C.	F	57	A	+	393	–
8 B.B.	F	43	I	–	11	–
9 D.M.	F	38	A	+	17	+
10 V.E.	F	53	A	–	53	–
11 G.M.	F	60	A	–	12	–
12 F.M.	F	58	A	+	17	+
13 M.B.	F	60	I	+	86	++
14 L.G.	M	35	I	–	132	+

n.t. = Not tested.

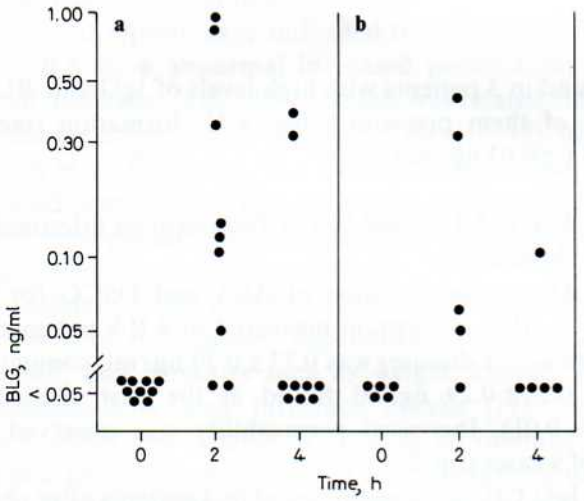

Fig. 1. Concentration of BLG in sera of RA patients collected before and 2 and 4 h after challenge with cow's milk. Both patients with active **(a)** and inactive **(b)** RA had been off treatment for 1 up to 4 weeks.

lenged again with cow's milk. A third oral challenge was given to the 9 RA patients after treatment with ASA only for a further fortnight. Sera were again collected and stored as described. Three normal controls were challenged after a single administration of 1 g of ASA.

Laboratory Tests

The following routine examinations were included at the beginning of the study for all patients: full blood count, erythrocyte sedimentation rate, serum electrophoresis, C-reactive protein, serum C3 and C4 levels, antinuclear antibodies, rheumatoid factor activity (latex agglutination and Waaler-Rose), and X-ray film of affected joints. Total immunoglobulin levels were measured by radial immunodiffusion (IgG, IgA, IgM) or paper radioimmunosorbent test (PRIST, Pharmacia, Sweden, for IgE).

IgG autoantibodies to IgE were determined on prechallenge samples by Elisa, as described [11]. Results were expressed as negative or positive with respect to 3 SD of mean background OD.

Circulating immune complexes (CIC) of the different Ig classes were studied in all samples by indirect immunofluorescence using fluorescein isothiocyanate (FITC)-conjugated isotype-specific antisera in the Raji cell method [12]. Results are expressed as highest positive serum dilution.

Intestinal permeability was determined by measurement of circulating immunoreactive BLG in all samples using a solid-phase RIA as described [13]. Results were expressed as ng/ml with reference to known standards of BLG diluted in antibody-negative sera. IgG antibodies to BLG were tested by Elisa, and considered to be positive when above 50 μg/ml.

Results

Serum IgE and IgG Anti-IgE in RA

IgE serum levels were within the normal range [14] in 12 of 14 RA patients. Arithmetical mean was 87 ± 141 IU/ml with a range of 1–426 IU/ml. Two patients had IgE levels above normal (393 and 426 IU/ml) and both sera were negative for IgG anti-IgE (table 1). These autoantiglobulins were present in 7 of 13 cases tested. IgM rheumatoid factor was present in 8 patients (5 with IgG anti-IgE and 3 negative). We observed a trend to inverse relationship between IgE levels and presence of IgG anti-IgE. In the 7 sera positive for anti-IgE mean serum values of IgE were 39 ± 49 IU/ml, versus a mean level of 148 ± 202 IU/ml in 6 sera without IgG anti-IgE. We found no correlation between disease activity and presence of IgG anti-IgE, rheumatoid factor or antinuclear antibodies (present in 2 cases at 1/80 and 1/160 dilutions, one with active and one with inactive disease).

