

# microRNA Regulation of Inflammatory Responses

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## Abstract

The mammalian inflammatory response is a rapid and complex physiological reaction to noxious stimuli including microbial pathogens. Although inflammation plays a valuable role in combating infection, its dysregulation often occurs in people and can cause a variety of pathologies, ranging from chronic inflammation, to autoimmunity, to cancer. In recent years, our understanding of both the cellular and molecular networks that regulate inflammation has improved dramatically. Although much of the focus has been on the study of protein regulators of inflammation, recent evidence also points to a critical role for a specific class of noncoding RNAs, called microRNAs (miRNAs), in managing certain features of the inflammatory process. In this review, we discuss recent advances in our understanding of miRNAs and their connection to inflammatory responses. Additionally, we consider the link between perturbations in miRNA levels and the onset of human inflammatory diseases.

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**Toll-like receptors**

**(TLRs):** a family of receptors that recognize molecules produced by microorganisms and whose signaling induces immune responses

**microRNA (miRNA):**

small single-stranded oligonucleotides that repress gene expression post-transcriptionally

**RNA-induced silencing complex**

**(RISC):** a protein complex that mediates repression of messenger RNA (mRNA) translation

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## INTRODUCTION

The often distinct clinical manifestations of chronic inflammation have allowed recognition of this process in humans for thousands of years (1). Some of the earliest reports on record were made by a Roman physician named Cornelius Celsus during the first century CE. He described patient symptoms of redness and swelling with heat and pain. Many centuries later, scientists began to advance our understanding of the physiological and cellular basis of inflammation. White blood cells and plasma components were found to emigrate from dilated blood vessels and enter into inflamed tissues. These inflammatory cells and other factors were not only important for host defense against pathogens, but also recognized by Elie Metchnikoff to play beneficial roles in tissue maintenance and homeostasis through the process of phagocytosis (2). However, inflammation can come at the expense of tissue damage and dysfunction, a major negative consequence of both acute and chronic inflammatory conditions.

In addition to these and other seminal discoveries of the inflammatory process, more recent work has shed light on how inflammatory responses are initially triggered. An important first step in this line of inquiry came from the germ theory of disease, pointing to microbes as the cause of human diseases including inflammation. For decades following the acceptance of this theory, many specific microbial components were studied and shown to trigger inflammation. These include such molecules as bacterial lipopolysaccharide (LPS), peptidoglycan, and viral double-stranded RNA. However, it was not until the end of the twentieth century that a major class of host receptors responsible for direct recognition of pathogen-associated molecular patterns (PAMPs) were identified and called Toll-like receptors (TLRs) (3, 4).

Like all multicellular organisms, mammals must live in the midst of a variety of microbial species. In some cases, these interactions can be mutually beneficial, as illustrated by commensal bacteria, whereas in other scenarios, microbes

can be pathogenic (5). In fact, the same microbe can be both. Thus, mammals require an immune system that can effectively and selectively survey the microbes they encounter and be ready to unleash a protective or inflammatory response when a pathogen or pathogenic process is detected. In healthy individuals, this sensitive system is extremely effective at detecting pathogens that are present, even at low levels, and eradicating them before they reach numbers that cause harm. However, the inflammatory response can also become defective and lead to immunodeficiency and susceptibility to infection. Alternatively, hypersensitive responses can cause untimely and unnecessarily high degrees of inflammation and host tissue damage (6). Therefore, the molecular networks that control the initiation, peak magnitude, and resolution of inflammation must all be properly tuned for optimal health. Recently these have become intense areas of investigation.

In parallel with this emerging interest in the molecular mechanisms that control the magnitude of inflammatory responses, a new and exciting aspect of gene regulation has become apparent in recent years with the discovery of mammalian microRNAs (miRNAs) (7, 8). miRNAs are small, evolutionarily conserved noncoding RNAs that are derived from much larger primary transcripts encoded in the genome. miRNA genes are most commonly transcribed by RNA polymerase II, and in some cases by RNA polymerase III. miRNAs can be found in the introns of protein-coding genes or as independent genes. Furthermore, a primary transcript can contain a single miRNA or multiple miRNAs that are processed out of the same transcript. Following initial processing by Drosha and DGCR8 in the nucleus, the pre-miRNA is exported to the cytoplasm where the miRNA hairpin is cleaved by Dicer resulting in a miRNA duplex. One of the RNA strands is loaded into the RNA-induced silencing complex (RISC) and guides this complex to the 3' untranslated regions (UTRs) of target messenger RNAs (mRNAs) leading to repressed target protein expression. This can occur from a variety of reported mechanisms including

decreased mRNA stability due to deadenylation and uncapping, or via inhibition of translation.

Approximately 500–1,000 miRNAs are expressed in human cells, and their expression signatures vary depending on the tissue and cell types examined. The first evidence that miRNAs may be important in mammalian physiology came with the observation that their expression levels are substantially perturbed in cancer cells (9). Since these initial findings, many studies have shown through both gain and loss of function approaches that miRNAs are indeed meaningful regulators of cellular function in a variety of different organ systems (10, 11). At the molecular level, miRNAs have been shown to impact target gene expression anywhere from 1.2- to 4-fold, indicating that they do not function as on-off switches for genes, but instead modulate and fine-tune expression levels of key regulatory proteins. Furthermore, miRNAs allow for more consistent protein expression levels between cells and species by limiting transcriptional noise, and this is hypothesized to have allowed for more complex phenotypes to evolve (12). From the perspective of inflammatory responses, miRNAs have recently been shown to be expressed in immune cells, to target proteins involved in regulating inflammation, and consequently to affect the magnitude of the response (13).

This review highlights some of the emerging concepts associated with miRNA control of inflammation and discusses their connection to human diseases impacted by inflammatory pathways. Overall, there is increasing evidence that miRNAs function as an effective system to regulate the magnitude of inflammatory responses through their effects on cellular development and acute cellular function.

