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High Speed P13K δ spams Germinal Centre GC T(fh)/GC B Cell Cross-Talk and misprogrammes B Cell Antibody coding

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In the study by Priete and research groups from the USA, and Italy¹, a mouse model with a mutation in the P13K gene that causes activation of P13K pathway, was used to demonstrate the cellular and molecular findings underlying the main biological and clinical findings of the rare human immunodeficiency disease called APDS or activated-P13k δ syndrome. In the human disease, over-activation of the P13K δ pathway causes recurrent respiratory infections with secondary bronchiectasis, lymphopenia, lymphoproliferation and poor vaccination response². The P13K δ mouse model has a gain of function (GOF) mutation of the P13K δ gene and also shows lymphopenia, lymphoproliferation and poor response to vaccination similar to the findings in the human disease. These findings suggested to the authors that the P13K δ -GOF mouse could be a useful tool to dissect out the cellular and molecular mechanisms and pathways that contribute to the similar human disease. The authors then probed the immune response/s in the P13K δ GOF mouse model using a variety of challenges and methods in order to identify where and how the P13K δ -GOF activated gene disturbs the cellular and molecular pathway of competent antibody formation and used comparisons with wild type mice (WT) and normal human donors to help define the APDS phenotype and the P13K δ -GOF mutant mouse model (MM).

What is the P13K signaling pathway?

The P13K δ mouse mutant mirrored the findings of the APDS disease and the authors went on to assess the effect of blocking the activation checkpoints in the P13K δ /AKT/mTOR pathway³, an intracellular signaling pathway directly related to cell proliferation, cancer, diabetes and immune disorders, by using immunological and confocal microscopical 'in vivo' and 'in vitro' methods. Each of the checkpoints at which changes in immune humoral immunity could occur in the P13K/ATK/mTOR pathway in the GOF P13K δ mutant mouse and the APDS patient are schematically outlined in Figure 1a.

The cell membrane receptor is triggered by growth factors that cause dimerization and phosphorylation of the P13K gene, which in the case of APDS and the GOF P13K δ mouse, is the combination of mutant catalytic subunit p110 δ and the regulator unit p85 α . This catalytic subunit and regulator come together inside the cell membrane with the support of 3 recycling phosphoinositol phosphate groups (called PIP3, 4, 5) to form activated P13K δ that phosphorylates protein kinase B (called AKT), which in turn phosphorylates and activates FOXO in the cell nucleus causing transcription of multiple factors associated with cell growth, including cancer and diabetes. The P13K pathway is normally highly regulated with many control mechanisms including the phosphatase and tensin homolog (PTEN) which blocks P13K activation through reverse PIP recycling, by molecular processes that cause AKT de-phosphorylation, and by mTOR (also known as mammalian target of rapamycin) that dephosphorylates FOXO causing it to move from the nucleus and be degraded through proteosomal/lysosomal mechanisms, thus preventing ongoing transcription and cell growth⁴. In this research the authors used these checkpoint inhibitors to explore how the molecular processes underlying P13K δ -GOF gene caused disruption of immune process leading to the phenotype of APDS disease –Figure 1a.

Germinal Centre Disorganisation in Mutant Mice

The microstructure of lymph nodes and spleen in P13K δ -GOF (MM) mice showed more active CD4+ T cells and fewer naïve CD4+ cells compared to WT mice, in a pattern similar to that found in blood from APDS patients. These findings prompted a deeper comparative investigation into the organisation of germinal centres (GCs) in P13K δ -GOF mutant mice and WT mice using high-dimensional immunofluorescence confocal microscopy. This showed marked disorganisation of the dark and light zones of germinal centres of lymph nodes in P13K δ -GOF mutant mice with T follicular helper (Tfh) cells, normally found in the light zone (LZ) also present in the dark zone (DZ), similar to findings in APDS patients- Figure 1c. Unlike normal germinal centres where many B cells with poor antibody characteristics do not survive, P13K δ -GOF mutant mice had both larger numbers of B cells and fewer Germinal Centre B (GC B) cells carrying caspase-3, the cell death marker⁵. This suggested that immune homeostasis was likely disturbed in germinal centres where cross-talk between Tfh and GC B cells was essential for the generation of normal and effective antibody responses.

