

# The Skin and the Gut in Psoriasis: The Number of Mast Cells and CD3+ Lymphocytes Is Increased in Non-involved Skin and Correlated to the Number of Intraepithelial Lymphocytes and Mast Cells in the Duodenum

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The aim of this work was to study tryptase+ mast cells and CD3+ T lymphocytes in non-involved skin in psoriasis and their possible relation to mast cells and lymphocytes in the duodenal mucosa. Skin biopsy specimens were obtained from 43 patients with psoriasis of variable severity and from 10 healthy subjects. Compared with the reference subjects, the number of mast cells in non-involved skin was clearly increased, most markedly in the papillary dermis. The increase was present both in mild, moderate and severe psoriasis. CD3+ lymphocytes were increased in non-involved skin in moderate and severe psoriasis.

Patients with an increased number of duodenal intraepithelial lymphocytes had significantly more mast cells in non-involved skin than those without such an increase, and there was a significant correlation between the number of mast cells in non-involved skin and score for intraepithelial lymphocytes. However, when the 14 patients with increased intraepithelial duodenal lymphocytes were excluded – as they may represent a separate type of psoriasis – another type of correlation between the skin and the duodenal mucosa was found, namely a highly significant inverse correlation between the number of CD3+ lymphocytes in non-involved skin and the number of duodenal mast cells, which is highly elevated in psoriasis. The results might indicate an interplay between skin and intestinal mast cells and lymphocytes in a hitherto unknown way.

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It has been known for many years that the number of mast cells is increased in psoriasis lesions (1). Mast cell degranulation, followed by endothelial hyperproliferation and later infiltration of mononuclear cells (2, 3), was considered a primary event in this development. More recently the distribution of tryptase- and tryptase-chymase positive mast cells in lesional psoriasis skin has been studied in detail with enzyme-histochemical and immunohistochemical methods (4–6).

In a preliminary study we reported on increased numbers of tryptase-positive mast cells in non-involved skin in psoriasis (7). The aim of the present work was to study, in a larger group of psoriasis patients, mast cells and CD3+ T lymphocytes in non-involved skin and their possible relation to mast cells and lymphocytes in the duodenal mucosa. We have recently found that a subgroup of psoriasis patients have an increased number of intraepithelial lymphocytes in the duodenal epithelium (8, 9) and that a majority of psoriasis patients

seem to have a pronounced increase of tryptase mast cells in the duodenal mucosa (10).

## PATIENTS AND METHODS

Forty-three patients with psoriasis (26 men, 17 women, aged 19–70 years, duration of psoriasis 1–42 years) were included. Nine had mild psoriasis (PASI mean  $\pm$ SD 2.8  $\pm$  2.3, range 0–6), 15 moderate (PASI mean  $\pm$ SD 3.8  $\pm$  1.6, range 1–17), and 19 severe disease (PASI mean  $\pm$ SD 9.6  $\pm$  5.3, range 1–20). Two patients were being treated with 10 mg of methotrexate orally once weekly and one was on etretinate 40 mg/day. All 3 had severe psoriasis. None of the patients were receiving UV light, topical steroids, dithranol or calcipotriol. None of them had any other skin disease. Three patients had a history of mild allergic rhinitis.

Serum IgA and IgG anti-gliadin antibodies (IgA AGA and IgG AGA) were determined with an ELISA method, as described previously (8). Patients with values  $\geq$  the 90th percentile value of a reference group of 99 blood donors (IgA AGA  $\geq$  51 U/ml and/or IgG AGA  $\geq$  12 U/ml) were considered to have elevated AGA values. The mean IgA AGA value was 81  $\pm$  78 U/ml (range 10–370) and the mean IgG AGA value 14  $\pm$  27 U/ml (range 1.2–110). Eight patients had normal levels for both IgA AGA and IgG AGA.

Forty of the 43 patients had previously undergone gastroscopy with duodenal biopsies. Sections from each of four paraffin embedded duodenal specimens were stained with hematoxylin and eosin (H&E) and Alcian blue-PAS, as used routinely. The H&E-stained sections had been evaluated semiquantitatively for the presence of mononuclear cells in the epithelium, and the patients were given a score of 0–3 as described in detail (9). One mononuclear cell or less per 4 epithelial cells in the H&E stainings was considered normal (=score 0). Twenty-six patients had a normal score of 0–1 (biopsies from non-involved skin for mast cells were available in 25/26 patients, Table I), 8 patients scored  $\geq$  1–2, 4 scored  $\geq$  2–3 and 2 scored  $\geq$  3.

