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BACKGROUND
Systemic sclerosis (SSc) is characterized by fibrosis, activation of the immune system and microvasculopathy. In recent years, regulatory B cells (Bregs) have been shown to play an important role in the development of autoimmune diseases. The breakdown of immunological tolerance is characterized by the predominance of autoreactive B cells and the lack of Bregs. Bregs include CD19 (+) CD24 (high) CD38 (high) (transitional Bregs) and memory Bregs. These cells suppress the activity of other cells mainly through the induction of interleukin-10 (IL-10). The role of Bregs has been investigated in murine models of experimental autoimmune diseases and human studies of various autoimmune diseases. In SSc there is a disturbance of homeostasis of B cells with evidence of hyperactivation of B cells producing pathogenic autoantibodies.

Our research group has recently showed insufficiency of Bregs in patients with SSc. In particular, both transitional Bregs and memory Bregs are numerically decreased, more profoundly in diffuse than limited cutaneous form of the disease. Interestingly, patients with SSc-associated pulmonary fibrosis have near total lack of transitional and memory Bregs. Furthermore, there is functional impairment of Bregs (inability to produce IL-10) in the fine balance between regulatory T cells (Tregs) and Th17 cells. It appears that in vitro Bregs induce T lymphocytes to express Tregs cell markers (CD25, FoxP3); leading them to produce IL-10. It seems that the reverse is also true, since Tregs induce the production of Bregs in vitro.

In some of our patients, we observed subpopulations of B cells which could produce small, but not negligible, IL-10. This leads us to the hypothesis that these populations may be potential reservoirs of immunoregulatory B lymphocytes. These cells were detectable even in patients with advanced diffuse SSc. We will isolate these subpopulations of B lymphocytes and attempt to differentiate them in inducible Bregs analogously with inducible Tregs.

AIMS AND SCOPE
The purpose of the study is twofold: after characterizing the cell subpopulations of Bregs, Tregs and Th17 in patients with SSc, we will study the ability of Bregs to induce Tregs in vitro.

In particular, the aims of the current proposal are: a) to assess Bregs (transitional and memory), Tregs and Th17 in patients with SSc; b) in vitro to induce Bregs; c) to study in vitro the effect of Bregs on Tregs (and vice versa) in patients with SSc.

MATERIAL AND METHODS
We will study Bregs, Tregs and Th17 from patients with SSc (early disease and established disease, limited or diffuse cutaneous form of the disease), and also from patients with rheumatoid arthritis-associated pulmonary fibrosis as disease controls, and healthy subjects as normal controls. The research protocol will be performed in accordance to the revised Helsinki declaration and is approved by the Ethical Committee of the

The anti-inflammatory/suppressory activity of IL-10 and the immunomodulatory action on B and T autoreactive cell populations has been documented. Recent studies have highlighted the key role played by Bregs in the fine balance between regulatory T cells (Tregs) and Th17 cells. It appears that in vitro Bregs induce T lymphocytes to express Tregs cell markers (CD25, FoxP3); leading them to produce IL-10. It seems that the reverse is also true, since Tregs induce the production of Bregs in vitro.

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Keywords: Tregs, Bregs, Th17, systemic sclerosis.
University General Hospital of Larissa, University of Thessaly. Patients undergo regular blood tests during follow up according to good clinical practice, and an additional 15-20 ml of blood sample is taken once for the purpose of this study. PBMCs are kept in liquid nitrogen until used. Isolated peripheral blood mononuclear cells (PBMCs) from patients and controls will be stored in liquid nitrogen and are readily available from the Biobanking Facility of the Department of Rheumatology and Clinical Immunology, University of Thessaly. Phenotypic characterization of B cell subpopulations will be performed by standard flow cytometry protocols using fluorochrome-conjugated monoclonal antibodies against surface markers such as CD19, CD24, CD38, and CD27. Negative selection based on magnetic beads will allow isolation of B or T cells to be used for the induction of regulatory subsets using non-specific and specific stimuli (polyclonal IgM, BCR activation; bacterial CpG, ODN2006:TLR-9 activation; bacterial LPS: TLR-4 activation), PMA and ionomycin which will be used in order to maximise induction of IL-10. Standard intracellular flow cytometric analysis will be used to measure cytokine expression. Cell cultures in the presence of immunomodulatory agents will assist the induction of Bregs and Tregs in addition to physiological agents, such as IL-2 and IL-15. Tregs will be identified by staining for cell surface CD25 and transcriptional factor FoxP3. B cells will be cultured with autologous CD4(+) T cells and the effect will be assessed by proliferation assays and/or cytokine production, such as IFNγ and IL-6.

**IMPORTANCE OF THE STUDY - ANTICIPATED RESULTS**

We anticipate that our study will obtain data that could elucidate the pathogenic mechanisms responsible for the development of SSc. The ultimate objective of the study is to find the most effective therapeutic strategy for SSc. This therapy will be based on autologous transfusion of inducible Bregs (alone or in combination with inducible Tregs). To this end, we will first see whether B lymphocytes in patients with SSc have the potential to convert into Bregs and to produce IL-10. Our research group is in an advantageous position with regard to the study of Bregs and their interaction with the Treg/Th17, since we have thoroughly studied these cells and were the first group to show the important role of Bregs in SSc.

**CONFLICT OF INTEREST**
The authors declare no conflict of interest.