Cell-intrinsic mechanisms to restrain inflammatory responses in T lymphocytes

Running Title: Limiting T cell functions

Emina Džafo[1,2], Niccolò Bianchi[1,2] & Silvia Monticelli[1]*

[1] Institute for Research in Biomedicine (IRB), Università della Svizzera italiana (USI), Via Vincenzo Vela 6, CH-6500, Bellinzona, Switzerland

[2] Equal contribution

* Correspondence to: silvia.monticelli@irb.usi.ch

Acknowledgements. Work in the lab on this topic is supported by the following grants to S.M.: Swiss National Science Foundation (n. 31003A_175569 and 310030L_189352); Swiss National Center of Competence in Research (NCCR) "RNA & Disease"; Novartis Foundation; Ceresio Foundation.

Summary

A mechanistic understanding of the regulatory circuits that control the effector responses of memory T helper lymphocytes, and in particular their ability to produce pro-inflammatory cytokines, may lead to effective therapeutic interventions in all immune-related diseases. Activation of T lymphocytes induces robust immune responses that in most cases lead to the complete eradication of invading pathogens or tumor cells. At the same time, however, such responses must be both highly controlled in magnitude and limited in time to avoid unnecessary damage. To achieve such sophisticated level of control, T lymphocytes have at their disposal an array of transcriptional and post-transcriptional regulatory mechanisms that ensure the acquisition of a phenotype that is tailored to the incoming stimulus while restraining unwarranted activation, eventually leading to the resolution of the inflammatory response. Here, we will discuss some of these cell-intrinsic mechanisms that control T cell responses and involve transcription factors, microRNAs and RNA-binding proteins. We will also explore how the same mechanisms can be involved both in anti-tumor responses and in autoimmunity.

Keywords (3-6)

T lymphocytes; transcription; post-transcriptional regulation; RNA-binding proteins, microRNAs

Main Text

1. Introduction

Initially thought to be a passive process, the resolution of inflammatory responses to an invading pathogen or noxious agent is now understood to be actively controlled, involving the upregulation of pathways with anti-inflammatory and reparatory functions, including anti-inflammatory cytokines, microRNAs (miRNAs) and inhibitory molecules and enzymes. Defects in these regulatory pathways can lead to sterile inflammatory processes and autoimmunity. Despite their importance in maintaining immune homeostasis, the mechanisms that restrain or dampen pro-inflammatory responses in T lymphocytes are not completely understood, and the therapeutic stimulation of resolution of inflammation may become attractive in the treatment of chronic inflammatory disorders such as multiple sclerosis and other autoimmune diseases. A number of cell-intrinsic factors can modulate T cell responses to an antigen, both at a transcriptional level through epigenetic mechanisms and transcription factors, and at a post-transcriptional level, for example through the action of miRNAs and RNA-binding proteins (RBPs) that collectively regulate mRNA stability and translation (***Figure 1***). An integrated network in which multiple layers of regulation cooperate in modulating the rapidity, intensity and length of an immune reaction is particularly important to achieve responses that are swift (for instance by maintaining 'pre-stored' mRNAs in a repressed state until they are needed), and at the same time sufficiently dynamic that they can be turned off before the onset of excessive damage.

Here, we will discuss how some of these mechanisms may intrinsically regulate the pro-inflammatory phenotype of T lymphocytes. We will focus primarily on regulatory networks that may influence the phenotype of inflammatory memory $CD4^+$ T helper (Th) lymphocytes, while for more in-depth discussions on the regulation of T regulatory (Treg) cells and the initial naïve-to-memory cell transition and differentiation, we refer the reader to some outstanding reviews on the topic (1-3).

2. Transcription Factors

T helper lymphocytes have a central role in the immune system thanks to their main effector function of secreting cytokines that affect the activity of other innate and adaptive immune cells. Upon activation, T lymphocytes undergo widespread changes in gene expression, allowing them to differentiate into effector phenotypes able to elicit efficient responses against invading pathogens. Many transcriptional regulators contribute to the induction and maintenance of a broad range of T cell phenotypes, from highly pro-inflammatory and potentially tissue damaging to regulatory or exhausted, the latter being associated with reduced effector functions (***Figure 2***). Among the transcription factors that are clearly associated with a pro-inflammatory phenotype, T-BET and RORγt are primarily expressed by the IFN-γ-producing and IL-17-producing subsets of T lymphocytes (Th1 and Th17 cells, respectively), contributing to their cellular identity and acting as master regulators of cytokine production. For instance, T-BET induces directly the expression of IFN-γ in Th1 cells, but it also limits the acquisition of the alternative IL-4-producing Th2 phenotype by sequestering GATA3 and cooperating with Runx-3 to silence IL-4 production (4-6). RORγt expression instead characterizes the highly pro-inflammatory Th17 and Th1* subsets, the latter being defined by a dual Th1/17 phenotype (7-11). Concordant with their role in the regulation of

inflammatory cells, these transcription factors are also involved in autoimmunity (12, 13). Among the factors that are now emerging as consistently associated with a high cytokine-producing and pro-inflammatory T cell signature is also BHLHE40, a transcription factor that appears to regulate gene expression acting primarily as a repressor of transcription (14-18). In both mouse and human T cells, expression of this transcription factor was associated with a pathogenic signature (14, 19), and its deletion led to reduced expression of several pro-inflammatory cytokines, most notably GM-CSF (14, 19-24). In human T cells, it was highly expressed by inflammatory Th1* cells (14), and *Bhlhe40-/-* mice were protected from experimental autoimmune encephalomyelitis (EAE) (19, 21, 22), pointing towards a crucial pro-inflammatory role for this transcription factor. Emphasizing the complex interplay between regulatory factors that can either favor or limit the acquisition of a pro-inflammatory phenotype by T lymphocytes, we found that BHLHE40 could bind to the promoter region of the *ZC3H12D* gene, encoding for Regnase-4, a negative regulator of cytokine mRNA stability and translation. BHLHE40-mediated regulation reduced *ZC3H12D* expression, ultimately leading to increased cytokine production (14).

While these and other factors have been consistently associated with an inflammatory phenotype, other transcription regulators are primarily linked to a regulatory or exhausted phenotype, and contribute to restraining T cell responses. In this regard, FOXP3 represents a prototypical example. This transcription factor orchestrates the genetic program regulating the differentiation and maintenance of Treg cells, and its deficiency in both mouse and human results in severe, often fatal multiorgan autoimmunity (25). However, human (but not mouse) conventional T cells also transiently upregulate FOXP3 expression upon activation (26). Although such transient FOXP3 expression is not sufficient to induce a full Treg differentiation program, for which stable and robust expression is required (27), this observation suggests that at least in human cells, limited FOXP3 expression may represent a contributing factor in reducing the activity of effector T lymphocytes in a cell-intrinsic manner. Expression of the transcription factor Helios (encoded by the *IKZF2* gene) also characterizes a subset of Treg cells, and at least in humans, loss of *IKZF2* expression resulted in impaired Treg functions (28, 29). Whether these factors also effectively contribute to the termination of the response in conventional T lymphocytes remains to be fully understood.

Other transcription factors, such as TOX, are instead primarily associated with T cell exhaustion, defined as a state of reduced T cell responsiveness in both $CD4^+$ and $CD8^+$ T lymphocytes (30-34). Following chronic antigenic stimulation, TOX promoted the acquisition of a dysfunctional phenotype by inducing widespread epigenetic and gene-expression changes in $CD8^+$ T cell (30-34). TOX expression was induced by activation of NFAT (30, 31, 33), a transcription factor with a crucial role in the regulation of T cell effector functions and in the induction of an anergic state (35). Importantly, NFAT activation also led to the induction of the NR4A transcription factor, a member of a family of nuclear receptors which is also critical for T cell exhaustion (33). Indeed, chimeric antigen receptor (CAR) T cells lacking all three members of the NR4A family (NR4A1-3) showed highly efficient tumor killing capacity and led to prolonged mouse survival compared to wild-type CAR T cells (36). While these results clearly identified TOX as a regulator of a dysfunctional program in T lymphocytes, its expression may not be exclusively associated with exhaustion. For example, circulating TOX^+ memory $CD8^+$ T cells were recently detected in humans. These cells targeted persistent viruses, while retaining the ability to produce high levels of effector molecules, such as

perforin and granzyme B (37), suggesting that some aspects of the regulation of the dysfunctional state may somewhat diverge in T cells from human and mouse origins. Further regulatory differences may also occur between $CD8^+$ and $CD4^+$ T lymphocytes. For instance, transcriptional profiling of $CD4^+$ and $CD8^+$ T cells during chronic viral infections identified both shared and unique factors potentially involved in regulating the hypofunctional state of these cells following chronic stimulation (38). Among these, expression of *Ikzf2* was specifically associated with exhausted conventional $CD4^+$, but not $CD8^+$ T cells, pointing towards some unique transcriptional aspects defining the dysfunctional state in these two cell populations (38).

Overall, a number of transcription factors and networks are emerging as key regulators of the phenotype of inflammatory T cells. Although some of these transcription factors are primarily associated to a particular T cell phenotype or state (for instance, Treg or exhausted cells), they might also more generally contribute to either restraining or favoring responses of conventional effector and memory cells. Adding to the possible combinatorial complexity in transcriptional regulation, many key factors, including for example FOXP3, form multi-protein complexes by interacting with a variety of binding partners. Depending on the recruited interacting partner, each factor can therefore influence transcription positively or negatively, potentially leading to very different transcriptional landscapes and cellular states (39).