### **microRNA BIOGENESIS AND FUNCTION AT THE MOLECULAR LEVEL DURING INFLAMMATION**

Following the exposure of immune cells to inflammatory cues, intracellular signals are sent to their nuclei that result in dynamic

transcriptional changes. This process culminates with the up- or downregulation of hundreds of immune response genes, an essential first step in coordinating inflammatory responses. As do protein-coding genes, miRNA-producing transcripts also change expression during this process. Among these, miR-155 and miR-146a were originally identified as inflammatory response miRNAs that are upregulated by NF- $\kappa$ B (14–16). Since these initial observations, several other miRNAs have also been shown to change their expression levels during inflammation and do so in a variety of different immune cell types (11, 13).

Although changes in miRNA expression during inflammation are controlled at the transcriptional level, there is emerging evidence that protein regulators of the miRNA biogenesis pathways can also be affected during inflammatory responses. A number of proteins have been shown to interact with the miRNA loop region and subsequently regulate processing. Among these, p53 and Smad are activated during inflammatory responses, suggesting that they impact miRNA biogenesis in selective settings (17, 18). KSRP is another loop-binding factor that regulates several miRNAs, including miR-155, and assists in the rapid increase in mature miR-155 levels seen during inflammation (19). Additionally, certain inflammatory cytokines, such as interferons (IFNs), can repress expression of biogenesis factors including Dicer (20). The miRNA sequence may also be altered during inflammation. Adar, an adenosine deaminase, is upregulated during inflammation and can convert A to I when a double-stranded RNA template is present. Consequently, studies have found that Adar can edit adenosines in the miRNA primary transcript sequence, affecting processing and target specificity (21). Taken together, there is growing evidence that the biogenesis of miRNAs is a highly regulated process that is substantially affected by the inflammatory process.

Once loaded into the RISC, mature miRNAs expressed in immune cells preferentially target signaling proteins, transcription factors, and regulators of cell death, leading

to reduced expression of these factors [mechanisms of repression are reviewed thoroughly elsewhere (22)]. These classes of proteins play instrumental roles in how extracellular signals are received and interpreted, and thus relatively modest reductions in their levels affect both the development and functional responses of inflammatory cell subtypes during inflammation. Investigators have questioned whether small differences in protein concentrations can have meaningful effects on physiological processes. One indication of the importance of small differences are cases where heterozygous loss of a gene, causing about a 50% loss of protein, had meaningful physiological consequences. One example is the transcription factor PU.1, a target of miR-155: Mice lacking a single copy of the gene encoding PU.1 show excess production of granulocytes at the expense of monocyte development (23). Another example is that mice heterozygous for the negative signaling regulator SOCS1, also a target of miR-155, display a heightened sensitivity to endotoxemia (24). These examples indicate that the miRNA binding sites found in the 3' UTRs of such genes, which mediate few-fold repressions, can be functionally important for an appropriately tuned inflammatory response. They highlight the need for accurate regulation of dosage-sensitive phenotypes.

The functional relevance of miRNAs expressed by immune cells during various types of inflammatory responses has become apparent over the past couple of years. Examples of how miRNAs impact inflammation at the cellular and organismal levels are provided throughout this review (Table 1).

## **INNATE IMMUNITY AND microRNAs**

Immunity is conventionally divided into two types, innate and adaptive. The former is the evolutionarily older and more widespread process, involving immune cells that respond to classes of pathogens by recognizing molecules found on these invading organisms. Adaptive immunity, mediated mainly by lymphocytes, is a process found only in vertebrates

and involves recognition of species-specific molecules on individual species of invaders. Adaptive immunity, involving clonal selection among lymphocytes, takes longer to develop but is able to completely clear an infection and provide the host with a learned memory, priming secondary responses.

The innate immune response constitutes the first line of defense against invading pathogens and is the primary initiator of inflammatory responses. It is a cellular response, involving monocyte/macrophages, granulocytes, and dendritic cells (DCs), and is initiated largely by the activities of signaling initiated by TLRs that are responsive to PAMPs found on different classes of pathogens (25). Thus, the TLRs mediate recognition of broad classes of pathogens; for example, TLR3 recognizes double-stranded RNA, which is usually associated with viruses, whereas TLR4 recognizes bacterial products, notably LPS (26). The interaction of a TLR with its ligand activates intracellular signaling cascades, of which MyD88-, Trif-, and TIRAP-mediated pathways are particularly important. In general, MyD88-mediated signaling results in transcriptional activation of inflammatory genes and Trif-mediated signaling results in the activation of the IFN response, giving rise to distinct patterns of immune activation at the cellular and organismal level. Both pathways activate NF- $\kappa$ B, a central mediator of the innate immune response leading to inflammation (27). Activation of these pathways must be finely controlled to prevent hyperactivation of the inflammatory response, which is powerful enough to kill the host organism acutely, as observed during bacterial sepsis.

Signaling by TLRs induces expression of not only many proteins but also numerous miRNAs (reviewed in 13), of which we discuss miR-155, miR-146a, and miR-21 in detail. For these miRNAs, two paradigms of function seem to emerge: One is the proinflammatory miRNAs, typified by miR-155, that precisely regulate the levels of their targets to promote the immune response; the other is the negative feedback regulators, typified by miR-146a and miR-21, that mute the immune response.

**Table 1 miRNAs and inflammatory conditions**

Inflammatory condition	miRNA	Cell type <sup>a</sup>	Animal model	Human tissue
Multiple sclerosis	miR-155	Th1 and Th17	x	x
	miR-326	Th17	x	x
	miR-124	Myeloid	x	
Rheumatoid arthritis	miR-155	B cell and Th17	x	x
	miR-223	T cells		
	miR-182	T cells	x	x
	miR-146a	T cells and Macs		x
Systemic lupus erythematosus	miR-146a	T cells	x	
	miR-182	T cells	x	
	miR-17-92	T cells	x	
	miR-21	T cells	x	x
	miR-155	B and T cells	x	
Type 1 diabetes	miR-510	Tregs		x
Type 2 diabetes	miR-146a	PBMCs		x
Sjögren's syndrome	miR-146a	Monocytes	x	x
Atopic dermatitis	miR-155	T cells		x
Allergic inflammation	let-7	T cells	x	
	miR-126	Th2	x	
Inflammatory bowel disease	miR-155		x	
IgA nephropathy	miR-155	Extracellular		x
	miR-146a	Extracellular		x
Endotoxemia	miR-146a	Myeloid	x	
	miR-155	Myeloid	x	
Bacterial infection	miR-155	Myeloid, B, and T cells	x	
Myeloproliferative disorders	miR-125b	HSPCs	x	
	miR-155	HSPCs	x	
	miR-146a		x	

