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Increase in FoxP3-positive Cells and Their Contacts with Mast Cells in Köbner-negative Patients with Psoriasis after Tape-stripping

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In psoriasis, regulatory T cells (Tregs) can modulate the function of effector Th cells and mast cells (MCs) (1, 2). Defined by their expression of CD4, CD25, and the transcription factor forkhead box P3 (FoxP3), Tregs are central in protecting an individual from autoimmune diseases. FoxP3 is a nuclear protein that is considered to be a master regulator in the development and function of Tregs (3, 4). There have been few studies on Tregs in psoriasis; however, it is known that the function of Tregs is impaired in psoriasis, in the lesional skin and in the peripheral blood, with respect to their ability to suppress effector T cells. Treatment of psoriasis may increase the numbers and activity of Tregs (5–7).

MC activation is one of the earliest morphological changes in the developing psoriatic lesion (2), but it is unknown to what extent pro-inflammatory MC cytokines are involved in the development of psoriatic lesions. The interaction between Tregs and MCs has been shown to be essential for immunosuppression in a variety of experimental models, such as hepatocarcinoma, colorectal carcinoma, and skin allografts (8–10).

The purpose of this study was therefore to investigate Tregs and their interaction with MCs in the early developing lesion in psoriasis. To this end, the tape-stripping technique was used to induce the Köbner reaction, and a series of skin biopsies was obtained at 0 days, 2 h, 1 day, 3 days and 7 days (11, 12). Patients who did not develop the Köbner reaction were the focus of this study, because we hypothesized that suppressive factors prevent the development of lesions compared with patients with positive Köbner reactions. These biopsies were analysed for expression of CD25/IL-2Rα and FoxP3 and for apparent morphological contacts (AMCs) between tryptase-positive MCs and FoxP3$^+$ cells by immunohistochemistry and double-staining. FoxP3 has been described as the marker of Tregs, but it may also be expressed by other cell types. This aspect must be remembered when interpreting the results.

MATERIALS AND METHODS

Mouse monoclonal anti-human FoxP3 (clone 236A/E7) was purchased from Abcam (Cambridge, UK). Mouse monoclonal anti-human CD25/IL-2Rα (clone 24204) was obtained from R&D Systems Europe Ltd (Oxon, UK). The substrate of tryptase, Z-Gly-Pro-Arg-4-methoxy-2-naphthylamide (Z-Gly-Pro-Arg-MNA), was obtained from Bachem (Bubendorf, Switzerland), and the chromogen, Fast Black K, was acquired from Sigma (St Louis, MO, USA). Reagents for immunohistochemistry were purchased from Vector Laboratories (Burlingame, CA, USA).

As described in our previous studies (11, 12), the Köbner reaction was studied by the tape-stripping technique on non-lesional arm skin in 18 patients with psoriasis (4 females and 14 males, age range 24–77 years). Four-mm punch biopsies were taken at 0 days, 2 h, and 3 days or at 0 days, 1 day, and 7 days. The Köbner phenomenon was evaluated at the follow-up visit 2–2.5 weeks later. Eight of 18 subjects showed an initial developing lesion (Köbner-positive group). The patients had not received any effective systemic or local treatment in the preceding month. The skin biopsies were embedded immediately in optimal cutting temperature (OCT) compound (Miles Scientific, Naperville, IL, USA) and frozen to prepare 5-µm thick cryosections (11, 12). The methods were approved by the ethics committee of Kuopio University Hospital, Kuopio, Finland.

For staining CD25 and FoxP3, the skin cryosections were fixed in cold acetone for 10 min and blocked with 1.5% normal horse serum in phosphate-buffered saline (PBS). The bound anti-FoxP3 (10 µg/ml) and anti-CD25 mAbs (5 µg/ml) were visualized with the avidin-biotin-peroxidase (ABC) technique using the Vectastain Elite ABC kit, 0.05% 3,3'-diaminobenzidine tetrahydrochloride, 0.04% nickel chloride, and 0.03% hydrogen peroxide. The numbers of CD25$^+$ and FoxP3$^+$ cells were counted in a blinded fashion on separate cryosections on a 0.2×0.2 mm ocular grid (Ella Graticules, Tonbridge, Kent, UK) in an area 0.4 mm (depth)×1.0 mm (width) immediately beneath the papillary dermis. The results are expressed as cells/mm^2.