3. RNA-binding proteins

Throughout the process of T cell activation and differentiation, tight control of cytokine production is necessary to orchestrate appropriate responses to incoming signals while avoiding excessive damage to healthy tissues. The expression of cytokines and other immune-relevant genes is therefore controlled not only at a transcriptional, but also at a post-transcriptional level, through the action of RBPs that modulate the stability and translation of target mRNAs (3, 40-42). For instance, the 3'-untranslated regions (3'UTRs) of many cytokine mRNAs contain multiple regulatory *cis*-acting elements, including stem-loop structures and adenine and uridine (AU)-rich elements (AREs) that influence mRNA stability and translation (43, 44). These *cis* elements are recognized in *trans* by RBPs that can positively or negatively affect mRNA decay and protein synthesis. Other factors that may strongly influence the stability and functionality of a given mRNA include miRNAs, which exert their functions in the context of miRNA-containing RNA silencing complexes (45), as well as the YTH domain-containing family of proteins YTHDF1-3, which recognize the N^6-methyladenosine (m^6A) modification in target transcripts (46). Therefore, although each RBP can individually affect mRNA translation or decay, mRNAs may contain binding sites for a multitude of factors (ARE and stem-loop binding proteins, miRNAs, m^6A-binding proteins), all potentially binding in a cooperative or competitive manner, and contributing to the final outcome on mRNA half-life and protein output. For instance, the cytokine transcripts *CSF1* (encoding G-CSF), *IL2* and *IL6* were shown to contain both ARE and stem-loop elements (47). Further increasing the complexity of studying the impact of post-transcriptional mechanisms on regulation of gene expression, each RBP-encoding mRNA can be subjected to cross-regulation by other RBPs, as illustrated by the fact that Regnase-1 is capable of repressing Roquin expression by targeting its 3'UTR (48). Because of these complexities and the number of possible regulatory combinations, here we will focus on specific examples of RBPs that

have been shown to impact primarily the pro-inflammatory phenotype of T helper lymphocytes, including tristetraprolin (TTP), Roquin and Regnase family members (***Table 1***).

In T cells, the importance of post-transcriptional regulation of cytokine mRNAs is highlighted by the recognition that CD28-mediated co-stimulation led to the stabilization of a number of transcripts, including *IL2*, *IFNG*, *TNF* and *CSF2* (encoding GM-CSF) (49), thereby ensuring that T lymphocytes produced cytokines shortly after activation, but only for a limited time. Interestingly, some of these regulatory mechanisms are cytokine-specific, and guarantee that individual cytokines are expressed at the appropriate time after activation. For instance, in murine effector and memory $CD8^+$ T cells, the immediate production of TNF-α was shown to be intense but transient, and primarily linked to the translation of pre-synthesized mRNAs, while IL-2 expression was dependent on *de novo* transcription upon antigen stimulation (50). Production of IFN-γ instead required both the initial translation of pre-formed mRNAs and *de novo* transcription, highlighting the importance of appropriate expression kinetics for each individual cytokine. IFN-γ expression was regulated post-transcriptionally via AREs present in the 3'UTR of the *Ifng* mRNA, and germline deletion of these elements led to uncontrolled, chronic cytokine production and tissue pathology with an autoimmune phenotype (51).

AREs are often found in short-lived mRNAs and usually confer rapid decay in a process known as ARE-mediated decay. There are several different domains, found in a variety of proteins, that were shown to bind AREs, although some of them may also recognize stem-loops (43). TTP (encoded by the *Zfp36* gene) represents one well-studied example of a destabilizing protein that recognizes ARE-containing inflammatory transcripts, such as *TNF*, *IL6*, *CSF2*, *IFNG*, *IL2*, but also the anti-inflammatory cytokine mRNA *IL10*, suggesting that RBPs contribute to modulating both the amplitude and duration of the initial inflammatory response, and also its decline and resolution (43, 52). TTP binds directly to target mRNAs (53), and induces their destabilization through deadenylation-dependent decay (54), by recruiting the CCR4-CAF1-NOT deadenylase complex and the exosome for RNA degradation (55, 56). Mice lacking TTP developed a severe and complex autoimmune syndrome (57), while stabilization of TTP expression protected mice against severe forms of inflammatory pathologies such as collagen-induced arthritis, imiquimod-induced dermatitis and EAE (58). To achieve such protection from a number of inflammatory diseases, it was sufficient to remove an ARE-containing region in the *Zfp36* mRNA that is responsible for TTP binding and destabilization of its own transcript. These results underscore the importance of tight post-transcriptional control in modulating immune responses.

Another RBP with key functions in immune cells is human antigen R (HuR, encoded by the *ELAVL1* gene). HuR also has preference for ARE sequences (59, 60), and its predominant role in the regulation of gene expression is linked to mRNA stabilization (61), although the final outcome on the phenotype in *in vivo* settings may be more complex (62, 63). Both TTP and HuR contribute to coordinated regulation of mRNA stability, as shown for example for *TNF*. Specifically, stimulation of macrophages by lipopolysaccharide induced phosphorylation of TTP, reducing its affinity for ARE sites in the *TNF* 3'UTR. This in turn allowed HuR binding and transcript stabilization, leading to increased translation and protein synthesis (64). Interestingly, since the *Zfp36* mRNA (encoding TTP) is under the same feedback control mediated by TTP itself, this mechanism may create a window of opportunity for HuR-mediated stabilization and translation of *TNF*, which would however be limited in time due, at least in part, to the concomitant stabilization of *Zfp36* (64).

Stem-loop-binding proteins strongly involved in the regulation of T cell functions and inflammation include the Roquin family members Roquin-1 and -2 (encoded by the *Rc3h1* and *Rc3h2* genes) (65), and the Regnase family members Regnase 1-4 (encoded by the *Zc3h12a-d* genes) (66). Roquin-1 and -2 are E3 ubiquitin ligase enzymes essential during development, as shown by the early postnatal lethality of both *Rc3h1* and *Rc3h2* deletions (67, 68). In T lymphocytes, the combined deletion of both enzymes led to hyperactivation of both $CD4^+$ and $CD8^+$ T cells, with development of splenomegaly and lymphadenopathy, pointing towards compensatory functions for these RBPs that are key to restrain effector cell functions (67, 69). Roquin ablation led to the aberrant differentiation of Th17 and T follicular helper cells, and also affected the phenotype and functionality of Treg cells, which became defective in their ability to suppress the activation of conventional T lymphocytes (70). The RNA-binding ROQ domain of Roquin proteins recognizes stem-loop structures (called constitutive decay elements or CDEs) characterized by a short stem and a 3-nucleotides loop (71-73). Similar to ARE-mediated mRNA decay, Roquin interacts with the CCR4-CAF1-NOT deadenylase complex, leading to mRNA decay upon deadenylation and decapping (73).

Regnase-1 was first shown to be involved in the direct destabilization and degradation of the *Il6* and *Il12* mRNAs (74). Such destabilization was dependent on the presence of a conserved region in the target 3'UTRs, and on the RNase, endoribonuclease activity of the Regnase-1 enzyme (74). Regnase-1 acts therefore as a strong negative regulator of inflammation. In its absence, mice developed a severe autoimmune pathology and died within 12 weeks after birth with severe splenomegaly, lymphadenopathy and hyperimmunoglobulinemia (74). Underlining the key role of Regnase-1 expression in T cells, deletion of this enzyme only in the $CD4^+$ T cell compartment was sufficient to induce an autoimmune phenotype similar to full *Zc3h12a*-deleted mice (75). Perhaps unsurprisingly, the expression of this potent modulator of inflammation is also regulated by multiple mechanisms. First, Regnase-1 is capable of degrading its own mRNA in a negative feedback loop. Second, the paracaspase MALT1 cleaves Regnase-1 (and also Roquin-1 and -2) protein upon T cell receptor (TCR) activation, essentially removing the "inflammation brake" which subsequently leads to a high production of cytokines (69, 75).

All members of the Regnase family share a conserved region containing RNase and deubiquitinase catalytic domains (76, 77). Regnase-2 (*ZC3H12B*) and Regnase-3 (*ZC3H12C*) are expressed at very low levels in human T lymphocytes (78), and they are therefore unlikely to have a relevant functional impact in these cells. Concordant with this observation, deletion of mouse *Zc3h12c* led to some lymphocyte abnormalities that were not cell-autonomous, but rather secondary to defects in the myeloid lineage (79). Both Regnase-1 and Regnase-4 were shown to degrade an overlapping set of mRNAs, such as *IL2*, *IL6*, *IL10*, and *TNF* in an RNase-dependent manner via the targets' 3'UTRs (***Table 1***). This observation raises the question about protein redundancy, and specifically whether these two enzymes may have fully overlapping or also unique functions in T cells. Pointing towards the possibility that these two proteins may not be fully redundant is the observation that the phenotype of mice lacking either *Zc3h12a* or *Zc3h12d* is different. Specifically, in contrast to Regnase-1, deletion of Regnase-4 did not lead to macroscopic changes in immune functions under steady state conditions. However, in models of EAE, these mice showed exacerbation of symptoms both at the peak of disease as well as in the resolution phase (80). These findings suggest that these two enzymes may in fact have functions that are not fully redundant, with Regnase-1 being potentially primarily

involved in regulating the initial activation of T lymphocytes, while Regnase-4 might be more important during the resolution phase of inflammation. Concordant with this hypothesis, the expression of Regnase-4 protein in mouse splenocytes was increased after three days of TCR stimulation with anti-CD3 antibodies, suggesting a role at later stages of activation (80), while *ZC3H12A* was most highly expressed in resting lymphocytes, suggesting a role in restraining initial T cell activation (14, 78) (***Figure 3***). An alternate explanation for these findings is that the apparent functional differences between these two proteins are actually due primarily to their relative expression levels, since Regnase-1 is more abundantly expressed than Regnase-4 in T lymphocytes. This might also explain the more severe phenotype observed upon Regnase-1 ablation, that could not be fully compensated by the more moderately expressed Regnase-4.

The relevance of the m^6A modification in T cell biology was revealed by studies showing that conditional deletion of the *Mettl3* gene (encoding for the m^6A methyltransferase enzyme Mettl3) in murine T lymphocytes compromised Treg cell functions (81) and led to the inability of naïve T cells to proliferate in response to cytokine signalling (82). Functional consequences of the m^6A marks on RNA are mediated by m^6A-binding proteins, including primarily members of the YTH family. Within the immune system, YTHDF1 impacted anti-tumor responses, and its ablation led to improved immunity and prolonged survival (83). Recent efforts to understand the level of redundancy between the different paralogs of the YTH family (YTHDF1-3) of m^6A-binding proteins recently reconciled apparently divergent results by showing that within individual cells, the three paralogs can fully compensate for each other in a gene dosage-dependent manner to mediate degradation of m^6A-containing mRNAs (84, 85). However, the phenotypes of the individual knock-out mice revealed differences due to the varying levels of expression of the YTH proteins across cell types (85). In other words, even though two proteins may be in reality fully redundant, deletion of a lowly expressed family member may have subtle consequences, for instance by affecting a subset of mRNAs highly sensitive to gene-dosage effects, while the deletion of a more highly expressed family member would affect mRNA stability or translation in a more widespread manner, leading to overall different phenotypes (84). Similar to the YTH proteins example, more studies investigating the details of Regnase-1 and -4 regulation and their mechanism of action will shed light on the level of functional overlap between these two enzymes, and specifically whether some of the differences observed when deleting individual proteins are due to unique functions and regulatory mechanisms, or they reflect instead hypomorphic phenotypes.