The study was undertaken during the winter months. The project was approved by the local Ethics Committee and all patients gave their informed consent.

### Skin biopsies

Punch biopsy specimens (3 mm diameter) were taken both from involved 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 10 patients, most of whom had mild psoriasis, lesions suitable for biopsy were not present and biopsy specimens were thus only obtained from non-involved skin. In 2 of the 43 patients specimens were not available from non-involved skin (Table I).

For comparison biopsy specimens from the gluteal region were obtained from 10 healthy subjects (3 women, 7 men, aged 22–65 years).

### Gastroscopy

Four mucosal biopsy specimens were taken from the duodenum distal to the papilla of Vater in conjunction with upper gastrointestinal

Table I. Means  $\pm$  SD for tryptase<sup>+</sup> mast cells (number/mm<sup>2</sup>) and CD3<sup>+</sup> lymphocytes (degree of infiltration, see text) in non-involved psoriasis skin and tryptase<sup>+</sup> mast cells (number/mm<sup>2</sup>) in the duodenal mucosa. Probability values are given in the text.

	Noninvolved skin				Duodenal mucosa	
	n	Mast cells	n	Lymphocytes	n	Mast cells
All patients	41	92 $\pm$ 73	35	4.1 $\pm$ 2.3	37	130 $\pm$ 58 <sup>a</sup>
Patients with mild psoriasis	9	78 $\pm$ 54	8	3 $\pm$ 2.4	6	138 $\pm$ 94
Patients with moderate and severe psoriasis	32	98 $\pm$ 76	27	4.4 $\pm$ 2.2	36	130 $\pm$ 51
Patients with duodenal score <1	25	80 $\pm$ 54	23	4.2 $\pm$ 2.4	21	134 $\pm$ 69
Patients with duodenal score $\geq$ 1	14	117 $\pm$ 90	12	3.8 $\pm$ 2.1	16	126 $\pm$ 43
Healthy controls	10	31 $\pm$ 25	10	2.8 $\pm$ 1.5		

<sup>a</sup> Irritable bowel syndrome patients (n=22) 28  $\pm$  18 tryptase+mast cells/mm<sup>2</sup> (10).

endoscopy, as previously described (9). The specimens were fixed in 4% buffered formaldehyde, and paraffin-embedded separately. As reported elsewhere, duodenal specimens with normal histology from 22 patients with irritable bowel syndrome (IBS) served as reference material with regard to the number of mast cells in the duodenal mucosa (10).

#### Immunohistology on frozen biopsies

Acetone-fixed, 6 mm thick, cryostat sections were stained using a three-step monoclonal antibody peroxidase antiperoxidase (PAP) technique. Endogenous peroxidase was blocked by incubation in 0.3% H<sub>2</sub>O<sub>2</sub> in phosphate-buffered saline (PBS) for 15 min. The sections were then allowed to react with normal rabbit serum (dilution 1/10) for 10 min to reduce non-specific staining. Thereafter they were incubated for 60 min with the primary mouse anti-human mast cell tryptase monoclonal antibody, dilution 1/5000 (MAB 1222, Chemicon, Temecula, CA, USA), which binds to mast cell tryptase or with anti Leu4, which binds to CD3<sup>+</sup> T lymphocytes (diluted 1/100; Becton-Dickinson, Oxnard, CA, USA). Rabbit anti-mouse IgG (diluted 1/40; Dakopatts, Copenhagen, Denmark) was used as a secondary antibody. Finally, the sections were incubated in a third step with horseradish peroxidase-mouse monoclonal anti-horseradish peroxidase (dilution 1/250; Dakopatts). The peroxidase reaction was developed with 3-amino-9-ethylcarbazole. The sections were counterstained with Mayer's hematoxylin. Controls without the primary antibodies gave no staining. All evaluations were made on coded sections. All tryptase<sup>+</sup> and CD3<sup>+</sup> cells in dermis were counted in three sections, the area of the whole section (epidermis included) was estimated by the number of grid squares for each section and the results presented as number of cells/mm<sup>2</sup>. In specimens with large numbers of CD3<sup>+</sup> cells, there were often difficulties in determining the exact number of cells. Therefore the number of CD3<sup>+</sup> cells was graded: 0–50 grade 1, 51–100 grade 2, and so on, although they had been counted in the same way as the mast cells.

