REVIEW

Enhancers in Autoimmune Arthritis

Implications and Therapeutic Potential

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Introduction

Genome-wide association studies (GWAS) have identified hundreds of single-nucleotide polymorphisms (SNPs) associated with autoimmune diseases, including autoimmune arthritis. So far, it has proven difficult to translate these findings into disease understanding and novel therapeutic approaches as the majority of these SNPs are not located in protein-coding regions. Recently, various studies found that a large number of autoimmune disease–associated SNPs affect DNA regulatory units, suggesting that altered epigenetic control of transcription is an important process in disease pathogenesis (1,2). Technical developments have allowed for detailed analysis of the epigenetic profile of disease-associated cells, creating new opportunities to study gene regulation in the context of disease. In this review, we will discuss these advances in the field of epigenetics, focusing on (super)enhancers associated with autoimmune arthritis. Furthermore, we will describe how enhancer profiling of disease-specific cells can contribute to better understanding of disease pathogenesis. Additionally, we will outline strategies that can target enhancer activity and discuss their potential use as therapeutic approaches in the treatment of autoimmune arthritis.

Genetic basis of autoimmune arthritis

The genetic basis of autoimmune arthritis has been studied extensively, especially in the last decade. Advances using high-throughput genome sequencing have identified multiple risk variants associated with various rheumatic diseases, including rheumatoid arthritis (RA), juvenile idiopathic arthritis (JIA), systemic lupus erythematosus (SLE), ankylosing spondylitis (AS), and psoriatic arthritis (3–8). Although these studies provide some important clues about the biologic pathways that might be affected, novel insights regarding the molecular function and role in disease pathogenesis remain limited. For example, genome-wide significant loci, including the major histocompatibility complex (MHC) loci, and regions with suggestive associations can only explain 18% of the risk for JIA (4).

This is mainly because of 2 different reasons, the first being the difficulty to define which SNP is the disease-causal variant. Disease-associated loci identified by GWAS contain numerous SNPs. This is due to linkage disequilibrium (LD), the nonrandom association between 2 alleles of different loci. Therefore, disease-causal variants are often surrounded by neutral or other disease-causal variants, making it difficult to pinpoint the candidate disease-causal SNP(s) (9). Improvements in identifying disease-causal variants have recently been made by several groups by developing algorithms that take into account either cell type or tissue-specific epigenomic information (e.g., probabilistic identification of causal SNPs [PICS], EPIGWAS, Risk Variant Inference

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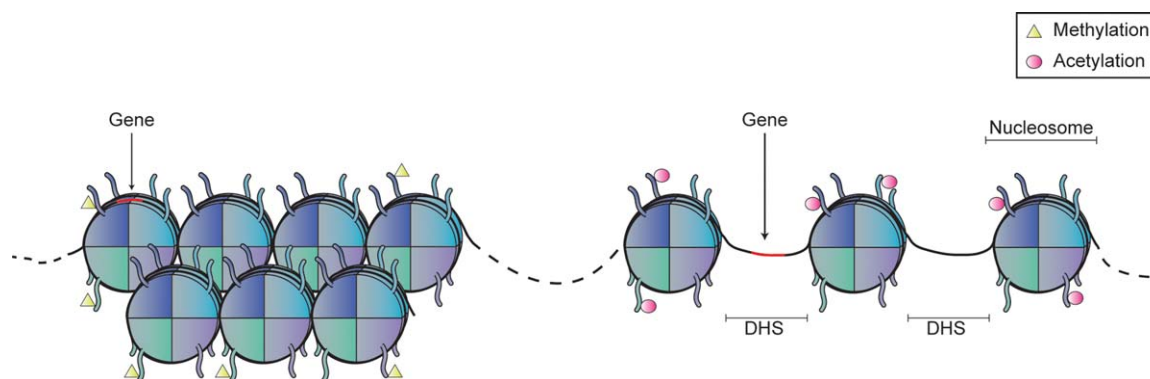
Figure 1. Schematic representation of chromatin structure. DNA is wrapped around an octamer of histone proteins, together forming a nucleosome. Nucleosomes can be tightly packed, rendering the DNA inaccessible (left), or can have a more loose structure, allowing transcription factors to bind (right). DHS = DNase I hypersensitivity site.

using Epigenomic Reference Annotation [RiVIERA], Robust Allele Specific QUAntitation and quality controL [RASQUAL]), (predicted) gene function and expression data (Data-driven Expression-Prioritized Integration for Complex Traits [DEPICT]), microRNA (miRNA)–target gene networks (miRNA–target gene enrichment analysis in GWAS [MIGWAS]), or genome-wide information from all SNPs, instead of a restricted SNP set, together with explicit modeling of LD (2,10–15). For example, the PICS algorithm demonstrates that only 5% of the SNPs that were originally thought to be disease-causing are actually assigned as being disease-causal variants (2). This indicates that genetic fine mapping of autoimmune disease variants will further unravel the genetic basis of autoimmunity.

The second reason that it has been difficult to translate GWAS findings into disease understanding is that ~90% of disease-associated SNPs are located outside of protein-coding regions, in regulatory DNA regions, making it difficult to understand which gene(s) is affected and how (1,2). As these regulatory DNA regions are epigenetically regulated, a better understanding of the epigenetic landscape is needed to understand the contribution of genetic variation to autoimmunity.