Overall, the overarching results paint a picture of a tightly controlled network of RBPs that regulate immune cell functions and responses both at resting state, and during the initiation and resolution phases of inflammation. However, mRNAs are subjected to regulation (and cross-regulation) by a large number of factors binding to a variety of sites in a cooperative or competitive manner, for which the regulatory logic remains for the most part to be examined.

4. The interplay between miRNAs and RBPs in the regulation of T lymphocytes

Through their ability to target a variety of mRNAs and limit their translation, miRNAs strongly influence T cell responses by modulating T cell differentiation, activation and proliferation (86, 87).

When investigating the role of miRNAs in T lymphocytes, the level of expression and the kinetic of miRNA expression in response to TCR triggering may already provide some information about their potential functional role. First, highly abundant miRNAs that are rapidly downregulated upon stimulation may be involved in restraining 'spurious' T cell activation that may occur in the absence of a genuine threat for the organism. One such example is provided by miR-125b, that was shown to be involved in the maintenance of the naïve state in human T cells (88). On the other hand, very lowly expressed miRNAs are very unlikely to achieve thresholds of expression that can lead to biologically relevant effects beyond stochastic noise (89, 90), if not in specific conditions of a few, very high-affinity target sites (potentially in cooperation with other miRNAs) and/ or in specialized T cell subsets. For instance, despite being an overall moderately expressed miRNA, miR-181a was expressed at relatively high levels in the Th17 subset of human T lymphocytes, where it contributed to define the threshold of TCR activation in these cells (91). Finally, modestly expressed miRNAs in resting cells that are strongly induced upon acute stimulation may achieve during this process an intracellular concentration sufficient to modulate the expression of mRNA targets important during T cell activation. Depending upon the kinetics of induction, categories of 'inducible miRNAs' might be divided into 'early' miRNAs (hours), potentially involved in favoring T cell activation and proliferation (e.g. miR-155 (92, 93)), and 'late' miRNAs (days), likely involved in the resolution of inflammation. A representative example of the latter category is provided by miR-146a, a negative regulator of NF-kB activation (94), whose deletion led to defective resolution of inflammation and development of T cell-associated autoimmunity (95).

Similar to protein-coding genes, miRNA expression is regulated at the transcriptional level by transcription factors. In contrast, there are not many examples in the literature regarding interactions between miRNAs and RBPs. However, one can easily envision various situations in which a) miRNA expression is regulated by RBPs; b) RBP transcripts are targeted and regulated by miRNAs; and c) both miRNAs and RBPs contribute to the regulation of the same target mRNA, in a cooperative, competitive or antagonist manner. The concept that miRNA expression is regulated post-transcriptionally by RBPs is exemplified by miR-146a, which was shown to be regulated by Roquin. In the absence of Roquin, miR-146a levels increased in T lymphocytes, due to the augmented stability of the mature miRNA (96). This stabilization was associated with enhanced ability of the Dicer enzyme to process the precursor pre-miR-146a (96). MiRNAs are also abundantly found in extracellular vesicles derived from many cell types, including activated primary human T lymphocytes (97, 98), which may have a role in cell-to-cell communication and also in the process of rapid 'elimination' of cytoplasmic factors that may hinder rapid T cell activation upon recognition of a specific antigen (99). Interestingly, hnRNPA2B1, a ubiquitously expressed RBP, was shown to be involved in the selection and loading of specific miRNAs into the exosomes of activated T cells, pointing towards an active sorting process in the loading of the exosomes' cargo and a further layer of interplay between RBPs and miRNAs (100).

As for examples of 'classic' miRNA regulation of RBP expression, miR-27b was shown to modulate TTP levels in macrophages, in a complex interplay with HuR binding (101). In addition, both miRNAs and RBPs may target the same site on a given mRNA, as shown for example by *Pten*. This mRNA is regulated both by miR-17~92 and by Roquin, although in this case Roquin was shown to limit the access to the miR-17~92 site through competitive binding at an overlapping site (70). A

further example is provided by HuR, which was shown to modulate miRNA-mediated mRNA targeting genome-wide in macrophages (101). Specifically, the presence of HuR-binding sites in the proximity of miRNA sites antagonized and attenuated miRNA activity resulting in increased gene expression, highlighting once more the complex interaction between different players in regulating mRNA stability and translation. In general, it is increasingly clear that both miRNAs and RBPs have a substantial impact of the regulation of gene expression during immune responses. However, the interplay between these different factors and their potential for cross-regulation remains less understood.

5. From autoimmunity to anti-tumor responses

Several of the molecular mechanisms discussed so far that affect lymphocyte responses are crucial in the context of their ability to limit T cell responses in autoimmunity, as shown by the many instances in which deletion of a miRNA or RBP led to spontaneous immune cell activation and pathology. However, the reverse can also be true, that unleashing the activity of these same factors might enhance T cell functions in the context of anti-tumor responses. Indeed, immune-related adverse effects such as autoimmunity also arise during immune cancer therapy, reflecting the removal of inhibitory brakes to T cell function (102). Understanding the mechanisms that regulate the balance between inflammation and tissue damage may be beneficial for both autoimmunity (where reduced inflammation is a desired outcome) and cancer immunotherapy, which instead would benefit of enhanced inflammatory and cytotoxic T cell responses.

The advent of immunotherapy has revolutionized prospects for cancer treatment, opening the possibility of inducing or reactivating anti-tumor immunity. Despite its effectiveness in many instances, the outcomes of immune checkpoint therapy are overall still highly heterogenous, and the basic biological knowledge that would reveal mechanistic insights about efficacy (or lack of thereof) in inducing anti-tumor responses is still lagging behind (103). The presence at tumor sites of infiltrating T lymphocytes retaining effector functions usually correlates with more favorable outcomes (104). However, tumor-infiltrating lymphocytes are very heterogenous regarding gene expression and functional properties, and especially cells of the $CD8^+$ subset can acquire a functionally impaired state that limits their ability to control tumor growth (104). Critical differences between human and mouse models also hinder the interpretation of exhausted states. For instance, markers of T cell exhaustion such as the transcription factor TOX are associated with dysfunction in the mouse but not in human cells, suggesting underlying mechanistic differences (37).

Several factors discussed so far that were associated with a cytokine-producing, potentially pathogenic T cell phenotype were also shown to impact T cell responses to tumors. For example, T cell-specific ablation of Regnase-1 in mouse models caused pathogenic activation of T lymphocytes, aberrant cytokine production and spontaneous autoimmune disease (75). However, Regnase-1-deficient $CD8^+$ T lymphocytes also showed markedly increased efficacy in models of cancer immunotherapy (105). Similarly, while the transcription factor BHLHE40 was associated to a pathogenic cytokine signature in both human and mouse (14, 19), a T cell subset defined by BHLHE40 expression was specifically expanded in colorectal cancer patients with favorable responses to treatment with immune-checkpoint therapy (106). These findings highlight how these

(and probably many other) factors may be involved in 'tipping the balance' of T cell responses, from beneficial for cancer therapy to pathogenic in autoimmunity.

One caveat to the apparently simple idea that anti-tumor responses should activate pathways that should instead be dampened in autoimmunity, is represented by the fact that the association between the two diseases is in reality bidirectional, and patients with autoimmune diseases are often also at increased risk of developing malignancies, at least in part because of the chronically altered inflammatory milieu (107). For instance, while the transcription factor TOX was clearly implicated in the acquisition of a dysfunctional, exhaustion program in tumor-specific $CD8^+$ T cells (30-34), it was also shown to promote $CD8^+$ T cell-mediated autoimmunity (108). These observations critically emphasize the importance of gaining a more comprehensive understanding of the intricacies of immune cell regulation.

One example of a potent pro-inflammatory cytokine at the crossroad between autoimmunity and cancer is provided by IL-17A. This cytokine plays a key role in the responses against infections with extracellular bacteria and fungi. However, its dysregulated expression is also strongly associated with autoimmunity, as shown by the effectiveness of neutralizing antibodies against IL-17A in the treatment of psoriasis (109). Dysregulated, chronic production of this cytokine is now emerging also as a player in tumorigenesis (110). Interestingly, IL-17-signaling was found to strongly affect the stability of different mRNAs, in part by inducing the expression of mRNA stabilizers such as HuR and Arid5a (111). Arid5a in particular binds to the 3'UTR of target mRNAs at stem-loop structures overlapping with Regnase-1 binding sites, thereby counteracting Regnase-mediated degradation and promoting mRNA translation (112). In T lymphocytes, Arid5a favored differentiation of naïve T cells towards the inflammatory Th17 subsets through the stabilization of the *Stat3* mRNA, which is required for Th17 cell differentiation (112). The *IL17A* mRNA is also itself a target of post-transcriptional regulation, as shown for instance by the reduced IL-17 expression observed in the absence of HuR in Th17 cells, and the consequent amelioration of EAE development (113). Whether some of these mechanisms may be harnessed in the context of anti-cancer therapies remains to be investigated. Overall, it is becoming clear that many of the abovementioned regulatory factors act at the interface between anti-tumor responses and autoimmunity, underlying a role that is most likely primarily linked to the maintenance of balanced immune responses.

6. Outstanding questions

Many questions remain open about the role of specific transcription factors in regulating T cell functions. Most notably, it will be important to better understand whether some of the factors that have been implicated primarily in the regulation of Treg cells or chronically activated, exhausted cells may actually have important physiological functions in conventional T lymphocytes. Such factors could for instance contribute to the attenuation of the response once a pathogen has been successfully eliminated. Other key issues that will have to be better understood in the future are related to potential regulatory differences between $CD4^+$ and $CD8^+$ T lymphocytes, and also whether some of the important findings that have been described using mouse models can be now recapitulated in humans.