#### Immunohistology on paraffin-embedded biopsies

Sections, 6 mm thick, from each of the four duodenal specimens were deparaffinized in xylene and then rehydrated. After blockage of endogenous peroxidase with 3% H<sub>2</sub>O<sub>2</sub> in methanol for 15 min the sections were allowed to react for 20 min with 10% normal rabbit serum to reduce non-specific staining. The sections were then incubated for 20 h with the primary antibody, MAB 1222, working dilution 1/5000, (Chemicon International Inc.), which binds to mast cell tryptase. Thereafter the same procedure as described for frozen biopsies was followed.

All tryptase<sup>+</sup> mast cells in three sections of the duodenal specimens were counted and the mean number/section was calculated. The area of a section was estimated by counting the number of grid squares covering the section; the number of mast cells/mm<sup>2</sup> is given.

#### Statistics

StatView II was used for calculation of mean  $\pm$  SD, and for paired and unpaired *t*-tests, for non-parametric tests and simple linear regression analysis.

## RESULTS

#### Mast cells in the skin

As shown in Table I the number of tryptase<sup>+</sup> cells was increased in non-involved skin in comparison with skin from the reference group ( $p=0.0045$ ). Mast cells were particularly abundant in the papillary dermis, but in many specimens an increase was also observed in the reticular dermis, around blood vessels and the skin appendages. Mast cells were not observed in the epidermis.

In the 10 patients in whom biopsy specimens were taken only from non-involved skin because of the absence of lesions or localization of lesions at sites unsuitable for biopsy (e.g. scalp or pubic area), the number of tryptase<sup>+</sup> cells/mm<sup>2</sup> was 72  $\pm$  53 (mean  $\pm$  SD,  $p=0.0156$  vs controls). Patients with mild psoriasis tended to have a smaller number of mast cells in non-involved skin than those with moderate or severe psoriasis, but the difference was not statistically significant. Patients with pruritic psoriasis did not have a larger mean number of mast cells in non-involved skin than those without pruritus.

The number of mast cells/mm<sup>2</sup> in involved skin was 111  $\pm$  67 ( $n=31$ ). There was a significant correlation between the number in non-involved and involved skin ( $p=0.007$ ). In 10 patients the number was similar in non-involved and involved skin. The difference in the number of mast cells between non-involved and involved skin was less pronounced in the subgroup of patients with a duodenal score of  $\geq 1$  than in the patients with a score of <1.

There was no significant correlation between the level of AGA in serum and the number of mast cells in non-involved and involved skin. In non-involved skin patients with a normal IgA AGA and IgG AGA level had 109  $\pm$  53 (mean  $\pm$  SD), ( $n=8$ ) tryptase<sup>+</sup> cells/mm<sup>2</sup> and in those with IgA AGA  $\geq 70$  U/l the corresponding number was 110  $\pm$  88 ( $n=14$ ).

The number of mast cells in non-involved skin was significantly correlated to the score for intraepithelial lymphocytes in the duodenal biopsy specimens ( $p=0.0237$ ), whereas no significant correlation was observed between the number of mast cells in involved skin and the duodenal score. The mean number of mast cells in non-involved skin was significantly

smaller in patients with a duodenal score of  $<1$  than in those with a score of  $\geq 1$  ( $p=0.0396$ ).

#### *Mast cells in the duodenal mucosa*

As reported elsewhere the number of mast cells in the duodenal mucosa in psoriasis patients is highly elevated in comparison with the number in histologically normal duodenal biopsy specimens from patients with IBS (10). There was no correlation between the number of mast cells in non-involved or involved skin and the number of mast cells in the duodenal mucosa.

