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Nature and nurture of tissue-specific macrophage phenotypes

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Abstract

Macrophages are key players in immunity and tissue homeostasis but can also contribute to a diverse range of human diseases, including cardiovascular diseases. Enhancers, *cis*-acting DNA elements regulating gene activity, have been shown to be crucial for control of macrophage development and function. The selection and activities of macrophage-specific enhancers are regulated by the combined actions of lineage determining transcription factors (LDTFs) and signal dependent transcription factors (SDTFs) that are specified by developmental origin and tissue-specific signals. As a consequence, each tissue resident macrophage population adopts a distinct phenotype. In this review, we discuss recent work on how environmental factors affect the activation status of enhancers and can lead to long-lasting epigenetic changes resulting in innate immune memory. Furthermore, we discuss how non-coding genetic variation affects gene expression by altering transcription factor binding through local and domain-wide mechanisms. These findings have implications for interpretation of non-coding risk alleles that are associated with human disease and efforts to target macrophages for therapeutic purposes.

Keywords

macrophage; enhancer; tissue environment; genetic variation

1. Introduction

Macrophages are key players in tissue homeostasis and inflammation. In addition to central roles in innate immunity and as modifiers of the adaptive immune response, tissue macrophages serve supportive functions to the tissues they reside in. For example, alveolar macrophages are necessary for the normal turnover of lung surfactant, while splenic macrophages and Kupffer cells play important roles in clearance of senescent red blood cells and iron homeostasis[1–4]. Systematic gene expression profiling of macrophages from diverse

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tissues indicate that each macrophage population expresses a distinct set of genes that is associated with the developmental and homeostatic demands of the corresponding tissue[5, 6]. New populations of specialized macrophages continue to be discovered, such as macrophages associated with sympathetic nerves in white adipose tissue that have roles in the uptake and clearance of sympathetic amines[7–9]. Dysregulation of macrophage activities, however, contributes to a diverse range of human diseases, including inflammatory, metabolic, and cardiovascular diseases[10, 11].

Although it was previously thought that tissue macrophages originate from monocytes, it is now clear that many tissue-resident macrophage populations arise from fetal progenitors and require minimal input from the adult hematopoietic system[12, 13]. Macrophages derived from yolk sac and erythromyeloid progenitors (EMP) during development are the primary sources of resident macrophages in the mouse brain, lung, liver, kidney, and represent self-renewing populations. In contrast, intestinal and dermal macrophages are continuously replaced by hematopoietic stem cell derived monocytes. Infiltration of monocyte derived macrophages is observed upon infection or injury and is commonly observed in a broad spectrum of chronic inflammatory diseases[10, 11, 14]. In atherosclerosis, for example, it is known that dyslipidaemia can lead to increased proliferation of tissue-resident macrophages in the vasculature that is combined with an increased influx of monocytes differentiating into macrophages/ foam cells[15–18]. Targeted inhibition of monocyte recruitment and subsequent differentiation into proinflammatory cells is thus a potential approach for prevention and treatment of atherosclerosis and other diseases.

The extent to which macrophage function in health and disease is dependent on relative contributions of their developmental origin and the stimuli present in the microenvironment is an active area of investigation. Recent studies indicate that environmental signals play major roles in determining macrophage phenotypes by regulating the expression and function of transcription factors that activate *cis*-regulatory enhancer elements[5, 6, 19]. These enhancers in turn promote gene expression of tissue specific genes and thereby play key roles in shaping tissue macrophage phenotypes[6, 19]. However, global gene expression profiling experiments indicate that monocyte-derived cells do not achieve the same patterns of gene expression as embryonically derived microglia or Kupffer cells following engraftment into the brain or liver, respectively, indicating that developmental origin restricts the regulatory potential of environmental signals[20–22].

An additional motivation for understanding transcriptional mechanisms that regulate cellspecific gene expression is the observation that ~90% of the common genetic variation associated with disease traits as defined by genome wide association studies (GWAS) resides in non-coding regions of the genome[23, 24]. Although non-coding variation can affect gene expression by several mechanisms, single nucleotide polymorphisms (SNPs), insertions and deletions (InDels) that alter transcription factor binding in enhancer regions are likely to account for a substantial fraction of causal alleles[25]. As enhancers function in a cell-specific manner, interpretation of non-coding variants requires definition of regulatory landscapes in the relevant cell types.

In this review, we discuss how environmental signals affect macrophage enhancers and macrophage phenotype. Furthermore, we will explore how genetic differences between individuals affect macrophage enhancers and how genetic variation can be used to study enhancer selection and function.

2. Macrophage enhancers and environmental signals

Cell-specific developmental programs and responses to internal and external signals require close collaboration between gene promoters and distantly located regulatory enhancer elements[26]. Promoter regions provide transcription start sites (TSS) and are marked by histone 3 lysine 4 trimethylation (H3K4me3), while enhancers are distal *cis*-regulatory regions that are preferentially marked by H3K4me1[27]. Both promoters and enhancers contain DNA motifs that are recognized by specific transcription factors and when activated they both gain H3K27 acetylation[28]. However, in comparison to promoters, enhancers are activated in a more cell-specific manner. Accordingly, promoter regions are mainly bound by general sequence-specific transcription factors, while enhancers are preferentially bound by combinations of lineage determining transcription factors (LDTFs) that frequently have pioneering functions, i.e., the ability to recognize and gain access to their binding sites in the context of closed chromatin. The binding of LDTFs to closed enhancer elements requires collaborative interactions with each other and additional classes of transcription factors, as discussed in further detail below (Figure 1). By driving the selection of regulatory enhancers, LDTFs play major roles in specifying the identity of a cell[29]. LDTF binding to an enhancer can lead to newly formed loops beteen enhancers and promoters or can activate enhancers that are already connected to a gene promoter[30]. Transcription factors PU.1, AP-1 and CEBPβ are among the LDTFs commonly present on enhancers of macrophages and are known to regulate macrophage differentiation and identity[29, 31, 32]. Subsequent recruitment of chromatin remodellers by these LDTFs results in stable local opening of the chromatin, thus priming the enhancer and making it competent for signal dependent transcription factors (SDTFs) binding[29] (Fig. 1).

