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INVESTIGATIVE REPORT

Gluten-free Diet in Psoriasis Patients with Antibodies to Gliadin Results in Decreased Expression of Tissue Transglutaminase and Fewer Ki67+ Cells in the Dermis

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Previous studies have shown that 16% of patients with psoriasis vulgaris have IgA and/or IgG antibodies to gliadin, but few have antibodies to endomysium. The increase in duodenal intraepithelial lymphocytes was mild. Still, highly significant clinical improvement was observed after 3 months on a gluten-free diet. This study surveys certain immunohistological aspects of involved and non-involved skin in 28 AGA-positive psoriasis patients before and after 3 months of a gluten-free diet. Staining was performed for CD4+ T lymphocytes, Langerhans' cells, endothelium, proliferating (Ki67) cells and tissue transglutaminase. In the entire group of patients, as well as in those on a gluten-free diet as the only treatment, Ki67+ cells in involved dermis were highly significantly decreased after the diet. There was a significant decrease in Ki67+ cells even in patients without increased intraepithelial lymphocytes. Tissue transglutaminase was highly overexpressed in involved skin in the papillary endothelium, and decreased by 50% after gluten-free diet. The possible role of tissue transglutaminase in the pathogenesis of psoriasis needs further investigation. ***Key words: endothelium; Langerhans' cells; lymphocytes.***

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Psoriasis patients (without arthritis) with a silent coeliac disease recover from their psoriasis when the coeliac disease is diagnosed and treated with a gluten-free diet (GFD). A good effect of 3 months of GFD on the psoriasis, with subsequent worsening when the ordinary diet was resumed, has also been observed in psoriasis patients with antibodies to gliadin but without antibodies to endomysium, and with only a very mild increase in intraepithelial lymphocytes in the duodenal epithelium (1). The mechanism for the effects of GFD on the skin is not yet known. The principal aim of this study was to overview the histopathological changes in involved and non-involved psoriasis skin after 3 months on GFD. A further aim was to see whether tissue transglutaminase (tTG) is expressed in the skin and whether the expression had changed after the GFD period. tTG is considered to be the main autoantigen in coeliac disease (2, 3).

PATIENTS AND METHODS

Thirty-seven psoriasis patients (22 men, 15 women, mean age 45 ± 13 years, range 18–70 years, with psoriasis for 19 ± 12 years, range 1–42 years), 31 of whom had IgA and 6 did not have IgA and/or IgG antibodies to gliadin (IgA AGA and/or IgG AGA), attempted to adhere to a gluten-free diet for 3 months. Twenty-one of the 31 patients had only IgA AGA (mean ± SD 90 ± 70 U/l), 5 had both IgA and IgG AGA (160 ± 118 and 59 ± 46 U/l, respectively) and 5 had only IgG AGA (25 ± 11 U/l).

An IgA AGA level >50 U/l and IgG AGA >12 U/l were considered elevated. Two patients with antibodies to gliadin had IgA antibodies to endomysium (EmA 1/160 and 1/10, respectively). Clinical and laboratory data for the 31 patients, including the degree of lymphocyte infiltration in the duodenal biopsy specimens before the diet, and the clinical effects of the diet, have recently been reported (1).

Briefly, before the diet 16 of the patients with AGA showed no increase in the number of intraepithelial lymphocytes (IEL) in the duodenum (<1 lymphocyte/4 epithelial cells), 7 showed a mild increase, and 6 showed a pronounced increase, with partial villous atrophy in 2. In patients without AGA there was no increase in IEL. A more detailed report on the duodenal biopsy results has also been published (4).

One patient with mild psoriasis also had palmo-plantar pustulosis. Three patients with and 3 without AGA also had psoriatic arthritis. Two women (both with antibodies to gliadin) had hypothyroidism, which was being treated with levothyroxine. During the diet period the patients remained on the same long-term treatment for their psoriasis as before the diet, but in some cases less treatment was required and allowed. There had been no changes in the treatment during the last month before the start of the trial. At the start, one AGA+ patient was receiving methotrexate 5 mg/week, 3 were on etretinate 40–50 mg/day, and 9 patients were having UV B twice weekly, whereas 18, among them the 2 EmA-positive patients, were only being given emollients.

