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Repositioning T_H cell polarization from single cytokines to complex help

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When helper T (T_H) cell polarization was initially described three decades ago, the T_H cell universe grew dramatically. New subsets were described based on their expression of few specific cytokines. Beyond T_H1 and T_H2 cells, this led to the coining of various T_H17 and regulatory (T_{reg}) cell subsets as well as T_H22, T_H25, follicular helper (T_{FH}), T_H3, T_H5 and T_H9 cells. High-dimensional single-cell analysis revealed that a categorization of T_H cells into a single-cytokine-based nomenclature fails to capture the complexity and diversity of T_H cells. Similar to the simple nomenclature used to describe innate lymphoid cells (ILCs), we propose that T_H cell polarization should be categorized in terms of the help they provide to phagocytes (type 1), to B cells, eosinophils and mast cells (type 2) and to non-immune tissue cells, including the stroma and epithelium (type 3). Studying T_H cells based on their helper function and the cells they help, rather than phenotypic features such as individual analyzed cytokines or transcription factors, better captures T_H cell plasticity and conversion as well as the breadth of immune responses in vivo.

T_H cell polarization is primarily geared toward responder cells that synergize, amplify and cooperate toward a distinct type of response, while repressing alternative responses at a certain time point of disease or infection. This is, to a large extent, achieved by a complex and tightly regulated network of activating and inhibiting cytokines. Aside from the cytokine pattern captured, helper properties are further expressed through surface molecules, pattern of migration and the ability to enter specific tissues. Here, we focus on what was traditionally used to define T_H cells, namely the individual cytokines proposed to categorize T_H cells. The expression of cytokines by T_H cells depends on upstream signals from the encounter with antigen-presenting cells (APCs). This combination of cytokines lays, together with specific transcription factors (TFs) that control their expression, the foundation for the current classification of T_H cell subsets. With the emergence of new technologies enabling us to simultaneously measure literally dozens of cytokines along with other markers such as TFs, integrins or chemokine receptors at the single-cell level[1], it is no longer feasible to categorize T_H cells based on a dominant cytokine or even a family of cytokines[2]. Also, by attempting to categorize every single T_H cell based on individual cytokines or TFs, we may overlook the actual complex biology of differential responses and other involved cell types. Here, we focus on how the expanding T_H cell universe can be reorganized based on the actual help provided toward the actual cellular targets, rather than on the momentary expression of certain cytokines and TFs.

Historical perspective

The categorization of T cells by their biological properties has provided us essentially with $CD8^+$ cytotoxic killer and $CD4^+$ T_H cells. In 1971, an inverse relationship between humoral and cell-mediated immunity was observed by Chris Parish and Eddy Liew and others[3], laying the foundation for T_H cell bifurcation[4–6]. Eventually, Mosmann and Coffman described in 1986 that T_H cells can be polarized to produce either interferon (IFN)-γ or interleukin (IL)-4, depending on their environment and stimulatory context[7]. Later, dominant TFs were found to drive this polarization program, namely T-bet for T_H1 cells and GATA-3 for T_H2 cells[8–10]. Importantly, one subset actively suppresses the other's ability to produce its characteristic cytokines and TFs[7].

Another, now well-established T_H subset comprises T_{reg} cells. Already in the early 1970s, experiments with thymectomized mice showed development of tissue damage in various organs, indicating the presence of a suppressive T cell subset developing in the thymus[11,12]. However, due to lack of reliable markers to distinguish these cells from other T cells, T_{reg} cells underwent a history from being defined as Tr1 cells, when secreting the suppressive cytokine IL-10 in vitro, to being termed T_H3 cells, when found to secrete transforming growth factor (TGF)-β upon induction of oral tolerance[13,14]. Nowadays, thymically hard-wired T_{reg} cells are characterized by high expression levels of the high-affinity IL-2 receptor α-chain CD25 (ref. [15]) and the TF FoxP3 (ref. [16]) and are known to

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be of particular importance for maintaining immune homeostasis and preventing autoimmunity[16].

