

Role of IRF4-Mediated Inflammation: Implication in Neurodegenerative Diseases

Mamun AA and Liu F*

¹Department of Neurology, McGovern Medical School, The University of Texas Medical School, USA

***Corresponding author:** Fudong Liu, Department of Neurology, University of Texas Medical School at Houston USA Phone, Tel: (713) 500-7038; E-mail: Fudong.Liu@uth.tmc.edu

Review Article

Volume 2 Issue 1

Received Date: November 14, 2016

Published Date: January 12, 2017

Abstract

Neuro-inflammation is a common feature of various central nervous system (CNS) disorders, including stroke, Alzheimer's disease, Multiple sclerosis, etc., and has a significant impact on the outcomes. Regulation of the immune response has therapeutic value. Interferon regulatory factor 4 (IRF4) is a hemopoietic transcription factor critical for activation of microglia/macrophages and modulation of inflammatory responses. The effects of IRF4 signaling on inflammation are pleiotropic, and vary depending on immune cell types and the pathological microenvironment that is regulated by both pro- and anti-inflammatory cytokines. Mechanistically, IRF4 is a quintessential 'context-dependent' transcription factor that regulates distinct groups of inflammatory mediators in a differential manner depending on their activation in different cell types including phagocytes, T-cell subtypes, and neuronal cells. In this review, we summarized the recent findings of IRF4 in the context of immune responses in different cell types with diverse pathological conditions. The primary goal of this review is to understand the signaling pathways and beneficial functions of IRF4, in hope of developing effective therapeutic strategies targeting the immune responses to neurodegenerative diseases.

Keywords: Autoimmunity; IRF4; Inflammation; Ischemic stroke; Neurodegeneration

Background

Inflammation is a fundamental pathological procedure in acute and chronic neurodegenerative disorders such as stroke, Alzheimer's disease (AD), multiple sclerosis (MS), etc. [1-3]. Mechanistically, inflammation is a complex patho-physiological procedure where immune cells and mediators orchestrate aspects of the inflammatory responses to pathogens and/or tissue injuries [4]. The immune response plays a critical role in host defenses against those pathological stimuli; however, it is a "double-wedge sword", and over-reaction of the immune system may exacerbate the tissue damage [5,6].

Therefore, it is crucial to regulate the immune response to promote/suppress the beneficial/detrimental effects respectively, so that better outcomes can be achieved.

A large number of proteins have been identified as potent regulators for inflammation. For the past decade, the interferon regulatory factors (IRFs) have been extensively studied in the context of immune responses and have been shown to have functionally regulatory roles [7,8]. IRFs were originally identified as transcription factors of type I IFN, including nine different forms from IRF1 to IRF9 based on their binding motifs [7]. IRF family shares the common N-terminal DNA-binding domain

consisting of 115 amino acids with five tryptophan repeats, but largely varies in C-terminal domain. IRF4, also known as multiple myeloma oncogene-1 (MUM1), is an IRF family transcription factor that is restricted in expression to the immune system and has critical roles in regulating immune responses [9,10]. Recent findings suggested that IRF4 not only determines the developmental fate of immune cells including T helper

(Th) cells, regulatory T (T-reg) cells, dendritic cells, macrophage/monocytes, and microglia, but also responds to changes of cellular micro-environment to manipulate the inflammatory process (Table 1). In this review, we summarized the recent advance in the understanding of cardinal features of IRF4 in inflammation and highlight the potential therapeutic value of the transcription factor in neuro-inflammation diseases.

Target gene	Expression pattern (celltype)	Role	Pathological condition	Reference
IL-6 and IL-17	Intestinal cells	Activate pro-inflammatory pathway	TNBS-induced colitis	[78,76]
Smad2/3	T cells	T cells induction,	Allergic Asthma	[97]
IL-21 and IL-17	T cells, T-reg in adipose tissue	Th17 cells maturation, proliferation, adipose tissue inflammation	RA, Obesity	[64,98,99]
IL1 β and TNF α	Adipose tissue	Obesity-induced inflammation		[30]
(NOD2), TRAF6 and RICK	Colonic lamina propria mononuclear cells	Down-regulation of NF- κ B activation.	CD	[84]
ROR γ t	Experimental colitis.	Th17-dependent colitis	IBD	[77]
FoxO1	alveolar macrophages	Anti-inflammatory response	Allergic asthma	[79]
Il-10 and IL-33	Dendritic cells	Promote dendritic cells differentiation to Th2 response	Inflammatory pulmonary disease	[100]
Blimp1 and icos	T-reg	Differentiation of effector T-reg cells and increase IL-10 levels	Auto-immune disease	[101]
Foxp3	T-reg			
ROCK2	T cells	IL-17 and IL-21 production	Auto-immune disease	[23,102,103]
Runx3	Thymic CD4/CD8	Recruitment of histone H3 and H4 in the Runx3 promoter region		[104]
IL-4	T cells	Interact with NFATc2 to regulate IL-4 expression	Auto-immune disease	[93]
IL-1 β , TNF α	Macrophage in adipose tissue	Decrease pro-inflammatory cytokines via M2 macrophage polarization	Obesity	[30]
CCL17	Macrophage	Regulate proinflammatory and algescic actions of GM-CSF	Arthritis and pain	[105]
TNF- α , IL-1 β , IL-6, IL-12/IL-23 (p40) and IL-10	Dendritic cells	Decrease pro-inflammatory and increase anti-inflammatory cytokines	Experimental Autoimmune Encephalomyelitis	[65]
DEF6 and SWAP-70	CD4+ T cells	T-cells and B-cells co-ordination in systemic lupus	(SLE)	[25]
		Erythematosus (SLE)		

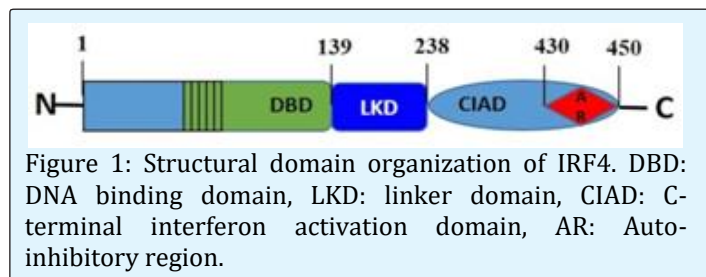
Table 1: Potential target genes of IRF4.

Structure of IRF4

Unlike other members of the IRF family, IRF4 has two conserved functional domains: an N-terminal helix-turn-

helix DNA binding domain (DBD) with a signature five conserved tryptophan residues and a C-terminal interferon activation domain (CIAD) including β -sheets and loops that serve as the binding site for its co-

regulators [11,12]. DBD binds with the interferon-stimulated response element (ISRE) consensus sequence within the target genes by recognizing GAAA sequence AANNNGAA sequence motifs [13]. By using a combination of X-ray and a small-angle X-ray scattering (SAXS) assay, previous studies have found that CIAD possesses a flexible 30 amino acid containing auto-inhibitory region (AR) that is not folded into the CIAD [12,14]. AR is required for binding of DBD with target genes and maintains IRF4 in an auto-inhibited state [15-17]. The structural domain organization is shown in Figure 1.



Activation and co-regulators of IRF4

The functional activity of IRF4 is primarily dependent on the pattern of phosphorylation that varies from cell type to cell type. IRF4 binds DNA with low affinity that however can be strengthened by interactions with binding partners, including other IRF family members, the leucine-zipper hetero dimer BATF-JunB, STAT6, PU.1 and PGC-1 α , etc [18-22]. Biswas *et al.* reported that the autoimmunity in mice largely depends on the phosphorylation of IRF4 by Rho-associated protein kinase 2 (ROCK2), which activates mouse T-cells to produce IL-17 and IL-21 [23]. The activation of IRF4 is crucial for IL-21 mediated induction, amplification, and stabilization of Th17 phenotype. On the other hand, virus-mediated transformation of IRF4 is triggered by c-Src-mediated tyrosine phosphorylation [24]. Moreover, it has been reported that the specificity of IRF4-DNA binding depends on lineage-specific transcriptional co-regulators. For example, in B cells, IRF-4 expression is strongly upregulated upon costimulation of B cells with CD40 and IL-4, where IRF4 acts as both the target and modulator of IL-4 signaling pathway [21,25].