Functional studies have shown that the dysregulation of miRNA expression can be causative in various diseases, leading to an interest in the development of therapeutics to harness miRNAs for

clinical benefit (114). One of the key issues in targeting these molecules to modulate immune cell functions is whether delivery systems can be designed that target efficiently and specifically the desired cell type, and at the same time can deliver sufficient quantities of miRNA mimic or antagonist that can reach biologically relevant intracellular concentrations. Moreover, the chemical modifications that are introduced in miRNAs to improve their stability *in vivo* can interfere with loading onto the silencing complex or with mRNA targeting (114). As a result, only few miRNA mimic molecules progressed to some initial clinical testing. An interesting development in this direction was recently described for a miR-146a mimic oligonucleotide conjugated to a Toll-like receptor 9 agonist. This conjugated molecule efficiently reached the cytoplasm of myeloid and leukemic cells and was effective in reducing NF-kB activation, in limiting leukemia progression and in dampening excessive inflammation in models of cytokine release syndrome. Whether this or similar systems can be exploited therapeutically will undoubtedly be explored in the near future (115).

Likewise, in the case of RBPs, and despite their emerging importance in the regulation of immune responses, a number of outstanding questions remain to be addressed. Specifically: which cytokines and genes are affected by the expression of individual RBPs? What is the extent of target overlap, if any, between RBPs belonging to the same family, such as Regnase-1 and Regnase-4? How are these rheostats of inflammation themselves regulated in T lymphocytes? And finally, what is the precise kinetic of events in the regulation of inflammatory mRNA stability and translation in response to environmental cues? Understanding the temporal relationship between different RBPs and mRNA expression may lead to a better understanding of the impact of many of these post-transcriptional regulators on the initiation and persistence of inflammatory responses, as well as their resolution.

7. Concluding remarks

If not appropriately controlled, the transcriptional program induced by pro-inflammatory stimuli or noxious agents has the potential to inflict significant damage to healthy tissues. The control of RNA stability and decay acts therefore in concert with transcription to delimit the amplitude and duration of an inflammatory response. Potentially, a combined approach that inhibits pro-inflammatory, pathogenic T cells and at the same time actively promotes the resolution of inflammation and tissue repair may become an attractive answer in the treatment of autoimmunity (116). On the other hand, the enhancement of some of these pathways, at least temporarily or targeted to antigen-specific cells (117), may improve strategies for cancer immunotherapy, highlighting the importance of advancing our understanding of the molecular and functional bases for immune activation and regulation. Many of the factors discussed heretofore (transcription factors, RBPs, miRNAs) act in a concerted, regulated manner to modulate the interface between insufficiently protective and excessively damaging immune responses, and may eventually become valuable targets for immunomodulation.

Conflict of Interest

The authors have no conflict of interest to declare.

References

1. Josefowicz SZ, Lu LF, Rudensky AY. Regulatory T cells: mechanisms of differentiation and function. Annual review of immunology. 2012;**30**:531-64.

2. Kanno Y, Vahedi G, Hirahara K, Singleton K, O'Shea JJ. Transcriptional and epigenetic control of T helper cell specification: molecular mechanisms underlying commitment and plasticity. Annual review of immunology. 2012;**30**:707-31.

3. Hoefig KP, Heissmeyer V. Posttranscriptional regulation of T helper cell fate decisions. J Cell Biol. 2018;**217**(8):2615-31.

4. Hwang ES, Szabo SJ, Schwartzberg PL, Glimcher LH. T helper cell fate specified by kinase-mediated interaction of T-bet with GATA-3. Science. 2005;**307**(5708):430-3.

5. Szabo SJ, Kim ST, Costa GL, Zhang X, Fathman CG, Glimcher LH. A novel transcription factor, T-bet, directs Th1 lineage commitment. Cell. 2000;**100**(6):655-69.

6. Djuretic IM, Levanon D, Negreanu V, Groner Y, Rao A, Ansel KM. Transcription factors T-bet and Runx3 cooperate to activate Ifng and silence Il4 in T helper type 1 cells. Nature immunology. 2007;**8**(2):145-53.

7. Zielinski CE, Mele F, Aschenbrenner D, et al. Pathogen-induced human TH17 cells produce IFN-gamma or IL-10 and are regulated by IL-1beta. Nature. 2012;**484**(7395):514-8.

8. Ciofani M, Madar A, Galan C, et al. A validated regulatory network for Th17 cell specification. Cell. 2012;**151**(2):289-303.

9. Zhang F, Meng G, Strober W. Interactions among the transcription factors Runx1, RORgammat and Foxp3 regulate the differentiation of interleukin 17-producing T cells. Nature immunology. 2008;**9**(11):1297-306.

10. Lazarevic V, Chen X, Shim JH, et al. T-bet represses T(H)17 differentiation by preventing Runx1-mediated activation of the gene encoding RORgammat. Nature immunology. 2011;**12**(1):96-104.

11. Annunziato F, Cosmi L, Santarlasci V, et al. Phenotypic and functional features of human Th17 cells. The Journal of experimental medicine. 2007;**204**(8):1849-61.

12. Ivanov, II, McKenzie BS, Zhou L, et al. The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17+ T helper cells. Cell. 2006;**126**(6):1121-33.

13. Bettelli E, Sullivan B, Szabo SJ, Sobel RA, Glimcher LH, Kuchroo VK. Loss of T-bet, but not STAT1, prevents the development of experimental autoimmune encephalomyelitis. The Journal of experimental medicine. 2004;**200**(1):79-87.

14. Emming S, Bianchi N, Polletti S, et al. A molecular network regulating the proinflammatory phenotype of human memory T lymphocytes. Nature immunology. 2020;**21**(4):388-99.

15. Li Y, Xie M, Song X, et al. DEC1 negatively regulates the expression of DEC2 through binding to the E-box in the proximal promoter. The Journal of biological chemistry. 2003;**278**(19):16899-907.

16. St-Pierre B, Flock G, Zacksenhaus E, Egan SE. Stra13 homodimers repress transcription through class B E-box elements. The Journal of biological chemistry. 2002;**277**(48):46544-51.

17. Sun H, Taneja R. Stra13 expression is associated with growth arrest and represses transcription through histone deacetylase (HDAC)-dependent and HDAC-independent mechanisms. Proceedings of the National Academy of Sciences of the United States of America. 2000;**97**(8):4058-63.

18. Cook ME, Jarjour NN, Lin CC, Edelson BT. Transcription factor Bhlhe40 in immunity and autoimmunity. Trends in immunology. 2020.

19. Lin CC, Bradstreet TR, Schwarzkopf EA, et al. IL-1-induced Bhlhe40 identifies pathogenic T helper cells in a model of autoimmune neuroinflammation. The Journal of experimental medicine. 2016;**213**(2):251-71.

20. Huynh JP, Lin CC, Kimmey JM, et al. Bhlhe40 is an essential repressor of IL-10 during Mycobacterium tuberculosis infection. The Journal of experimental medicine. 2018;**215**(7):1823-38.

21. Lin CC, Bradstreet TR, Schwarzkopf EA, et al. Bhlhe40 controls cytokine production by T cells and is essential for pathogenicity in autoimmune neuroinflammation. Nature communications. 2014;**5**:3551.

22. Martinez-Llordella M, Esensten JH, Bailey-Bucktrout SL, et al. CD28-inducible transcription factor DEC1 is required for efficient autoreactive CD4+ T cell response. The Journal of experimental medicine. 2013;**210**(8):1603-19.

23. Sun H, Lu B, Li RQ, Flavell RA, Taneja R. Defective T cell activation and autoimmune disorder in Stra13-deficient mice. Nature immunology. 2001;**2**(11):1040-7.

24. Yu F, Sharma S, Jankovic D, et al. The transcription factor Bhlhe40 is a switch of inflammatory versus antiinflammatory Th1 cell fate determination. The Journal of experimental medicine. 2018;**215**(7):1813-21.

25. Rudensky AY. Regulatory T cells and Foxp3. Immunol Rev. 2011;**241**(1):260-8.

26. Gavin MA, Torgerson TR, Houston E, et al. Single-cell analysis of normal and FOXP3-mutant human T cells: FOXP3 expression without regulatory T cell development. Proceedings of the National Academy of Sciences of the United States of America. 2006;**103**(17):6659-64.

27. Honaker Y, Hubbard N, Xiang Y, et al. Gene editing to induce FOXP3 expression in human CD4(+) T cells leads to a stable regulatory phenotype and function. Science translational medicine. 2020;**12**(546).

28. Baine I, Basu S, Ames R, Sellers RS, Macian F. Helios induces epigenetic silencing of IL2 gene expression in regulatory T cells. Journal of immunology. 2013;**190**(3):1008-16.

29. Ng MSF, Roth TL, Mendoza VF, Marson A, Burt TD. Helios enhances the preferential differentiation of human fetal CD4(+) naive T cells into regulatory T cells. Science immunology. 2019;**4**(41).

30. Scott AC, Dundar F, Zumbo P, et al. TOX is a critical regulator of tumour-specific T cell differentiation. Nature. 2019;**571**(7764):270-4.

31. Khan O, Giles JR, McDonald S, et al. TOX transcriptionally and epigenetically programs CD8(+) T cell exhaustion. Nature. 2019;**571**(7764):211-8.

32. Alfei F, Kanev K, Hofmann M, et al. TOX reinforces the phenotype and longevity of exhausted T cells in chronic viral infection. Nature. 2019;**571**(7764):265-9.

33. Seo H, Chen J, Gonzalez-Avalos E, et al. TOX and TOX2 transcription factors cooperate with NR4A transcription factors to impose CD8(+) T cell exhaustion. Proceedings of the National Academy of Sciences of the United States of America. 2019;**116**(25):12410-5.

34. Yao C, Sun HW, Lacey NE, et al. Single-cell RNA-seq reveals TOX as a key regulator of CD8(+) T cell persistence in chronic infection. Nature immunology. 2019;**20**(7):890-901.