Three patients with antibodies to gliadin dropped out of the study (two early in the study for practical reasons and one just before the end of the trial despite good improvement). Two patients on methotrexate without antibodies to gliadin also dropped out because of the need for a higher dosage of methotrexate. The ability to adhere strictly to the diet was not perfect in all patients; for example, there were difficulties during travelling.

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Skin biopsies

Punch biopsy specimens (3 mm diameter) were taken from involved (the same lesion before/after diet) and non-involved skin in the majority of the patients and snap-frozen. The specimens from non-involved skin were usually taken at a distance of 4–5 cm from the edge of a lesion. In nine patients (8 AGA+), most of whom had mild psoriasis, lesions suitable for biopsy were not present and paired specimens – one before and one after diet – were only obtained from non-involved skin. Paired samples from involved skin before/after diet were obtained from 19 AGA+ patients.

The project was approved by the local Ethics Committee and all patients had given their informed consent.

Processing of the specimens

An overview of antibodies and processing procedures is given in Table I. The monoclonal mouse tTG antibody has been shown to have no cross-reactivity with factor XIIIa or TG1 (keratinocyte TG). Endothelial cells are used as positive controls by the manufacturer. Standard peroxidase-antiperoxidase (PAP, DAKO) or avidin-biotin complex (ABC) (Vector Laboratories Inc., Burlingame Calif., USA) methods were used on 6 μm thick sections.

For staining of the endothelium, the monoclonal antibodies anti CD31 and Q-Bend 10 were diluted 1/40 and mixed before incubation. Double staining with MIB 1 and CD31/Q-Bend was performed on 18 μm thick, cryostat sections fixed in 3.7% cold formalin for 10 min. The sections were blocked in 0.5% H_2O_2 and 10% normal horse serum (Vector) and thereafter incubated with MIB 1 for 20 h at 4°C . Biotinylated anti-mouse IgG (dilution 1/200; Vector) was used as secondary antibody. Finally, the sections were incubated with Vectastain ABC kit. The peroxidase reaction was developed with 3,3′-diaminobenzidine. Subsequently the sections were incubated with 10% normal rabbit serum for 10 min. The antibodies anti CD31/Q-Bend 10 were diluted, mixed and incubated for 20 h. Rabbit anti-mouse IgG (dilution 1/40, Dakopatts) was used as secondary antibody. Finally, the sections were incubated with alkaline-phosphatase-anti-alkaline phosphatase (APAAP, Dako) diluted 1/25. The sections were developed with FAST Red Substrate Kit.

Microscopy

The coded specimens were analysed with a Leica Q Win computerized image system with a digital camera, DC 200. All CD4+ T lymphocytes in the epidermis in each section (usually 3) were counted and related to the length of the surface epidermis. The number of CD4+ cells in dermis was estimated semiquantitatively. The amounts of CD1a+ and Ki67+ cells in the epidermis were estimated by calculating the percentage area that was stained. In the dermis, the percentage [amount] of positive staining down to 300 μm below the basal cell layer was calculated for CD1a, CD31/Q-Bend and tTG.

The results of the double staining for endothelium and Ki67+ cells were evaluated by counting cells positive for both Ki67 and CD31/Q Bend (dark brown nucleus and red cytoplasm) and cells positive only for Ki67.

Statistics

The degree of significance was tested with the non-parametric Wilcoxon signed-rank test for paired two-group comparison.