2.1 Tissue macrophage enhancers

Systematic analysis of diverse macrophage populations indicated that differential selection and activation of *cis* regulatory elements primarily occurred at enhancers, providing evidence that enhancer regions are the main drivers of differences in transcription and cellular function[6, 19]. A core of macrophage enhancers are likely to be shared by all tissue macrophages and depend on shared lineage determining transcription factors like PU.1. A comparison of enhancers defined in microglia and large peritoneal macrophages, for example, indicates that about 80% of the enhancers are shared and these enhancers are proximal to similarly expressed genes. However, about 20% of the enhancers are specific for each cell type[19], and these are associated with corresponding differences in gene expression (Fig. 2A). Studies on these cells provided evidence that tissue-specific enhancer selection is the result of signals from the environment where the macrophage resides in (Fig. 2B). These signals regulate the expression and activities of additional transcription factors, which results in a refinement of the enhancer repertoire specifically set up for the tissue environment[33]. For instance, in the peritoneal cavity, retinoic acid acts as a signal that induces the tissue-

specific peritoneal macrophage phenotype through the induction of transcription factor GATA6[34–36]. The retinoic acid–GATA6 pathway in peritoneal macrophages, in turn, can promote IgA class switching in peritoneal B-1 cells, illustrating the importance of tissue-specific functions of tissue macrophages[6, 19, 34, 35]. Similarly, SDTFs associated with tissue-specific enhancers have been identified for a variety of different tissue macrophages, including LXR-α in splenic macrophages[37], PPAR-γ in alveolar macrophages[38] and SMADs and SALL1 in microglia[19, 39, 40].

In relation to atherosclerosis, not much is known on aortic macrophage enhancer function thusfar. This in part reflects the technical difficulty of assessing enhancer function in the small number of macrophages that reside within atherosclerotic lesions. However, it is thought that anti-inflammatory signals like interleukin 4 (IL-4), in contrast to pro-inflammatory signals, are benificial for atherosclerosis outcome. For instance, the expression of the pro-fibrotic gene *Arg1*, a marker of IL-4 activation, is inversely correlated with atherosclerosis progression. Levels of the SDTF LXR-α were shown to be highly expressed in regressive plaques and promote the expression of *Arg1* through increased PU.1 and IRF8 binding to its promoter[41]. In contrast, proinflammatory signals like interferon-γ (IFN-γ) are thought to worsen atherosclerosis outcome, which fits with our current understanding that blocking pro-inflammatory pathways reduce cardiovascular events[42]. Stimulation of macrophages with pro- and anti-inflammatory mediators result in different macrophage phenotypes that affect atherosclerosis which are extensively reviewed before[43, 44]. As a model of different macrophage phenotypes and the differences in enhancer activation, we will discuss some of the recent work on how proinflammatory IFN-γ and anti-inflammatory IL-4 affect enhancers below.

2.2 Macrophage enhancers and in vitro stimuli

Using *in vitro* models, it has been shown that different stimuli are able to activate distinct repertoires of macrophage enhancers. These enhancers can be already present and further activated by the specific stimuli (poised enhancers) or they can be newly formed (*de novo* or latent enhancers). The repertoire of *de novo* enhancers is highly stimulus-specific and acquire H3K4 methylation and H3K27 acetylation that depends on the collaboration of SDTFs with LDTFs and histone modifying enzymes[45–47]. Once induced, many de novo enhancers did not return to a latent state when stimulation was withdrawn and were associated with faster and stronger responses when cells were restimulated[45]. These studies provided some of the initial evidence that enhancers could encode an epigenetic memory of an environmental perturbation.

Recent studies on macrophage responses to stimuli have focussed on how different signals interact on the epigenomic level. Stimulation of macrophages with pro-inflammatory IFN-γ and anti-inflammatory IL-4 led to opposing transcriptional functional programs. In mouse macrophages, it was demonstrated that these signals were found to mutually inhibit the epigenomic and transcriptional changes induced by each cytokine alone[48]. In accordance with this, Czimmerer et al. recently showed that the IL-4 activated transription factor STAT6 can act as a transcriptional repressor, next to its known functions of activating IL-4 dependent genes[49]. IL-4 STAT6-bound repressed enhancers were associated with reduced

LDTF, p300 and RNA polymerase II binding and H3K27 acetylation. These enhancers seem to regulate inflammatory genes, providing an explanation of the inhibitory effects on inflammatory responsiveness, including inflammasome activation[49]. In human macrophages, it was recently demonstrated that IFN-γ suppresses expression of IL-4 target genes by Enhancer of zeste homolog 2 (Ezh2)mediated H3K27 trimethylation at a small subset of IL-4 target gene promoters, leading to gene repression. This included repression of the gene encoding the transcription factor peroxisome proliferator-activated receptor γ (PPARγ), which is a SDTF associated with anti-inflammatory characteristics[50]. Moreover, IFN-γ also downregulates expression of transcription factor MAF and loss of MAF binding was observed at IFN-γ repressed enhancers, which coincides with LDTF loss of binding[51]. These IFN-γ repressed enhancers were mostly associated with IL-4 target genes. At IFN-γ primed enhancers IRF1, STAT1 and LDTF binding is increased, resulting in more open chromatin and enhancer activation. Consistently, in synovial macrophages, MAF expression and IRF1 and STAT1 expression are respectively negatively and posititively associated with the systemic inflammatory disease reumatoid arthritis[51].

