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ANB032, a BTLA Checkpoint Agonist Antibody, Attenuates Dendritic Cell Maturation and Function: A Novel Mechanism Addressing Atopic Dermatitis Pathophysiology

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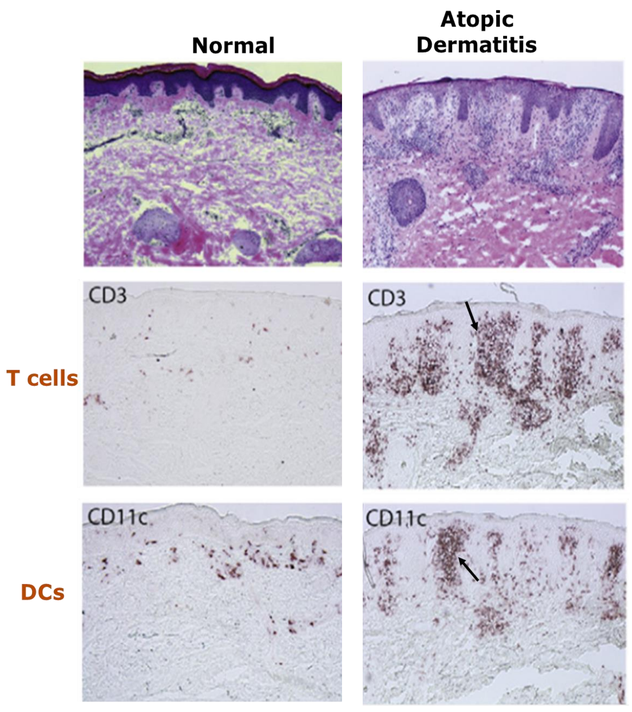
ABSTRACT

Introduction & Objectives: Atopic Dermatitis (AD) is characterized by heterogeneous immunologic drivers including broad T cell and DC activity. Modulating co-inhibitory checkpoint receptors is a promising strategy to regulate immune cells for the treatment of systemic, heterogeneous autoimmune and inflammatory disorders such as AD. One co-inhibitory checkpoint receptor that modulates the activity of T cells, B cells, and DCs is B and T Cell Lymphocyte Attenuator (BTLA). ANB032 is an investigational BTLA checkpoint agonist antibody that has been shown to reduce T cell proliferation and reduce secretion of inflammatory cytokines (Th1, Th2, Th17, Th22) in AD patient-derived peripheral blood mononuclear cells (PBMCs). Since DCs represent a heterogeneous population of myeloid cells that play a pivotal role in the initiation of adaptive immune responses and the maintenance of immune tolerance, the role of BTLA agonism in modulating DC activation and maturation was investigated.
Materials & Methods: Purified monocytes from healthy PBMCs were differentiated to DCs and stimulated with lipopolysaccharide (LPS) to determine the expression of BTLA on the DCs. DCs were then stimulated with LPS in the presence or absence of ANB032 and the maturation state of the DCs was assessed. MHC II expression and costimulatory molecule expression were measured by FACS. ANB032-treated DCs were washed and then co-cultured with allogeneic naïve CD4 T cells in a mixed lymphocyte reaction (MLR) for an additional five days to evaluate the frequency of differentiated FOXP3+ regulatory T cells and secretion of inflammatory cytokines by FACS and MSD, respectively.
Results: LPS induced rapid DC maturation and expression of high levels of BTLA on mature DCs. ANB032, included in the DC culture during LPS stimulation, reduced the absolute number of mature DCs by 53%. Additionally, ANB032 reduced HLA-DR expression and costimulatory molecule expression of CD80, CD86, CD40, and OX40L. When co-cultured with allogeneic naïve CD4 T cells, ANB032-treated DCs increased the frequency of FOXP3+ Tregs and reduced the secretion of Th1 and Th2 cytokines in the MLR. **Conclusion:** These data demonstrate the induction of BTLA expression on mature DCs by a stimuli relevant in AD and provides additional insight regarding the effect of BTLA agonism on DC maturation and function. BTLA agonism by ANB032 has the potential to restore immune balance by impacting a broad range of pathogenic immune cells, including T cells and DCs, while inducing Tregs, which may provide therapeutic value in the treatment of autoimmune and inflammatory disorders, including AD. These data, additional in vitro data and results from a Phase 1 healthy volunteer study support the ongoing double-blind, placebo-controlled, global Phase 2b study of ANB032 in moderate-to-severe AD (NCT05935085).

