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Identification of regulatory T cells in systemic lupus erythematosus

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ABSTRACT

The concept that regulatory T cells (Treg) play a key role in both development and maintenance of autoimmune response in rheumatic diseases is well accepted. In recent years, several studies analyzed Treg cell phenotype and function in systemic lupus erythematosus (SLE), the prototypical systemic autoimmune disorder in humans. Although qualitative and/or quantitative abnormalities of Treg cells have been shown, data are often conflicting. This may depend on the selection of patients with different degrees of disease activity or on immunosuppressive treatments that can alter Treg cell findings. Among several proposed surface or intracellular Treg cell markers, CD25 at high level of expression and the transcription factor Foxp3 are the two most investigated in SLE. Despite the glucocorticoid-induced TNF receptor-related protein (GITR) represents a reliable phenotypic marker of murine Treg cells, little is known about its role in humans, in particular in the course of systemic autoimmune disorders. Preliminary data seems to suggest that this marker may represent a good tool to identify cell populations included within Treg cell subsets.

Contents

1. Introduction

Regulatory T cells (Treg) are specialized $CD4^+$ lymphocyte subsets able to control destructive immune responses to pathogens as well as to prevent immune responses against inappropriate targets, including self antigens or non-harmful external antigens [1]. It is thought that they play a key role in sustaining peripheral tolerance by controlling the small proportion of circulating T cells escaped from thymic central deletion. The concept of T cell-mediated suppression of autoimmunity, initially described about 40 years ago, recovered strength in the mid-90s when Sakaguchi et al demonstrated that a T-cell subset, expressing high levels of the IL-2 receptor α-chains (CD25), was able to prevent the onset of systemic autoimmune diseases in thymectomized mice [2]. The subsequent isolation of these cells also in human peripheral blood led to investigations not only in animal models with autoimmune disorders, but also in patients with autoimmune diseases [3,4].

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Natural Treg cells are $CD4^+CD25^+$ T cells generated in the thymus in early years of life with the ability to bind self antigens by their T-cell receptor. They are distinguished from adaptive Treg cells that are induced in the peripheral blood by conversion of $CD4^+CD25^-$ naïve T cells in the presence of a particular microenvironment [5]. Although Treg cells could be basically considered suppressor T cells, it has been elucidated that they are more heterogeneous and able to play a pivotal role in the maintenance of immune tolerance [6]. They can suppress both autoreactive effector cells and T cells specific for foreign antigens in a cell–cell contact manner or by secreting cytokines, such as IL-10 and TGF-β, and/or other soluble molecules. Moreover, Treg lymphocytes are involved in a more complex scenario, which includes effector Th1 and Th2 cells as well as the recently discovered Th17 cells, whose balance determinates the development of immune response or the maintaining of tolerance.

In earlier studies, Treg cells were identified primarily by their surface expression of CD4 and high level of expression of CD25 to distinguish them from effector T cells. Additional surface molecules, including cytotoxic T lymphocyte antigen-4 (CTLA-4) and glucocorticoid-induced TNF receptor-related protein (GITR), have been subsequently proposed as markers of Treg cells. Unfortunately, CD25, CTLA-4, and GITR are also upregulated on the surface of activated T cells, complicating interpretation of some experimental data. In addition, recent acquisitions showed that also a small population of $CD4^+CD25^-$ T cells retains suppressive activity. The discovery of the forkhead winged-helix transcription factor Foxp3 (forkhead box p3) as master regulator for Treg cells added a key marker for this T cell subset. Foxp3, in fact, is constitutively expressed at high levels in both natural and adaptive $CD4^+CD25^{high}$ Treg cells in human beings and mice. It is required for the natural Treg lineage commitment in the thymus and is essential in stabilizing and amplifying a Treg program, inclusive of anergy and defective IL-2 production, induced by interaction between Treg precursors and stromal cells in the thymus [7]. Furthermore, it has been recently shown, by investigations on induced Foxp3 expression in activated $CD4^+CD25^-$ T cells, that the presence at a high level as well as the persistence of expression of Foxp3 are important for maintaining suppressor function [8]. Interestingly, it is now well accepted that Foxp3, despite being a distinctive marker for Treg cells, can also be expressed by human effector T cells after activation. However, its expression on these cells is transient and never reaches the expression levels displayed by Treg cells. Recently, expression of the CD127 molecule was found to be inversely correlated with that of Foxp3, so that, at the moment, Treg cells are known to be $CD4^+CD25^{high}Foxp3^+CD127^{low}$ [9].