3. Immune memory

Although previously thought to be specific for adaptive immune cells, it has become clear that also monocytes and macrophages have memory, as they remember signals they have seen before. These signals lead to long-lasting epigenetic changes at both promoters and enhancers, including latent enhancers[30]. Depending on the signal and the dose of the first signal the monocyte/macrophage senses, a second stimulation can result in tolerance, leading to reduced inflammatory responses, or trained immunity, resulting in an increased responses[52, 53].

3.1 Tolerance

Tolerance can occur when lipopolysaccharide (LPS) activated macrophages are prestimulated with a high dose LPS. It leads to gene-specific chromatin modifications that result in silencing of one class of (tolerizable) genes, which includes pro-inflammatory mediators on one hand, thereby preventing tissue damage[54]. While on the other hand, a second (non-tolerizable) class, which includes antimicrobial effectors, is primed or trained[54].

Pretreatment with interferon γ (IFN-γ) can prevent tolerization of primary human monocytes and restores TLR4-mediated induction of HLA-DR expression and various proinflammatory cytokines *in vitro*[55] and in septic patients[56], possibly through nucleosome remodeling by Brahma-related gene 1 (Brg1)[55]. *Brg1* expression was recently shown to be negatively regulated by miR-221 and miR-222, two micro-RNAs highly expressed in septic mice and patients. The increased expression causes the transcriptional silencing of a subset of inflammatory genes that depend on chromatin remodelling and STAT1/2, which in turn leads to a defect in the induction of these inflammatory genes, thereby promoting tolerance[57]. Also type I IFNs (IFN-α/β) were shown to prevent silencing of TNF-tolerized inflammatory genes, not by attenuating TLR4 signaling pathways, but rather by changes at the level of chromatin to reprogram inflammatory responses[58].

Besides IFNs, *Candida albicans* cell wall component β-glucan was recently shown to reverse LPS tolerance for 60% of the tolerizeable genes *in vitro*. Also, *ex vivo* β-glucan treatment of monocytes isolated from volunteers undergoing experimental endotoxemia reversed tolerance, leading to transcriptional reactivation of otherwise unresponsive genes[59]. Together, these findings suggest a strategy by which the tolerized phenotype might be reversed in sepsis patients to prevent morbidity and mortality as a result of immunosuppression[60].

3.2 Trained immunity

In trained immunity, a term first proposed by Netea and colleagues[61], an initial challenge of monocytes or macrophages with pathogen-associated molecular patterns (PAMPs) results in an enhanced response to a second challenge[52, 53]. Bacille Calmette-Guérin (BCG) and β-glucan are classical examples of PAMPs that are known to cause trained immunity. BCG vaccination, a live attenuated vaccine against tuberculosis, also protects against a wide variety of other infections[62]. In healthy volunteers, BCG vaccination increased the production of various cytokines in response to unrelated pathogens, which was accompanied with longlasting increased H3K4 trimethylation on the *TNF*, *IL6*, and *IL1B* promoters[63, 64] Also β-glucan enhances the production of proinflammatory cytokines through increased H3K4 trimethylation at these cytokine promoters and a gain of H3K27 acetylation at both promoter and enhancer regions[52, 65]. Moreover, β-glucan training was shown to change metabolism to an increased aerobic glycolysis through the mTOR–HIF-1α pathway[66]. These findings correspond with our current thinking on the cross talk between immune cell metabolism and inflammation[67]. Also, many epigenetic enzymes make use of substrates that are intermediates of metabolic pathways, providing additional links between metabolism and the epigenetic regulation of gene expression[68–70].

Trained immunity has been suggested to play an important role in diseases. In a mouse experimental Alzheimer's disease model, for example, a previous inflammatory trigger that activates microglia worsens the later onset of amyloid-β deposition, accelerating Alzheimer's disease[71]. In relation to atherosclerosis, it was described that atherogenic danger-associated molecular patterns (DAMPs) oxidized low density lipoprotein (oxLDL) and lipoprotein(a) will induce trained innate immunity. Initial challenges with these atherogenic DAMPs elucidate an enhanced inflammatory response when stimulated with LPS[72, 73]. Although these effects are not as strong as with the before mentioned PAMPs BCG and β-glucan, they do fit with the current hypothesis that inflammation is an important component of atherosclerosis. In vivo, training with a low dose of LPS resulted in increased inflammatory monocytes and aggravated atherosclerosis in *Ldlr*−/− mice[74]. The effects of BCG training on mouse has resulted in different outcomes and remains to be inconclusive, as recently discussed elsewhere[75].

In mouse atherosclerosis models it has been shown that high fat diet induces long-lasting transcriptomic and epigenomic reprogramming of myeloid progenitor cells, which leads to increased proliferation and enhanced innate immune responses[76, 77]. Interestingly, *Tet2* and *Tlr4* enhancers were found to be more open in granulocyte monocyte precursor cells as a result of a high fat diet based on ATAC (Assay for Transposase-Accessible Chromatin)

sequencing[77]. Although from this study it is not clear how these enhancers affect gene activity, both *Tlr4* and *Tet2* have clear and distinct roles in atherosclerosis onset and progression. Lack of *Tlr4* was previously shown to reduce atherogenesis by reducing inflammation[78], while mutations in *Tet2* were recently shown to result in clonal hematopoiesis and increased inflammasome activation[79].

In conclusion, these studies showed that the monocyte/macrophage memory is regulated at the level of histone modifications in promoters and enhancers resulting in long-lasting effects on the transcription profile and inflammatory potential of monocytes and macrophages. The model of trained immunity can be helpful to further study in a variery of disease states and can help us better understand the influence of environmental signals on our immune system.