Enhancers and superenhancers

To fit the approximately 2-meter–long DNA strand in the nucleus, DNA is tightly packed. DNA is wrapped around the histone proteins H2A, H2B, H3, and H4, 2 of each type, thereby forming a nucleosome and creating the chromatin structure. The N-terminal tail of histones can be covalently modified. Generally, methylation allows tight packing of nucleosomes, rendering the DNA inaccessible. Acetylation reduces the positive charge of the histone tail, thereby reducing the

interaction with the negatively charged DNA, allowing enzymes and transcription factors to bind (16) (Figure 1). Regulatory DNA regions are characterized by DNase I hypersensitivity sites, meaning that in these regions DNA can be cleaved by DNase I, indicating a loose chromatin structure. Of the SNPs located in regulatory DNA regions, the majority localize to so-called enhancers (10,17). Enhancers are *cis*-regulatory DNA elements to which transcription factors and cofactors can bind, and they are crucial for transcriptional regulation. By recruitment of RNA polymerase II and mediator complex to the promoter of their target gene, enhancer elements regulate gene expression (Figure 2A).

Enhancers are generally a few hundred basepairs in size, contain multiple transcription factor binding sites, and can be located up to 1,000 kb upstream or downstream of the promoter of their target gene (16). The dispersion of enhancers throughout the genome and the 3-dimensional chromatin conformation make it difficult to define which gene a certain enhancer is regulating. Generally, epigenomic studies assume that enhancers regulate the gene whose transcriptional start site is closest to the enhancer. For a more precise understanding of the gene regulated by a certain enhancer, chromosome conformation capture (3C) techniques (3C-based technologies) are available that enable capturing of the physical interactions between enhancers and promoters (18). These technologies indicate that 27–40% of the active enhancers indeed interact with their nearest promoter, suggesting that 3C-based technologies are pivotal for understanding the epigenetic landscape (19,20).

Enhancers that are permissive for transcriptional regulation but that are not active (i.e., inactive/poised enhancers) are characterized by monomethylation of histone H3 at lysine 4 (i.e., H3K4me), while active
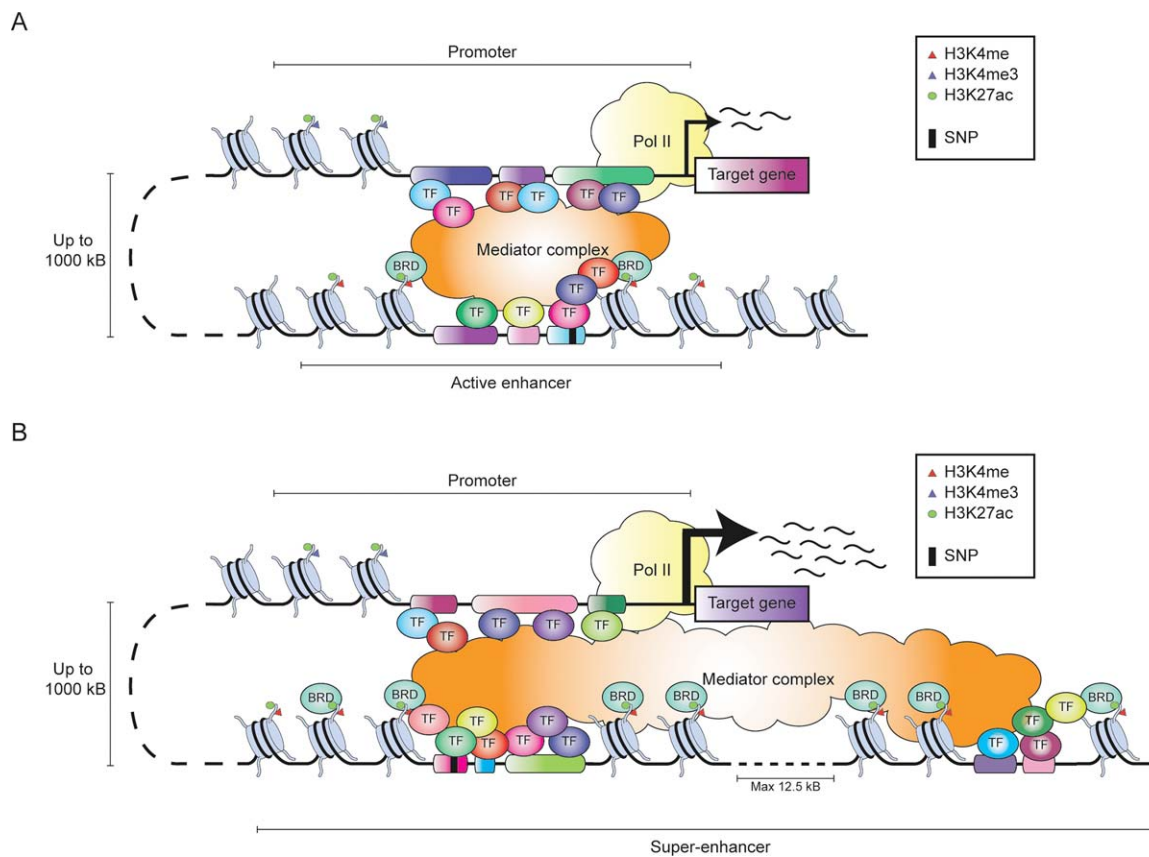
Figure 2. Schematic representation of transcriptional regulation by enhancers and superenhancers. **A,** Gene expression of a target gene driven by an active enhancer. Active enhancers are characterized by monomethylation of histone H3 at lysine 4 (H3K4me) and acetylation of histone H3 at lysine 27 (H3K27ac). Enhancers facilitate transcription by transcription factor (TF) binding, recruitment and binding of bromodomain and extraterminal proteins, consisting of bromodomain-containing proteins (BRDs), the mediator complex, looping of the DNA, and RNA polymerase II (Pol II) recruitment. Single-nucleotide polymorphisms (SNPs) are enriched in regulatory DNA regions, for example, enhancers. Enhancers can be located up to 1,000 kb from their target gene. **B,** Gene expression of a target gene driven by a superenhancer. Superenhancers are large enhancers characterized by extensive acetylation of histone H3 at lysine 27 and increased binding of the mediator complex and transcription factors. Active enhancers within 12.5 kb of each other can together form a superenhancer, leading to increased gene expression. Superenhancers are enriched for SNP localization compared to active enhancers. H3K4me3 = trimethylation of histone H3 at lysine 4.