Whereas the simple T_H1–T_H2 paradigm provided an easy explanation of immune responses toward intracellular and extracellular pathogens, respectively, numerous open questions emerged in the context of chronic inflammation and autoimmunity. The path for extending the T_H family was cleared after it was noted that the IFN-γ-inducing cytokine IL-12 was not the critical factor for the induction of autoimmune pathology in preclinical models of chronic tissue inflammation, mimicking diseases such as multiple sclerosis (MS), rheumatoid arthritis and others. Instead, IL-23, which shares the p40 subunit with IL-12, was actually the main driver of the inflammatory response[17–19]. In addition to being pivotal for the development of pathogenic $CD4^+$ T cells in neuroinflammation, IL-23 also triggers IL-17 expression[20,21]. Thus, it was recognized that T_H1 cells were not the sole driving force for autoimmune pathology, at least in the context of experimental autoimmune encephalomyelitis (EAE), and the call was out for the identification of the true (pathogenic) T_H cell subset(s) in this disease.

In 2005, IL-17-producing T_H cells were described as a new entity[22,23]. This subset was readily accepted as an independent T_H subset, probably due to its clear segregation from T_H1 and T_H2 cells, the induction of which seemed to antagonize the production of IL-17 (ref. [22]). The definition of TGF-β and IL-6 as differentiation factors for these T cells in vitro[24–26] and the identification of RAR-related orphan receptor γ (RORγt) as a critical TF for IL-17 secretion solidified the standing of an independent T_H17 subset[27]. Even though the role of T_H17 cells in tissue inflammation in general has been heavily debated, IL-17-producing cells have been clearly implicated in a number of chronic inflammatory diseases such as psoriasis, rheumatoid arthritis and Crohn's disease (reviewed in ref. [28])[29,30]. Of note, neutralization of IL-17 in patients triggers fungal infection as a major frequent side effect, demonstrating the importance of IL-17 and IL-17-producing cells (such as T_H17 cells) in anti-fungal control in mucosal tissues.

Already in 2000, another new subset was proposed, when two groups showed that B cell help in follicles was provided by specific T_H cells that reside close to the B cell zone in secondary lymphoid structures[31,32]. These T_H cells express the CXC chemokine receptor 5 (CXCR5) that is also expressed on mature B cells and were termed T_{FH} cells. However, it was not until 2009 that BCL-6 was identified as the TF necessary for the generation of T_{FH} cells[33]. Even then, the acceptance of T_{FH} cells as an independent entity was strongly debated. This was partly due to the observation that the expression of canonical T_H1, T_H2 or T_H17 cytokines such as IFN-γ, IL-4 and IL-17, respectively, was necessary to induce a proper class-switching reaction in B cells[34–36]. Although the regulation of the expression of these cytokines in T_{FH} cells is not yet clear, it has been proposed that T_{FH} cells differentiate independently of other T_H subsets from naive $CD4^+$ T cells when interacting with B cells upon initial activation by dendritic cells (DCs)[37]. Interestingly, the generation and retention of T_{FH} cells depends on the same antagonistic TFs needed for germinal center B cell differentiation, namely BCL-6 and BLIMP1 (ref. [33]), which may hint toward a role of specific niches as drivers for T cell diversity and plasticity.

The addition of new cytokines in the analysis workflow of immunology laboratories led to the description of additional T_H subsets, such as T_H9 (refs. [38,39]), T_H22 (refs. [40–42]) and T_H25 (ref. [43]). To then adjust to this single-cytokine-based view on T_H cells in immunity, even more subsets were coined. These include pathogenic versus non-pathogenic T_H17, $T_H17.1$, $T_H17.2$ and T_H5 cells, among others[44–46]. During this expanding discovery phase of new T_H subsets, several voices warned against the idea that the identification of an individual cytokine expressed by T_H cells should not automatically deliver a newly coined subset and that immunologists should keep an eye on the biology of these T cells and their role in immune responses[47,48]. The same holds true for the definition of a dominating TF needed to allow the 'discovery' of a new T_H subset, especially as most of the subsequent findings were based on in vitro studies in which specific cytokine cocktails were applied to either naive or activated purified T cells.

Furthermore, the distinction of subsets requires not only 'private' master TFs but also, and maybe more importantly, stability and the ability to form memory. Stability is largely granted through epigenetic imprinting, which ensures the maintenance of the cells' identity even after an extended period of time and without persistent antigenic threat. Even though there is some evidence that T_{reg} cells can develop into T_{FH} cells[49] or intestinal intraepithelial cells[50], genetic stability has been best described in T_{reg} cells[51]. Some level of stability has been observed in T_H1 and T_H2 subsets[52,53]; however, not so much in T_H17 cells[54] or any of the other described subsets. At the present day, it is needless to say that the diversity of coined T_H subsets has become exceedingly complex and also increasingly controversial among immunologists, as the designation of T_H subsets beyond T_H1, T_H2 and T_H17 cells remains debated.