<sup>a</sup>PBMC, peripheral blood mononuclear cell; HSPC, hematopoietic stem/progenitor cell.

miR-155 targets and downregulates the negative regulators SHIP1 and SOCS1, thus leading to increased activation of AKT and IFN response genes (28, 29). These pathways play important roles in mediating cell survival, growth, and migration (29–31), as well as antiviral responses (32). In the absence of miR-155, the expression of SHIP1 and SOCS1 is increased, whereas in miR-155-overexpressing cells, they are further downregulated (28–32). These effects of miR-155 are subtotal but nonetheless have profound effects on the cellular and organismal levels. Mice deficient in miR-155 have decreased immune responses, whereas miR-155-overexpressing mice develop

a myeloproliferation resembling chronic inflammation and hematopoietic cancers in some cases (30, 33–35). Interestingly, hematopoietic reconstitution of mice with bone marrow expressing a small interfering RNA against SHIP1, or genetic deletion of the gene encoding SHIP1, results in a similar hematopoietic phenotype to that caused by sustained expression of miR-155 (29, 36). Deletion of *Socs1* in mice also results in a profound hematopoietic effect, implying that it is part of the miR-155 overexpression phenotype (37). Taken together, these findings suggest that the levels of SHIP1 and SOCS1 are critically important in controlling the inflammatory response and

that SHIP1 and SOCS1 are tightly regulated by miR-155 expression.

miR-146a was also among the first set of miRNAs discovered to play a role in TLR signaling (15). Unlike miR-155, which acts to potentiate the immune response and carry it forward, miR-146a is a negative regulator of the immune response. This functional role for miR-146a occurs through its inhibition of expression of the mRNAs encoding TRAF6 and IRAK1, two proteins that are involved in the transduction of TLR signaling and lead to NF- $\kappa$ B activation (15, 38, 39). During and following innate immune cell activation, miR-146a dampens the production of proinflammatory mediators such as IL-6 and TNF- $\alpha$  (38, 39). miR-146a is also induced during viral infection, pointing to a general role for this miRNA as a feedback negative regulator of the innate immune response (40). The kinetics of this miRNA's action may explain how it is uniquely suited for this role: Being induced by NF- $\kappa$ B, miR-146a prevents the further production of signal transducer proteins. However, TRAF6 and IRAK1 proteins already synthesized will continue to transduce signals, producing a built-in delay to the action of miR-146a that would be predicted to cause a gradual downregulation of the inflammatory signaling cascades in innate immune cells.

More recent data suggest that such negative feedback regulation may also apply to miR-21 (41). miR-21, like miR-146a, is induced by NF- $\kappa$ B during TLR4 induction of macrophages in a MyD88-dependent manner. Once induced, it targets the mRNA encoding PDCD4, a tumor suppressor proinflammatory protein that seems to activate NF- $\kappa$ B by unknown mechanisms. This leads to downregulation of NF- $\kappa$ B signaling and a switch to the so-called anti-inflammatory response, dependent on secretion of IL-10. Once again, the kinetics of PDCD4 degradation show that although miR-21 is rapidly induced and *Pdcd4* mRNA is rapidly downregulated, PDCD4 protein persists and is downregulated in a delayed manner. Similar to what we have suggested for miR-146a, these data provide evidence that miRNAs

can serve as delay switches in negative feedback circuits.

Other miRNAs that are important in controlling innate immune responses include miR-125b and let-7. Both miR-125b and let-7 are downregulated by LPS stimulation of macrophages; miR-125b targets TNF- $\alpha$  mRNA, whereas let-7 targets IL-6 mRNA (42, 43). These miRNAs appear to be inhibited during the proinflammatory phase of the immune response, and this results in derepression of inflammatory cytokine mRNAs and enhanced inflammatory responses.

By targeting signal transduction proteins involved in the transmission of intracellular signals following initial pathogen recognition, and by directly targeting mRNAs that encode specific inflammatory cytokines, miRNAs can have a significant impact on the magnitude of the ensuing inflammatory response.

## ADAPTIVE IMMUNE CELLS AND microRNAs

T lymphocytes play an essential role in both managing and directly carrying out specific types of inflammatory responses. Their activation is dependent upon presentation of specific antigens in the context of major histocompatibility complex by antigen-presenting cells, a function carried out predominately by innate immune cells that have made first contact with the infectious agent. Because antigen-specific T cell responses are part of the second wave of the immune response, they tend to play more prominent roles in chronic inflammatory situations or during secondary responses to an inflammatory cue. Early studies looking at global deletion of miRNAs in T cells, accomplished by deleting Dicer specifically in T cells, revealed a clear involvement of miRNAs in T lymphocyte biology. Dicer-deficient mice had a hypocellular T cell compartment, with the severity being based upon the stage of development when Dicer was removed (44, 45). Since these initial observations, specific miRNAs involved in T cell development, effector, and regulatory functions have been identified.

During thymic development, immature T lymphocytes are selected for further differentiation into mature cells and exit into the periphery if they recognize self-antigens with an appropriate degree of affinity. If antigen binding by a cell's TCR is too weak or strong, then that T cell is eliminated. Recent studies have shown that miR-181a has a significant impact on positive selection by gauging the strength of TCR signaling during thymic development (46). This is accomplished through repression of specific phosphatases that function to dampen the TCR signal. Inhibition of miR-181a led to overt reactions to self-peptides normally sufficient only for positive selection (47). Thus, miR-181a contributes to proper clonal selection and to preventing autoreactive T cell clones from reaching the periphery and potentially causing autoimmunity.