enhancers contain both H3K4me and acetylation of histone H3 at lysine 27 (i.e., H3K27ac) (21,22). In any mammalian cell type, tens of thousands of enhancers can be found, exceeding the number of protein-coding genes (~20,000), indicating that a single gene can be regulated by multiple enhancers within one cell and that the enhancer(s) regulating a certain gene can differ between cells (19,23).

In addition, a number of studies have recently identified extremely large enhancer domains, containing clusters of individual enhancers, termed superenhancers or stretched enhancers (24–26). Active enhancers within 12.5 kb of each other can together form a superenhancer (Figure 2B). Superenhancers are identified by extensive H3K4me, H3K27ac, p300 binding, mediator complex

occupancy, and increased DNase I hypersensitivity sites and are characterized by increased transcription factor binding. Superenhancer-regulated genes are expressed to a higher extent than genes driven by a regular enhancer (26). This increase in gene expression seems to be due to synergy between individual enhancer constituents within a superenhancer, rather than being the sum of individual enhancers (27–29). However, as some superenhancers are single enhancers, and enhancers within 12.5 kb of each other do not necessarily form a superenhancer, it remains questionable whether superenhancers are a novel concept in gene regulation, or whether they are more a reflection of the characteristics of their individual enhancer constituents, such as a different sequence composition leading to increased

transcription factor binding and increased transcriptional activity (30). Superenhancers preferentially regulate genes important for cell identity as well as genes associated with disease. For example, in multiple cancer subtypes, superenhancers are associated with tumor oncogenes (24,31). Taken together, this suggests that understanding the role of (super)enhancers in immune cell function will help in defining their function in disease pathogenesis. For autoimmune arthritis, it seems logical to analyze the importance of (super)enhancers in cells from both the adaptive and innate immune systems.

Enhancers in immune cell function

The diverse properties of different cell types within the immune system are reflected within their enhancer landscape, demonstrating that enhancers can be highly cell type specific. This is illustrated by the distinct enhancer landscape of naive CD4+ T cells versus differentiated T cell subsets (32,33). STAT proteins are pivotal for establishing subset-specific enhancers, with STAT-1 and STAT-4 being important for the generation of Th1 cell–specific enhancers, while STAT-6 shapes Th2 cell–specific enhancers (33). T cell superenhancers are preferentially associated with cytokines and cytokine receptors; for instance, the T cell subtype–specific genes *Ifng*, *Il13*, and *Il17a* are associated with a superenhancer in mouse Th1, Th2, and Th17 cells, respectively (34,35). The strongest superenhancer in CD4+ Th1, Th2, and Th17 cells is linked to *Bach2*, a suppressor of T effector cell differentiation. *Bach2* deletion reduces expression of other T cell superenhancer-associated genes, suggesting the presence of a key regulatory node in T cells driven by BACH2 (35). Also, in B cells BACH2 is associated with a superenhancer, highlighting the important role of this gene in adaptive immune cell regulation (24).

The innate immune response, with monocytes and macrophages being important mediators, is characterized by the rapid expression of a subset of genes 0.5–2 hours after stimulation (i.e., primary response genes) and by expression of another gene subset 2–8 hours after stimulation (i.e., secondary response genes) (36). These characteristics are reflected within the enhancer landscape of innate cells. Namely, Toll-like receptor 4 (TLR-4) stimulation leads to the acquisition of H3K27ac by some poised enhancers, reduction or complete loss of certain enhancers, and reduction of the strength of a subset of enhancers already present in unstimulated conditions (37,38). These epigenetic alterations are rapid and thus likely to be associated with expression of early primary response genes. TLR-4 stimulation also induces the formation of ~1,000 de novo enhancers. As de novo

enhancer formation takes time and involves nucleosome remodeling, which is known to be required for secondary response gene expression, these enhancers are linked to secondary response genes (39).

