Treg17 cells are programmed by Stat3 to suppress Th17 responses in systemic lupus

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Systemic lupus erythematosus (SLE) is a complex and potentially fatal autoimmune disorder. Although Th17 cells are thought to be central mediators of SLE, mechanisms underlying their counter regulation remain largely unknown. To help define this, we studied the function of the newly defined Stat3-dependent Th17-specific regulatory T cells (Treg17). Treg-specific deletion of Stat3 was achieved by generating $Foxp3^{Cre} \times Stat3^{fl/fl}$ mice and SLE was induced by intraperitoneal injection of pristane. Lack of Treg17 cells in these mice caused selectively enhanced peritoneal Th17 inflammation. Importantly, Treg17 deficiency also resulted in aggravated pulmonary vasculitis with increased percentages of Th17 cells and significantly higher mortality. Similarly, 4 and 9 months after pristane injection, analysis of renal and systemic immunity showed overshooting Th17 responses in the absence of Treg17 cells, associated with the aggravation of lupus nephritis. Expression of the Th17 characteristic trafficking receptor CCR6 was strikingly reduced on Tregs of $Foxp3^{Cre} \times Stat3^{fl/fl}$ mice, resulting in impaired renal Treg infiltration. Thus, Stat3-induced Treg17 cells are novel antiinflammatory mediators of SLE. One mechanism enabling Treg17 cells to target pathogenic Th17 responses is shared expression of the chemokine receptor CCR6.

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Systemic lupus erythematosus (SLE) is a common autoimmune disease causing high morbidity and mortality in a rather young collective of patients.[1–3] Multiple tissues can be affected including vital organ systems such as the central nervous system, lungs, and kidneys. In particular, development of lupus nephritis has been identified as a major risk factor for a poor prognosis.[4] Despite intensive research, the etiopathology of SLE remains widely elusive. As a consequence, therapeutic options are unspecific and insufficient, which highlights the need to identify novel therapeutic targets.[5–7]

One such promising target could be Th17 cells. Multiple studies have supported an important role for interleukin (IL)-17 and T helper (Th)17 cells in human SLE[8–10] and show their presence in lupus nephritis.[11] Moreover, in various experimental rodent models, the IL-17/Th17 axis has been demonstrated to be critical for the development of autoimmunity and renal pathology in SLE.[12–17] It is of note, however, that one recent study in two genetic mouse models of SLE did not find a vital contribution of IL-17 to the development of lupus nephritis.[18] This once more underlines the complex nature of SLE disease pathogenesis and highlights that a deeper understanding of Th17 biology is needed. In particular, the mechanisms responsible for effective downregulation of Th17 responses remain widely unknown to date. Generally, a T cell lineage with a highly regulatory phenotype termed regulatory T cells (Tregs) has been identified as crucial for counteracting pro-inflammatory Th cell responses. Tregs are commonly defined by expression of the transcription factor Foxp3[19–21] and until recently were regarded as a homogenous population. However, given the wide array of highly specialized and diverse T effector cell subpopulations, potent counter regulation by one singular anti-inflammatory Treg population seems unlikely.[22] In this respect, the intriguing concept of lineage-specific Tregs was proposed. According to this concept, Th1 responses are downregulated by Treg1 cells, whereas Th17 responses are under the control of Treg17 cells. Lineage specificity was suggested to be induced by sharing some of the same transcription factors with the respective effector cell population.[23–26] Recent studies have linked activation of the Th1-specific transcription factor

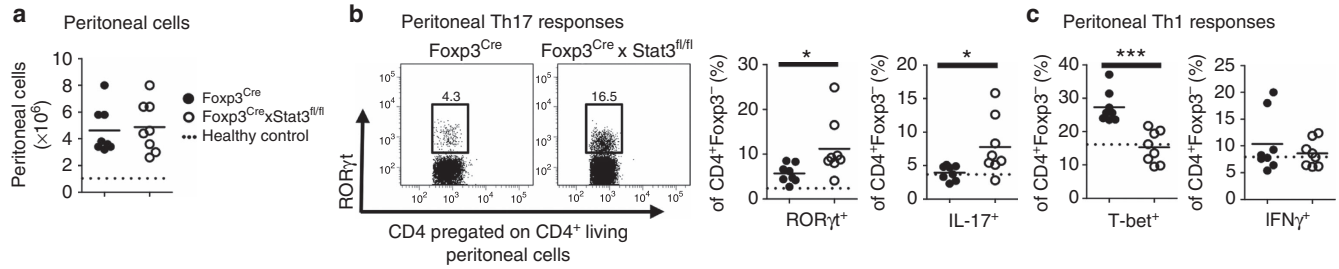

Figure 1 | Peritoneal immunity is skewed towards Th17 in Treg17-deficient mice. (**a**) Quantification of total peritoneal cells in Foxp3^{Cre} and Foxp3^{Cre} × Stat3$^{fl/fl}$ mice at day 7 after pristane administration (dotted line represents $n = 3$ untreated controls). (**b**) Representative FACS plots of peritoneal Th17 cells in Foxp3^{Cre} and Foxp3^{Cre} × Stat3$^{fl/fl}$ mice (left, gating strategy as indicated). Flow cytometric quantification of peritoneal Th17 cells (right). (**c**) Flow cytometric quantification of peritoneal Th1 responses. Numbers in FACS plots indicate percentages of gated cells. Circles represent individual mice, horizontal lines indicate mean values. *$P < 0.05$, ***$P < 0.001$. FACS, fluorescence-activated cell sorting; Th, T helper cells; Treg, regulatory T cells.