4. Macrophage enhancers and genetic variation

Besides changes in enhancer activity, driven by environmental stimuli, also individual genetic differences affect the activation status of enhancers. GWAS studies have shown that the majority of disease associated SNPs reside in non-coding DNA-regions[24]. In human autoimmune diseases, it was shown that 90% of the causal disease variants are non-coding, with 60% of the causal variants mapping to enhancer elements[23]. These findings imply that phenotypic consequences of such variation causing disease are largely caused by an altered regulation of gene expression and not by variation in protein-encoding DNA. Studying various immune cell types exposed to inflammatory triggers, the Immune Variation project and others recently demonstrated that the gene expression of thousands of inflammatory genes is regulated by thousands of genetic variants[80–84]. Most of these genetic variants were found in *cis* and are specific for both cell-type and environmental exposure, which justifies a need to study genetic variation in enhancer elements in many cellular states[25].

A better understanding of mechanisms by which genetic variation influences disease risk therefore requires knowledge of its impact on the functioning of enhancer elements. Using natural genetic variation between mouse C57Bl/6 and BALB/c macrophages as a 'mutagenesis' approach, it was found that mutations in the LDTF C/EBPβ binding motifs abolishes not only the binding of C/EBPβ but also that of PU.1 bound to nearby PU.1-recognition motifs where no mutations were observed. Conversely, mutations in PU.1 motifs abolished binding of PU.1 and that of C/EBPβ to C/EBP motifs without mutations. These findings suggested a mechanism of collaborative DNA binding in which the genome-wide distributions of these proteins are determined by mutually required interactions with each other and additional transcription factors. Moreover, confirming the pioneering functions of LDTFs PU.1 and C/EBPβ in macrophages, it was shown that if LDTF binding was disturbed by a local mutation, the corresponding enhancer lacks active enhancer histone marks, like H3K4 methylation and H3K27 acetylation. Additionally, binding of SDTF NF-κB was shown to be dependent on LDTF binding, as mutations in PU.1 or C/EBPβ motifs abolished signal dependent binding of NF-κB[85]. However, at most NF-κB binding sites, mutations in the κB recognition motif did not alter the binding of PU.1 and C/EBPβ. These findings provided evidence for a hierarchical model for enhancer selection and function at many genomic locations, in which the binding of SDTFs is determined by prior binding of LDTFs.

Based on the principal of collaborative binding, effects of natural genetic variation on transcription factor binding motifs were further exploited to identify collaborative binding partners for PU.1 in peritoneal macrophages and microglia. In addition to mutations in sites that are recognized by PU.1 itself, the most highly significant motifs in both cell types corresponded to sequences recognized by C/EBPβ and interferon regulatory factors. However, tissue macrophage-specific motifs were also identified using this approach, including the GATA and AP-1 motifs in peritoneal macrophages and the SMAD motif in microglia. These motifs are recognized by factors that are responsive to the specific environmental signals in each macrophage population, i.e., retinoic acid in peritoneal macrophages, which induces GATA6 expression, and TGFβ in the brain, which activates SMAD transcription factors[19].

In our most recent study[86], we expanded the use of genetic variation by studying five different inbred mouse strains that collectively provide more than 60 million SNPs and InDels, ranging from approximately 5 million for the pairwise comparison of C57BL/6 to BALB/c, to more than 45 million for the pair wise comparison of C57BL/6 to SPRET (Fig. 3A). As expected, we observed increased differential gene expression that scaled with the extent of genetic variation between two mouse strains (Fig. 3B). At a 4-fold cutoff, approximately 100 genes are differentially expressed in comparing C57Bl/6 and BALB/c bone marrow derived macrophages, whereas there are more than 1500 genes differentially expressed when comparing C57Bl/6 to SPRET. These differences indicate tolerance for a broad range of gene expression changes associated with macrophage function. Differentially expressed genes segregated into different modules that were enriched for distinct biological functions (Fig. 3C) that predicted a defect in type I IFN response genes in SPRET macrophages. This prediction was confirmed by global gene expression profiling and is also consistent with a prior report on the incapability of SPRET macrophages to produce IFN-β[87]. Thus, the variation in gene expression among these five strains of mice is likely to be associated with biologically significant phenotypes. A major goal going forward will be to understand the basis for these strain-specific differences. By generating F1 hybrids and analyzing allele-specific transcripts, nearly all variation in gene expression is explainable by differences in *cis* regulatory elements.

As an example of strain-specific LDTF binding that is associated with strain-specific presence of active histone marks and gene expression, we observed at the *Apoe* locus that loss of PU.1 biding results in decreased H3K27 acetylation and *Apoe* gene expression in the strains where PU.1 does not bind (Fig. 4A). Remarkably, the variation found in the binding of PU.1 and other LDTFs, greatly exceeded the variation observed on the gene expression level (Fig. 3B). While mutations in the motifs for LDTFs PU.1, C/EBPβ and AP-1 are significantly associated with strain-specific LDTF binding, the majority of the strain-specific differences we observed are explained by mutations in nearby collaborative transcription factor motifs (Fig. 4B). Evaluation of genetic variation suggested that many transcription factors are involved in shaping the macrophage enhancer landscape as over 80 different transcription factor motifs were found at these strain-specific sites. Unexpectedly, still a substantial fraction of strain-specific LDTF binding cannot be explained by mutations in the LDTF motif nor in neighboring collaborative transcription factor motifs. Based on the strain-specific absence or presence of multiple transcription factor bindings in specific DNA

domains, we were able to identify hundreds of domains that are associated with differences in strain-specific transcriptional activity. These domains, driven by *cis*-acting genetic variation, were initially observed in lymphoblastoid cell lines and are currently referred to as *cis*-regulatory domains (CRDs)[88, 89]. These CRDs demarcate the regions where active enhancer elements are found and gene transcription takes place[90]. Further analysis using assays of chromatin interactions showed that most of the CRDs found in mouse macrophages reside within topologically associated domains (TADs) and are highly intra- and inter-connected. This phenomenon suggests the possibility that strain specific differences in transcription factor binding at sites without local mutations result from 3 dimensional interactions that alter transcription factor concentrations or DNA accessibility (Fig. 5), and adds an additional layer of complexity to the chromatin organization of cells that needs further investigation.