In addition to lipopolysaccharide (LPS), the proinflammatory cytokine tumor necrosis factor (TNF), via activation of NF-κB, can shape the enhancer repertoire (40). This occurs mainly via de novo superenhancer formation and drives proinflammatory gene expression. Since some of these proinflammatory genes are cytokines, this implies a (positive) feedback loop whereby cytokines can affect the enhancer profile in an autocrine and paracrine manner. Similar effects of the (local) microenvironment on the enhancer landscape are described for tissue-resident macrophages. For example, transplantation of macrophages into distinct tissues of recipient mice leads to the acquisition of an enhancer profile comparable to that of recipient tissue-resident macrophages (41,42). Taken together, these studies illustrate that the microenvironment drives selection and function of enhancers and thereby regulates cellular identity and plasticity. This implies that it might be crucial to analyze the enhancer profile of cells directly after isolation from their tissue, as this reflects their enhancer landscape within the microenvironment. This is especially important to take into account for autoimmune arthritis, in which the microenvironment is one of the main drivers of the inflammatory cellular phenotype.

Enhancer regulation in autoimmune arthritis

Given the critical role of (super)enhancers for proper immune cell function, it is almost inevitable that they play a significant role in autoimmune arthritis as well. However, the role of enhancers in immune-related diseases has hardly been investigated. The importance of enhancers for autoimmunity is underscored by the enrichment of autoimmune disease–associated SNPs in enhancer regions of immune cells from healthy controls, with the highest enrichment in superenhancers compared to regular enhancers (2,24,25,35). For example, RA-associated noncoding SNPs are 3.2-fold more enriched in superenhancers and 2.2-fold more enriched in regular enhancers compared to other DNA regulatory regions. In addition, disease-associated variants preferentially map to (super)enhancers that are specific for disease-relevant cell types (24,25,35). For instance, the majority of RA-associated SNPs map to T cell, natural killer cell, and B cell (super)enhancers (43). For SLE, SNPs are predominantly located within B cell superenhancers, underscoring their important role in SLE pathogenesis (24). It might therefore be informative to

map disease-associated SNPs to the (super)enhancer profile of different immune cell (subsets) for not-so-well-characterized diseases, as this can reveal the cell type(s) involved in the disease.

Since ~90% of disease-associated SNPs are located in noncoding regions and 60% of these SNPs map to enhancers, disease-associated SNPs might affect gene transcription (1,2). Indeed, SNPs present in DNase I hypersensitivity sites are 4 times more likely to have an effect on gene expression compared to SNPs located outside DNase I hypersensitivity sites (44). Nonetheless, due to the presence of multiple SNPs in LD in a risk locus and because of the difficulty of defining which of these SNPs is causal for the disease, it is difficult to determine exactly the transcriptional effect of a SNP within a regulatory region. The effect of genetic enhancer variants on transcription is thought to be relatively small, with a reported 1.3–2-fold difference in target gene expression (45). Although modest, these differences can play important roles in disease pathogenesis.

For example, disease-associated SNPs are enriched at expression quantitative trait loci (eQTLs), which are genomic regions containing DNA variants that affect gene expression and that thus might alter the immunophenotype (46). A large proportion of eQTL SNPs are present around the transcription start site and are therefore likely to affect the promoter region (47). So far, eQTL studies have mostly been focused on promoter biology, but enhancer eQTL data are starting to emerge. For example, eQTL SNPs are enriched in DNase I hypersensitivity sites and regions characterized by active histone marks, with a strong enrichment for H3K27ac regions 5–100 kb upstream from the transcription start site, suggesting enrichment within enhancer regions (44). A more recent study (2), looking at SNPs associated with heritable differences in peripheral blood gene expression, shows that ~9% of the eQTL SNPs are located within promoters and ~14% within enhancers, suggesting that enhancer eQTL SNPs are important to take into account. However, considering that 60% of noncoding variants map to enhancers, this also suggests that a large proportion of enhancer SNPs do not map to eQTLs, and it remains to be investigated if and how these variants affect gene expression. There are some suggestions (e.g., for RA and SLE) that these diseases cannot be explained by one SNP, but that genetic variants within clusters of enhancers present at risk loci together affect gene expression and therefore confer disease susceptibility; however, this needs to be further investigated (48).

For autoimmune vitiligo, 3 SNPs are located within an MHC class II superenhancer, which also corresponds to an eQTL for HLA–DR and HLA–DQ expression. The presence of these SNPs correlates with increased HLA–DR and HLA–DQ surface expression and increased cytokine production, illustrating how SNP localization in a specific genomic region can contribute to the development of autoimmunity (49). Similarly, 2 SLE-associated SNPs are located in an enhancer element downstream of the *TNFAIP3* promoter (50). *TNFAIP3* encodes for A20, an inhibitor of NF-κB signaling. The presence of these 2 variants impairs NF-κB binding to the enhancer, thereby reducing promoter–enhancer interaction, leading to reduced A20 expression (50). This results in increased NF-κB signaling and thus supports a causal role for the SNP pair in SLE pathogenesis. For AS, a possible disease-causal SNP has been identified within an enhancer between *IL23R* and *IL12RB2* (51). The presence of this SNP corresponds to reduced H3K4me of the enhancer region in CD4+ T cells, impaired binding of nuclear proteins to the SNP-containing DNA region, and reduced reporter activity. However, expression of *IL23R* and *IL12RB2* is not affected, although the frequency of Th1 cells, which express *IL12RB2*, is altered. A possible explanation for this discrepancy could be that the SNP-containing enhancer is not regulating *IL23R* and *IL12RB2* expression but has another, yet unidentified, target gene affecting Th1 cell numbers. The 3C-based technologies could help in unraveling the biologic effect of this particular SNP and other disease-associated SNPs to which a molecular function has not so far been ascribed.

