

Lumpers and splitters: Birth of Th17 cells

 Brigitta Stockinger 

Th17 cells were born as a new subset of CD4 T cells to complement Th1, Th2, and T reg cells. From their identification as a distinct subset, they quickly became the paradigm for the astonishing plasticity that CD4 T cells can exhibit depending on tissue environment and circumstances.

Some time in 2004, Dan Cua visited the National Institute for Medical Research for a seminar in their Immunity and Infection series. He talked about his recent discovery (published in *JEM* in 2005; [Langrish et al., 2005](#)) around the cytokine IL-23, a heterodimer consisting of a unique p19 subunit and a common p40 subunit shared with IL-12. He had found that this cytokine seemed to promote a unique T cell population characterized by the secretion of IL-17A and F, which had highly inflammatory potential in the mouse model of multiple sclerosis, termed EAE (experimental autoimmune encephalomyelitis). These cells seemed clearly distinct from Th1 and Th2 cells, and Cua's findings prompted speculation of a new T cell subset, albeit their developmental origin remained obscure as they could not be cultured from naive precursors ([Harrington et al., 2005](#)). A very talented postdoc in my laboratory, Marc Veldhoven, was so intrigued by Cua's seminar that he immediately bought an antibody for the detection of IL-17A to set out and determine how this novel "subset" might be generated. In the immunology department, there was some grumbling about the notion of yet another T cell subset, and someone decreed that immunologists were either lumpers (focusing on shared features of different immune cell types) or splitters (promoting the existence of distinct subsets). Marc was unconcerned by being

labeled a splitter and pursued a particular line of investigation.

Marc had been puzzled by a paper published in 2003 in *Science* by the group of Ruslan Medzhitov ([Pasare and Medzhitov, 2003](#)), which showed that, in the presence of inflammatory stimuli via TLR stimulation of dendritic cells (DCs), the suppressive effect of regulatory T (T reg) cells on effector CD4 T cells was blocked, allowing normal proliferation of effector T cells. Marc, however, found that although the proliferative response of effector CD4 T cells was restored, they were still profoundly suppressed for secretion of effector cytokines such as IL-2 and IFN γ . This made little sense physiologically, as an effector cell that just proliferates but is unable to secrete cytokines seemed pointless. So, Marc set up co-cultures of naive CD4 T cells with T reg and DCs stimulated by LPS and, after 3–4 d of co-culture, tested the supernatant for cytokines such as IL-2, IFN γ , and, in addition, IL-17. The result was one of those rare eureka moments in science. As described in the *Science* paper, the proliferation of CD4 T cells co-cultured with T reg cells was heavily suppressed unless DCs were stimulated by LPS, in which case proliferation normalized. However, in none of these conditions was there any sign of IL-2 or IFN γ . Instead, cultures of CD4 T cells with T reg cells and LPS-stimulated DCs yielded a whopping proportion of T cells that secreted IL-17.



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Marc quickly established that the contribution of T reg cells to this effect consisted of the provision of TGF β , and blocking with anti-TGF β antibodies abolished the generation of IL-17 producing T cells. It was somewhat more problematic to figure out what the factor produced by LPS-stimulated DC was. Of course, the choice of IL-6 was obvious, but Marc was at first unable to block IL-6 with antibodies, and none of the other inflammatory cytokines such as IL-1 β or TNF seemed to do the job. Eventually it turned out that DCs make such a high concentration of IL-6 that the amount of blocking antibody he had used was simply insufficient.

So in the end, Marc had established that a combination of IL-6 and TGF β drives development of naive T cells into Th17 cells. This can further be enhanced by IL-1 β and TNF, but IL-23 has no impact on the initial

The Francis Crick Institute, London, UK.

 Brigitta Stockinger: Brigitta.Stockinger@crick.ac.uk.

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differentiation, but promotes further expansion of established Th17 cells. Retrospectively, this fits well with the fact that the receptor for IL-23 is not expressed on naive T cells, but gradually appears during Th17 cell differentiation (McGeachy et al., 2009).