#### *CD3<sup>+</sup> T lymphocytes in the skin*

The number of CD3<sup>+</sup> lymphocytes was increased in non-involved skin in those with moderate and severe disease, as shown in Table I ( $p=0.0354$  vs controls). In lesional skin most patients had an increased number of lymphocytes (degree of lymphocyte infiltration  $7.3 \pm 3.0$ ,  $n=31$ ). There was no significant relation between the number of lymphocytes in non-involved and involved skin or between the number of mast cells and lymphocytes in non-involved or in involved skin. Nor was there any significant correlation between the number of lymphocytes in non-involved or involved skin and the score for intraepithelial lymphocytes in the duodenal biopsy specimens.

There was, however, a highly significant inverse relationship between the number of CD3<sup>+</sup> lymphocytes in non-involved skin and the number of mast cells in the duodenal mucosa in patients with normal score ( $<1$ , no increase of lymphocytes in the duodenal epithelium), ( $p=0.0015$ ). In patients with score  $\geq 1$  there was no significant correlation between these parameters.

## DISCUSSION

There is an increase in the number of mast cells in several inflammatory skin diseases, e.g. lesional psoriasis, chronic urticaria, pemphigoid and lichen ruber planus (12). In this study our preliminary results (7) have been confirmed, namely that tryptase-positive mast cells are increased in number not only in involved but also in non-involved skin in psoriasis. In recent studies on mast cells in psoriasis the distribution of tryptase- and tryptase-chymase-positive mast cells was studied in different dermal layers both in non-involved and involved skin (4–6, 14). In those studies significantly increased numbers of mast cells were only observed in lesional skin. Possible explanations for the discrepancy between the results might be differences in the type and severity of disease. If the biopsy specimens are taken in the vicinity of lesions, particularly if the lesion is not stable, the number might also be larger, as discussed by Goodfield et al. in their report on vascular proliferation in relation to mast cell increase (15). However, in our study the numbers of mast cells were also increased in specimens taken from skin areas several decimeters away from any lesions.

CD3<sup>+</sup> T lymphocytes were also significantly increased in number in non-involved skin in moderate and severe disease, whereas – in contrast to mast cells – the increase was not significant in mild disease. The increase in the number of T cells in non-involved skin is in accordance with the report by De Rie et al. (16). There was no significant correlation between the number of CD3<sup>+</sup> lymphocytes and that of mast cells in non-involved skin. It may be assumed, however, that

there is a close and complicated interrelationship between lymphocytes and mast cell activation.

Other factors that might be associated with mast cells and lymphocytes in the skin may be the presence of antibodies to gliadin in the serum and increased lymphocyte infiltration in the duodenal epithelium as well as the degree of mast cell infiltration in the duodenal mucosa. However, we found no evidence of higher mast cell numbers in non-involved skin in patients with mildly or moderately elevated AGA levels without a concomitant increase in the intraepithelial duodenal lymphocytes, compared with the number in those ( $n=8$ ) without antibodies.

Little is known about mast cells and lymphocytes in the skin in relation to these cells in the gastro-intestinal tract. A significant correlation was found between the number of mast cells in non-involved skin and the score for intraepithelial lymphocytes in duodenal biopsy specimens. The observed association between skin mast cells and raised duodenal intraepithelial lymphocytes might also be a chance observation, linked to the fact that these patients had a more severe psoriasis than those with normal numbers of intraepithelial lymphocytes.

However, the majority of the patients had no increase in the intraepithelial duodenal lymphocytes and in these patients with a normal score (but not in those with an abnormal score  $\geq 1$ ) another unexpected correlation was found, namely a strong inverse relation between the number of CD3<sup>+</sup> lymphocytes in non-involved skin and the number of mast cells in the duodenal mucosa, which we have recently reported is highly elevated (10). These results may seem contradictory but anyhow indicate that there may be a communication between the skin and the intestinal tract mast cells and lymphocytes in psoriasis in hitherto unknown ways. The mechanisms for a possible interplay between the gut lymphocytes and mast cells and the skin mast cells and lymphocytes are unknown, but it may be speculated that the same (auto)antigen(s) may be present both in the intestinal mucosa and in the skin. Alternatively, mast cell- and T lymphocyte-promoting factors with systemic effects might be produced in psoriasis, e.g. it is known that IL3-like cytokines can be synthesized by keratinocytes (17) and that there are factors in psoriatic sera with considerable chemokinetic effects (18).

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