2. Treg cells in systemic lupus erythematosus (SLE)

The concept that Treg cells play a key role in preventing autoimmune disorders is now well defined. Several data are available in human beings on the presence and function of Treg in different autoimmune diseases. SLE represents the classical prototype of systemic autoimmune disease in which loss of immune tolerance to self antigens leads to activation and expansion of autoreactive lymphocytes, uncontrolled production of several autoantibodies and release of inflammatory mediators that ultimately damage multiple organs. It is generally assumed that the derangement of immune system in this disorder takes place from a dysregulation of immune T-cell tolerance in both human and murine SLE. However, since the mechanisms of central tolerance appear relatively unaffected in murine models of SLE, it is usually thought that the breakdown of tolerance in SLE is essentially a peripheral event. In this context, the hypothesis that this may involve a significant defect in Treg cell number and/or function is appealing as this may provide new ways for the treatment of this devastating disorder.

The reported findings on Treg cells in SLE, however, are not always consistent, in part because investigations were performed in different disease phases and with ongoing immunosuppressive treatments. Most of the earlier studies focused only on phenotypic characterization of circulating T cells, but they were limited by difficulties in distinguishing Treg cells from simply activated T cells bearing the CD25 surface molecule, as Treg cells in humans are more represented in $CD25^{high}$ cell fraction, that, however, is difficult to be unequivocally defined. It has been demonstrated, in fact, that the higher is CD25 surface expression the higher is suppressor activity. In order to more accurately discriminate between Treg and activated T cells, additional evaluations of Foxp3 mRNA expression within the $CD4^+CD25^+$ cell population have been performed in some studies in SLE patients. Recently, a more extensive analysis of Foxp3 expression has been possible with the availability of new tools for Foxp3 detection by flow cytometry that have improved data reliability. Functional evaluations of isolated Treg from peripheral blood of SLE subjects have been described only in few studies. The results obtained in the main studies about number and/or function of $CD25^+/Foxp3^+$ cells in SLE are summarized in Table 1. It is evident from the analysis of the different studies that the data arising from the evaluation of the global $CD4^+CD25^+$ cell population are inconclusive, probably because of the extreme heterogeneity of this T-cell subset. Indeed, since the percentage of circulating Treg in humans should be less than 2–3%, the very high numbers and the wide variability of the reported percentages of the cell subpopulations considered in these studies, ranging from 6.5% to 31.3% in normal controls and from 6% to 37.8% in SLE patients, appear to support this hypothesis. On the opposite, there is a general agreement in considering usually reduced the number of $CD4^+CD25^{high}$ cells circulating in the peripheral blood of SLE patients as compared to aged- and sex-matched healthy controls. It is to note that the percentages of $CD4^+CD25^{high}$ cells described in the Suarez's study, reporting higher numbers in SLE than in controls [16], are the highest among these investigations, while the increase of this T-cell subset in SLE, recently reported by Azab et al., refers to mean fluorescence intensity rather than cell percentages [21].

Analysis of the results about Foxp3 cell expression in SLE is more complex, since the evaluation of its expression has been carried out with different methods and it has been analyzed within different cell subsets. However, it is of interest the observation that Foxp3 expression, when evaluated in both $CD25^+$ and $CD25^-$ cell subsets, seems to be reduced in the cell population bearing the CD25 and increased in the T-cell subset lacking this surface molecule. The very recent description of an increased prevalence in SLE peripheral blood of circulating $CD25^-/^{low}$ $CD4^+$ $Foxp3^+$ T cells, that could be considered Treg cells, appears to support these findings [29]. Interestingly, similar results have been reported in a cohort of new-onset SLE patients [23,30]. Taken together, these observations suggest