Epigenetic regulation of IRF4 can determine the ultimate fate of immune cells, usually through the interaction of IRF4 with Jumonji domain containing 3 (Jmjd3), a histone 3 Lys 27 (H3K27) demethylase, by which macrophages are polarized towards M2 phenotype [26]. Jmjd3 removes an inhibitory trimethyl group from H3K27 at the promoter region of IRF4 to induce IRF4 expression, a process regulated by IL-4-induced STAT6 signaling. After IL-4 stimulation, STAT6 is increased and

binds with the consensus sequence of Jmjd3 promoter, resulting in increased Jmjd3 activity and decreased methylation of H3K27 [27,28]. The role of Jmjd3-IRF4 axis in M2 macrophage polarization has been proven in studies of multiple inflammatory diseases, including helminth infection and periodontitis [26,29].

Synergistic binding with co-regulators increases IRF4 DNA-binding affinity [30]. A number of co-regulators have been identified by means of biochemical, genetic, molecular, and cellular strategies. For example, PU.1 is a hematopoiesis-specific family transcription factor expressed in myeloid and B cells [26] that binds to DNA and recruits its trans-activation partner, IRF4 [31]. Absence of PU.1 results in weak interaction of IRF4 with template DNA [22]. Furthermore, phosphorylation of S148 in the PEST (proline, glutamine, serine, and threonine rich) domain of PU.1 is essential for strong DNA binding of IRF4 *in vitro* [32]. In conditional PU.1 knockout mice, disruption of PU.1 in mature B cells by the CD19-Cre locus did not affect B-cell maturation [33]. Similarly, IRF4^{-/-} mice showed a relatively normal B and T lymphocyte distribution and cellularity compared with wild type controls at early age [34]. The above two findings suggested that the sole deficiency of either PU.1 or IRF4 had no significant detrimental effect on B cell maturation. In contrast, cooperative interaction of PU.1/IRF-4 promotes antigen presenting functions, activation and differentiation of B lymphocytes as well as antibody diversification in chicken B cells [35,36]. Another co-regulator, switch-associated protein 70 (SWAP-70), has been shown to control IRF4 protein expression and thereby regulate the initiation of plasma cell differentiation [37]. Rebecca *et al.* reported that nuclear factor κ B (NF- κ B) and RelB were also transcriptional co-activators of IRF4 [38], and the complex is essential in establishing a pattern of gene expression that promotes cell proliferation, survival, and differentiation of lymphocytes. Gupta *et al.* have isolated a human cDNA gene (Def6) that encodes IRF4-binding protein (IBP), another potential co-regulator of IRF4 sharing substantial homology with SWAP-70 [21]. Collectively, functional activation of IRF4 requires recruitment of intrinsic factors that vary depending on cell types.

The Role of IRF4 Signaling in Neurodegenerative Diseases

Role of IRF4 in stroke

Ischemic stroke triggers a complex cascade of events that interplay with innate and adaptive immune systems. Microglia, the resident immune cells in the brain, is the key initiator and the most potent regulator of immune

responses to stroke [39]. The activation of microglia is largely dependent on the microenvironment of the ischemic brain. The classically activated microglia (M1) release destructive pro-inflammatory mediators while alternatively activated microglia (M2) clear cellular debris and trigger anti-inflammatory processes, a mechanism strictly controlled by endogenous transcription factors. M1 microglia are characterized by expression of signature proteins such as TNF, iNOS, IL-6 and MHC-II. By contrast, M2 populations are characterized by arginase-1, IL-10, TGF- β and CD206, etc. Studies of the peripheral inflammation suggested that the macrophage M1 phenotype is regulated by IRF5 mediated pro-inflammatory pathway whereas the M2 phenotype is regulated by IRF4-driven anti-inflammatory signaling pathway [26,40-42]. Initially, IRF4 was considered a lymphocyte-restricted member of IRF family that regulates the lymphocytic response exclusively; however, recent findings have suggested that IRF4 is also expressed in myeloid cells and possesses a broad functional spectrum in insulin resistance, cardiac pathology, cell survival, and oncogenesis [30,43-45]. Furthermore, the latest studies reported that IRF4 exerts neuroprotection in adult and neonatal stroke mice [46,47]. IRF4 competes with IRF5 for binding to the adaptor MyD88 that transmits TLR outside-in signaling to NF- κ B and other pro-inflammatory transcription factors [48]. Like other transcription factors, IRF4 also needs to be phosphorylated by its co-regulator to translocate into the nucleus [23,24]. By examining mRNA in sorted microglia

from mice stroke brains with flow cytometry (FC), we observed an increased IRF4 mRNA level at 3d of stroke that declined by day 7 (Figure 2A). Interestingly, the microglial expression of CD206, an anti-inflammatory cytokine, showed the same pattern as that of IRF4 mRNA (Figure 2B), suggesting IRF4 is important in inducing M2 microglial activation. Studies from other groups also found an evident link between IRF4 and anti-inflammatory responses. For example, over expression of IRF4 resulted in increased IL-4 and IL-10 expression and decreased IL-9 production in Th2 cell type [49]; IRF4^{-/-} mice were defective in producing anti-inflammatory cytokines (i.e IL-4 and IL-10) and there was a high propensity of pro-inflammatory cytokines like TNF α and IL-6 in LPS-induced sepsis [50]. One recent study also reported that the expression of IRF4 was strongly induced in bone marrow derived macrophages upon priming with IL-4, suggesting an important role of IL-4 in shaping M2 phenotype through IRF4 [28].

Interestingly, neuronal expression of IRF4 also assists in improving outcomes from stroke [46]. IRF4 over-expressed mice had reduced infarct size and improved neurological deficits than wild type mice; whereas conditional KO of IRF4 in neurons induced exacerbating effects. IRF4 was found to directly activate and up-regulate the expression of serum response factor (SRF) that counteracts programmed cell death in ischemic brains [46].

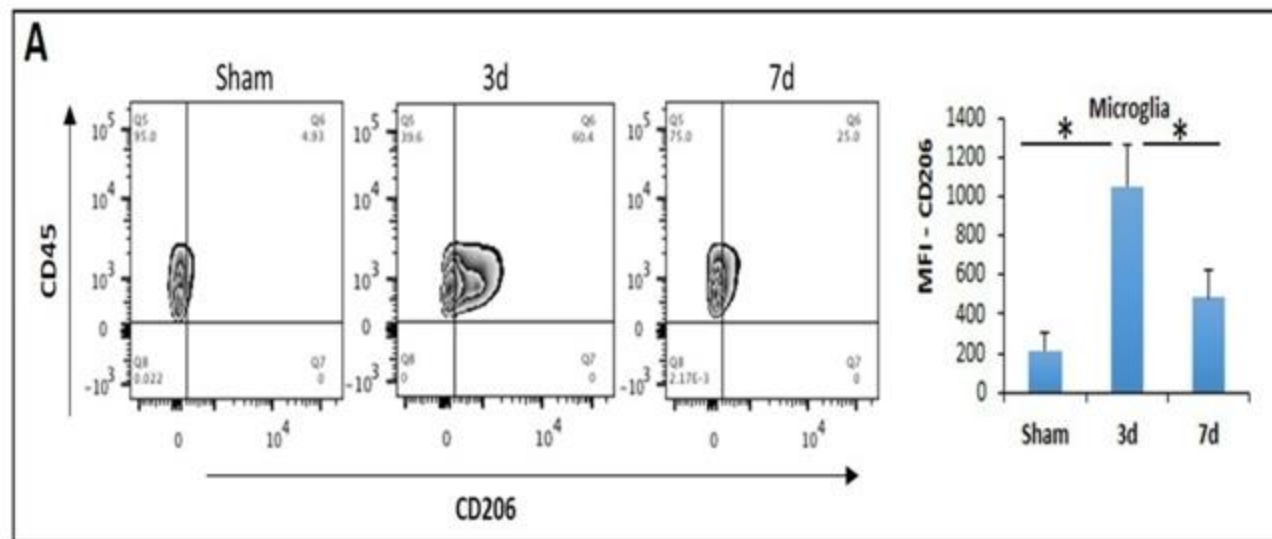


Figure 2: Flow cytometric (FC) characterization of M2 microglia activation after stroke.

