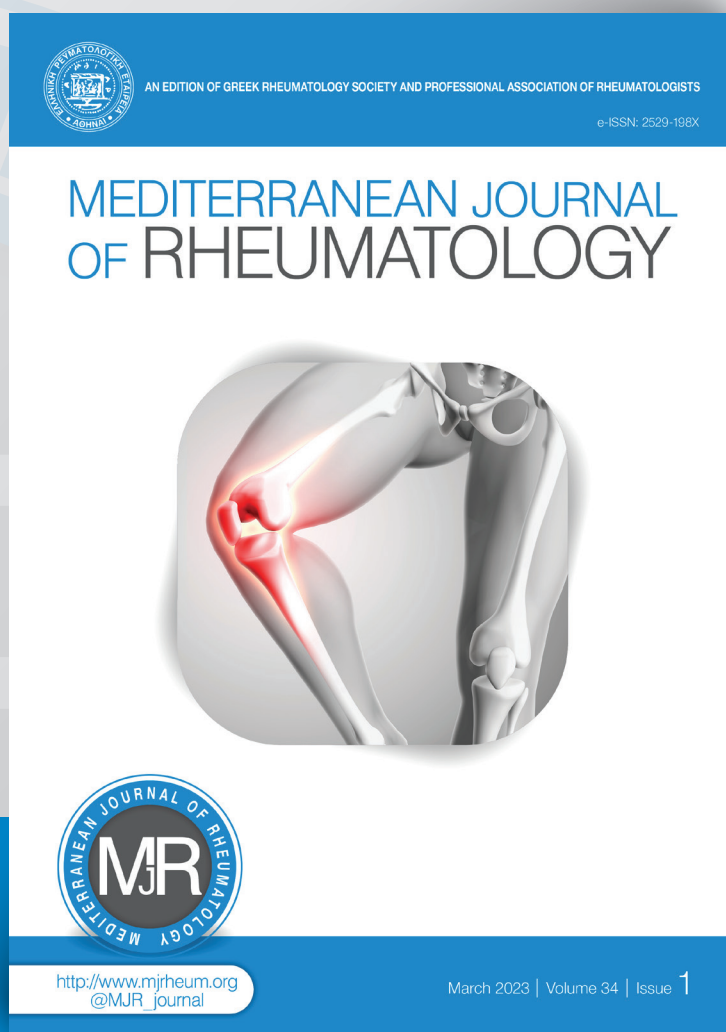

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Investigating the Role of T-bet⁺ B Cells (ABCs/DN) in the Immunopathogenesis of Systemic Lupus Erythematosus

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ABSTRACT

Background: Age-associated B cells (ABCs) constitute a B cell subset, defined as CD19⁺CD21⁻CD11c⁺, that expands continuously with age and accumulates strongly in individuals with autoimmune and/or infectious diseases. In humans, ABCs are principally IgD⁻CD27⁻ double-negative (DN) B cells. Data from murine models of autoimmunity, implicate ABCs/DN in the development of autoimmune disorders. T-bet, a transcription factor which is highly expressed in these cells, is considered to play a major role in various aspects of autoimmunity, such as the production of autoantibodies and the formation of spontaneous germinal centres. **Aims of the study:** Despite the available data, the functional features of ABCs/DN and their exact role in the pathogenesis of autoimmunity remain elusive. This project focuses on the investigation of the role of ABCs/DN in the pathogenesis of systemic lupus erythematosus (SLE) in humans, as well as the effects that various pharmacological agents may have on these cells. **Methods:** Samples from patients with active SLE will be used to enumerate and immunophenotype - via flow cytometry - the ABCs/DN found in the peripheral blood of the patients. Transcriptomic analysis and functional assays for the cells, both before and after in vitro pharmacological treatments, will also be performed. **Anticipated benefits:** The results of the study are expected to allow characterization of the pathogenetic role of ABCs/DN in SLE and could probably contribute, following careful association with the clinical state of the patients, towards the discovery and validation of novel prognostic and diagnostic markers of disease.

