Strategy A

Use "Scientific Premise" in section label only, without trying to fit the scientific premise into a single sentence

Example 2020_G1107

Scientific premise and rigor of the prior research: The AA is a site of AD. Our prior studies demonstrate a collapse in AD in the AA prior to the development of an HH, leading to perifollicular trafficking of BB and CC cells. To identify specific gene pathways involved in AA, we conducted global gene expression studies using affected AB from both patients with AA and the C3H/HeJ (C3H herein) AA mouse model. This work revealed an increase in gene signatures indicative of immune activation, including elevated levels of DD and EE pathways (10), both of which signal via FF-dependent pathways. Subsequently, our preclinical studies in the C3H AA mouse model demonstrated that treatment with GG, a small-molecule pan-FF inhibitor, or HH, a JJ inhibitor, prevented the development of AA and ameliorated established disease. As a proof of concept, we treated three AA patients with oral HH and observed KK regrowth in each of them (10), illustrating that studies conducted in the C3H mouse model of AA can predict the efficacy of a novel class of drugs for human AA. Numerous follow-up phase II clinical trials and case reports of FF inhibitors conducted by us (11-14) and others (15-18) have strengthened the rationale for continued development of agents in this class for treatment of AA. However, FF kinases are involved in transducing signals downstream of many cytokines and extracellular proteins and, as a result, may have unintended consequences when administered systemically for treatment purposes. As an example, a second black-box warning was added to the FF inhibitor drug class describing increased rates of potentially-fatal KKs as well as all-cause mortality among those being treated with FF inhibitors. Therefore, establishing a greater understanding of the specific cell types and pathogenic circuits that give rise to AA will help identify inhibitors of these pathways that exhibit higher levels of specificity and have improved side effect profiles, thus enabling more effective treatments for this disease.

AD at the AA is thought to be established due to the production of LL by MMs. However, the rigor of the work that supports this finding is weakened by the use of LL-deficient mice on a selective and separately bred C3H substrain ("C3H/HeJBir") with distinct phenotypic characteristics from AA-prone, C3H controls (19). Nevertheless, LL-producing MMs are known for their ability to dampen or prevent WW cell responses (20,21), and their propensity to cluster around intact AAs (22) is suggestive that LL and MMs indeed play a role in maintaining AD. In support of this, our preliminary data from our C3H AA mouse model show that blocking LL receptor signaling increases the rate at which disease develops, suggesting it plays a pivotal role in preventing the development of immune reactions to the AA.

In various AD cell types, including BB cells, CC cells, and MMs, production of LL is stimulated by NN (23-25). NN is a member of the PP family of cytokines and is a heterodimer composed of QQ and RR subunits. It binds to the NN receptor, which is also a heterodimer consisting of the SS and TT subunits (26). The NN receptor is expressed on cells of the AD system as well as epithelial and endothelial cells (27). Although initial studies indicated NN had AC and CC-polarizing effects, subsequent work has demonstrated pleiotropic VV functions for this cytokine. Preliminary data using our C3H mouse model of AA, as well as findings on the impact of NN in VV (28) and HH diseases (29), including those at barrier surfaces (30), provides evidence that NN potently ameliorates pathogenic VV responses to reduce VV pathology. Specifically, our preliminary data demonstrate that adeno-associated virus (AAV)-mediated delivery of NN in the C3H model effectively prevents the development of AA (detailed in Preliminary Data section below). Previous studies showed that NN-mediated VV regulation is dependent on upregulation of LL production in WW cells, including CC cells and BB cells (23,25,31,32). Consistent with this, our preliminary studies in mice demonstrate that NN-mediated suppression of AA is accompanied by increased numbers of LL-producing cells (see Preliminary Data). However, it is unclear if LL is required for NN-mediated AA suppression, and if NN modulates MM function, including production of LL, to prevent the development of

Commented [BJY1]: Funded in 2021.

To put this into context, the subsections of Significance in this proposal were:

- ***Importance of the problem***
- ***Scientific premise and rigor of the prior research***
- ***How the proposed project will improve scientific knowledge***

Note that although the current NIH questions focus on "Rigor of prior research," we still recommend including a subsection within Significance that includes this terminology ("***Scientific premise and rigor of prior research***") as is shown here because what's being evaluated in this subsection is actually the scientific premise.

AA. In addition, it is unknown what protective mechanisms are invoked by NN that lead to changes in WW cell biology or the microenvironment that surrounds and is associated with the AA, including the constellation of YY cells and ZZ. Therefore, our *objective* in this proposal is to determine the mechanisms by which exogenous NN prevents an HH against the AA. Based on our preliminary data, *we hypothesize* that exogenous NN maintains AA AD by promoting a WW cell phenotype in BB, CC, and MM populations to restrain AE and by directly suppressing AF signals from the AA microenvironment. We will address this using our novel mouse model system, which enables us to control NN expression in a temporal and cell-type specific manner.

Example 2017_G1107

Scientific premise: X is a member of the G protein-coupled receptor (GPCR) family and is expressed throughout the nervous system, including in the Y[4,5]. In animal models of Y, treatment with agonists of X induces Y whereas treatment with antagonists as well as genetic knockout (KO) of X reduces Y[6-12]. Although GPCRs are known for converting extracellular signals into intracellular responses, some like X are present not only on the cell surface but also on intracellular membranes, where their function is not understood. Notably, in the case of X, which is expressed in the D, E, and F, 70–90% of the protein is found on nuclear membranes[13-18]. Similarly, more than 60% of X is expressed on nuclear membranes in neurons of the A[6,7]. We recently tested whether nuclear X contributes to Y using B, an *in vivo* model of Y, and found that intrathecal injection of a X antagonist that is cell-impermeable and not transported (L)[19] had only a small effect on various Y responses, whereas another that is cell-permeable (K) blocked Y responses in a dose-dependent fashion[2]. These exciting results support the notion that nuclear X is a key mediator of B-induced Y. Caveats to this interpretation exist, however. These include studies showing that (1) at high concentrations L can block other X receptors[19]; (2) K has a very short half-life (~30 min) *in vivo*[20,21]; and (3) evaluation of the relative contributions of nuclear versus surface receptors to function depends on the pharmacological properties of the agent used and on its ability to permeate the membrane. Thus, definitive resolution of the roles that nuclear and cell surface-localized X receptors play will require tools that enable testing of location-specific X signaling and the restriction of receptor function to one location or the other.