BACKGROUND & OBJECTIVE

- Atopic dermatitis (AD) is a systemic, heterogenous inflammatory disease with pathogenesis driven by Th1, Th2, Th17, Th22, and dendritic cells (DCs) both in skin and the periphery
- There are significantly more DCs in the skin of AD patients, with up to 10-fold increase in the epidermis and up to 3.5-fold increase in the dermis[1]
- BTLA is a co-inhibitory checkpoint receptor expressed preferentially on activated T cells, B cells, and DCs, key contributors to inflammatory diseases, and preclinical studies have shown that BTLA dysregulation is linked to the development of autoimmune diseases, including dermatitis[2,3]
- Although the expression of BTLA on subsets of DCs has been reported, BTLA's role in modulating DC maturation and function has not been thoroughly investigated
- ANB032 is an investigational non-depleting BTLA agonist that does not compete with the binding of BTLA to herpesvirus entry mediator (HVEM), its ligand (**Figure 1**)



Guttman-Yassky et al, JACI, 2011

Proposed Mechanism of Action for ANB032

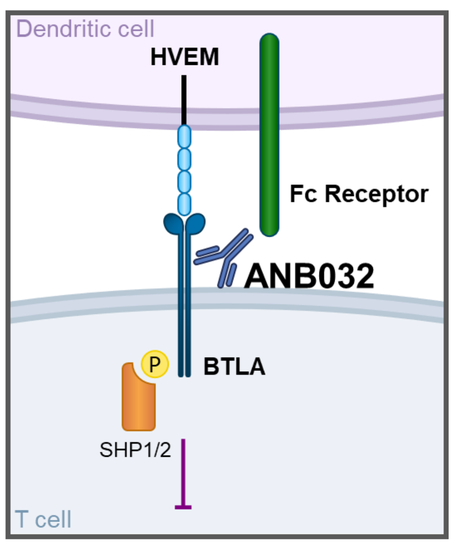


Figure 1. Schematic of proposed MoA

ANB032: IgG4 antibody (non-depleting)

- Binds to BTLA on membrane proximal epitope
- Fc receptor binding profile contributes to differentiated potency
- Non-blocking of HVEM engagement

ANB032's agonist signal modulates immune cells

- Inhibits activated T cell proliferation
- Reduces inflammatory cytokine secretion
- Modulates DC function, including inducing Tregs

Objective: Investigate the role of BTLA and effect of ANB032 on DC maturation and activation in a preclinical model

METHODS

BTLA expression on DCs

- Purified monocytes from healthy PBMCs were differentiated to DCs
- Differentiated DCs were either stimulated with lipopolysaccharide (LPS) or rested in fresh medium, then stained for MHC II and CD11c, and the BTLA expression was evaluated on immature and mature DCs

Effect of ANB032 on DCs

- Differentiated DCs were treated with either ANB032 or isotype control, then stimulated with LPS and evaluated by flow cytometry to assess the maturation state of DCs, absolute number of mature DCs, expression of MHC II and costimulatory molecules