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ABBREVIATIONS

ABCs: age-associated B cells
DN: double-negative B cells
SLE: Systemic Lupus Erythematosus

BACKGROUND/RATIONALE

Transcription factor T-bet was first identified as a Th1 lineage commitment regulator.¹ However, it soon became clear that it is also expressed in other

cell types, such as NK cells, dendritic cells and B cells, and orchestrates their immune functions.^{2,3,4} In B cells, T-bet seems to be responsible for isotype switching to IgG2a (in mice) and the appearance of IgG2a-expressing memory B cells.^{5,6}

Age-associated B cells (ABCs) is a CD19⁺CD21⁺CD11c⁺ B cell population that accumulates with age, and in autoimmune and infectious diseases, which has recently been described by several research groups.^{7,8,9,10} ABCs display an elevated expression of the transcription factor T-bet, as a result of a synergistic triggering of their BCR receptor, the TLR7 receptor and a third receptor, either IFN γ R or IL21R.^{8,11} The exact role and the functions of these cells in aging, pathogenesis of autoimmunity and immune responses against pathogens are still elusive, and most intriguingly, some contrasting findings emerged.

Data from murine models of autoimmunity, implicate ABCs in the production of autoreactive IgG, the enhanced antigen presentation to T cells and the formation of spontaneous germinal centers.^{8,12,13,14} T-bet is considered to be the master regulator of all these processes,¹² although recently published data, questions the importance of the transcription factor in the generation of functional ABCs.¹⁵

In humans, ABCs are mainly IgD⁻CD27⁻ double-negative (DN) B cells.^{16,17,18} DN have further been divided in two subgroups, based on the expression of CXCR5 chemokine, which serves as a follicular homing marker.¹⁸ The CXCR5⁺ subgroup (DN1) is expanded in the elderly healthy individuals and lacks T-bet expression, whereas the CXCR5⁻ subgroup (DN2) expresses T-bet and is more marked in systemic lupus erythematosus (SLE).^{16,18,19} The DN2 cells are hyper-responsive to TLR7 signalling and thus are poised to generate autoreactive antibody-secreting plasmablasts.¹⁸ In general though, their role in the development of autoimmunity remains elusive.

The expansion of ABCs/DN2 is pronounced in African-American SLE patients,¹⁸ a fact which indicates that genetic burden is a crucial factor for the generation of these cells. It is well known that ethnicity - among other factors - is linked to the severity of SLE manifestations,²⁰ and a better understanding of the differences among the various ethnic groups could probably enable better management of the patients. To highlight this, it is important to mention that in a clinical trial, in which IFN γ was targeted via an anti-IFN γ monoclonal antibody, no therapeutic impacts were produced in a cohort of non-African-American SLE patients with lupus nephritis,²¹ probably due to the fact that ABCs/DN2 - which require IFN γ for their activation - are more marked in African-American patients. Taking into account these data, we find it important to assess whether SLE patient populations with different genetic backgrounds, such as a population from Greece, differs

from an African-American population, in terms of ABC/DN2 immunophenotype.

AIMS OF THE STUDY (OBJECTIVES)

There are three specific aims:

1st aim: Enumerate and immunophenotype ABCs/DN in the peripheral blood of healthy donors and patients with active systemic lupus erythematosus (SLE), in order to identify correlations between the cell populations and the clinical profiles of the subjects. These correlations could evolve into useful prognostic and/or diagnostic markers of the disease.

2nd aim: Evaluate the effects of “traditional” and “modern” pharmacological agents on ABC/DN's percentage and functionality. The process shall be based on primary cell cultures combined with in vitro pharmacological treatments.

3rd aim: Perform functional assays and transcriptomic analysis for ABCs/DN.

RESEARCH STRATEGY (PATIENTS AND METHODS)

The project will be implemented in three work packages: Work package 1 deals with the 1st aim of the project. We will be collecting blood samples from healthy individuals (n=15) and SLE patients (n=25). To be eligible for inclusion as a patient, the subject should not have been diagnosed with an active infectious disease and/or an autoimmune disorder, other than SLE. Moreover, as far as exclusion criteria are concerned, the treatments of the patients should not include biologics, as these pharmaceutical agents dramatically affect the B cell percentages. Please observe that the relatively small number of patients in our research protocol is dictated by the rarity of the disease. However, these rather small patient groups are common in the field and are in line with other published studies.