(A) CD206 expression on gated microglia at 3d and 7d of stroke (left three plots). Mean fluorescence intensity (MFI) of CD206 on microglia showed CD206 significantly increased at 3d but decreased to baseline at 7d. $P^* < 0.05$; $n = 4 \sim 6$ /gp.

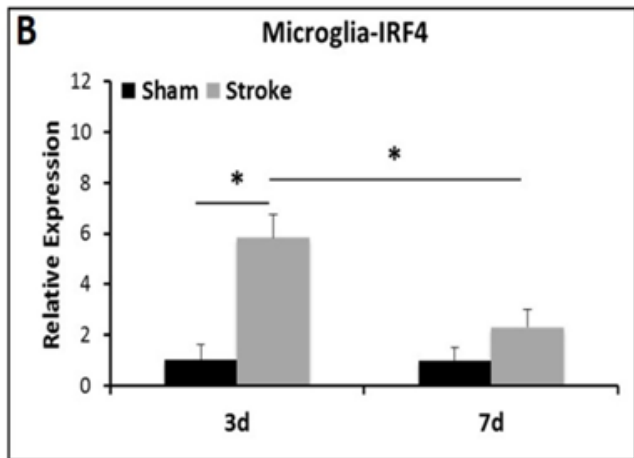


Figure 2: Flow cytometric (FC) characterization of M2 microglia activation after stroke.

(B) Levels of IRF4 mRNA in FC sorted microglia at 3d and 7d after stroke showing the same pattern as that of CD206 expression. $P^* < 0.05$; $n = 4 \sim 6$ /gp

IRF4-mediated neuroprotection in Alzheimer's disease.

IRF4 may be important to regulate immune responses to all CNS disorders and confer neuroprotection. Alternatively activated (M2) macrophages/microglia have been reported to have neuroprotective effects not only in stroke [51], but also in traumatic brain injury [52], spinal cord injury [53] and AD [54]. In AD, most of activated microglia was associated with dense-core plaques; however, a few were found in the vicinity of diffused A β deposits [55-57]. Reactive microglia clear the A β deposits via phagocytosis [58] and are a primary source of inflammatory factors [59,60]. Recent research suggested the IRF4-mediated neuroprotection in CNS disorders is induced through altering IRF5/4 ratio in microglia [47,54]. In a rat AD model, intracerebroventricular injection of A β_{1-42} switched M2 microglia to M1 and increased levels of IRF5/4 ratio. Notably, transplantation of M2 macrophages attenuated inflammation in the brain, reversed A β_{1-42} -induced changes in the IRF5/4 ratio, and ameliorated cognitive impairment via M2 microglia polarization [54]. These studies suggest that IRF4 is a key player in developing an anti-inflammatory micro-environment in AD brains.

Role of IRF4 in Experimental autoimmune encephalomyelitis

Multiple sclerosis (MS) is an autoimmune disease that is characterized by recurrent episodes of T-cell-mediated immune attack on the protective myelin and the spinal cord, leading to axonal damage and progressive disability

[61,62]. One recent study demonstrated that the imbalance between different immune cell subsets, such as Th1/Th2 or Th17/T-regs, greatly contributes to the axonal damage induced by the experimental autoimmune encephalomyelitis (EAE), an animal model of MS [63]. It has been found that IRF4 was critical for generation of the autoimmunity in EAE. Selective inhibition of IRF4 with siRNA decreases EAE scores, reduces the infiltration and differentiation of Th1 and Th17 cells, and down-regulates Th-17 response in EAE. Furthermore, in a DC-T-cell co-culture system, siIRF4-treated DCs resulted in significantly less IFN- γ and IL-17 production in T cells. Notably, transfer of CD11c⁺ DCs from siIRF4-treated mice into recipient mice induced significantly reduced expression of pro-inflammatory cytokines TNF α , IL-1b, IL-6, and IL-12/IL-23 (p40), with a corresponding increase in anti-inflammatory IL-10 expression. These data suggest that IRF4 deficient mice are resistant to the development of EAE [64,65].

Apart from the role of IRF4 in Th17 cells, the etiology of EAE also involves IRF4-mediated inflammation triggered by IL-17-producing CD8⁺T (Tc17) cells [66]. Tc17 cells are detectable in multiple sclerosis (MS) lesions and CSF of patients with early-stage MS [67]. For the first time Huber *et al.* reported that Tc17 differentiation is controlled by IRF4-mediated expression of ROR γ t, Eomes, and Foxp3 in CD8⁺ cell [68]. In the absence of IRF4, Tc17 development was abolished in vivo. After immunization of myelin oligodendrocyte glycoprotein 37-50 (MOG₃₇₋₅₀) peptide, IRF4^{-/-} mice showed a complete EAE resistance [68]. Surprisingly, adoptive transfer of WT CD8⁺ T cells and subsequent immunization of MOG₃₇₋₅₀ in IRF4^{-/-} mice led to development of Tc17 cells in the periphery that however were not able to infiltrate on their own into the CNS and not enough to restore EAE pathology in IRF4^{-/-} mice [68]. Next the authors tried to evaluate whether the CD8⁺T cell function are dependent on CD4⁺ T cell mediated activation for EAE pathology, and found that CD8⁺T cells mutually interact with CD4⁺ T cells to induce EAE in IRF4^{-/-} mice [68]. These data suggested that Tc17 cells directly interplay with CD4⁺ T cells, leading to the production of IL-17A in IRF4 deficient microenvironment and the induction of EAE pathology [68,69]. The fact that the IRF4 signaling has different effects in Th17 vs. Tc17 cells suggests that the role of IRF4 in inflammatory responses varies depending on cell types.

IRF4 in other inflammatory processes

Inflammatory bowel diseases (IBDs)

IBDs are characterized by chronic relapsing inflammation in the gastrointestinal tract independent of

specific pathogens. There are two major forms of IBDs: Crohn disease (CD) and ulcerative colitis. In IBDs, the activation of the mucosal immune system is characterized by the production of pro-inflammatory cytokines [70-72]. IL-6 is a pleiotropic cytokine secreted mainly by T cells and macrophages that has both pro- and anti-inflammatory properties. Importantly, pro-inflammatory properties of IL-6 signaling are the major driving force for the pathogenesis of IBDs [73,74].

Role of IRF4 in mucosal inflammation in CD: CD is a heterogeneous disease characterized by aggressive T cell responses. The onset and reactivation of the disease is triggered by multiple factors such as infections/vaccinations, genetic susceptibility, environmental triggers (smoking, diet, etc.) that transiently break the mucosal barrier and stimulate immune responses [75]. Recently, IRF4 has been found to be necessary for IL-6 and IL-17 dependent pathogenesis of CD [76,77]. IRF4^{-/-} deficient T cells fail to induce colitis in adoptive transfer experiments in RAG-knockout mice which have no mature B and T lymphocytes, suggesting that the protective capacity of IRF4 deficiency is at least partially due to the effects of T lymphocyte inactivation [76]. Mechanistically, IL-6 expression was strongly up-regulated in the inflamed colon of WT but not IRF4-deficient mice. In contrast to mucosal IL-6 levels, splenic cell-derived IL-6 production in colitis was not different between WT and IRF4^{-/-} knockout mice. These results suggest that IRF4 regulates mucosal rather than systemic IL-6 levels in the experimental colitis [76,78]. Furthermore, patients with either CD or ulcerative colitis exhibited an increased IRF4 expression in lamina propria CD3⁺ T cells and elevated levels of mucosal IL-6 compared with controls [76]. It is likely that the involvement of IL-6 and IRF4 is a common feature of immune responses, as other inflammatory processes also showed the similar characteristic, e.g. in allergic asthma [79]. Beside IL-6, IL-17 also interacts with IRF4 to impact the chronic intestinal inflammation and autoimmunity [80]. The development of inflammatory Th17 cells and IL-17 production are dependent on IRF4 [64]. IRF4 deficiency was protective in T-cell-dependent transfer colitis associated with reduced ROR α / γ t levels and impaired IL-17 production, suggesting that IRF4 acts as a master regulator of mucosal Th17 cell differentiation in CD [77]. The roles of IRF4 in mucosal T-cell mediated inflammatory responses have been illustrated in Figure 3A.