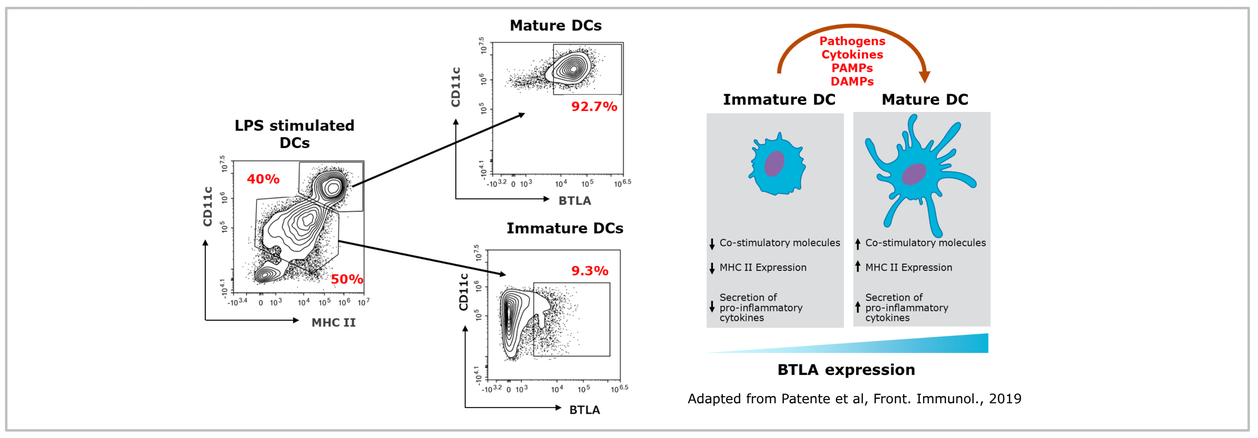
Effect of ANB032 on Tregs and inflammatory cytokines

- Differentiated DCs were treated with either ANB032 or isotype control
- ANB032-treated DCs were washed and then co-cultured with allogenic naïve CD4 T cells, then T cells were evaluated for CD4, CD25, and intracellular Foxp3 expression to identify inducible regulatory T cells (iTreg)
- The frequency of differentiated iTregs and secretion of inflammatory cytokines were evaluated by FACS and MSD, respectively

RESULTS

BTLA was Highly Expressed on Mature DCs with LPS Stimulation

- LPS-stimulated DCs resulted in 40% mature and 50% immature DCs
- BTLA expression was seen in 92.7% of mature DCs versus 9.3% of immature DCs (**Figure 2**)

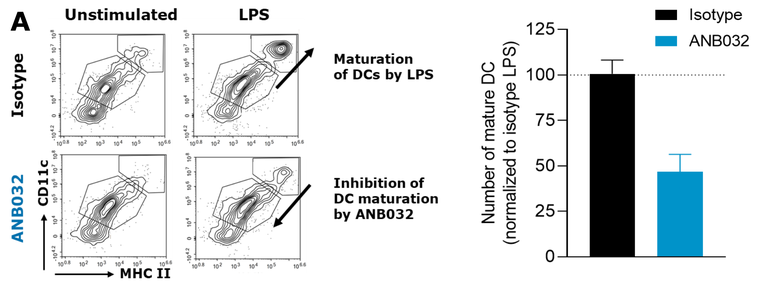


Adapted from Patente et al, Front. Immunol., 2019

Figure 2. BTLA expression on DCs

ANB032 Inhibits DC Maturation and Reduces Antigen Presentation & Co-Stimulatory Molecules

Maturation of LPS-stimulated DCs was inhibited by ANB032 resulting in reduced absolute numbers of mature DCs compared to isotype control treatment



ANB032-treated LPS-stimulated DCs had lower expression of MHC II and reduced expression of co-stimulatory molecules, CD80, CD86, and CD40, compared to the isotype control

