mucosal barrier protection, Godinho-Silva et al.[2] discovered that central circadian-clock-tuned signals inform the ILC3 intrinsic circadian machinery to regulate the migration of ILC3s to the gut (Fig. 1).

Notably, a study under review by Talbot et al.[10] reports that food-induced VIP–VIPR2-mediated activation of chemokine receptor CCR6$^+$ ILC3s inhibits IL-22 production. In turn, reduced IL-22 levels inhibit the production of the antimicrobial peptide RegIIIγ by intestinal epithelial cells (IECs), which affects epithelial barrier function. Interestingly, Talbot et al.[10] show that such VIP–VIPR2-mediated inhibition of IL-22 production enhances the expression of lipid transporters by IECs to expedite intestinal lipid absorption. Currently, it is not possible to reconcile the differences of these findings with those of Seillet et al. due to distinctions in experimental methods, such as ILC3 gating strategies and the circadian timepoints used. Therefore, it will be of great interest to explore the underlying mechanisms that determine the opposite roles of VIP–VIPR2 signaling in ILC3s following food consumption.

In addition, the complete circuitry connecting the local and systemic neuronal pathways with the intrinsic signaling cascades in ILC3s remains to be determined. It is still unclear how the suprachiasmatic nuclei communicates with peripheral ILC3s. VIPR2 is also highly expressed in the suprachiasmatic nuclei, where it conveys extrinsic cues to regulate central circadian oscillations[11]. It would be interesting to address whether central VIP signaling has a role in orchestrating the intrinsic clock of ILC3s. Although Seillet et al. and Godinho-Silva et al. have clearly demonstrated fluctuations in IL-22 activity in enteric ILC3s in response to feeding or diurnal changes, it remains to be elucidated whether similar regulatory mechanisms are present in ILCs in other systems. Visceral white adipose tissue is highly enriched in ILCs, which contribute to inflammation in obesity[12]. Future studies could therefore explore whether the ILC-derived cytokines in adipose tissue fluctuate with food intake, and thus coordinate adipose function with feeding behavior via immune regulation.

There is increasing interest in the impact of circadian rhythms on intestinal physiology. Disrupted circadian rhythms have been reported to cause profound gastrointestinal tract inflammation and result in several digestive pathologies and metabolic disorders; however, the underlying mechanisms are still unclear. The data of Seillet et al.[1] and Godinho-Silva et al.[2] have shed new light on the molecular mechanisms through which circadian disruptions induce inflammatory bowel diseases, metabolic syndrome and bowel cancer, and these studies undoubtedly open new possibilities for targeting this neuroimmune axis for novel therapeutic strategies. ❐

Gitalee Sarker[1], Chelsea M. Larabee[1] and Ana I. Domingos[1,2]*

[1]*Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford, UK.* [2]*The Howard Hughes Medical Institute (HHMI), New York, NY, USA.*

**e-mail: ana.domingos@dpag.ox.ac.uk*

Published online: 3 January 2020
https://doi.org/10.1038/s41590-019-0580-1

References

1. Seillet, C. et al. *Nat. Immunol.* https://doi.org/10.1038/s41590-019-0567-y (2019).
2. Godinho-Silva, C. et al. *Nature* **574**, 254–258 (2019).
3. Spits, H. et al. *Nat. Rev. Immunol.* **13**, 145–149 (2013).
4. von Burg, N., Turchinovich, G. & Finke, D. *Front. Immunol.* **6**, 416 (2015).
5. Hughes, M. E. et al. *PLoS Genet.* **8**, e1002835 (2012).
6. Mukherji, A., Kobiita, A., Ye, T. & Chambon, P. *Cell* **153**, 812–827 (2013).
7. Mackley, E. C. et al. *Nat. Commun.* **6**, 5862 (2015).
8. Nussbaum, J. C. et al. *Nature* **502**, 245–248 (2013).
9. Ibiza, S. et al. *Nature* **535**, 440–443 (2016).
10. Talbot, J. et al. Preprint at *bioRxiv* https://doi.org/10.1101/721464 (2019).
11. Maywood, E. S. et al. *Proc. Natl Acad. Sci. USA* **110**, 9547–9552 (2013).
12. O'Sullivan, T. E. et al. *Immunity* **45**, 428–441 (2016).

Competing interests

The authors declare no competing interests.

MR1-RESTRICTED T CELLS

'Bohemian Rhapsody' of MR1T cells

A T cell recognizing the MHC class I–related molecule MR1, expressed by a wide range of cancer cell types, might have great potential for adoptive cell therapy in cancer.

Lucia Mori and Gennaro De Libero

Like 'Bohemian Rhapsody', a kind of mock opera outside the norm of rock songs, which only became famous with time, MHC class I–related molecule–restricted T cells (MR1T cells) appeared outside the box and are now being appreciated for their functions and great potential in cancer immunotherapy. In the current issue of *Nature Immunology*, Crowther et al.[1] describe an MR1-restricted T cell clone with possible importance in translational anti-cancer responses.

