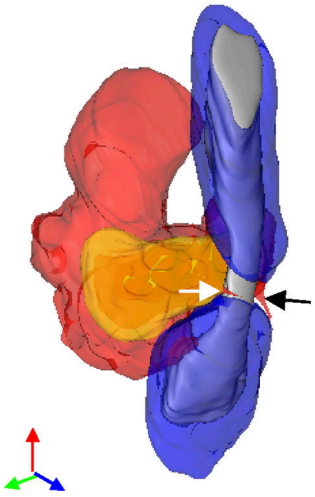
Macrophages derived from infiltrating monocytes mediate autoimmune myelin destruction

Insight from Michael Heneka

Macrophages mediate myelin destruction in multiple sclerosis (MS), but the origin of these cells (whether derived from tissue-resident microglial cells or infiltrating monocytes) has been widely debated. Now, Yamasaki and colleagues distinguish these cells in a mouse model of MS and show that monocyte-derived macrophages (MDMs) mediate myelin destruction, whereas microglia-derived macrophages (MiDMs) clear up the debris.

Previous attempts to decipher the nature and role of cells involved in autoimmune demyelination have proven challenging. Although ontogenetically distinct, it has not been possible to distinguish macrophages derived from tissue-resident or -infiltrating cells based on morphological features (by light microscopy) or surface phenotype. Previous attempts to address this problem include parabiosis and bone marrow transplantation after irradiation, both strategies with substantial technical problems and limitations.



Nodes of Ranvier represent a prime site of attack for MDMs at the onset of EAE. This 3D reconstruction of SBF-SEM images shows a monocyte-derived macrophage encircling the node of Ranvier, as shown by the two primary processes (white and black arrows).

Yamasaki et al. studied double chemokine receptor (CCR2-RFP$^+$; CX3CR1-GFP$^+$) mice in the experimental autoimmune encephalomyelitis (EAE) mouse model of MS. Inflammatory lesions were filled with both MDMs and MiDMs. Confocal immunohistochemistry, serial block-face scanning electron microscopy (SBF-SEM), and subsequent 3D reconstruction revealed that myelin destruction was initiated by MDMs, often at the nodes of Ranvier, whereas MiDMs were not detected at this site. Disruption of MDM infiltration by CCR2 deficiency completely abolished the presence of macrophages at the nodes of Ranvier. Gene expression profiling of both cell types at disease onset revealed substantial differences, which correlated well with the observations obtained by SBF-SEM. MDMs expressed genes attributable to effector functions, including those involved in phagocytosis and cell clearance. In contrast, MiMD gene expression patterns at disease onset were characteristic of a repressed metabolic state.

This paper sets a new standard for further studies in the field. For the first time, MDMs and MiDMs have been clearly differentiated and their morphological relationship to axoglial structures has been analyzed. The finding that MDMs rather than MiDMs initiate myelin destruction at disease onset should enable this cell population to be targeted more effectively in future. The next stage is to verify these findings in human tissue. Future research should also assess further time points over the entire disease course, in particular to exclude that MiDMs do not join MDMs at the node of Ranvier at later stages of disease. A precise distinction between local and infiltrating cell populations may also contribute to a better understanding of pathogenesis in other CNS disorders such as stroke and brain trauma and will hopefully lead to the development of new therapeutic strategies.

Yamasaki, R., et al. 2014. *J. Exp. Med.* http://dx.doi.org/10.1084/jem.20132477.

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Critical role for CX_3CR1^+ mononuclear phagocytes in intestinal homeostasis

Insight from Giorgio Trinchieri

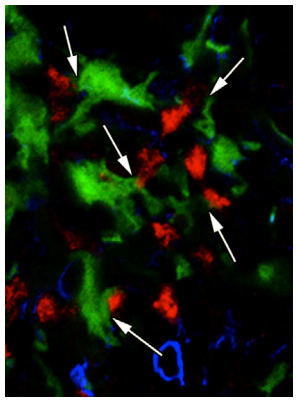
A key challenge at the intestinal barrier is to minimize responses to commensal bacteria, which can lead to inflammatory bowel disease (IBD) in genetically predisposed individuals, but retain the ability to recognize and control the growth of infectious pathogens. Group 3 innate lymphoid cells (ILC3) help maintain intestinal homeostasis by producing the cytokine IL-22, which promotes mucosal healing and maintains barrier integrity. Microbial signals trigger production of IL-23 and IL-1β, which stimulate ILC3s to produce IL-22, leading to the induction of antibacterial peptides and epithelial cell regeneration.

