# Vasoactive Intestinal Peptide alters TCR proximal signaling in naïve T cells

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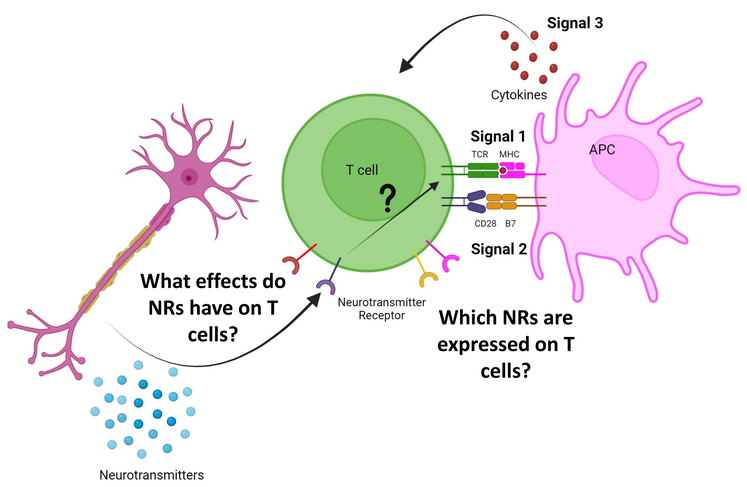
## Abstract

Communication between the nervous and immune systems is critical for regulating the immune response. Neuropeptides have been observed to exert modulatory effects on many different lymphocyte subsets. In mouse models, we found peripheral T cells expressed a set of 26 neurotransmitter receptors and exhibited distinct patterns across T cell subsets. The Vasoactive Intestinal Peptide receptor (VIPR1) had an intriguing pattern with high expression in naïve peripheral CD4 T cells and none in Tregs. Upon T cell receptor (TCR) stimulation, VIPR1 expression rapidly declines, suggesting endogenous VIP primarily affects resting naïve T cells. Functionally, treatment with VIP dampened the early activation of naïve T cells, as measured by reductions in proliferative responses and IL-2 secretion in response to antigenic stimulation. Mechanistically, VIP-exposure directly affected signaling via the TCR. Although the early events in the signaling cascade, such as the phosphorylation of ZAP70 was intact, downstream activation of ERK was reduced. Endogenous VIP has a very short lifespan and therefore localized production is likely to impact naïve T cell activation. To map the cellular sources of VIP we generated strains of VIP-lineage-tracing mice. Preliminary analysis shows strong expression in multiple neurons. These data suggest that VIP secreted by neurons has the potential to influence T cell response in a broad manner at the early stage of an immune response.

## Introduction




- Neurons are known to regulate immune function
- T cells can respond to neuronal signals via their neurotransmitter receptors
- We examined which neurotransmitter receptors (NR) are expressed on T cells and are studying their functional consequences
- Here, we focus on one of these NRs, Vasoactive Intestinal Peptide Receptor 1 (VIPR1), and how it allows VIP to regulate T cells

## Results

### T cells express a discrete set of neurotransmitter receptors

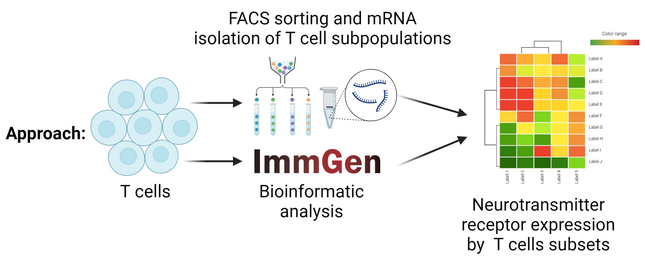



Figure 1. To determine which of the 185 transcripts encode neuroreceptors in T cells, we took two parallel approaches – one using a bioinformatics strategy to mine publicly available gene expression data for different T cell subpopulations and the other to validate these signatures using a combination of FACS sorting and mRNA isolation.

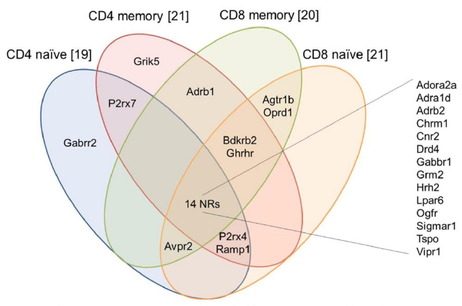



Figure 2. (A) Overview of NR expression by different T cell subsets, summarized from the analysis of ImmGen datasets. Importantly, while all T cells express a core group of 14 NRs, many others are subset-specific