A confounding factor in linking GWAS data with epigenomics data is that the epigenetic data being used are predominantly based on cells from healthy controls and might not represent the epigenetic landscape of disease-relevant, patient-derived cells. Additionally, for most diseases it is more informative to profile cells from the affected tissue than cells from the peripheral circulation. Indeed, JIA (super)enhancer profiling of synovial-derived CD4+ T cells revealed a different profile compared to peripheral blood cells from healthy controls (52). Similar observations have been made in SLE-derived monocytes (53). Expression QTL mapping is generally also performed using samples from healthy controls; however, eQTLs were recently mapped using an RA cohort (54). This revealed that RA GWAS hits are enriched in RA-identified eQTLs compared to healthy control eQTLs and that RA eQTLs are enriched in enhancer regions. Furthermore, combination of these eQTL data with GWAS and epigenomics data identified novel disease-relevant genes (54).

Consistent with these observations, arthritis-associated SNPs are more enriched in JIA patient superenhancers compared to healthy control superenhancers (52).

These findings underscore the importance of using patient-derived cells and illustrate that integrating multiple data sets can be more informative than a single data set in identifying autoimmune arthritis–associated molecular mechanisms. In addition, comparing the enhancer profile of different autoimmune diseases can be useful in unraveling autoimmune disease pathogenesis. This is of particular interest since autoimmune disease–associated SNPs are profoundly enriched in T cell superenhancers in JIA, while this is not the case for non–autoimmune disease–associated SNPs (52). Correspondingly, it has been reported that autoimmune diseases have shared risk loci, suggesting overlap in disease pathogenesis (2). In addition, enhancer data may also define distinct pathogenic processes between diverse types of autoimmune arthritis. For example, RA synovial fibroblasts (RASFs) are distinguishable from osteoarthritis-derived synovial fibroblasts based on DNA methylation and transcriptome data (55). Furthermore, these data can also discriminate between RASFs isolated from different joints. These observations suggest that joint-specific epigenetic signatures exist, and they therefore indicate that molecular pathways affected in the disease might differ from one joint to another. Epigenetic profiling can be used to identify these differences, and, more importantly, these observations raise the question of whether epigenetic knowledge can be translated into novel therapeutic strategies for the treatment of autoimmune arthritis.

Inhibition of enhancer activity in relation to autoimmune arthritis

The observations that autoimmune arthritis–associated SNPs are preferentially located in enhancers and that superenhancers are associated with disease-relevant genes strongly suggest that enhancers contribute directly to disease pathogenesis. Therefore, there is a growing body of interest in the development of therapeutic strategies aimed at targeting (super)enhancer activity. Enhancer regions are critically dependent on chromatin regulators (e.g., reader proteins recognizing histone modifications), which recruit transcription factors and other proteins to facilitate transcription initiation and elongation. Important reader proteins at enhancers are bromodomain and extraterminal (BET) proteins, consisting of bromodomain-containing protein 2 (BRD-2), BRD-3, BRD-4, and bromodomain testis-specific protein, each containing 2 bromodomains (56)

(Figure 2). The bromodomain allows BET proteins to bind to acetylated histone and nonhistone proteins. BRD-4 is most extensively studied and is present at promoters and active enhancers, with increased localization at superenhancers (31,57). Binding of BET proteins to acetylated transcription factors might contribute to their preferential localization in (super)enhancer regions (58).

Recently, a wide range of small-molecule inhibitors of BET proteins has been developed (59) (Table 1). The therapeutic potential of BET inhibitors has been demonstrated in numerous in vitro and in vivo tumor models, in which BET inhibitors inhibit the expression of different oncogenes, including c-*myc* (58,60). Inhibition of c-*myc* is linked to the disruption of c-*myc*–associated superenhancer activity (31). This is probably related to the high levels of BRD-4 present at superenhancers and the transcriptional dependency thereof and thus contributes to the preferential inhibition of superenhancer-driven gene expression by BET inhibitors.

BET inhibitors are highly effective in shaping the adaptive immune response. For example, the BET inhibitors JQ1 and I-BET762 significantly impair differentiation of naive CD4+ T cells into T effector cell subsets, both in vitro and in vivo (61,62). Furthermore, BET inhibition of differentiated CD4+ T cells has a profound effect on production of cytokines, such as interleukin-17 (IL-17). BET inhibition also impairs B cell function by inhibiting Ig class-switch recombination and B cell expansion and proliferation (63,64). Additionally, BET inhibitors can affect innate immune responses. For instance, JQ1, I-BET762, and I-BET151 all inhibit proinflammatory cytokine production by LPS-stimulated monocytes and macrophages in vitro and are effective in different in vivo animal models (65–69). Genes affected by I-BET762 in LPS-stimulated macrophages belong to the secondary response genes. Given the epigenetic differences underlying the expression of these genes, this suggests that I-BET762, and possibly other BET inhibitors as well, preferentially acts on de novo enhancers (68). This corresponds to the observation that de novo superenhancers, induced by TNF and LPS, are highly susceptible to BET inhibition, resulting in decreased superenhancer-mediated gene expression (37,40).

