UNIVERSITÀ
DEGLI STUDI
FIRENZE

FLORE

Repository istituzionale dell'Università degli Studi di Firenze

The Treg/Th17 cell ratio is reduced in the skin lesions of patients with pyoderma gangrenosum.

Questa è la Versione finale referata (Post print/Accepted manuscript) della seguente pubblicazione:

Original Citation:

The Treg/Th17 cell ratio is reduced in the skin lesions of patients with pyoderma gangrenosum / Caproni M; Antiga E; Volpi W; Verdelli A; Venegoni L; Quaglino P; Fabbri P; Marzano AV.. - In: BRITISH JOURNAL OF DERMATOLOGY. - ISSN 0007-0963. - STAMPA. - 173:(2015), pp. 275-278.

Availability:

This version is available at: 2158/969192 since: 2015-11-09T11:05:10Z

Terms of use:

Open Access

La pubblicazione è resa disponibile sotto le norme e i termini della licenza di deposito, secondo quanto stabilito dalla Policy per l'accesso aperto dell'Università degli Studi di Firenze (<https://www.sba.unifi.it/upload/policy-oa-2016-1.pdf>)

Publisher copyright claim:

(Article begins on next page)

These articles have been accepted for publication in the *British Journal of Dermatology* and are currently being edited and typeset. Readers should note that articles published below have been fully refereed, but have not been through the copy-editing and proof correction process. Wiley-Blackwell and the British Association of Dermatologists cannot be held responsible for errors or consequences arising from the use of information contained in these articles; nor do the views and opinions expressed necessarily reflect those of Wiley-Blackwell or the British Association of Dermatologists

This article is protected by copyright. All rights reserved.

Accepted Date : 27-Dec-2014

Article type : Research Letter

The Treg/Th17 cell ratio is reduced in the skin lesions of patients with pyoderma gangrenosum

M. Caproni,^{1,*} E. Antiga,^{1,*} W. Volpi,¹ A. Verdelli,¹ L. Venegoni,² P. Quaglino,³ P. Fabbri,¹ A.V. Marzano.²

¹Department of Surgery and Translational Medicine, Section of Dermatology, University of Florence, Florence, Italy

²Dipartimento di Fisiopatologia Medico-Chirurgica e dei Trapianti, Università degli Studi di Milano, Unità Operativa di Dermatologia, IRCCS Fondazione Ca' Granda, Ospedale Maggiore Policlinico – Milan, Italy

³Department of Biomedical Sciences and Human Oncology, 1st Dermatologic Clinic, University of Turin, Turin, Italy

*These authors contributed equally to the preparation of the manuscript

Corresponding author:

Dr. Emiliano Antiga

Department of Surgery and Translational Medicine, Section of Dermatology

University of Florence

Viale Michelangelo 41

This article is protected by copyright. All rights reserved.

50125 Florence, Italy

Phone: +39 055 6939664

Fax: +39 055 6939598

e-mail: emiliano.antiga@unifi.it

Funding: The study was funded by a grant Ministry of Instruction, University and Research of the Italian Government within the funding program PRIN 2008 (project code: 2008EW3FHK).

Conflict of interests: None

Keywords: pyoderma gangrenosum, Sweet's syndrome, regulatory T cells, T helper 17 cells

Pyoderma gangrenosum (PG) is a rare, inflammatory skin disease that, together with other conditions such as Sweet's syndrome (SS), is included within the group of neutrophilic dermatoses.¹ Although its pathogenesis remains poorly understood,² the treatments with the best clinical evidence are tumor necrosis factor (TNF)-inhibitors, high-dose systemic corticosteroids and cyclosporine, suggesting the pivotal role of inflammatory pathways in the development of PG.

Interestingly, recent studies highlighted the role of T helper 17 (Th17) cells in neutrophilic dermatoses,² and an increase of IL-17³ and IL-23⁴ expression was found in PG lesions.

Together with Th17 cells, regulatory T cells (Tregs) play a major role in human disease.⁵ Accordingly, recent reports suggest that controlling the balance between Treg and Th17 cells may be a promising therapeutic strategy for inflammatory diseases.⁶ However, no data are present in the literature about Tregs in PG.

This article is protected by copyright. All rights reserved.

In this study, we investigated the proportions of Tregs and Th17 cells in the skin of 15 PG patients (7 males, 8 females; age range 27-69 years), not on immunosuppressive treatment nor topical steroids for at least 4 weeks prior to entering the study.

