

2019 AAAAI Faculty Development Program:

Class Switch Recombination Defects in B Cells with Activated PIK3CD

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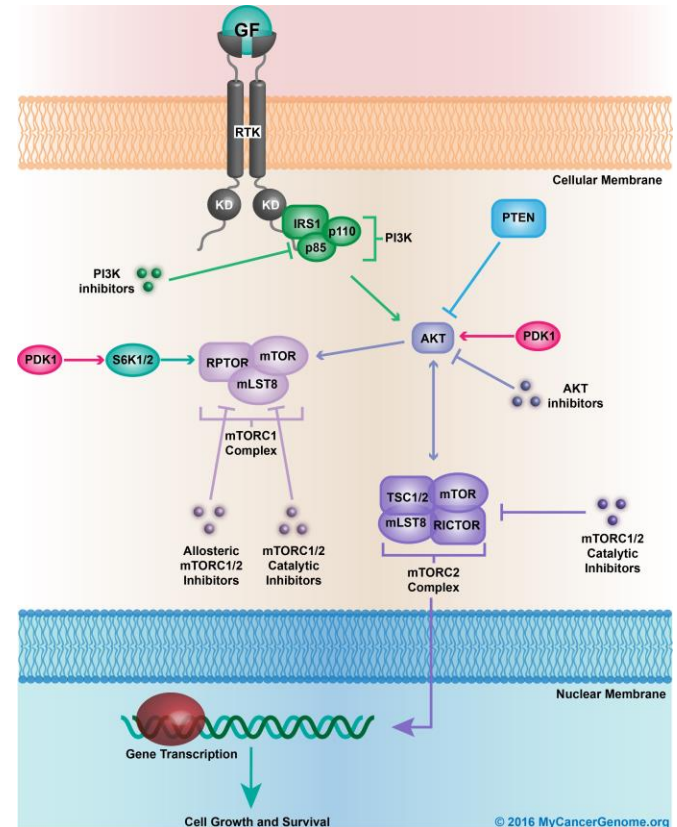
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Mentor: Tom Fleisher, MD



Activated PI3K δ Syndrome (APDS)

- Phosphoinositide-3-kinase δ (PI3K δ)
 - Catalytic subunit p110 δ
 - Src homology 2 (Src2)-containing regulatory unit p85 α
- *PIK3CD* encodes p110 δ subunit and is expressed in hematopoietic cells
- First reported in 2013 by Angulo et al (Science) and Lucas et al (Nature Immunology)
- Germline mutations in *PIK3CD* (APDS1), *PIK3R1* (APDS2), or *PTEN* (APDS-L)
- >200 patients described in literature