The family of MR1-restricted T cells has been growing since the first cells were described by Porcelli and colleagues in 1993[2] and by Lantz and colleagues in 1999[3]; these cells were named mucosal-associated invariant T (MAIT) cells[4]. MAIT cells typically express a semi-invariant T cell antigen receptor (TCR)[5,6], display an oligoclonal TCRβ repertoire[7] reminiscent of antigen-driven expansion, and respond to antigens derived from microbial riboflavin metabolism[8,9]. Other MR1-restricted T cells have been described that do not express the typical MAIT TCR. One such cell type responds to bacteria producing riboflavin as well as to bacteria lacking the riboflavin pathway[10]. Rare cells react to 6-formylpterin, a photodegradation product of folic acid, without responding to typical MAIT bacterial antigens[11]. More recently, T cells expressing a conserved TCRα chain were identified by costaining with both the microbial metabolite 5-(2-oxopropylideneamino)-6-D-ribitylaminouracil (5-OP-RU)-loaded tetramers (which stain MAIT cells) and 6-formylpterin-loaded MR1 tetramers. Their function and antigen specificity remain to be studied[12].

A population of MR1-restricted T cells, which respond to tumor-associated antigens but not to microbial antigens or folate derivatives, have also been described[13]. These have been referred to as MR1T cells.

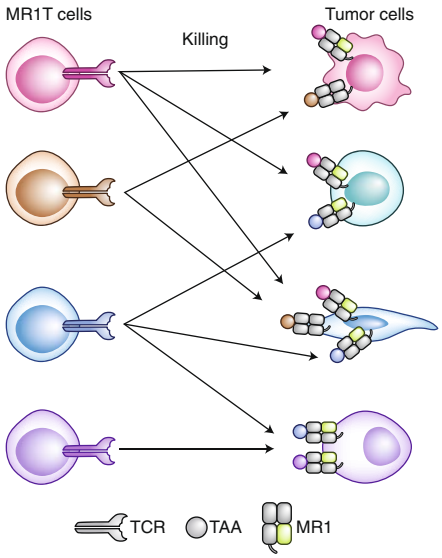

Fig. 1 | Broad tumor recognition by MR1T cells. MR1T cells are a population of MR1-restricted T cells that recognize and kill tumor cells of various tissue origins[13]. The target recognition is mediated by the T cell antigen receptor (TCR) and is directed to a complex containing MR1 and tumor-associated antigens (TAA), whose nature is still unknown. The MR1T TCR may recognize unique TAA or widely expressed TAA, thus leading to T cells that recognize and kill only one tumor type or multiple tumors sharing the same TAA. The possibility exists that the same TCR could cross-react with several TAA.

MR1T cells are heterogeneous, express variable TCRαβ or γδ (L.M. and G.D.L., unpublished observations) and respond to a variety of tumor cells of varying tissue origin that constitutively express low levels of surface MR1[13] (Fig. 1).

Crowther et al. describe a T cell clone that does not express a TCRαβ heterodimer typical of MAIT cells, does not respond to riboflavin precursors or folate derivatives, and is not stained by 5-OP-RU-loaded MR1 tetramers. This T cell kills several human cancer cell lines expressing low levels of MR1 on their cell surface. The T cell clone, named MC.7.G5, is restricted by the nonpolymorphic MR1 molecule, thus it can kill tumor cells across the major histocompatibility barrier. Remarkably, despite MR1 being ubiquitously expressed, this clone remains inert to noncancerous cells. This latter aspect is what is widely described as the gold standard for a safe cancer immunotherapy. The authors tested a variety of resting, activated, stressed and infected cells from various tissues of healthy donors and none were killed by the MC.7.G5 T cell clone. Based on these results, the obvious next step was to prove the therapeutic potential of these cells in vivo. In a xenograft model of leukemia, the administration of MC.7.G5 T cells to immunodeficient NSG mice 7 days after leukemia engraftment mediated leukemia regression and prolonged survival of the mice. After this in vivo proof of concept experiment in mice, MC.7.G5 TCR transduction into T cells from a patient with melanoma also showed efficacy in a flow-based, 36-hour killing of autologous and non-autologous melanoma cells. These findings convincingly suggest that the MC.7.G5 T cell clone is a prototype of a T cell population with great potential as a cell therapy for a wide variety of cancers.

What we've learned from these findings with the MC.7.G5 clone is that several tumor cell lines can activate a single T cell in an MR1-restricted manner, but the data are far too preliminary to conclude that this TCR is truly pan-cancer reactive. In fact, it remains to be clarified whether MC.7.G5 T cells kill some tumors in a TCR-independent manner, that is, involving natural killer cell-activating receptors, FAS ligand or through TNF release. It is unusual that killing assays last 36–72 hours or even 7 days, raising the question of which killing mechanism was involved.