Apoptosis is a common mechanism used during T cell development to get rid of unwanted T cell clones, and miRNAs have been clearly linked to the regulation of apoptosis. In T cells, miRNAs in the miR-17 to miR-92 cluster are involved in managing cell survival by repressing BIM and PTEN, both of which potentiate cell death (48). Overproduction of miRs-17–92 in transgenic mice results in T cell populations that are hyperproliferative and resistant to cell death, yielding a lymphoproliferative disease.

Once mature T cells reach the periphery, if they sense their cognate antigens, they unleash an immune response against that target. A hallmark of T cell-driven inflammation is the proper skewing of mature naive T cells into distinct subtypes with the capacity to elicit unique inflammatory outcomes. For instance, Th2 cells drive allergic inflammation, whereas Th1 cells trigger inflammatory responses intended to eradicate viral and intracellular bacteria infections. There have been several reports that specific miRNAs regulate T cell skewing by targeting specific transcription factors that influence these decisions.

T cells deficient in miR-155, a miRNA expressed in activated CD4<sup>+</sup> T cells, exhibit a Th2 bias under neutral conditions *in vitro* (16, 49).

miR-155<sup>-/-</sup> mice are defective in their capacity to produce Th1 and Th17 cells during autoimmune inflammation. This has been shown in mouse models of experimental autoimmune encephalomyelitis (EAE), colitis, and collagen-induced arthritis (CIA) (34, 50, 51). Although the mechanistic basis for miR-155's role in T cell differentiation is still being unraveled, there is evidence that one of its direct targets, cMaf, is involved (49). cMaf is expressed in Th2 cells and promotes the development of this lineage. Thus, the higher levels of cMaf in miR-155 knockouts might hinder development of Th1 or Th17 cell types. Another promoter of Th17 responses is miR-326 (52). Like miR-155, miR-326 promotes EAE in mice by enhancing Th17 cell development. miR-326 targets the transcription factor Ets1, which is an established repressor of Th17 development. Because Th17 cells play a prominent role during tissue inflammation, miRNAs that regulate this cell type can have a significant impact on a range of inflammatory conditions.

When a T cell locates its cognate antigen and receives proper costimulation, it undergoes clonal expansion, greatly increasing its numbers. For this to be possible, the T cell must reduce the expression of factors that limit its rate of cell division. A recent report found that miR-182, which is upregulated in T cells by IL-2, plays an important role during clonal expansion by repressing Foxo1 (53). MiR-214 is another miRNA that is upregulated during costimulation; it promotes T cell expansion by repressing PTEN, an established inhibitor of the PI3K pathway (54). These are two examples of miRNAs that exhibit context-dependent expression and function specifically during productive immune responses, expanding T cell numbers.

In addition to producing effector T cells, the thymus also generates a subset of T cells, called regulatory T cells (Tregs), that function to repress inflammatory responses. Interestingly, global deletion of miRNAs specifically in Tregs, accomplished by eliminating Dicer in Foxp3-expressing cells, leads to a systemic and lethal inflammatory condition due to a failure of

Tregs to develop properly (55–57). miR-146a-deficient mice also succumb to a chronic inflammatory disorder that shortens their life span, although this phenotype is not as aggressive as that displayed by mice with Dicer-deficient Tregs (39). miR-146a is expressed in Tregs and conventional T cells, and its absence leads to elevated expression of one of its targets, STAT1, and an unchecked Th1 response, suggesting its involvement in the Dicer-null Treg phenotype (58). Our recent unpublished data also suggest that miR-146a is expressed in CD4<sup>+</sup> and CD8<sup>+</sup> effector T cells and functions to control the resolution of antigen-specific T cell responses by regulating NF- $\kappa$ B activation (L. Yang, M. Boldin, Y. Yu, C. Liu, C.-K. Ea, and D. Baltimore, unpublished observations).

In the course of developing specific, long-lasting immunity against pathogens, traditional B cells (B-2 B cells) play a central role by secreting highly specific immunoglobulins. Following development in the bone marrow where they acquire a surface immunoglobulin receptor using the mechanisms of V(D)J recombination, B cells are subject to activation in secondary and tertiary lymphoid organs during inflammatory responses. This process of activation involves antibody secretion, class switch recombination, and somatic hypermutation [the latter two are mediated by the activation-induced cytidine deaminase (AID) enzyme], all of which involve interactions with CD4<sup>+</sup> T cells (particularly the recently described Tfh cells) and follicular DCs (59, 60). Antigen binding by the immunoglobulin receptor on the B cell stimulates activation of the cell; costimulatory signals from B cells are needed for its full activation. The strength of signaling from antigen receptors is believed critical in mediating the cellular output from the B cell.

The global importance of miRNAs in B cell development was established by the conditional deletion of *Dicer* in early B cell development, which led to an arrest of development at the pro-B cell stage (61). More recently, *Dicer* ablation in mature B cells was shown to skew the cells toward transitional and marginal zone phenotypes in the spleen with a concomitant

reduction of follicular B cells (62). Investigators also determined that these mice had an increased titer of autoimmune antibodies with autoimmune sequelae in female mice. It remains to be determined which miRNAs are critical to this overall anti-inflammatory function in B cells because some miRNAs certainly have roles in promoting inflammation.

In B-2 cells, miR-155 plays a major role in regulating the response in germinal center cells. miR-155 is induced during LPS activation of B cells in mice and during the germinal center response *in vivo*. miR-155-deficient mice show marked defects in both antibody secretion and class switch recombination upon immunization (16, 63). The mechanism of this action seems to be miR-155-mediated repression of more than 60 target genes, including Pu.1, SHIP1, and AID (63). The latter target, which encodes the protein responsible for somatic hypermutation and class switch recombination, was subjected to an exacting study by two groups who generated disruption of the miR-155 target site in the *Aicda* 3' UTR (64, 65). Disruption of the interaction led to increased somatic hypermutation, abnormal translocations, and decreased high-affinity antibodies in immunized mutant mice. This was postulated to occur via ongoing somatic hypermutation, which disrupted the normal cycle of mutation and positive selection, implying a so far undiscovered mechanism that precludes cells that are in the process of undergoing mutation from being subjected to positive selection. Although not explicitly addressed in these studies, miR-155 inhibition of *Aicda* may represent a delayed negative regulatory switch that allows for the proper spacing in time of somatic hypermutation and positive selection that occur in the germinal center. Of note, AID's specialized role in lymphocyte biology indicates that it is a target important in B cells and not myeloid cells, making it an example of a cell type-specific target of miR-155.