Limitations of the current T_H classification

The current T_H subset classification reaches its meaningful limits when trying to categorize T_H cells involved in the induction of pathologies. One prominent example is EAE, a preclinical model for the neuroinflammatory disease MS, in which the responsible T_H subset was not fully elucidated despite decades of research (reviewed in ref. [55]). For simplicity, we will here focus on tissue inflammation rather than immunity elicited by pathogens. As a frequently studied preclinical model for tissue inflammation, EAE was believed to be a T_H1-mediated disease model because of the abundant IFN-γ-expressing T_H cell infiltration in the central nervous system[56,57]. However, the observation that loss of IL-12 and IFN-γ signaling, respectively, led to EAE aggravation[17,58,59] suggested that T_H1 cells were not required for encephalitogenicity but may even have, at least partly, a protective role.

Shortly after, it was discovered that IL-23 signaling was pivotal for EAE induction and simultaneously was a potent inducer of numerous cytokines including IL-17 (ref. [21]). This observation coincided with the claim that T_H17 cells represent an independent T_H cell subset[22,23]. This association in turn suggested that T_H17 cells may represent the pathogenic, disease-initiating population in EAE. However, there are contradicting reports on the effect of canonical T_H17 cytokines IL-17A and IL-17F on EAE. While one study described a milder course of EAE upon the depletion of IL-17A[60], others failed to observe a tangible effect on the progression of EAE upon loss of IL-17A or IL-17F[61], making conclusions on the involvement of T_H17 cells in EAE more difficult. Only recently, it was shown that the effects of IL-17 on the disease course, aside from direct effects on the blood–brain barrier and perhaps astrocytes[62,63], stem from its ability to shape the microbiome in the gut, thereby indirectly acting on central nervous system inflammation by shaping the systemic immune compartment[64]. The same study showed that exclusive IL-17 production by neuroantigen-specific T cells was dispensable for their pathogenic potential. Moreover, although the use of IL-17 fate-mapping mice showed that the use of complete Freund's adjuvant does favor the formation of IL-17-expressing T_H cells, upon initiation of immunopathology, these cells showed a high degree of plasticity[65]. After tissue invasion, many of them produced high levels of IFN-γ, thereby raising the idea of an intermediate T_H17–T_H1 phenotype covering the 'pathogenic' T_H cell subset.

An essential key-player cytokine of this pathogenic T_H cell subset is the granulocyte–macrophage colony-stimulating factor (GM-CSF). In the context of EAE, GM-CSF is mainly produced by T_H cells[66] and has a dominant function in the development of the inflammatory cascade, as GM-CSF-deficient mice are completely resistant to EAE[66–68]. Furthermore, patients suffering from

MS have elevated frequencies of GM-CSF-expressing T_H cells[69]. It appears that GM-CSF, similar to tumor necrosis factor (TNF), can be transiently expressed by several T_H subsets upon T cell receptor (TCR)-mediated stimulation (reviewed in ref. [70]), making it difficult to allocate GM-CSF to one of the established T_H subsets. Due to the inability to clearly define T_H1 or T_H17 cells as a pathogenic entity in EAE, a new GM-CSF-expressing T_H subset was discussed[66,71,72]. This idea was supported by the observation that, while GM-CSF was clearly coexpressed with IFN-γ, coexpression with IL-17 was rarely observed[73]. However, GM-CSF expression was shown to be regulated by a complex transcriptional network downstream of the TCR, including the activity of TFs such as RORγt, NFAT, NF-κB, JNK–AP-1, PU.1 and BHLHE40 (reviewed in ref. [70]); thus no individual dominant TF for GM-CSF expression has been identified so far. Regulation by different pathways might also indicate the need of tight control of GM-CSF expression to avoid accidental activation of this potent pro-inflammatory cytokine. Regardless of whether GM-CSF-expressing cells represent a new and independent cellular subset, the present categorization of T_H cells is not able to unravel the bundle of distinct and overlapping T_H subsets but rather limits the possibilities to define specific (disease-related) processes without colliding with the established nomenclature.