What is currently known about MR1-restricted T cells that recognize endogenous cellular antigens? They express TCRαβ heterodimers not typical of MAIT cells, which unequivocally mediate the recognition of tumor cells from various tissue origins expressing low surface levels of MR1. MR1T cells represent a consistent part of the normal human repertoire, having a frequency between 1 in 2,500 and 1 in 5,000 in circulating T cells. Remarkably, their functional response is of a varied nature that includes killing and T_H1, T_H2 and T_H17 cytokine release and is directed toward antigens present not only in in vitro cultured tumor cell lines but also in freshly resected tumors. Some MR1T cells recognize in vitro differentiated monocyte-derived dendritic cell (DCs), promoting their maturation, while others respond to transformed goblet-like intestinal cell lines by releasing cytokines that promote mucin expression, and thus possibly contribute to epithelial cell function. Finally, upon target recognition, individual MR1T cell clones show different transcription signatures, confirming the existence of functionally different cell types. These findings are described in a published study[13] and indicate that MR1T cells are a bona fide new population of adaptive-like T cells[14].

What are the similarities of the MC.7.G5 T cell clone with previously described MR1T cells? It can be considered as belonging to the MR1T 'family' owing to its capacity to recognize various tumor cells expressing physiological levels of MR1. It does not respond to monocyte-derived DCs, and such DC-non-reactive cells were already described by Lepore and colleagues[13]. The T cell clone described by Crowther et al. differs from the previously described MR1T cells because it only recognizes wild-type MR1, whereas the previously described MR1T cell clones also recognize Lys43 MR1 mutants. Perhaps it displays a rare antigen specificity, thus confirming the heterogeneity of MR1T cells.

Apart from semantic diatribes, which often bring no scientific advancement, what are the open questions whose answers will pave the way for a safe and efficacious broad-spectrum cancer immunotherapy? The identification of the antigens expressed by tumor cells in association with MR1 molecules is key. Are there several families of antigens shared by many tumor cells and do these antigens show conserved chemical structures? Why are tumor cells and not normal cells stimulatory? Only the presence of common ligands may explain the broad tumor cross-reactivity of MR1T cells. Perhaps tumor cells and not normal cells are programmed to express MR1T-stimulatory ligands.

What is the TCR repertoire of such pan-cancer T cells? Do private or public TCRs recognize such antigens? A truly polyclonal TCR repertoire together with the presence of junctional convergence will represent an additional piece of evidence of the adaptive nature of MR1T cells.

Are the tumor-reactive MR1T cells naive or mostly effector/memory cells, like MAIT cells? Being found in healthy donors, it is unlikely that MR1T cells could have been primed by tumor cells. Perhaps they are primed by cross-reactive antigens of microbial origin, or by normal cells undergoing cellular stresses similar to those of many tumor cells. In the case of MC.7.G5 cells, such stresses seem not to involve reactive oxygen species production or cell damage or death. Thus, what is the primary physiological function of MR1T cells? They might represent a cell population with homeostatic capacities, recognizing aberrant accumulation of MR1 self ligands.

Do MR1T cells express unique or common costimulatory molecules, and are they subject to modulation by check-point inhibitory molecules? This information will shed light on the activation requirements of MR1T cells and the mechanisms tuning their response. Lastly, what is the range of MR1T cell frequencies in healthy individuals, in patients with cancer and in autoimmune and infectious diseases, and are they present in larger amounts in tissues than in circulation? These studies

are needed to disclose MR1T cell relevance in diseases and their functions at the site of immune responses.

Crowther et al., by describing the example of the T cell clone MC.7.G5, have added a drop of water into the ocean of MR1-restricted T cells. However, *gutta cavat lapidem* ('a water drop hollows the stone') and many more examples will surely be reported in future studies. We are very excited about the immunological functions of this new T cell population and the potential use of their TCRs in tumor cell therapy. It will take time for its application, and its translation will be challenging. Nonetheless, it may just be a matter of skill, perseverance and a bit of luck! ❐

Lucia Mori* and Gennaro De Libero
Experimental Immunology, Department of Biomedicine, University of Basel and University Hospital Basel, Basel, Switzerland.
**e-mail: lucia.mori@unibas.ch*