BLG Absorption and CIC Formation after Oral Challenge

Circulating BLG was detected both in normal and in RA patients after challenge, with a detection limit of 0.1–0.05 ng/ml, and maximal levels were mostly observed in samples collected 2 h after challenge (fig. 1). The mean of maximal values found in 10 normal subjects was 0.18 ± 0.26 ng/ml, with only 2 cases showing levels higher than 0.3 ng/ml. These figures

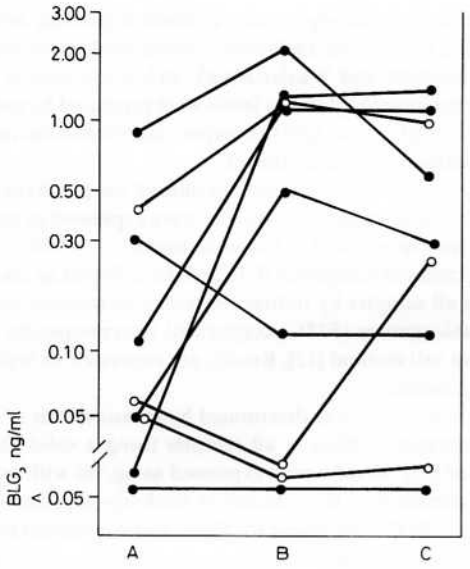

Fig. 2. Individual maximal values of circulating BLG in patients with active (●) and inactive (○) RA in the three separate oral challenges with cow's milk. A = After the washout period; B = after treatment with DSCG and ASA; C = after treatment with ASA only.

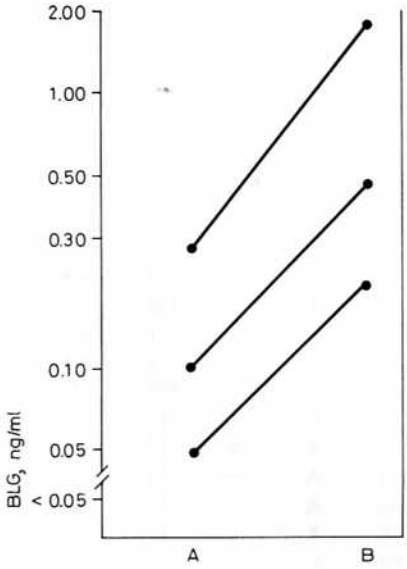

Fig. 3. Maximal serum BLG levels in 3 normal controls after challenge of cow's milk, with (B) and without (A) the administration of 1 g of ASA.

compare well with our previous findings in normals, taking into account the lower amount of milk given in this study [13]. One case out of 5 tested had a rise of CIC belonging to both IgG and IgM classes, and IgG anti-BLG antibodies were present in high titre in only 1 other subject of the 8 tested.

Mean levels of maximal BLG detectable in RA patients were 0.24 ± 0.29 ng/ml (p = n.s. compared to normals), but levels were higher in patients with active disease (mean 0.28 ± 0.34 ng/ml, n = 9) compared to those with inactive disease (mean 0.16 ± 0.16 ng/ml, n = 5; $p < 0.01$ by Wilcoxon's test). CIC of the IgA class were never found before or after challenge, 1 patient showed a rise of CIC-IgM and in 7 of 13 tested we observed the presence of CIC-IgG. Three of these 7 had high levels of IgG anti-BLG, compared to none of 6 negative for CIC-IgG. Two of the 7 cases positive for CIC also had IgG anti-IgE, which were present in 5 of 6 RA cases without IgG CIC. This seems to rule out the possible involvement of IgG anti-IgE in IgG CIC formation. Values of BLG absorption were also higher in patients negative for CIC after challenge (mean value 0.31 ± 0.28 ng/ml) versus positive (mean 0.18 ± 0.29 ng/ml). IgG CIC were detected in 6 of 9 active RA compared with 1 of 5 inactive cases. Very low values of BLG absorption were found in 3 patients with high levels of IgG anti-BLG, all of them presenting IgG CIC formation (mean 0.11 ± 0.03 ng/ml).

Effect of ASA and DSCG Treatment on Intestinal Permeability

After administration of ASA and DSCG for 14 days, BLG absorption measured in 9 RA patients (6 with active disease) was 0.73 ± 0.79 ng/ml, compared to 0.21 ± 0.29 ng/ml found at the first challenge ($p < 0.01$). Increased permeability was observed in 5 of 9 cases (fig. 2).

IgG CIC were again found in 4 patients after challenge, 3 of whom had high BLG absorption values, but none had IgG to BLG and 1 only had high levels of IgE but without IgG anti-IgE.

