Noncoding RNAs and chronic inflammation: Micro-managing the fire within

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Inflammatory responses are essential for the clearance of pathogens and the repair of injured tissues; however, if these responses are not properly controlled chronic inflammation can occur. Chronic inflammation is now recognized as a contributing factor to many age-associated diseases including metabolic disorders, arthritis, neurodegeneration, and cardiovascular disease. Due to the connection between chronic inflammation and these diseases, it is essential to understand underlying mechanisms behind this process. In this review, factors that contribute to chronic inflammation are discussed. Further, we emphasize the emerging roles of microRNAs (miRNAs) and other noncoding RNAs (ncRNA) in regulating chronic inflammatory states, making them important future diagnostic markers and therapeutic targets. Copyright Line: 2015 The Authors *BioEssays* Published by Wiley-VCH Verlag GmbH & Co. KGaA.

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Introduction

The mammalian inflammatory response is a double-edged sword. Although immune responses are necessary for efficient pathogen clearance, symbiosis with commensal microbes, wound repair and overall tissue homeostasis, these responses can become dysregulated and initiate a chronic reaction that lacks resolution [1]. This condition, referred to as chronic low-grade inflammation, can fester for long periods of time and adversely contribute to, or possibly even cause, many diseases associated with the aging including obesity [2], type 1 diabetes (T1D) [2], rheumatoid arthritis (RA) [3], systemic lupus erythematosus (SLE) [4], neurodegeneration [5], and cardiovascular diseases (CVD) [6]. In many of these cases chronic inflammatory symptoms, such as elevations in inflammatory cytokines and autoantibodies in the serum, can serve as a prognostic indicator of later disease manifestation and overall morbidity and mortality [7].

As the world's elderly population continues to grow at an alarming rate, there is tremendous need to predict which individuals are at the highest risk for developing many of the disorders mentioned above. However, because chronic inflammation does not typically cause obvious clinical symptoms, and screening for inflammatory markers is not a test performed during routine medical exams, the potentially predictive power of one's chronic inflammatory state is not currently being harnessed. This is in part due to the fact that elevated inflammatory factors in the serum are not markers specific to chronic inflammation, and thus their diagnostic value is currently limited. Further, until a better understanding of the mechanisms underlying this deleterious condition is obtained, therapeutic inhibition of chronic inflammation will remain challenging.

In this article, we review our current understanding of the known causes of chronic low-grade inflammation with a focus on factors distinct from chronic infection. We will also focus on cells of the immune system, although we recognize that non-immune cells also contribute to this state. We also discuss recent evidence that mammalian microRNAs and long noncoding RNAs (lncRNAs) have evolved to regulate chronic

inflammatory states including those that occur during the aging process, and describe how they provide both diagnostic and therapeutic opportunities moving forward.

Causes of chronic inflammation

During chronic inflammation the resolution phase of the inflammatory response does not occur. This can be a result of either a persisting stimulus and/or the perturbation of molecular mechanisms involved in the resolution of inflammation. Thus, normal "healthy" immune responses can progress to chronic inflammatory states in instances where either of these events takes place.

Immune responses involve a resolution phase where the inflammatory response is shut down once the stimulus, such as a pathogen, is cleared. Mechanisms of resolution have been an intense area of study in recent years, and many important steps in this process have been discovered. Specialized cell types, such as T regulatory cells (Tregs), carry out critical immune repressive functions that are essential in preventing autoimmunity [8]. At the molecular level, the cytokine IL-10 [9, 10], the signaling molecule A20 [11], the signaling receptor PD1 [12], the signaling molecule CTLA4 [13], and the secreted factor IL1RA [14] are all examples of molecules that have evolved to balance and ultimately shut down immune cell activation. In some cases, these pathways are already being exploited therapeutically for such applications as cancer therapy. For instance, antibody disruption of the PD1 pathway, which enhances the immune response against tumors, is proving to be an effective therapy for melanoma [15]. Further, as we will describe below, specific miRNAs such as miR-146a have also evolved to keep immune responses in check.

Initial causes of some forms of chronic inflammatory states are also incompletely understood, yet their identification and prevention is key to avoiding the disease process. Recently, several distinct contributing factors have been described (Fig. 1) and include the following: (i) chronic inflammation can be primarily initiated by immune responses to self-tissues. The recognition of self-antigens by the immune system can result in diseases such as RA [16], SLE [17], multiple sclerosis (MS) [18], and T1D [19], and this self-recognition may or may not have a microbial component; (ii) in other instances, the immune response appears to be a secondary event that emerges in response to damage associated molecular patterns (DAMPs) that are produced following a breakdown in tissue homeostasis where the ensuing immune response driven by DAMPs alters tissue function [20]. Examples of diseases associated with DAMPs include obesity – where nutrient excess and hypertrophic adipocytes are the primary drivers [21], CVD – where lipoprotein buildup initially seeds the pathology [22], and certain neurodegenerative disorders such as Alzheimer's disease (AD) – mediated by protein aggregation [5]; (iii) contributions by the microbiota are also documented in some types of chronic inflammation, as their metabolites can influence both gut and peripheral tissues [23]. Obesity has been associated with alterations to the gut microbiota composition [24]; (iv) finally, the aging process itself leads to changes in immune system phenotypes and correlates with increasing inflammatory status as we grow older [25]. In this section, we will expand on each of these aspects of chronic inflammation.

### Self-antigens produce autoimmune responses

A common cause of inflammatory conditions involves the inappropriate immune response to self-tissues, as is the case for RA [16], SLE [17], MS [18], and T1D [19]. For instance, in RA auto-reactive leukocytes attack joint tissues through a variety of mechanisms including production of autoantibodies, reactive oxygen, and nitrogen species as well as secretion of pro-inflammatory cytokines that recruit additional immune cells to the site of tissue damage [16]. Although this phenotype is fairly well characterized, and thought to arise from an inappropriate initial response by the immune system, the underlying triggers of diseases such as RA are still being deciphered. Studies have found a link between a person's genetics, such as MHC type, and disease risk and this is consistent with antigen presentation playing a critical role in the triggering of disease [26, 27]. Additionally, self-antigen responses might also ensue as a result of cleaning up dead or dying cells that may trigger responses against antigens from the tissue where these cells were derived [28].

Additional contributions may also be made by microbial pathogens that produce antigens that are similar to host proteins [29]. This type of molecular mimicry is an attractive hypothesis; however, the identity of pathogens that trigger these responses in different autoimmune settings remains largely elusive. One example of an infectious agent that triggers chronic arthritis is *Borrelia burgdorferi* [30]. Although most people return to health after clearing the infection, about 10% of infected individuals develop chronic arthritis in their joints even after the infection is cleared [31]. Whether this is working through molecular mimicry or a persistent, hard to detect microbial reservoir is unclear and future work is needed to better define this process.

Another interesting theory is that human endogenous retroviruses may also play a role in driving disease onset. Human endogenous retrovirus-K has been implicated in the development of RA [32]. It is thought that this retrovirus impacts the development of RA through molecular mimicry of self antigens. Additionally, human endogenous retrovirus type W envelope expression has been associated with MS [33]. Along with these examples, there have been several implications for human endogenous retroviruses in the development of inflammatory diseases [34].