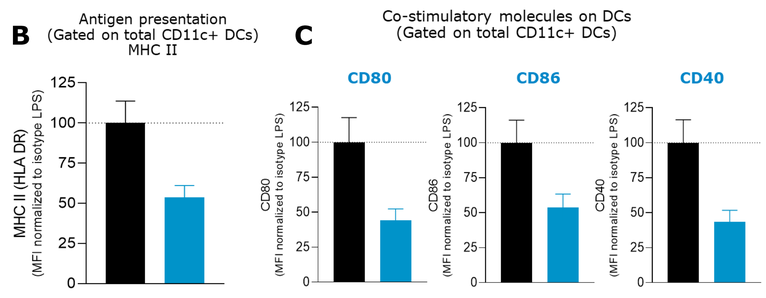
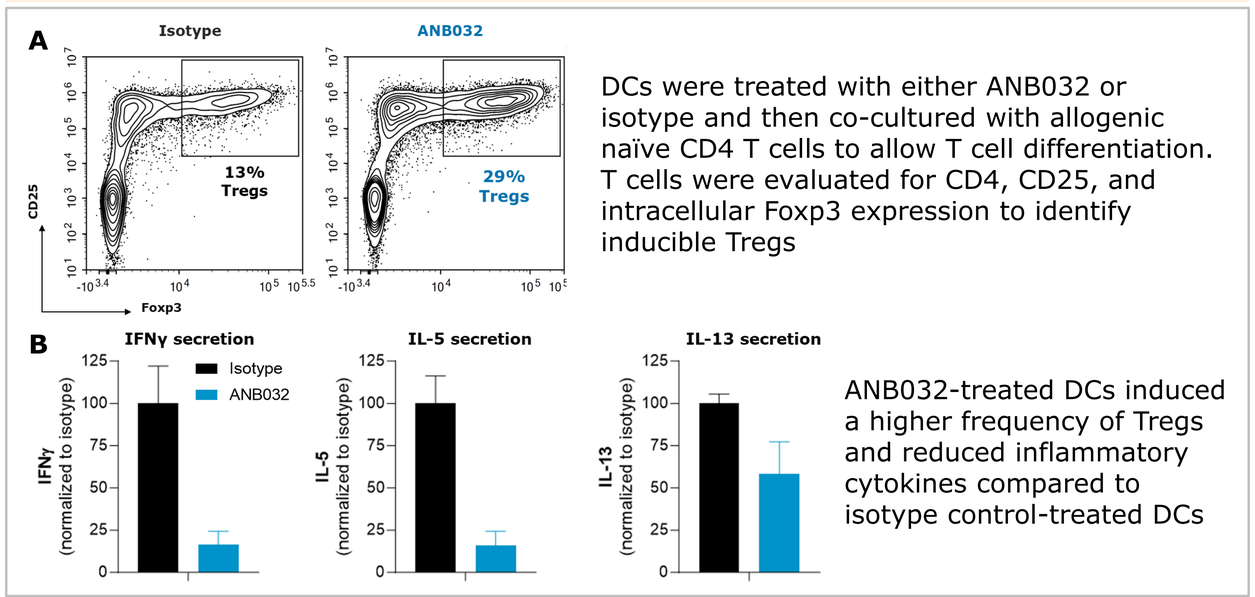


Figure 3. Effect of ANB032 on DC maturation (A), MHC II presentation (B) and co-stimulatory molecule expression (C)

ANB032-treated DCs Induce Functional Tregs

DCs were treated with either ANB032 or isotype and then co-cultured with allogenic naïve CD4 T cells to allow T cell differentiation. T cells were evaluated for CD4, CD25, and intracellular Foxp3 expression to identify inducible Tregs



ANB032-treated DCs induced a higher frequency of Tregs and reduced inflammatory cytokines compared to isotype control-treated DCs

Figure 4. Effect of ANB032-treated DCs on functional Tregs (A) and cytokine secretion (B)

CONCLUSIONS

- BTLA is highly expressed on mature DCs
- Preclinical evaluation of ANB032 demonstrated:
  - Inhibition of DC maturation and reduction of antigen presentation and co-stimulatory molecule expression
  - Modulation of DC function that results in increased Foxp3+ Tregs that contribute to reduced inflammatory cytokine production
  - Inhibition of a broad range of T cell subsets and DCs, while inducing Tregs, supports a potential for restoring immune balance
- Based on these findings, ANB032 may provide therapeutic value in the treatment of autoimmune and inflammatory diseases, including AD
- ANB032 is being evaluated in an ongoing Phase 2 study in moderate-to-severe AD (NCT05935085)

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