To enumerate and immunophenotype the T-bet⁺ B cells (ABCs/DN), we shall perform flow cytometric staining of whole blood samples. For this multi-parameter flow cytometric analysis, we will be using markers carefully selected from the literature, allowing characterization of both ABCs and DN. Despite the fact that, concerning DN cells, an emphasis will be placed on DN2 subset, as it is a discrete T-bet⁺ B cell population which is implicated in the pathogenesis of SLE,¹⁸ DN will be further examined for the T-bet, CD11c, CD21 and CXCR5 statuses, in order to investigate whether populations other than DN1 and DN2 exist in the individuals and probably play a role in SLE pathogenesis. The frequency detection of all these populations may contribute, following careful correlation with the clinical state of the subjects, towards the discovery and validation of novel prognostic and diagnostic markers of SLE.

Work package 2 will focus on the 2nd aim, which refers to the evaluation of the effects that various pharmaco-

logical agents (may) have on ABCs/DN. The process shall be based on cell cultures combined with in vitro pharmacological treatments. Different concentrations of the agents will be used. We will be performing assays with both “traditional” molecules, such as hydroxychloroquine, and more “intelligent”, such as belimumab. Hydroxychloroquine is an antimalarial drug, which accumulates in the lysosomes, altering their pH and inhibiting several cellular processes. It is widely used in SLE, however its mechanism of action has not been fully elucidated and is believed to be associated with inhibition of TLR7 and TLR9 receptors and resulting signalling events, as well as the lysosomal degradation autophagic pathway.²² Belimumab, on the other hand, is a human monoclonal antibody, which binds with high affinity the B-cell activating factor BAFF, thus preventing BAFF interaction with its receptors (BR3, TAC1, BCMA) on the surface of B cells. Interaction of BAFF with its receptors is of vital importance for the survival and differentiation for B cells and belimumab was until recently the only biological agent approved for the treatment of SLE.^{23,24}

To evaluate the effects of hydroxychloroquine, belimumab and others on T-bet⁺ B cells, we will be exposing primary cultures of whole blood (whole blood assay/WBA) or peripheral blood mononuclear cells (PBMCs) from SLE and healthy individuals to clinically relevant concentrations of the agents for varying periods and evaluate the effects on the T-bet⁺ B cell populations, using flow cytometry with ABC/DN markers. We believe that such an approach will provide direct evidence on the effects these agents have on T-bet⁺ B cells and could evolve into a valuable precision medicine tool, allowing prognosis of the patients’ response, even before the actual treatment begins. Moreover, these data could provide useful insight in the pathogenetic mechanisms of SLE and the involvement of T-bet⁺ B cells, thus contributing to the discovery and validation of novel druggable targets.

Work package 3 focuses on transcriptomic analysis and functional assays for ABCs/DN. The transcriptomic analysis shall be performed, at the single-cell level (scRNA seq), via a 10X Genomics platform,²⁵ according to which a number of 10.000 cells per individual can be read. An SLE patient and a healthy donor will be examined. The results of the analysis are expected to allow characterisation of the genes and pathways associated with ABC/DN biology, paving the way for identification of novel genes of interest in SLE. With the aim of gaining as much sequencing information as possible for the ABCs and DN, cell sorting or magnetic beads isolation of CD19⁺ cells will be carried out prior to RNA isolation and sequencing. Of note, in order to examine more subjects than one patient and one healthy donor, we may alternatively (or additionally) perform a T-bet⁺ B cell transcriptomic meta-analysis. In such case, data shall be derived from already published scRNA seq studies.

As far as functional assays are concerned, we will use ELISA to estimate serum levels of cytokines (such as TNF α and IFN γ) and also autoantibodies. We will be collecting serum samples; along with the blood samples (see Work package 1). Moreover, we will use nephelometry to determine the levels of IgG isotypes. All these data shall then be used to identify possible correlations with the ABC/DN percentages.

All three work packages are expected to be completed until 2023.

