Published in final edited form as:
J Invest Dermatol. 2009 December ; 129(12): 2747–2750. doi:10.1038/jid.2009.290.

Regulation gone wrong: a subset of Sézary patients have malignant regulatory T cells

Rachael A. Clark[1]

[1] Harvard Skin Disease Research Center and the Department of Dermatology, Brigham and Women's Hospital, Boston, Massachusetts, USA

Abstract

Cutaneous T cell lymphomas are a heterogeneous group of non-Hodgkin's lymphomas derived from the population of T cells that home to and inhabit the skin. There have been conflicting reports as to whether CTCL may represent a malignancy of regulatory T cells, a particular T cell subset that can suppress local immune reactions. In this issue of the JID, authors Heid *et al.* present convincing evidence that the malignant T cells in a specific subgroup of Sézary patients are FOXP3$^+$ regulatory T cells. Clonal malignant T cells in these patients have increased expression of the Treg associated transcription factor FOXP3, demethylation of the FOXP3 gene locus, and T cells from at least some of these patients could suppress T cell proliferation *in vitro*.

By virtue of their ability to inhibit T cell activation, proliferation, cytokine production and cytotoxicity, regulatory T cells (Tregs) can suppress immune reactions in their local vicinity (Sakaguchi, 2005). Tregs play a critical role in inducing and maintaining immune self tolerance; humans and mice with mutations in the Treg associated transcription factor FOXP3 gene lack functional Tregs and develop widespread and lethal automimmunity (Bacchetta et al., 2006; Brunkow et al., 2001; Khattri et al., 2003; Powell et al., 1982; Satake et al., 1993). In addition to their normal role in maintaining self tolerance, Tregs are either expanded or locally recruited in many human cancers and act to impair anti-tumor immunity (Baecher-Allan and Anderson, 2006).

Despite their functional importance, identification of Tregs can be problematic. Both Tregs and recently activated T cells express the high affinity IL-2 receptor chain CD25, making it impossible to discriminate Tregs from recently activated T cells using this marker alone. Tregs can be identified definitively by demonstrating their functional ability to suppress T cell proliferation *in vitro*. However, Tregs are often present in low numbers, making functional assays difficult or impossible. The next most reliable method of identifying Tregs is by demonstrating high and constant expression of the transcription factor FOXP3. Expression of the FOXP3 protein is critical for the development and functional activity of Tregs. As noted previously, mutations in this gene lead to decreased regulatory T cell function in both mice and humans and expression levels of the FOXP3 protein correlate with the ability of Tregs to suppress (Fontenot et al., 2003; Tao et al., 2007). In mice, FOXP3 is expressed exclusively by Tregs. In humans, FOXP3 is transiently expressed by all T cells after activation, although expression levels do not reach those observed in true Treg (Clark and Kupper, 2007; Gavin et al., 2006; Pillai et al., 2007). Before this biology was fully understood, small increases in FOXP3 protein expression or mRNA levels were

Correspondence should be addressed to: Dr. Rachael A. Clark, Brigham and Women's Hospital Department of Dermatology, EBRC Room 505A, 221 Longwood Avenue, Boston, MA, 02115. Phone: (617) 525-8512, Fax: (617) 264-5123, rclark1@partners.org.

Conflict of Interest: Participant, Scientific Advisory Board meeting, Therakos, Inc.

misinterpreted as indicating a Treg phenotype. One bright spot in the field of Treg biology is the recent finding that demethylation at the FOXP3 promoter site can be used to discriminate true Tregs from activated non-regulatory T cells (Baron et al., 2007; Janson et al., 2008).