These studies exploiting the natural genetic variation between different mouse strains will help us better understand how genetic variation leads to phenotypical differences. Although the positions of enhancers in mice and humans are poorly conserved, the mechanisms driving cell specific gene expression are thought to be very similar[91, 92]. For example, it was shown in mice that differences in genetically determined binding of PPARγ to adipocyte enhancers result in strain-specific transcriptional effects of the antidiabetic drug rosiglitazone[93]. Also in humans, SNPs determining genomic binding of PPARγ were associated with changes in nearby genes and metabolic phenotypic differences[93], illustrating that studying genetic variation in mice can substantially advance our efforts to understand the influences of non-coding genetic variation on gene expression and phenotypes in humans. By further studying the interplay between genetic variation and environmental stimuli, we can better understand the role of genetic variations in disease. As an example, in human macrophages it was recently shown that enhancers undergo major oxLDL-induced changes, orchestrated by the LDTF CEBPβ. Interestingly, cardiovascular disease-associated genetic variants are enriched in regulatory DNA sites altered by oxLDL and one of the genetic variants was shown to affect enhancer activity and expression of the *PPAP2B* gene[94]. Together, these studies will help us better understand how genetic variation among individuals affects leads to disease-relevant differences.

5. Conclusions and future perspectives

Recent progress in genomics have greatly increased our understanding on the importance of enhancer function in relation to macrophage differentiation and responses to external stimuli. The interplay between environmental and genetic factors influence the macrophage enhancer landscape by recruitment of LDTFs and SDTFs to specific loci in the genome. Although the activation of these transcription factors depend on external stimuli, genetic factors will determine whether a factor can bind to a specific locus or not. The collaborative and hierarchical binding of LDTFs and SDTFs required for selection and activation of enhancers is therefore crucial for macrophage differentiation and specific stimuli-induced responses.

Future efforts will help us better understand how enhancers affect corresponding gene transcription. Using new techniques like HiChIP or PLAC-seq, one can now determine

which enhancers interact with which promoters and study how different disease relevant external signals create new or abolish existing interactions. Furthermore, using genetic variation, the requirements for LDTF or SDTF binding should be further investigated to understand how genetic variation affects macrophages and other disease-relevant cell types. Finally, a better understanding of when transcription factor binding will lead to enhancer activation or repression instead will be key to determine the roles of individual LDTFs and SDTFs in macrophages.

Acknowledgements

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Highlights

- Macrophage enhancers are selected as a result of origin and tissue signals

- Selection of enhancer differences result in tissue-specific macrophage phenotypes

