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14. ABSTRACT

The main goals of our research project have not changed and consist in studying the defective central B cell tolerance in MISTRG6 humanized mice engrafted with hematopoietic stem cells (HSCs) isolated from the bone marrow of patients with systemic lupus erythematosus (SLE). We also proposed to test whether PTPN22 blockade may prevent the production of developing autoreactive B cells in humanized mice engrafted with HSCs from patients with SLE injected with LTV-1 PTPN22 inhibitor.

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1. Major accomplishments

The main goals of our research project have not changed and consist in studying the defective central B cell tolerance in MISTRG6 humanized mice engrafted with hematopoietic stem cells (HSCs) isolated from the bone marrow of patients with systemic lupus erythematosus (SLE). We also proposed to test whether PTPN22 blockade may prevent the production of developing autoreactive B cells in humanized mice engrafted with HSCs from patients with SLE injected with LTV-1 PTPN22 inhibitor.

PTPN22 blockade corrects the impaired B cell tolerance in SLE. Since the rare loss-of-function 263Q PTPN22 variant was reported to confer protection against SLE and RA, we hypothesized that the inhibition of PTPN22 enzymatic activity by small molecules or its expression by RNA interference may strengthen BCR signaling and increase the removal of developing autoreactive B cells, thereby resetting defective central B cell tolerance in SLE. We therefore proposed to analyze the frequency of autoreactive B cells in humanized mice engrafted with SLE HSCs with or without inhibition of PTPN22 enzymatic activity using the LTV-1 PTPN22 inhibitor or by inhibiting PTPN22 expression using shRNA-expressing lentiviruses. We have previously shown that both methods correct the impaired removal of autoreactive clones in humanized mice transplanted with HSCs carrying the *PTPN22 T* risk allele associated with the development of most autoimmune diseases including SLE. In addition, we initially reported that NSG mice that received HSCs isolated from the bone marrow of a T1D patient or a RA patient displayed new emigrant/transitional B cells that expressed high frequencies of polyreactive and HEp-2 reactive antibodies, indicating that central B cell tolerance was not functional. However, the engraftment efficiency of NSG mice with HSCs isolated from adults was very low and led us to use an alternative humanized mouse model using MISTRG6 mice obtained from Dr. Richard Flavell at Yale University (Fig. 1).

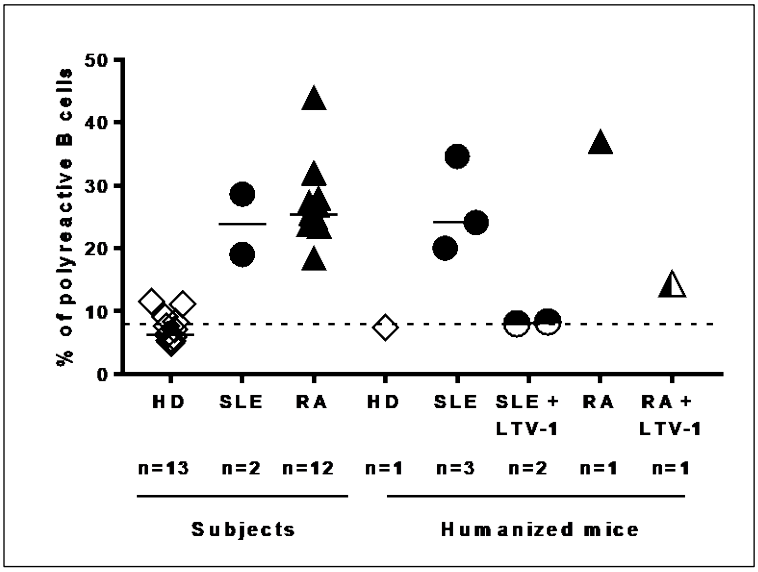

Fig. 1. The defective central B cell tolerance in a humanized mouse engrafted with HSCs from either SLE or an RA patient is corrected by LTV-1 PTPN22 inhibitor. The frequencies of polyreactive clones in new emigrant/transitional B cells isolated from the blood of healthy donors, SLE patients and an RA patient and those from humanized mice transplanted with their respective HSCs were determined by ELISA using dsDNA, insulin and LPS. Antibodies were considered polyreactive when they bound all three different antigens. Each symbol represents an individual/mouse.

MISTRG6 (M-CSFh;IL-3/GM-CSFh; hSIRPAtg;TPOh;Rag2-;γc-;IL-6h) are Rag2-deficient, Il2rg-deficient mice with human versions of five cytokines important for myeloid and lymphoid immune cell development and allow better engraftment with HSCs isolated from adult donors. We have now enrolled three patients with SLE and generated three MISTRG6 humanized mice per patient, one of which was injected with the LTV-1 PTPN22 inhibitor twice daily for one week as previously reported. All mice were well-reconstituted and allowed the isolation of single new emigrant/transitional B cells isolated from the spleen of these animals for assessment of antibody reactivity. We found that all three humanized mice engrafted with SLE patient's HSCs produced B cells that contained elevated frequencies of polyreactive clones compared to MISTRG6 mice

engrafted with HSCs from an adult control donor (Fig. 1). These autoreactive B cell frequencies were similar to those determined with new emigrant/transitional B cells isolated from the peripheral blood of respective SLE patients and healthy donor (Fig. 1 and ongoing experiments). Thus, our humanized mouse model recapitulates the impaired central B cell tolerance checkpoint characteristic of patients with SLE and other autoimmune diseases.