The power of plasticity

There is evidence that all T_H cells, with the exception perhaps of T_{reg} cells, retain a certain degree of plasticity upon differentiation into effector cells. This is a fortuitous feature, as it enables immune responses to adapt to changing circumstances based on incoming stimulating or inhibitory cues. Experiments regarding the stability of the single subsets showed that even fully differentiated T_H1 and T_H2 cells were able to switch their transcriptional signature when challenged under the respective conditions within the first 5 days of stimulation[74,75]. Prolonged stimulation, however, induced a more stabilized T_H1 or T_H2 program[74]. This indicates that polarized T_H cells retain flexibility with regard to their transcriptional signature for several rounds of expansion, giving them enough time to adjust their response to stimulation. Especially T_H17 cells have a particularly unstable lineage commitment, thus readily converting into T_H1-like or T_{reg}-like phenotypes (reviewed in ref. [76]). The conversion of T_H17 cells into T_H1-like cells has especially been associated with the occurrence of organ-specific autoimmune diseases. Importantly, a high degree of T_H flexibility cannot only be observed in laboratory animals under strictly defined experimental conditions but also in the human immune system. One example is the development of different vaccine-specific T_H subsets that were not only diverse directly upon immunization but even able to change their 'fate' with following rounds of expansion[77].

In sum, the flexibility of T_H cells makes their classification based on cytokine patterns alone opaque and bulky. In a review article by O'Shea and Paul[78], the authors acknowledged this challenge and proposed a continuum model in which T_H cells are positioned across an orbital shape of states with the three TFs RORγt, T-bet and GATA-3 as the three extreme positions.

This 'continuum model' was certainly a step in the right direction, but, with increasing numbers of TFs and cytokines analyzed simultaneously, the anchor points of this orbital model extend into multidimensional space and can no longer help the visualization and conceptualization of T cell states. Therefore, we believe that the continual bifurcation of T_H subsets no longer contributes to the understanding of the plasticity and functionality that these cells adduce but rather unnecessarily complicates our appreciation of dynamic immune responses. Current state-of-the-art methods such as single-cell RNA sequencing, assay for transposase-accessible chromatin with sequencing (ATAC-seq) and high-dimensional cytometry also failed to capture canonical polarized T_H cells, particularly in vivo[2,79]. Instead, the data support the notion that T_H cell-driven immune responses in mammals are highly diverse and complex. Kiner et al. recently also challenged the utility of T_H archetypes in that unbiased analysis of intestinal T_H cells shows that their phenotype is molded by the microbes they encounter[79]. This apparent breadth of T_H cell states could be explained by the following: (1) T_H cells are primed toward a certain lineage but then retain a high level of plasticity, or (2) T_H cells are primed toward a diverse continuum, and there are no dedicated canonical lineages. Either way, dividing T_H cells into increasing numbers of subsets, based on the cytokine production measured, may only apply to specific experimental conditions at a certain time point but does not contribute substantially to a better understanding of T_H cell biology. Hence, we propose to take one step back and focus again on the actual helper function of T_H cells and consider their polarization based on the target cells they 'help', akin to the designation of T_{reg} and T_{FH} cells, designations based on function rather than phenotype.

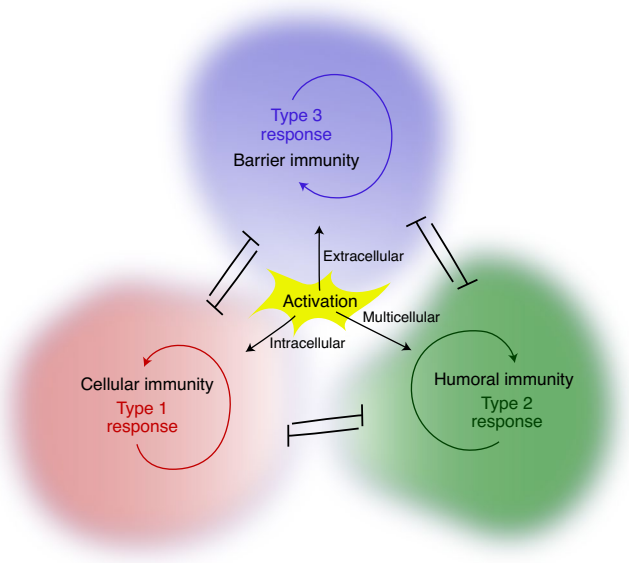

Fig. 1 | Cross-inhibition model. From the perspective of a pathogenic insult, type 1 immune responses are typically triggered by intracellular pathogens. Multicellular organisms that cannot easily be phagocytosed induce type 2 responses that support the development of humoral immunity. Type 3 responses are initiated upon extracellular activation at barrier sites such as the skin, gut and other mucosal tissue. In this model, the three types of immune responses inhibit each other and are strengthened by auto-amplification.