APDS1 Clinical Phenotype

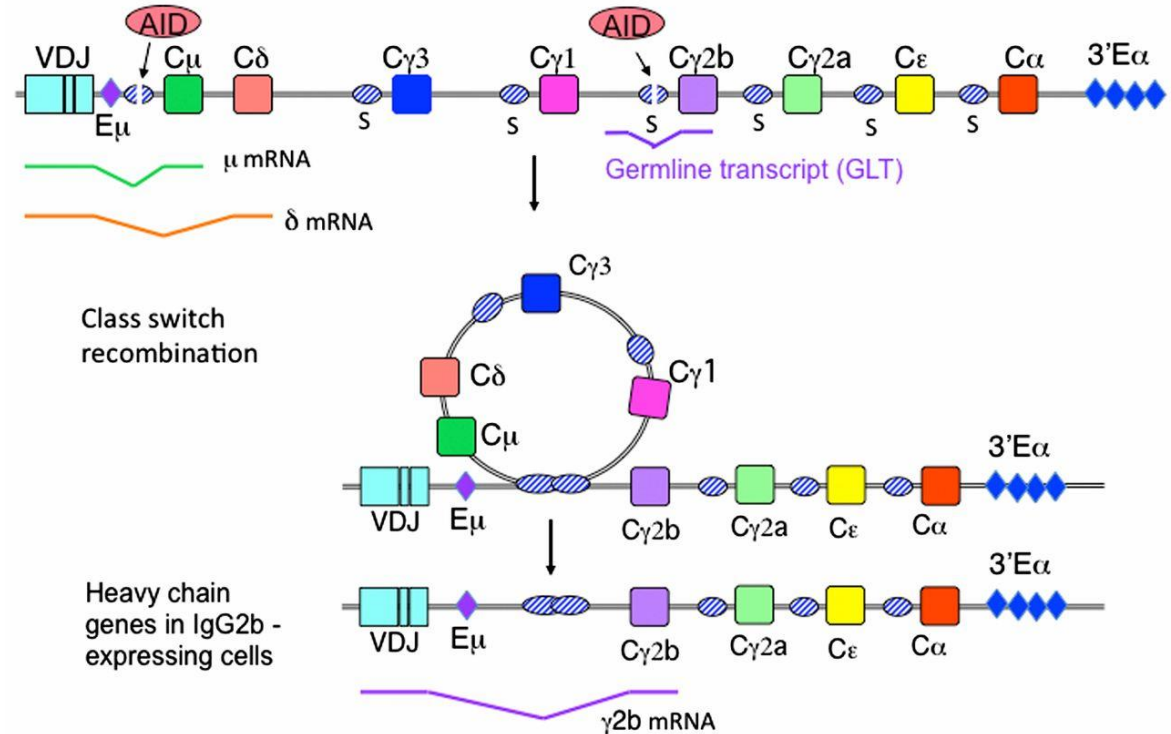
- Common variable immunodeficiency (CVID) or combined immunodeficiency (CID)
- Recurrent sinopulmonary infections (*S. pneumoniae*, *H. influenzae*) – 70%
 - Bronchiectasis – 60%
- Herpesviridae (CMV and EBV) – 41-49%
 - Viremia, EBV reactive lymphoproliferation, CMV lymphadenitis
- Conjunctivitis – 20%
- Bacterial lymphadenitis – 20%
- *S. aureus* cutaneous and dental abscesses
- Mucocutaneous candidiasis – 13%
- Lymphoproliferation – 75%
 - Lymphadenopathy, splenomegaly, hepatomegaly
 - Nodular lymphoid hyperplasia of airways, GI with enteropathy and IBD-like
- Autoimmune cytopenias
- B-cell lymphomas – 13%
- Neurodevelopmental delay – 19%

APDS1 Immunological Phenotype

B cells

- Progressive B-cell lymphopenia
- IgA low, IgM normal or increased, IgG variable
- Heterogenous response to protein antigens
- No response to polysaccharide antigens
- Increased transitional cells (CD10⁺CD24⁺CD38⁺IgM⁺)

Heavy chain genes in IgM expressing cells



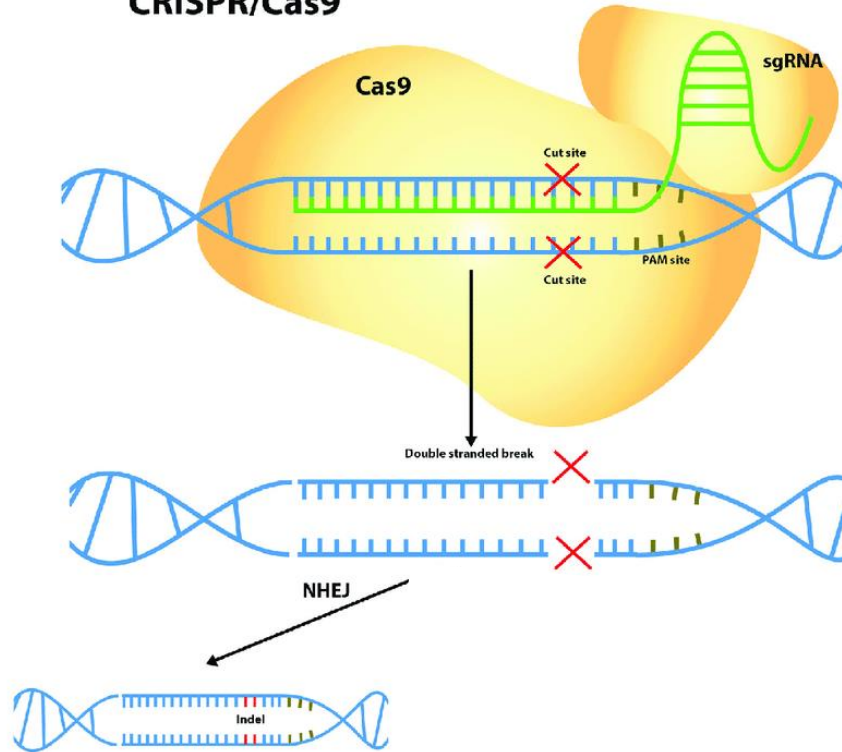
Stavener and Schrader. *J Immunol.* 2014, 193(11):5370-5378.