- Danger signals result in long-term epigenetic changes leading to immune memory

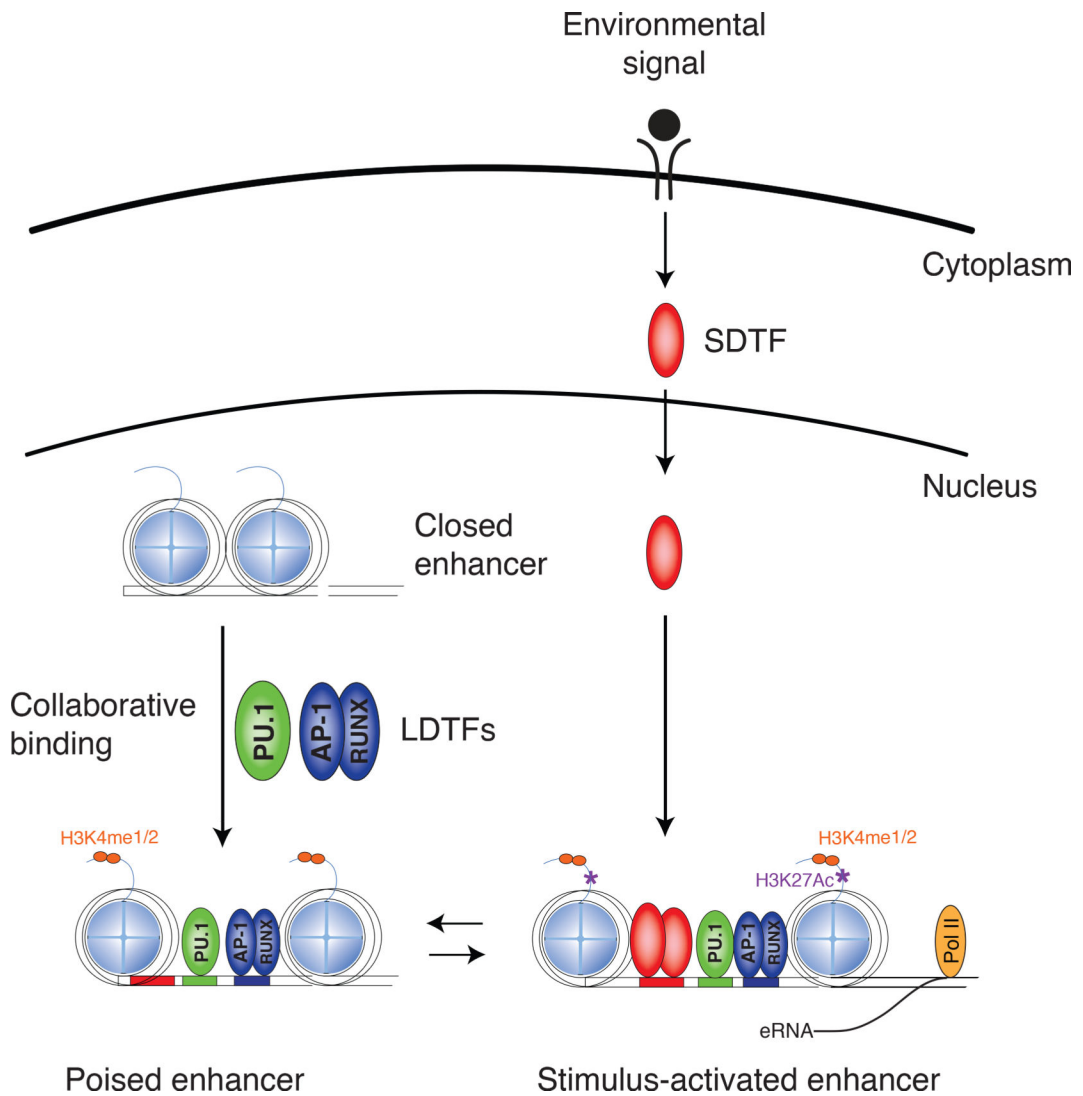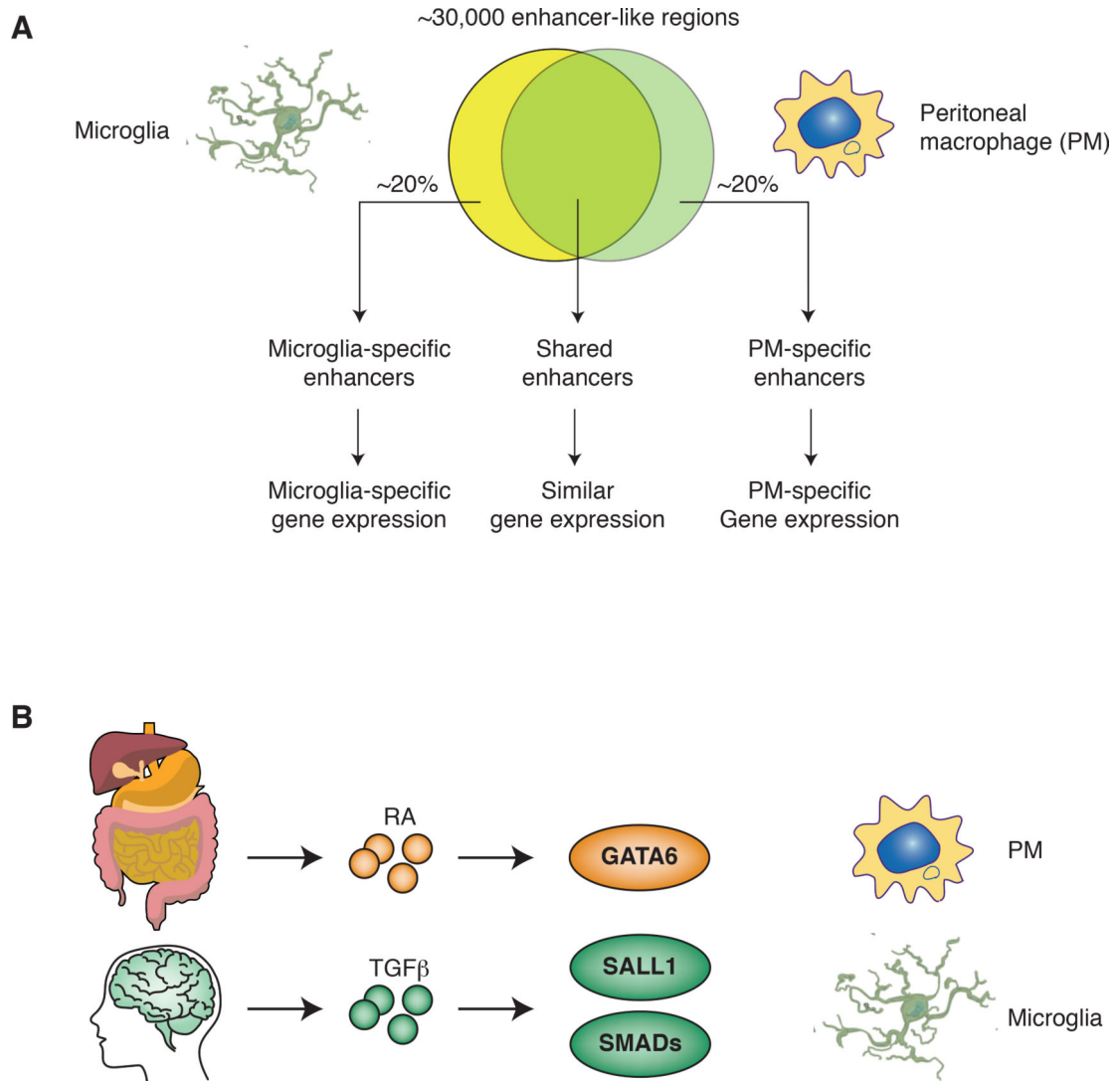- Genetic variation alters enhancer selection and activation

Fig 1.

Stimulus-dependent macrophage enhancer activation.

Macrophage enhancers marked by H3K4me1/2 require macrophage lineage determining transcription factors (LDTFs) like PU.1 and collaborating transcriptions like AP-1 and RUNX1. Enhancers frequently require signal-dependent transcription factor (SDTF) binding to gain H3K27 acetylation before they become active and transcribe enhancer RNAs (eRNA) and/or interact with target gene promoters.