The technique of double-staining tryptase$^+$ MCs and FoxP3$^+$ cells has been developed to analyse the morphological relationships between tryptase$^+$ MCs and sensory nerves (13). The 5-µm thick skin cryosections were fixed in cold acetone for 10 min and blocked with diluted normal horse serum in PBS. The sections were first treated with anti-FoxP3 and then with biotin-conjugated secondary Ab. Next, tryptase$^+$ MCs were identified histochemically using 1 mM Z-Gly-Pro-Arg-MNA and Fast Black K, resulting in dark-blue to violet MCs. Finally, immunolabelled FoxP3$^+$ cells were visualized with the ABC technique, generating a black-stained product. AMCs were identified in an area of 0.4 mm (depth) × 1.0 mm (width) immediately beneath the papillary dermis, and the results are expressed as the percentage of tryptase$^+$ MCs in AMCs with at least one FoxP3$^+$ cell. Only clearly stained tryptase$^+$ MCs, FoxP3$^+$ cells, and AMCs were counted. The cryosections were blinded before the cells were counted.

Statistical analysis

The results were analysed by paired or unpaired 2-tailed *t*-test, and $p<0.05$ was considered statistically significant.

RESULTS

In the Köbner-negative group, the number of FoxP3$^+$ cells increased significantly in the 3–7-day and 7-day biopsies (Table SI[1], Fig. S1b[1]) compared with day 0. No

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statistically significant changes in the number of CD25+ or FoxP3+ cells (Fig. S1[1]) were found between the 2 Köbner groups or in CD25+ cells within the Köbner groups (Table SI[1]).

With regard to the percentage of tryptase+ MCs in AMCs with at least one FoxP3+ cell between and within the Köbner groups, a statistically significant increase was noted in the Köbner-negative group in the 7-day vs. 0-day biopsies (Table SII[1], Fig. S1c[1]).

DISCUSSION

Using the same biopsy material, we have previously reported that a positive Köbner reaction is associated with a rapid, but transient, decrease in IL-33 immunostaining in the epidermis, higher MC IL-6 expression, and the appearance of dermal cells that express IL-6R and IL-33. However, the number of IL-6R+ cells was even higher in Köbner-negative skin on days 3–7 (11, 12). Goodman et al. (14) have demonstrated that IL-6 can enhance the resistance of effector T cells to Treg-mediated suppression, a mechanism that may contribute to this failure in regulation.

The essential findings of this study are that the number of FoxP3+ cells and the AMCs between tryptase+ MCs and FoxP3+ cells increased within 3–7 days in the Köbner-negative, but not in the Köbner-positive, group. It is possible that at least some of these FoxP3+ cells expressed IL-6R, as detected earlier in this same Köbner-negative group (11). In contrast, no significant changes were observed in CD25+ cells. The increase in AMCs is probably due to an increase in FoxP3+ cells, because the number of tryptase+ cells did not change significantly in either Köbner group (11).

Although Tregs have been characterized by their CD25 expression, it has been shown that CD25 is also expressed by recently activated effector T cells (1), probably resulting in the inclusion of CD25+ effector T cells in the CD25+ cell population considered in this study. Also, the expression of CD25 does not necessarily correlate with that of FoxP3 (15). Recently, a new marker, CD127 (IL-7Rα), has been found to identify Tregs. Liu et al. (15) have shown that CD127 correlates inversely with FoxP3 and the suppressive function of human CD4+ Treg cells.

In conclusion, the increase in FoxP3+ cells and their contact with MCs within 3–7 days in the Köbner-negative group suggests that they express suppressive factors that consequently prevent the development of the Köbner reaction. This hypothesis is supported by a variety of animal models (8–10).

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