Reframing T_H cell subsets

In 2018, Eberl and Pradeu proposed a unifying theory that takes the bigger physiological picture into account[80]. They started by picking up on the idea that the immune system is not activated by recognizing non-self per se but by the change in 'normality': the so called 'discontinuity theory'[81] (that builds upon the danger model that was proposed by Polly Matzinger in the 1990s[82]). The new theory considers three levels of immune responses: activation of the immune system by different means (for example, intracellular, tissular, extracellular), regulation of the immune response by cross-inhibition of different types of immune response (Fig. 1) and integration of the immune response into other vital processes necessary for maintaining homeostasis at the level of the whole organism[80]. The three types of responses that they described are loosely associated with the known concept of type 1, 2 and 3 immunity[83]. Accordingly, type 1 responses are induced by intracellular discontinuities, type 2 responses are involved in tissue-repair mechanisms to prevent

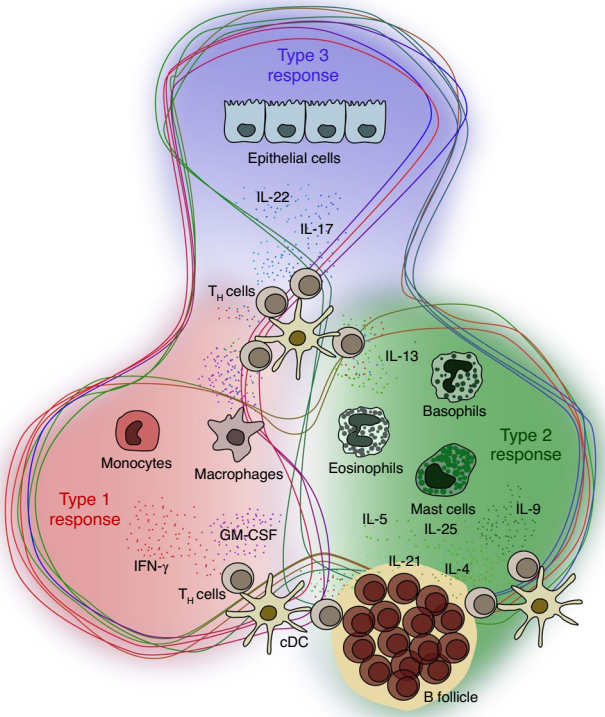

Fig. 2 | Orbital model based on T_H cell targets. T_H cells can be classified by the primary target cells engaged. Type 1 responses target mononuclear phagocytes including macrophages and monocytes. The responding cells of type 2 immunity are predominantly mast cells, eosinophils and basophils, as well as B cells (particularly in germinal centers). Type 3 cytokines engage predominantly non-immune cells, such as epithelial cells across barrier tissues. In this model, the three types of immunity are interconnected, plastic and allow cross-talk when necessary. cDC, conventional dendritic cells.

entrance of pathogens, and type 3 responses are activated by discontinuities affecting extracellular space, such as fungi and bacteria in barrier tissues[84]. Such a simple classification would mirror that of other lymphocytes with helper function, namely ILCs (for review, see ref. [85]).

We propose to extend this concept toward the initial definition of T_H cells, namely their primary function: to provide help. T_H cells are not predominantly killers or cleaners, but, as their name indicates, they support and enable other cells in the execution of their tasks. Depending on the context of activation, T_H cells interact with different other cell types and produce a variety of cytokines, probably in varying concentrations and for a certain duration. This in turn acts on a palette of cell types including macrophages, DCs, monocytes, B cells or non-immune cell subsets that cross-regulate each other to achieve the desirable or adequate type of response. Therefore, we propose to consider T_H cells by the type of responding cells that they target (Fig. 2). This classification, based on function rather than phenotype, is then further refined by the continuum model of O'Shea and Paul[78] to acknowledge the plastic nature of T_H cell states. However, while plasticity can be extensive, it is also limited by two major principles: first, cross-inhibitory interaction between type 1, type 2 and type 3 responses (as also suggested by Eberl[80]) and second, auto-amplification of established T_H cell responses. Auto-amplification loops have been described for type 1, type 2 and type 3 responses, mostly based on T cell-derived cytokines that directly add back on their sources, re-enforcing their functional phenotype. IFN-γ[86], IL-4 (ref. [87]) and IL-21 (ref. [88]) are examples of such autocrine feed-forward loop drivers for type 1, type 2 and type 3 responses, respectively.