As control, skin samples from 5 SS patients (2 males, 3 females; age range 31-64 years) and 6 normal subjects (NS) (3 males, 3 females; age range 28-59 years) were collected.

The trial was approved by ethical committee and conducted according to the Declaration of Helsinki. All the patients and controls provided written informed consent.

Treg and Th17 markers were analysed by immunohistochemistry using monoclonal antibodies (mAb) anti-CD4 (1:20; Dako, Copenhagen, Denmark), anti-CD25 (1:25; Histo-Line Laboratories, Milan, Italy), anti-CD161 (1:80; AbD Serotec, Oxford, UK), anti-FoxP3 (1:80; Abcam, Cambridge, UK), anti-IL10 (1:300; Dako), anti-IL-17 (1:1000; Abcam), anti-ROR γ t (1:2000; R&D Systems, Minneapolis, MN, USA), anti-TGF β 1 (1:2000; Abcam), as described previously.⁷

The stained cells were counted in three consecutive microscopic fields (400 $\times$). Furthermore, FOXP3⁺/CD4⁺, TGF- β ⁺/CD4⁺, IL-10⁺/CD4⁺ and ROR γ t⁺/CD4⁺ cell ratios were calculated. The results were analysed with Mann-Whitney *U* test and considered significant with a *p* value <0.05.

In PG and SS, CD4⁺ and CD25⁺ cells were located in the whole dermis with some cells scattered in the epidermis (Fig. 1a,b,d,e). The number of CD4⁺ cells in PG was significantly higher than in SS (*p*=0.0003), while no differences were found for CD25⁺ cells. By contrast, CD4⁺ and CD25⁺ cells were significantly less represented in NS than in the other two groups (*p*<0.0001) (Fig. 2a).

Some FOXP3⁺ cells were detected within the superficial dermis of patients with PG (Fig. 1g); their number was higher than in NS (*p*=0.004), but lower than in SS (*p*=0.0001) (Fig. 2a). Interestingly, the FOXP3⁺/CD4⁺ cell ratio was significantly lower in PG compared to SS (*p*<0.0001) and NS (*p*<0.0001) (Fig. 2a).

Some IL-10⁺ cells were found in PG superficial dermis (Fig. 1j); their number was significantly lower than in SS ($p < 0.0001$) and higher than in NS ($p < 0.0001$). Moreover, the IL-10/CD4⁺ cell ratio was reduced in PG than in SS and NS ($p < 0.0001$ and $p = 0.03$, respectively) (Fig. 2a).

TGF- β staining was diffusely distributed within the superficial and medium dermis in PG and SS. Moreover, some TGF- β ⁺ cells could be detected in the same areas (Fig. 1m,n). Their number was similar in both PG and SS. By contrast, the TGF- β ⁺/CD4⁺ cell ratio was significantly reduced in PG ($p = 0.01$). Moreover, although NS showed a lower number of TGF- β ⁺ cells than PG ($p < 0.0001$) and SS ($p < 0.0001$), their TGF- β ⁺/CD4⁺ cell ratio was significantly higher (NS vs PG: $p = 0.001$; NS vs SS: $p = 0.0001$) (Fig. 2a).

Regarding Th17 markers, ROR γ t⁺ cells were distributed in the upper dermis in PG (Fig. 1p); their number was similar to that found in SS. By contrast, the ROR γ t⁺/CD4⁺ ratio was significantly lower in PG than in SS ($P = 0.008$) (Fig. 2a). As expected, no ROR γ t expression was found in NS. The numbers of both CD161⁺ and IL-17⁺ cells, that were predominantly distributed in the superficial and medium dermis (Fig. 1s,t), were similar in PG and SS, while no CD161 nor IL-17 expression was detected in NS (Fig. 2a).

Finally, in order to quantify the balance between Tregs and Th17 cells, we calculated the ratio between FOXP3 and ROR γ t, that was significantly lower in PG than in SS ($p < 0.0001$) (Fig. 2b).

Our study demonstrated a reduced proportion of Tregs in PG skin, that may be responsible for an impairment of the suppressive activity, leading to the development of the lesions. Interestingly, Treg reduction was not found in SS, where the skin inflammation is less strong and the tissue damage milder.