DAMPs are initiators of chronic inflammatory states

In other instances, sustained inflammatory responses can be driven by DAMPs produced as a result of tissue damage or stress, or other events that disrupt tissue homeostasis. Examples include lipoproteins in the vasculature that drive atherosclerosis [35], and protein aggregates in the CNS that are associated with AD [36]. In such cases, DAMPs are produced and are recognized by Toll-like receptors (TLRs) or Nod-like
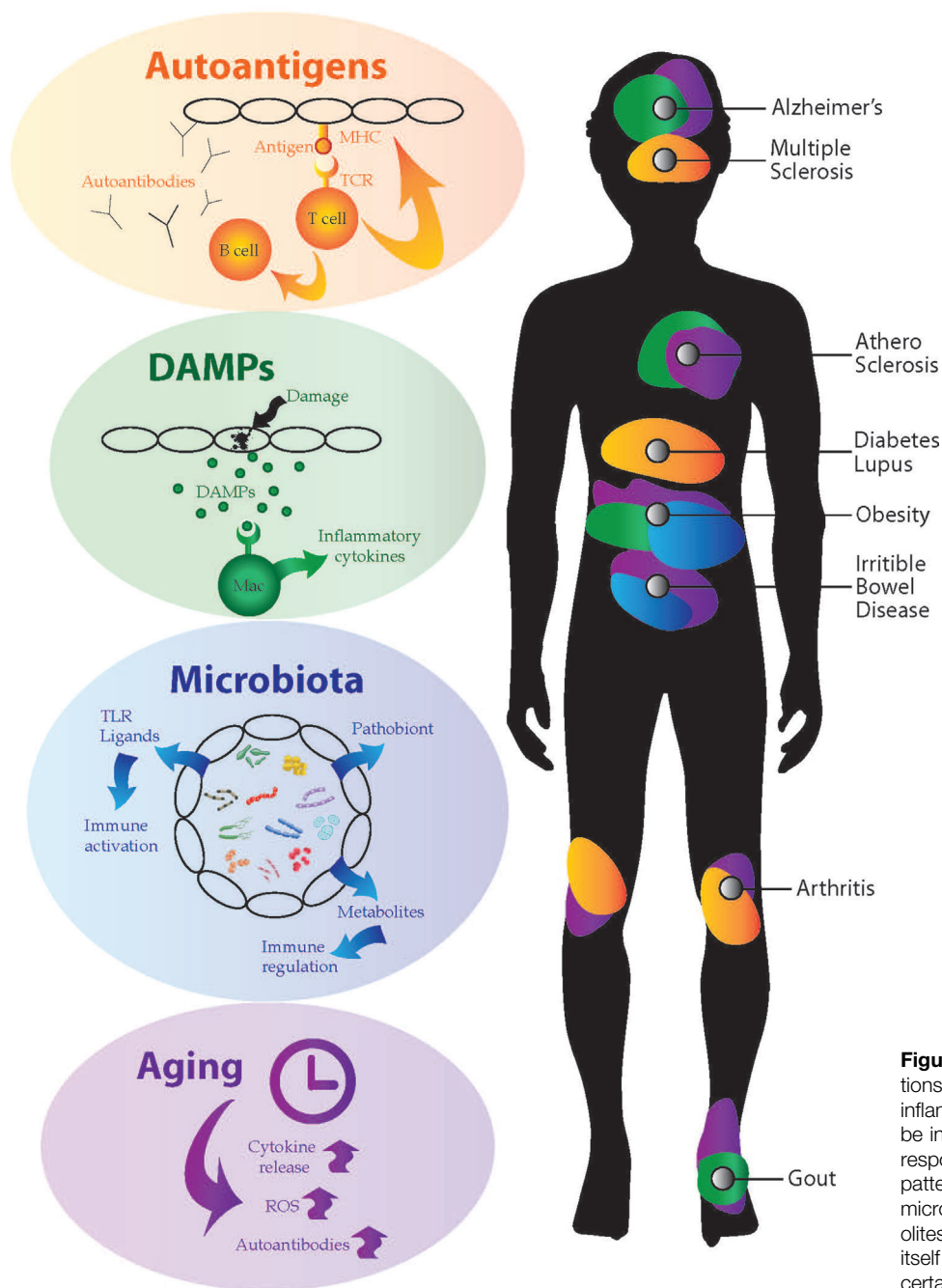
Figure 1. Mechanisms, anatomical locations, and disease types involving chronic inflammation. Chronic inflammation can be initiated by autoantigens or in response to damage associated molecular patterns (DAMPs). Furthermore, the microbiota – via the action of their metabolites, etc. – as well as the aging process itself have been shown to be involved in certain types of chronic inflammation.

receptors (NLRs) on innate immune cells [37]. In these instances, the innate immune system plays a secondary role as it responds to the inappropriate buildup or localization of certain molecules that signal disruptions to tissue homeostasis.

In the case of metabolic syndromes, such as obesity and diabetes, nutrient excess drives adipocyte hypertrophy, production of adipokines as well as inflammatory cytokines leading to eventual necrosis [38]. As adipocytes begin to die, their contents are taken up by tissues macrophages that are activated by products such as secreted cytokines and fatty acids that are detected by macrophage TLRs [39]. This response can change the nature of the tissue macrophage from an M2 to an M1 subtype, which subsequently initiates low-grade inflammation within adipose tissues. This includes the recruitment of a variety of immune cell mediators that reinforce the inflammatory state and promote insulin resistance, an early step in the development of type 2 diabetes (T2D). This is a good example of how a stressed tissue can induce an inflammatory response that is self-sustaining.

The microbiota and their products impact chronic inflammatory states

It is now widely recognized that the human microbiome, most of which resides in the intestinal tract, has an enormous

impact on our health. Thus, it is not surprising that commensal bacteria have been linked to a variety of chronic inflammatory conditions including inflammatory bowel disease (IBD), T1D, obesity, and neuroinflammatory diseases such as MS [40]. In each case, the microbiome of diseased individuals differs from that of healthy controls. Further, animal models have shown that the microbiota can cause or inhibit disease, based on its composition. This influence is mediated, at least in part, through the production and delivery of TLR ligands and other metabolites to either intestinal or extra-intestinal tissues that alter tissue homeostasis [41]. Many factors can influence the microbiota including diet, exposure to animals and agriculture, stress, and geographical location, indicating that lifestyle choices play an important role in microbiota composition [42]. However, while the importance of the microbiota in human health, including chronic inflammation, has become clear in recent years, the contribution of different microbial members to disease phenotypes is still under intense study as it is a complex mixture of distinct species.

The immune system changes with aging

Like other systems in our body, our immune system undergoes a variety of alterations as we grow older. Our thymus produces fewer naïve T lymphocytes [43], restricting one's ability to adequately respond to novel antigens and form memory against new pathogens or in response to vaccination. There is also an elevated amount of serum autoantibodies against self-tissues [44] and memory phenotype T cells can produce higher levels of inflammatory cytokines as they respond to persistent/chronic viral infections [45]. Hematopoietic output of innate immune myeloid cells becomes more prevalent [46], and senescent macrophages secrete higher amounts of inflammatory cytokines and produce ROS spontaneously [47]. Thus, the aging process creates an immune system that is less specific and more deregulated leading to a higher prevalence of autoimmunity in older versus younger individuals. It is also not surprising that a majority of diseases that emerge in the elderly are correlated with high levels of chronic inflammatory markers during middle age. As the world's aging population continues to grow larger, the need to prevent or treat disease in the elderly has become vital.

The regulatory potential of noncoding RNAs in the immune system

With the long-term goal of being able to prevent or reverse the pathological outcomes of chronic inflammatory states, much work has gone into understanding how these debilitating conditions are initially triggered, as we have just discussed. Further, we have developed a good understanding of various mechanisms that provide resolution to immune responses following necessary and protective responses to infection. This knowledge has led to the emergence of a wide range of possible therapeutic targets that are either currently being exploited clinically or still being tested and developed to

reduce inflammation. Examples of therapeutic targets include anti-TNFα treatment for inflammatory arthritis [48], anti-IL-1 as a therapy for gout [49], anti-IL1R as treatment for a wide range of inflammatory diseases [50], anti-PD1 and anti CTLA4 for cancer treatment [15], and steroids for a variety of inflammatory conditions [51].

However, while these approaches hold much promise, they are based almost exclusively on targeting, activating, or inhibiting cellular protein factors that we know are involved in chronic inflammatory responses. Yet, approximately 3/4 of the human genome is transcribed into RNA, with only about 1% of these transcripts encoding proteins. Thus, most of the RNA diversity in our cells is made up of ncRNA. In recent years, it has become clear that different types of ncRNAs play important regulatory roles, not only in the immune system, but in all mammalian organ systems. In particular, miRNAs and lncRNAs have emerged as critical regulators of immune system development and function [52–54], including several new studies that have linked specific miRNA and lncRNA species to the control of chronic inflammatory conditions (Table 1). The role of lncRNAs in the immune system is reviewed further by Heward and Lindsay [54].

