Atopic Dermatitis is Characterized by Enrichment in a BTLA Transcriptomic Signature

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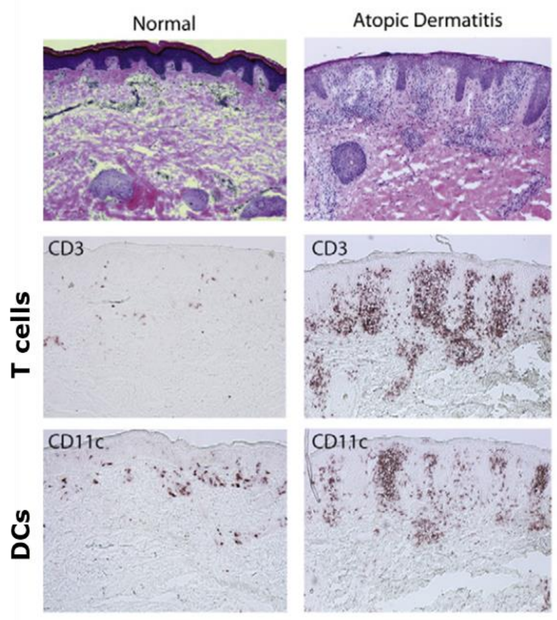
ABSTRACT

BTLA (B and T lymphocyte attenuator) is a co-inhibitory immune checkpoint receptor present on T cells, B cells, and dendritic cells (DCs). ANB032 is an investigational BTLA agonist antibody that is currently under investigation in a Phase 2 study in atopic dermatitis (AD) and has previously been shown to inhibit the proliferation of activated T cells, reduce cytokine secretion by T helper cells, and modulate DC function in vitro. In this study, we aimed to use transcriptomic data obtained from preclinical models to study the biologic consequences of ANB032 engagement, develop a molecular signature for BTLA agonism, and apply this signature to better understand the role of BTLA in inflammatory disorders, including AD.

BACKGROUND & OBJECTIVE

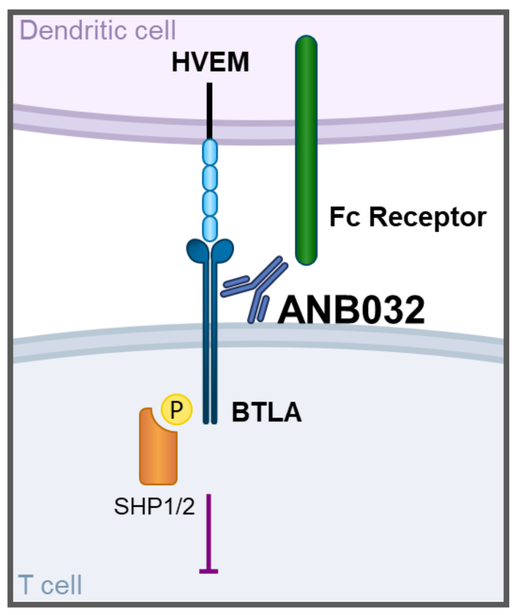
- AD is a systemic, heterogenous inflammatory disease driven by Th1, Th2, Th17, Th22, and DCs both in skin and the periphery
- There are significantly more DCs in the skin of AD patients, with up to 10-fold increase in the epidermis and up to 3.5-fold increase in the dermis[1]
- BTLA is a co-inhibitory checkpoint receptor expressed preferentially on activated T cells, B cells, and DCs, which are key contributors to inflammatory diseases
- ANB032 is an investigational BTLA agonist that has been shown to reduce activated T cell proliferation, reduce inflammatory cytokine secretion (Th1, Th2, Th17, Th22), and modulate DC function while inducing regulatory T cells in vitro[2]



Guttman-Yassky et al, JACI, 2011

- In this study, transcriptomic data from humanized mouse models of graft-versus-host-disease (GvHD) were evaluated to study the immune effects of ANB032-mediated BTLA agonism, with a focus on T cell genes. This signature was applied to transcriptomic data from AD tissues