There have been conflicting reports regarding the role of Tregs in cutaneous T cell lymphoma (CTCL). A report that malignant T cells from CTCL patients stimulated *in vitro* with immature dendritic cells (DC) loaded with apoptotic T cell debris adopted a Treg like phenotype led to assertions that CTCL was a malignancy of Tregs (Berger et al., 2005). However, even normal T cells co-cultured with immature DC presenting peptides from apoptotic cells have been shown to adopt a regulatory T cell phenotype (Steinman et al., 2003). This is a major mechanism for the formation of peripheral tolerance, a mechanism that may explain maternal fetal tolerance and is currently being exploited to treat allograft rejection (Adams et al., 2007; Morelli and Thomson, 2007). Subsequent histologic studies found low or negative expression of FOXP3 in the malignant T cells of CTCL skin lesions (Gjerdrum et al., 2007). However, CTCL skin lesions did contain benign infiltrating $FOXP3^+$ Tregs and the increased numbers of benign Tregs were actually associated with improved survival. A second study also found no evidence for preferential FOXP3 expression among malignant T cells in the skin lesions of Sézary syndrome and in fact found an overall reduction in $FOXP3^+$ Treg numbers in both the skin and blood of Sézary patients (Klemke et al., 2006). Studies of peripheral blood in advanced stage CTCL found no clear differences in blood Treg numbers in CTCL patients versus normal controls but found that Tregs in patients with high tumor burdens had decreased suppressive function (Tiemessen et al., 2006). Taken together, these studies suggest that Treg function is not always a feature of the malignant T cells in CTCL and that maintenance of normal Treg function may actually be a positive prognostic indicator. Recent findings that extracorporeal photopheresis (ECP) increases Treg numbers in patients with CTCL, as it does in other inflammatory diseases, supports this observation (Shiue et al., 2009). However, one recent study did find convincing Treg function in the malignant T cells from a subset of patients with Sézary syndrome. Malignant T cells in these patients produced IL-10 and TGFβ and could suppress T cell proliferation *in vitro* (Krejsgaard et al., 2008). Interestingly, suppressive function in these cells differed from that of normal Tregs in that it was driven by Jak3/STAT5 signaling as opposed to FOXP3. In short, there are conflicting findings as to whether CTCL represents a malignancy of Treg and indeed, whether Treg function is part of the problem in CTCL or part of the solution.

Authors of the current manuscript have done a thorough job of demonstrating that the malignant T cells in a subset of Sézary patients are true $FOXP3^+$ Tregs. Malignant T cells in these patients expressed elevated levels of both FOXP3 mRNA and protein, had demethylation at the FOXP3 promoter locus and most critically, were able to suppress the proliferation of other T cells *in vitro*.

Ongoing controversies in CTCL aside, most groups would agree that CTCL is a malignancy of T cells that home to and populate the skin. Normal human skin is populated by 20 billion T cells (Clark et al., 2006). 70–80% of these T cells are diverse, polarized effector memory T cells, including Th1, Th2, and Th17 cells, as well as recently described populations of T cells producing IL-13 (Calarese et al., 2009) and TNFα alone (Clark et al., 2009). 5–10% of the T cells in normal human skin are $FOXP3^+$ Tregs (Clark and Kupper, 2007). These cells have high and stable expression of FOXP3 and suppress the proliferation on autologous T cells *in vitro*. Skin resident effector T cells lack expression of the lymph node homing addressins CCR7 and L-selectin, and thus would be expected to be excluded from LN (Clark et al., 2006). Moreover, few are found in blood (Campbell et al., 2001). Elegant studies in mice have recently shown that skin resident effector memory T cells not only survive long

term within the skin but also remain in a fixed location (Gebhardt et al., 2009). This characteristic may explain why lesions of fixed drug eruption (which are infiltrated by IFNγ producing T cells) and psoriasis lesions (infiltrated by Th17 cells) remain in fixed locations and tend to recur at the same sites when suppressive therapy is discontinued (Lowes et al., 2008; Teraki and Shiohara, 2003). In contrast to these populations of effector and regulatory T cells, the remaining 20% of T cells in human skin express co-express skin homing addressins (CLA, CCR4) as well as lymph node homing addressins (CCR7 and L-selectin). Co-expression of L-selectin and CCR7 has been used to identify central memory T cells, a type of T cell found in high numbers in blood and thought to represent a long term repository for immunologic memory (Campbell et al., 2001). The addressin expression pattern of these normal skin T cells suggests they are an intermediate phenotype capable of accessing the skin, blood and secondary lymphoid organs.

Human skin therefore contains 20 billion T cells that are exposed throughout our lives to multiple insults, including environmental toxins, UV light and potentially mutagenic viral infections. Studies utilizing spectral karyotyping, comparative genomic hybridization (CGH) and TCR γ chain rearrangements have demonstrated that chromosomally abnormal T cells are present in the skin lesions of even the earliest stages of CTCL and that in later stages in which malignant T cells can be clearly identified, these genetically damaged cells are identical to those bearing the clonal malignant TCR (Kaltoft et al., 1994; Marcus Muche et al., 2004; Padilla-Nash et al., 2007; Whang-Peng et al., 1982). Thus, even in its earliest stages, CTCL is a malignancy of genetically unstable, chromosomally damaged T cells (Thestrup-Pedersen and Kaltoft, 1994). Given the mixed population of T cells in skin, it should not be surprising that one observes a range of phenotypes depending on the characteristics of the transformed cell type. Malignantly transformed cells often maintain some characteristics of their benign precursors. For example, T cells in stable patch plaque CTCL lack expression of CCR7, L-selectin and CD27, all characteristics of polarized effector memory T cells (Campbell et al., 2009). The fact that malignant T cells in MF are found only in skin and often remain localized for decades to fixed plaques is consistent with the sessile nature of skin resident effector memory T cells as demonstrated in mice (Gebhardt et al., 2009). In contrast, malignant T cells in Sézary syndrome express the central memory T cell markers L-selectin, CCR7 and CD27 as well as variable expression of the skin homing addressins CCR4 and CLA (Campbell et al., 2009). In this way, Sézary syndrome T cells resemble the 20% of T cells in normal skin that coexpress CLA, CCR4, L-selectin and CCR7 (Clark et al., 2006). From what we understand of homing receptor expression, these T cells should be capable of entering and migrating through the blood, skin and lymph nodes. Indeed, the malignant T cells in Sézary syndrome migrate throughout the skin, producing erythroderma, and accumulate in the blood and lymph nodes.