MicroRNAs, immune responses, and the regulation of cellular physiology

MicroRNAs modulate immune cell differentiation and responses

MicroRNAs are small, single-stranded ncRNAs that were first discovered in *C. elegans* [55] approximately one decade before they were appreciated in mammalian cells [56, 57]. Since then, research involving miRNAs has exploded over the past 10–15 years and much has been learned regarding their biogenesis, expression patterns and functions at the molecular, cellular and organismal levels. miRNAs clearly function to repress gene expression and influence virtually all organ systems in vertebrates [58]. Much of this has been extensively reviewed elsewhere [59]. However, there are certain fundamental attributes of miRNAs that make them ideally suited to regulate chronic inflammatory conditions.

Through their ability to modulate gene expression networks by adjusting the levels of dosage sensitive target genes, miRNAs are able to shift thresholds that dictate whether a cellular response will occur or not, how strong it will be, and if it will be resolved (Fig. 2) [60]. For instance, miR-146a is induced in response to TLR signaling and forms a negative feedback loop that inhibits Traf6 and Irak1, two critical upstream TLR-signaling mediators that promote macrophage activation (Fig. 2A). While miR-146a is a repressor of immune cell signaling [61], miR-155 and miR-181a are activators of inflammation. miR-155 is induced in activated myeloid cells and represses both Socs1 and Ship1 to enhance cytokine production by dendritic cells and macrophages (Fig. 2B) [62]. miR-155 has also been shown to enable CD8+ T cells to respond to limiting doses of γ-chain cytokines, which enables robust immune responses in lymphoreplete hosts [63]. T cell receptor (TCR) signaling strength is regulated by miR-181a, which modulates expression of several phosphatases

Table 1. Selected examples of ncRNAs with roles in regulating inflammation

Species	Type	Disease	Cell types	Targets	Reference
miR-155	miRNA	CVD, viral infection, MS, RA, SLE, tumor immunity, chronic low-grade inflammation	Tfh, Th17, Th1, Th2, Macs, B cells, Treg, DCs	SHIP1, SOCS1, BACH1, PU.1, JARID2, PELI1, FOSl2, ETS1	[62, 63, 66–70, 77–79, 88, 106–108]
miR-146a	miRNA	Autoimmunity, dermatitis, chronic low-grade inflammation	Th1, Tfh, Treg, B cell, Macs, DCs, HSC	TRAF6, IRAK1, STAT1	[61, 68, 86, 89, 108, 109]
miR-17∼92	miRNA	Tumor immunity asthma, MS, viral Infection	Tfh, Th17, Th1, Th2, Treg, B cell	PTEN, PHLPP2, SOCS1, RORA, A20, IKZF4	[65, 80–82, 110, 111]
miR-181a	miRNA	Autoimmunity, aging-related inflammation	T cells	DUSP6, SHP2, DUSP5, PTPN22	[64, 112]
miR-182	miRNA	Tissue inflammation	T cells	FOXO1	[113]
miR-29a	miRNA	Crohn's disease	Th1, DC	TBET, EOMES, IL-12p40	[114, 115]
miR-125	miRNA	IBD, SLE	Macs	KLF13, IRF4	[71, 72]
miR-223	miRNA	Inflammatory lung pathology	Macs, granulocytes	Mef2c, Pknox1	[73, 74]
miR-124	miRNA	Neuro-inflammatory	Microglia	C/EBP-α, PU.1	[116]
LincRNA-Cox2	LncRNA	–	Macs	CCL5, IL-6	[117]
NeST	LncRNA	Microbial infection	T cells, NK cells	IFNG	[118]
LncDC	LncRNA	–	Macs	STAT3 target genes	[119]
CCR2	LncRNA	–	TH2	TH2 genes	[120]
E330013P06	LncRNA	Diabetes	Macs	–	[103]
Thril	LncRNA	Kawasaki disease	Macs	TNFα, IL-8, CXCL10, CCL1, CSF1	[121]

DC, dendritic cells; HSC, hematopoietic stem cell; Macs, macrophages; NK cell, natural killer cell; Tfh, T follicular helper cells; Tregs, regulatory T cells.

that inhibit TCR-induced signaling pathways resulting in activation of T cell genes (Fig. 2C) [64]. Thus, miRNAs can both enhance or hinder signaling pathways that control innate and adaptive immune responses that underlie inflammation.

Further, miRNAs have also been shown to confer robustness to cellular states. In these scenarios, specific miRNAs play important roles in determining the extent to which differentiation occurs. For example, the miR-17∼92 cluster of miRNAs repress Pten and Phlpp2, inhibitors of Icos signaling in activated T cells, resulting in the skewing of cells into T follicular helper cells (Tfh cells) (Fig. 2D) [65]. In the absence of the miR-17∼92 cluster, these proteins are at higher levels and reduce the amount of Tfh cells produced during inflammatory responses. Another example is miR-155, a miRNA that is necessary for both T cell homeostasis and optimal differentiation of multiple T cell types including Th17 [66, 67], Tfh [68], Th2 [69], and Th1 cells [70]. In these cases, miR-155 appears to be working through repression of multiple targets including Jarid2, Socs1, Ship1, Ets1, Peli1, Fosl2 (and possibly others). However, additional work is needed to determine if unique target/s are used by miR-155 depending on the Th cell type produced. Because T cells are central regulators of inflammatory responses, their modulation by miRNAs is of significant relevance to chronic inflammatory states, as described below.

Additionally, several miRNAs have been implicated in regulating macrophage lineage skewing during inflammatory responses. Macrophages can be skewed toward either proinflammatory subtypes (M1), or toward more reparative and less inflammatory subtypes (M2). miR-125 has been shown to repress M1 skewing while promoting the M2 fate [71, 72]. miR-223 has also been implicated in macrophage skewing where

miR-223 promotes macrophage polarization toward the M2 subtype [73]. Additionally, miR-223 has been implicated in control of granulocyte activation, and miR-223-/- mice display overactive immune responses and develop inflammatory lung pathology [74].

Further highlighting the importance of miRNAs in human systems, greater numbers of miRNAs have emerged throughout evolution, in addition to increased target diversity [75]. This suggests that miRNAs are among the regulatory mechanisms that enable increased human complexity despite a genome size that is similar to less complex organisms. This appears to include critical roles in establishing proper inflammatory set points and facilitating optimal responses and resolution by our immune system. In the next section, we will assess our current understanding of how miRNAs influence distinct types of chronic inflammatory conditions.