Recent studies have also implied a significant role for miRNAs in B-1 B cells. miR-150-deficient mice showed an expansion of B-1 B cells accompanied by dramatic increases



in steady-state immunoglobulin levels. However, miR-150-deficient mice also showed an enhanced response to immunization with T-dependent antigens, indicating an effect on follicular B cells. These findings, supported by gain-of-function studies that demonstrated a block in B cell development, were largely explained by alterations in the amount of c-Myb, which is a critical target of miR-150. Indeed, examining transgenic mice with differing levels of expression of miR-150, Xiao and colleagues (66, 67) demonstrated a dosage effect on both c-Myb levels and the ensuing phenotype. These findings elegantly illustrate a recurring theme in miRNA biology—that fine control of target gene expression levels can be attained by miRNA function and that small changes in critical target genes can result in marked phenotypic consequences.

## INFECTIOUS DISEASES AND microRNAs

With the broad range of regulatory roles that miRNAs play in immune cells, it is not surprising that miRNAs are intimately involved in regulating the signals and physiological outcomes that result from host-pathogen interactions. The encounter with a broad range of infectious agents, from bacteria to viruses to parasites, leads to alterations in the expression of specific miRNAs in immune cells. Some of these miRNAs, like miR-155, potentiate the immune response against the infectious agent following their upregulation. This has been demonstrated for immunity against *Salmonella typhimurium* in that wild-type but not miR-155<sup>-/-</sup> mice can be vaccinated against this pathogen (49). Some miRNAs restrict specific aspects of immunity against infectious microbes. For instance, mice deficient in miR-223 have increased responses to fungal pathogens owing to hyperactivation of granulocytes (68). Thus, miRNAs expressed by immune cells can both positively and negatively regulate inflammatory reactions against pathogens, consistent with their role in establishing a properly balanced response.

A common consequence of uncontrolled bacterial infection is the onset of sepsis, which can result in death in a relatively short period of time. Both miR-155 and miR-146a have been shown to impact the severity of sepsis in a murine model of endotoxemia, albeit in opposite directions. Inhibition of miR-155 using an antisense oligonucleotide reduced the severity, whereas miR-146a<sup>-/-</sup> mice exhibited increased sensitivity to administration of *Escherichia coli* LPS (28, 39). Mechanistically, this is likely explained by the targets repressed by each of these miRNAs. miR-155 repression of SHIP1 and SOCS1, two negative regulators of inflammation, leads to a heightened response to LPS, as mice deficient in either of these genes are more susceptible to endotoxemia (24, 69). In contrast, repression of the miR-146a targets IRAK1 and TRAF6, both signaling proteins in the TLR/IL-1R pathways, reduces the magnitude of this reaction (39). Again, this dichotomy indicates that miRNAs are in place to provide a properly balanced response of the appropriate magnitude.

Although the host's miRNAs are important for coordinating responses against infectious agents, some pathogens such as viruses also benefit from miRNAs encoded by the host or their own genomes. Epstein Barr virus (EBV) is capable of upregulating host miRNAs in infected B cells and this is thought to contribute to transformation (70). In the case of Marek's disease, host miR-155 is essential for its oncogenic potential, demonstrating its role as a link between inflammation and cancer following infection (38). Interestingly, Kaposi's sarcoma-associated herpesvirus (KSHV) encodes an ortholog of miR-155 that has an identical seed sequence and targets overlapping gene subsets (71). DNA viruses in general encode many different miRNAs, and the relevance of these miRNAs during infection is currently under investigation by several groups.

There has also been some success exploiting the interplay between viral infections and miRNAs for therapeutic purposes. Hepatitis C virus's (HCV's) lifecycle in hepatocytes is dependent upon miR-122. Preclinical models

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**Epstein Barr virus (EBV):** a virus that infects human B lymphocytes and causes infectious mononucleosis

**Kaposi's sarcoma-associated herpesvirus (KSHV):** a human cancer-causing virus

**Hepatitis C virus (HCV):** a human virus that infects and destroys hepatocytes

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### Multiple sclerosis

**(MS):** an autoimmune condition that results from immune responses against myelin proteins

### Systemic lupus erythematosus

**(SLE):** a systemic autoimmune condition with common joint swelling and pain

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have indicated that inhibition of miR-122 using an antisense oligonucleotide is effective in reducing HCV titers, and this therapy is now in clinical trials (72). As we continue to understand the relationships between specific types of pathogens and miRNAs, researchers will undoubtedly attempt additional therapeutic strategies.

## microRNAs AND AUTOIMMUNE INFLAMMATION

Many of the same molecular and cellular responses that are required for host defense against infectious pathogens can also become inappropriately activated. This can cause damage to host tissues, and in some situations these responses can mistakenly target self-antigens, causing autoimmune inflammation. A number of recent reports have found that the expression of specific miRNAs is perturbed in many types of human autoimmune disorders, whereas mouse models are beginning to demonstrate their functional importance in some situations.

Both miR-155 and miR-326 are overexpressed in human multiple sclerosis (MS) (73), and the mouse model mentioned above (EAE) indicates that they may be functionally relevant to the disease process. With their influences on Th17 development, an important driver of tissue inflammation, these miRNAs are clear examples of how miRNAs can affect inflammation by controlling inflammatory cell development. miR-124a also affects EAE in mice (74). miR-124 is expressed in microglia, but not other peripheral monocytes or macrophages, and functions to enforce cellular quiescence by repressing the transcription factor C/EBP- $\alpha$ . This, in turn, restricts inflammation in the CNS under steady-state conditions. During the onset of EAE, miR-124 is downregulated in microglia, resulting in their activation and contributing to neuroinflammation. This latter example demonstrates the capacity of miRNAs to regulate the functional capacity of mature cells, an additional way in which miRNAs can modulate the magnitude of inflammatory responses.

miRNAs have also been implicated in rheumatoid arthritis (RA), an inflammatory condition that damages the synovium, cartilage, and ultimately the bone at joints. Human samples have increased expression of miR-155, miR-146, and miR-223 (51, 75, 76). Mouse models of arthritis have demonstrated the functional relevance of miR-155 and miR-182 for regulating B and T cell function during disease (51, 53). Systemic lupus erythematosus (SLE) is another systemic autoimmune condition that is driven by antibody-immune complex formation. Although a functional role for miRNAs during SLE is just beginning to be explored, perturbations in miRNA expression have been described (77–80). Links between miRNAs and other autoimmune conditions, including diabetes, atopic dermatitis, Sjögren's syndrome, and inflammatory bowel disease (IBD), are emerging (50, 81–84), and some of the same miRNAs described above likely impact these conditions as well.