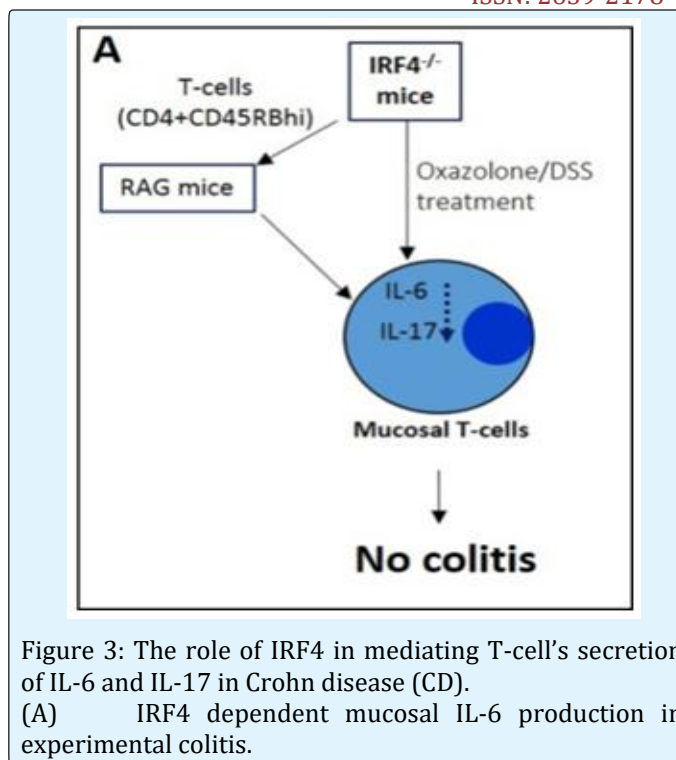


Figure 3: The role of IRF4 in mediating T-cell's secretion of IL-6 and IL-17 in Crohn disease (CD).

(A) IRF4 dependent mucosal IL-6 production in experimental colitis.

Epigenetic regulation of IRF4 in CD: IRF4-mediated inflammatory responses are associated with nucleotide-binding oligomerization domain 2 (NOD2) gene functions, the major epigenetic risk factor in CD. Loss-of-function of NOD2 leads to abolition of microbial sensing of bacteria-derived muramyl dipeptide (MDP) and increased pro-inflammatory cytokines in CD driven by excessive NF- κ B activation [81,82]. It is well established that MDP acts as a high-affinity ligand for and a stimulant of Nod2 [82,83]. In a colonic inflammation model, MDP activation of NOD2 resulted in an increased IRF4 expression; and subsequently, IRF4 bound MyD88, TRAF6 and receptor interacting serine-threonine kinase (RICK), leading to IRF4-mediated inhibition of Lys63-linked polyubiquitination of TRAF6 and RICK followed by down-regulation of TLR-MyD88-induced NF- κ B activation [84]. Previous studies have identified a key regulatory role of IRF4 in mediating IL-6 production by mucosal T cells and subsequently T cell apoptosis [76]. IRF4 expression in IBD patient was augmented in the presence of active inflammation in contrast to other forms of IRFs (IRF1, IRF5, and IRF8) [77]. The possible roles of IRF4 in CD-induced inflammation are complicated and summarized in Figure 3B.

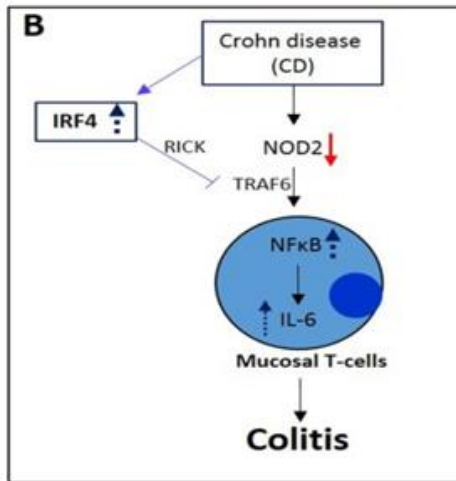


Figure 3(B): Relationship between IRF4 and epigenetic regulation of CD.

Rheumatoid arthritis

IL-17 is a pro-inflammatory cytokine produced by Th17-lymphocytes lineage in response to injuries, and has a key role in inflammatory responses not only in EAE and IBD, but also in other autoimmune diseases such as RA [85], large-vessel vacuities [86] and autoimmune encephalomyelitis [87]. Emerging data have highlighted the immune modulatory effect of IRF4 in autoimmune diseases like rheumatoid arthritis (RA), and the effect is mainly controlled by IL-17 signaling [85,88]. Despite the well-known role of IRF4 in promoting the differentiation and maturation of Th17 cells, the signaling pathways used by T cells to regulate IL-17 production in RA are not clear. Biswas *et al.* have reported that the phosphorylation of IRF4 is one of the key mechanisms by which ROCK2 regulates the development of autoimmunity in mice and induces the production of IL-17 and IL-21 [23]. Therefore, the inhibition of ROCK2 signaling pathway can be used to test the role of IRF4 in regulating pro-inflammatory pathways in T cell subsets [89]. It has been found that the downstream proteins of ROCK2 signaling are primarily STAT3, IRF4, and RORγt. Selective inhibition of ROCK2 (treatment with KD025) under Th17-skewing conditions down-regulates the phosphorylation of STAT3 and leads to reduced levels of IRF4 and RORγt protein in a dose-dependent manner in human CD4 T cells, suggesting ROCK2 regulates IL-17 secretion via STAT3, IRF4, and RORγt transcription factors [89]. It was suggested that the ROCK2 mediated phosphorylation of IRF4 is required for IL-17 and IL-21 production in autoimmune responses [23].

The immune modulatory roles of IRF4 in RA are also cell-type based. Studies on RA have shown that Th1 cells

are pathogenic and Th2 cells are protective [90-92]. IRF4 plays a critical role in RA by mediating Th2 cell response, and Th2 cells secrete anti-inflammatory cytokines including IL-4 and play differential roles depending on cell types [93, 94]. IRF4 inhibits the production of IL-4 by naive CD4+ T cells but promotes the production of IL-4 by effector/memory CD4+ T cells [95]. Moreover, IRF4 can directly activate the il4 promoter to stabilize the Th2 cell developmental program through affecting the expression of Gfi1, a transcriptional repressor required for Th2 cell differentiation [94]. Indeed, the il4 promoter contains multiple IRF4 binding sites that are located adjacent to the transcription factor binding sites for known nuclear factor of activated T cells (NFAT). NFAT is a family of transcription factors critical in regulating early genes in response to T cell receptor-mediated signals [96]. Among the four known NFAT transcription factors, NFATc1 and NFATc2 have been shown to cooperate with IRF4 in transactivating reporter constructs driven by the IL-4 promoter [93]. Interestingly, the data of chromatin immune precipitation assay suggested that IRF4 interacts with NFATc2 to regulate IL-4 gene regulation [93].