Immunisation Responses of P13K δ -GOF cells

The authors first investigated the differences between WT and mutant PK13k δ mice in their antigen-specific antibody responses to immunisation using the T cell-dependent antigen NP-OVA. Both WT and mutant PK13k δ mice showed similar early increases in Tfh cells and GC B cells but as P13K δ mutant mice aged, there was no further increase, in contrast to WT mice. Importantly there was a lower ratio of antigen-specific binding NP+ GC B cells and a lower frequency of IgG1+ cells in mutant mice compared to WT mice, suggesting impairment of effective immunoglobulin class-switching in P13K δ mutant mice.

P13k δ -GOF T cell immunisation outcomes

In comparative assessments of the competence of mutant P13K δ T-cell intrinsic role in immunisation outcomes, the authors transferred naïve P13K-OT-11 cells or mutant GOF P13K δ -OT-11 cells (OT11 cells with an OVA-specific T-cell antigen receptor) into a new wild-type hosts and then immunised with NP-OVA-Figure 1b(i). This immunisation regime showed that mutant GOF P13K δ OT-11 cells produced more Tfh cells in association with IL-21, compared to naïve P13K-OT-11 counterparts. Furthermore mutant GOF P13K δ OT-11 cells were able to provide help to B cells similar to naïve P13K-OT-11 cells, suggesting that the GOF P13K δ mutation could drive a cell-intrinsic expansion of Tfh cells. The addition of ICOS-L blocking antibody (anti-ICOS ligand), did not reduce numbers of mutant P13K δ GOF Tfh cells, meaning that the P13K δ GOF mutation could bypass the normal necessity to use ICOS receptor's direct path of activation of P13K-Figure 1a.

P13K δ B cell immunisation outcomes.

In further innovative immunisation experiments the research group investigated the B-cell intrinsic roles associated with the activated P13K δ mouse phenotype by transferring native P13K-MD4 B cells and mutant GOF P13K δ -MD4 B cells (MB4 cells with transgenic hen-egg lysosome (HEL)-specific B cell receptor), together with naïve OT-11 cells into new wild-type mice, followed by immunisation with a fusion antigen combining HEL and OVA peptides-Figure 1b(ii). The combined antigen challenge produced greater numbers of GC B cells associated with the GOF-P13K δ phenotype compared to the P13K phenotype, indicating integrity of the GC B cell role for each phenotype; however the mutant P13K δ showed impaired capacity of B cells to produce NP-OVA antigen-specific responses. These changes were reversed in an 'in vitro'

preparation by direct P13K inhibition, again implicating over activation of P13K δ gene in directing the APDS-type phenotypic change.

P13K δ , auto-antibodies and the gut microbiome

Many patients with APDS develop auto-antibodies and auto-immune diseases such as glomerulonephritis and rheumatoid arthritis⁶. It was of interest that the authors found higher serum IgM and IgG anti-nuclear antibodies in mutant compared to wild-type mice. In a follow-on study they studied gut germinal nodes (Peyers patches) and the gut bacteria (microbiome) and showed increased cell numbers and disorganisation in mutant mice compared to wild-type mice, similar to findings in gut lymph nodes of APDS patients-Figure 1c. P13K δ MM mice showed increased free faecal IgA and IgA-coated bacteria⁷, compared to WT mice but less diversity of bacterial types (taxa). The significance of the predominant commensal bacterium *Akkermansia muciniphilia* in mutant P13K δ mice has yet to be explored. Another interesting finding was the increase in auto-immune antigens and markers of systemic inflammation in P13K δ mice compared to WT mice, and that an antibiotic cocktail could reduce splenic cellularity, decrease activated CD4 T and Tfh cells and reduce IgG and auto-immune antibodies to levels seen in WT mice.