It is also becoming clear that miRNAs can be found in extracellular compartments, although the purpose of such a location is not yet clear. A study analyzing patients with IgA nephropathy—inflammation of the glomeruli in the kidney associated with deposition of IgA in the kidney—found increased levels of intrarenal and urinary miR-155 and miR-146a (85). This finding suggests that an analysis of miRNA levels in different body fluids might serve an important diagnostic purpose.

Allergic inflammation represents another category of inflammatory response triggered by exposure to allergens and driven by Th2 cells. Although a role for miRNAs during allergic reactions is just emerging, recent reports indicate that administration of a let-7 mimic to mice can repress production of IL-13, a Th2 cytokine, resulting in reduced airway inflammation (86). Blocking miR-126 also resulted in a reduced asthmatic response to the house dust mite antigen (87). These studies were carried out in vivo and thus the nature of the responding cell types requires further exploration. However, these findings suggest that therapeutic

targeting of specific miRNAs might be effective in reducing human allergic responses.

## INFLAMMATORY HEMATOPOIESIS AND CANCERS OF THE HEMATOPOIETIC SYSTEM

In recent years, investigators have increasingly appreciated a distinct developmental program activated in the bone marrow during inflammation. This program is characterized by changes in the output of the marrow with a skewing toward myeloid differentiation and relative decreases in lymphocytic and erythroid differentiation. Classically, this program was thought to be activated by the hematopoietic stem cell (HSC) sensing depletion of downstream hematopoietic cells, but it is now becoming clear that there are more direct ways in which HSCs are regulated. Recent studies have demonstrated that HSCs respond to IFN signaling by proliferating and that this constitutes an important response to infection (88). Additionally, TLR expression has been found in hematopoietic stem and progenitor cells and TLR4 activation can lead to activation of MyD88 signaling and myeloid differentiation of these cells (89). NF- $\kappa$ B is also highly important in inflammatory hematopoiesis (90, 91). Several miRNAs seem to be involved in the regulation of this program, including miR-155, miR-223, miR-146a, and miR-125b.

The overexpression of miR-155 produces a myeloproliferative disorder (MPD) that is highly reminiscent of inflammatory hematopoiesis, with reductions in the numbers of erythroid and B cells and a profound myeloid expansion (35). This phenotype is thought to involve hyper-repression of the SHIP1 phosphatase. A mouse model with transgenic expression of miR-155 under control of the B cell-specific E $\mu$  promoter also demonstrated increased myeloid cells in addition to B cell leukemia/lymphoma (30, 31). At the level of the HSC, miR-155 is enriched compared with more mature hematopoietic cells, and its overexpression increases the ability of bone

marrow to repopulate the hematopoietic system (92).

Manipulation of another miRNA, miR-223, profoundly affects hematopoiesis. Initially, investigators thought that miR-223 was a factor that promoted granulocytic differentiation based on cell culture studies with leukemic cell lines (93, 94). However, when miR-223<sup>-/-</sup> mice were generated, these mice had increased granulocytes that showed signs of hyperfunctionality (68). This study also demonstrated that deletion of a target of miR-223, *Mef2c*, resulted in a partial correction of the phenotype with restored numbers of granulocytes but ones that retained the activated phenotype. Recent studies have implicated *Mef2c* as an important factor in cell lineage choice between B lymphocytes and myeloid cells (95). Further work is required to determine if the skewing of lineage choice in inflammatory hematopoiesis is also dependent on *Mef2c* and miR-223.

Mice deficient in miR-146a develop a constellation of hyperinflammatory and immunoproliferative phenotypes caused by loss of negative feedback regulation of NF- $\kappa$ B (38, 39, 58). Indeed, it was possible to partially correct the phenotype by deletion of *Nfkb1*, the gene encoding NF- $\kappa$ B p50, implying that constitutive expression of NF- $\kappa$ B is an important driver of these phenotypes (38). The early phenotype is reminiscent of that observed following miR-155 activation, with myeloid proliferation and decrease in the output of B cells and erythroid cells. A similar phenotype was independently observed when miR-146a was repressed using a sponge strategy, and in that case the phenotype seemed largely dependent on a target of miR-146a, TRAF6 (96). It will be of interest to delineate how precisely miR-146a activity regulates the expression levels of TRAF6 and whether different levels of TRAF6 can lead to distinct differentiation patterns during inflammatory hematopoiesis.

The miR-125 family members miR-125a and miR-125b are enriched in HSCs (92, 97, 98). Overexpression of miR-125 in HSC-enriched bone marrow potentiated hematopoietic engraftment in the setting of a

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**Hematopoietic stem cell (HSC):** a stem cell that gives rise to all blood cells

**Myeloproliferative disorder (MPD):** an unregulated overproduction of myeloid cells

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competitive reconstitution of lethally irradiated mice. In some settings, increased myeloid output has been observed during these experiments (92). This phenotype might depend upon high levels of miR-125b overexpression, albeit amounts that have been observed in some types of human leukemia (99, 100). Because miR-125b can affect hematopoiesis and increase the output of myeloid cells as seen during inflammation, it might play a role in inflammatory hematopoiesis. However, this role remains to be directly examined.

