was evident only in the presence of the ERK inhibitor, and when the drug was removed, *BRAF*-amplified subclones showed a decrease in copy number, implying that increased pathway activity is detrimental to growth in the absence of other pressures. This is consistent with a previous report in which the cessation of BRAF-inhibitor treatment of a xenografted *BRAF*-mutant melanoma after the emergence of resistance, surprisingly, resulted in tumor regression when compared to continued treatment[8]. Xue and colleagues propose that the degree of *BRAF* amplification needed to maintain pathway activity under drug treatment defines a 'fitness threshold' (**Fig. 1**).

By combining BRAF, MEK, and ERK inhibitors, Xue and colleagues found that the triple-agent treatment of *BRAF*-mutant tumors was remarkably efficacious, yielding tumor regression in 75% of PDX models, including those with either intrinsic or acquired ERK-inhibitor resistance. Moreover, after treatment with two-drug or three-drug combinations, followed by a brief drug withdrawal to enable tumor re-expansion, only tumors that had been treated with the triple-drug combination lacked any evidence of *BRAF* gene amplification. Thus, the efficacy of t he three-drug treatment seems to reflect the imposition of an unsurpassable fitness barrier on *BRAF* amplification as a resistance mechanism, thereby leading to a more durable treatment response than two-drug treatment.

These findings suggest that sequential treatment with BRAF, MEK, and ERK inhibitors enables a probabilistically more favorable evolutionary trajectory for tumors, i.e., a 'shallow' gradient, thereby enabling the cell population to overcome the fitness barrier (**Fig. 1**). This situation contrasts with other paradigms of resistance to targeted therapies; such as the use of EGFR-kinase inhibitors in non-small-cell lung cancer, in which case sequential treatment with monotherapies can yield relatively durable effects because the predominant mechanisms that drive tumor resistance are distinct to the various inhibitors being used[9].

Xue and colleagues explored various treatment schedules using the three-drug regimen in the xenographs, and identified an intermittent '3 d on, 4 d off' dosing scheme that yielded near maximal tumor-growth inhibition while reducing toxicity relative to a continuous treatment scheme. The reduced toxicity could reflect both the reduced total drug exposure as well as the known 'paradoxical' activation of ERK by BRAF-kinase inhibitors that can adversely affect cells with wild-type *BRAF*—that is, in the normal, nontumorous tissue of individuals with *BRAF*-mutant cancers[10].

These findings have potentially important implications for the use of multiple targeted agents as cancer therapy. For one, the fitness-threshold model suggests that in cases in which several targeted agents can be defeated by the same resistance mechanism, such as *BRAF* amplification, sequential monotherapy treatment will generally be ineffective at managing drug resistance. Rather, combining drugs at time of treatment initiation should be more effective at preventing any 'escape' above the fitness threshold. These findings also highlight the potential role of alternative treatment schedules for managing toxicities associated with combination drug treatment. Notably, however, it remains unclear to what extent treatment schedules in mice will translate meaningfully to the context of human studies. Furthermore, there are likely to be logistical challenges associated with testing multiple treatment schedules in parallel in patients. These observations also raise the question as to whether the fitness-threshold model applies more generally to other treatment-combination contexts. Nevertheless, in light of the availability of agents targeting all three of these BRAF-pathway kinases, clinical testing of these observations is currently feasible, and the results could prove illuminating.

COMPETING FINANCIAL INTERESTS
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1. Pratilas, C.A., Xing, F. & Solit, D.B. *Curr. Top. Microbiol. Immunol.* **355**, 83–98 (2010).
2. Shi, H. *et al. Nat. Commun.* **3**, 724 (2012).
3. Larkin, J. *et al. N. Engl. J. Med.* **371**, 1867–1876 (2014).
4. Long, G.V. *et al. N. Engl. J. Med.* **371**, 1877–1888 (2014).
5. Xue, Y. *et al. Nat. Med.* **23**, 929–937 (2017).
6. Bock, C. & Lengauer, T. *Nat. Rev. Cancer* **12**, 494–501 (2012).
7. Brioli, A., Melchor, L., Cavo, M. & Morgan, G.J. *Br. J. Haematol.* **165**, 441–454 (2014).
8. Das Thakur, M. *et al. Nature* **494**, 251–255 (2013).
9. Skoulidis, F. & Papadimitrakopoulou, V.A. *Clin. Cancer Res.* **23**, 618–622 (2017).
10. Holderfield, M., Nagel, T.E. & Stuart, D.D. *Br. J. Cancer* **111**, 640–645 (2014).