Obesity-Induced Inflammation

In recent years, much research effort has been devoted to elucidating mechanisms underlying IRF4-mediated inflammatory responses to obesity, particularly the release of anti-inflammatory cytokines from macrophages. Functionally, IRF4 is one of the endogenous regulators of adipogenesis and adipocyte gene expression in obesity [44,99,106]. It has been shown that IRF4 mediates obesity-induced inflammation through regulation of adipose tissue macrophage polarization where macrophages play a significant role in the metabolic response to obesity [30]. It was concluded that IRF4 promotes insulin sensitivity through actions in at least two distinct cell types: adipocytes and macrophages. IRF4 expression is strictly regulated in primary macrophages and in adipose tissue macrophages of high-fat diet-induced obese mice [106]. IRF4 knockout (*Ir4*^{-/-}) macrophages produce higher levels of pro-inflammatory cytokines, e.g. IL-1β and TNF-α, in response to fatty acids *in vitro* compared to WT macrophages. In adipocytes, IRF4 promotes lipolysis and represses lipogenesis in a nutritionally regulated manner. Mice lacking IRF4 specifically in adipose tissue have enhanced weight gain and insulin resistant [44]. Consistently, mice lacking IRF4 specifically in myeloid cells also show reduced insulin sensitivity, even in the absence of adiposity [30].

There is overwhelming evidence from human and preclinical studies that suggest the adipose tissue T-reg cells are important in the development of obesity-linked

insulin resistance and inflammation [107-109]. The development and function of T-reg cells are largely depends on Foxp3 (forkhead box P3) [110], and are also regulated by IRF4 [111]. IRF4 deficient T-reg cells failed to differentiate into effector T-reg cells and lacked Blimp1 expression, indicating that IRF4 functions upstream of Blimp-1 in the differentiation of effector T-reg cells [101]. Additionally, IRF4-deficient T-reg cells not only lack inducible T-cell co-stimulator (ICOS) and IL-10, but are also impaired in the function to regulate key molecules required for homing, such as CD62L, CD103 and CCR6 [101]. Zheng *et al.* have used *Icos* as a model gene to test the synergistic binding of IRF4 and Foxp3 in transcriptional regulation [64]. By using the transcription factor binding site prediction algorithm, the authors found a putative IRF4 binding site within the *Icos* promoter corresponding to the Foxp3 binding sites [112]. Therapeutically, adoptive transfer of CD4+FoxP3+ T-reg cells significantly improved insulin sensitivity and diabetic nephropathy in obese mice [107]. Foxp3+ T-reg cells exert immunosuppressive effect by activating IL-10 mediated JAK/STAT pathway and blocking I κ B activity [113] or by inducing tyrosine phosphorylation of STAT-3 [114]. All together, these data suggest that the regulatory role of IRF4 in T-reg cells might be crucial for the T-reg-

mediated protective effect in obesity-induced inflammation.

Conclusion

Previous data from studies on signaling pathways and gene profiles have demonstrated that IRF4 has pleiotropic effects in immune responses. IRF4 plays a critical role in multiple pathophysiological procedures, including inflammatory balance, development of T and B cells, differentiation of T cell subsets (Th1, Th2 and Th17), regulation of T-reg cells, adipose tissue immune response, and macrophage/microglia polarization, etc. Abnormal expression of IRF4 is related with exacerbating pathologies in stroke, AD, and MS. Although IRF4 is widely considered the mediator of anti-inflammatory signaling pathways in microglia/macrophage response to neurodegenerative diseases, the mechanisms by which IRF4 coordinates the whole inflammatory response require further elucidation (Figure 4) given its multi-factorial effects in other inflammatory diseases. Effective manipulation of IRF4 signaling pathways might provide new insights into the pathology of these diseases, and hopefully will help develop new therapeutic strategies.

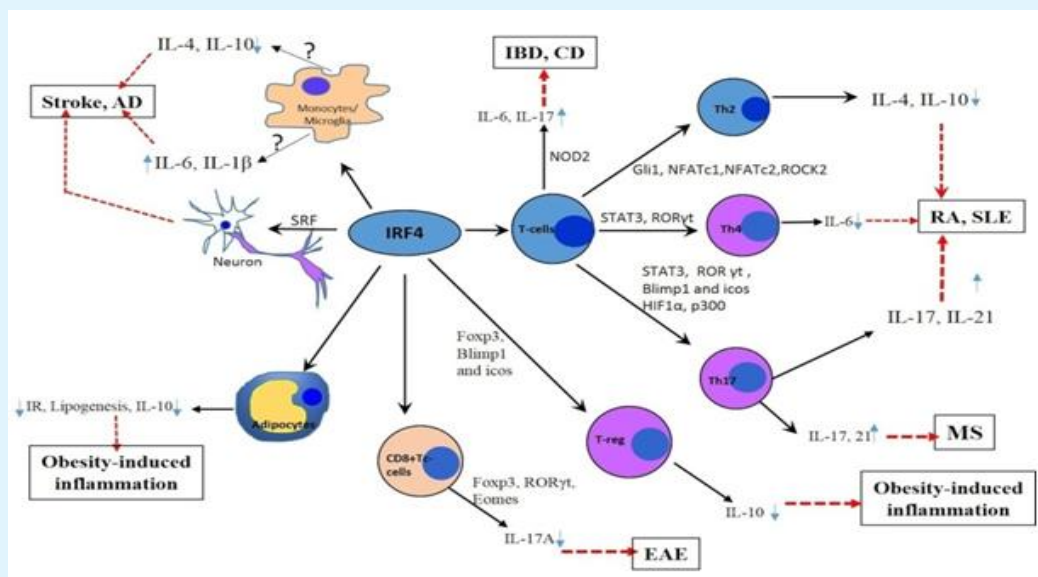


Figure 4: Role of IRF4 in inflammatory disease on immune cells.

IRF4 affects development and differentiation of T-cells subtypes such as Th2 and Th17. IRF4 differentially regulates the pro- and anti-inflammatory cytokine levels in IBD via NOD2/ STAT3/ROR γ t signaling in naïve T-cells, and mediates the differentiation of Th2 and Th17 cells via G α i1/NFATc1/NFATc2 and STAT3/ROR γ t/Blimp1/ICOS pathway respectively in autoimmune diseases in a lineage specific manner. IRF4 promotes differentiation of T-reg cells via Foxp3/Blimp1/ICOS signaling in obesity induced inflammation. In CNS, IRF4-SRF and Jmjd3-IRF4 regulatory axis regulate the neuronal degeneration and microglial activation respectively in ischemic stroke and AD. IBD; Inflammatory bowel diseases, RA; Rheumatoid arthritis, CD; Crohn disease, EAE; Experimental Autoimmune Encephalomyelitis, SLE; systemic lupus Erythematosus (SLE), MS; Multiple sclerosis, AD; Alzheimer's disease.

Acknowledgement

This work was supported by the NIH funding (grants NS093042/NS091794 to Fudong Liu).