Type 1 response. Type 1 responses are executed primarily by mononuclear myeloid cells, such as monocytes, macrophages and DCs. The most canonical type 1 cytokines produced by T_H cells are IFN-γ and GM-CSF. Effects of IFN-γ in responder cells depend on the nature of the responding cell type[89]. The IFN-γ receptor (IFNGR) is a tetramer of two ligand-binding IFNGR1 chains and two signal-transducing IFNGR2 chains. While IFNGR1 is constitutively expressed on the surface of most cell types, IFNGR2 expression is more tightly regulated and predominantly found in phagocytes. More than 2,000 IFN-γ-responsive genes have been identified, including those encoding major histocompatibility complex (MHC)-I, MHC-II, nitric oxide synthase (NOS)2, various cell adhesion molecules such as vascular cell adhesion molecule (VCAM)1 and CD44, interferon regulatory factor (IRF)1–IRF9 and different tripartite motif (TRIM) genes[90]. IFN-γ is particularly important for APCs, as it not only induces the upregulation of MHC-I and MHC-II molecules but also slows lysosomal function in macrophages to enhance antigen processing[91,92]. Interestingly, other pro-inflammatory stimuli such as type I IFN, lipopolysaccharide and TNF can initiate a signaling cascade similar to that of IFN-γ[93,94], thereby modulating the IFN-γ response but also possibly accounting for the mild phenotype of *Ifng*$^{-/-}$ and *Ifngr*$^{-/-}$ mice[95]. However, loss of IFN-γ signaling in mice leads to impaired clearance of several intracellular pathogens and a shift in the T_H1–T_H2 response (reviewed in (ref. [96])).

GM-CSF similarly acts as a potent communication conduit between T cells and myeloid cells[97–100]. The GM-CSF receptor is a heterodimer composed of the cytokine-specific α-chain and a β-chain that is shared with receptors for IL-3 and IL-5 (reviewed in ref. [101]). Its cellular expression is even more restricted than the expression of the IFNGR, as the GM-CSF receptor is almost exclusively expressed by myeloid cells. In vitro stimulation with GM-CSF initiates the differentiation of DCs, granulocytes and macrophages, depending on the concentration of the cytokine[102]. The situation in vivo is more complex, although there is evidence that GM-CSF also has dose- and time-dependent effects in vivo[103]. In general, GM-CSF promotes survival, differentiation and activation of monocytes, macrophages and other phagocytes by engaging the Janus kinase (JAK)2–signal transducer and activator of transcription (STAT)5 and extracellular signal-regulated kinase (ERK) pathways[104]. Under certain inflammatory conditions, GM-CSF can be regarded as a pro-inflammatory mediator between T_H cells and phagocytes (reviewed in ref. [105]) and may also act on astrocytes to promote central nervous system pathology[106,107]. Hence, it is not surprising that GM-CSF-blocking antibodies are prominently used in clinical trials, for example, recently in the context of coronavirus disease 2019 (ref. [108]).

Of note, among others, GM-CSF expression is induced by IL-23, which was also shown to be important for the modulation of 'T_H17' responses[20,66] and other type 3 immune responses (see below), making IL-23 both a type 1 and type 3 response-inducing cytokine, depending on circumstances (perhaps linked to its ability to signal through both STAT4 and STAT3). In this regard, it will be interesting to decipher additional factors causing a mainly destructive GM-CSF-driven type 1 response versus a protective IL-17-mediated type 3 response upon IL-23 exposure. Although it was argued that GM-CSF might serve as a marker for 'destructive or pathogenic' T_H17 (or T_H1–T_H17, or $T_H17.1$) cells, GM-CSF-producing cells preferably coexpress IFN-γ over IL-17 (refs. [66,69,71,73]). Nevertheless, the relationship with IFN-γ appears to be a complex one, because both IFN-γ and its driver IL-12 effectively suppress GM-CSF production in T cells[66]. Of note, whereas T cells can sense IFN-γ, which has long been considered to aid in the maintenance of the T_H1 phenotype,

GM-CSF is not sensed by lymphocytes themselves. In spite of the apparent contradictions that emerge when T_H cells are categorized by individual cytokine expression, the categorization of T_H cells by the target cells that they help alleviates that problem and permits a better understanding of the actual biology of T_H cells in type 1 immunity.

In sum, in type 1 responses, T_H cells mainly target and activate phagocytic cells. While this communication aids in the elimination of intracellular pathogens, aberrant (dysregulated) type 1 responses, through persistent recruitment of phagocytes, can be drivers of immunopathology.