Resolving a chronic inflammation mystery

Ben Roediger & Wolfgang Weninger

Using interleukin (IL)-9-deficient mice, Rauber and colleagues unveil a crucial role for group 2 innate lymphoid cells (ILC2s) in the resolution phase of arthritic inflammation, opening up new therapeutic avenues for chronic inflammatory disease.

Chronic unresolved inflammation underpins the pathology of many common and uncommon inflammatory diseases, including asthma, atherosclerosis and diabetes. The archetypical chronic inflammatory disease is rheumatoid arthritis (RA), one of the most common autoimmune diseases in the world, affecting 24 million people globally[1]. RA has a complex but well-studied etiology, arising from breakdowns in T cell and B cell tolerance and dysregulated innate inflammatory pathways. Inflammatory cytokines, particularly tumor necrosis factor (TNF), IL-6 and IL-17, have crucial roles and have been successfully targeted therapeutically in the clinic. The morbidity of RA, similar to that of other inflammatory diseases, varies widely between individuals. And although genetic and animal studies have shed light on the molecular and cellular mechanisms leading to RA and many other chronic inflammatory conditions, why lesions resolve in some individuals and persist in others remains largely unknown.

The resolution of disease in chronic inflammation is not dependent solely on the cessation of proinflammatory stimuli. Rather, restoration of tissue homeostasis is achieved through the induction of active immunosuppressive molecular pathways elaborated by regulatory T (T_{reg}) cells, macrophages and resident stromal cell populations. The mechanisms underpinning

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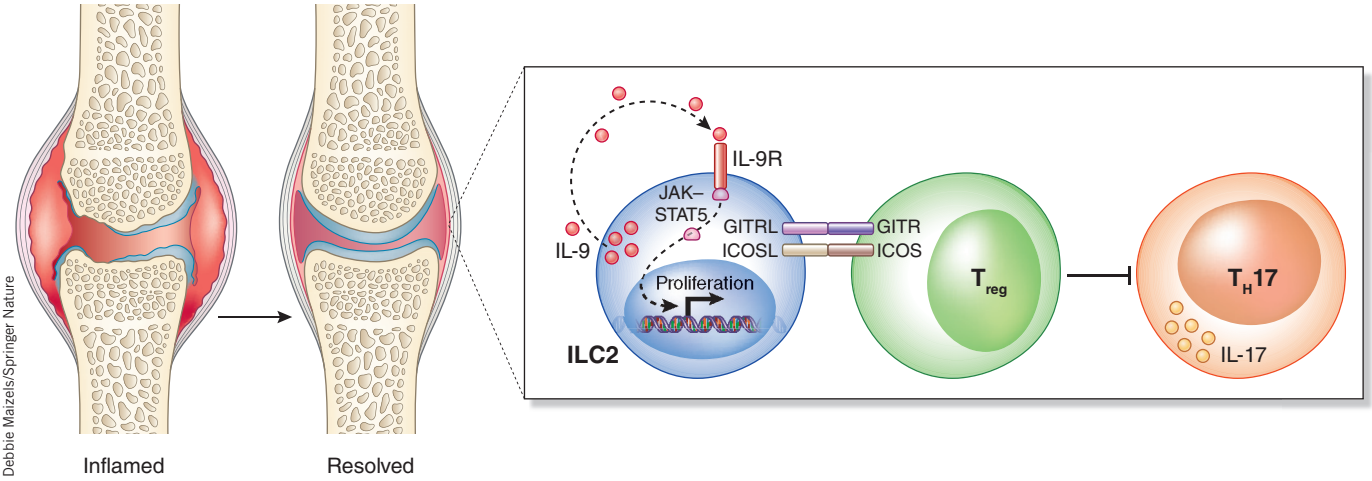