Given this view of CTCL, it is logical that the Tregs in skin are equally vulnerable to malignant transformation. When this occurs, a malignancy of T cells is produced that may maintain the ability to suppress T cell activation. Indeed, the suppressive characteristics of Tregs may give lymphomas derived from these cells a survival advantage, allowing them to suppress tumor specific cytotoxic T cells and thereby evade immune destruction. Infection is the most common cause of death in advanced stage CTCL (Axelrod et al., 1992). In the subset of Sézary patients in whom malignant T cells are derived from the Treg lineage, these cells may contribute to immune suppression. However, one does not have to invoke Treg mediated suppression to explain the profound immune defects in patients with other subtypes of advanced CTCL. In most advanced stage patients and even some with earlier stages, there is profound loss of T cell diversity and compensatory expansion of surviving clones (Yamanaka et al., 2005; Yawalkar et al., 2003) suggesting widespread death of T cells on a par with that observed in HIV disease. T cell losses of this magnitude represent a massive loss of critical immune memory accumulated over a lifetime of pathogen exposures.

Why T cell death is so widespread in CTCL, particularly in the subset of early stage patients with only limited skin disease, remains unknown.

Human skin is populated by a varied collection of T cells with differing functional, proliferative and migratory capacities. The diversity of clinical presentations in CTCL likely reflects the functional diversity of these T cells. Authors of the current manuscript have identified a Treg subtype of Sézary syndrome. As our understanding of both CTCL and normal skin T cell biology improves, additional functional subtypes of CTCL will likely be identified, for example, Th17 or Th1 CTCL. In this way, we will move from a description of the reaction pattern (folliculotropic, granulomatous) to a description of the pathogenic T cell of origin. This improved understanding of the biology of malignant T cells will in turn allow us to tailor our therapies to address the specific problems raised by each subtype. For example, therapies that suppress cytokine production and effector functions may be most appropriate in stable patch plaque MF, where malignant T cells make up a small but vocal minority of the T cells in skin lesions and primarily cause trouble by engendering and supporting inflammation. In contrast, therapies that aim to kill malignant T cells may be advantageous in Sézary syndrome, where the problem is more the sheer numbers of malignant T cells infiltrating the skin, blood and lymph nodes and the resistance of these T cells to normal mechanisms of T cell death. Until such time, well executed investigations such as the current study shed much needed light on the dimly lit field of CTCL immunobiology.

Acknowledgments

The author would like to thank Dr. Thomas Kupper, Adam Calarese, Dr. Michael Lichtman and Dr. Jessica Teague for helpful suggestions and editorial comments. The author is supported by NIH grants 1R01AR056720-01A1, 1K08AI060890-01A1, 1R01CA122569-01A2, a New Investigator Award from the Scleroderma Foundation and a Clinical Investigator Award from the Damon Runyon Cancer Research Foundation.

Abbreviations used

CLA	cutaneous lymphocyte antigen
CCR	CC chemokine receptor
Tregs	regulatory T cells

References

Adams KM, Yan Z, Stevens AM, Nelson JL. The changing maternal “self” hypothesis: a mechanism for maternal tolerance of the fetus. Placenta 2007;28:378–382. [PubMed: 16934327]

Axelrod PI, Lorber B, Vonderheid EC. Infections complicating mycosis fungoides and Sezary syndrome. Jama 1992;267:1354–1358. [PubMed: 1740857]

Bacchetta R, Passerini L, Gambineri E, Dai M, Allan SE, Perroni L, et al. Defective regulatory and effector T cell functions in patients with FOXP3 mutations. The Journal of clinical investigation 2006;116:1713–1722. [PubMed: 16741580]