Inflammation-associated diseases are characterized by proinflammatory mediators present at the site of inflammation, suggesting that BET inhibition might be a way to specifically target cytokine-induced, and thus disease-associated, (super)enhancers. Indeed, JQ1 treatment of CD4+ T cells derived from synovium of JIA patients preferentially reduces expression of disease-associated genes, with the majority being involved in

Table 1. Overview of small molecules affecting enhancer activity*

Small molecule	Target	Effect on immune system (ref.)
CP1–0610	BRD-2, BRD-3, BRD-4, bromodomain testis-specific protein; targeted bromodomain: BD1	Not assessed
DRB	CDK-9	Inhibition of T cell priming under Th2 and Th17 conditions (62)
I-BET151	BRD-2, BRD-3, BRD-4, bromodomain testis-specific protein; targeted bromodomains: BD1 and BD2	Inhibition of proinflammatory gene expression in LPS-stimulated monocytes and macrophages (66,69); inhibition of inflammatory genes and matrix-degrading enzymes in RASFs (71); suppression of inflammation-induced arthritis, TNF-induced bone resorption, and EAE (66,74)
I-BET762	BRD-2, BRD-3, BRD-4, bromodomain testis-specific protein; targeted bromodomains: BD1 and BD2	Inhibition of macrophage and CD4+ T cell cytokine production (62,67,68); suppression of EAE (62)
JQ1	BRD-2, BRD-3, BRD-4, bromodomain testis-specific protein; targeted bromodomains: BD1 and BD2	Inhibition of proinflammatory cytokine production in macrophages and T cells (61,65,75); inhibition of DC maturation (86); inhibition of T cell differentiation (61); inhibition of Ig class-switch recombination and mitogenesis in B cells (63,64); inhibition of cytokine production in CD4+ T cells in JIA (52,70); suppression of CIA, EAE, psoriasis, and endotoxic shock in mouse models (61,65,72,73)
LY294002	BRD-2, BRD-3, BRD-4, bromodomain testis-specific protein; targeted bromodomain: BD1	Inhibition of inflammation in LPS-stimulated PBMCs (87)
MS417	BRD-2, BRD-3, BRD-4, bromodomain testis-specific protein; targeted bromodomains: BD1 and BD2	Inhibition of HIV-associated kidney disease (88)
Olinone	BRD-2, BRD-3, BRD-4, bromodomain testis-specific protein; targeted bromodomain: BD1	Not assessed
OTX015	BRD-2, BRD-3, BRD-4, bromodomain testis-specific protein; targeted bromodomains: BD1 and BD2	Disruption of HIV-1 latency (89)
PC579	CDK-9	Suppression of CIA (80)
PC585	CDK-9	Suppression of CIA (80)
PFI-1	BRD-2, BRD-3, BRD-4, bromodomain testis-specific protein; targeted bromodomains: BD1 and BD2	Inhibition of IL-1β–induced inflammation in airway epithelial cells (90)
RVX-208	BRD-2, BRD-3, BRD-4, bromodomain testis-specific protein; targeted bromodomain: BD2	Not assessed
RX-37	BRD-2, BRD-3, BRD-4, bromodomain testis-specific protein; targeted bromodomains: BD1 and BD2	Not assessed
TEN-010	BRD-2, BRD-3, BRD-4, bromodomain testis-specific protein; targeted bromodomains: BD1 and BD2	Not assessed
THZ1	CDK-7	Not assessed
Tofacitinib	JAK	Inhibition of RA-associated risk genes in CD4+ T cells from healthy controls (35); inhibition of TLR-induced cytokine gene expression in macrophages (67)

* BRD-2 = bromodomain-containing protein 2; CDK-9 = cyclin-dependent kinase 9; LPS = lipopolysaccharide; RASFs = rheumatoid arthritis synovial fibroblasts; TNF = tumor necrosis factor; EAE = experimental autoimmune encephalomyelitis; DC = dendritic cell; JIA = juvenile idiopathic arthritis; CIA = collagen-induced arthritis; PBMCs = peripheral blood mononuclear cells; IL-1β = interleukin-1β; TLR = Toll-like receptor.

proinflammatory and cytokine-related processes (52,70). Correspondingly, I-BET151 treatment of RASFs suppresses the production of matrix metalloproteinases and cytokines upon stimulation with TNF, IL-1β, or TLR ligands, resulting in reduced proliferation and chemoattractant properties (71). Similar observations are obtained by genetically silencing BET proteins in RASFs (72).

These findings indicate that BET inhibition can be a putative therapeutic approach for the treatment of autoimmune diseases. This has been tested using several in vivo autoimmune disease models. For instance, JQ1, I-BET151, or I-BET762 treatment of experimental autoimmune encephalomyelitis (EAE; an animal model of multiple sclerosis) and an in vivo model of psoriasis significantly reduces the onset and severity of disease symptoms (61,62,66,73). These results are associated with the preferential suppression of IL-6 and IL-17 production and Th17 cell numbers. The protective effect of BET inhibitors has also been explored in mouse models of autoimmune arthritis. For example, in serum-induced and collagen-induced arthritis (CIA) models, JQ1 and I-BET151 dramatically reduce disease progression and plasma and serum cytokine levels (61,72,74). Furthermore, I-BET151 inhibits the differentiation of monocytes toward osteoclasts in vitro and reduces TNF-induced bone resorption in vivo. This is consistent with observations that JQ1 inhibits RANKL-induced

up-regulation of osteoclast-associated genes (75). Since osteoclast formation and bone resorption are related to autoimmune arthritis, this implies a dual role for BET inhibitors in the treatment of autoimmune arthritis. As TNF and IL-6 can induce osteoclast differentiation, the effect of BET inhibition on osteoclast differentiation is likely to result from a combination of direct and indirect mechanisms of action, the latter via inhibition of TNF and IL-6 production by cells in the synovial compartment (76). Of note, 3 SNPs associated with RA are present in BRD-2 (77). It might be informative to study the effects of these SNPs on the regulation of gene transcription, disease, and susceptibility to BET inhibitors.