Interestingly, constitutive NF- $\kappa$ B activation has been noted in human acute myeloid leukemia CD34<sup>+</sup> cells and in other myeloid malignancies, including myelodysplastic syndromes and myeloproliferative neoplasms (reviewed in 101). It is possible that pathways involved in inflammatory hematopoiesis are

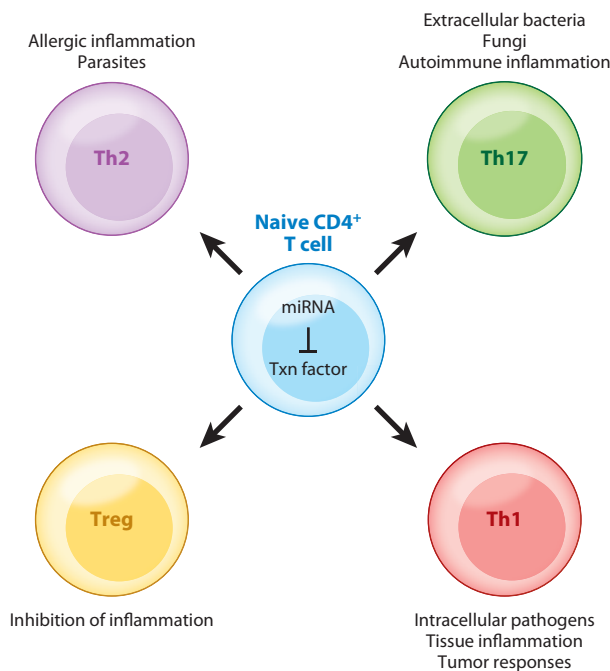
constitutively “on” in myeloid malignancies. miRNAs are no doubt part of these disruptions, and hence therapeutic manipulation of miRNAs may be important in both chronic inflammatory conditions and in myeloid malignancies.

## CONCLUDING REMARKS

It is now clear that a variety of inflammatory responses can occur. These inflammatory subtypes can differ regarding the cause of the inflammatory insult, the tissue type impacted, the identity of cells that carry out the response, and the overall duration of the reaction. At this point, experimental data indicate that miRNAs are involved in most types of inflammatory responses and have a significant impact on their magnitude. This is accomplished primarily in two major ways: First, by impacting the development of inflammatory cell subsets (e.g., Th2 versus Th17) (**Figure 1**) and, second, by establishing the level of immune cell function (e.g., controlling how much cytokine is made by DCs) (**Figure 2**).

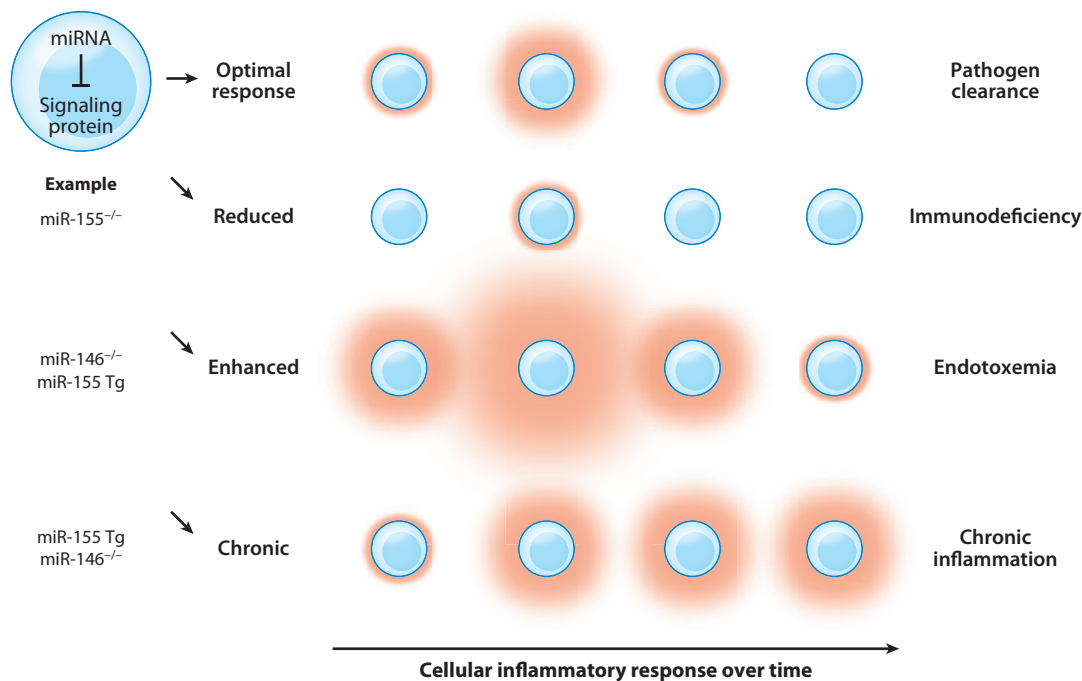
Since first being recognized as inflammation-induced miRNAs, miR-155 and miR-146a have proven to play important roles in various inflammatory responses. However, these miRNAs appear to have opposing impacts on inflammatory responses, as described above. This indicates that, like other systems regulated by miRNAs, the immune system utilizes multiple miRNAs to properly balance its functional capacity, creating a tension between activation and repression that can be precisely tuned.

Moving forward, several important areas of investigation in the field of miRNAs and inflammation remain. First, an improved understanding of how multiple miRNAs can collaborate to properly balance inflammatory responses is needed, and this will provide a better understanding of how miRNA networks can work together to optimize responses. Second, the relevance of extracellular miRNAs found in different tissue fluids should be determined. It is tantalizing to think that extracellular miRNAs might function in a manner similar



**Figure 1**

microRNAs (miRNAs) affect lineage choices made by inflammatory T cell subsets. Certain miRNAs are expressed in T lymphocytes following cellular activation. These particular miRNAs repress their targets, which are often lineage-determining transcription factors, and this affects the type of inflammatory T cell that is produced during inflammation. The types of immune responses regulated by each subtype of T cell is shown.