Baecher-Allan C, Anderson DE. Regulatory cells and human cancer. Seminars in cancer biology 2006;16:98–105. [PubMed: 16378733]

Baron U, Floess S, Wieczorek G, Baumann K, Grutzkau A, Dong J, et al. DNA demethylation in the human FOXP3 locus discriminates regulatory T cells from activated FOXP3(+) conventional T cells. European journal of immunology 2007;37:2378–2389. [PubMed: 17694575]

Berger CL, Tigelaar R, Cohen J, Mariwalla K, Trinh J, Wang N, et al. Cutaneous T-cell lymphoma: malignant proliferation of T-regulatory cells. Blood 2005;105:1640–1647. [PubMed: 15514008]

Brunkow ME, Jeffery EW, Hjerrild KA, Paeper B, Clark LB, Yasayko SA, et al. Disruption of a new forkhead/winged-helix protein, scurfin, results in the fatal lymphoproliferative disorder of the scurfy mouse. Nat Genet 2001;27:68–73. [PubMed: 11138001]

Calarese AW, Lichtman M, Kupper TS, Clark RA. Skin resident T cells producing IL-13 and TNFα: a possible role in the initiation of cutaneous sclerosis. The Journal of investigative dermatology 2009;129:S14.

Campbell JJ, Murphy KE, Kunkel EJ, Brightling CE, Soler D, Shen Z, et al. CCR7 Expression and Memory T Cell Diversity in Humans. J Immunol 2001;166:877–884. [PubMed: 11145663]

Campbell JJ, Soler D, Clark RA, Kupper TS. Malignant clonal T cells in leukemic (L-)CTCL/Sezary syndrome (SS) exhibit a unique cell surface profile: high and uniform expression of CCR4 and central memory markers CCR7, L-selectin, and CD27. The Journal of investigative dermatology 2009;129:S54.

Clark RA, Chong B, Mirchandani N, Brinster NK, Yamanaka K, Dowgiert RK, et al. The vast majority of CLA+ T cells are resident in normal skin. J Immunol 2006;176:4431–4439. [PubMed: 16547281]

Clark RA, Kupper TS. IL-15 and dermal fibroblasts induce proliferation of natural regulatory T cells isolated from human skin. Blood 2007;109:194–202. [PubMed: 16968902]

Clark RA, Mollet IG, Hijnen D, Calarese AW, Purwar R, Lichtman M, et al. Skin resident T cells producing TNFα but not Th1, Th2, or Th17 cytokines: Their possible role as immune sentinels in skin. The Journal of investigative dermatology 2009;129:S110.

Fontenot JD, Gavin MA, Rudensky AY. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. Nature immunology 2003;4:330–336. [PubMed: 12612578]

Gavin MA, Torgerson TR, Houston E, DeRoos P, Ho WY, Stray-Pedersen A, et al. Single-cell analysis of normal and FOXP3-mutant human T cells: FOXP3 expression without regulatory T cell development. Proceedings of the National Academy of Sciences of the United States of America 2006;103:6659–6664. [PubMed: 16617117]

Gebhardt T, Wakim LM, Eidsmo L, Reading PC, Heath WR, Carbone FR. Memory T cells in nonlymphoid tissue that provide enhanced local immunity during infection with herpes simplex virus. Nature immunology 2009;10:524–530. [PubMed: 19305395]

Gjerdrum LM, Woetmann A, Odum N, Burton CM, Rossen K, Skovgaard GL, et al. FOXP3+ regulatory T cells in cutaneous T-cell lymphomas: association with disease stage and survival. Leukemia 2007;21:2512–2518. [PubMed: 17713545]

Janson PC, Winerdal ME, Marits P, Thorn M, Ohlsson R, Winqvist O. FOXP3 promoter demethylation reveals the committed Treg population in humans. PloS one 2008;3:e1612. [PubMed: 18286169]

Kaltoft K, Hansen BH, Thestrup-Pedersen K. Cytogenetic findings in cell lines from cutaneous T-cell lymphoma. Dermatol Clin 1994;12:295–304. [PubMed: 8045039]

Khattri R, Cox T, Yasayko SA, Ramsdell F. An essential role for Scurfin in CD4+CD25+ T regulatory cells. Nature immunology 2003;4:337–342. [PubMed: 12612581]

Klemke CD, Fritzsching B, Franz B, Kleinmann EV, Oberle N, Poenitz N, et al. Paucity of FOXP3+ cells in skin and peripheral blood distinguishes Sezary syndrome from other cutaneous T-cell lymphomas. Leukemia 2006;20:1123–1129. [PubMed: 16557241]