We propose to test the *in vivo* "location dependence" of X using the CRISPR technology. We will generate a mouse strain in which a short sequence from the C is attached to the C-terminus of X to target it to the nuclear membrane[22], limiting its expression to this location. In a second strain, the N-terminus of X will be tagged with a short sequence from the N-terminus of the rhodopsin receptor[23] to target X solely to the cell surface. These unique strains are expected to allow us to ascribe – for the first time – cell surface versus nuclear functions of X *in vivo*. We expect that they will validate our recent report that nuclear X is key to the induction of Y by B, an established model system[2]. We hypothesize that blocking cell surface-restricted X will not improve Y behaviors, whereas blocking nuclear-membrane-restricted X will do so.

Commented [CMB2]: Funded in 2017.

To put this into context, the subsections of Significance in this proposal were:

- ***Importance of the problem***
- ***Scientific premise***
- ***How the proposed project will improve scientific knowledge***

This is an older proposal, based on the previous NIH format that did not focus on "Rigor of prior research." We currently recommend including a subsection within Significance that includes the terminology ("***Scientific premise and rigor of prior research***") because what's being evaluated in this subsection is actually the scientific premise.

Strategy B

Use "*scientific premise*" in a sentence or sentences that briefly summarize the premise or multiple aspects of premise

2021_G1029

Need to establish D therapies as options for treating V. In the clinical setting, X is prevented by the inhibition of Y proliferation through the release of A and B inhibitors. However, a limitation of this approach, that is especially relevant clinically, is that Z is also inhibited. Currently, the published evidence of S function in F cells is very limited. However, given that deletion of S strongly affected D *in vitro* in several cell types (3, 23-25, 29) (35), and that global S deletion is lethal (35), general inhibition of S would likely have severe unintended side effects, for example, reduced Z or impairment of H. *If S inhibition is to become a viable approach for combatting X, delivery of the S inhibitor will have to be selective for Ys.* Tools for Y-selective delivery include RNA-based aptamers, synthetic, structured oligonucleotides that recognize targets with a specificity and affinity for their epitopes that are similar to those of antibodies. Aptamers are already in use as therapeutic tools; for example, an aptamer against R is FDA-approved for the treatment of W. The cell-selectivity and being RNA-based makes RNA aptamers an ideal delivery vehicle for siRNAs. (36) Thus, aptamer-siRNA chimeras (AsiCs) have been successfully synthesized and tested as novel therapies for cancer and HIV (37-39). However, the feasibility of delivering AsiCs in V models has not been explored. We used an iterative process termed J to generate Y-specific aptamers, some of which have intrinsic anti-proliferative properties, even without an siRNA (40). The Y-selective aptamers have been successfully deployed in previous studies (41). *These findings are the premise for investigating the extent to which delivery of siS by Y blocks Y-specific aptmers and X (Aim 3).*

Commented [CMB3]: Funded in 2021.

To put this into context, the subsections of Significance in this proposal were:

- **Importance of the problem**
- **Scientific premise and rigor of prior research**
  - ***Need to establish ...***
  - ***Need to establish ...***
  - ***Need to establish ...*** (premise statement falls at end of this section)
- **Significance of the expected research contribution**

Ideally, the same kind of statement would be made for all three needs paragraphs (not just the final one).

Alternatively, an overall premise statement could be made for the entire grant, in a wrap-up statement for the "Scientific premise and rigor of prior research" subsection, with reference to all the data above, e.g., "Collectively, these results represent the premise for testing X, Y, and Z in this grant", or something a bit more subtle as in the example below from another project.

2020_G1046

Consistent with prior work (xx, xx, xx), we showed that AA patients had a 53% BB cell loss, compared with a 67% loss in the CC (Fig 1). Although DD has been the focus of much AA research (xx,), our data clearly demonstrate that BBs are also decimated by AA. BBs send rich projections to the JJ and the MM, with the latter playing a critical role in PPs such as working memory, attention, behavioral flexibility, and timing (xx, xx, xx). These PPs are powerfully modulated by EE (xx, xx), suggesting deficits in BBs may contribute to PP symptoms in AA (xx, xx, xx). Indeed, prior work by our group and others demonstrates that disrupting FF in the NN or MM powerfully affects PP (xx, xx, xx, xx, xx). However, to our knowledge, HH projections from the NN to the MM *have never been studied* in the context of AA-related PP. Combined with our data, the fact that FF may be affected early in AA (xx) leads to our *premise* that HH projections are required for PP, which has implications for AA-related PP dysfunction.

Commented [BJY4]: Funded in 2021

To put this into context, the subsections of Significance in this proposal were:

- **Importance of the problem**
- **Scientific premise**
  - ***Need to establish***
  - ***Need to establish...*** (premise statement falls at end of this section)
  - ***Data to support main method used***
- **Significance of the expected research contribution**

Note on the structure: in some cases it may be ideal to put the data supporting the main method used in the paper *before* stating the scientific premise (or this could even go in the Approach), but in this particular example, this work was central to the proposal, and could only be understood by readers if it came after the details of the premise were stated.