Figure 1 The role of the ILC2–T_{reg} cell axis in resolving inflammatory lesions. Rauber *et al.*[3] find that in mouse models of rheumatoid arthritis, autocrine IL-9 drives ILC2 expansion to enable optimal engagement with regulatory T cells in the inflamed synovium. The resultant T_{reg} cell activation facilitates their suppression of IL-17-producing effector T cells within the lesion, and resolves inflammation. IL-9R, IL-9 receptor.

these pathways remain poorly understood, with the exception of transforming growth factor (TGF)-β and the resolvins, the latter of which are lipid-based mediators that act through G-protein-coupled receptors on immune cells to suppress their activity. New therapeutics for chronic inflammatory disease have been developed on the basis of these[2].

Nevertheless, there is a great deal that we do not understand about the cellular and molecular mechanisms that determine whether and how inflammatory lesions will subside. In this issue of *Nature Medicine*, Rauber *et al.*[3] identify a crucial role for the cytokine IL-9 and ILC2s in the resolution phase of rheumatic inflammation, thereby providing not only a potential avenue of therapy for chronic inflammatory disease, but also shedding new light on an enigmatic cytokine and an enigmatic immune cell.

IL-9 is a cytokine best known for its role in mast cell expansion in mucosal tissues. It is expressed by helper T cells and has additionally been shown to inhibit the growth of melanoma, possibly also through mast cells[4]. IL-9 can also be expressed by ILC2s, which use it in an autocrine fashion to drive their proliferation and expansion *in situ*[5]. ILC2s are well known for their capacity to promote allergic inflammation at epithelial–environmental interfaces through the production of IL-5 and IL-13, but our understanding of their functional repertoire is diversifying; for example, recent discoveries have demonstrated their additional roles in fat metabolism and eosinophil homeostasis[6,7].

Rauber *et al.*[3] studied antigen-induced arthritis, a classic mouse model of acute inflammation, and demonstrate that, in contrast to wild-type animals, mice lacking IL-9 are incapable of resolving the acute inflammatory response, leading to prolonged joint swelling and bone loss. They found that inflammation could be resolved through the restoration of IL-9 in the mice. They then show that, in a chronic model of arthritic inflammation, delivery of IL-9 was sufficient to alleviate inflammation and reduce bone loss, thereby demonstrating the potential utility of IL-9 or IL-9 receptor agonists in rheumatic disease.

The authors found that unresolved inflammation in mice lacking IL-9 was associated with persistent IL-17 production by helper T cells, and that T_{reg} cells—the predominant and crucial suppressor cell population for curbing autoimmunity—were functionally impaired and unable to suppress T cell expansion *in vitro* as compared to those from wild-type mice. Although there were no numerical deficits in T_{reg} cells, which were present in similar numbers in both mice lacking IL-9 and in wild-type mice, they expressed decreased levels of the surface molecules GITR and ICOS, which are important for activating T_{reg} cells to optimally suppress effector T cells. These functional and phenotypic deficiencies in T_{reg} cells likely explain the dysregulated IL-17 production in IL-9-deficient mice.

The authors identified ILC2s as the major source of IL-9 within inflamed joints by labeling cells expressing *Il9*, and found that helper T cells were only a minor contributor. Consistent with the aforementioned role of IL-9 in ILC2 proliferation, both the total number and the proportion of ILC2s in cell cycle were markedly reduced within the inflamed joints of mice lacking IL-9 relative to those of wild-type mice. These experiments suggested a crucial role for ILC2s in resolving inflammation in the joint.