## Functional roles for miRNAs during chronic inflammation

MicroRNAs regulate antigen specific responses

There has been a substantial amount of work to date assessing how miRNAs control different types of acute inflammatory responses following infection, immunization, tumor challenge, and induction of several antigen-dependent autoimmune conditions that are all rooted in inflammation [76]. In each case, specific miRNAs have been shown to play pivotal roles during disease onset, peak magnitude, and rate of resolution by influencing the immune cell populations that mediate these steps (Table 1). Examples include a pro-
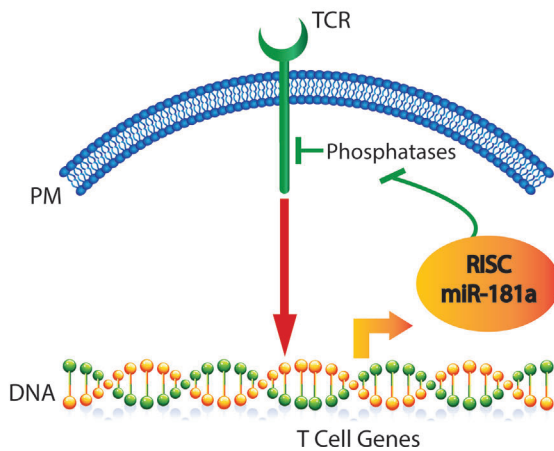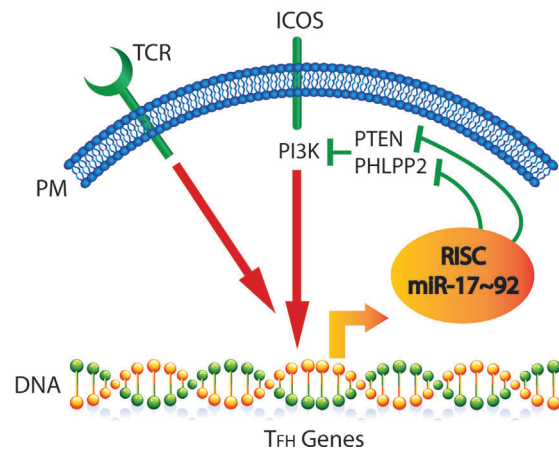
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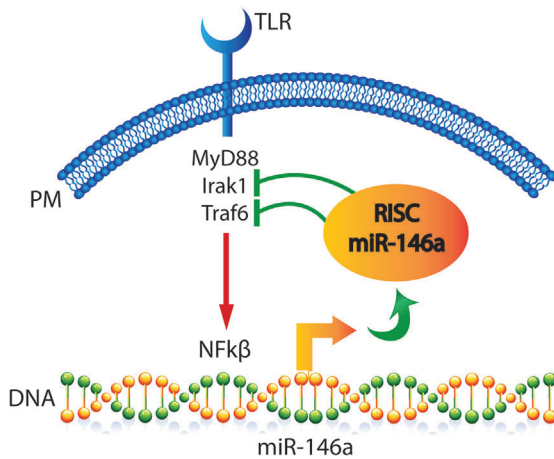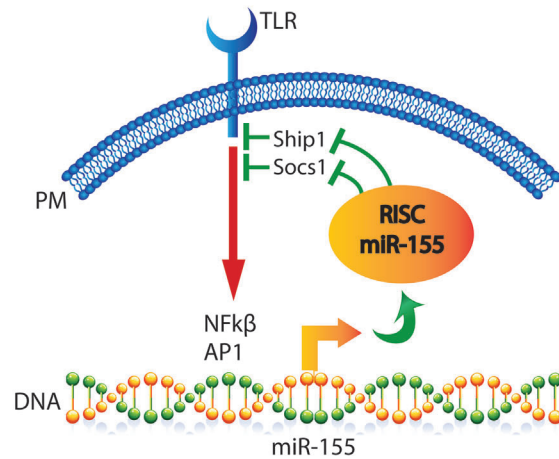
Figure 2. Mechanisms of miRNA function in the immune system. miRNAs can have various functions within the immune system and can act in different manners. **A:** For example, miR-146a acts as an anti-inflammatory miRNA via feedback inhibition of an inflammatory pathway. **B:** miR-155, on the other hand, is a pro-inflammatory miRNA that acts in a feed-forward manner bolstering the immune response. **C:** miR-181a is also a pro-inflammatory miRNA that acts by inhibiting phosphatases that block the activation of T cell genes thus activating T cells. **D:** Finally, the miR-17~92 cluster is involved in promoting the differentiation of a subset of inflammatory T cells called T follicular helpers cells (T$_F$H).

inflammatory role for miR-155 in T cells during antigen-induced experimental autoimmune encephalomyelitis (EAE) in mice [70], in B and T cells during collagen-induced arthritis in mice [77], in licensing CD8+ T cell responses against viruses and tumors [78], and in B cells during murine lupus [79]. miR-17~92 has been shown to enhance antibody responses against viral infections through its promotion of Tfh cell differentiation [65, 80], to promote Th1 cell responses against solid tumors [81], and provoke asthma through its augmentation of Th2 cell development [82]. miR-146a has been shown to play a critical role in preventing the onset of arthritis following infection by *Borrelia burgdorferi* by influencing

macrophage responses [83]. Importantly, this body of work strongly implicates miRNAs in the human iterations of these disorders where their altered expression is often observed. It is also relevant to note that while many of these studies are based upon induced disease states in mice, several of these disorders have been linked to pre-conditions of low-grade inflammation characterized by elevated titers of self-reactive antibodies and/or pro-inflammatory cytokines.

Although studies continue to unravel roles for different miRNAs in autoimmune disease states mediated by auto-antigens, far fewer studies have determined the role of miRNAs during chronic low-grade inflammation triggered by DAMPs, aging and the microbiota, and the diseases that emerge as a result of these triggers. We next turn to these emerging areas.

MicroRNAs are involved in inflammaging

As described above, the aging process itself is associated with inflammatory phenotypes. Early studies in *C. elegans* have functionally linked miRNAs to lifespan, perhaps offering a first clue that miRNAs are involved in different aspects of the aging process. For instance, lin-4 loss-of-function mutants
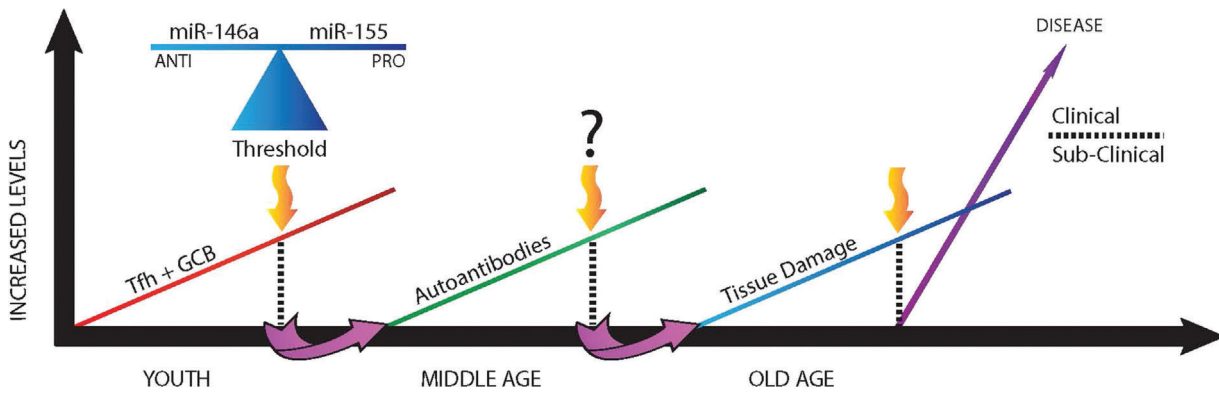
Figure 3. Sequential threshold model of age-dependent diseases linked to miRNA regulated chronic inflammation. During youth, the immune system has little chronic inflammation; however, as the aging process occurs, levels of inflammatory cells, such as T follicular helper cells (T$_F$H) and germinal center B cells (GCB), start to rise. Once the levels of these cells reach a certain threshold, they trigger increased inflammation and the production of autoantibodies. As levels of autoantibodies rise this might trigger tissue damage, which can accumulate and result in the clinical manifestation of chronic inflammatory diseases.

have shortened lifespans while lin-14 loss-of-function mutants have increased lifespans [84]. Additionally, mouse studies have shown that certain miRNAs alter expression patterns with aging. For example, in the mouse brain miR-22 and miR-101a are up-regulated in aged mice [85]. These studies reveal that miRNAs can both affect the aging process as well as be affected by it.

Recently, the roles of miRNAs in age-dependent inflammatory phenotypes have started to be explored. In particular, it has been discovered that mice lacking miR-146a develop an age-dependent, chronic inflammatory disease that is spontaneous, life-shortening, associated with inflammatory cytokines and autoantibodies, and that involves a variety of hematopoietic abnormalities and/or malignancies typically associated with the aging process [68]. Further, the condition has been shown to involve activated lymphocytes and be largely dependent upon NF-kB [86].