Specific Aims

- *PIK3CD* p.E1021K (aPIK3CD) accounts for >2/3 APDS1
 1. We will test whether B cell intrinsic expression of aPIK3CD is sufficient to inhibit class switch recombination (CSR).
 2. We will test how T cell intrinsic expression of aPIK3CD will effect T_{fh} interaction with B cells with and without intrinsic expression of aPIK3CD.

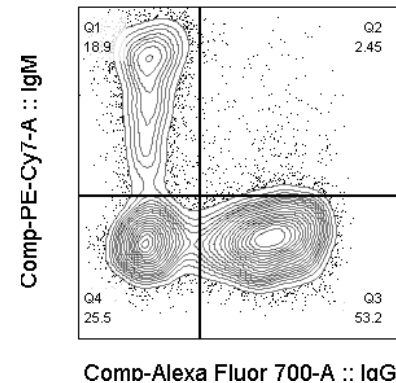
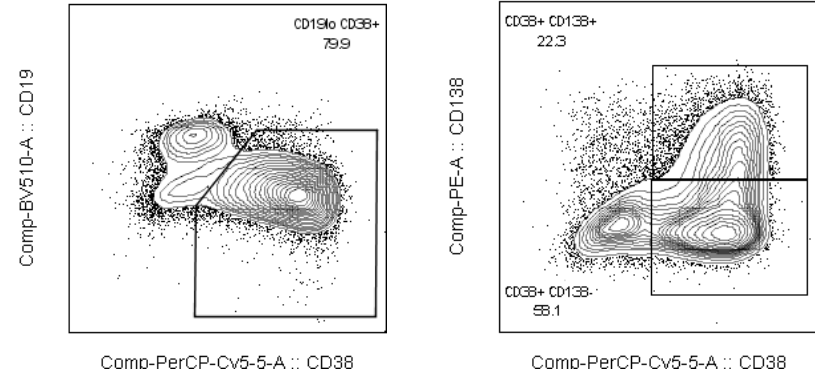
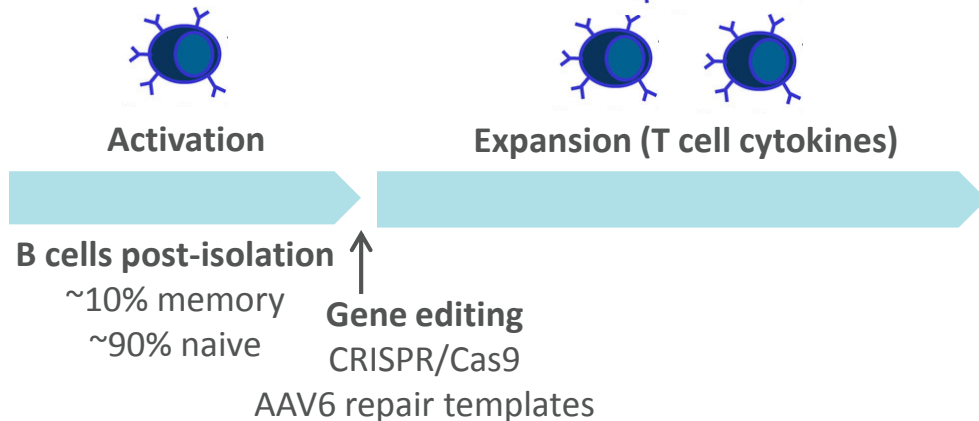
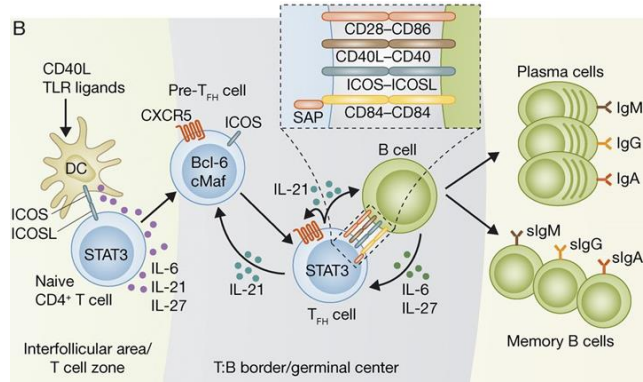
Gene Editing