35. Bandyopadhyay S, Soto-Nieves N, Macian F. Transcriptional regulation of T cell tolerance. Semin Immunol. 2007;**19**(3):180-7.

36. Chen J, Lopez-Moyado IF, Seo H, et al. NR4A transcription factors limit CAR T cell function in solid tumours. Nature. 2019;**567**(7749):530-4.

37. Sekine T, Perez-Potti A, Nguyen S, et al. TOX is expressed by exhausted and polyfunctional human effector memory CD8(+) T cells. Science immunology. 2020;**5**(49).

38. Crawford A, Angelosanto JM, Kao C, et al. Molecular and transcriptional basis of CD4(+) T cell dysfunction during chronic infection. Immunity. 2014;**40**(2):289-302.

39. Kwon HK, Chen HM, Mathis D, Benoist C. Different molecular complexes that mediate transcriptional induction and repression by FoxP3. Nature immunology. 2017;**18**(11):1238-48.

40. Anderson P. Post-transcriptional control of cytokine production. Nature immunology. 2008;**9**(4):353-9.

41. Fu M, Blackshear PJ. RNA-binding proteins in immune regulation: a focus on CCCH zinc finger proteins. Nature reviews Immunology. 2017;**17**(2):130-43.

42. Diaz-Munoz MD, Turner M. Uncovering the Role of RNA-Binding Proteins in Gene Expression in the Immune System. Frontiers in immunology. 2018;**9**:1094.

43. Turner M, Galloway A, Vigorito E. Noncoding RNA and its associated proteins as regulatory elements of the immune system. Nature immunology. 2014;**15**(6):484-91.

44. Villarino AV, Katzman SD, Gallo E, et al. Posttranscriptional silencing of effector cytokine mRNA underlies the anergic phenotype of self-reactive T cells. Immunity. 2011;**34**(1):50-60.

45. Bartel DP. Metazoan MicroRNAs. Cell. 2018;**173**(1):20-51.

46. Patil DP, Pickering BF, Jaffrey SR. Reading m(6)A in the Transcriptome: m(6)A-Binding Proteins. Trends Cell Biol. 2018;**28**(2):113-27.

47. Brown CY, Lagnado CA, Goodall GJ. A cytokine mRNA-destabilizing element that is structurally and functionally distinct from A+U-rich elements. Proceedings of the National Academy of Sciences of the United States of America. 1996;**93**(24):13721-5.

48. Cui X, Mino T, Yoshinaga M, et al. Regnase-1 and Roquin Nonredundantly Regulate Th1 Differentiation Causing Cardiac Inflammation and Fibrosis. Journal of immunology. 2017;**199**(12):4066-77.

49. Lindstein T, June CH, Ledbetter JA, Stella G, Thompson CB. Regulation of lymphokine messenger RNA stability by a surface-mediated T cell activation pathway. Science. 1989;**244**(4902):339-43.

50. Salerno F, Paolini NA, Stark R, von Lindern M, Wolkers MC. Distinct PKC-mediated posttranscriptional events set cytokine production kinetics in CD8(+) T cells. Proceedings of the National Academy of Sciences of the United States of America. 2017;**114**(36):9677-82.

51. Hodge DL, Berthet C, Coppola V, et al. IFN-gamma AU-rich element removal promotes chronic IFN-gamma expression and autoimmunity in mice. J Autoimmun. 2014;**53**:33-45.

52. Ogilvie RL, Sternjohn JR, Rattenbacher B, et al. Tristetraprolin mediates interferon-gamma mRNA decay. The Journal of biological chemistry. 2009;**284**(17):11216-23.

53. Carballo E, Lai WS, Blackshear PJ. Feedback inhibition of macrophage tumor necrosis factor-alpha production by tristetraprolin. Science. 1998;**281**(5379):1001-5.

54. Akira S. Regnase-1, a ribonuclease involved in the regulation of immune responses. Cold Spring Harb Symp Quant Biol. 2013;**78**:51-60.

55. Chen CY, Gherzi R, Ong SE, et al. AU binding proteins recruit the exosome to degrade ARE-containing mRNAs. Cell. 2001;**107**(4):451-64.

56. Sandler H, Kreth J, Timmers HT, Stoecklin G. Not1 mediates recruitment of the deadenylase Caf1 to mRNAs targeted for degradation by tristetraprolin. Nucleic acids research. 2011;39(10):4373-86.

57. Taylor GA, Carballo E, Lee DM, et al. A pathogenetic role for TNF alpha in the syndrome of cachexia, arthritis, and autoimmunity resulting from tristetraprolin (TTP) deficiency. Immunity. 1996;**4**(5):445-54.

58. Patial S, Curtis AD, Lai WS, et al. Enhanced stability of tristetraprolin mRNA protects mice against immune-mediated inflammatory pathologies. Proceedings of the National Academy of Sciences of the United States of America. 2016;**113**(7):1865-70.

59. Levine TD, Gao F, King PH, Andrews LG, Keene JD. Hel-N1: an autoimmune RNA-binding protein with specificity for 3' uridylate-rich untranslated regions of growth factor mRNAs. Molecular and cellular biology. 1993;**13**(6):3494-504.

60. Lebedeva S, Jens M, Theil K, et al. Transcriptome-wide analysis of regulatory interactions of the RNA-binding protein HuR. Molecular cell. 2011;**43**(3):340-52.

61. Mukherjee N, Corcoran DL, Nusbaum JD, et al. Integrative regulatory mapping indicates that the RNA-binding protein HuR couples pre-mRNA processing and mRNA stability. Molecular cell. 2011;**43**(3):327-39.

62. Katsanou V, Papadaki O, Milatos S, et al. HuR as a negative posttranscriptional modulator in inflammation. Molecular cell. 2005;**19**(6):777-89.

63. Yiakouvaki A, Dimitriou M, Karakasiliotis I, Eftychi C, Theocharis S, Kontoyiannis DL. Myeloid cell expression of the RNA-binding protein HuR protects mice from pathologic inflammation and colorectal carcinogenesis. The Journal of clinical investigation. 2012;**122**(1):48-61.

64. Tiedje C, Ronkina N, Tehrani M, et al. The p38/MK2-driven exchange between tristetraprolin and HuR regulates AU-rich element-dependent translation. PLoS Genet. 2012;**8**(9):e1002977.

65. Vinuesa CG, Cook MC, Angelucci C, et al. A RING-type ubiquitin ligase family member required to repress follicular helper T cells and autoimmunity. Nature. 2005;**435**(7041):452-8.

66. Jeltsch KM, Heissmeyer V. Regulation of T cell signaling and autoimmunity by RNA-binding proteins. Curr Opin Immunol. 2016;**39**:127-35.

67. Vogel KU, Edelmann SL, Jeltsch KM, et al. Roquin paralogs 1 and 2 redundantly repress the Icos and Ox40 costimulator mRNAs and control follicular helper T cell differentiation. Immunity. 2013;**38**(4):655-68.

68. Bertossi A, Aichinger M, Sansonetti P, et al. Loss of Roquin induces early death and immune deregulation but not autoimmunity. The Journal of experimental medicine. 2011;**208**(9):1749-56.

69. Jeltsch KM, Hu D, Brenner S, et al. Cleavage of roquin and regnase-1 by the paracaspase MALT1 releases their cooperatively repressed targets to promote T(H)17 differentiation. Nature immunology. 2014;**15**(11):1079-89.

70. Essig K, Hu D, Guimaraes JC, et al. Roquin Suppresses the PI3K-mTOR Signaling Pathway to Inhibit T Helper Cell Differentiation and Conversion of Treg to Tfr Cells. Immunity. 2017;**47**(6):1067-82 e12.

71. Tan D, Zhou M, Kiledjian M, Tong L. The ROQ domain of Roquin recognizes mRNA constitutive-decay element and double-stranded RNA. Nat Struct Mol Biol. 2014;**21**(8):679-85.

72. Schlundt A, Heinz GA, Janowski R, et al. Structural basis for RNA recognition in roquin-mediated post-transcriptional gene regulation. Nat Struct Mol Biol. 2014;**21**(8):671-8.

73. Leppek K, Schott J, Reitter S, Poetz F, Hammond MC, Stoecklin G. Roquin promotes constitutive mRNA decay via a conserved class of stem-loop recognition motifs. Cell. 2013;**153**(4):869-81.

74. Matsushita K, Takeuchi O, Standley DM, et al. Zc3h12a is an RNase essential for controlling immune responses by regulating mRNA decay. Nature. 2009;**458**(7242):1185-90.

75. Uehata T, Iwasaki H, Vandenbon A, et al. Malt1-induced cleavage of regnase-1 in CD4(+) helper T cells regulates immune activation. Cell. 2013;**153**(5):1036-49.

76. Liang J, Saad Y, Lei T, et al. MCP-induced protein 1 deubiquitinates TRAF proteins and negatively regulates JNK and NF-kappaB signaling. The Journal of experimental medicine. 2010;**207**(13):2959-73.

77. Xu J, Peng W, Sun Y, et al. Structural study of MCPIP1 N-terminal conserved domain reveals a PIN-like RNase. Nucleic acids research. 2012;**40**(14):6957-65.

78. Schmiedel BJ, Singh D, Madrigal A, et al. Impact of Genetic Polymorphisms on Human Immune Cell Gene Expression. Cell. 2018;**175**(6):1701-15 e16.

79. von Gamm M, Schaub A, Jones AN, et al. Immune homeostasis and regulation of the interferon pathway require myeloid-derived Regnase-3. The Journal of experimental medicine. 2019;**216**(7):1700-23.

80. Minagawa K, Wakahashi K, Kawano H, et al. Posttranscriptional modulation of cytokine production in T cells for the regulation of excessive inflammation by TFL. Journal of immunology. 2014;**192**(4):1512-24.

81. Tong J, Cao G, Zhang T, et al. m(6)A mRNA methylation sustains Treg suppressive functions. Cell research. 2018 Feb;**28**(2):253-256.

82. Li HB, Tong J, Zhu S, et al. m(6)A mRNA methylation controls T cell homeostasis by targeting the IL-7/STAT5/SOCS pathways. Nature. 2017;**548**(7667):338-42.

83. Han D, Liu J, Chen C, et al. Anti-tumour immunity controlled through mRNA m(6)A methylation and YTHDF1 in dendritic cells. Nature. 2019;**566**(7743):270-4.