T-bet in Foxp3$^+$ Tregs to a Th1-specific Treg1 phenotype.[23,24] Independently, another landmark study provided evidence for the presence of Treg17 cells that depend on the Th17 transcription factor Stat3. A selective deletion of Stat3 in murine Tregs caused Treg17 deficiency, which resulted in overshooting Th17 responses.[25] In line, our group recently identified Stat3-dependent Treg17 cells as important mediators of renal tissue protection in a model of acute glomerulonephritis. In addition, we were able to identify Treg17 cells in kidney biopsies from human patients with antineutrophil cytoplasmic antibody (ANCA) associated vasculitis and to provide evidence that human Treg17 cells also depend on the activation of Stat3.[27] As one of the major mechanisms for specific suppression of Th17 cells, we could identify expression of the Th17 characteristic trafficking receptor CCR6 on Treg17 cells, which enables them to traffic into areas of Th17-mediated inflammation.

Although our study revealed an important role for Treg17 cells during acute renal inflammation, data with respect to chronic disease are completely lacking. This aspect is of special clinical importance, because many patients suffer from chronically progressive renal inflammation, requiring life-long treatment. Likewise, nothing is known about the impact of Treg17 cells on the complex development of autoimmunity and the clinical course of SLE. In order to address these important issues, we decided to study the murine model of pristane-induced lupus. Peritoneal injection of the naturally occurring hydrocarbon oil pristane (2,6,10,14-tetramethylpentadecane) leads to chronic inflammation with development of lupus-like autoimmunity. This particularly includes the formation of SLE characteristic antibodies as well as immune-complex nephritis and pulmonary vasculitis with a high degree of similarity to human SLE.[28,29] Importantly, we and others could recently show that the development of pristane-induced SLE crucially depends on the IL-17/Th17 axis, which makes a role for Treg17 cells quite likely.[15,16] In the present study, we therefore used this well-established model of murine SLE to define the role of Treg17 cells with special focus on the development of autoimmunity and end organ damage.

RESULTS

Peritoneal immunity is skewed towards Th17 responses in Foxp3^{Cre} × Stat3$^{fl/fl}$ mice

Peritoneal immune responses were analyzed in Foxp3^{Cre} × Stat3$^{fl/fl}$ and Foxp3^{Cre} control mice at 1 week after pristane injection. Our studies showed enhanced peritoneal cell numbers in both strains of mice as compared with untreated controls (Figure 1a). Analysis of peritoneal leukocytes showed slightly higher frequencies of the CD4$^+$ Th subset in Foxp3^{Cre} × Stat3$^{fl/fl}$ mice while all other populations were similar between the groups (Supplementary Figure S1A online). Importantly, we observed significant skewing towards Th17 responses in Foxp3^{Cre} × Stat3$^{fl/fl}$ mice with selective enhancement of both RORγt$^+$ and IL-17$^+$ Th17 cells (Figure 1b). Conversely, infiltration of T-bet$^+$ Th1 cells was already observed in Foxp3^{Cre} controls but not yet in Th17-skewed Foxp3^{Cre} × Stat3$^{fl/fl}$ mice. Interferon (IFN)γ production by Th1 cells was still at baseline levels in both strains of mice (Figure 1c). IL-17 production by the CD3$^+$ CD4$^-$ T cell population, including CD4$^-$CD8$^-$ double negative and γδ T cells, was indistinguishable between the groups (Supplementary Figure S1B online) as were Th2 responses (Supplementary Figure 1C online).

Increased mortality from pulmonary vasculitis in Foxp3^{Cre} × Stat3$^{fl/fl}$ mice

We next assessed the impact of Treg17 deficiency on the clinical course of pristane-induced SLE. Untreated Foxp3^{Cre} × Stat3$^{fl/fl}$ control mice remained healthy over time and did not show any signs of diarrhea or wasting. Pristane treatment, however, resulted in excessive early mortality of Foxp3^{Cre} × Stat3$^{fl/fl}$ mice (Figure 2a). Our search for the underlying cause of death revealed pristane-induced pulmonary hemorrhage with pronounced neutrophilia and leukocytoclastic capillaritis. Lungs of untreated Treg17-deficient Foxp3^{Cre} × Stat3$^{fl/fl}$ mice did not show any signs of inflammation (data not shown). After application of pristane, however, Foxp3^{Cre} × Stat3$^{fl/fl}$ mice developed severe pulmonary vasculitis, which was significantly aggravated in comparison with controls (Figure 2b). In line, pulmonary