**Figure 2**

microRNAs (miRNAs) impact the magnitude of inflammatory responses. Specific miRNAs expressed in inflammatory cells, such as miR-155 and miR-146a, target signaling proteins that regulate the strength of the inflammatory signal. Under optimal conditions, a properly gauged signal results in a transient inflammatory response that clears infection without causing host tissue damage. In other cases, a lack of certain miRNAs, such as miR-155, can reduce the magnitude of the immune response, and this results in an immunodeficiency. Other miRNA alterations, such as a lack of miR-146a or overexpression of miR-155, can result in a hyperactive response to infection. This can be extremely harmful to the host and result in endotoxemia. Finally, constant overexpression of miR-155 or deletion of miR-146a can cause a chronic inflammatory state where inflammation is not resolved.

to secreted cytokines—being able to target and regulate inflammatory cells through autocrine, paracrine, and endocrine mechanisms. Third, a deeper understanding of how miRNAs can affect cellular plasticity should point to how miRNAs can be used to shape the population of inflammatory cells and, in so doing, alleviate unwanted inflammatory responses and tissue damage. Finally, continued work in the area of miRNA target prediction and validation is necessary owing to the large number of predicted targets for a given miRNA. With the advent of biochemical techniques such as Argonaute HITS-CLIP, miRNA:mRNA-containing protein complexes can be directly studied, and

such approaches should be included during target validation (102).

In just the first decade of this century, it is has become clear that miRNAs are instrumental players in the arena of mammalian inflammatory responses. As a consequence of these studies, miRNAs are exposing novel paradigms, including the need for precise expression levels of regulatory proteins to mediate efficient pathogen clearance without the onset of inflammatory disease. Although there are few success stories to date owing to the infancy of the field, therapeutic modulation of miRNAs is a promising new approach to treating human inflammatory disorders.

## DISCLOSURE STATEMENT

R.M.O. and D.B. are on a patent application (#13/230,695) entitled “Treatment of Autoimmune Inflammation using miR-155.” D.B. is a director and scientific advisor to Regulus Therapeutics, a company devoted to the commercialization of antagonists of microRNAs.

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# Contents

Decisions About Dendritic Cells: Past, Present, and Future <i>Ralph M. Steinman</i> .....	1
The Basel Institute for Immunology <i>Fritz Melchers</i> .....	23
Regulation of Immune Responses by mTOR <i>Jonathan D. Powell, Kristen N. Pollizzi, Emily B. Heikamp, and Maureen R. Horton</i> .....	39
Sphingosine-1-Phosphate and Lymphocyte Egress from Lymphoid Organs <i>Jason G. Cyster and Susan R. Schwab</i> .....	69
Selection of Self-Reactive T Cells in the Thymus <i>Gretta L. Stritesky, Stephen C. Jameson, and Kristin A. Hogquist</i> .....	95
Adaptive Immunity to Fungi <i>Marcel Wüthrich, George S. Deepe, Jr., and Bruce Klein</i> .....	115
Microbial Translocation Across the GI Tract <i>Jason M. Brenchley and Daniel C. Douek</i> .....	149
The Response to and Repair of RAG-Mediated DNA Double-Strand Breaks <i>Beth A. Helmink and Barry P. Sleckman</i> .....	175
VLR-Based Adaptive Immunity <i>Thomas Boehm, Nathanael McCurley, Yoichi Sutoh, Michael Schorpp, Masanori Kasahara, and Max D. Cooper</i> .....	203
Immune Regulatory Function of B Cells <i>Claudia Mauri and Anneleen Bosma</i> .....	221
Lung Dendritic Cells in Respiratory Viral Infection and Asthma: From Protection to Immunopathology <i>Bart N. Lambrecht and Hamida Hammad</i> .....	243
Tolerance of Infections <i>Janelle S. Ayres and David S. Schneider</i> .....	271
microRNA Regulation of Inflammatory Responses <i>Ryan M. O'Connell, Dinesh S. Rao, and David Baltimore</i> .....	295

Reflex Principles of Immunological Homeostasis <i>Ulf Andersson and Kevin J. Tracey</i> .....	313
Chromatin Topology and the Regulation of Antigen Receptor Assembly <i>Claudia Bossen, Robert Mansson, and Cornelis Murre</i> .....	337
Siglecs and Immune Regulation <i>Shiv Pillai, Ilka Arun Netravali, Annaiah Cariappa, and Hamid Mattoo</i> .....	357
Monogenic Autoimmunity <i>Mickie H. Cheng and Mark S. Anderson</i> .....	393
Germinal Centers <i>Gabriel D. Victora and Michel C. Nussenzweig</i> .....	429
Neutrophil Function: From Mechanisms to Disease <i>Borko Amulic, Christel Cazalet, Garret L. Hayes, Kathleen D. Metzler, and Arturo Zychlinsky</i> .....	459
Signaling by Myeloid C-Type Lectin Receptors in Immunity and Homeostasis <i>David Sancho and Caetano Reis e Sousa</i> .....	491
Regulatory T Cells: Mechanisms of Differentiation and Function <i>Steven Z. Josefowicz, Li-Fan Lu, and Alexander Y. Rudensky</i> .....	531
Pathogenesis of Human B Cell Lymphomas <i>Arthur L. Shaffer III, Ryan M. Young, and Louis M. Staudt</i> .....	565
Autophagy and the Immune System <i>Petric Kuballa, Whitney M. Nolte, Adam B. Castoreno, and Rannik J. Xavier</i> .....	611
Innate Lymphoid Cells: Emerging Insights in Development, Lineage Relationships, and Function <i>Hergen Spits and Tom Cupedo</i> .....	647
Cancer and Inflammation: An Old Intuition with Rapidly Evolving New Concepts <i>Giorgio Trinchieri</i> .....	677
Transcriptional and Epigenetic Control of T Helper Cell Specification: Molecular Mechanisms Underlying Commitment and Plasticity <i>Yuka Kanno, Golnaz Vabedi, Kiyoshi Hirabara, Kentner Singleton, and John J. O'Shea</i> .....	707
Induced CD4 <sup>+</sup> Foxp3 <sup>+</sup> Regulatory T Cells in Immune Tolerance <i>Angelina M. Bilate and Juan J. Lafaille</i> .....	733
The Microbiome in Infectious Disease and Inflammation <i>Kenya Honda and Dan R. Littman</i> .....	759