References

1. Amor S, Puentes F, Baker D, van der Valk P (2010) Inflammation in neurodegenerative diseases. *Immunology* 129(2): 154-169.
2. Pohl D, Benseler S (2013) Systemic inflammatory and autoimmune disorders. *Handb Clin Neurol* 112: 1243-1252.
3. Gilroy DW, Lawrence T, Perretti M, Rossi AG (2004) Inflammatory resolution: new opportunities for drug discovery. *Nat Rev Drug Discov* 3(5): 401-416.
4. Libby P (2007) Inflammatory mechanisms: the molecular basis of inflammation and disease. *Nutr Rev* 65(12 Pt 2): S140-S146.
5. Stoll G, Jander S, Schroeter M (2002) Detrimental and beneficial effects of injury-induced inflammation and cytokine expression in the nervous system. *Adv Exp Med Biol* 513: 87-113.
6. Ekdahl CT, Claassen JH, Bonde S, Kokaia Z, Lindvall O (2003) Inflammation is detrimental for neurogenesis in adult brain. *Proc Natl Acad Sci U S A* 100(23): 13632-13637.
7. Paun A, Pitha PM (2007) The IRF family. *Biochimie* 89(6-7): 744-753.
8. Taniguchi T, Ogasawara K, Takaoka A, Tanaka N (2001) IRF family of transcription factors as regulators of host defense. *Annu Rev Immunol* 19: 623-55.
9. Falini B, Fizzotti M, Pucciarini A, Bigerna B, Marafioti T, et al. (2000) A monoclonal antibody (MUM1p) detects expression of the MUM1/IRF4 protein in a subset of germinal center B cells, plasma cells, and activated T cells. *Blood* 95(6): 2084-2092.
10. Gualco G, Weiss LM, Bacchi CE (2010) MUM1/IRF4: A Review. *Appl Immunohistochem Mol Morphol* 18(4): 301-310.
11. Fujii Y, Shimizu T, Kusumoto M, Kyogoku Y, Taniguchi T, et al. (1999) Crystal structure of an IRF-DNA complex reveals novel DNA recognition and cooperative binding to a tandem repeat of core sequences. *EMBO J* 18(18): 5028-5041.
12. Qin BY, Liu C, Lam SS, Srinath H, Delston R, et al. (2003) Crystal structure of IRF-3 reveals mechanism of autoinhibition and virus-induced phosphoactivation. *Nat Struct Biol* 10(11): 913-921.
13. Escalante CR, Yie J, Thanos D, Aggarwal AK (1998) Structure of IRF-1 with bound DNA reveals determinants of interferon regulation. *Nature* 391(6662): 103-106.
14. Remesh SG, Santosh V, Escalante CR (2015) Structural Studies of IRF4 Reveal a Flexible Autoinhibitory Region and a Compact Linker Domain. *J Biol Chem* 290(46): 27779-27790.
15. Perkel JM, Atchison ML (1998) A two-step mechanism for recruitment of Pip by PU.1. *J Immunol* 160(1): 241-252.
16. Yee AA, Yin P, Siderovski DP, Mak TW, Litchfield DW, Arrowsmith CH (1998) Cooperative interaction between the DNA-binding domains of PU.1 and IRF4. *J Mol Biol* 279(5): 1075-10783.
17. Brass AL, Zhu AQ, Singh H (1999) Assembly requirements of PU.1-Pip (IRF-4) activator complexes: inhibiting function in vivo using fused dimers. *EMBO J* 18(4): 977-991.
18. Li P, Spolski R, Liao W, Wang L, Murphy TL, et al. (2012) BATF-JUN is critical for IRF4-mediated transcription in T cells. *Nature* 490(240): 543-546.
19. Glasmacher E, Agrawal S, Chang AB, Murphy TL, Zeng W, et al. (2012) A genomic regulatory element that directs assembly and function of immune-specific AP-1-IRF complexes. *Science* 338(6109): 975 980.
20. Tussiwand R, Lee WL, Murphy TL, Mashayekhi M, Kc W, et al. (2012) Compensatory dendritic cell development mediated by BATF-IRF interactions. *Nature* 490(7421): 502-507.
21. Gupta S, Jiang M, Anthony A, Pernis AB (1999) Lineage-specific modulation of interleukin 4 signaling by interferon regulatory factor 4. *J Exp Med* 190(12): 1837-1848.
22. Pongubala JM, Nagulapalli S, Klemsz MJ, McKercher SR, Maki RA, et al. (1992) PU.1 recruits a second nuclear factor to a site important for immunoglobulin

- kappa 3' enhancer activity. *Mol Cell Biol* 12(1): 368-378.
23. Biswas PS, Gupta S, Chang E, Song L, Stirzaker RA, et al. (2010) Phosphorylation of IRF4 by ROCK2 regulates IL-17 and IL-21 production and the development of autoimmunity in mice. *J Clin Invest* 120(9): 3280-3295.
 24. Wang L, Ning S (2013) Interferon regulatory factor 4 is activated through c-Src-mediated tyrosine phosphorylation in virus-transformed cells. *J Virol* 87: 9672-9679.
 25. Biswas PS, Gupta S, Stirzaker RA, Kumar V, Jessberger R, et al. (2012) Dual regulation of IRF4 function in T and B cells is required for the coordination of T-B cell interactions and the prevention of autoimmunity. *J Exp Med* 209(17): 581-596.
 26. Satoh T, Takeuchi O, Vandenbon A, Yasuda K, Tanaka Y, et al. (2010) The Jmjd3-Irf4 axis regulates M2 macrophage polarization and host responses against helminth infection. *Nat Immunol* 11(10): 936-944.
 27. Ishii M, Wen H, Corsa CA, Liu T, Coelho AL, et al. (2009) Epigenetic regulation of the alternatively activated macrophage phenotype. *Blood* 114(15): 3244-3254.
 28. El Chartouni C, Schwarzfischer L, Rehli M (2010) Interleukin-4 induced interferon regulatory factor (Irf) 4 participates in the regulation of alternative macrophage priming. *Immunobiology* 215(9-10): 821-825.
 29. Xuan D, Han Q, Tu Q, Zhang L, Yu L, et al. (2016) Epigenetic Modulation in Periodontitis: Interaction of Adiponectin and JMJD3-IRF4 Axis in Macrophages. *J Cell Physiol* 231(5): 1090-1096.
 30. Eguchi J, Kong X, Tenta M, Wang X, Kang S, et al. (2013) Interferon regulatory factor 4 regulates obesity-induced inflammation through regulation of adipose tissue macrophage polarization. *Diabetes* 62(10): 3394-3403.
 31. McKercher SR, Lombardo CR, Bobkov A, Jia X, Assa-Munt N (2003) Identification of a PU.1-IRF4 protein interaction surface predicted by chemical exchange line broadening. *Proc Natl Acad Sci USA* 100(2): 511-516.
 32. Klemsz MJ, McKercher SR, Celada A, Van Beveren C, Maki RA (1990) The macrophage and B cell-specific transcription factor PU.1 is related to the ets oncogene. *Cell* 61(1): 113-124.
 33. Iwasaki H, Somoza C, Shigematsu H, Duprez EA, Iwasaki-Arai J, et al. (2005) Distinctive and indispensable roles of PU.1 in maintenance of hematopoietic stem cells and their differentiation. *Blood* 106(5): 1590-1600.
 34. Mittrucker HW, Matsuyama T, Grossman A, Kundig TM, Potter J, et al. (1997) Requirement for the transcription factor LSIRF/IRF4 for mature B and T lymphocyte function. *Science* 275(5299): 540-543.
 35. van der Stoep N, Quinten E, Marcondes Rezende M, van den Elsen PJ (2004) E47, IRF-4, and PU.1 synergize to induce B-cell-specific activation of the class II transactivator promoter III (CIITA-PIII). *Blood* 104(9): 2849-2857.
 36. Luo H, Tian M (2010) Transcription factors PU.1 and IRF4 regulate activation induced cytidine deaminase in chicken B cells. *Mol Immunol* 47(7-8): 1383-1395.
 37. Chopin M, Chacon-Martinez CA, Jessberger R (2011) Fine tuning of IRF-4 expression by SWAP-70 controls the initiation of plasma cell development. *Eur J Immunol* 41(10): 3063-3074.
 38. Boddicker RL, Kip NS, Xing X, Zeng Y, Yang ZZ, et al. (2015) The oncogenic transcription factor IRF4 is regulated by a novel CD30/NF-kappaB positive feedback loop in peripheral T-cell lymphoma. *Blood* 125(20): 3118-3127.
 39. Patel AR, Ritzel R, McCullough LD, Liu F (2013) Microglia and ischemic stroke: a double-edged sword. *Int J Physiol Pathophysiol Pharmacol* 5(2): 73-90.
 40. Gunthner R, Anders HJ (2013) Interferon-regulatory factors determine macrophage phenotype polarization. *Mediators Inflamm* 2013: 731023.
 41. Krausgruber T, Blazek K, Smallie T, Alzabin S, Lockstone H, et al. (2011) IRF5 promotes inflammatory macrophage polarization and TH1-TH17 responses. *Nat Immunol* 12(3): 231-238.
 42. Takaoka A, Yanai H, Kondo S, Duncan G, Negishi H, et al. (2005) Integral role of IRF-5 in the gene induction programme activated by Toll-like receptors. *Nature* 434(7030): 243-249.

