Zonulin May Not be a Marker of Autoimmunity in Patients with Psoriasis

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The pathogenesis of psoriasis is not fully understood, but many immunological mechanisms known to occur in psoriasis can also be found in autoimmunity (1). The classical understanding of autoimmune pathogenesis involves a genetic susceptibility, in combination with environmental trigger factors, leading to a dysregulation of immune responses. A third element has recently been added to this paradigm: the loss of intestinal barrier function (2, 3). A major barrier component between the external environment and the human immune system is the intestinal mucosa. Proteins leading to an antigen-specific immune response usually pass the intestinal mucosa via a paracellular pathway, which is, in part, regulated by intercellular tight junctions (TJ) (3). Zonulin (also known as pre-haptoglobin 2), a human protein analogue to the *Vibrio cholera*-derived Zonula occludens toxin, is involved in the regulation of these TJ, and can reversibly open TJ in the small intestine (2, 4, 5). The involvement of zonulin in intestinal barrier function has been shown in different diseases with an autoimmune pathophysiology, such as coeliac disease and type 1 diabetes (6–8). In both conditions zonulin serum concentrations are increased and in autoimmune (type 1) diabetes high serum zonulin levels correlated with increased intestinal permeability. The authors suggested that up-regulation of zonulin precedes the diagnosis of type 1 diabetes, and re-establishing the mucosal integrity may be a treatment approach in autoimmune-mediated diseases (3).

In coeliac disease it has been shown that the gliadin fraction of wheat gluten is strongly associated with the development of intestinal damage, as preformed zonulin is released in the presence of gliadin, leading to barrier dysfunction, which could be prevented by a zonulin antagonist (FZ1/0) *in vitro* (9). Thus, zonulin may be a key protein in mediating autoimmunity.

Furthermore, patients with coeliac disease are at increased risk of developing any other kind of autoimmune disease (10). Therefore, an association of coeliac disease with psoriasis has been proposed. In a Swedish cohort of patients with psoriasis, antibodies to gliadin were present in 16% of the patients (11), and a gluten-free diet was able to improve skin lesions in this subgroup of patients (12). In a recent case-control study in Israel involving 12,502 patients with psoriasis and 24,285 control subjects, it was shown that patients with psoriasis have a greater prevalence of coeliac disease than matched controls (13). Association analysis of single-nucleotide variants in the interleukin-23 receptor (IL23R) region showed a genetic link between psoriasis and coeliac disease (14). The linkage of this gene region to psoriasis and inflammatory bowel disease (IBD) has been demonstrated previously (15). The pathophysiological mechanism of Crohn's disease is similar to mechanisms found in psoriasis underlying the similarity between these two diseases of the gut, on the one hand, and the skin, on the other hand.

The objective of this study was to investigate a possible role of zonulin in the pathogenesis of psoriasis and to determine whether zonulin could act as a marker of autoimmunity in patients with psoriasis.

MATERIALS AND METHODS

Serum samples from 80 patients with the clinical diagnosis of plaque psoriasis from two different centres (Department of Dermatology, University Medical Center Schleswig-Holstein, Campus Kiel and Asklepios-Nordseeklinik, Westerland/Sylt, Germany) were analysed for serum zonulin concentrations. After obtaining informed consent 20 ml samples of venous blood were collected from inpatients and outpatients. The study was approved by the ethics committee of the University of Kiel. Epidemiological data were documented using a standardized questionnaire. Data on age, sex, height, weight and severity of psoriasis using the Psoriasis Area and Severity Index (PASI) were documented.

A control group of 80 healthy volunteers matched for age and sex was recruited and epidemiological data were collected using the same questionnaire, as appropriate.

No diagnosis of any kind of autoimmune disease, including coeliac disease or diabetes type 1, was documented for any of the patients with psoriasis or the healthy controls.

For quantification of zonulin-serum levels a highly sensitive enzyme-linked immunoassay (ELISA)-based assay was used (Immunodiagnostik AG, Bensheim, Germany). The lower limit of detection was 8.6 ng/ml. Each experiment was carried out with one patient sample and one control group sample. Positive and negative controls, as well as standard solutions, were used in duplicate. The ELISA was carried out in accordance with the manufacturer's instructions.

Statistical analyses were performed using GraphPad Prism® software (Version 3.0; GraphPad Software Inc., La Jolla, CA, USA). Means, medians and standard deviations were calculated where appropriate. To compare data from patients and controls an unpaired two-tailed *t*-test was performed where appropriate.

RESULTS

The mean age of patients and controls was almost the same (Table I). There was no statistically significant difference in the age ($p = 0.44$) and sex between both groups. The mean body mass index (BMI) was statistically significantly higher in the patient population than in the control group (BMI patients: 28.4, SD

Table I. *Comparison between patients with plaque psoriasis and control subjects*

	Patients ($n=80$)	Controls ($n=80$)	p-value	Type of test
Age, years, mean ± SD	47.7 ± 1.5	48 ± 1.4	0.44	Unpaired t-test
Sex				
Male	40	41	na	na
Female	40	39	na	na
PASI				
Mean	16	na	na	na
Median	12.2	na	na	na
BMI, mean ± SD	28.4 ± 0.6	26.7 ± 0.55	0.03	Unpaired t-test
Zonulin, ng/ml	Below level of detection	Control 1: 38.7 Control 2: 326.4 Control 3: 363.3 Control 4: 432.5		

p-value <0.05 is statistically significant.
SD: standard deviation; na: not applicable; BMI: body mass index; PASI: Psoriasis Area and Severity Index.

0.6; BMI controls: 26.7, SD 0.55; $p=0.03$). The mean PASI of patients with psoriasis was 16 (median 12.2), which indicates a moderate-to-severe form of plaque psoriasis (Table I).

Zonulin was below the limit of detection (8.6 ng/ml) in all serum samples collected from patients with psoriasis. In the control group only four subjects showed detectable levels of zonulin in serum (Table I). Thus, zonulin levels are not elevated in patients with moderate to severe forms of plaque psoriasis.

DISCUSSION

The regulation of paracellular trafficking of immunogenic molecules is a pivotal function of the gastrointestinal system. Intercellular TJ have attracted attention, as under physiological conditions trafficking of proteins larger than approximately 3.5 kDa can be controlled. A dysfunction of intestinal TJ has been described in the pathogenesis of autoimmune diseases (2).

As psoriasis is regarded as an immune-mediated inflammatory disease in which mechanisms found in autoimmunity are present, it was of interest to evaluate zonulin levels as a marker for the regulation of TJs in this patient cohort.

In contrast to other diseases with an autoimmune background we could not detect zonulin in the serum of patients with psoriasis and only in four out of 80 healthy controls. Therefore, our study does not support a role of zonulin in the pathogenesis of psoriasis.

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