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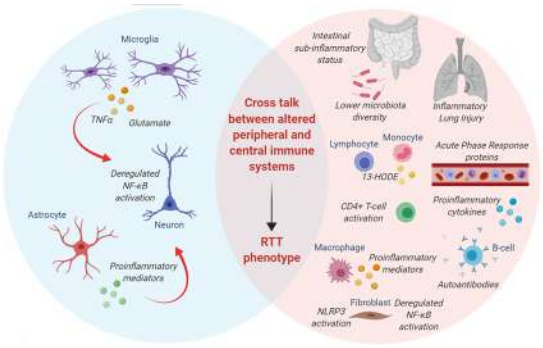

Compromised immune/inflammatory responses in Rett syndrome

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Abstract

Mutations in X-linked gene methyl-CpG-binding protein 2 (*MECP2*), a key transcriptional regulator, account for most cases of Rett syndrome (RTT), a devastating neurodevelopmental disorder with no known cure. Despite extensive research to elucidate MeCP2 functions, the mechanisms underlying RTT pathophysiology are still unclear. In addition to a variety of neurological symptoms, RTT also includes a plethora of additional phenotypical features including altered lipid metabolism, redox imbalance, immune dysfunction and mitochondrial abnormalities that explain its multisystemic nature. Here, we provide an overview of the current knowledge on the potential role of dysregulated inflammatory and immune responses in RTT. The findings show that abnormalities of humoral and cell-mediated immunity together with chronic low-grade inflammation in multiple organs represent not only clinical manifestations of RTT but rather can contribute to its development and deteriorating course. A future research challenge could be to target therapeutically immune dysfunction as a novel means for RTT management.

Keywords: neurons; cytokines; NF-κB; hydroxyoctadecadienoic acids; inflammasome; autoantibodies.

Rett syndrome a multisystem disorder

Rett syndrome (RTT; OMIM 312750) is a complex neurodevelopmental disorder characterized by a wide range of neurological and physical impairments with an incidence ranging from 1/10,000 to 1/15,000 live births, making RTT the second most frequent cause of intellectual disability in females after Down Syndrome. In approximately 90% of patients, classic RTT is due to a *de novo* mutation in the X-linked methyl-CpG-binding protein 2 gene (*MECP2*), a multifunctional protein implicated in the regulation of transcriptional activity, chromatin organization, microRNA processing, and RNA splicing [1].

Typically, after a period of normal development ranging from 6 to 18 months of life, RTT patients experience a rapid deterioration of the acquired psychomotor skills that leads to a characteristic clinical phenotype that includes repetitive hand movements, absent or very limited speech, seizures, irregular breathing, cardiac abnormalities, ataxia, and autistic features [2].

Although the hallmarks of RTT are mainly neurological, in the last few years, several alterations in other tissues and organs including lungs, bones, heart, microvascular system, and gastrointestinal tract have been identified [3]. In addition, RTT patients show compromised signaling pathways and metabolic processes including unbalanced redox homeostasis, dysfunctional mitochondrial bioenergetics, perturbed lipid metabolism and abnormal immune-inflammatory responses [4,5]. These combined abnormalities can explain the complexity and the global nature of RTT. Despite significant progress in the understanding of MeCP2 functions, to date it remains to be clarified how the mutation of a single protein can cause such a wide variety of clinical manifestations.

Immune dysfunction and chronic subclinical inflammation as pathophysiological-contributing factors to RTT development and progression

Over the last decade, it has become increasingly clear that immune dysfunction and chronic subclinical inflammation can be involved in RTT [4,5]. While RTT is primarily a central nervous system (CNS) disorder, a dysregulated function of immune cells and a propagation of pro-inflammatory signals within the brain as well as in the body's periphery could contribute to the development and progression of some clinical features of RTT, thus also explaining its multi-systemic nature [6]. On the other hand, an abnormal immune function occurs also in other neurodevelopmental disorders such as Autism Spectrum Disorder (ASD), in which category was also classified RTT until few years ago [7].

Involvement of RTT microglia in CNS inflammation

In mammals, MeCP2 is widely expressed throughout the body, especially in the nervous system but also in other tissues. Within the brain, MeCP2 is reported at high levels in neurons, but is also expressed by all types of glial cells, including microglia, the primary brain-resident macrophages.

