INFLAMMATION

Handing down ancient traits

Surviving an infection requires either the activation of vigorous, energy-demanding immune responses that resist and clear the microorganism, often causing collateral damage, or the induction of more energy-efficient, homeostatic mechanisms that tolerate the microorganism and repair any damage. A new study published in *Nature Immunology* describes genetic variants present in archaic hominins and modern humans that tune the host's resistance versus tolerance strategies.

One molecule known to temper immune responses and promote microbial tolerance is the ubiquitin-editing enzyme A20, which inhibits nuclear factor- κ B (NF- κ B) signalling. Zammit et al. identified three variants of A20, each affecting its OTU domain and activation. A variant comprising amino acid substitutions T108A and I207L was frequent among indigenous people of Maori and

Pacific Islander ancestry but not found in modern humans elsewhere in the world. This T108A/I207L variant originated from Denisova hominins: archaic humans who migrated from Siberia to Southeast Asia and interbred with modern humans ~50,000 years ago. The retention and high frequencies of this haplotype in modern human populations in Island Southeast Asia and Oceania suggested it was beneficial to human survival.

Clues to understanding the possible beneficial effect of the Denisovan A20 variant came from analysis of a second variant, identified in a mutagenesis screen in mice. This I325N allele, also affecting the A20 OTU domain, led to reduced levels of the NF- κ B inhibitor I κ B α in most immune cells, enhanced inflammatory responses and increased NF- κ B signalling, which suggests diminished A20-mediated inhibition. Indeed, transfer of bone marrow cells harbouring the I325N allele

into wild-type mice confirmed a cell-autonomous increase in NF- κ B activation downstream of multiple immune receptors. Consistent with this heightened immune activation, mice heterozygous or homozygous for the I325N allele had greater resistance to coxsackievirus. However, I325N homozygous mice also showed low-grade inflammation in various tissues, such as the pancreatic islets, suggesting that better immunity comes at the cost of loss of tissue homeostasis in peripheral tissues.

The third, rare, variant of A20, C243Y, lacked the beneficial effects of the T108A/I207L and I325N variants and was found in a Japanese family with a history of Behçet's disease (an inflammatory disorder affecting multiple tissues). Biochemical comparison revealed that the three A20 variants showed varying degrees of phosphorylation at Ser381 by IKK β . The C243Y allele showed the most severe loss of Ser381 phosphorylation, I325N had intermediate phosphorylation levels and the Denisovan T108A/I207L variant had a slight reduction in

“genetic variants present in archaic hominins and modern humans that tune the host's resistance versus tolerance strategies”

T CELLS

Naive T cells can be primed for tissue residency

CD8⁺ tissue-resident memory T (T_{RM}) cells in the epidermis are part of a highly sensitive sentinel system that can rapidly respond to previously encountered pathogens. Their formation requires transforming growth factor- β (TGF β), an abundant cytokine with broad functions in the immune system. It had been assumed that CD8⁺ T_{RM} cells develop locally in the skin from uncommitted CD8⁺ memory precursor T cells. However, a report in *Science* now shows that T cells are preconditioned for epithelial T_{RM} fate at a much earlier developmental stage: that is, as naive T cells, which are presented with TGF β during encounters with migratory dendritic cells (mDCs) in lymph nodes.

TGF β is secreted in a latent form in a complex that includes latency-associated peptide (LAP). It becomes activated when LAP binds to members of the α V class of integrin molecules, which facilitate the release of active TGF β . Mani et al. generated

mice that lacked α V integrin expression in DCs (α V- Δ DC mice) and observed a selective loss of epithelial CD8⁺ T_{RM} cells, indicating that the activation of TGF β by DCs is crucial for the formation of these cells.

Interestingly, when ex vivo activated ovalbumin (OVA)-specific effector CD8⁺ T cells (OT-I cells) were injected into these mice, they were able to migrate to epidermal sites of inflammation and differentiate into T_{RM} cells. This indicated that DC-activated TGF β is not required for the differentiation of effector CD8⁺ T cells into T_{RM} cells.

Instead, the authors hypothesized that DC-activated TGF β may act at an earlier stage and precondition naive CD8⁺ T cells in secondary lymphoid organs for enhanced T_{RM} cell formation. They tested this by injecting α V- Δ DC and wild-type mice with naive OT-I cells and then vaccinating them with an OVA-expressing plasmid. They found that vaccination straight after cell transfer resulted in equal numbers

of epithelial T_{RM} cells in wild-type and α V- Δ DC mice, whereas vaccination 3 weeks after cell transfer resulted in lower numbers of epithelial T_{RM} cells in the latter. This indicated that the capacity of naive CD8⁺ T cells to form epithelial T_{RM} cells decreases over time when DCs are unable to activate TGF β .

Further analysis of naive CD8⁺ T cells from wild-type and α V- Δ DC mice

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Journal Club

EFFECTS OF STROKE BEYOND THE BRAIN

In late 2000, after finishing my PhD and starting an overseas postdoctoral position, I was ready to move on from the topic of stroke. I was eagerly examining how immune cells in the liver are crucial for host antibacterial defence. However, I came across a paper that provided the first experimental proof of brain-mediated peripheral immune impairment in stroke, and I was back in the field of stroke once again.