43. Takaoka A, Tamura T, Taniguchi T (2008) Interferon regulatory factor family of transcription factors and regulation of oncogenesis. *Cancer Sci* 99(3): 467-478.
44. Eguchi J, Wang X, Yu S, Kershaw EE, Chiu PC, et al. (2011) Transcriptional control of adipose lipid handling by IRF4. *Cell Metab* 13(3): 249-259.
45. Jiang DS, Bian ZY, Zhang Y, Zhang SM, Liu Y, et al. (2013) Role of interferon regulatory factor 4 in the regulation of pathological cardiac hypertrophy. *Hypertension* 61(6): 1193-1202.
46. Guo S, Li ZZ, Jiang DS, Lu YY, Liu Y, et al. (2014) IRF4 is a novel mediator for neuronal survival in ischaemic stroke. *Cell Death Differ* 21(6): 888-903.
47. Mehwish AM, Xu Y, McCullough LD, Liu F (2015) Role of IRF5-IRF4 Regulatory Axis in Microglial Polarization After Neonatal Stroke. *Stroke* 46: A20.
48. Negishi H, Fujita Y, Yanai H, Sakaguchi S, Ouyang X, et al. (2006) Evidence for licensing of IFN-gamma-induced IFN regulatory factor 1 transcription factor by MyD88 in Toll-like receptor-dependent gene induction program. *Proc Natl Acad Sci USA* 103(41): 15136-15141.
49. Ahyi AN, Chang HC, Dent AL, Nutt SL, Kaplan MH (2009) IFN regulatory factor 4 regulates the expression of a subset of Th2 cytokines. *J Immunol* 183(3): 1598-1606.
50. Honma K, Udono H, Kohno T, Yamamoto K, Ogawa A, et al. (2005) Interferon regulatory factor 4 negatively regulates the production of proinflammatory cytokines by macrophages in response to LPS. *Proc Natl Acad Sci USA* 102(44): 16001-16606.
51. Hu X, Li P, Guo Y, Wang H, Leak RK, et al. (2012) Microglia/macrophage polarization dynamics reveal novel mechanism of injury expansion after focal cerebral ischemia. *Stroke* 43(11): 3063-3070.
52. Wang G, Zhang J, Hu X, Zhang L, Mao L, et al. (2013) Microglia/macrophage polarization dynamics in white matter after traumatic brain injury. *J Cereb Blood Flow Metab* 33(12): 1864-1874.
53. Kigerl KA, Gensel JC, Ankeny DP, Alexander JK, Donnelly DJ, et al. (2009) Identification of two distinct macrophage subsets with divergent effects causing either neurotoxicity or regeneration in the injured mouse spinal cord. *J Neurosci* 29(43): 13435-13444.
54. Zhu D, Yang N, Liu YY, Zheng J, Ji C, et al. (2016) M2 Macrophage Transplantation Ameliorates Cognitive Dysfunction in Amyloid-beta-Treated Rats Through Regulation of Microglial Polarization. *J Alzheimers Dis* 52(2): 483-495.
55. Akiyama H, Mori H, Saido T, Kondo H, Ikeda K, et al. (1999) Occurrence of the diffuse amyloid beta-protein (Abeta) deposits with numerous Abeta-containing glial cells in the cerebral cortex of patients with Alzheimer's disease. *Glia* 25(4): 324-331.
56. Mackenzie IR, Hao C, Munoz DG (1995) Role of microglia in senile plaque formation. *Neurobiol Aging* 16:797-804.
57. Akiyama H, McGeer PL (1990) Brain microglia constitutively express beta-2 integrins. *J Neuroimmunol* 30(1): 81-93.
58. Lee CY, Landreth GE (2010) The role of microglia in amyloid clearance from the AD brain. *J Neural Transm (Vienna)* 117(8): 949-60.
59. Graeber MB, Li W, Rodriguez ML (2011) Role of microglia in CNS inflammation. *FEBS Lett* 585(23): 3798-3805.
60. Aguzzi A, Barres BA, Bennett ML (2013) Microglia: scapegoat, saboteur, or something else? *Science* 339(6116): 156-161.
61. Keegan BM, Noseworthy JH (2002) Multiple sclerosis. *Annu Rev Med* 53: 285-302.
62. Salmaggi A, Lamperti E, Eoli M, Venegoni E, Bruzzone MG, et al. (1994) Spinal cord involvement and systemic lupus erythematosus: clinical and magnetic resonance findings in 5 patients. *Clin Exp Rheumatol* 12(4): 389-394.
63. Steinman L (2008) Nuanced roles of cytokines in three major human brain disorders. *J Clin Invest* 118(11): 3557-3563.
64. Brustle A, Heink S, Huber M, Rosenplanter C, Stadelmann C, et al. (2007) The development of inflammatory T(H)-17 cells requires interferon-regulatory factor 4. *Nat Immunol* 8(9): 958-966.
65. Yang C, He D, Yin C, Tan J (2015) Inhibition of Interferon Regulatory Factor 4 Suppresses Th1 and Th17 Cell Differentiation and Ameliorates Experimental Autoimmune Encephalomyelitis. *Scand J Immunol* 82(4): 345-351.

66. Constantinescu CS, Farooqi N, O'Brien K, Gran B (2011) Experimental autoimmune encephalomyelitis (EAE) as a model for multiple sclerosis (MS). *Br J Pharmacol* 164(4): 1079-1106.
67. O'Brien K, Gran B, Rostami A (2010) T-cell based immunotherapy in experimental autoimmune encephalomyelitis and multiple sclerosis. *Immunotherapy* 2(1): 99-115.
68. Huber M, Heink S, Pagenstecher A, Reinhard K, Ritter J, et al. (2013) IL-17A secretion by CD8+ T cells supports Th17-mediated autoimmune encephalomyelitis. *J Clin Invest* 123(1): 247-260.
69. Fletcher JM, Lalor SJ, Sweeney CM, Tubridy N, Mills KH (2010) T cells in multiple sclerosis and experimental autoimmune encephalomyelitis. *Clin Exp Immunol* 162(1): 1-11.
70. Blumberg RS, Saubermann LJ, Strober W (1999) Animal models of mucosal inflammation and their relation to human inflammatory bowel disease. *Curr Opin Immunol* 11(6): 648-656.
71. Fuss IJ, Neurath M, Boirivant M, Klein JS, Strong SA, et al. (1996) Disparate CD4+ lamina propria (LP) lymphokine secretion profiles in inflammatory bowel disease. Crohn's disease LP cells manifest increased secretion of IFN-gamma, whereas ulcerative colitis LP cells manifest increased secretion of IL-5. *J Immunol* 157(3): 1261-1270.
72. Strober W, Fuss IJ, Blumberg RS (2002) The immunology of mucosal models of inflammation. *Annu Rev Immunol* 20: 495-549.
73. Ito H (2003) IL-6 and Crohn's disease. *Curr Drug Targets Inflamm Allergy* 2(2): 125-130.
74. Ito H (2003) Anti-interleukin-6 therapy for Crohn's disease. *Curr Pharm Des* 9(4): 295-305.
75. Sartor RB (2006) Mechanisms of disease: pathogenesis of Crohn's disease and ulcerative colitis. *Nat Clin Pract Gastroenterol Hepatol* 3(7):390-407.
76. Mudter J, Amoussina L, Schenk M, Yu J, Brustle A, et al. (2008) The transcription factor IFN regulatory factor-4 controls experimental colitis in mice via T cell-derived IL-6. *J Clin Invest* 118(7): 2415-2426.
77. Mudter J, Yu J, Zufferey C, Brustle A, Wirtz S, et al. (2011) IRF4 regulates IL-17A promoter activity and controls RORgammat-dependent Th17 colitis in vivo. *Inflamm Bowel Dis* 17(6): 1343-1358.
78. Mudter J, Yu J, Amoussina L, Weigmann B, Hoffman A, et al. (2009) IRF4 selectively controls cytokine gene expression in chronic intestinal inflammation. *Arch Immunol Ther Exp (Warsz)* 57(5): 369-376.
79. Chung S, Lee TJ, Reader BF, Kim JY, Lee YG, et al. (2016) FoxO1 regulates allergic asthmatic inflammation through regulating polarization of the macrophage inflammatory phenotype. *Oncotarget* 7(14): 17532-17546.
80. Yen D, Cheung J, Scheerens H, Poulet F, Mc Clanahan T, et al. (2006) IL-23 is essential for T cell-mediated colitis and promotes inflammation via IL-17 and IL-6. *J Clin Invest* 116(5): 1310-1316.
81. Strober W, Fuss I, Mannon P (2007) The fundamental basis of inflammatory bowel disease. *J Clin Invest* 117(3): 514-521.
82. Strober W, Kitani A, Fuss I, Asano N, Watanabe T (2008) The molecular basis of NOD2 susceptibility mutations in Crohn's disease. *Mucosal Immunol* 1 (S1): S5-S9.
83. Grimes CL, Ariyananda Lde Z, Melnyk JE, O'Shea EK (2012) The innate immune protein Nod2 binds directly to MDP, a bacterial cell wall fragment. *J Am Chem Soc* 134(33): 13535-13537.
84. Watanabe T, Asano N, Meng G, Yamashita K, Arai Y, et al. (2014) NOD2 downregulates colonic inflammation by IRF4-mediated inhibition of K63-linked polyubiquitination of RICK and TRAF6. *Mucosal Immunol* 7(6): 1312-1325.
85. Biswas PS, Bhagat G, Pernis AB (2010) IRF4 and its regulators: evolving insights into the pathogenesis of inflammatory arthritis? *Immunol Rev* 233(1): 79-96.
86. Ciccia F, Rizzo A, Guggino G, Cavazza A, Alessandro R, et al. (2015) Difference in the expression of IL-9 and IL-17 correlates with different histological pattern of vascular wall injury in giant cell arteritis. *Rheumatology (Oxford)* 54(9): 1596-1604.
87. Noubade R, Kremmentsov DN, Del Rio R, Thornton T, Nagaleekar V, et al. (2011) Activation of p38 MAPK in CD4 T cells controls IL-17 production and autoimmune encephalomyelitis. *Blood* 118(12): 3290-3300.

