

I κ B ζ as a Therapeutic Target for Murine Rheumatoid Arthritis

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INTRODUCTION: Rheumatoid Arthritis (RA) is a chronic inflammatory autoimmune disease considered as one of the leading causes of disability worldwide. Elevated levels of inflammatory cytokines and catabolic enzymes, expressed by a variety of synovial cells in RA, perpetuate a vicious inflammatory and destructive cycle in the joint tissue, leading to the demise of articular cartilage and subchondral bone. Current pharmacologic and biologic therapies have targeted these cytokines individually or in combination, albeit with limited therapeutic efficacy owing to cytokine redundancies, short half-life of the biologics used and many adverse side effects on normal physiology. NF- κ B signaling plays a crucial role in the production and function of these inflammatory cytokines. Hence, its logical that selective and strategic targeting of NF- κ B activation possibly be more effective to plummet the production and function of inflammatory culprits compared with the established therapies employing individual cytokine neutralization strategies. However, given the essential role of NF- κ B in physiologic processes, a more effective approach is needed to target pathway downstream of NF- κ B, which regulates expression of inflammatory cytokines in RA. In our recent study in chondrocyte, we have identified I κ B ζ (gene: *Nfkbiz*) as a unique inflammatory signature of NF- κ B that controls transcription of inflammatory cytokines only under pathologic conditions. Therefore, this study was designed to investigate I κ B ζ as a potent inflammatory mediator of NF- κ B signaling which can be pharmacologically targeted to decrease the inflammation burden and joint pathology in RA animal models.

METHODS: (1) To investigate the role I κ B ζ on physiological skeletal homeostasis we compared the bone phenotype in LysM-Cre^{+/+}: *Nfkbiz*^{-/-} knockout (*Nfkbiz*^{ALysM}) with littermate WT control mice. (2) To study the role I κ B ζ as a central modulator of RA pathogenesis, we employed global tamoxifen-inducible deletion of *Nfkbiz* in KBxN serum transfer induced arthritis (STIA) mouse model. More specifically, we used *CAGG-Cre*^{+/+}-*ERT2:Nfkbiz*^{-/-} KO (*Nfkbiz*^{ACAGG}) and *Ubc-Cre*^{+/+}-*ERT2:Nfkbiz*^{-/-} KO (*Nfkbiz*^{Ubc}) mice along with littermate WT control (*Nfkbiz*-flox) mice. This study was approved by IACUC. To monitor RA progression in STIA, we performed paw and ankle thickness measurement, behavioral analysis (to correlate with RA pathology), micro-CT analysis, histological analysis, qPCR, ELISA and FACS analysis from synovial cells isolated from arthritic WT and *Nfkbiz*-null mice. (3) For various *in vitro* biochemical studies, we utilized WT and *Nfkbiz*-null, bone marrow derived macrophages (BMDM), osteoclast (OC), osteoblast (OB), chondrocyte, neutrophil and T cells. For *in vitro* analysis, the cells were treated with or without LPS followed by qPCR, ELISA and western blotting to identify and quantify changes in genes expression and signaling pathways. (4) Finally, using biochemical and *in silico* analysis we identified Dimethyl Itaconate (DI) and 8-Hydroxyquinoline (8HQ) as selective pharmacological inhibitors of I κ B ζ . To investigate the efficacy of DI and 8HQ as WT mice subjected to STIA were administered with DI and/or HQ followed by paw and ankle thickness measurement, micro-CT, and histological analysis. Statistical analyses were performed by using Student t-test. Multiple treatments were analyzed by one-way ANOVA followed by post hoc Newman-Keuls test of significance.