84. Zaccara S, Jaffrey SR. A Unified Model for the Function of YTHDF Proteins in Regulating m(6)A-Modified mRNA. Cell. 2020;**181**(7):1582-95 e18.

85. Lasman L, Krupalnik V, Viukov S, et al. Context-dependent functional compensation between Ythdf m(6)A reader proteins. Genes & development. 2020.

86. Baumjohann D, Ansel KM. MicroRNA-mediated regulation of T helper cell differentiation and plasticity. Nature reviews Immunology. 2013;**13**(9):666-78.

87. Monticelli S. MicroRNAs in T helper cell differentiation and plasticity. Semin Immunol. 2013;**25**(4):291-8.

88. Rossi RL, Rossetti G, Wenandy L, et al. Distinct microRNA signatures in human lymphocyte subsets and enforcement of the naive state in CD4+ T cells by the microRNA miR-125b. Nature immunology. 2011;**12**(8):796-803.

89. Jens M, Rajewsky N. Competition between target sites of regulators shapes post-transcriptional gene regulation. Nat Rev Genet. 2015;**16**(2):113-26.

90. Denzler R, McGeary SE, Title AC, Agarwal V, Bartel DP, Stoffel M. Impact of MicroRNA Levels, Target-Site Complementarity, and Cooperativity on Competing Endogenous RNA-Regulated Gene Expression. Molecular cell. 2016;**64**(3):565-79.

91. Mele F, Basso C, Leoni C, et al. ERK phosphorylation and miR-181a expression modulate activation of human memory TH17 cells. Nature communications. 2015;**6**:6431.

92. O'Connell RM, Kahn D, Gibson WS, et al. MicroRNA-155 promotes autoimmune inflammation by enhancing inflammatory T cell development. Immunity. 2010;**33**(4):607-19.

93. Thai TH, Calado DP, Casola S, et al. Regulation of the germinal center response by microRNA-155. Science. 2007;**316**(5824):604-8.

94. Taganov KD, Boldin MP, Chang KJ, Baltimore D. NF-kappaB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. Proceedings of the National Academy of Sciences of the United States of America. 2006;**103**(33):12481-6.

95. Yang L, Boldin MP, Yu Y, et al. miR-146a controls the resolution of T cell responses in mice. The Journal of experimental medicine. 2012;**209**(9):1655-70.

96. Srivastava M, Duan G, Kershaw NJ, et al. Roquin binds microRNA-146a and Argonaute2 to regulate microRNA homeostasis. Nature communications. 2015;**6**:6253.

97. de Candia P, Torri A, Gorletta T, et al. Intracellular modulation, extracellular disposal and serum increase of MiR-150 mark lymphocyte activation. PLoS One. 2013;**8**(9):e75348.

98. Torri A, Carpi D, Bulgheroni E, et al. Extracellular MicroRNA Signature of Human Helper T Cell Subsets in Health and Autoimmunity. J Biol Chem. 2017;**292**(7):2903-15.

99. Chiou NT, Kageyama R, Ansel KM. Selective Export into Extracellular Vesicles and Function of tRNA Fragments during T Cell Activation. Cell Rep. 2018;**25**(12):3356-70 e4.

100. Villarroya-Beltri C, Gutierrez-Vazquez C, Sanchez-Cabo F, et al. Sumoylated hnRNPA2B1 controls the sorting of miRNAs into exosomes through binding to specific motifs. Nature communications. 2013;**4**:2980.

101. Lu YC, Chang SH, Hafner M, et al. ELAVL1 modulates transcriptome-wide miRNA binding in murine macrophages. Cell Rep. 2014;**9**(6):2330-43.

102. June CH, Warshauer JT, Bluestone JA. Is autoimmunity the Achilles' heel of cancer immunotherapy? Nat Med. 2017;**23**(5):540-7.

103. Sharma P, Allison JP. Dissecting the mechanisms of immune checkpoint therapy. Nat Rev Immunol. 2020;**20**(2):75-6.

104. van der Leun AM, Thommen DS, Schumacher TN. CD8(+) T cell states in human cancer: insights from single-cell analysis. Nat Rev Cancer. 2020;**20**(4):218-32.

105. Wei J, Long L, Zheng W, et al. Targeting REGNASE-1 programs long-lived effector T cells for cancer therapy. Nature. 2019;**576**(7787):471-6.

106. Zhang L, Yu X, Zheng L, et al. Lineage tracking reveals dynamic relationships of T cells in colorectal cancer. Nature. 2018;**564**(7735):268-72.

107. Giat E, Ehrenfeld M, Shoenfeld Y. Cancer and autoimmune diseases. Autoimmunity reviews. 2017;**16**(10):1049-57.

108. Page N, Klimek B, De Roo M, et al. Expression of the DNA-Binding Factor TOX Promotes the Encephalitogenic Potential of Microbe-Induced Autoreactive CD8(+) T Cells. Immunity. 2018;**48**(5):937-50 e8.

109. Hueber W, Patel DD, Dryja T, et al. Effects of AIN457, a fully human antibody to interleukin-17A, on psoriasis, rheumatoid arthritis, and uveitis. Science translational medicine. 2010;**2**(52):52ra72.

110. Zhao J, Chen X, Herjan T, Li X. The role of interleukin-17 in tumor development and progression. The Journal of experimental medicine. 2020;**217**(1).

111. McGeachy MJ, Cua DJ, Gaffen SL. The IL-17 Family of Cytokines in Health and Disease. Immunity. 2019;**50**(4):892-906.

112. Masuda K, Ripley B, Nyati KK, et al. Arid5a regulates naive CD4+ T cell fate through selective stabilization of Stat3 mRNA. The Journal of experimental medicine. 2016;**213**(4):605-19.

113. Chen J, Cascio J, Magee JD, et al. Posttranscriptional gene regulation of IL-17 by the RNA-binding protein HuR is required for initiation of experimental autoimmune encephalomyelitis. Journal of immunology. 2013;**191**(11):5441-50.

114. Rupaimoole R, Slack FJ. MicroRNA therapeutics: towards a new era for the management of cancer and other diseases. Nat Rev Drug Discov. 2017;**16**(3):203-22.

115. Su YL, Wang X, Mann M, et al. Myeloid cell-targeted miR-146a mimic inhibits NF-kappaB-driven inflammation and leukemia progression in vivo. Blood. 2020;**135**(3):167-80.

116. Buckley CD, Gilroy DW, Serhan CN, Stockinger B, Tak PP. The resolution of inflammation. Nature reviews Immunology. 2013;**13**(1):59-66.

117. Bluestone JA, Anderson M. Tolerance in the Age of Immunotherapy. N Engl J Med. 2020;**383**(12):1156-66.

118. Yosef N, Shalek AK, Gaublomme JT, et al. Dynamic regulatory network controlling TH17 cell differentiation. Nature. 2013;**496**(7446):461-8.

119. Raghavan A, Robison RL, McNabb J, Miller CR, Williams DA, Bohjanen PR. HuA and tristetraprolin are induced following T cell activation and display distinct but overlapping RNA binding specificities. The Journal of biological chemistry. 2001;**276**(51):47958-65.

120. Mino T, Iwai N, Endo M, et al. Translation-dependent unwinding of stem-loops by UPF1 licenses Regnase-1 to degrade inflammatory mRNAs. Nucleic acids research. 2019;**47**(16):8838-59.

121. Wawro M, Wawro K, Kochan J, et al. ZC3H12B/MCPIP2, a new active member of the ZC3H12 family. RNA. 2019;**25**(7):840-56.

122. Huang S, Liu S, Fu JJ, et al. Monocyte Chemotactic Protein-induced Protein 1 and 4 Form a Complex but Act Independently in Regulation of Interleukin-6 mRNA Degradation. The Journal of biological chemistry. 2015;**290**(34):20782-92.

123. Wawro M, Kochan J, Krzanik S, Jura J, Kasza A. Intact NYN/PIN-Like Domain is Crucial for the Degradation of Inflammation-Related Transcripts by ZC3H12D. Journal of cellular biochemistry. 2017;**118**(3):487-98.

124. Glasmacher E, Hoefig KP, Vogel KU, et al. Roquin binds inducible costimulator mRNA and effectors of mRNA decay to induce microRNA-independent post-transcriptional repression. Nature immunology. 2010;**11**(8):725-33.

125. Zhang Q, Fan L, Hou F, Dong A, Wang YX, Tong Y. New Insights into the RNA-Binding and E3 Ubiquitin Ligase Activities of Roquins. Sci Rep. 2015;**5**:15660.

126. Maruyama T, Araki T, Kawarazaki Y, et al. Roquin-2 promotes ubiquitin-mediated degradation of ASK1 to regulate stress responses. Sci Signal. 2014;**7**(309):ra8.

127. Fenger-Gron M, Fillman C, Norrild B, Lykke-Andersen J. Multiple processing body factors and the ARE binding protein TTP activate mRNA decapping. Molecular cell. 2005;**20**(6):905-15.

128. Carballo E, Lai WS, Blackshear PJ. Evidence that tristetraprolin is a physiological regulator of granulocyte-macrophage colony-stimulating factor messenger RNA deadenylation and stability. Blood. 2000;**95**(6):1891-9.

129. Jing Q, Huang S, Guth S, et al. Involvement of microRNA in AU-rich element-mediated mRNA instability. Cell. 2005;**120**(5):623-34.

130. Durie D, Lewis SM, Liwak U, Kisilewicz M, Gorospe M, Holcik M. RNA-binding protein HuR mediates cytoprotection through stimulation of XIAP translation. Oncogene. 2011;**30**(12):1460-9.

131. Valencia-Sanchez MA, Liu J, Hannon GJ, Parker R. Control of translation and mRNA degradation by miRNAs and siRNAs. Genes & development. 2006;**20**(5):515-24.

132. Kawai T, Lal A, Yang X, Galban S, Mazan-Mamczarz K, Gorospe M. Translational control of cytochrome c by RNA-binding proteins TIA-1 and HuR. Molecular and cellular biology. 2006;**26**(8):3295-307.