A

~30,000 enhancer-like regions

Microglia

Peritoneal macrophage (PM)

~20%

~20%

Microglia-specific enhancers

Shared enhancers

PM-specific enhancers

Microglia-specific gene expression

Similar gene expression

PM-specific Gene expression

B

RA → GATA6

PM

TGFβ → SALL1 / SMADs

Microglia

Fig 2.
Environmental factors shape the macrophage enhancer landscape.
(A) Tissue macrophages like microglia and peritoneal macrophages (PM) share the majority of macrophage core enhancers, while a subset of enhancers is specific for the tissue they reside in. (B) Environmental factors present in the tissue activate signal dependent transcription factors (SDTFs). In the peritoneum, retinoic acid (RA) activates GATA6 in peritoneal macrophages (PM), while in the brain, factors like TGF-β induce transcription factors SMADs and SALL1 that are specific for microglia.
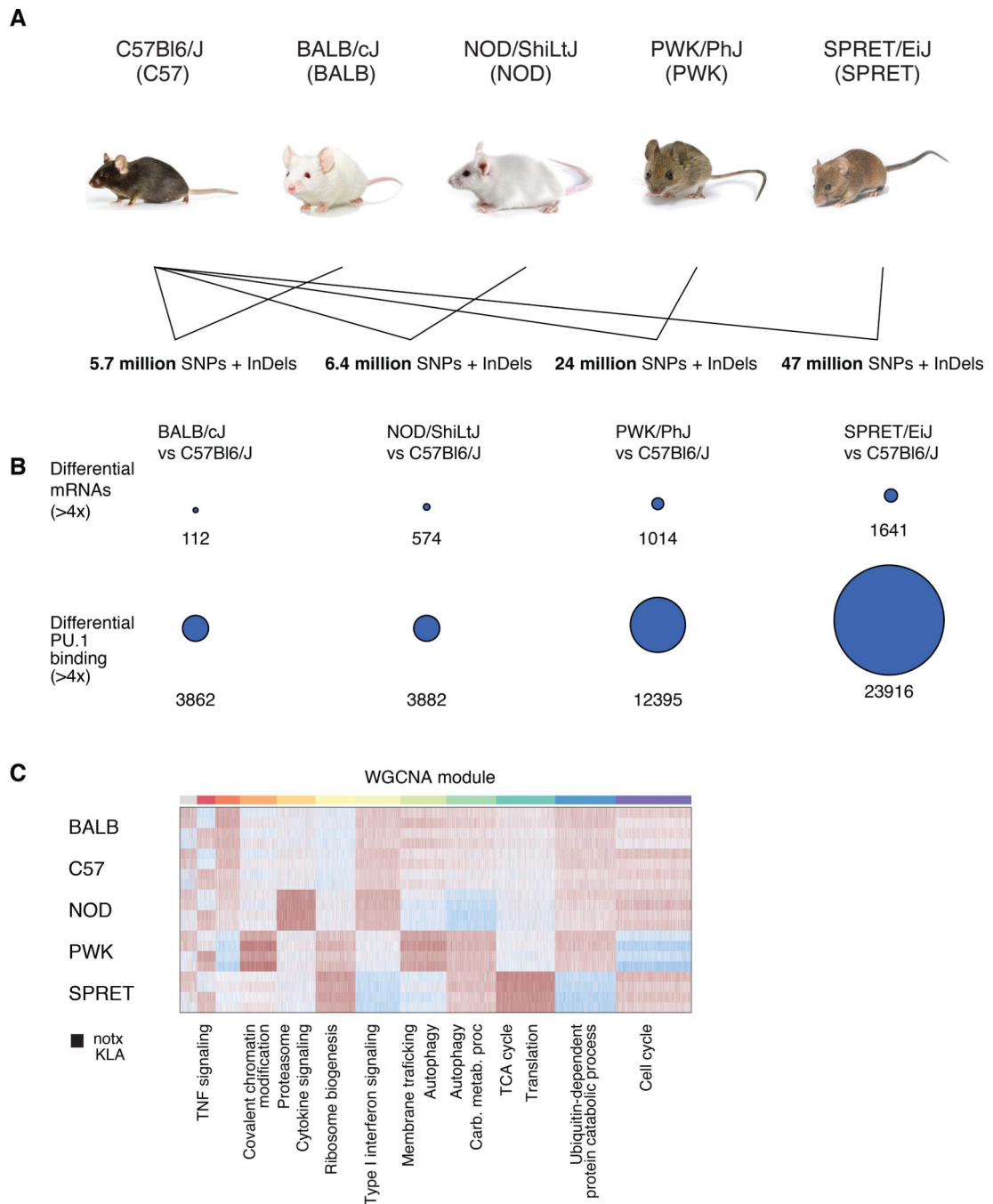
Fig 3.

Different strains of mice as a tool to study the effects of genetic variation on enhancer activation and gene expression.