RESULTS: (1) MicroCT analysis showed no difference in basal bone mass parameters between WT control and *Nfkbiz*^{ALysM} mice. Deletion of *Nfkbiz* did not affect OC and OB differentiation as observed by no significant changes in *Trap* and *Nfat1* gene expression in OC and *Alp* and *Col1* expression in OB, respectively. These findings suggest that *Nfkbiz* is not activated in physiological conditions and hence deletion of I κ B ζ does not appear to impact normal skeletal physiology. (2) Compared to WT BMDM, *Nfkbiz*-null BMDM failed to show any significant increases in the expression of inflammatory genes such as *Il1 β* , *Il6*, *Il18*. Similarly, *Nfkbiz*-null committed OC precursors (pre-OC) failed to differentiate to pathologically activated osteoclast (PAOC). In addition, *Nfkbiz* deletion significantly inhibits LPS induced of *Il1 β* expression in neutrophils. These findings show that I κ B ζ controls expression of inflammatory genes in response to pathological stimulation in various cell types involved in RA pathology. (3) Global *Nfkbiz* null mice (*Nfkbiz*^{ACAGG} and *Nfkbiz*^{Ubc}) were used to delete *Nfkbiz* from all the cell types involved in STIA. *Nfkbiz* deficient mice displayed significant low paw and ankle swelling, negligible bone erosion and osteolysis, decreased number of infiltrating immune cells and OC, higher frequency of synovial cells with anti-inflammatory and regenerative properties, decreased expression of inflammatory genes (*Lcn2*, *Mmp9*, *Mmp3*, *Rankl*, *S100A*, *Tnfa*, *Il1a* and *Il1 β*) from synovial cells, and improved grip-strength and tolerance to thermal analgesia. These findings confirm that *Nfkbiz* deletion mitigates joint inflammation and attenuates experimental mouse STIA. (4) We have identified DI and 8HQ as candidate I κ B ζ inhibitors. *In vitro* analysis using macrophages showed that DI and HQ specifically inhibited inflammatory signals downstream to I κ B ζ activity without affecting physiological NF- κ B activity. Finally using STIA model we showed that systemic administration of DI and HQ attenuated STIA progression in mice. In summary, we have identified I κ B ζ as a potent inflammatory mediator of NF- κ B signaling which can be selectively targeted to decrease the inflammation burden and joint pathology in RA.

DISCUSSION:

Major strides have been made using NSAIDs and biologics targeting inflammatory cytokines to treat RA. However, despite their disease modifying effect, no interventions have had remarkable and lasting effect, and some approaches displayed severe adverse effects limiting their utility. Among the primary challenges are the multi-cellular and multi-factorial pathogenicity of various subtypes of RA, rendering targeting individual cells or factors ineffective. Although the inflammatory response is orchestrated by the transcription factor NF- κ B, targeting NF- κ B is also futile since it hinders its physiologic function which is essential for cell survival. Our study identified I κ B ζ an NF- κ B downstream transcription factor, as an inflammation mediator and amplifier, which when disabled genetically or pharmacologically resulted with significant attenuation of murine RA. Equally important, ablation of I κ B ζ appears to have negligible effect on normal physiology. At the gene and cellular level, our findings demonstrate that genetic deletion of *Nfkbiz* attenuates expression of a group of prominent inflammatory, senescence, catabolic, and osteoclastogenic factors in synovial cells. Our finding that the cell-permeable DI inhibits I κ B ζ -induced IL-1 β expression and protects against joint inflammation and destruction, positions DI as a potential therapeutic target for RA. Using *in-silico* analysis, we also identified the anti-bacterial compound 8HQ that potently mimics DI function; inhibiting I κ B ζ and inflammatory arthritis. In summary, this work identifies I κ B ζ as an attractive RA therapeutic target sparing essential NF- κ B physiologic machinery. One major limitation of our work is that it does not elucidate any mechanism by which DI or HQ inhibit I κ B ζ function.

SIGNIFICANCE/CLINICAL RELEVANCE: (1) The most innovative concept of our research is identification of I κ B ζ , inhibition of which can decrease the inflammation burden in all synovial cells involved in RA progression. (2) We have identified DI and 8HQ as novel pharmacological inhibitors of I κ B ζ function which inhibits RA progression in mouse model of arthritis.

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