In this careful study the authors suggest that the GOF P13K δ mutation interferes with proper cross-talk between Tfh and GC B cells in the germinal centres and this inadequate programming of GC B cells results in an excessive proliferation of B cells, not competent in making effective antibodies, so putting carriers of GOF P13K δ gene mutation, such as APDS patients, at risk of recurrent infections and cancer. A further interesting finding in the follow-on study is the suggestion that alterations in gut bacteria (microbiome) may drive the auto-immune diathesis evident in the GOF P13K δ mutant mice and often seen in APDS patients. However the P13K/ATK/mTOR signalling pathway is involved in the regulation of a vast number of cellular physiological processes that serve important roles in the cell cycle, growth, proliferation and immunity and is linked with the NF- κ B cytokine pathway and inflammation, where the balance between inflammation damage and repair is very finely balanced⁸. Over activation of the P13K pathway is present not only in human APDS but also in many malignancies and anti-P13K drugs such as Idelalisib have been used in the treatment of cancers, including chronic lymphatic leukaemia⁹. This drug also causes autoimmune side effects and so fits with the authors' finding that P13K δ

GOF increases auto-immune diathesis. What was unexpected was that the cocktail of antibiotics should return the auto-immune changes to baseline and perhaps the next interesting question is how and why? This detailed and multi-faceted investigation by Dr Preite and her research team¹ has provided further understanding of the molecular and immunological processes underlying the P13K δ pathway and should help to provide increased treatment options for not only APDS patients but also for cancer, diabetic and immune disorder patients.

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Legends for Figure 1

1a-P13K δ /AKT/mTOR pathway pathway

Cell membrane receptor when triggered by growth factors causes dimerization and phosphorylation of the P13K gene, a combination of mutant catalytic subunit p110 δ and regulator unit p85 α . P13K is activated by phosphorylation by phosphoinositol phosphate groups (PIP₃, 4, 5). Activated P13K δ phosphorylates protein kinase B (AKT), which phosphorylates and activates FOXO in the cell nucleus causing protein transcription and cell growth. Control mechanisms on the P13K δ pathway are-1] phosphatase and tensin homolog (PTEN) which blocks P13K activation through reverse PIP recycling, 2] molecular processes causing AKT de-phosphorylation, 3] mTOR (mammalian target of rapamycin) that dephosphorylates FOXO causing it to be degraded in lysosome.

1b-P13K δ Immunisation responses and outcomes

1b(i)-Shows P13K (WT) and P13K δ (MM) cells **with OVA-TCR antigen receptor** transferred into new wild-type mice and injected intraperitoneally (ip) with NP-OVA antigen at day 0. The mutant P13K δ phenotype produced more Tfh cells (\approx 15%) with OT-11 markers than the WT P13K (\approx 12%) at 8 days.

1b(ii)-Shows the transfer of P13K (WT) and P13K δ (MM) MD4 cells **with HEL-BCR antigen receptor** and OT-11 cells into new wild-type mice and injected with HEL-OVA fusion peptide at day 0. At 8 days the mutant P13K δ phenotype produced more MD4 GC B cells (\approx 60%) compared to WT P13K (\approx 40%).

1b-P13K δ , gut microbiome and auto-antibodies

i-Germinal Centres in P13K wild-type (WT) mice and mutant P13K δ mice (MM), showing highly disorganised germinal centres (GC) in P13K δ mice (MM). Yellow centre of GC is light zone (LZ) and Tfh and GC-B cell cross-talk area. Dark orange of GC is the dark zone area (DZ) of B cell proliferation.

ii-Gut mucosal wall showing commensal bacteria types providing antigen stimulation to Tfh cells which move to GC to cross-talk with GC B cells, produce plasma cells that secrete IgA immunoglobulin antibodies.

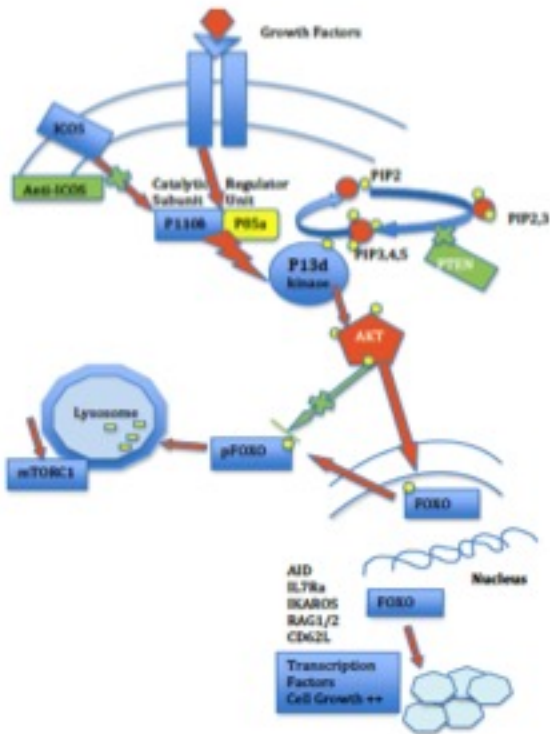
iii-Increased faecal IgA and increased IgA-coated bacteria in mutant P13K δ mice (MM) compared to P13K wild-type (WT) mice in gut