CRISPR/Cas9



Leads to a loss of function/deletion

Does aPIK3CD affect CSR and IgG production?

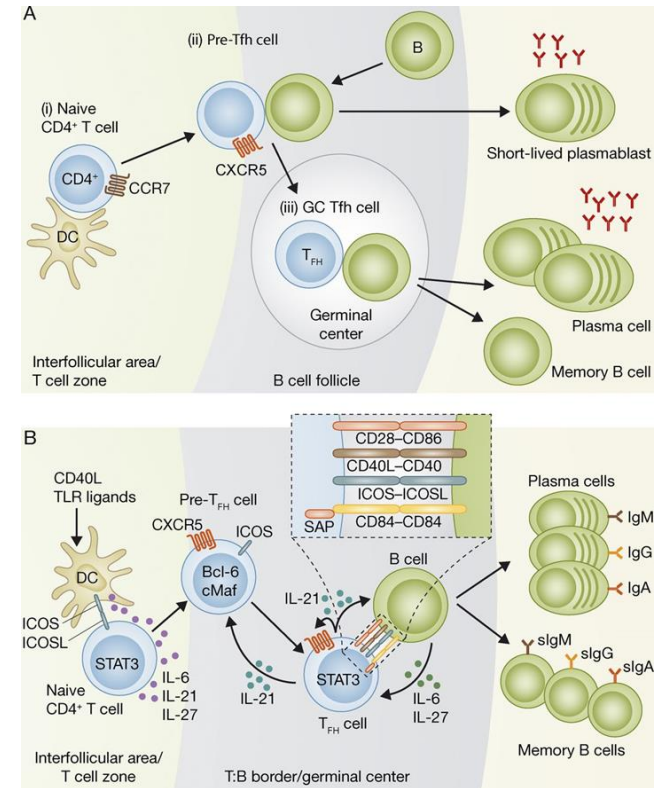


What is the mechanism of the decreased IgG level?

- Dependent on results of initial phase
 - Is isotype switching occurring and the cells are incapable of surviving?
 - Will look for upregulation of caspase 3 and other proteins of the apoptotic pathway
 - Is isotype switching unable to occur?
 - Will compare expression via RNA-seq versus single-cell sequencing of WT versus aPIK3CD cells before and after CSR occurs

Do T_{fh} cells play a role in APDS?

- Determine involvement of T_{fh} in CSR
 - Use activin A and IL-12 to induce CD4 differentiation to become T_{fh}
 - Compare cytokine signatures and surface marker expression and their influence on CSR and plasma cell development



Ma et al. *J Exp Med.* 2012, 209:1241-1253.

Hypotheses

- Cell survival in aPIK3CD B-cells is poor following CSR
- aPIK3CD T_{fh} likely contributes to inadequate CSR and to B-cell apoptosis

Future Directions

- CSR and plasma cell behavior *in vivo*
 - Murine models of aPIK3CD specifically expressed in B-cells
- Compare CSR and plasma cell survival in APDS2 with APDS1
- Investigate possible DNA repair defects in APDS



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Thank you!



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References

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