Type 2 response. Type 2 immune responses were initially described to primarily foster humoral immunity, and T_H-derived type 2 cytokines predominantly help the B cell compartment and the involved intricacies to generate potent high-affinity antibodies. However, here again, the pure categorization of T_H cell by their cytokine profile makes it much harder to capture the function of IL-4-secreting T_H2 cells and T_{FH} cells alike. As such, type 2 T_H cells include not only T_H2 and T_{FH} cells but also T_H1 cells, as all of them were shown to be necessary for humoral (type 2) immunity[109,110]. Typical type 2 cytokines are IL-4, IL-5 and IL-13. IL-4 was the first factor that was recognized to be crucial for B cell maturation and class switching, therefore recognizing T_H2 cells as main providers of B cell help[111]. However, the deletion of T_H2-associated genes did not cause loss of germinal centers. Later, it became apparent that IL-4 was solely needed for immunoglobulin (Ig)E class-switch recombination[112] and that additional factors such as CD40 ligand (CD40L) and IL-21 were needed for fully functional B cell responses, which were attributed to T_{FH} cells (reviewed in ref. [109]). Of course, there are various flavors of T_{FH} cells, which may warrant a T_{FH} cell-specific nomenclature as suggested by Eisenbarth et al.[113]. Nevertheless, in this perspective, we consider their target, namely B cells, the reason why T_{FH} cells are primarily type 2 T_H cells.

Another important function of type 2 immunity beyond the engagement of B lymphocytes is the attraction and activation of eosinophils, mast cells and basophils during inflammatory responses. This is mainly achieved by the cytokines IL-5 and IL-13, which induce eosinophilia and goblet cell hyperplasia during helminth infections[114]. However, eosinophils, mast cells and basophils are not only type 2 effector cells, but they are also involved in the amplification of type 2 immunity by producing IL-4 and other type 2 mediators themselves. Eosinophil recruitment, for instance, can occur before infiltration of T_H cells, which in turn stimulates APCs to initiate a type 2-promoting T_H phenotype[115,116]. Although it is not fully understood which cell types induce the initial attraction of eosinophils, tissue-resident group 2 ILCs (ILC2 cells) might be involved, as they can react before the adaptive response is initiated[117], making them important early-phase type 2 players. Furthermore, it was shown that the presence of ILC2 cells was required for a complete T_H response, at least in the context of allergic inflammation[118,119].

The alarmin IL-25, also known as IL-17E, was first reported to be secreted by T_H2 cells and subsequently led to the coining of T_H25 cells as an IL-25-producing entity that boosts type 2 responses by enhancing IL-4, IL-5 and IL-9 production via STAT5 activation[120]. Now we know that it can be produced by many different hematopoietic and non-hematopoietic cell types, such as mast cells, alveolar epithelial cells, brain capillary endothelial cells and others (reviewed in ref. [121]). The exact mechanisms by which these cells induce and enhance type 2 responses are not fully understood yet; however, there is strong evidence that ILC2 cells act as type 2-response amplifiers[122–124].

Another type 2 cytokine that has defined an independent T_H subset is IL-9 (refs. [38,39]). Initially believed to be a T cell growth factor[125], IL-9 was soon recognized to be crucial for mast cell expansion and recruitment[126]. In this context, it is involved in the clearance of parasitic infections but may also play a role in promoting allergic inflammation (reviewed in ref. [127]).

In sum, type 2 T cells, including T_{FH} cells, primarily target B cells to aid in germinal center formation and class switch, whereas dysregulated type 2 immunity leads to allergic inflammation involving eosinophils, mast cells and basophils.

Type 3 response. Type 3 responses have been very well defined as barrier tissue-specific reactions to extracellular disturbances. Receptors for the critical cytokines IL-17 and IL-22 are expressed throughout the stromal and immune compartment, but dysregulated expression of these cytokines (IL-17A, IL-17F, IL-22, etc.) leads to dramatic immunopathology across barrier tissues (skin, lung, gut), with little to no signs of internal organ-specific effects[61,128–130]. Ectopic IL-17 expression has the most dramatic effect upon engagement of the IL-17 receptor complex in epithelial cells of the skin[131]. Apart from the production of antimicrobial peptides, IL-17-activated keratinocytes produce a set of chemokines and cytokines that in turn attract neutrophils into the skin (reviewed in ref. [132]). Dysregulation of IL-17 in mammals also triggers psoriasiform inflammation, characterized by the cellular expansion of keratinocytes, and the influx of neutrophils. Targeting the type 3 immune response in patients suffering from psoriasis through neutralization of IL-17 or IL-23 dramatically alleviates clinical symptoms (reviewed in ref. [28]). Strikingly, IL-23 is critical for both GM-CSF and IL-17 production in inflammatory conditions (as discussed above). This poses interesting questions about the regulation of IL-23 receptor signaling within different inflammatory conditions and cell types. In line with this, IL-23 was also shown to be released in response to nociceptor activation[133,134], linking the immune system with the neuronal network. The notion that there is more to the immune system than simple host defense applies not only to type 3 immunity and pain sensation. A growing scientific field has attempted to decipher the interplay of the immune system and other physiological processes such as the neuronal network and the enteric system (reviewed in ref. [135]).