Accordingly, we found significantly reduced IL-10⁺/CD4⁺ and TGF- β ⁺/CD4⁺ cell ratios in PG. IL-10 and TGF- β are involved in the biology of Tregs, and their defective signaling is associated with

inflammatory conditions.⁸⁻¹⁰ In agreement with our results, PBMCs from patients with PAPA syndrome (pyogenic sterile arthritis, PG, and acne) showed a diminished production of IL-10 after stimulation, suggesting an impairment of IL-10 pathway.¹¹

As a result, in PG, the impaired T cell regulation due to the reduction of Tregs and of the regulatory cytokines may allow the uncontrolled activation of effector T cells, such as Th17 cells.

Interestingly, in PG skin, we found augmented numbers of Th17 cells, that may play a role in the disease *via* the recruitment of neutrophils and monocytes,¹² and the induction of MMPs.¹³

Accordingly, some Authors reported the effectiveness of therapies targeting the Th17 pathway in the treatment of PG.^{4,14} In this view, our findings may pave the way to the use of novel Th17-oriented therapies such as IL-17 antagonists for PG therapy.

Finally, we found an impairment of the balance between Tregs and Th17 cells in PG but not in SS, that could explain the most severe clinical course of the former. Interestingly, a reduced ratio of Tregs to Th17 cells was recently found even in patients with inflammatory bowel diseases,¹⁵ that are often associated to PG, suggesting that common mechanisms could be implicated in immune system dysregulation in both conditions.

References

1. Marzano AV, Ishak RS, Saibeni S, et al. Autoinflammatory skin disorders in inflammatory bowel diseases, pyoderma gangrenosum and Sweet's syndrome: a comprehensive review and disease classification criteria. *Clin Rev Allergy Immunol* 2013; **45**:202–10.
2. Butler D, Shinkai K. What do autoinflammatory syndromes teach about common cutaneous diseases such as pyoderma gangrenosum? A commentary. *Dermatol Clin* 2013; **31**:427–35.

3. Marzano AV, Cugno M, Trevisan V, et al. Role of inflammatory cells, cytokines and matrix metalloproteinases in neutrophil-mediated skin diseases. *Clin Exp Immunol* 2010; **162**:100–7.
4. Guenova E, Teske A, Fehrenbacher B, et al. Interleukin 23 expression in pyoderma gangrenosum and targeted therapy with ustekinumab. *Arch Dermatol* 2011; **147**:1203–5.
5. Buckner JH. Mechanisms of impaired regulation by CD4(+)CD25(+)FOXP3(+) regulatory T cells in human autoimmune diseases. *Nat Rev Immunol* 2010; **10**:849–59.
6. Niu YH, Yin DL, Liu HL, et al. Restoring the Treg cell to Th17 cell ratio may alleviate HBV-related acute-on-chronic liver failure. *World J Gastroenterol* 2013; **19**:4146–54.
7. Caproni M, Torchia D, Antiga E, et al. The comparative effects of tacrolimus and hydrocortisone in adult atopic dermatitis: an immunohistochemical study. *Br J Dermatol* 2007; **156**:312–9.
8. Caproni M, Torchia D, Antiga E, et al. The effects of tacrolimus ointment on regulatory T lymphocytes in atopic dermatitis. *J Clin Immunol* 2006; **26**:370–5.
9. Antiga E, Quaglino P, Bellandi S, et al. Regulatory T cells in the skin lesions and blood of patients with systemic sclerosis and morphoea. *Br J Dermatol* 2010; **162**:1056–63.
10. Glocker EO, Kotlarz D, Boztug K, et al. Inflammatory bowel disease and mutations affecting the interleukin-10 receptor. *N Engl J Med* 2009; **361**:2033–45.
11. Demidowich AP, Freeman AF, Kuhns DB, et al. Brief report: genotype, phenotype, and clinical course in five patients with PAPA syndrome (pyogenic sterile arthritis, pyoderma gangrenosum, and acne). *Arthritis Rheum* 2012; **64**:2022–7.

12. Marzano AV, Fanoni D, Antiga E, et al. Expression of cytokines, chemokines and other effector molecules in two prototypic autoinflammatory skin diseases, pyoderma gangrenosum and sweet's syndrome. *Clin Exp Immunol* 2014; **178**:48–56.
13. Agarwal S, Misra R, Aggarwal A. Interleukin 17 levels are increased in juvenile idiopathic arthritis synovial fluid and induce synovial fibroblasts to produce proinflammatory cytokines and matrix metalloproteinases. *J Rheumatol* 2008; **35**:515–9.
14. Goldminz AM, Botto NC, Gottlieb AB. Severely recalcitrant pyoderma gangrenosum successfully treated with ustekinumab. *J Am Acad Dermatol* 2012; **67**:e237-8.
15. Eastaff-Leung N, Mabarrack N, Barbour A, et al. Foxp3+ regulatory T cells, Th17 effector cells, and cytokine environment in inflammatory bowel disease. *J Clin Immunol* 2010; **30**:80-9.

