Preprint watch

Reservoirs of allergic memory

Allergic disease is driven by plasma cells that produce allergen-specific IgE, which binds to high-affinity receptors on mast cells and basophils and causes their degranulation after allergen cross-linking. Allergies can be lifelong, but IgE-producing plasma cells are short-lived and IgE^+ memory B cells (MBCs) are very rare. The current consensus is that allergen-specific B cell memory is maintained by non-IgE MBCs, but their phenotype is not well described. Two simultaneous preprints (not peer-reviewed) now characterize the primary reservoir of allergen-specific IgE-producing plasma cells.

Using new and pre-existing single-cell datasets in human allergy, Koenig et al. identify a type 2-polarized MBC subset they term 'MBC2s'. MBC2s are $CD23^{hi}IL\text{-}4R\alpha^{hi}CD32^{low}$ and express IgE germline transcript (eGLT). These cells are polarized by IL-4, have gene signatures of adaptive immune activation, and have a transcriptional programme that retains them in a memory state. The authors report that MBC2s are enriched in IgG1 and IgG4 expression and, based on the expression of eGLT, seem poised to switch to IgE.

In individuals with allergic rhinitis and food allergy, Koenig et al. found that the allergen-specific MBCs were mainly MBC2s, whereas this was not the case for SARS-CoV-2-specific MBCs. They also identified mouse homologues of human MBC2s in allergy models. Finally, they provide in vivo evidence that human MBC2s are primary clonal relatives of allergen-specific IgE-producing plasma cells after 1 month of allergen sublingual immunotherapy in individuals allergic to birch.

The preprint by Ota et al. describes a similar allergen-specific MBC reservoir using single-cell transcriptomics of children with peanut allergies. They show that these MBCs also express CD23, IL-4R and eGLT, and produce mainly IgG1 and IgG4. Sorted peanut-specific B cells were consistent with the type 2-polarized MBC population. In addition, the peanut allergen-specific reservoir of MBCs had highly mutated B cell receptors (BCRs). Furthermore, in a subset of highly sensitized, peanut-allergic individuals, they could identify MBCs with convergent high-affinity BCRs specific for the main peanut allergen, the conglutin Ara h 2.

Together, these preprints provide compelling evidence of a new phenotype of type 2-polarized MBCs that can retain allergen-specific IgE memory. Future work to understand how these cells develop and generate IgE-producing plasma cells could help to enable reprogramming of allergen-specific memory for therapeutic benefit.

Michelle Tran & **Cecilia Berin**
Preprint Club, Icahn School of Medicine at Mount Sinai, New York, NY, USA.
e-mail: highlights@preprintclub.com

Original articles: Koenig, J. et al. A distinct phenotype of polarized memory B cell holds IgE memory. Preprint at *bioRxiv* https://doi.org/10.1101/2023.01.25.525495 (2023); Ota, M. et al. The memory of pathogenic IgE is contained within $CD23^+IgG1^+$ memory B cells poised to switch to IgE in food allergy. Preprint at *bioRxiv* https://doi.org/10.1101/2023.01.25.525506 (2023)

Related article: Tran, M. Preprint Journal Club. *PreprintClub* https://www.preprintclub.com/preprint-reviews/2023-feb-koenig (2023)

Preprint watch

Adaptive meets innate: $CD8^+$ T cells kill MHC-I-negative tumour cells

The success of cancer immunotherapies, including immune checkpoint blockade (ICB), has generally been attributed to their ability to augment tumour-specific $CD8^+$ T cell responses. Yet, for certain tumours, low or absent surface expression of MHC class I (MHC-I), often resulting from inactivating mutations or deletions in the β_2-microglobulin (β_2M) gene, is associated with retained ICB responsiveness despite reduced antigen presentation to $CD8^+$ T cells. Other immune effectors, including natural killer (NK) cells and γδ T cells, have been proposed to compensate for the absence of conventional cytotoxic T cell activity. In this preprint (not peer reviewed), Lerner et al. offer an alternative explanation by showing that conventional $CD8^+$ T cells can kill tumour cells lacking MHC-I expression.

The authors examined responses to combined PD1 blockade plus 4-1BB agonism (αPD1/α4-1BB) in C57BL/6 mice implanted with murine glioma or melanoma cells. These tumour cells were either wild-type ($B2M^{WT}$) or deficient ($B2M^{KO}$) for β_2M expression and were engineered to express tyrosinase-related protein 2 (TRP2) or ovalbumin (OVA), which harbour the MHC-I-restricted model antigens $TRP2_{180-188}$ and $OVA_{257-264}$, respectively.

They found that whereas TRP2–$B2M^{KO}$ and OVA–$B2M^{KO}$ tumour cells (lacking MHC-I) were resistant to killing by antigen-specific $CD8^+$ T cells in vitro, they were rejected upon implantation in immune-competent mice in response to αPD1/α4-1BB treatment, in a manner dependent on $CD8^+$ T cells but not NK cells. The in vivo phenotype could be recapitulated in vitro when the cognate antigen was presented by $B2M^{WT}$ tumour cells or antigen-loaded macrophages, suggesting that prior antigen-cognate T cell receptor activation is necessary and sufficient to license killing of $MHC\text{-}I^{null}$ tumours.

Transcriptomic analysis of $OVA_{257-264}$-specific $CD8^+$ T cells stimulated with OVA-loaded macrophages revealed that T cell expression of *KLRK1* (which encodes the activating killer cell lectin-like receptor NKG2D) was markedly upregulated when co-cultured with $MHC\text{-}I^{null}$ tumour cells. Blocking NKG2D abrogated killing of $MHC\text{-}I^{null}$ tumour cells in vitro and in vivo, implying a key role for NKG2D–ligand interactions in regulating MHC-I-independent, cell-mediated anti-tumour immunity — a finding also recently described for γδ T cells.

Overall, these findings uncover a novel mechanism of conventional $CD8^+$ T cell-mediated cytotoxicity, whereby antigen-activated $CD8^+$ T cells kill $MHC\text{-}I^{null}$ cells in an NKG2D-dependent manner, which further obscures the boundaries between the adaptive and innate immune systems.

Bas W. A. Peeters & **Geraldine M. Gillespie**
Preprint Club, Nuffield Department of Medicine, University of Oxford, Oxford, UK.
e-mail: highlights@preprintclub.com

Original article: Lerner, E. et al. A novel MHC-independent mechanism of tumor cell killing by $CD8^+$ T cells. Preprint at *bioRxiv* https://doi.org/10.1101/2023.02.02.526713 (2023)

Related article: Peeters, B. W. A. Preprint Journal Club. *PreprintClub* https://www.preprintclub.com/preprint-reviews/2023-feb-lerner (2023)