Preprint watch

BRD4 acts as a pH sensor to tune inflammation

Inflammation is crucial for responding to infections but can also have detrimental effects on tissue health. As such, cells need to adapt to local environmental signals and tune the inflammatory response to avoid excessive damage.

In this preprint (not peer-reviewed), Wu et al. examine the impact of environmental pH on the expression of lipopolysaccharide (LPS)-driven inflammatory genes. Focusing on macrophages, the authors investigated the effects of environmental pH on responses to LPS stimulation. They identified several Toll-like receptor 4 (TLR4)-responsive genes that show rapid changes in expression in an acidified environment, but failed to identify roles for established pH sensors. Moreover, acidification did not affect signalling downstream of TLR4. However, bulk RNA-sequencing analysis comparing macrophages stimulated with LPS at pH 7.4 and pH 6.5 revealed significant changes in the expression of genes related to antigen presentation, chemotaxis, and cytokine signalling, implicating alterations in histone modification and chromatin accessibility. Using ATAC sequencing, the authors confirmed that chromatin accessibility was altered under different pH conditions. Therefore, pH-driven changes in chromatin accessibility may control gene expression through the modification of chromatin-modulating proteins.

Drawing inspiration from reports of pH-sensitive chromatin modulators in yeast, the authors defined criteria for pH-regulated proteins. A bioinformatics screen for similar proteins in mice identified BRD4, a known binding partner of acetylated histones, as a potential chromatin regulator with pH-dependent functions.

BRD4 was shown to form transcriptional condensates within the nuclei of cells at pH 7.4 and act as a direct, nuclear sensor of an acidified environment. Notably, the number of observed BRD4 condensates decreased when the external pH was lowered from 7.4 to 6.5. Acidification blocked the association of BRD4 with BRD9, preventing formation of the chromatin-remodelling complexes needed to activate enhancers at NF-κB- and IRF3-binding motifs.

Importantly, BRD4 transcriptional condensates were also observed in primary human macrophages, human fibroblasts, and epithelial cell lines and were reduced by acidification, which suggests a universal mode of gene regulatory adaptation to pH.

In summary, Wu et al. report a mode of inflammation-driven gene regulation that integrates an acidified environment as a regulatory signal through chromatin modulation.

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Related article: Mortha, A. Preprint Journal Club. *PreprintClub* https://www.preprintclub.com/preprint-reviews/2025-june-wu

Preprint watch

Spatial immunometabolic zonation in tuberculosis granulomas

A hallmark of *Mycobacterium tuberculosis* (*Mtb*) infection is the formation of spatially organized granulomas composed of diverse immune cell and stromal cell populations. Tuberculosis (TB) granulomas are central to bacterial control and host survival but the spatial and functional dynamics that govern their organization and immune competence are incompletely understood. Previous work suggested the presence of an immune-tolerant macrophage phenotype in human TB granulomas marked by expression of PD-L1, IDO1, TGFβ and IL-10; lymphocytes that express IFNγ were preferentially absent from this tolerogenic niche.

In a recent preprint (not peer-reviewed), McCaffrey and Delmastro et al. expand on these observations in non-human primates and human lung tissue. Using an integrative multimodal approach that combines multiplexed antibody imaging, spatial transcriptomics, single-cell RNA sequencing, chromatin profiling and an in situ hypoxia sensor, the authors delineate distinct immunometabolic zones of the necrotic core and surrounding areas of TB granulomas that are linked to cellular function and bacterial burden.

They find that the inner caseous necrotic zone, containing debris from dead macrophages and neutrophils as well as *Mtb*, is encircled by a hypoxic zone characterized by peri-necrotic macrophages with increased expression of glycolytic markers such as GLUT1. This region is also enriched for neutrophils, fibronectin-positive macrophages, and CD11c$^+$ and CD68$^+$ macrophages, but has a marked exclusion of T cells. Surrounding this inner myeloid region is an outer myeloid zone enriched for expression of IDO1, a rate-limiting enzyme of tryptophan catabolism that usually has potent immunosuppressive effects. Notably, this IDO1$^+$ region comprises CD14$^+$CD11c$^+$ macrophages and T cells, and exhibits interferon signalling, the production of reactive oxygen species and epigenetic signatures of immune activity. The outermost region (cuff) is dominated by lymphocytes. Importantly, bacterial burden is highest in the hypoxic, glycolysis-dominant zone, and TB granulomas with high *Mtb* burden show an expansion of this area. Hence, the authors suggest that hypoxia is the core driver for zonation and immune subversion in TB granulomas that enables bacterial growth.

This observational study lays a foundation for future functional and mechanistic investigations by spatial metabolomics and metabolic isotope tracing. The work marks a notable step forward in our understanding of granuloma biology, emphasizing the spatial immunometabolic organization, which could guide the development of host-directed therapies.

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Related articles: Sinha, A. et al. Preprint Journal Club. *PreprintClub* https://www.preprintclub.com/preprint-reviews/2025-june-mccaffrey (2025)