Legend to figures:

Figure 1. Immunohistochemical staining for CD4, CD25 and FOXP3 as markers of Tregs (a-i), for the regulatory cytokines TGF- β and IL-10 (j-o), as well as for the Th17 cell markers ROR γ t, CD161 and IL-17 (p-x) in skin biopsy specimens from patients with pyoderma gangrenosum (PG) and Sweet's syndrome (SS). Scale bar = 100 μ m

Figure 2. (a) Numbers of CD4⁺, CD25⁺, FOXP3⁺, TGF- β ⁺, IL-10⁺, ROR γ t⁺, CD161⁺ and IL-17⁺ cells expressed as medians, as well as FOXP3/CD4, TGF- β /CD4, IL-10/CD4 and ROR γ t/CD4 cell ratios expressed as % of CD4⁺ cells in the inflammatory infiltrate of skin biopsy specimens from patients with pyoderma gangrenosum (PG), Sweet's syndrome (SS) and normal subjects (NS).

(b) FOXP3/ROR γ t cell ratios in the inflammatory infiltrate of skin biopsy specimens from patients with PG and SS. PG showed a significantly reduced ratio than SS. *: p<0.05.

Supplementary table 1. Clinical findings in 15 patients with pyoderma gangrenosum.

N	Sex	Age (years)	Disease duration (months)	Disease severity (BSA%)	Associated diseases	Family history
1	M	65	6	12	Klinefelter	Negative
2	M	61	3	8	-	Negative
3	F	28	12	18	Cystic fibrosis	Negative
4	M	30	18	6	IBD	Negative
5	F	35	10	5	-	Negative
6	F	30	4	8	IBD	Negative
7	M	67	3	20	-	Negative
8	M	62	24	8	-	Negative
9	M	57	18	3	-	Negative
10	F	44	48	2	-	Negative
11	F	69	40	3	-	Negative
12	M	42	6	6	-	Negative
13	F	48	16	1	-	Negative
14	F	53	36	10	-	Negative
15	F	38	36	6	-	Negative

IBD: inflammatory bowel disease

Supplementary table 2. Quantitative analysis on the numbers of positive cells for field (400X) in skin lesions of patients with pyoderma gangrenosum and Sweet's syndrome, as well as in normal subjects as assessed by immunohistochemistry

	PG	SS	NS
<i>CD4</i>	103 [90.7-143.5]	69 [54-77]	4.5 [2-7]
<i>CD25</i>	18.5 [14-23.2]	22 [17.5-27]	1.5 [0.25-2]
<i>FOXP3</i>	11 [2.2-16]	19 [18-23.5]	1 [0-2]
<i>% FOXP3/CD4</i>	8.5 [3-12.7]	28 [26-29]	25 [6.2-33.3]
<i>TGF-β</i>	28 [23.7-35.2]	28 [24.5-31]	3 [1.25-4]
<i>% TGF-β/CD4</i>	23.9 [18.9-27.3]	36.8 [32.64-43]	63.6 [50-73.2]
<i>IL-10</i>	16 [11.2-18]	24 [18-27]	1 [0-1.75]
<i>% IL-10/CD4</i>	14.3 [12.1-16.6]	30.4 [22.4-39.5]	20 [11.7-38]
<i>RORγt</i>	15 [13-18]	16 [11.2-32.2]	0 [0-0]
<i>% RORγt/CD4</i>	12.1 [8.3-22.1]	23.3 [17.4-40.8]	0 [0-0]
<i>CD161</i>	32 [23.5-65.5]	27 [17.5-31.5]	0 [0-0]
<i>IL-17</i>	20.5 [17-25.2]	17.5 [13.2-23.5]	0 [0-0]
<i>% FOXP3/RORγt</i>	32.4 [13.1-76.4]	122.1 [67.3-156.2]	0 [0-0]

Positive-cell counts are expressed as medians [25th–75th percentile].

PG, pyoderma gangrenosum; SS, Sweet's syndrome; NS, normal subjects.