133. Masuda K, Ripley B, Nishimura R, et al. Arid5a controls IL-6 mRNA stability, which contributes to elevation of IL-6 level in vivo. Proceedings of the National Academy of Sciences of the United States of America. 2013;**110**(23):9409-14.

134. Mino T, Murakawa Y, Fukao A, et al. Regnase-1 and Roquin Regulate a Common Element in Inflammatory mRNAs by Spatiotemporally Distinct Mechanisms. Cell. 2015;**161**(5):1058-73.

135. Lai WS, Carballo E, Strum JR, Kennington EA, Phillips RS, Blackshear PJ. Evidence that tristetraprolin binds to AU-rich elements and promotes the deadenylation and destabilization of tumor necrosis factor alpha mRNA. Molecular and cellular biology. 1999;**19**(6):4311-23.

136. Myer VE, Fan XC, Steitz JA. Identification of HuR as a protein implicated in AUUUA-mediated mRNA decay. The EMBO journal. 1997;**16**(8):2130-9.

137. Hanieh H, Masuda K, Metwally H, et al. Arid5a stabilizes OX40 mRNA in murine CD4(+) T cells by recognizing a stem-loop structure in its 3'UTR. European journal of immunology. 2018;**48**(4):593-604.

138. Li M, Cao W, Liu H, et al. MCPIP1 down-regulates IL-2 expression through an ARE-independent pathway. PloS one. 2012;**7**(11):e49841.

139. Zhang H, Wang WC, Chen JK, et al. ZC3H12D attenuated inflammation responses by reducing mRNA stability of proinflammatory genes. Molecular immunology. 2015;**67**(2 Pt B):206-12.

140. Ogilvie RL, Abelson M, Hau HH, Vlasova I, Blackshear PJ, Bohjanen PR. Tristetraprolin down-regulates IL-2 gene expression through AU-rich element-mediated mRNA decay. Journal of immunology. 2005;**174**(2):953-61.

141. Lee HH, Yoon NA, Vo MT, et al. Tristetraprolin down-regulates IL-17 through mRNA destabilization. FEBS Lett. 2012;**586**(1):41-6.

142. Kim HH, Kuwano Y, Srikantan S, Lee EK, Martindale JL, Gorospe M. HuR recruits let-7/RISC to repress c-Myc expression. Genes & development. 2009;**23**(15):1743-8.

143. Yeh CH, Hung LY, Hsu C, et al. RNA-binding protein HuR interacts with thrombomodulin 5'untranslated region and represses internal ribosome entry site-mediated translation under IL-1 beta treatment. Mol Biol Cell. 2008;**19**(9):3812-22.

144. Kuwano Y, Kim HH, Abdelmohsen K, et al. MKP-1 mRNA stabilization and translational control by RNA-binding proteins HuR and NF90. Molecular and cellular biology. 2008;**28**(14):4562-75.

145. Pullmann R, Jr., Kim HH, Abdelmohsen K, et al. Analysis of turnover and translation regulatory RNA-binding protein expression through binding to cognate mRNAs. Molecular and cellular biology. 2007;**27**(18):6265-78.

146. Lin FY, Chen YH, Lin YW, et al. The role of human antigen R, an RNA-binding protein, in mediating the stabilization of toll-like receptor 4 mRNA induced by endotoxin: a novel mechanism involved in vascular inflammation. Arterioscler Thromb Vasc Biol. 2006;**26**(12):2622-9.

147. Ford LP, Watson J, Keene JD, Wilusz J. ELAV proteins stabilize deadenylated intermediates in a novel in vitro mRNA deadenylation/degradation system. Genes & development. 1999;**13**(2):188-201.

148. Ma WJ, Chung S, Furneaux H. The Elav-like proteins bind to AU-rich elements and to the poly(A) tail of mRNA. Nucleic acids research. 1997;**25**(18):3564-9.

149. Amatya N, Childs EE, Cruz JA, et al. IL-17 integrates multiple self-reinforcing, feed-forward mechanisms through the RNA binding protein Arid5a. Sci Signal. 2018;**11**(551).

150. Iwasaki H, Takeuchi O, Teraguchi S, et al. The IkappaB kinase complex regulates the stability of cytokine-encoding mRNA induced by TLR-IL-1R by controlling degradation of regnase-1. Nature immunology. 2011;**12**(12):1167-75.

151. Huang L, Yu Z, Zhang Z, Ma W, Song S, Huang G. Interaction with Pyruvate Kinase M2 Destabilizes Tristetraprolin by Proteasome Degradation and Regulates Cell Proliferation in Breast Cancer. Sci Rep. 2016;**6**:22449.

152. Brook M, Tchen CR, Santalucia T, et al. Posttranslational regulation of tristetraprolin subcellular localization and protein stability by p38 mitogen-activated protein kinase and extracellular signal-regulated kinase pathways. Molecular and cellular biology. 2006;**26**(6):2408-18.

153. von Roretz C, Gallouzi IE. Protein kinase RNA/FADD/caspase-8 pathway mediates the proapoptotic activity of the RNA-binding protein human antigen R (HuR). The Journal of biological chemistry. 2010;**285**(22):16806-13.

154. Guha A, Ahuja D, Das Mandal S, et al. Integrated Regulation of HuR by Translation Repression and Protein Degradation Determines Pulsatile Expression of p53 Under DNA Damage. iScience. 2019;**15**:342-59.

155. Nyati KK, Masuda K, Zaman MM, et al. TLR4-induced NF-kappaB and MAPK signaling regulate the IL-6 mRNA stabilizing protein Arid5a. Nucleic acids research. 2017;**45**(5):2687-703.

156. Katsanou V, Milatos S, Yiakouvaki A, et al. The RNA-binding protein Elavl1/HuR is essential for placental branching morphogenesis and embryonic development. Molecular and cellular biology. 2009;**29**(10):2762-76.

157. Ghosh M, Aguila HL, Michaud J, et al. Essential role of the RNA-binding protein HuR in progenitor cell survival in mice. The Journal of clinical investigation. 2009;**119**(12):3530-43.

158. Rozwarski DA, Diederichs K, Hecht R, Boone T, Karplus PA. Refined crystal structure and mutagenesis of human granulocyte-macrophage colony-stimulating factor. Proteins. 1996;**26**(3):304-13.

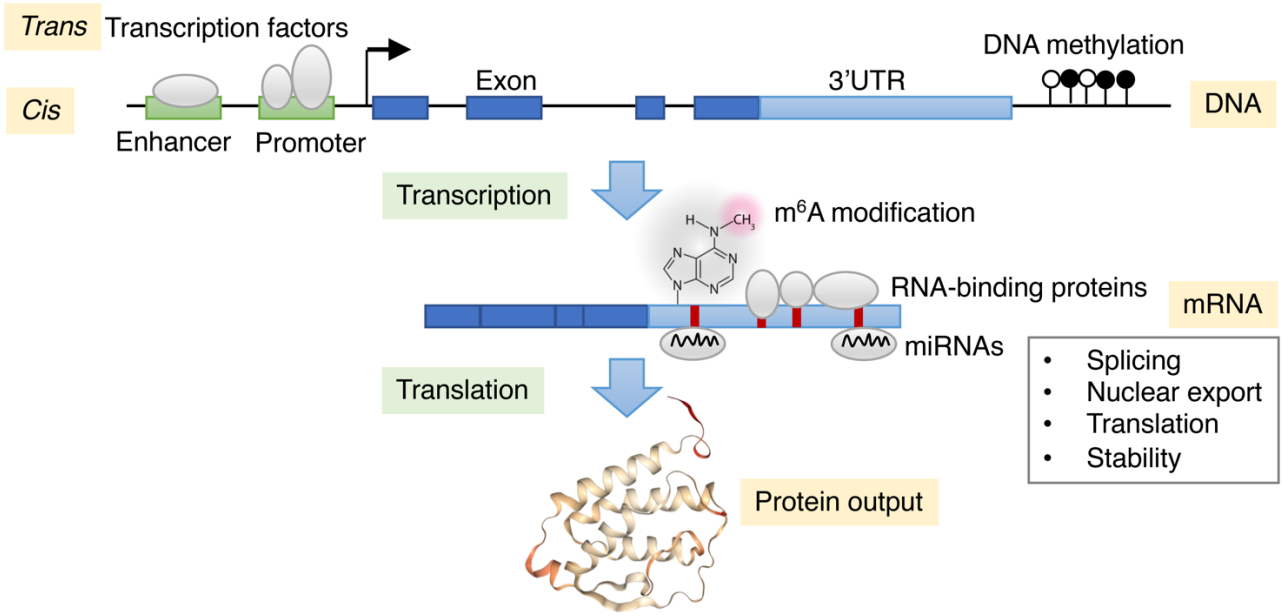

Figure 1. An integrated regulatory network in the transcriptional and post-transcriptional control of gene expression. Gene expression is a complex process with many regulatory layers. Transcription is controlled by *trans*-acting elements (e.g. transcription factors) binding to *cis* regulatory regions on the DNA (e.g. promoters and enhancers), but also by DNA methylation, histone modifications (not depicted) and in general all the mechanisms that influence chromatin topology and DNA accessibility. Once an mRNA transcript is generated, its maturation, intracellular localization, stability and translation are regulated by an array of RBPs, possibly acting in a cooperative or antagonistic, redundant or unique manner, and potentially also with modes of action that are defined in time and space by environmental triggers. All of these mechanisms (and more that are not depicted for simplicity), contribute to modulate the final output on protein synthesis. Arrow: transcriptional start site; open and closed circles, unmethylated and methylated cytosines in DNA; red vertical lines: RBP binding regions; the protein shown is human GM-CSF (image from the RCSB PDB (rcsb.org), PDB ID 2GMF (158)).