Our group and others have recently explored this miR-146a deficiency phenotype further and found that it involves the spontaneous development of T follicular helper cells that play a pivotal role in facilitating germinal center (GC) B cell development, production of high affinity, class-switched antibodies, and formation of B cell memory [68]. Consistent with this, both Tfh and GC B cells begin to spontaneously arise in younger miR-146a-/- animals, and this precedes most other phenotypes in this model. Upon reaching middle age, these animals begin to produce anti-dsDNA autoantibodies as a consequence of their deregulated GC response, and begin to display inflammation in a variety of different peripheral tissues. Of relevance, this phenotype was largely dependent on T cell expression of miR-155 indicating that these two miRNAs counter-regulate chronic inflammation. Although more work remains, it is plausible that these autoantibodies contribute to tissue stress and ultimately the onset of disease upon reaching old age (Fig. 3).

MicroRNAs are involved in other chronic inflammatory contexts

A role for miRNAs in controlling commensal bacteria populations and their production of metabolites that influence inflammatory conditions is beginning to emerge [87]. This has important implications both within the gut and in peripheral tissues. miRNA specific knockout mice have been shown to have altered gut microbiota. For example, miR-155-/- mice have increases in pathobionts within the gastrointestinal tract due to defective humoral immunity [88]. This suggests that the function of miRNA within host immune cells can help shape the composition and control of commensal microbes. However, it remains unclear if miRNAs shape populations that influence chronic inflammatory disease states.

Although specific miRNAs have been shown to regulate metabolic syndromes, there is little functional evidence thus far linking miRNAs, inflammation, and obesity/diabetes. However, based upon our understanding of miRNA functions in other contexts, it is highly likely that miRNAs regulate low-grade inflammatory conditions that influence weight gain and insulin resistance. Further, clinical evidence is beginning to emerge. For instance, there have been reports linking altered miR-146a expression and T2D [89].

MicroRNAs are emerging biomarkers and therapeutic targets in chronic inflammation

As miRNAs have been functionally connected to the development of chronic inflammation, it follows that alterations to miRNA levels could be a reasonable way to detect the presence of chronic inflammatory states in patients. miRNAs are currently being used as diagnostics for at least some types of diseases, including some forms of chronic inflammation such as colitis and IBD [90, 91]. With some diseases, miRNAs can even be used for both diagnosis and prognosis [92]. The appeal of using miRNAs as diagnostic markers comes from the high sensitivity that miRNA biomarkers possess as well as the ability to use miRNA profiles to stratify distinct downstream disease outcomes. The hope is that miRNAs associated with chronic inflammation can be used to diagnose chronic inflammation before clinical manifestations appear. This

would allow for preventative treatment of diseases that stem from chronic, low-grade inflammatory states.

Recently, the identification of miRNAs in blood serum as well as other biological fluids has opened the door for diagnosis of various diseases using these samples obtained through non-invasive methods. Serum miRNAs can either exist cell-free in association with the RISC complex or within small lipid vesicles such as exosomes [93]. Recently, there has been significant interest in utilizing these extracellular miRNAs as biomarkers because of their specificity and sensitivity of detection. Specific secreted miRNAs in the serum can be used to diagnose chronic inflammatory diseases such as IBD [94] where each disease has a unique profile of secreted miRNAs. This could also be an approach to diagnosing other forms of chronic inflammatory states, such as those described above.

The presence of extracellular miRNAs raises questions regarding the biological role of these secreted miRNAs. One current theory of the role of extracellular miRNAs, especially those within exosomes, is that they constitute a novel form of intercellular communication [95, 96]. This idea is supported by several reports providing evidence that secreted miRNAs are functionally passed between various cell types including immune cells [97–99]. It is possible that these extracellular miRNAs could play a role in the development, advancement, or inhibition of chronic inflammatory states. However, further investigation is needed to determine the role of extracellular miRNAs during chronic inflammation and diseases derived from this condition.

miRNAs have also begun to emerge as therapeutic targets. Currently, several anti-miRNA therapeutics are in clinical trials: most notably antisense inhibitors of miR-122 are being used to combat HCV infections [100, 101]. The therapeutic targeting of miRNAs is discussed in further detail in Li and Rana's 2014 review [100]. Based on the success of these approaches, it is our view that miRNAs that regulate chronic inflammation, such as miR-155 or miR-146a, could also be targeted therapeutically with optimal doses of anti-miRs or miRNA mimics. Ideally, chronic inflammatory disease could be diagnosed early using miRNA detection in blood serum samples, and then possibly treated with specific cocktails targeting the particular miRNAs that are dysregulated. However, there are some barriers to utilizing miRNAs as therapies. One of the biggest hurdles is targeting the miRNA therapeutics to the cells of interest. Exosomes and other lipid carriers have received a lot of attention lately as possible ways by which miRNAs and other therapies may be delivered to specific cell types [102]. However, there is still additional understanding that is required before these approaches can be effectively used in the clinic to provide specificity and sufficient dosing.

Long non-coding RNAs are involved in chronic inflammation

MicroRNAs are currently the best characterized ncRNAs involved in chronic inflammation; however, there are also other emerging classes of ncRNAs, such as lncRNAs, that are also involved (Table 1) [54]. LncRNAs appear to function through a variety of different molecular mechanisms, and most commonly play a scaffolding role to promote proper recruitment and positioning of protein regulators both in the nucleus and in the cytoplasm. Loss-of-function approaches have found that lncRNAs regulate the biology of both innate and adaptive immune cells during inflammatory responses. For example, there have recently been reports linking macrophage lncRNAs and obesity [103], as well as other studies that have implicated certain lncRNAs in macrophage and DC inflammatory functions [104]. LncRNA have also been connected to the regulation of T cell homing and differentiation into effector subtypes [105]. These and other classes of ncRNAs must be further characterized to better understand their roles in chronic inflammation. The role of lncRNAs in the immune system is reviewed further by Heward and Lindsay [54].

Conclusions and outlook

The association of chronic inflammation with a variety of diseases emphasizes the importance of gaining a deeper understanding of the underlying mechanisms behind this phenomenon. In this review, we have highlighted factors that contribute to chronic inflammation emphasizing the newly identified roles of miRNAs and other ncRNAs. As we move forward, it will be essential to consider contributions by both coding and noncoding factors in order to formulate an optimal approach for diagnosing, treating, and/or preventing diseases associated with chronic inflammation.

Despite the potential that ncRNAs pose as therapeutic reagents or targets, there are significant barriers that must be overcome in order to achieve therapeutic efficacy. Cell-specific delivery of reagents to manipulate ncRNAs remains a significant challenge. Although progress has been made on cell targeting therapies utilizing lipid vesicles and nonlipid carriers containing antisense miRNAs or miRNA mimics, this remains a substantial challenge facing the field of ncRNA therapy. Additionally, delivery of effective doses of ncRNAs or their inhibitors is also a major hurdle for therapy. For diseases brought on by chronic inflammation, it may also be difficult to determine when to begin treatment, although the use of ncRNA biomarkers in patients' biofluids should held to indicate when treatment should begin. Despite these barriers, the manipulation of ncRNAs represents a potential way to treat chronic inflammatory diseases, and hopefully surmounting these barriers will lead to more efficacious uses of ncRNAs as therapeutics.

References

1. **Medzhitov R.** 2008. Origin and physiological roles of inflammation. *Nature* **454**: 428–35.
2. **Xu H, Barnes GT, Yang Q, Tan G**, et al. 2003. Chronic inflammation in fat plays a crucial role in the development of obesity- related insulin resistance. *J Clin Invest* **112**: 1821–30.
3. **Choy EH, Panayi GS.** 2001. Cytokine pathways and joint inflammation in rheumatoid arthritis. *N Engl J Med* **344**: 907–16.
4. **Arbuckle MR, McClain MT, Rubertone MV, Scofield RH**, et al. 2003. Development of autoantibodies before the clinical onset of systemic lupus erythematosus. *N Engl J Med* **349**: 1526–33.