In addition to BET inhibitors, some other compounds could be used to inhibit enhancer activity, for instance, cyclin-dependent kinase (CDK) inhibitors specific for CDK-7 or CDK-9. CDK-7 and CDK-9 are both implicated in transcription initiation and elongation by phosphorylation of RNA polymerase II (78). CDK-7 inhibition, via the small molecule THZ1, affects superenhancer-driven gene expression in c-Myc–dependent tumor models (79). A recent study provides evidence for the use of CDK inhibitors in autoimmune diseases as well, as CDK-9 inhibition delays disease onset and reduces disease symptoms in a CIA model (80). Whether this effect is mediated via (preferential) inhibition of superenhancers has not been investigated and remains to be further explored. Comparison of the effect of CDK-9 inhibition and BET inhibition has revealed some differential effects on T cell priming, suggesting that different inhibitors of enhancer activity can have distinct properties (62). Therefore, the therapeutic potential of these compounds might differ between different disease types, probably reflecting differences in the underlying disease mechanisms.

The observed enrichment of STAT proteins at T cell (super)enhancers prompted researchers to investigate the effect of the JAK inhibitor tofacitinib, which is also approved for the treatment of RA as second-line therapy. In vitro treatment of T cells with tofacitinib dramatically alters gene expression, with a more drastic effect on genes driven by superenhancers compared to those driven by regular enhancers (35). Since the majority of RA-associated SNPs are located within T cell superenhancers, tofacitinib treatment of T cells in RA could result in selective targeting of RA-associated risk genes, underscoring its potential for the treatment of RA. Considering the (high) levels of acetylation and methylation associated with (super)enhancer activity, it could be speculated that targeting enzymes affecting acetylation and methylation of specific histone sites might be an alternative way to impair enhancer activity. For example, GSK-J4, an inhibitor of the H3K27me3 demethylases KDM6A and KDM6B, impairs the LPS-induced inflammatory response in macrophages derived from both healthy controls and RA patients (81). Furthermore, GSK-J4 suppresses in vitro Th17 cell differentiation and has been demonstrated to suppress EAE via the induction of tolerogenic dendritic cells (82,83). A possible explanation for this observation could be that decreased demethylation of H3K27 in enhancer regions renders these enhancers inactive, reducing the likelihood of acquiring acetylation and thus becoming active. Therefore, the immunomodulatory effect of GSK-J4 could be mediated via reduction of enhancer activity, but whether this is actually the case needs to be further explored.

Future perspective

In this review, we have discussed the recent advances in the field of epigenetics, focusing on enhancers and their implications for autoimmune arthritis. Epigenetic regulation of immune cells is essential for proper cell function and therefore crucial for mounting an appropriate immune response. It is becoming more and more evident that alterations related to (super)enhancers are linked to autoimmunity, including autoimmune arthritis.