But the identity of the cell type producing IL-23 in response to microbial signals is unclear and has been the subject of much debate; resident mononuclear phagocytes, inflammatory monocytes, and conventional dendritic cells have all been implicated. In this issue, Longman et al. provide compelling evidence, both in mouse following *Citrobacter rodentium* infection and in patients with colitis, that CX_3CR1^+ mononuclear phagocytes (MNPs) are the most potent producers of IL-23 and IL-1β and are very efficient in inducing IL-22 production by ILC3.

The authors demonstrated the importance of microbial stimulation in IL-22 induction in patients with surgical diversion of the fecal stream—IL-22 production by ILC3 was lower in the sites unexposed to the gut microbiota compared with exposed sites. Microbial TLR4 and TLR9 agonists were particularly efficient at inducing IL-23 and IL-1β production by CX_3CR1^+ MNPs. The authors also discovered that a gene significantly associated with both ulcerative colitis and Crohn's disease in GWAS, TNF-like ligand 1A (TL1A or TNFSF15), is overexpressed in mouse CX_3CR1^+ MNPs and synergizes with IL-23 and IL-1β to induce IL-22 production in both human and mouse ILC3.

The study does not completely exclude the role of other cell types in the production of IL-23 and induction of IL-22 production but in the conditions studied (mouse infection with *C. rodentium* and human colitis), CX_3CR1^+ MNPs were crucial in mediating this mucosal protective loop. In basal conditions or in other types of inflammatory or preneoplastic conditions, and under stimulation by different microbial TLR agonists (e.g., flagellin), it is quite possible that other cell types, including conventional dendritic cells, may play an important role, as suggested by published studies. However, the study by Longman et al. greatly contributes to our understanding of the mechanisms of human IBD and reassures us that when mouse data are carefully combined with experimental human studies and GWAS, they are powerful in providing mechanistic evidence that explains human pathology.

Longman, R.S., et al. 2014. *J. Exp. Med.* http://dx.doi.org/10.1084/jem.20140678.



Confocal immunofluorescence image of mouse colon shows juxtaposition (white arrows) of CX_3CR1^+ MNPs (green) and $ROR\gamma t^+$ ILC3 (red).

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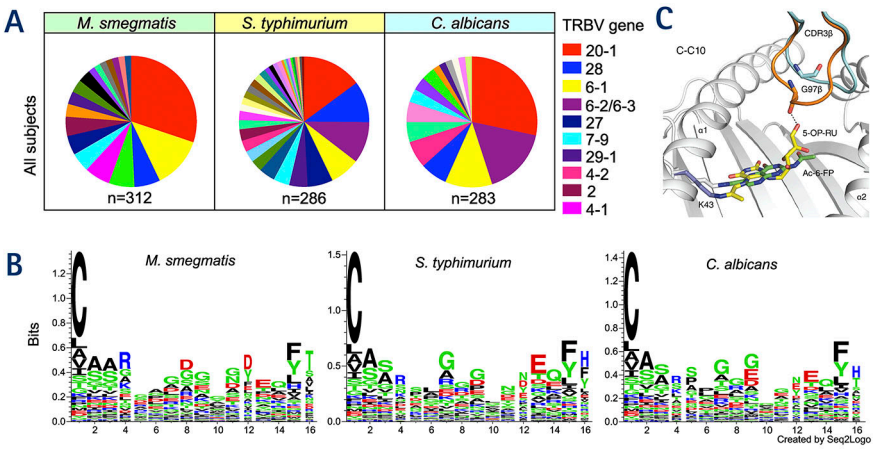
The in-betweeners: MAIT cells join the innate-like lymphocytes gang

Insight from David Margulies

Recent studies of mucosal-associated invariant T (MAIT) cells indicate that they respond to a range of pathogen-derived vitamin metabolites presented at the cell surface by the evolutionarily conserved and nonpolymorphic MHC-related class I-like molecule, MR1. Two papers in this issue describe how the structure of the T cell receptors (TCRs) of MAIT cells adapts to recognize metabolites from different intracellular infections.