ANTICIPATED BENEFITS

In general, the results of the study are expected to allow characterization of the pathogenetic role of ABCs/DN in SLE and could probably contribute, following careful association with the clinical state of the patients, towards the discovery and validation of novel prognostic and diagnostic markers of the disease. Moreover, the elucidation of the role of ABCs/DN in the pathogenesis of SLE could lead to the discovery of novel druggable targets.

STUDY APPROVAL

The study has been approved by the Ethics Committee of Hippokraton General Hospital, School of Medicine A.U.Th., Thessaloniki, Greece.

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ΕΛΛΗΝΙΚΗ ΡΕΥΜΑΤΟΛΟΓΙΚΗ ΕΤΑΙΡΕΙΑ
& ΕΠΙΓΕΓΡΑΜΜΕΝΗ ΕΝΩΣΗ ΡΕΥΜΑΤΟΛΟΓΩΝ ΕΛΛΑΔΟΣ

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Szabo SJ, Kim ST, Costa GL, Zhang X, Fathman CG, Glimcher LH. A novel transcription factor, T-bet, directs Th1 lineage commitment. *Cell* [Internet] 2000;100(6):655–69. Available from: [http://dx.doi.org/10.1016/s0092-8674\(00\)80702-3](http://dx.doi.org/10.1016/s0092-8674(00)80702-3)
2. Townsend MJ, Weinmann AS, Matsuda JL, Salomon R, Farnham PJ, Biron CA, et al. T-bet regulates the terminal maturation and homeostasis of NK and Valpha14i NKT cells. *Immunity*