Stroke-induced systemic immunosuppression and the subsequent development of infection were first described in the 1970s. These early observations challenged the accepted idea that fatal bacterial pneumonia following stroke arises simply due to aspiration secondary to dysphagia (difficulty swallowing). In 2003, Prass et al. provided a mechanistic basis for these observations and elegantly demonstrated that catecholamine-mediated lymphocyte dysfunction was the key factor in the impaired antibacterial immune response after stroke. Their study revealed that impaired natural killer cell and T cell function, particularly in their ability to produce IFN γ , is the crucial stroke-induced deficit in host antibacterial defence. Adoptive transfer of IFN γ -producing lymphocytes or early treatment with recombinant IFN γ was effective in halting bacteraemia and pneumonia after stroke, with both strategies clearly restoring immunity independent of aspiration.

It turns out that the injured post-stroke brain transmits signals of immunosuppression via stress pathways, namely the sympathetic arm of the autonomic nervous system. Inhibitors of the sympathetic adrenergic receptors efficiently limit lymphocyte dysfunction and bacterial infections after stroke. This discovery triggered subsequent efforts to reveal novel mechanisms and potentially harness neuroendocrine pathways to improve stroke outcomes. In addition, this study has broad implications in the field of immunology. Physiological systems have long been studied in isolation from each other. This study highlighted that immune cells are not only self-regulated but also function in close association with many other cell types, such as neurons, to maintain homeostasis and provide effective host responses to injury.

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ORIGINAL ARTICLE Prass, K. et al. Stroke-induced immunodeficiency promotes spontaneous bacterial infections and is mediated by sympathetic activation reversal by poststroke T helper cell type 1-like immunostimulation. *J. Exp. Med.* **198**, 725–736 (2003)

RELATED ARTICLES Emsley, H. C. & Hopkins, S. J. Acute ischaemic stroke and infection: recent and emerging concepts. *Lancet Neurol.* **7**, 341–353 (2008) | Chamorro, Á. et al. The immunology of acute stroke. *Nat. Rev. Neurol.* **8**, 401–410 (2012)

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Ser381 phosphorylation. The graded phosphorylation status between the different variants consequently caused a graded reduction in A20 function and control of NF- κ B (T108A/I207L > I325N > C243Y).

By introducing the human A20 mutations I207L and C243Y into C57BL/6 mice (which already have T108A) using CRISPR–Cas9 genome editing, the authors could study the physiological impact of the variants. Consistent with the biochemical data, the graded reduction in A20 phosphorylation led to correspondingly graded protective immunity to coxsackievirus. For example, mice with C243Y or I325N variants were fully protected from an otherwise lethal dose of the virus in wild-type mice, and T108A/I207L mice were partially

protected. However, the opposite was found in a model of sepsis: mice homozygous for C243Y and I325N had higher mortality and IL-6 concentrations following injection with lipopolysaccharide than wild-type mice and T108A/I207L homozygous mice.

So, tuning A20, through variation in phosphorylation levels, to increase immunity is balanced by corresponding loss of bacterial tolerance. Specific alleles encoding A20 may have been crucial for genetic selection of indigenous populations in earlier environmental conditions.

Lucy Bird

ORIGINAL ARTICLE Zammit, N. W. et al. Denisovan, modern human and mouse *TNFAIP3* alleles tune A20 phosphorylation and immunity. *Nat. Immunol.* **20**, 1299–1310 (2019)

revealed differences in chromatin accessibility, particularly in regions enriched for binding motifs of certain transcription factors. In naive CD8⁺ T cells from wild-type mice, these regions were enriched in binding motifs for the RUNX family of transcription factors, including RUNX3, which is known to be a positive regulator of epithelial T_{RM} cell differentiation. This implied that epigenetic changes driving epithelial T_{RM} cell differentiation occur before T cell activation.

In order to determine where this epigenetic preconditioning takes place, the authors analysed mice that lack lymph nodes and mice deficient in mDCs, a type of DC that, at steady state, migrates from peripheral tissues into draining lymph nodes but is absent from the spleen. In both cases, the preconditioning of naive CD8⁺ T cells for epithelial T_{RM} cell fate was impaired. This was also the case in mice where DCs did not co-express MHC class I (MHC-I) molecules and α V integrin, leading to the conclusion that the preconditioning of naive CD8⁺ T cells involves non-cognate, but MHC-I-dependent and α V integrin-dependent, physical

interactions of the T cells with mDCs in lymph nodes, during which the naive T cells are presented with active TGF β . This also exemplifies how a widely abundant cytokine can have very localized and specific functions.

In contrast to the general assumption that the naive T cell population is relatively uniform in its potential to differentiate into different effector and memory cell subsets, the study shows that naive CD8⁺ T cells are preconditioned during steady state to efficiently form epidermal T_{RM} cells upon antigen encounter. The authors point out that this may have important implications for vaccine development, where optimized preconditioning might enhance vaccine-induced protection. Conversely, in conditions such as psoriasis where epithelial T_{RM} cells play a pathogenic role, one could envisage therapeutic approaches that interfere with preconditioning for T_{RM} cell fate.

Alexandra Flemming

ORIGINAL ARTICLE Mani, V. et al. Migratory DCs activate TGF- β to precondition naive CD8⁺ T cells for tissue-resident memory fate. *Science* **366**, eaav5728 (2019)