5. **Ross CA, Poirier MA.** 2004. Protein aggregation and neurodegenerative disease. *Nat Med* **10**: 10–7.
6. **Libby P, Ridker PM, Maseri A.** 2002. Inflammation and atherosclerosis. *Circulation* **105**: 1135–43.
7. **Ferrucci L, Corsi A, Lauretani F, Bandinelli S**, et al. 2005. The origins of age-related proinflammatory state. *Blood* **105**: 2294–9.
8. **Fontenot JD, Gavin MA, Rudensky AY.** 2003. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. *Nat Immunol* **4**: 330–6.
9. **Fiorentino DF, Zlotnik A, Mosmann TR, Howard M**, et al. 1991. IL-10 inhibits cytokine production by activated macrophages. *J. Immunol.* **147**: 3815–22.
10. **Sakaguchi S.** 2004. Naturally arising CD4+ regulatory t cells for immunologic self-tolerance and negative control of immune responses. *Annu Rev Immunol* **22**: 531–62.
11. **Lee EG, Boone DL, Chai S, Libby SL**, et al. 2000. Failure to regulate TNF-induced NF-kappaB and cell death responses in A20-deficient mice. *Science* **289**: 2350–4.
12. **Schwab JM, Chiang N, Arita M, Serhan CN.** 2007. Resolvin E1 and protectin D1 activate inflammation-resolution programmes. *Nature* **447**: 869–74.
13. **Wing K, Onishi Y, Prieto- Martin P, Yamaguchi T**, et al. 2008. CTLA-4 control over Foxp3+ regulatory T cell function. *Science* **322**: 271–5.
14. **Ohlsson K, Björk P, Bergenfeldt M, Hageman R**, et al. 1990. Interleukin-1 receptor antagonist reduces mortality from endotoxin shock. *Nature* **348**: 550–2.
15. **Hamid O, Robert C, Daud A, Hodi FS**, et al. 2013. Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma. *N Engl J Med* **369**: 134–44.
16. **Schaller M, Burton DR, Ditzel HJ.** 2001. Autoantibodies to GPI in rheumatoid arthritis: linkage between an animal model and human disease. *Nat Immunol* **2**: 746–53.
17. **Muñoz LE, Janko C, Schulze C, Schorn C**, et al. 2010. Autoimmunity and chronic inflammation—two clearance-related steps in the etiopathogenesis of SLE. *Autoimmun Rev* **10**: 38–42.
18. **Genain CP, Cannella B, Hauser SL, Raine CS.** 1999. Identification of autoantibodies associated with myelin damage in multiple sclerosis. *Nat Med* **5**: 170–5.
19. **Wilson SB, Kent SC, Patton KT, Orban T**, et al. 1998. Extreme Th1 bias of invariant Valpha24JalphaQ T cells in type 1 diabetes. *Nature* **391**: 177–81.
20. **Bianchi ME.** 2007. DAMPs, PAMPs and alarmins: all we need to know about danger. *J Leukoc Biol* **81**: 1–5.
21. **Cinti S, Mitchell G, Barbatelli G, Murano I**, et al. 2005. Adipocyte death defines macrophage localization and function in adipose tissue of obese mice and humans. *J Lipid Res* **46**: 2347–55.
22. **Gordon DJ, Probstfield JL, Garrison RJ, Neaton JD**, et al. 1989. High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. *Circulation* **79**: 8–16.
23. **Nicholson JK, Holmes E, Kinross J, Burcelin R**, et al. 2012. Host-gut microbiota metabolic interactions. *Science* **108**: 1262–8.
24. **Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V**, et al. 2006. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* **444**: 1027–31.
25. **Dorshkind K, Montecino-Rodriguez E, Signer RAJ.** 2009. The ageing immune system: is it ever too old to become young again? *Nat Rev Immunol* **9**: 57–62.
26. **De Bakker PIW, McVean G, Sabeti PC, Miretti MM**, et al. 2006. A high-resolution HLA and SNP haplotype map for disease association studies in the extended human MHC. *Nat Genet* **38**: 1166–72.
27. **Wandstrat A, Wakeland E.** 2001. The genetics of complex autoimmune diseases: non-MHC susceptibility genes. *Nat Immunol* **2**: 802–9.
28. **Mevorach D, Zhou JL, Song X, Elkon KB.** 1998. Systemic exposure to irradiated apoptotic cells induces autoantibody production. *J Exp Med* **188**: 387–92.
29. **Wucherpfennig KW.** 2001. Mechanisms for the induction of autoimmunity by infectious agents. *J Clin Invest* **108**: 1097–104.
30. **Aberer E, Brunner C, Suchanek G, Klade H**, et al. 1989. Molecular mimicry and lyme borreliosis: a shared antigenic determinant between *Borrelia burgdorferi* and human tissue. *Ann Neurol* **26**: 732–7.
31. **Brown CR, Reiner SL.** 1998. Clearance of *Borrelia burgdorferi* may not be required for resistance to experimental lyme arthritis. *Infect Immun* **66**: 2065–71.
32. **Freimanis G, Hooley P, Ejtehadi HD, Ali HA**, et al. 2010. A role for human endogenous retrovirus-K (HML-2) in rheumatoid arthritis: investigating mechanisms of pathogenesis. *Clin Exp Immunol* **160**: 340–7.
33. **Perron H, Germi R, Bernard C, Garcia-Montojo M**, et al. 2012. Human endogenous retrovirus type W envelope expression in blood and brain cells provides new insights into multiple sclerosis disease. *Mult Scler J* **12**: 1721–36.
34. **Urnovitz HB, Murphy WH.** 1996. Human endogenous retroviruses: nature, occurrence, and clinical implications in human disease. *Clin Microbiol Rev* **9**: 72–99.
35. **Tabas I, Williams KJ, Borén J.** 2007. Subendothelial lipoprotein retention as the initiating process in atherosclerosis: update and therapeutic implications. *Circulation* **116**: 1832–44.
36. **Yvette G.** 2010. Prion-like transmission of protein aggregates in neurodegenerative diseases. *Nat Rev Mol Cell Biol* **11**: 301–7.
37. **Allen IC, TeKippe EM, Woodford R-MT, Uronis JM**, et al. 2010. The NLRP3 inflammasome functions as a negative regulator of tumorigenesis during colitis-associated cancer. *J Exp Med* **207**: 1045–56.
38. **Ouchi N, Parker JL, Lugus JJ, Walsh K.** 2011. Adipokines in inflammation and metabolic disease. *Nat Rev Immunol* **11**: 85–97.
39. **Hotamisligil GS.** 2006. Inflammation and metabolic disorders 1. *Nature* **444**: 860–7.
40. **Kosiewicz MM, Zirnheld AL, Alard P.** 2011. Gut microbiota, immunity, and disease: a complex relationship. *Front Microbiol* **2**: 1–11.
41. **Round JL, Mazmanian SK.** 2009. The gut microbiota shapes intestinal immune responses during health and disease. *Nat Rev Immunol* **9**: 313–23.
42. **Benson AK, Kelly SA, Legge R, Ma F**, et al. 2010. Individuality in gut microbiota composition is a complex polygenic trait shaped by multiple environmental and host genetic factors. *Proc Natl Acad Sci USA* **107**: 18933–8.
43. **Lynch HE, Goldberg GL, Chidgey A, van den Brink MR**, et al. 2009. Thymic involution and immune reconstitution. *Trends Immunol* **30**: 366–73.
44. **Weksler ME.** 2000. Changes in the B-cell repertoire with age. *Vaccine* **18**: 1624–8.
45. **Haynes L, Swain SL.** 2006. Why aging T cells fail: implications for vaccination. *Immunity* **24**: 663–6.
46. **Geiger H, de Haan G, Florian MC.** 2013. The ageing haematopoietic stem cell compartment. *Nat Rev Immunol* **13**: 376–89.
47. **Desai A, Grolleau-Julius A, Yung R.** 2010. Leukocyte function in the aging immune system. *J Leukoc Biol* **87**: 1001–9.
48. **Elliott MJ, Maini RN, Feldmann M, Long-Fox A**, et al. 1993. Treatment of rheumatoid arthritis with chimeric monoclonal antibodies to tumor necrosis factor alpha. *Arthritis Rheum* **36**: 1681–90.
49. **So A, De Smedt T, Revaz S, Tschopp J.** 2007. A pilot study of IL-1 inhibition by anakinra in acute gout. *Arthritis Res Ther* **9**: R28.
50. **McIntyre KW, Stepan GJ, Kolinsky KD, Benjamin WR**, et al. 1991. Inhibition of interleukin 1 (IL-1) binding and bioactivity in vitro and modulation of acute inflammation in vivo by IL-1 receptor antagonist and anti-IL-1 receptor monoclonal antibody. *J Exp Med* **173**: 931–9.
51. **Barnes PJ.** 2006. How corticosteroids control inflammation: Quintiles Prize Lecture 2005. *Br J Pharmacol* **148**: 245–54.
52. **Xiao C, Rajewsky K.** 2009. MicroRNA control in the immune system: basic principles. *Cell* **136**: 26–36.
53. **O'Connell RM, Rao DS, Chaudhuri AA, Baltimore D.** 2010. Physiological and pathological roles for microRNAs in the immune system. *Nat Rev Immunol* **10**: 111–22.
54. **Heward JA, Lindsay MA.** 2014. Long non-coding RNAs in the regulation of the immune response. *Trends Immunol* **35**: 408–19.
55. **Lee RC, Feinbaum RL, Ambros V.** 1993. The *C. elegans* heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell* **75**: 843–54.
56. **Calin GA, Sevignani C, Dumitru CD, Hyslop T**, et al. 2004. Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. *Proc Natl Acad Sci USA* **101**: 2999–3004.
57. **Takamizawa J, Konishi H, Yanagisawa K, Tomida S**, et al. 2004. Reduced expression of the let-7 microRNAs in human lung cancers in association with shortened postoperative survival. *Cancer Res* **64**: 3753–6.
58. **He L, Hannon GJ.** 2004. MicroRNAs: small RNAs with a big role in gene regulation. *Nat Rev Genet* **5**: 522–31.
59. **Ambros V.** 2004. The functions of animal microRNAs. *Nature* **431**: 350–5.
60. **Ebert MS, Sharp PA.** 2012. Roles for microRNAs in conferring robustness to biological processes. *Cell* **149**: 505–24.
61. **Taganov KD, Boldin MP, Chang K, Baltimore D.** 2006. NF-kB-dependent induction of miR-146a, an inhibitor targeted to signaling proteins of innate immune responses. *Proc Natl Acad Sci USA* **33**: 6–11.