- 2004;20:477–94.
3. Lugo-Villarino G, Ito S, Klinman DM, Glimcher LH. The adjuvant activity of CpGDNA requires T-bet expression in dendritic cells. *Proc Natl Acad Sci USA* 2005;102:13248–53.
 4. Peng SL, Li J, Lin L, Gerth A. The role of T-bet in B cells. *Nat Immunol* [Internet] 2003;4(11):1041; author reply 1041. Available from: <http://dx.doi.org/10.1038/ni1103-1041a>
 5. Gerth AJ, Lin L, Peng SL. T-bet regulates T-independent IgG2a class switching. *Int Immunol* [Internet] 2003;15(8):937–44. Available from: <http://dx.doi.org/10.1093/intimm/dxg093>
 6. Wang NS, Mcheyzer-Williams LJ, Okitsu SL, Burris TP, Reiner SL, Mcheyzer-Williams MG. Divergent transcriptional programming of class-specific B cell memory by T-bet and RORalpha. *Nat Immunol* 2012;13:604–11.
 7. Hao Y, O'Neill P, Naradikian MS, Scholz JL, Cancro MP. A B-cell subset uniquely responsive to innate stimuli accumulates in aged mice. *Blood* [Internet] 2011;118(5):1294–304. Available from: <http://dx.doi.org/10.1182/blood-2011-01-330530>
 8. Rubtsov AV, Rubtsova K, Fischer A, Meehan RT, Gillis JZ, Kappler JW, et al. Toll-like receptor 7 (TLR7)-driven accumulation of a novel CD11c B-cell population is important for the development of autoimmunity. *Blood* 2011;118:1305–15.
 9. Rubtsova K, Rubtsov AV, Van Dyk LF, Kappler JW, Marrack P. T-box transcription factor T-bet, a key player in a unique type of B-cell activation essential for effective viral clearance. *Proc Natl Acad Sci USA* 2013;110:E3216–3224.
 10. Rubtsova K, Rubtsov AV, Halemans K, Li SX, Kappler JW, Santiago ML, et al. T cell production of IFN γ in response to TLR7/IL-12 stimulates optimal B cell responses to viruses. *PLoS One* 2016;11.
 11. Naradikian MS, Myles A, Beiting DP, Roberts KJ, Dawson L, Herati RS, et al. Cutting Edge: IL-4, IL-21, and IFN- γ Interact To Govern T-bet and CD11c Expression in TLR-Activated B Cells. *J Immunol* 2016;197:1023–8.
 12. Rubtsov PAV, Marrack K. T-bet expressing B cells—Novel target for autoimmune therapies? *Cell Immunol* 2017;321:35–9.
 13. Rubtsov AV, Rubtsova K, Kappler JW, Jacobelli J, Friedman RS, Marrack P. CD11c-expressing B cells are located at the T cell/B cell border in spleen and are potent APCs. *J Immunol* [Internet] 2015;195(1):71–9. Available from: <http://dx.doi.org/10.4049/jimmunol.1500055>
 14. Domeier PP, Chodisetti SB, Soni C, Schell SL, Elias MJ, Wong EB, et al. IFN- γ receptor and STAT1 signaling in B cells are central to spontaneous germinal center formation and autoimmunity. *J Exp Med* 2016;213:715–32.
 15. Du SW, Arkatkar T, Jacobs HM, Rawlings DJ, Jackson SW. Generation of functional murine CD11c+ age-associated B cells in the absence of B cell T-bet expression. *Eur J Immunol* [Internet] 2019;49(1):170–8. Available from: <http://dx.doi.org/10.1002/eji.201847641>
 16. Colonna-Romano G, Bulati M, Aquino A, Pellicano M, Vitello S, Lio D, et al. A double-negative (IgD-CD27-) B cell population is increased in the peripheral blood of elderly people. *Mech Ageing Dev.* 2009;130:681–90.
 17. Wei C, Anolik J, Cappione A, Zheng B, Pugh-Bernard A, Brooks J, et al. A new population of cells lacking expression of CD27 represents a notable component of the B cell memory compartment in systemic lupus erythematosus. *J Immunol* [Internet] 2007;178(10):6624–33. Available from: <http://dx.doi.org/10.4049/jimmunol.178.10.6624>
 18. Jenks SA, Cashman KS, Zumaquero E, Marigorta UM, Patel AV, Wang X, et al. Distinct Effector B Cells Induced by Unregulated Toll-like Receptor 7 Contribute to Pathogenic Responses in Systemic Lupus Erythematosus. *Immunity* 2018;49:1–15.
 19. Zumaquero E, Stone SL, Scharer CD, Jenks SA, Nellore A, Mousseau B, et al. IFN γ induces epigenetic programming of human T-bet^{hi} B cells and promotes TLR7/8 and IL-21 induced differentiation. *Elife* [Internet] 2019;8. Available from: <http://dx.doi.org/10.7554/elife.41641>
 20. González LA, Toloza SMA, McGwin G Jr, Alarcón GS. Ethnicity in systemic lupus erythematosus (SLE): its influence on susceptibility and outcomes. *Lupus* [Internet] 2013;22(12):1214–24. Available from: <http://dx.doi.org/10.1177/0961203313502571>
 21. Boedigheimer MJ, Martin DA, Amoura Z, Sánchez-Guerrero J, Romero-Diaz J, Kivitz A, et al. Safety, pharmacokinetics and pharmacodynamics of AMG 811, an anti-interferon- γ monoclonal antibody, in SLE subjects without or with lupus nephritis. *Lupus Sci Med* 2017 Sep 14;4(1):e000226.
 22. Ponticelli C, Moroni G. Hydroxychloroquine in systemic lupus erythematosus (SLE). *Expert Opin Drug Saf* [Internet] 2017;16(3):411–9. Available from: <http://dx.doi.org/10.1080/14740338.2017.1269168>
 23. Burness CB, McCormack PL. Belimumab: in systemic lupus erythematosus. *Drugs* [Internet] 2011;71(18):2435–44. Available from: <http://dx.doi.org/10.2165/11208440-000000000-00000>
 24. Blair HA, Duggan ST. Belimumab: A review in systemic lupus erythematosus. *Drugs* [Internet] 2018;78(3):355–66. Available from: <http://dx.doi.org/10.1007/s40265-018-0872-z>
 25. Nehar-Belaid D, Hong S, Marches R, Chen G, Bolisetty M, Baisch J, et al. Mapping systemic lupus erythematosus heterogeneity at the single-cell level. *Nat Immunol* [Internet] 2020;21(9):1094–106. Available from: <http://dx.doi.org/10.1038/s41590-020-0743-0>