We then tested if the *in vivo* inhibition of PTPN22 enzymatic activity could restore central B cell tolerance. LTV-1 is a compound that was identified to selectively inhibit human PTPN22 enzymatic activity and the injection of LTV-1 twice daily for a week restored central B cell tolerance in mice engrafted with HSCs carrying *1858T PTPN22* allele(s). We found that PTPN22 inhibition resulted in an increase in lambda chain usage in all three humanized mice engrafted with HSCs from each SLE patient (Fig. 2A). Since kappa light chain Ig genes rearrange before lambda light chain genes, our data suggest that ongoing light chain gene recombination that mediate receptor editing, the main mechanism of central B cell tolerance, is likely responsible for increase lambda chain usage. In agreement with active ongoing recombination of the light chain loci, lambda chains expressed in new emigrant/transitional B cells from LTV-1 injected humanized mice favored more downstream Jλ3 gene segment usage combined to more upstream Vλ gene usage (Fig. 2B and data not shown). The injection of 0.75mg of TLV-1 twice daily for a week was not only modify the BCR repertoire of new emigrant/transitional B cells but also prevented the production of autoreactive B cells in MISTRG6 mice engrafted with HSCs isolated from SLE patients. Indeed, the frequencies of new emigrant/transitional B cells expressing polyreactive antibodies in LTV-1 injected MISTRG6 humanized mice engrafted with HSCs from SLE patients were strongly decreased compared to those of un-injected matched humanized mice and similar to those observed in healthy donors or in humanized mice engrafted with HSCs from healthy donors (Fig. 1). Of note, similar results were obtained in MISTRG6 mice were humanized with HSCs from an RA patient (Fig. 1). Of note, neither the three patients with SLE nor the RA patient harbored the *PTPN22 T* risk allele, revealing that PTPN22 inhibition may also be beneficial for non-carrier patients. Hence, PTPN22 blockade restores the impaired central B cell tolerance characteristic of patients with SLE and RA. Thus, PTPN22 inhibition may represent a novel strategy to prevent autoreactive B cell production and potential thwart the development of autoimmune manifestations.

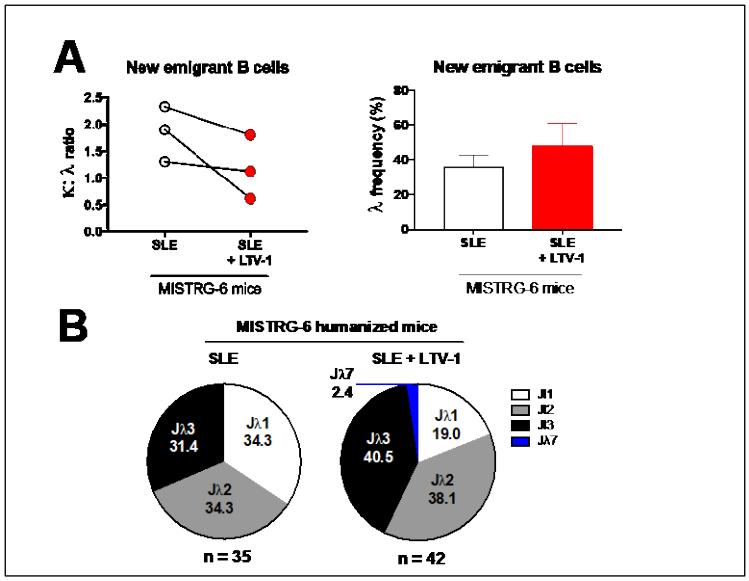

Fig. 2. PTPN22 inhibition may favor receptor editing as evidenced by increased lambda light chain expression. (A) Kappa/lambda light chain ratio (left) and lambda chain frequencies (right) in MISTRG6 mice engrafted with HSCs from three distinct SLE patients and injected or not twice daily with LTV-1 PTPN22 inhibitor for a week. (B) PTPN22 blockade favors downstream Jλ3 and Jλ7 gene segment usage. The frequencies of each Jλ gene segment expressed by new emigrant/transitional B cells from un-injected or LTV-1 injected MISTRG6 mice engrafted with HSCs from SLE patients is represented as pie charts. The total numbers of lambda sequences pooled from the three humanized mice analyzed are listed below.

2. Impact of the research

Our research application explores a novel strategy that may prevent SLE by inhibiting PTPN22 enzymatic activity and re-establishing proper thresholds for the removal of autoreactive B cells defective in the disease.
Preliminary data already suggest the feasibility of such approach and the restoration of a normal B cell repertoire that should no longer favor the development of SLE and potentially other autoimmune diseases. Hence, treatment with PTPN22 inhibitor of recently diagnosed SLE patients or asymptomatic subjects with autoantibodies predicting SLE development may ameliorate disease or at least delay disease onset and potentially block the development of many comorbidities if given in combination with anti-B cell therapy.
In addition, PTPN22 blockade may also prevent the development of many other autoimmune diseases such as rheumatoid arthritis, sjögren's syndrome and scleroderma. If successful, our research would therefore have a great impact on patient's life span and quality of life.

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