The importance of cross-talk between innate lymphoid and adaptive immune cells is garnering more appreciation. Rauber *et al.*[3] then investigated whether there existed a relationship between ILC2s and T_{reg} cells. Using histological staining of inflamed joints from wild-type mice, they found that ILC2s expressing IL-9 could be observed in close contact with T_{reg} cells, and that ILC2s upregulated the ligands for ICOS and GITR in response to IL-9 *in vitro*. The authors then demonstrated that, *in vitro*, ILC2s were able to restore the capacity of T_{reg} cells lacking IL-9 to suppress effector T cell proliferation in a cell-contact-, GITR- and ICOS-dependent fashion. Critically, both the adoptive transfer of T_{reg} cells stimulated with anti-ICOS/GITR and the transfer of IL-9-sufficient ILC2s were sufficient to restore resolution of inflammation in mice lacking IL-9. Collectively, these data support a model in mice in which ILC2s, in response to autocrine IL-9, expand and upregulate the ICOS and GITR ligands (ICOSL and GITRL) to promote the suppressive capacity of T_{reg} cells in the joint to suppress T_H17 cells and alleviate inflammation (**Fig. 1**).

Finally, to extend the relevance of their preclinical findings to the clinic, the authors examined the presence of IL-9$^+$ ILC2s in the blood and synovial tissues of humans with active RA and patients in remission, as well as patients longitudinally, before and after 6 months of anti-inflammatory treatment. Strikingly, in patients with RA who were in clinical remission, levels of synovial IL-9$^+$ ILC2s were elevated as compared to those in both normal control individuals and patients with active RA. Furthermore, the prevalence of ILC2s in the blood in individuals with RA inversely correlated with disease severity and, in longitudinal studies, was elevated in patients who went into remission but lowered in patients experiencing a flare-up of disease.

Thus, these data support an important role for IL-9 and ILC2s in the resolution phase of inflammation of the joint in RA in humans, which is potentially true for other inflammatory conditions.

What remains unclear is how IL-9 production is regulated in ILC2s. In contrast to IL-5 and IL-13, which are produced by ILC2s universally across all tissues, IL-9 production is not a universal feature. Skin ILC2s, for example, do not seem to produce IL-9 (ref. 8). It has been previously demonstrated that IL-2 can induce *Il9* expression in ILC2s (ref. 9), but other pathways are likely important. Given that in both mice lacking IL-9 and in mice lacking resolvins, there is an inability to resolve arthritic inflammation[10], it is tempting to speculate that resolvins themselves may regulate IL-9 production in ILC2s—particularly because the resolvin lipoxin A4 has been previously demonstrated to regulate human ILC2 function directly through the G-protein-coupled receptor FPR2 (ref. 11).

This study also has implications for our understanding of T_{reg} cell function within the periphery. The role of T_{reg} cells in suppressing naive T cell responses in lymphoid tissues is well appreciated, but their function in inflammatory sites remains poorly understood. This study hints at complex and likely highly dynamic cellular crosstalk between numerous cell subtypes within inflammatory lesions.

Rauber and colleagues have expanded our concept of how inflammatory lesions resolve, beyond mere lipid signaling and immunosuppressive cytokines, bringing cellular immunology back into the limelight as an important and underexplored element of this clinically relevant process.

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1. GBD 2015 Disease and Injury Incidence and Prevalence Collaborators. *Lancet* **388**, 1545–1602 (2016).
2. Serhan, C.N. *Nature* **510**, 92–101 (2014).
3. Rauber, S. *et al. Nat. Med.* **23**, 938–944 (2017).
4. Purwar, R. *et al. Nat. Med.* **18**, 1248–1253 (2012).
5. Turner, J.E. *et al. J. Exp. Med.* **210**, 2951–2965 (2013).
6. Brestoff, J.R. *et al. Nature* **519**, 242–246 (2015).
7. Nussbaum, J.C. *et al. Nature* **502**, 245–248 (2013).
8. Schlapbach, C. *et al. Sci. Transl. Med.* **6**, 219ra8 (2014).
9. Wilhelm, C. *et al. Nat. Immunol.* **12**, 1071–1077 (2011).
10. Perretti, M., Cooper, D., Dalli, J. & Norling, L.V. *Nat. Rev. Rheumatol.* **13**, 87–99 (2017).
11. Barnig, C. *et al. Sci. Transl. Med.* **5**, 174ra26 (2013).