RESULTS

Patients with antibodies against gliadin

The results obtained in involved and non-involved skin in all paired samples are summarized in Table II. Before the GFD there was no evidence of lower values for any of the parameters in patients receiving long-term systemic treatment compared with values in those without systemic treatment. In fact there was a tendency for these patients to have somewhat higher

Table II. *Overview of the parameters studied in involved and non-involved skin in psoriasis patients with IgA and/or IgG antibodies to gliadin before and after 3 months on a gluten-free diet (n=19; mean±SD)*

	Before	After	p value
No. of CD4+ T lymphocytes/mm epidermis			
- *involved* epidermis	15±10	11±7	0.027
- *non-involved* epidermis	5±5	3±3	
Percent CD1+ positive area			
- *involved* epidermis	1.94±1.97	2.33±1.60	n.s.
- *non-involved* epidermis	4.29±2.73	4.57±2.19	n.s.
- *involved* papillary dermis	1.20±1.13	0.90±0.67	n.s.
- *non-involved* papillary dermis	0.43±0.57	0.39±0.29	n.s.
Percent CD31/Q-Bend+ area			
- *involved* dermis	2.84±1.35	2.10±0.84	n.s.
- *non-involved* dermis	0.93±0.47	0.97±0.49	n.s.
Percent Ki67+ area			
- *involved* epidermis	3.13±2.44	2.58±1.61	n.s.
- *non-involved* epidermis	0.74±0.55	0.14±1.64	0.059
No. of Ki67+ cells in dermis /mm epidermal length			
- *involved* skin	30±21	16±17	0.0079
- *non-involved* skin	4±5	2±3	0.043
Percent tTG+ area			
- *involved* dermis	5.16±3.73	2.65±1.40	0.0079
- *non-involved* dermis	0.76±0.55	0.73±0.49	n.s.

Table I. *Antibodies and procedures used.*

Antigen	Antibody	Species	Visualizing	Dilution	Fixative	Technique	Source
CD4	Leu-3a	Mouse	T cells	1/40	Acetone	PAP	Becton Dickinson, Franklin Lakes, USA
CD1a	Leu-6	Mouse	Langerhans cells	1/50	Acetone	PAP	Becton Dickinson, Franklin Lakes, USA
Q-Bend 10	Q-Bend 10	Mouse	Endothelial cells	1/80	Acetone	ABC	Skybio,Wyboston, Bedfordshire,UK
CD31	CD31	Mouse	Endothelial cells	1/80	Acetone	ABC	Dakopatts, Glostrup, Denmark
Ki67	MIB I	Mouse	Proliferating cells	1/50	Formalin	ABC	Immunotech, Marseille, France
Tissue transglutaminase	TGase II	Mouse	Transglutaminase	1/200	Acetone	ABC	NeoMakers, Fremont, USA

PAP: peroxidase-antiperoxidase; ABC: avidin-biotin complex.

values indicating lack of response to the ongoing treatment (data not shown).

Involved skin

CD4+ T lymphocytes. The number of positive cells in the epidermis decreased significantly during the period of GFD diet. The two EmA-positive patients showed the most pronounced decrease, one patient from 12 cells to 1 cell/mm, the other from 13 to 3 cells/mm. In the dermis there was no marked change in the number as estimated semiquantitatively.

Langerhans' cells. Before the diet, the percent *CD1a+* area was smaller in the involved than in the non-involved epidermis ($p=0.011$, $n=13$), whereas it was larger in the involved than in the non-involved dermis ($p=0.002$, $n=13$). The percentage CD1a+ area in the epidermis and dermis was not significantly changed after GFD, although the mean positive epidermis area was increased and the mean positive area in dermis was decreased after the diet period.

Endothelium. CD31/Q-Bend endothelium was highly increased in lesional skin. There was a tendency to a lower mean value after the diet period. In some of the patients with clinical improvement there was a marked decrease in the endothelium after the GFD (Fig. 1a, b) and in all four patients with the most pronounced duodenal changes a lower percent positive area was observed after the diet (3.81 ± 2.05 versus 1.80 ± 0.52).