(A and B) Variation in gene expression scales with underlying genetic variation, variation in LDTF PU.1 binding exceeds the differences seen on the gene expression level. (C) Weighted correlation network analysis (WGCNA) analysis of RNA-seq on macrophages from different strains treated with or without Kdo2-Lipid A (KLA) shows that differentially expressed genes are associated with diverse cellular functions, adapted from Link et al.[86].
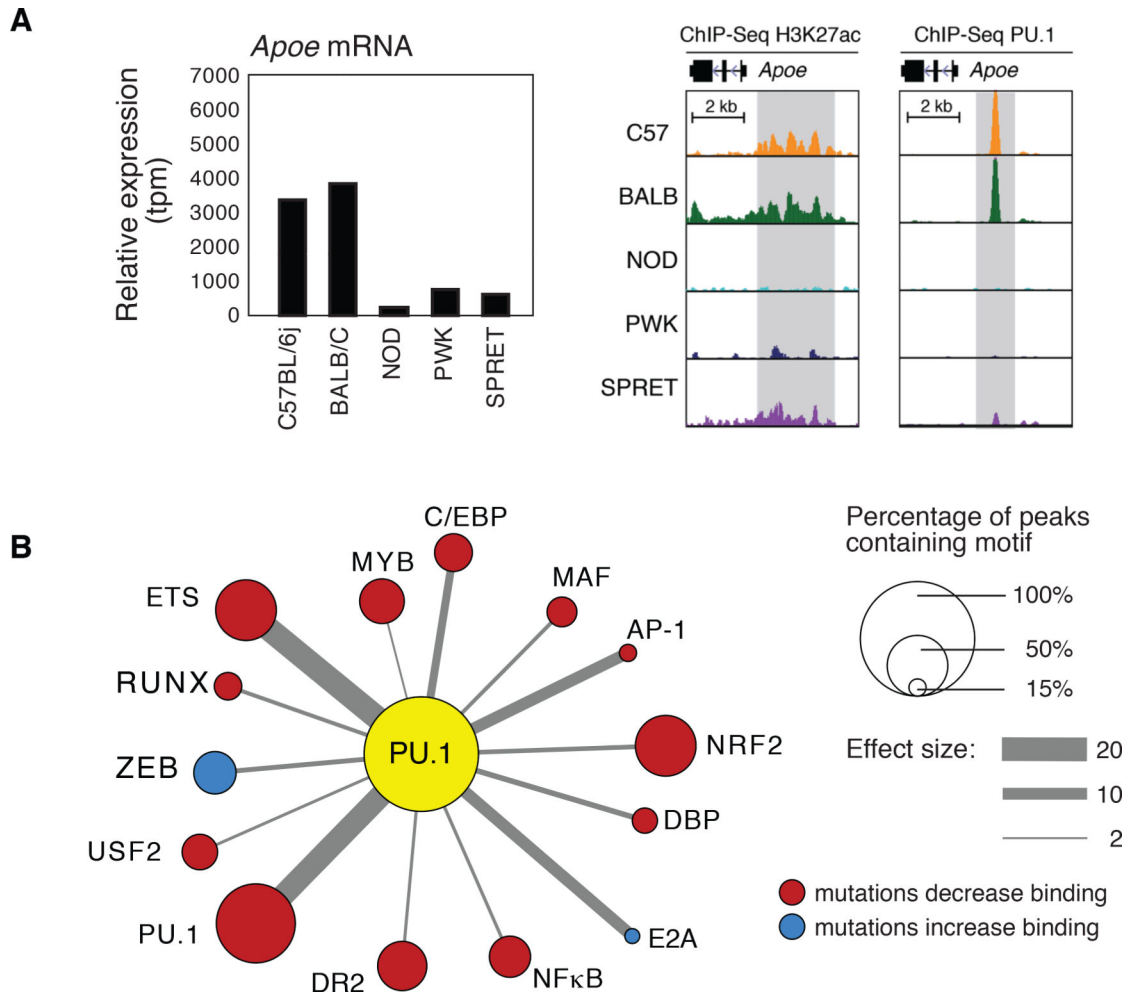
A

B

Fig 4.

Effects of collaborating transcription factor motif mutations on LDTF binding.

(A) *Apoe* gene expression, as measured with RNA-seq in bone marrow derived macrophages from five strains and strain specific PU.1 binding at the *Apoe* locus with corresponding H3K27 acetylation in the strains where PU.1 binds. (B) Top 14 of 48 motifs correlated with binding of PU.1 as determined by motif mutation analysis. The node size is the fraction of PU.1 peaks containing the indicated motif, and edge thickness is proportional to the effect size of motif mutations. Nodes indicate motifs in which mutations result in reduced PU.1 binding (red) or in which mutations result in increased PU.1 binding (blue), adapted from Link et al.[86].
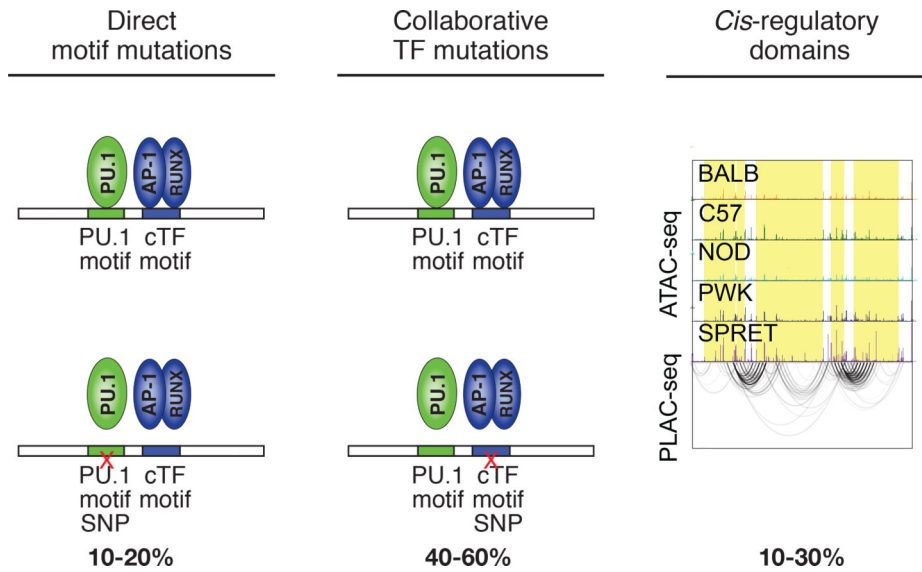
Fig 5.
Mechanisms underlying strain-specific transcription factor binding patterns.
Only 10–30% of the strain-specific LDTF binding can be explained by mutations in the actual LDTF (PU.1) motif itself, the majority of the variation is explained by mutations in collaborating transcription factor (cTF) motifs. The remaining strain-specific LDTF binding are mostly located in *cis*-regulatory domains.