iv-Differences in bacterial species varieties and numbers in mutant P13K δ mice (MM) compared to P13K wild-type (WT) mice in gut. *Akkermansia muciniphilia* bacteria, increased in P13K δ mice (MM).

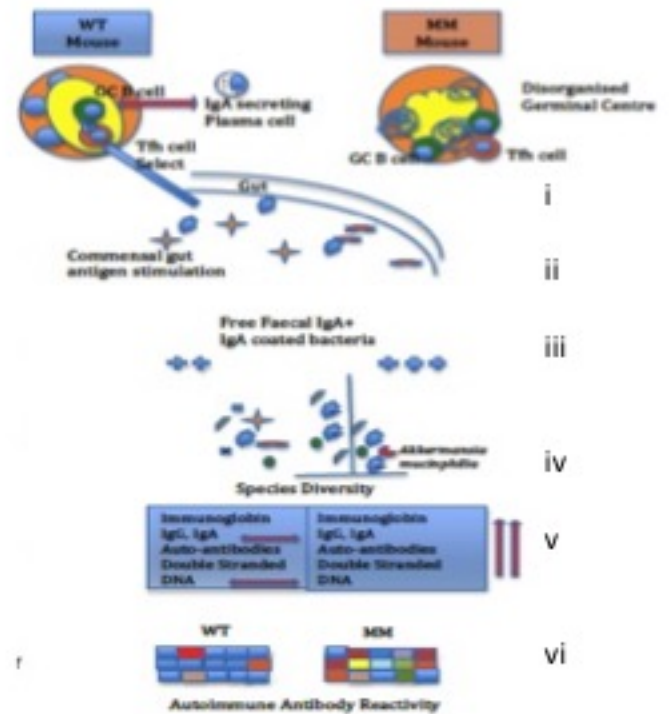
v-Serum Immunoglobins IgG and IgA, auto-antibodies and double-stranded DNA increased in P13K δ mice (MM) compared to P13K wild-type (WT) mice.

vi-Auto-immune reactivity on antigen array increased in P13K δ mice (MM) compared to P13K wild-type (WT) mice.

1a

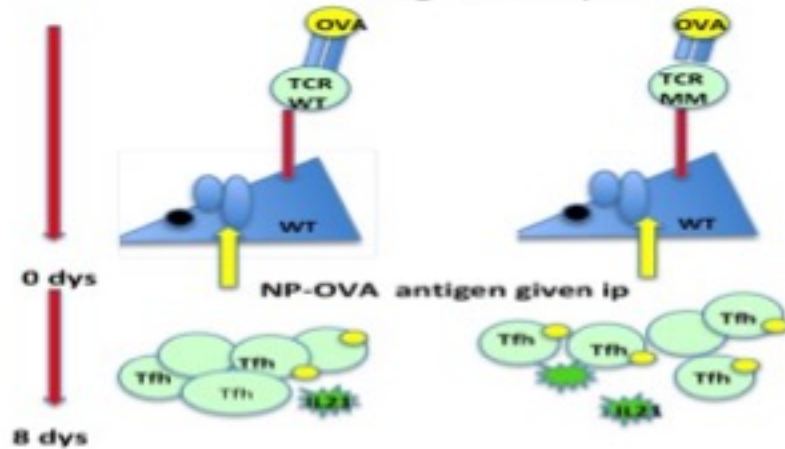


1c



1b(i)

Transfer of WT or MM OT11 cells with OVA-TCR antigen Receptor



1b(ii)

Transfer of WT or MM MD4 cells with HEL BCR antigen receptor and OT11 cells