Our paper was published on Valentine's Day 2006 (Veldhoen et al., 2006), closely followed by two further publications that confirmed these results (Bettelli et al., 2006; Mangan et al., 2006). The description of the in vitro differentiation conditions for Th17 cells paved the way for Dan Cua and Dan Littman's seminal study describing ROR γ t as the lineage-driving transcription factor for Th17 cells (Ivanov et al., 2006), which gave the final blessing to the new CD4 T cell subset.

Nevertheless, the story does not end here. However pure and well defined in vitro-generated Th17 cells may have appeared, researchers working in human immunology questioned the existence of a pure Th17 cell subset, and there also were reports of divergent cytokines displayed by Th17 cells in inflammatory mouse models such as EAE. In human samples, e.g., from the gut of Crohn's patients, Th17 cells coexpressed IL-17 and IFN γ (Annunziato et al., 2007) or showed a Th1-like phenotype in inflammatory settings in vivo, suggesting a high potential for plasticity (Murphy and Stockinger, 2010). To get around the ongoing complication of heterogeneous cultures or mixed populations found in vivo, we generated an IL-17 fate reporter that permanently marked any cell that had initiated the Th17 program by a fluorochrome, irrespective of whether or not the cell subsequently shut off IL-17 production (Hirota et al., 2011). This reporter system established first that in vitro differentiation of Th17 cells remains incomplete with maximally a third of cells reporting via eYFP fluorochrome, whereas

reporting in vivo is more concordant between intracellular staining for IL-17 and eYFP expression. Importantly, however, it became clear that in inflammatory conditions such as EAE, Th17 cells rapidly switch off IL-17 and acquire additional cytokines across the inflammatory spectrum. Thus, without a fate reporter, it would be difficult to establish that the inflammatory cells accumulating in the CNS of a mouse with EAE pathology had anything to do with Th17 cells, whereas the fate reporter indicated that the majority of cells secreting a plethora of cytokines other than IL-17 had all started their existence as Th17 cells and then switched to a much broader spectrum of cytokines as well as transcription factors. There are no comparable fate reporters for Th1 or Th2 cells, so it is difficult to say whether Th17 cell plasticity is the exception or the norm for CD4 effector T cell subsets.

Taken together, this experience probably emphasizes that not all conditions encountered in an intact organism can be accurately mimicked in vitro. Work on cell cultures can provide valuable mechanistic insights, but the differentiation and effector potential of T cells is critically shaped by the environment they find themselves in vivo. Using Th17 cells as an example again, there is an integral paradox in the fact that in vivo Th17 cells naturally reside in the intestine of unperturbed mice elicited by a commensal microbiota constituent, segmented filamentous bacteria (Ivanov et al., 2009), without causing any inflammatory unrest. On the other hand, many inflammatory conditions, from CNS inflammation to psoriasis, uveitis, and rheumatoid arthritis, are at least partially driven by highly inflammatory Th17 cells. This has made Th17 cells a prime therapeutic target, and blockade of inflammatory Th17 responses by targeting cytokines (IL-17, IL-23) has proven highly effective, for instance, in the case of psoriasis. In contrast, anti-IL-17 treatment in

Crohn's had to be terminated because of side effects (reviewed in Stockinger and Omenetti, 2017).

It appears that the indigenous Th17 population in the gut is noninflammatory and remains so even in a challenged environment, whereas inflammatory Th17 cells with a highly plastic phenotype are readily induced de novo by intestinal infection (Omenetti et al., 2019), in line with prior data that showed that infected apoptotic cells phagocytosed by neutrophils are critical for induction of Th17 cells (Torchinsky et al., 2009). By and large, the main concepts around Th17 cells—particularly inflammatory Th17—discovered in the mouse also hold true for humans. However, there is more to learn about the development and function of intestinal Th17 cells in humans.

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