### VIPR1 expression is higher in naïve T cells than activated subsets

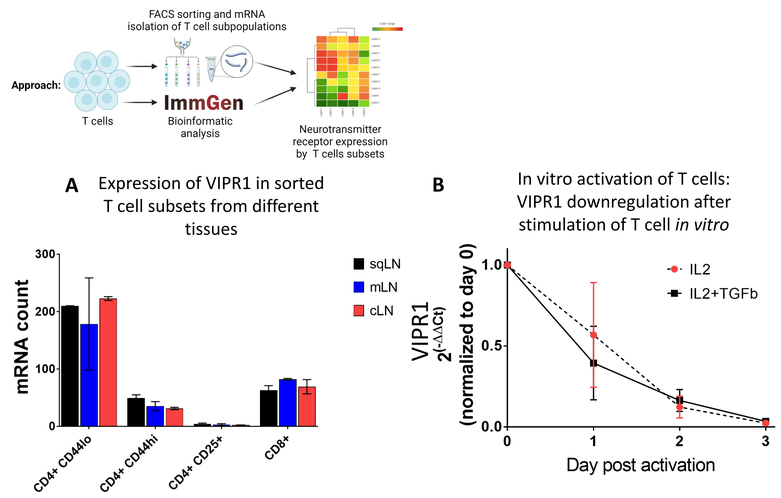



Figure 3. (A) VIPR1 mRNA levels are higher in naïve T cells and is consistent throughout different Lymph nodes. Vipr1 expression in subcutaneous (sqLN), mesenteric (mLN), and cervical (cLN) collected from four C57BL/6 mice analyzed by qRTPCR. (B) VIPR1 levels in T cells activated *ex vivo* with anti-CD3/28 over 3 days

### Does Vasoactive Intestinal Peptide regulate T cell activation?

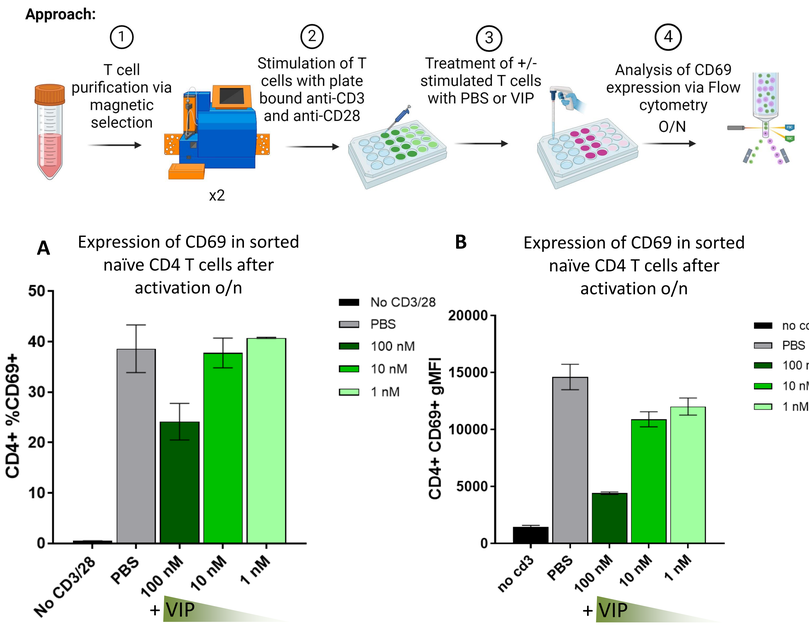



Figure 4. Purified T cells activated O/N with anti-CD3/28 in the absence (gray bars) or presence (shades of green) of different concentrations of VIP, were analyzed for CD69 expression after gating on CD4+TCR+ cells. Data is presented as percentage positive (A) or the mean expression levels in the positive population (B).