Published online: 20 January 2020
https://doi.org/10.1038/s41590-019-0588-6

References

1. Crowther, M. D. et al. *Nat. Immunol.* https://doi.org/10.1038/s41590-019-0578-8 (2019).
2. Porcelli, S., Yockey, C. E., Brenner, M. B. & Balk, S. P. *J. Exp. Med.* **178**, 1–16 (1993).
3. Tilloy, F. et al. *J. Exp. Med.* **189**, 1907–1921 (1999).
4. Treiner, E. et al. *Nature* **422**, 164–169 (2003).
5. Reantragoon, R. et al. *J. Exp. Med.* **210**, 2305–2320 (2013).
6. Gold, M. C. et al. *J. Exp. Med.* **211**, 1601–1610 (2014).
7. Lepore, M. et al. *Nat. Commun.* **5**, 3866 (2014).
8. Kjer-Nielsen, L. et al. *Nature* **491**, 717–723 (2012).
9. Corbett, A. J. et al. *Nature* **509**, 361–365 (2014).
10. Meermeier, E. W. et al. *Nat. Commun.* **7**, 12506 (2016).
11. Gherardin, N. A. et al. *Immunity* **44**, 32–45 (2016).
12. Koay, H. F. et al. *Nat. Commun.* **10**, 2243 (2019).
13. Lepore, M. et al. *eLife* **6**, e24476 (2017).
14. Lepore, M., Mori, L. & De Libero, G. *Front. Immunol.* **9**, 1365 (2018).

Competing interests

The University of Basel has filed a patent on MR1T cells.

TRANSCRIPTIONAL REGULATION

Gene regulatory networks STARR-ing B cells

A genome-wide screening of functionally active enhancers, combined with analyses of chromatin features, transcription factor binding and gene expression, reveals general principles of gene regulatory networks in activated B cells.

Alexia Martínez de Paz and Steven Zvi Josefowicz

Cell fate transitions, both during development and in response to external stimuli, depend upon the establishment of specific transcriptional programs by gene regulatory networks (GRNs). These complex networks are based on the interplay between (1) '*trans*-acting' factors: transcription factors (TFs) and chromatin regulatory factors, including coactivators and corepressors; (2) '*cis*-acting' regulatory DNA elements, including enhancers and promoters; and (3) biophysical features of the chromatin itself, namely the accessibility of DNA in chromatin and post-translational modifications (PTMs) of histones and DNA. With their varied differentiation from a common hematopoietic progenitor and a shared capacity for rapid responses to damage or pathogen sensing, cells of the mammalian immune system represent ideal subjects for studying the complex interplay between *trans*-acting factors, regulatory DNA, and chromatin characteristics of GNRs. B cells are especially interesting because they balance developmental programming and rapid response capability with germline B cell antigen receptor (BCR) immunoglobulin rearrangement and remarkable rates of cellular proliferation in the germinal center reaction, additional processes involving extensive chromatin regulation. In this issue of *Nature Immunology*, Chaudhri et al.[1] leverage multiple '-omics' approaches to untangle the complexity of GRNs in B cells upon bacterial lipopolysaccharide (LPS) stimulation, an activation method broadly used to model B cell differentiation and germinal center entry programs.

In part because of the suitability of B cells for GRN studies, the first 'endogenous' (non-viral) tissue-specific enhancer was discovered in B cells in 1983 by Susumu Tonegawa and Walter Shaffner and their colleagues[2,3]. The enhancer, at the immunoglobulin heavy chain (*Igh*) locus, is positioned preceding the Cμ coding sequence, well downstream of the promoter and V, D and J sequences, and influences transcription only following receptor rearrangement, when it is brought into proximity with the promoter. These early findings set the stage for modern studies of regulatory DNA in general, but notably also highlighted the importance of location, physical proximity and higher-order chromatin architecture in regulatory events involving DNA elements and their associated transcriptional and chromatin regulatory factors. Beyond early recognition of the importance of spatially linked control of regulatory DNA elements in immune receptor rearrangement and oncogenic translocations[4], recent awareness of the dynamics of chromatin architecture in 3D space makes these concepts broadly applicable.

One principal challenge of studying GRNs is the accurate identification of functional enhancer regions, DNA regulatory elements that can promote gene expression independent of the distance and orientation to the transcriptional start site. During the last decade, the development of whole-genome sequencing technologies and collaborative efforts such as the Encyclopedia of DNA Elements (ENCODE[5]) or the Immunological Genome Project (ImmGen[6]) consortia have substantially improved our ability to detect putative regulatory regions by genome-wide profiling of transcription factors and histone modifications through chromatin immunoprecipitation and sequencing (ChIP-seq), chromatin accessibility by formaldehyde-assisted isolation of regulatory elements and sequencing (FAIRE-seq[7]), DNase I hypersensitive site sequencing (DHS-seq[8]), or assay for transposase accessible chromatin and sequencing (ATAC-seq[9]). However, despite the utility of these datasets, a major limitation to understanding GRNs at a global genomic level has been the inability to define which regulatory elements are active — capable of causally driving transcriptional changes at associated genes — rather than simply associated with descriptive features of 'active' chromatin, including accessibility, TF binding and the 'active' histone PTMs