Naturally occurring regulatory T cells in mucous membrane pemphigoid lesions

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ABSTRACT

Background: A growing body of evidence suggests the involvement of naturally occurring CD4+ CD25+ regulatory (nTreg) T cells in autoimmune diseases.

Objective: To evaluate the expression of some nTreg markers in mucous membrane pemphigoid (MMP) lesions.

Methods: Lesional biopsies from six patients with untreated MMP were stained immunohistochemically with anti-CD25, -FoxP3, -CD103, and -CCR5.

Results: All of the stained cells, both in MMP lesions and controls, were observed in the interstitial lamina propria or dermis. Positive cell counts of all the markers studied were low or very low in all sections, and significantly higher in MMP specimens than in healthy controls.

Conclusions: The expression of CCR5 and CD103, which mediate recruitment into peripheral tissues, indicates that CD25+ FoxP3+ nTreg cells may be present in MMP lesions according to a specific homing. nTreg cells may contribute to directing the MMP immunoinflammation towards chronicity, and thus favor the cicatricial evolution of lesions.

KEY WORDS

CCR5, CD103, CD25, FoxP3, mucous membrane pemphigoid, regulatory T cells

Introduction

Mucous membrane pemphigoid (MMP) is a group of putative autoimmune, chronic inflammatory, subepithelial blistering diseases predominantly affecting mucous membranes that is characterized by linear deposition of IgG, IgA, or C3 along the epithelial basement membrane zone (BMZ) (1). Circulating autoantibodies that recognize several BMZ components such as BP180, BP230, and laminin 5, induce blister formation in experimental models *in vivo* and are likely to also be pathogenic in human disease. Nevertheless, several studies suggested the involvement of immune cells and related cytokines, such as interleukin (IL)-4 and transforming growth factor (TGF)-β, in the pathogenesis of MMP (2–5).

Regulatory T (Treg) cells play a key role in the modulation of allergic and autoimmune responses. Two

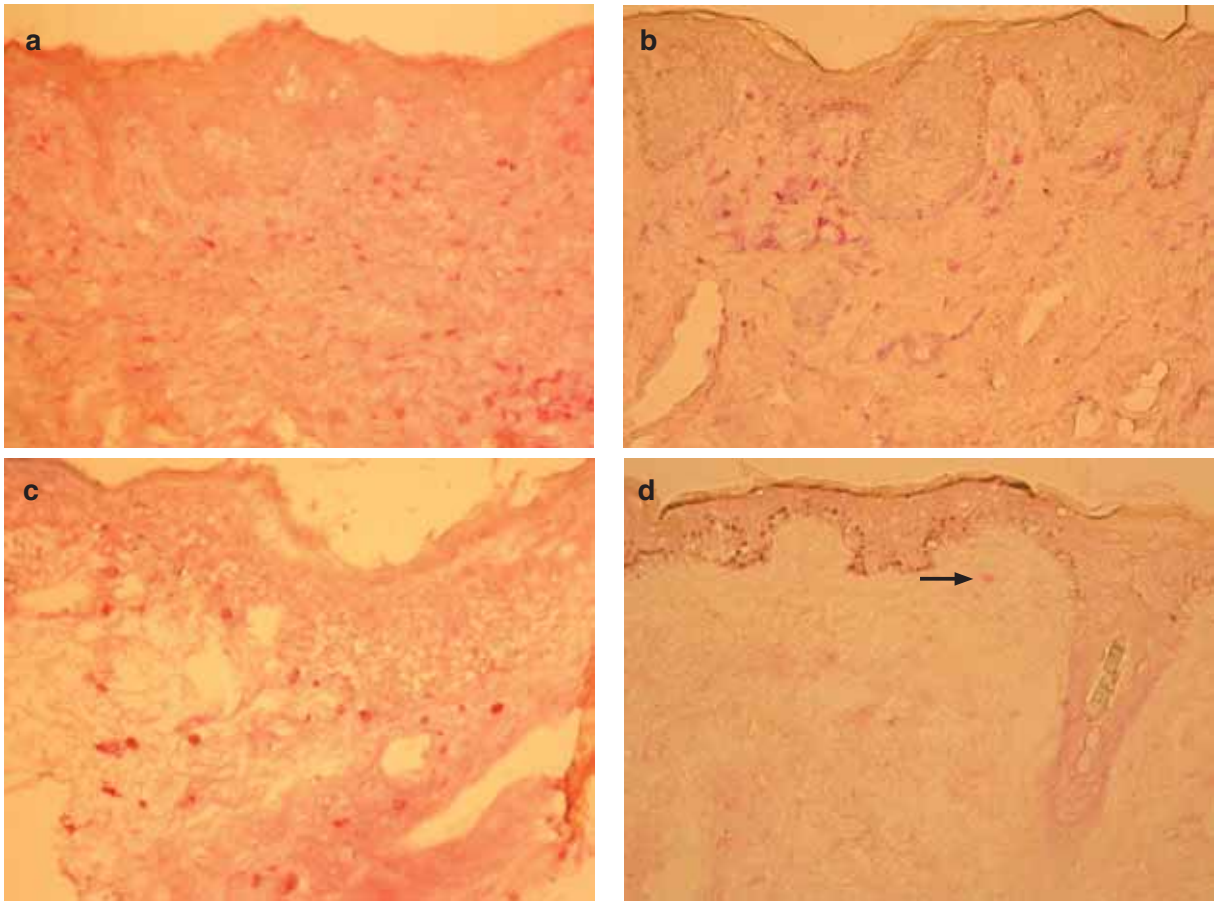

Figure 1. Immunohistochemistry, original magnification ×100.
a) oral mucosa: CD25+ cells in the interstitial lamina propria.
b) CD25+ cells in dermis of healthy skin control.
c) CD103+ cells in conjunctiva.
d) a single CD103+ cell , arrowhead highlight positive cell.