Proliferating cells. Ki67+ cells in the dermis were scattered, but seemed to be more common in association with groups of inflammatory cells. Double staining for Ki67 and endothelium (Fig. 1) performed on 6 pairs of specimens – 6 before and 6 after the diet period – showed that 35–50% of the Ki 67+ cells were localized to the endothelium or close to it. After 3 months there was a highly significant decrease in the number of Ki67+ cells in the papillary dermis, with the most pronounced decrease in patients with good clinical response. The number of Ki67+ cells/mm dropped from 33 to 1 in the patient with an EmA titre of 1/160, who also had a duodenal score of 3 with partial villous atrophy. In the other EmA-positive patient (titre 1/10) and a duodenal score of 1.13, there was a corresponding reduction from 20 to 8.

The patients with no other treatment than GFD had a highly significant decrease in the number of Ki67+ dermal cells from 23 ± 10 to 9 ± 5/mm ($n=11$, $p=0.0099$). Patients with a duodenal score of <1 also showed a significant decrease in the number of dermal Ki67+ cells (36 ± 15 vs. 16 ± 14, $p=0.016$, $n=10$). The number of Ki67+ cells decreased to a similar degree both in the CD31/Q-Bend positive and negative areas in the dermis; the non-endothelial Ki67 positive cells have not yet been identified.

In contrast to the decrease in the number of Ki67+ cells in the dermis, the percentage area of epidermis expressing Ki67 was not significantly different after 3 months of GFD, even when the decreased acanthosis was taken into account.

Tissue transglutaminase (tTG). Before the diet period, tTG expression was considerably higher in involved dermis than in non-involved skin (5.06 ± 3.80 vs. $0.67 \pm 0.54\%$, $n=13$, $p=0.0002$). tTG was localized to the endothelium, but was also present in some other structures (lymph vessels?, eccrine glands and some other cells not yet identified). In some specimens from lesional skin the epidermis was also stained. The positive tTG staining covered a higher percentage of the papillary dermis than did CD31/Q-Bend (mean values 5.16% and 2.84%, respectively). After the diet

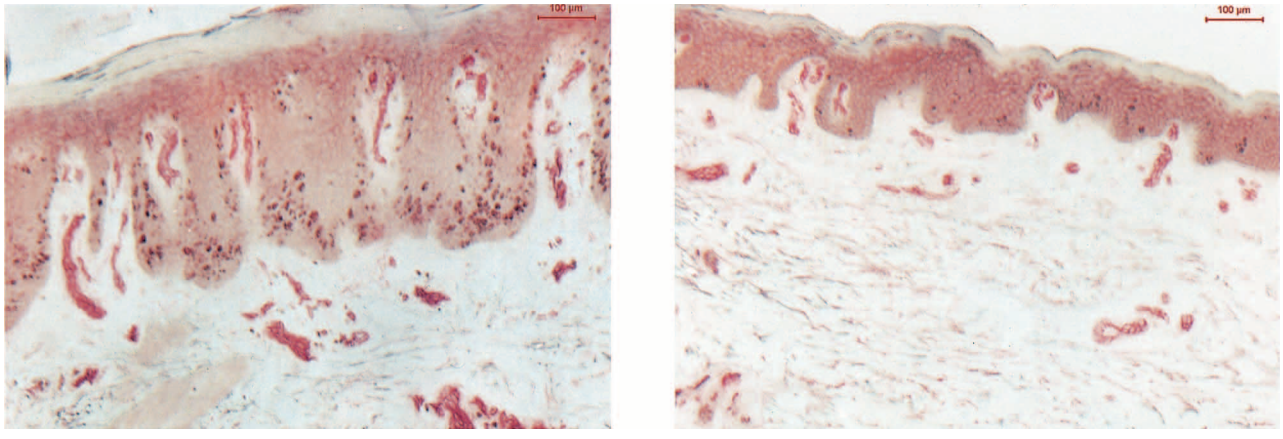

Fig. 1. Endothelial and Ki67 double-staining (a) before and (b) after the gluten-free diet (GFD). Note the decreased endothelium and fewer Ki67+ cells both in the dermis and epidermis after GFD. The specimens are from a 35-year-old woman with IgA AGA and IgA EmA (titre 1/160), a duodenal lymphocyte score of 3, and partial villous atrophy (previously unknown). No other treatment than GFD was given. PASI score decreased from 6.8 to 3.0 during 3 months on GFD and increased to 13.1 within 3 months after resumption of an ordinary diet. When GFD was resumed, her psoriasis cleared.