After an additional 15 days on ASA only, BLG absorption was only marginally modified in the 9 cases studied (mean levels 0.54 ± 0.58 ng/ml, p = n.s. versus the second oral challenge). BLG absorption was still significantly higher than detected after the first oral challenge ($p < 0.01$). IgG CIC were detected in 2 of 6 cases tested.

Three normal controls underwent a second challenge, preceded by administration of 1 g of ASA, to study if the effect of ASA was also present in normal

conditions. BLG absorption was 0.82 ± 0.85 ng/ml compared to 0.2 ± 0.18 without ASA (fig. 3). None developed CIC after challenge.

Discussion

We found no evidence of increased intestinal permeability in patients with RA when off treatment for 1–4 weeks. Individual cases, however, showed some increased absorption 2 h after a standardized challenge with cow's milk, and they were mostly with active disease. After 2 weeks of treatment with ASA we observed a significant increase in gut permeability in 9 patients which was not prevented by administration of DSCG and persisted with slight modifications after 2 more weeks of ASA only.

Pretreatment with 1 g of ASA increased BLG absorption also in 3 normal subjects with similar values to those observed in RA patients, showing that alterations of the gastrointestinal barrier are directly related to the drug and occur in normals too.

Several reports have indicated that some patients with RA have abnormal increased permeability to small molecules (PEG 400) and this was related either to disease activity or to the type of diet [15–18]. Bjarnason et al. [17] using ^{51}Cr-EDTA observed that increased permeability was present in patients treated with nonsteroidal anti-inflammatory agents, a finding which is confirmed by our study.

Different results may be obtained in the assessment of intestinal permeability depending on which tracer is used, and variables such as weight, charge, and hydrophobicity may influence uptake [19]. Since macromolecules capable of eliciting an immune response have been implicated in food allergy, we used immunoreactive BLG as a probe for gut absorption. It is, however, not surprising that our findings are largely in agreement with those of other studies [15–19].

It has been difficult to reconcile these results with the clinical effects of restricted diets in RA [2]. Manipulation of the diet suggested that individual patients may benefit from withdrawal of specific foods [2, 4, 5, 20] or fasting [7]. Changes in fatty acid composition in the diet and EPA-rich fish oil seemed also to ameliorate symptoms in some of the cases [21]. Aberrant immune responses to cow's milk has been reported in 1 patient by Panush et al. [5], but only occasionally has IgE-mediated hypersensitivity to foods been observed [6] and quite certainly unrelated to RA. Total IgE levels in RA are within the normal range in 85% of our cases, a figure which is very similar to that reported by O'Driscoll et al. [6]. Autoantibodies to IgE have been postulated to play a role in atopic syndromes [11, 22] and their presence has been found also in patients with 'allergic arthralgia' and with RA [23, 24]. About 50% of our patients had IgG anti-IgE, despite the absence of high IgE levels and/or atopic symptoms. There was no correlation between the presence of anti-IgE and rheumatoid factor activity, and IgG anti-IgE did not seem to be involved in IgG-CIC formation in RA patients. These observations seem to indicate that anti-IgE in RA should be considered as an additional sign of deranged immune response [22].

Formation of immune complexes usually follows oral challenge in individuals with preformed antibodies to food antigens [9]. However, their participation to immunopathological damage in patients with food allergy is controversial [22, 24].

We used an antigen-nonspecific method for detection of CIC with different Ig isotypes, and 8 of 13 RA patients studied showed an increase of IgG CIC after oral challenge. This variation may in part be due to cow's milk BLG-specific CIC, and seems to be reflected by higher intestinal absorption. Moreover, the increased permeability to milk proteins does not seem secondary to the release of anaphylactic mediators from gut mast cells, since it was not modified by pretreatment with DSCG.

In the course of chronic RA some co-factors may be important for both self-perpetuation and progression of the disease. The patients' diet may be one such co-factor, with dramatic improvement in some cases after elimination of certain foods or dietary restriction [2, 5, 7, 20].

We propose that prolonged treatment with anti-inflammatory agents induces a continuous increase of gut permeability, resulting in secondary damage with clinical effects. The roles of gastrointestinal lesions and local or systemic prostaglandin inhibition cannot be distinguished from our data, but the latter possibility is favored [17] and also suggested by our findings in normal subjects.

Oral challenges for assessment of intestinal absorption may be useful to individuate a subgroup of RA patients who would benefit from dietary restriction, but only a prospective study will assess the role of diet therapy in these patients.

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