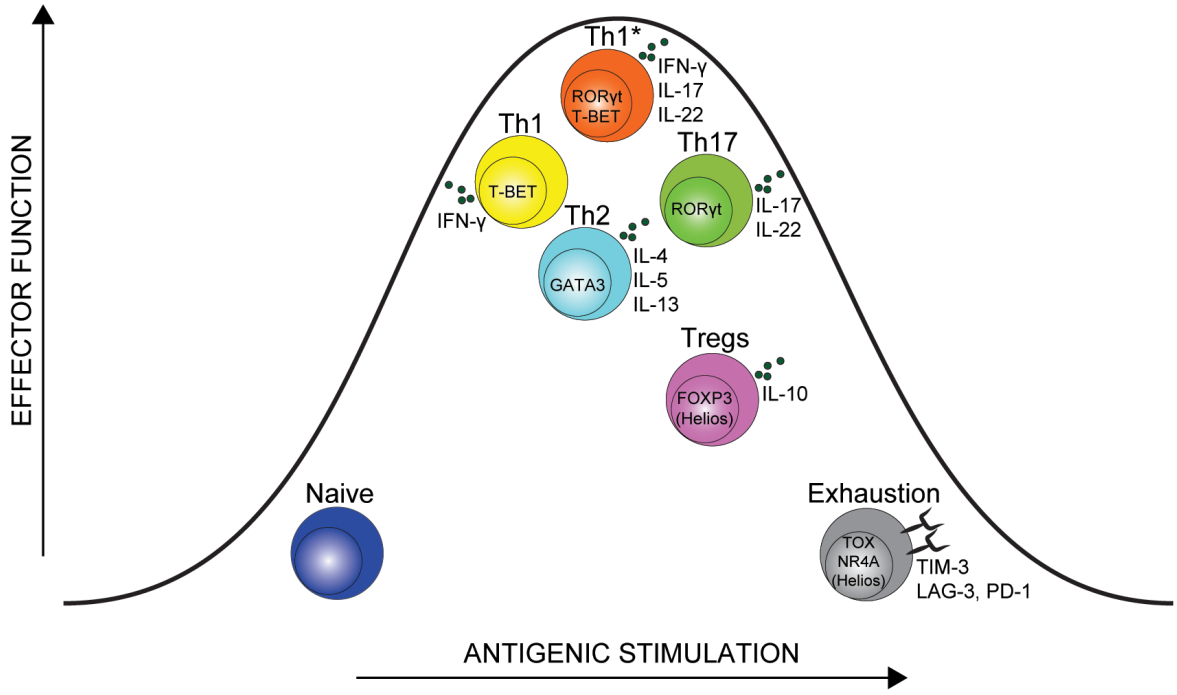

Figure 2. T helper lymphocyte differentiation upon antigenic stimulation. Following TCR engagement, T lymphocytes differentiate toward effector subsets characterized by specific functions, determined by a finely tuned transcriptional profile. Specifically, Th1, Th17 and Th1* T cell subsets display a more inflammatory profile, characterized by the expression, among others, of the transcription factors T-BET and RORγt and the cytokines IFN-γ, IL-17 and IL-22. Similarly, Th2 cells are characterized by GATA3 and IL-4 expression, while the hallmark of Treg cells is the expression of the transcription factor FOXP3. However, chronic TCR stimulation may lead to the loss of functional effector responses and to the induction of inhibitory surface receptors such as PD-1, LAG-3 and TIM-3. The transcription factors TOX and NR4A recently emerged as key factors associated with the exhausted phenotype, while Helios expression was associated to a subset of Treg cells and chronically activated $CD4^+$, but not $CD8^+$ T cells.

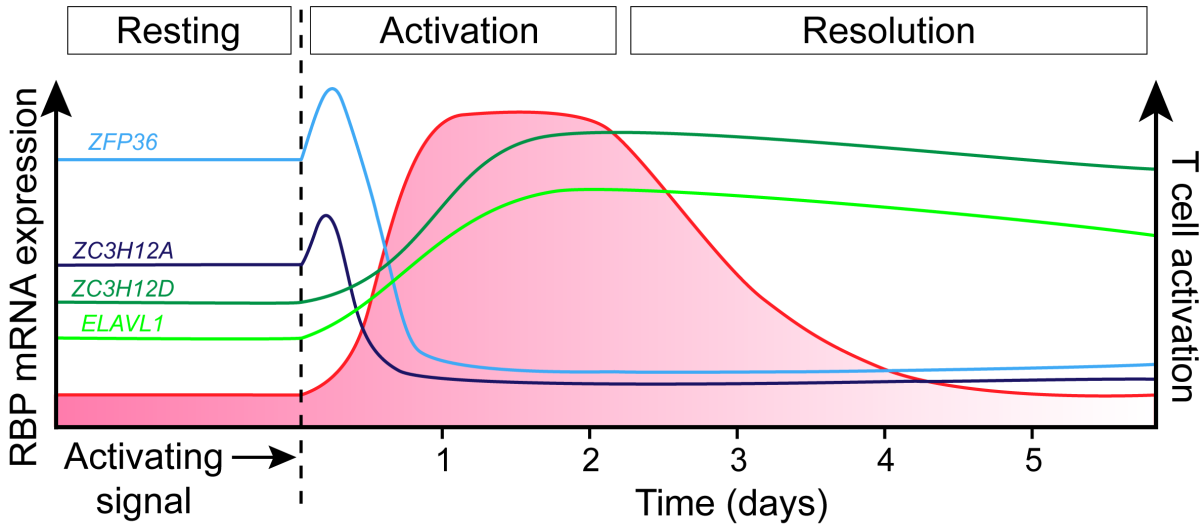

Figure 3. Dynamic expression of specific RBPs at different stages of T cell activation. Following T cell activation, the expression of selected RBPs relevant for the regulation of inflammation exhibit dynamic changes in mRNA expression. Specifically, based on our own data (14) and on the Database of Immune Cells (DICE) (78), *ZFP36* (TTP) was the most highly expressed RBP in resting lymphocytes, followed by *ZC3H12A* (Regnase-1), *ZC3H12D* (Regnase-4) and *ELAVL1* (HuR). Upon stimulation, *ZFP36* and *ZC3H12A* are transiently upregulated, at least at the mRNA level, followed by a strong downregulation, thereby potentially enabling cells to produce high levels of cytokines. In contrast, *ZC3H12D* and *ELAVL1* are gradually induced during the course of activation. Expression data for activated T cells were based on microarray data published in (118), as well as our own unpublished data. Similar changes in expression upon activation (downregulated TTP and Regnase-1, upregulated Regnase-4 and HuR) were reported also at protein level (75, 80, 119).

Table 1. Comparison of selected RBPs with a regulatory role in T lymphocytes.

	Anti-inflammatory RBPs								Pro-inflammatory RBPs
	Regnase-1	**Regnase-2**	**Regnase-3**	**Regnase-4**	**Roquin-1/2**	**TTP**	**HuR**		**ARID5A**
Encoding gene	*ZC3H12A*	*ZC3H12B*	*ZC3H12C*	*ZC3H12D*	*RC3H1/2*	*ZFP36*	*ELAVL1*		*ARID5A*
Mechanism of action	RNase activity Catalyzed by four aspartic acid residues (77, 120) Deubiquitinase activity catalyzed by at least one cysteine residue (76)	Probable RNase, aspartic acid residues conserved (121) Deubiquitinase activity unknown, cysteine residue conserved	Unknown, aspartic acid and cysteine residues conserved	Probable RNase, aspartic acid residues conserved (122, 123) Deubiquitinase activity unknown, cysteine residue conserved	Recruitment of CCR4-NOT and EDC4 for deadenylation and decapping (73, 124) Ubiquitination of targets (125, 126)	Recruitment of CCR4-NOT and DCP1/2 for deadenylation and decapping, recruitment of exosome and RISC (55, 56, 127-129)	Promotes translation by binding to 5'UTR mRNA (130) Blocks binding of the silencing complex RISC (131) Recruits translational inhibitor TIA-1 (132)		Competes with Regnase-1 and Roquin-1/2 for *cis* element (133)
Cis-acting element	Stem loop (74)	Stem loop (121)	Not described	Stem loop (123)	Stem loop (73, 134)	ARE (135)	ARE (136)		Stem loop (137)
Targeted mRNAs	*IL1B, IL12B, IL2, IL6, ICOS, INOS, TNF, TNFRSF4 (OX40), CTLA4, CREL, CCL2, NFKBID, NFKBIZ, IRF4, TFRC, PTGS2 (COX2), ZC3H12A* (74, 75, 120, 138)	*IL6, IER3, ZC3H12A* (121)	*VCAM1, ICAM1, LECAM2, IL-8, CCL2, ZC3H12A* (79, 121)	*CFOS, IL1B, IL10, IL17A, IL2, IL2, IL6, IER3, INOS, NFKBIZ, TNF, ZC3H12A* (75, 80, 122, 123, 139)	*ICOS, NKFBIZ, NKFBID, TNF, TNFRSF4, PTGS2* (67, 73, 134)	*IL1B, L2, IL3, L6, IL10, IL27, TNF, TTP, IL17A, CSF2, CXCL1, IFNG, PTGS2, ZPF36* (67, 140, 141)	*CDKN1B, IGF1R, THBD, WNT5A, MYC* (142)	*CFOS, IL6, ELAVL1, CSF2, TGFB, TNF, TLR4, PTGS2* (143-148)	*IL6, TBX21, TNFRSF4, STAT3, CXCL1, CXCL5, NFKBIZ* (112, 149)
Regulation	Cleaved by MALT1 paracaspase at site of one arginine residue (69, 75) Proteasomal degradation upon phosphorylation of DSGXXS motif (150)	Unknown; arginine residue conserved, DSGXXS motif not conserved	Proteasome degradation, but mechanism unknown, DSGXXS motif not conserved, arginine residue conserved (79)	Unknown; arginine residue conserved, DSGXXS motif not conserved	Cleaved by MALT1 paracaspase at site of one arginine residue (69)	Proteasomal degradation upon interaction with pyruvate kinase M2 (151, 152)	Cleaved by caspase-3/8 (153) Proteasomal degradation mediated by E3-ubiquitin-ligase TRIM13 (154)		Proteasomal degradation upon phosphorylation by MAPK (155)
Knockout mouse phenotype	Systemic autoimmunity, lethal within 12 weeks after birth (74, 75)	Unknown	Macrophage-dependent sublethal lympha-denopathy (79)	No effect under steady state; severe paralysis with induced EAE (75)	Perinatal lethality (68)	Systemic inflammation, TNFα-mediated cachexia, dermatitis, arthritis (57)	Embryonic lethality; atrophy of bone marrow, thymus (156, 157)		Resistant to EAE induction (133)