period both parameters had decreased, but the decrease in mean tTG staining (Fig. 2) was highly significant and more pronounced (50%) than that in the CD31/Q-Bend + staining (25%). The patients with GFD as only treatment had a highly significant decrease in the expression of tTG in involved skin from $4.42 \pm 1.60\%$ to $2.13 \pm 1.46\%$ ($n = 11$, $p = 0.0076$).

There was no obvious relation between the levels of serum antibodies to gliadin, either before or after the diet, and any of the parameters studied.

Non-involved skin

In the epidermis there was a nearly significant decrease in the percentage Ki67+ area, and in the dermis there was a significant decrease in the number of Ki67+ cells (Table II).

Patients without antibodies against gliadin

Before GFD the mean percentage tTG positive area in lesional skin was 3.54 ± 0.991 ($n = 5$). No significant changes were observed in these patients after the diet period, but the group was small and only two of the patients were free from systemic treatment.

DISCUSSION

The results of this study showed that the clinical improvement in patients with antibodies to gliadin was accompanied by several significant histological changes, particularly in the dermis, in both involved and non-involved skin. The changes were significant also in patients with no other treatment than GFD – some of whom with no response to previous regimens – indicating that the effects were induced by the diet, furthermore as there was a clinical worsening when ordinary diet was resumed (1).

There is much evidence to indicate that development of a psoriasis lesion starts in the dermis, but the mechanisms are still unknown. In particular, proliferation of the endothelium has been shown to occur at an early stage (5, 6) and possibly in parallel with the accumulation of mast cells, lymphocytes and macrophages. In the present study the most pronounced changes at the end of the GFD period were observed in the dermis. For example, the number of Ki67+ cells, which represent proliferating cells, was reduced both in involved and non-involved dermis after the diet. Furthermore, there was a pronounced decrease in the expression of tissue transglutaminase in involved dermis.

In a study of the microvasculature in involved psoriasis Creamer et al. (7) confirmed the occurrence of proliferation of endothelial cells. In our study we found that in six pairs of double-stained specimens taken before and after the diet 35–48% of the observed Ki67+ cells were localized to the endothelium, in the papillae or just below the papillae. The remaining cells seemed to be inflammatory cells, but have not yet been identified. The decrease in Ki67+ dermal cells seen after the diet was observed both in the endothelium and in the unidentified cells, in similar proportions. As the Ki67+ endothelial cells were reduced after the diet, it might be expected that the endothelium will also decrease. Although the mean percentage area of endothelium was lower after GFD, the difference was not significant. If the proliferating endothelial cells are localized mainly in the papillae and superficial plexus, a decrease might be concealed if the deeper parts of the papillary dermis are also included in the evaluation. Possibly the results might have differed if only the area of the endothelium within the papillae had been evaluated.