88. Xu WD, Pan HF, Ye DQ, Xu Y (2012) Targeting IRF4 in autoimmune diseases. *Autoimmun Rev* 11: 918-924.
89. Zanin-Zhorov A, Weiss JM, Nyuydzefe MS, Chen W, Scher JU, et al. (2014) Selective oral ROCK2 inhibitor down-regulates IL-21 and IL-17 secretion in human T cells via STAT3-dependent mechanism. *Proc Natl Acad Sci USA* 111(47): 16814-16819.
90. Lafaille JJ (1998) The role of helper T cell subsets in autoimmune diseases. *Cytokine Growth Factor Rev* 9(2): 139-151.
91. Nicholson LB, Kuchroo VK (1996) Manipulation of the Th1/Th2 balance in autoimmune disease. *Curr Opin Immunol* 8(6): 837-842.
92. Liblau RS, Singer SM, Mc Devitt HO (1995) Th1 and Th2 CD4+ T cells in the pathogenesis of organ-specific autoimmune diseases. *Immunol Today* 16(1): 34-38.
93. Rengarajan J, Mowen KA, McBride KD, Smith ED, Singh H et al. (2002) Interferon regulatory factor 4 (IRF4) interacts with NFATc2 to modulate interleukin 4 gene expression. *J Exp Med* 195(8): 1003-1012.
94. Tominaga N, Ohkusu-Tsukada K, Udono H, Abe R, Matsuyama T, et al. (2003) Development of Th1 and not Th2 immune responses in mice lacking IFN-regulatory factor-4. *Int Immunol* 15(1): 1-10.
95. Honma K, Kimura D, Tominaga N, Miyakoda M, Matsuyama T, et al. (2008) Interferon regulatory factor 4 differentially regulates the production of Th2 cytokines in naive vs. effector/memory CD4+ T cells. *Proc Natl Acad Sci USA* 105(41): 15890-15895.
96. Ranger AM, Gerstenfeld LC, Wang J, Kon T, Bae H, et al. (2000) The nuclear factor of activated T cells (NFAT) transcription factor NFATp (NFATc2) is a repressor of chondrogenesis. *J Exp Med* 191(1): 9-22.
97. Tamiya T, Ichiyama K, Kotani H, Fukaya T, Sekiya T, et al. (2013) Smad2/3 and IRF4 play a cooperative role in IL-9-producing T cell induction. *J Immunol* 191(5): 2360-2371.
98. Huber M, Brustle A, Reinhard K, Guralnik A, Walter G, et al. (2008) IRF4 is essential for IL-21-mediated induction, amplification, and stabilization of the Th17 phenotype. *Proc Natl Acad Sci USA* 105(52): 20846-20851.
99. Fabrizi M, Marchetti V, Mavilio M, Marino A, Casagrande V, et al. (2014) IL-21 is a major negative regulator of IRF4-dependent lipolysis affecting Tregs in adipose tissue and systemic insulin sensitivity. *Diabetes* 63(6): 2086-2096.
100. Williams JW, Tjota MY, Clay BS, Vander Lugt B, Bandukwala HS (2013) Transcription factor IRF4 drives dendritic cells to promote Th2 differentiation. *Nat Commun* 4: 2990.
101. Cretney E, Xin A, Shi W, Minnich M, Masson F, et al. (2011) The transcription factors Blimp-1 and IRF4 jointly control the differentiation and function of effector regulatory T cells. *Nat Immunol* 12(4): 304-311.
102. Chen Z, Laurence A, O'Shea JJ (2007) Signal transduction pathways and transcriptional regulation in the control of Th17 differentiation. *Semin Immunol* 19(6): 400-408.
103. Ivanov, Zhou L, Littman DR (2007) Transcriptional regulation of Th17 cell differentiation. *Semin Immunol* 19(6): 409-417.
104. Cao Y, Li H, Sun Y, Chen X, Liu H, et al. (2010) Interferon regulatory factor 4 regulates thymocyte differentiation by repressing Runx3 expression. *Eur J Immunol* 40(11): 3198-3209.
105. Achuthan A, Cook AD, Lee MC, Saleh R, Khiew HW, et al. (2016) Granulocyte macrophage colony-stimulating factor induces CCL17 production via IRF4 to mediate inflammation. *J Clin Invest* 126(9): 3453-3466.
106. Eguchi J, Yan QW, Schones DE, Kamal M, Hsu CH, et al. (2008) Interferon regulatory factors are transcriptional regulators of adipogenesis. *Cell Metab* 7(1): 86-94.
107. Eller K, Kirsch A, Wolf AM, Soppor S, Tagwerker A, et al. (2011) Potential role of regulatory T cells in reversing obesity-linked insulin resistance and diabetic nephropathy. *Diabetes* 60(11): 2954-2562.
108. Deiluiis J, Shah Z, Shah N, Needleman B, Mikami D, et al. (2011) Visceral adipose inflammation in obesity is associated with critical alterations in regulatory cell numbers. *PLoS One* 6(1): e16376.
109. Matarese G, Procaccini C, De Rosa V, Horvath TL, La Cava A (2010) Regulatory T cells in obesity: the leptin connection. *Trends Mol Med* 16(6): 247-256.

110. Sakaguchi S, Miyara M, Costantino CM, Hafler DA (2010) FOXP3+ regulatory T cells in the human immune system. *Nat Rev Immunol* 10(7): 490-500.
111. Ohkura N, Sakaguchi S (2011) Maturation of effector regulatory T cells. *Nat Immunol* 12(4): 283-284.
112. Zheng Y, Chaudhry A, Kas A, deRoos P, Kim JM, et al. (2009) Regulatory T-cell suppressor program co-opts transcription factor IRF4 to control T(H)2 responses. *Nature*(7236) 458(7236): 351-356.
113. Schottelius AJ, Mayo MW, Sartor RB, Baldwin AS (1999) Interleukin-10 signaling blocks inhibitor of kappaB kinase activity and nuclear factor kappaB DNA binding. *J Biol Chem* 274(45): 31868-31874.
114. Lumeng CN, Bodzin JL, Saltiel AR (2007) Obesity induces a phenotypic switch in adipose tissue macrophage polarization. *J Clin Invest* 117(1): 175-184.