Proposed Mechanism of Action for ANB032



BTLA is a key node of immune regulation

- BTLA is a potent co-inhibitory checkpoint receptor
- Expressed only on immune cells and preferentially on activated immune cells
- Dysregulation of BTLA pathway accelerates onset and exacerbates disease

ANB032: IgG4 antibody (non-depleting)

- Binds BTLA proximal to immune cell
- Fc receptor binding contributes to differentiated potency
- Non-blocking of HVEM engagement

METHODS

Humanized Mouse Model of Graft-versus-Host Diseae (GvHD)

- Purified human peripheral blood mononuclear cells (PBMCs) were adoptively transferred into irradiated NODscid IL2Rγ^{null} (NSG) mice and cohorts were treated with ANB032 or isotype control
- At the study midpoint, gene signatures of sorted human T cells were profiled with bulk RNA-sequencing to capture the differential transcriptome signals between ANB032-treated and control groups

Overlap of ANB032 MOA and AD Disease Pathology

- Differential expression analysis and pathway enrichment analysis were performed for both our internal GvHD model (T cells from ANB032 vs. isotype control-treated mice) and from a published data set (lesional skin from 52 AD patients and 20 healthy controls)[3]
- Statistically significant pathways from both analyses were then evaluated for overlap in drug and disease mechanisms

Evaluation of BTLA Transcriptomic Signature

- The most differentially expressed genes in the GvHD study were combined with curated differentially regulated genes important in AD pathology, along with previously identified genes altered by BTLA agonism in B and T lymphocytes[4] to derive a robust BTLA signature
- Gene set variation analysis (GSVA) was performed using the BTLA signature in skin from AD patients and healthy controls from a published data set[3]

RESULTS

ANB032 downregulated multiple inflammatory pathways in the GvHD model. ANB032-treated cohorts decreased key immune pathways and cytokines when compared to isotype control cohorts, including but not limited to T cell activation and proliferation, T helper cell differentiation, Toll-Like Receptor signaling, microbial and defense response, and Type 1 IFN signaling (**Figure 1**). These same inflammatory pathways were significantly upregulated in AD lesional skin compared to healthy skin. In AD skin, BTLA levels were elevated and HVEM, the natural agonist ligand, levels were decreased when compared to control skin (**Figure 2**). GSVA using the BTLA signature showed significant enrichment in AD patients compared to healthy controls (p-value = 6.58 x 10^{-14})