Review essays

62. **O'Connell RM, Chaudhuri AA, Rao DS, Baltimore D.** 2009. Inositol phosphatase SHIP1 is a primary target of miR-155. *Proc Natl Acad Sci USA* **106**: 7113–8.

63. **Ji Y, Wrzesinski C, Yu Z, Hu J**, et al. 2015. miR-155 augments CD8+T-cell antitumor activity in lymphoreplete hosts by enhancing responsiveness to homeostatic γ c cytokines. *Proc Natl Acad Sci USA* **112**: 476–81.

64. **Li QJ, Chau J, Ebert PJR, Sylvester G**, et al. 2007. miR-181a is an intrinsic modulator of T cell sensitivity and selection. *Cell* **129**: 147–61.

65. **Kang SG, Liu W-H., Lu P, Jin HY**, et al. 2013. MicroRNAs of the miR-17~92 family are critical regulators of T(FH) differentiation. *Nat Immunol* **14**: 849–57.

66. **Hu R, Huffaker TB, Kagele DA, Runtsch MC**, et al. 2013. MicroRNA-155 confers encephalogenic potential to Th17 cells by promoting effector gene expression. *J Immunol* **190**: 5972–80.

67. **Escobar TM, Kanellopoulou C, Kugler DG, Kilaru G**, et al. 2014. MiR-155 activates cytokine gene expression in Th17 cells by regulating the DNA-binding protein Jarid2 to relieve Polycomb-mediated repression. *Immunity* **40**: 865–79.

68. **Hu R, Kagele DA, Huffaker TB, Runtsch MC**, et al. 2014. miR-155 promotes T follicular helper cell accumulation during chronic, low-grade inflammation. *Immunity* **41**: 605–19.

69. **Material SO, Web S, Web ISI, Press H**, et al. 2008. Regulation of the germinal center response by microRNA-155. *Science* **604**: 604–8.

70. **O'Connell RM, Kahn D, Gibson WSJ, Round JL**, et al. 2010. MicroRNA-155 promotes autoimmune inflammation by enhancing inflammatory T cell development. *Immunity* **33**: 607–19.

71. **Chaudhuri AA, So AY-L, Sinha N, Gibson WSJ**, et al. 2011. MicroRNA-125b potentiates macrophage activation. *J Immunol* **187**: 5062–8.

72. **Banerjee S, Cui H, Xie N, Tan Z**, et al. 2013. MiR-125a-5p regulates differential activation of macrophages and inflammation. *J Biol Chem* **288**: 35428–36.

73. **Zhuang G, Meng C, Guo X, Cheruku PS**, et al. 2012. A novel regulator of macrophage activation: MiR-223 in obesity-associated adipose tissue inflammation. *Circulation* **125**: 2892–903.

74. **Johnnidis JB, Harris MH, Wheeler RT, Stehling-Sun S**, et al. 2008. Regulation of progenitor cell proliferation and granulocyte function by microRNA-223. *Nature* **451**: 1125–9.

75. **O'Connell RM, Baltimore D.** 2012. MicroRNAs and hematopoietic cell development. *Curr Top Dev Biol* **99**: 145–74.

76. **O'Connell RM, Rao DS, Baltimore D.** 2012. microRNA Regulation of Inflammatory Responses. *Annu Rev Immunol* **30**: 295–312.

77. **Kurowska-Stolarska M, Alivernini S, Ballantine LE, Asquith DL**, et al. 2011. MicroRNA-155 as a proinflammatory regulator in clinical and experimental arthritis. *Proc Natl Acad Sci USA* **108**: 11193–8.

78. **Dudda JC, Salaun B, Ji Y, Palmer DC**, et al. 2013. MicroRNA-155 is required for effector cd8+ t cell responses to virus infection and cancer. *Immunity* **38**: 742–53.

79. **Thai T-H, Patterson HC, Pham D-H, Kis-Toth K**, et al. 2013. Deletion of microRNA-155 reduces autoantibody responses and alleviates lupus-like disease in the Faslpr mouse. *Proc Natl Acad Sci USA* **110**: 20194–9.

80. **Baumjohann D, Kageyama R, Clingan JM, Morar MM**, et al. 2013. The microRNA cluster miR-17~92 promotes TFH cell differentiation and represses subset-inappropriate gene expression. *Nat Immunol* **14**: 840–8.

81. **Jiang S, Li C, Olive V, Lykken E**, et al. 2011. Molecular dissection of the miR-17-92 cluster's critical dual roles in promoting Th1 responses and preventing inducible Treg differentiation. *Blood* **118**: 5487–97.

82. **Simpson LJ, Patel S, Bhakta NR, Choy DF**, et al. 2014. A microRNA upregulated in asthma airway T cells promotes T H 2 cytokine production. *Nat Immunol* **15**: 1162–70.

83. **Lochhead RB, Ma Y, Zachary JF, Baltimore D**, et al. 2014. MicroRNA-146a provides feedback regulation of lyme arthritis but not carditis during infection with *Borrelia burgdorferi*. *PLoS Pathog* **10**: e1004212.

84. **Boehm M, Slack F.** 2005. A developmental timing microRNA and its target regulate life span in *C. elegans*. *Science* **310**: 1954–7.

85. **Dimmeler S, Nicotera P.** 2013. MicroRNAs in age-related diseases. *EMBO Mol Med* **5**: 180–90.