In line with the notion that type 3 immune responses predominantly involve barrier tissues, physiological amounts of type 3 cytokines (such as IL-17A, IL-17F and IL-22) are involved in the control of mucosal pathogens, in particular, fungi (reviewed in ref. [136]). However, IL-22-producing cells can be easily 'reprogrammed' into IFN-γ- or IL-4-expressing T_H cells, illustrating one more time the dynamics of T_H cell plasticity and indicating the importance of a flexible and collaborative environment for a functional immune system[137].

Importantly, IL-17 and IL-22 production is readily observed in ILC3 cells and thymic educated γδ T cells, which are prominent and early responders in barrier tissue immunity, supporting the idea that a major portion of type 3 immunity is an evolutionary hard-wired mechanism of barrier protection[138].

In summary, in contrast to type 1 and type 2 responses, type 3 responses are less targeted to distinct immune effector cells but activate and regulate non-immune cells. The code, which is used to induce type 3 responses (through, for example, IL-17 and the IL-20 family of cytokines), is likely phylogenetically old and is used by tissue-resident immune cells (such as ILC3 cells and γδ T cells) to communicate with their non-hematopoietic environment. Eventually, it has been co-opted by the adaptive immune system for host defense at lining tissues.

Summary and conclusion

The establishment of advanced single-cell analysis tools such as single-cell RNA-seq and high-dimensional cytometry revealed that the hitherto known classification of the T_H cell universe, based on previously established cytokine patterns[1,2,79], does not adequately

capture the diversity and complexity of the mammalian immune system. For example, it was shown recently that T_H cells isolated from the lamina propria could not be attributed to 'classical' T_H1 or T_H17 subsets but rather expressed a continuum of different (signature) cytokines[79]. Hence, we propose to take a step back to acknowledge the bigger picture, instead of focusing on small T_H subsets that might simply represent an intermediate stage in their differentiation. By expanding the concept initially proposed by Eberl and Pradeu[80] and integration of the until-now described subsets into a more comprehensive capture of immunity based on target cells of the T_H response (Fig. 2), we propose the following nomenclature:

- Type 1 T_H cells that primarily activate and attract mononuclear phagocytes such as monocytes, macrophages and DCs
- Type 2 T_H cells targeting B cells and polymorphonucleated granulocytes such as mast cells, basophils and eosinophils
- Type 3 T_H cells acting on non-hematopoietic cells at barrier tissue sites, including epithelial cells and stromal cells.

This categorization is, in our opinion, superior to the coining of ever-new T_H subsets and sub-subsets. We acknowledge that this concept is, however, also imperfect in that it does not capture all possible cellular states and their individual role in immune responses. Furthermore, we would hope to have solid molecular markers of T_H cell states to better describe their biology. In lieu of such a 'super-marker' or molecular pattern of T_H cell states, this simplified contextual 'help' framework proposed here is also not overly rigid. While polarized T_H cells will in general fall into one of the three categories, this does not mean that their role in immunity is by any means inflexible. There is solid evidence of plasticity in memory T_H cells and the ability to respond to different challenges with speed and agility. Hence, all attempts to categorize single T_H cells observed during a snapshot within a complex immune response cannot truly give an account of the actual function and the role of individual T_H cell in the development of a dynamic immune response. The physiological importance of T_H differentiation must be the outcome of the response: the activation, attraction or modulation of responder cells. We hope that this perspective may help to establish a more intuitive classification of T_H cell function, which will help to understand the growing complexity in this field. Lastly, this perspective here is not meant to cast a new nomenclature for T_H cells but instead is meant to initiate the discussion to consider help function over phenotype as a potential stratifier for a more function-based categorization of T_H cells.

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Competing interests

The authors declare no competing interests.

Additional information

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