main groups of Treg cells have been defined (6). One comprises natural Treg (nTreg) cells, which originate in the thymus, are characterized by their CD4+ CD25+ phenotype, and highly express the forkhead/winged helix transcription factor (FoxP3). The other group ("adaptive" Treg) is induced in the periphery after encounters with foreign antigens and pathogens. There is ample evidence that nTreg cells actively suppress self-reactive T cells. Once they leave the thymus, they survive in the periphery poised for normal surveillance of autoantigens, and prevent potential autoimmune responses (7). Many surface molecules can be considered nTreg markers because they are present on nTreg cells but are absent, or far less abundant, on conventional T cells. Forkhead box transcription factor (FoxP3) expression is now considered the gold standard property of nTreg cells and one that is actually involved in the differentiation of T cells into a regulatory function (7). The glucocorticoid-induced tumor necrosis factor and the cytotoxic T lymphocyte-associated receptor 4 may both be involved in nTreg function. In contrast to conventional CD4+ T cells, nTreg cells preferentially express the receptor 5 for CC chemokines (CCR5), which enables them to migrate in response to the CCR5 ligands in vitro (8). In addition, expression of the migration molecules CD62 ligand (CD62L) and the aE integrin CD103 may help distinguish between nTreg cells that seek lymph nodes (CD62L+) and those that home to tissue sites (CD103+) (9). The actual mechanisms by which nTreg cells mediate suppression remain poorly understood at least *in vivo* (7). The suppression of IL-2 mRNA transcription and proliferation of CD4+ and CD8+ T effector cells, as well as the cytotoxicity mediated by the secretion of perforin and granzyme B, may represent important functions of nTreg cells.

A growing body of evidence suggests the involvement of nTreg cells in human health and diseases, including autoimmune, allergic, and neoplastic processes (7).

The aim of our study was to evaluate the expression of some nTreg markers in the lesional mucous membranes or skin of subjects with MMP.

Table 1. Cell counts, expressed as means ± standard deviations, in MMP specimens and controls.

	MMP	Healthy controls	*p*
CD25	9.93 ± 5.59	5.44 ± 2.26	0.004
FOXP3	7.42 ± 3.29	1.25 ± 1.06	< 0.0001
CD103	5.80 ± 4.35	1.0 ± 1.21	0.001
CCR5	3.25 ± 2.22	0.92 ± 1.24	0.004

Material and methods

Upon written informed consent, lesional mucous membrane or skin biopsies were taken and studied from six patients (1 male, 5 females, mean age 73.5 years, range 63–92) that were diagnosed according to widely accepted criteria for MMP[1] and untreated. The biopsy sites were clinically non-bullous lesions of the conjunctiva (2 cases), oral cavity (2), and skin (2). The normal-appearing skin of six healthy subjects (3 males, 3 females, mean age 68.5 years, range 54–83) served as control.

Tissue specimens were immediately frozen at -80 °C in liquid nitrogen and then cut into 5-μm thickness sections, which were stained immunohistochemically with the alkaline phosphatase/anti-alkaline phosphatase method as described previously (5). The monoclonal antibodies included those to CD25 (1:10; DAKO, Copenhagen, Denmark), FoxP3 ab10563 (1:80; Abcam Ltd., Cambridge, UK), CD103 (1:50; Serotec, Oxford, UK), and CCR5 (1:100; R & D Systems, Minneapolis, MN, USA).

Two independent "blind" observers evaluated serial sections. For quantitative analysis, the stained cells were counted in three consecutive microscopic fields ×250 and the mean was calculated. Statistical significance ($p < 0.05$) was assessed using Student's *t*-test.

Results

All of the stained cells, both in MMP lesions and controls, were observed in the interstitial lamina propria or dermis (Fig. 1). The positive cell counts of all of the markers studied were low or very low in all sections, and significantly higher in MMP specimens than in healthy controls (Table 1).

Discussion

Our findings show that some markers of nTreg cells are expressed in MMP lesions. The membrane receptor of IL-2 (i.e., CD25) is mainly expressed on activated T cells. In agreement with previous studies (3), we found that CD25+ cells, mainly belonging to the CD4+ subset (3), were present in MMP lamina propria/dermis. Some of these cells expressed FoxP3, and thus had to be considered nTreg cells. The study of cell surface markers involved in tissue location showed that CCR5 and CD103, which mediate recruitment into peripheral tissues, were expressed on some infiltrating cells, thus indicating that nTreg cells may be present in MMP lesions according to a specific homing. The presence of a higher number of nTreg cells in a site of autoimmune phlogosis, such as MMP lesions, compared with healthy specimens, is in agreement with a recent study, which showed an infiltration of CD4+CD25+FOXP3+ T cells in lesional bullous pemphigoid (i.e., the prototypic variant of autoimmune subepidermal bullous disorders), but not healthy skin (10).

We hypothesize that the inhibitory functions of nTreg cells may contribute to directing the MMP immunoinflammation towards chronicity. This phenomenon, together with the anatomical distribution of the autoantigens and the action of profibrotic molecules, may favor the cicatricial evolution of MMP lesions. In addition, a direct role of nTreg cells in the activation of fibroblasts (e.g., via the membrane form of the fibrogenic cytokine TGF-β) also cannot be excluded. Future studies will help clarify these issues.

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A U T H O R S ' A D D R E S S E S

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