86. **Zhao JL, Rao DS, Boldin MP, Taganov KD**, et al. 2011. NF-kappaB dysregulation in microRNA-146a-deficient mice drives the development of myeloid malignancies. *Proc Natl Acad Sci USA* **108**: 9184–9.

87. **Runtsch MC, Round JL, O'Connell RM.** 2014. MicroRNAs and the regulation of intestinal homeostasis. *Front Genet* **5**: 1–10.

88. **Clare S, John V, Walker AW, Hill JL**, et al. 2013. Enhanced susceptibility to citrobacter rodentium infection in microRNA-155-Deficient mice. *Infect Immun* **81**: 723–32.

89. **Balasubramanyam M, Aravind S, Gokulakrishnan K, Prabu P**, et al. 2011. Impaired miR-146a expression links subclinical inflammation and insulin resistance in Type 2 diabetes. *Mol Cell Biochem* **351**: 197–205.

90. **Ahmed FE, Jeffries CD, Vos PW, Flake G**, et al. 2009. Diagnostic microRNA markers for screening sporadic human colon cancer and active ulcerative colitis in stool and tissue. *Cancer Genomics Proteomics* **296**: 281–95.

91. **Archanioti P, Gazouli M, Theodoropoulos G, Vaiopoulou A**, et al. 2011. Micro-RNAs as regulators and possible diagnostic bio-markers in inflammatory bowel disease. *J Crohn's Colitis* **5**: 520–4.

92. **Yanaihara N, Caplen N, Bowman E, Seike M**, et al. 2006. Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. *Cancer Cell* **9**: 189–98.

93. **Turchinovich A, Weiz L, Langheinz A, Burwinkel B.** 2011. Character-ization of extracellular circulating microRNA. *Nucleic Acids Res* **39**: 7223–33.

94. **Iborra M, Bernuzzi F, Correale C, Vetrano S**, et al. 2013. Identification of serum and tissue micro-RNA expression profiles in different stages of inflammatory bowel disease. *Clin Exp Immunol* **173**: 250–8.

95. **Mittelbrunn M, Sánchez-Madrid F.** 2012. Intercellular communication: diverse structures for exchange of genetic information. *Nat Rev Mol Cell Biol* **5**: 328–35.

96. **Robbins PD, Morelli AE.** 2014. Regulation of immune responses by extracellular vesicles. *Nat Rev Immunol* **14**: 195–208.

97. **Okoye IS, Coomes SM, Pelly VS, Czieso S**, et al. 2014. MicroRNA-containing T-regulatory-cell-derived exosomes suppress pathogenic T helper 1 cells. *Immunity* **41**: 89–103.

98. **Montecalvo A, Larregina AT, Shufesky WJ, Stolz DB**, et al. 2012. Mechanism of transfer of functional microRNAs between mouse dendritic cells via exosomes. *Blood* **119**: 756–66.

99. **Alexander M, Hu R, Runtsch MC, Kagele DA**, et al. 2015. Exosome-delivered microRNAs modulate the inflammatory response to endo-toxin. *Nature Comm* **6**: 7321.

100. **Li Z, Rana TM.** 2014. Therapeutic targeting of microRNAs: current status and future challenges. *Nat Rev Drug Discov* **13**: 622–38.

101. **Janssen HLA, Reesink HW, Lawitz EJ, Zeuzem S**, et al. 2013. Treatment of HCV infection by targeting microRNA. *N Engl J Med* **368**: 1685–94.

102. **Ohno S, Takanashi M, Sudo K, Ueda S**, et al. 2013. Systemically injected exosomes targeted to EGFR deliver antitumor microRNA to breast cancer cells. *Mol Ther* **21**: 185–91.

103. **Reddy M, Chen Z, Park JT, Wang M**, et al. 2014. Regulation of inflammatory phenotype in macrophages by a diabetes-induced long non-coding RNA. *Diabetes* **63**: 4249–61.

104. **Carpenter S, Aiello D, Atianand MK, Ricci EP**, et al. 2014. A long noncoding RNA mediates both activation and repression of immune response genes. *Science* **341**: 789–92.

105. **Pang KC, Dinger ME, Mercer TR, Malquori L**, et al. 2009. Genome-wide identification of long noncoding RNAs in CD8+ T cells. *J Immunol* **182**: 7738–48.

106. **O'Connell RM, Taganov KD, Boldin MP, Cheng G**, et al. 2007. MicroRNA-155 is induced during the macrophage inflammatory response. *Proc Natl Acad Sci USA* **104**: 1604–9.

107. **O'Connell RM, Rao DS, Chaudhuri AA, Boldin MP**, et al. 2008. Sustained expression of microRNA-155 in hematopoietic stem cells causes a myeloproliferative disorder. *J Exp Med* **205**: 585–94.

108. **Huffaker TB, Hu R, Runtsch MC, Bake E**, et al. 2012. Epistasis between microRNAs 155 and 146a during T cell-mediated antitumor immunity. *Cell Rep* **2**: 1697–709.

109. **Boldin MP, Taganov KD, Rao DS, Yang L**, et al. 2011. miR-146a is a significant brake on autoimmunity, myeloproliferation, and cancer in mice. *J Exp Med* **208**: 1189–201.

110. **Xiao C, Srinivasan L, Calado DP, Patterson HC**, et al. 2008. Lymphoproliferative disease and autoimmunity in mice with increased miR-17-92 expression in lymphocytes. *Nat Immunol* **9**: 405–14.

111. **Mogilyansky E, Rigoutsos I.** 2013. The miR-17/92 cluster: a comprehensive update on its genomics, genetics, functions and increasingly important and numerous roles in health and disease. *Cell Death Differ* **20**: 1603–14.

112. **Li G, Yu M, Lee W-W, Tsang M**, et al. 2012. Decline in miR-181a expression with age impairs T cell receptor sensitivity by increasing DUSP6 activity. *Nat Med* **18**: 1518–24.

113. **Stittrich A- B, Haftmann C, Sgouroudis E, Kühl AA**, et al. 2010. The microRNA miR-182 is induced by IL-2 and promotes clonal expansion of activated helper T lymphocytes. *Nat Immunol* **11**: 1057–62.

Review essays

114. **Bottoni A, Cox GM, Satoskar AR, Croce CM**, et al. 2012. miR-29ab1-deficiency identifies a negative feedback loop controlling Th1 bias that is dysregulated in multiple sclerosis. *J Immunol* **189**: 1567–76.

115. **Wu F, Zikusoka M, Trindade A, Dassopoulos T**, et al. 2008. MicroRNAs are differentially expressed in ulcerative colitis and alter expression of macrophage inflammatory peptide-2α. *Gastroenterology* **135**: 1624–35.

116. **Ponomarev ED, Veremeyko T, Barteneva N, Krichevsky AM**, et al. 2011. MicroRNA-124 promotes microglia quiescence and suppresses EAE by deactivating macrophages via the C/EBP-α-PU.1 pathway. *Nat Med* **17**: 64–70.

117. **Carpenter S, Aiello D, Atianand MK, Ricci EP**, et al. 2013. A long noncoding RNA mediates both activation and repression of immune response genes. *Science* **341**: 789–92.

118. **Gomez JA, Wapinski OL, Yang YW, Bureau JF**, et al. 2013. The NeST long ncRNA controls microbial susceptibility and epigenetic activation of the interferon-γ locus. *Cell* **152**: 743–54.

119. **Wang P, Xue Y, Han Y, Lin L**, et al. 2014. The STAT3-binding long noncoding RNA lnc-DC controls human dendritic cell differentiation. *Science* **344**: 310–3.

120. **Hu G, Tang Q, Sharma S, Yu F**, et al. 2013. Expression and regulation of intergenic long noncoding RNAs during T cell development and differentiation. *Nat Immunol* **14**: 1190–8.

121. **Li Z, Chao T-C, Chang K-Y, Lin N**, et al. 2014. The long noncoding RNA THRIL regulates TNFα expression through its interaction with hnRNPL. *Proc Natl Acad Sci USA* **111**: 1002–7.