Unlike classical αβ T cells, which recognize a broad range of peptide antigens in complex with polymorphic MHC class I or MHC class II molecules, nonclassical T cells have a more restricted repertoire, recognizing nonpeptide antigens presented by nonpolymorphic MHC class I-like molecules. These nonclassical T cells include NKT cells that detect glycolipid antigens presented by CD1d, germline-encoded mycolyl-lipid reactive (GEM) T cells that recognize CD1b, and the MAIT cells that are the subject of these papers.

To date, MAIT cells have been considered to be innate cells in that they recognize a limited number of conserved antigens in the context of the nonpolymorphic MR1 molecule. Gold et al. characterized the TCR repertoire of MAIT cells reactive with an epithelial cell line infected with either *M. smegmatis*, *S. typhimurium*, or *C. albicans*. Although the use of Vα genes was restricted, Vβ usage was more varied, reflecting differences in the organisms to which the particular MAIT TCR responded. This suggests that MAIT cells have adaptive capacity within a pattern-like recognition system.



(A and B) Distribution of TRBV gene usage in MAIT cells stimulated ex vivo by intracellular pathogens with summaries of the CDR3β sequences. (C) Structural plasticity of the TCR β-chain CDR3 loop of MAIT TCR C-C10 comparing TCR-MR1-antigen complexes with an antagonist (green) or agonist (yellow) ligand.

Eckle et al., in a technological tour de force, report the crystallographic structures, biophysical properties, and functional recognition of MAIT TCRs, as well as ternary structures of MAIT TCRs bound to MR1-antagonist or MR1-agonist vitamin B–derived antigens. They show that different TCRs bind the same MR1–antigen complexes slightly differently, with different affinities and with structural accommodations

including a general preservation of TCR docking mode on the MR1, conserved interaction of a key TCR α-chain CDR3-encoded residue (Tyr95α) with the antigen, and significant interactions of the hypervariable CDR3β loop with the antigen. The characterization of a strong antagonist of MAIT TCR recognition reveals plasticity of the MR1 binding pocket to accommodate the ligand and shows how the ligand itself can affect MR1 stability.

Collectively, these structures indicate that although the conserved TCR Vα-chain plays a major role in MR1 interaction, it also contributes to recognition of stimulatory antigens, whereas Vβ differences may accommodate subtle differences among distinct antigens. These studies support the view that MAIT cells co-evolved with their MR1-presenting element to recognize metabolic products from distinct intracellular microorganisms, conserving the Vα and Jα much like innate pattern recognition receptors, but allowing the Vβ-chains to vary according to the particular pathogen, following the scheme of adaptive immunity.

So, MAIT cells seem to be an "in-between" type of T cell with semi-invariant TCRs, recognizing antigens (at least those we now know) derived from metabolic products of lower organisms, and utilizing conserved, invariant presentation molecules. However, the questions that remain are provocative. How varied are the MR1-presented antigens? How do the metabolite-derived antigens load onto MR1 molecules, and how do they cross organelle barriers from the intracellular sites of infection? Are there self-antigens that mimic the vitamin metabolites for MR1 binding and MAIT cell selection? Can we find ancestral invariant TCR-like molecules that bind MR1-like predecessors that still respond to metabolite antigens?

Although many questions remain, these papers not only enhance our understanding of the evolution and function of MAIT cells and their novel TCRs, but also provide a new perspective on the many immunological solutions that nature has developed to combat pathogens.

Eckle, S.B.G., et al. 2014. *J. Exp. Med*. http://dx.doi.org/10.1084/jem.20140484.
Gold, M.C., et al. 2014. *J. Exp. Med*. http://dx.doi.org/10.1084/jem.20140507.

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