Krejsgaard T, Gjerdrum LM, Ralfkiaer E, Lauenborg B, Eriksen KW, Mathiesen AM, et al. Malignant Tregs express low molecular splice forms of FOXP3 in Sezary syndrome. Leukemia 2008;22:2230–2239. [PubMed: 18769452]

Lowes MA, Kikuchi T, Fuentes-Duculan J, Cardinale I, Zaba LC, Haider AS, et al. Psoriasis vulgaris lesions contain discrete populations of Th1 and Th17 T cells. The Journal of investigative dermatology 2008;128:1207–1211. [PubMed: 18200064]

Marcus Muche J, Karenko L, Gellrich S, Karhu R, Kytola S, Kahkonen M, et al. Cellular coincidence of clonal T cell receptor rearrangements and complex clonal chromosomal aberrations-a hallmark of malignancy in cutaneous T cell lymphoma. The Journal of investigative dermatology 2004;122:574–578. [PubMed: 15086537]

Morelli AE, Thomson AW. Tolerogenic dendritic cells and the quest for transplant tolerance. Nat Rev Immunol 2007;7:610–621. [PubMed: 17627284]

Padilla-Nash HM, Wu K, Just H, Ried T, Thestrup-Pedersen K. Spectral karyotyping demonstrates genetically unstable skin-homing T lymphocytes in cutaneous T-cell lymphoma. Exp Dermatol 2007;16:98–103. [PubMed: 17222222]

Pillai V, Ortega SB, Wang CK, Karandikar NJ. Transient regulatory T-cells: a state attained by all activated human T-cells. Clinical immunology (Orlando, Fla 2007;123:18–29.

Powell BR, Buist NR, Stenzel P. An X-linked syndrome of diarrhea, polyendocrinopathy, and fatal infection in infancy. J Pediatr 1982;100:731–737. [PubMed: 7040622]

Sakaguchi S. Naturally arising Foxp3-expressing CD25+CD4+ regulatory T cells in immunological tolerance to self and non-self. Nature immunology 2005;6:345–352. [PubMed: 15785760]

Satake N, Nakanishi M, Okano M, Tomizawa K, Ishizaka A, Kojima K, et al. A Japanese family of X-linked auto-immune enteropathy with haemolytic anaemia and polyendocrinopathy. Eur J Pediatr 1993;152:313–315. [PubMed: 8482279]

Shiue LH, Aakhus E, Arias-Mendoza P, Duvic M, Ni X. Increased levels of CD4+25high regulatory T cells in patients with cutaneous T-cell lymphoma after extracorporeal photopheresis. The Journal of investigative dermatology 2009;129:S46.

Steinman RM, Hawiger D, Nussenzweig MC. Tolerogenic dendritic cells. Annual review of immunology 2003;21:685–711.

Tao R, de Zoeten EF, Ozkaynak E, Chen C, Wang L, Porrett PM, et al. Deacetylase inhibition promotes the generation and function of regulatory T cells. Nature medicine 2007;13:1299–1307.

Teraki Y, Shiohara T. IFN-gamma-producing effector CD8+ T cells and IL-10-producing regulatory CD4+ T cells in fixed drug eruption. The Journal of allergy and clinical immunology 2003;112:609–615. [PubMed: 13679823]

Thestrup-Pedersen K, Kaltoft K. Genotraumatic T cells and cutaneous T-cell lymphoma. A causal relationship? Archives of dermatological research 1994;287:97–101. [PubMed: 7726642]

Tiemessen MM, Mitchell TJ, Hendry L, Whittaker SJ, Taams LS, John S. Lack of suppressive CD4+CD25+FOXP3+ T cells in advanced stages of primary cutaneous T-cell lymphoma. The Journal of investigative dermatology 2006;126:2217–2223. [PubMed: 16741512]

Whang-Peng J, Bunn PA Jr, Knutsen T, Matthews MJ, Schechter G, Minna JD. Clinical implications of cytogenetic studies in cutaneous T-cell lymphoma (CTCL). Cancer 1982;50:1539–1553. [PubMed: 6981450]

Yamanaka K, Yawalkar N, Jones DA, Hurwitz D, Ferenczi K, Eapen S, et al. Decreased T-cell receptor excision circles in cutaneous T-cell lymphoma. Clin Cancer Res 2005;11:5748–5755. [PubMed: 16115912]

Yawalkar N, Ferenczi K, Jones DA, Yamanaka K, Suh K-Y, Sadat S, et al. Profound loss of T-cell receptor repertoire complexity in cutaneous T-cell lymphoma. Blood 2003;102:4059–4066. [PubMed: 12829591]