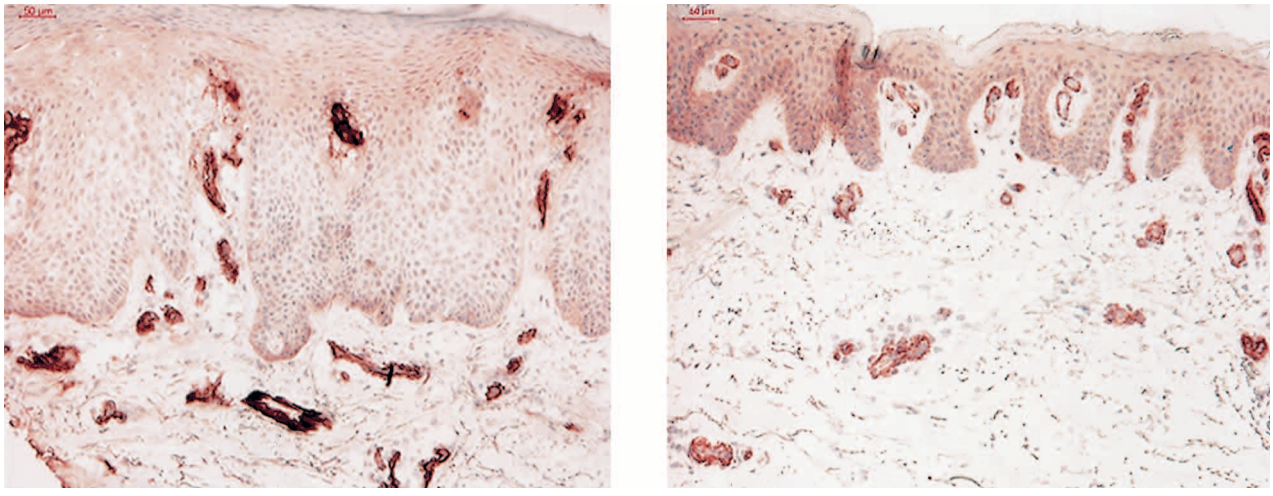

Fig. 2. Tissue transglutaminase staining in involved skin (a) before and (b) after 3 months on the gluten-free diet. The staining was decreased after 3 months. The specimens are from the same patient as in Fig. 1.

A previously unknown feature of involved dermis was the strong over-expression of tTG in comparison with that in non-lesional skin. tTG has been identified as the major target of autoantibodies in coeliac disease (2, 3). A majority of patients with coeliac disease with the most pronounced changes in the intestinal mucosa have circulating IgA antibodies to endomysium and tTG, whereas these serum antibodies are less frequent in those with increased intraepithelial lymphocytes but no change in the mucosal architecture (8).

tTG is an enzyme which is closely associated with proliferating blood vessels. Lesional psoriasis epidermis has also been reported to express tTG (9) and a weak epidermal staining was observed in the present study in some lesions. In healing rat skin wounds, tTG was expressed at the sites of neovascularization within 24 h of wounding (10) and the tTG antigen was increased 4-fold to 5-fold 3 days after the wounding. Another proposed implication for tTG is a role in programmed cell death, cell growth and cell adhesion.

In our AGA+ patients the mean percentage tTG positive area in the dermis before the diet was about eight times higher in involved than in non-involved skin. Also AGA+ patients with ongoing treatment (methotrexate, etretinate, UVB) had this increase in expression of tTG before GFD, indicating that these regimens had not been able to influence the tTG expression. The fact that the percentage tTG area in dermis displayed a highly significant decrease after the diet is noteworthy. Interestingly, in the dermis the expression of tTG decreased to the same degree as the number of Ki67+ cells.

The effect of GFD on the tTG expression raises the question as to whether it is non-specific or specific. In addition to clinical trials with GFD other methods have to be used before any conclusions concerning tTG as a possible autoantigen can be drawn.

In psoriatic lesions the dermal lymphocyte infiltrate is dominated by CD4+ T lymphocytes and migration of these cells into the epidermis is also observed. The fact that there were significantly fewer CD4+ cells in the epidermis after GFD, particularly in the EmA+ patients, indicates a less "activated" epidermis. There may be many explanations for this, one of which might be a decrease of epidermal tTG when the patient is on the GFD.

The results of this study have further confirmed that GFD can influence psoriasis. The pathogenetic role in psoriasis of tTG (TG2) as well as of several other transglutaminases present in the skin and with an abnormal expression in psoriatic epidermis, for example TG 1 (11) and TG 5 (12), deserves further study.

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