Top Pathways Downregulated by ANB032 were Upregulated in AD Lesional Skin

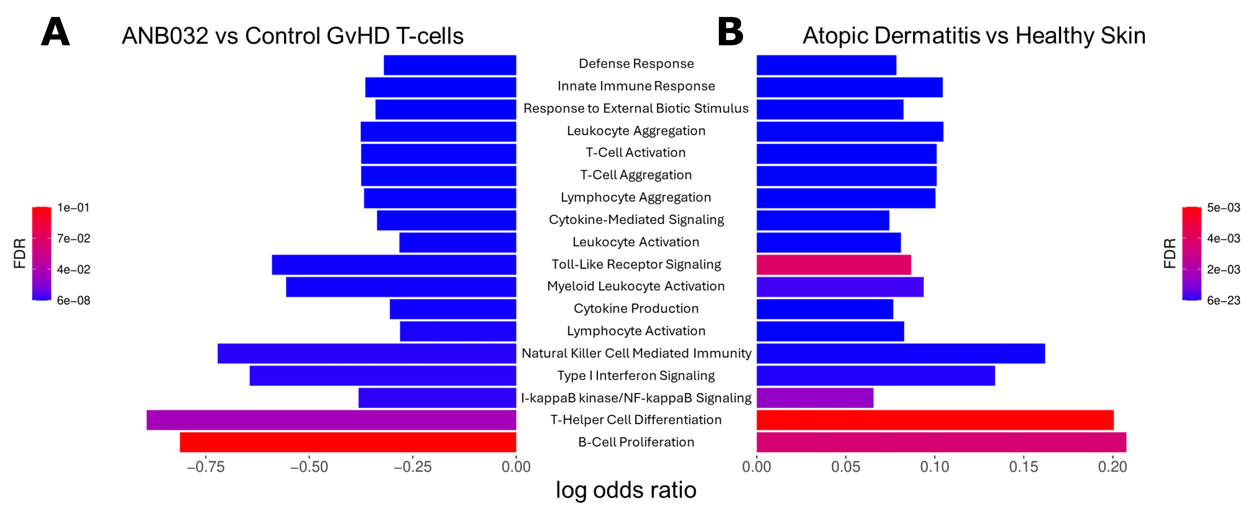


Figure 1. Top downregulated (log odds ratio < 0) pathways from a Gene Ontology (GO) pathway enrichment analysis for ANB032 versus isotype control treated GvHD cohorts were identified (A) and compared against the same pathways in AD lesional skin versus healthy skin (B)

Both BTLA Expression and Signature were Upregulated and HVEM was Downregulated in AD Lesional Skin

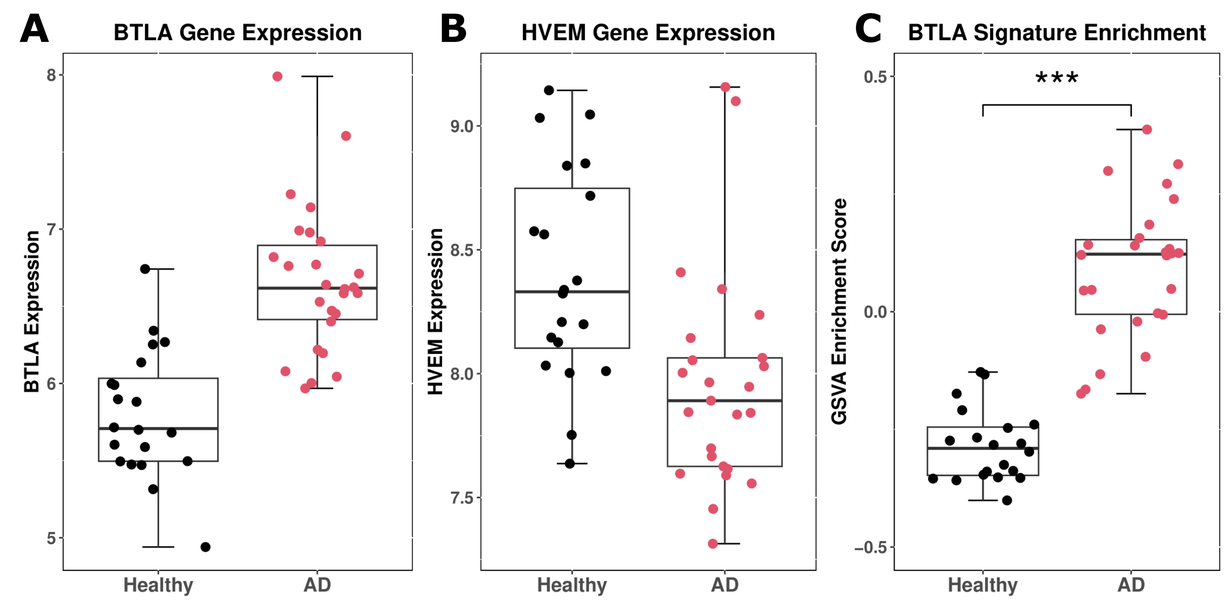


Figure 2. Microarray gene expression compared healthy and AD skin for BTLA (A) and HVEM (B). GSVA using the internally developed gene set evaluated healthy and AD skin for BTLA signature enrichment (C)

CONCLUSIONS

Generation of a BTLA transcriptomic signature from this preclinical study were compared to pathways in human AD lesional skin showed:

- Genes modulated by BTLA agonism via ANB032 significantly overlapped with dysregulated immune genes/pathways in AD
- AD lesional skin had enrichment of the derived BTLA signature when compared to healthy skin, supporting the role of the BTLA pathway in the pathogenesis of AD

These human translational data combined with data from other preclinical studies and a Phase 1 healthy volunteer study support the rationale for the ongoing Phase 2b study of ANB032 in moderate-to-severe AD (NCT05935085)

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