For a better understanding of enhancers in the context of autoimmune arthritis, it is crucial to perform enhancer profiling of disease-relevant, patient-derived cells. As enhancers are highly cell type specific, it is pivotal that the enhancer landscape is defined in specific cell types and not in a mixed population of cells, as this will affect interpretation of the results. Since patient material is often limited, one of the challenges will be to obtain a sufficient number of cells. Another important factor to take into account is patient stratification, as distinct disease subtypes can have different enhancer landscapes. From another perspective, enhancer profiling could actually assist in classifying patients into existing disease subtypes and may be used for identifying novel disease subtypes. For example, this might be useful in the case of JIA, in which the designation of disease subtypes is still clinically based. Furthermore, it is important to know exactly what the target gene(s) of each enhancer is. Integration of chromosome conformation information with GWAS data will help in ascribing a molecular function to GWAS hits. This will contribute to better insight into the (shared) pathogenesis of autoimmune arthritis and might lead to the identification of novel therapeutic targets. In addition, enhancer profiling might reveal novel biomarkers that could be used to
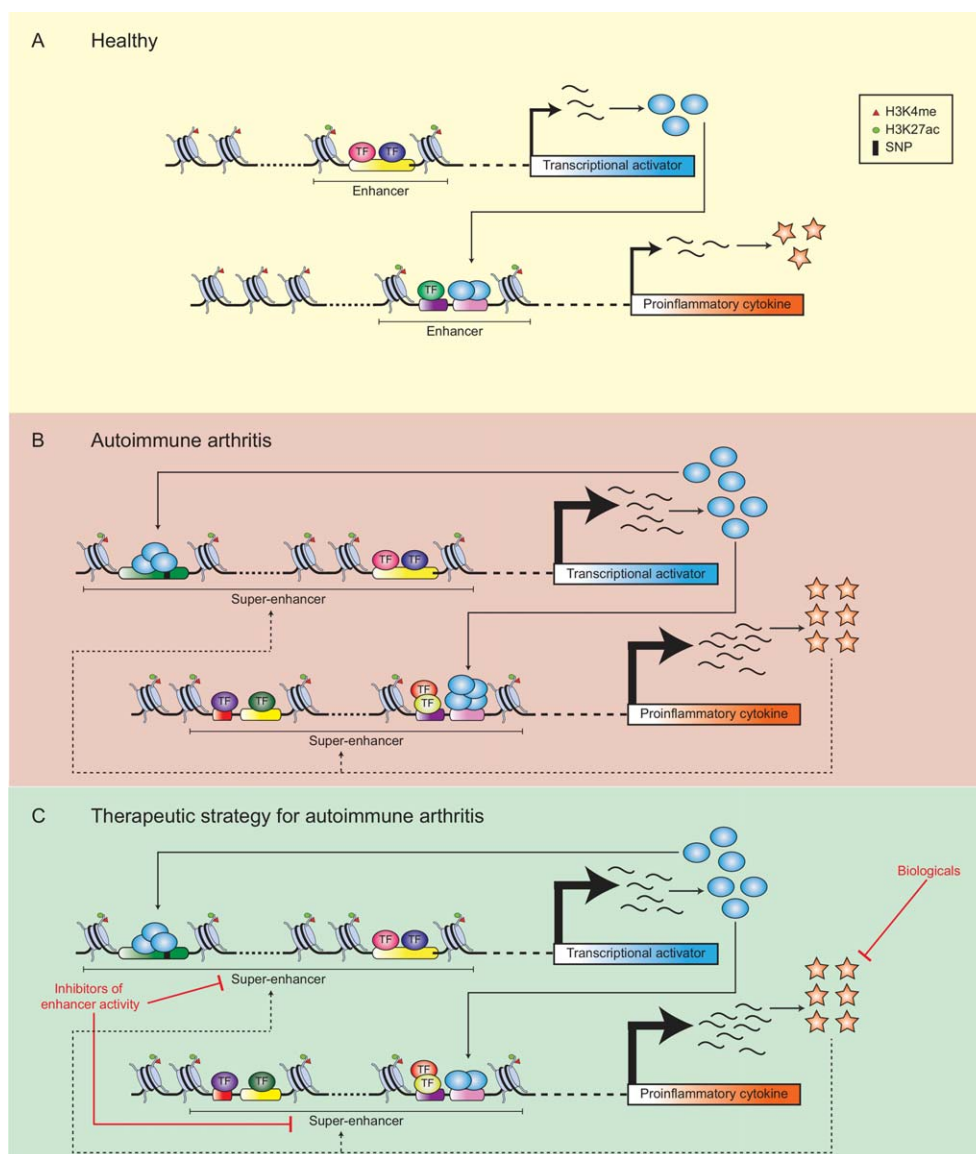
Figure 3. Schematic representation of the role of (super)enhancers in autoimmune arthritis. **A,** Under healthy conditions, enhancers regulate gene expression of proinflammatory cytokines and transcriptional activators, leading to controlled gene transcription. **B,** In autoimmune arthritis, superenhancers contribute to proinflammatory cytokine expression. Proinflammatory cytokines can shape the (super)enhancer repertoire, thereby creating self-regulatory loops and stimulating expression of transcriptional activators. Transcriptional activators can function in a regulatory feedback loop by regulating their own expression and that of proinflammatory cytokines. **C,** Inhibitors of enhancer activity can inhibit (super)-enhancers, thereby reducing proinflammatory gene expression. Biologic agents can inhibit the mechanism of action of proinflammatory cytokines, thereby disrupting the regulatory feedback loop. See Figure 2 for definitions.

predict therapeutic responses. This information could be extremely relevant when aiming for more personalized or stratified treatment regimens for autoimmune arthritis.

The increased localization of SNPs in enhancer regions and the observations that proinflammatory cytokines can shape the enhancer repertoire suggest that autoimmune arthritis–associated enhancer alterations can be a cause as well as a consequence of the disease,

and that it might be difficult to distinguish the one from the other. Furthermore, this indicates that (self)regulatory feedback loops can contribute to disease pathogenesis and thus suggests that it is important to disrupt these regulatory loops (Figure 3). This can be achieved by inhibiting (super)enhancer activity (e.g., by BET inhibitors). Compared to treatment with biologic agents, BET inhibitors might be favorable, as they preferentially inhibit

the expression of numerous disease-associated genes at once and inhibit the production of proinflammatory cytokines instead of inhibiting or blocking them after production. It could also be argued that combination therapy of BET inhibitors and biologic agents might be an even more powerful strategy, as biologic agents will prevent (super)enhancer formation by the inflammatory environment and thereby contribute to disruption of the regulatory feedback loop.

Although BET inhibitors seem highly effective in in vivo models of autoimmune arthritis, it remains to be established whether they will be as efficacious in a setting of autoimmune disease in humans. So far, the use of BET inhibitors in clinical trials for the treatment of severe cancers seems to be promising and to have limited side effects, such as thrombocytopenia, gastrointestinal adverse effects, and fatigue (84,85). However, it needs to be determined whether these side effects are acceptable for patients with autoimmune arthritis. Consistent with this, development of compounds with a higher selectivity for individual BET proteins, bromodomains, or novel targets will allow for more selective modulation of (super)-enhancer activity and thus for a more specific therapeutic application.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published.

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