**Summary: Early T cell activation is dampened in the presence of VIP**

## Hypothesis: VIP signals interfere with early TCR proximal signaling

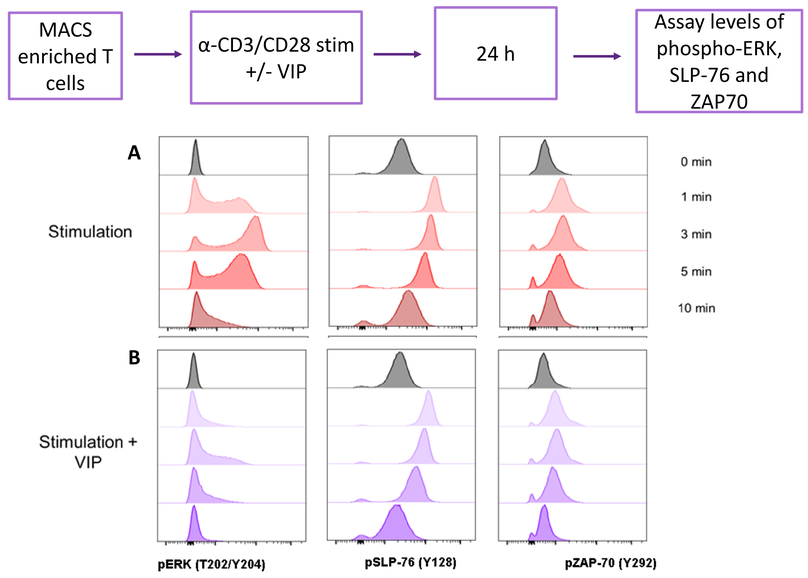
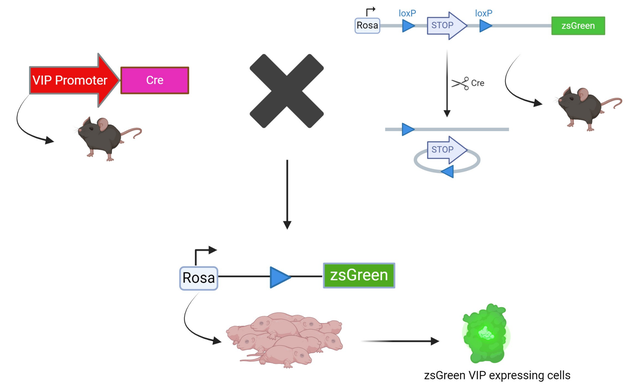


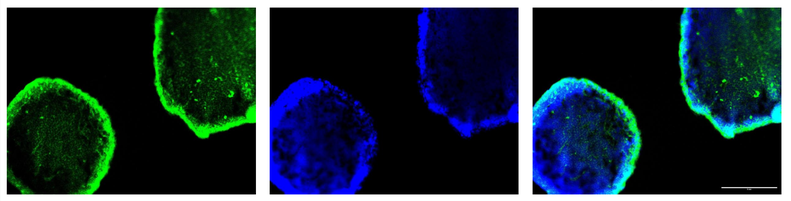

Figure 5. Time course analysis of purified T cells activated *ex vivo* with anti-CD3/28 in the (A) presence or (B) absence of VIP, analyzed for phosphorylation of ERK (left column), SLP-76 (middle column) and ZAP-70 (right column).

## Are there cellular sources of VIP in the Lymph Nodes?

Lineage tracing of VIP-expressing cells using a Cre-reporter model




Immuno-fluorescence analysis of VIP-lineage cells in the Axillary Lymph nodes




## T cells localize near VIP-lineage cells in the lymph nodes

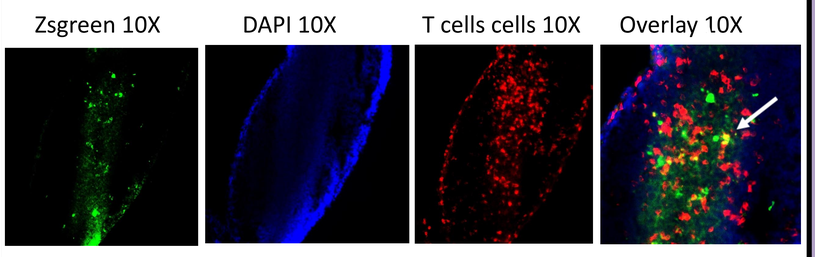



Figure 6. To visualize population of cells producing VIP within secondary lymphoid tissues, a lineage tracing model (Hatter, 2023) was used to track VIP producing cells. A. In this model, VIP-Cre crossed to Rosa-Flox-STOP-ZsGreen cells making VIP to be lineage marked subsequently. B & C. Lymph node tissues were collected from this model, embedded in a tissue mold with OCT and flash frozen using liquid nitrogen. 7um sections were made from the tissue. Slides were stained with ProLong Diamond Antifade Mountant with DAPI and TCR-beta H57-597 conjugated to APC. Images were collected with ECHO Revolve Microscope at 10X.

## Summary

- Of the 179 possible NR genes examined, fewer than 30 are expressed in sorted peripheral T cells
- VIPR1 has a unique pattern of expression, being high on naïve T cells but lower after activation
- Incubation with VIP reduced T cell activation as shown by CD69 expression (as well as IL-2 & Proliferation – not shown).
- VIP dampens early signaling downstream of the TCR
- VIP-lineage marked cells can be found in the SLOs – suggesting a source of VIP for naïve T cells in vivo; but further studies would be needed to establish their identity & engagement with T cells.

## References

Rosenberg, K. M., & Singh, N. J. (2019). Mouse T cells express a neurotransmitter-receptor signature that is quantitatively modulated in a subset- and activation-dependent manner. Brain, Behavior, and Immunity, 80. https://doi.org/10.1016/j.bbi.2019.04.002

Hatter, J. A., & Scott, M. M. (2023). Selective ablation of VIP interneurons in the rodent prefrontal cortex results in increased impulsivity. PLOS ONE, 18(6). https://doi.org/10.1371/journal.pone.0286209