Research over the last 10 years highlighted a possible role for an altered microglia function in RTT during brain development (Table 1) [8]. In an *in vitro* study, microglia from *Mecp2*-null mice demonstrated to be involved in the release of abnormal high levels of glutamate with neurotoxic effects on hippocampal neurons including damage to dendrites and synapses [9]. In a successive study, the mechanism for the over-release of glutamate by RTT microglia and neurotoxicity has been clarified. Indeed, Jin et al. [10] shown that MeCP2 acts as transcriptional repressor for the glutamine transporter SNAT1. As a direct consequence of SNAT1 aberrant

expression, *Mecp2*-deficient microglia show increased glutamine uptake that induces, at the same time, overproduction of glutamate and mitochondrial oxidative stress [10]. Interestingly, in an *in vivo* study, restoring wild type microglia by bone marrow transplantation was able to prolong lifespan and to rescue RTT phenotype in both male and female RTT mice [11]. However, a study by Wang et al. [12] was not able to replicate these results, showing that using bone marrow transplantation or genetics to restore Mecp2 in microglia did not improve the pathological status in *Mecp2*-null mice. In addition, Schafer et al. [13] demonstrated that the involvement of microglia in the impairment of neuronal circuits by engulfing the presynaptic inputs in late phenotypic *Mecp2*-null mice is a secondary event, independent of microglia-specific loss of Mecp2 expression.

Nevertheless, other evidence supports a possible contribution of altered inflammatory and phagocytic functions of microglia in RTT. Indeed, in *Mecp2*-null mice, microglia develop an inflammatory activation, showing increased mRNA expression of the pro-inflammatory cytokine TNFα, and then they are lost with disease progression [14]. In addition, a transcriptomic study on microglia from heterozygous *Mecp2*-null female mice at pre-phenotypic and phenotypic stages revealed a dysregulated expression of genes associated with innate immunity and cellular stress responses, suggesting that an increased vulnerability of microglia to cellular stressors during the pre-phenotypic phase could be the prelude to a later onset of neurological symptoms [15]. In a study aimed to evaluate the therapeutic efficacy of dendrimer-N-acetyl-L-cysteine conjugates (D-NAC) in counteracting RTT immune dysfunction by targeting activated glial cells, the authors found that mixed astrocyte-microglia cultures from *Mecp2*-null mice show an increased release of pro-inflammatory cytokines in resting conditions, which worsens further after lipopolysaccharides (LPS) stimulation [16]. It is interesting to note that, after systemic

administration, the specific accumulation of D-NAC dendrimers in microglia was coupled with an improved behavioral outcome of *Mecp2*-null mice, highlighting the possible participation of these cells in the pathogenic mechanisms of RTT [16]. Moreover, the pathological role of *MeCP2*-null microglia on neuronal toxicity was also proven by the improvement in disease phenotype and survival of *Mecp2*-null mice following the ablation of CX3CR1, a cytokine receptor involved in neuron–microglia interaction [17]. Finally, in a recent paper, analyzing the DNA methylation in post-mortem cerebral cortex from patients affected by RTT, ASD, and Dup15q syndrome, the authors [18] identified a convergent epigenomic signature between the three neurodevelopmental disorders. In particular, epigenetic modifications affected neuronal and microglial genes known to be essential for both early nervous system development and immune system, specifically microglia and, therefore, suggesting a role for the immune system in mediating epigenetic influences on neuronal development [18].

Based on these evidences it is possible to assume that any alteration of the microglial activity, as a consequence of *MECP2* mutation, during the early postnatal period could profoundly affect neural development, leading to defective maturation of synaptic circuits and, thus, contributing to RTT pathogenesis and progression [19].

Contribution of peripheral immune cells to RTT

In addition to the CNS, an aberrant activated immune system in RTT have been also described in the periphery (Table 1). Since the body's immune system is known to actively interact with CNS and *vice versa*, it is possible that any imbalances and dysfunctions of innate and adaptive immune cells may then be translated into alterations in brain development and function [20].

Based on the report of Cronk et al. [14], Mecp2 deficiency affected not only microglia function but also induced abnormalities in several peripheral macrophage populations and correlated with an increased number of circulating neutrophils. In this study, *Mecp2*-null mice in the pre-phenotypic phase showed a loss of resident monocytes and intestinal macrophages, while other peripheral macrophage populations were lost progressively at the late-phenotypic stage of the disease. By using RNAseq analysis, the authors also revealed that peritoneal macrophages isolated from *Mecp2*-null mice displayed changes in genes related to glucocorticoid signaling and hypoxia responses. Furthermore, when stimulated *in vivo* with TNFα, peritoneal macrophages from *Mecp2*-null mice exhibited an altered inflammatory response characterized by up- or down-regulated expression of several TNFα responsive genes [14,21]. Based on these results, Mecp2 could play an important role in regulating macrophage functions, the alteration of which could contribute in part to RTT pathophysiology, also considering that the postnatal Mecp2 re-expression in different populations of tissue-resident macrophage and monocyte has been shown to increase the lifespan of *Mecp2*-null mice [14,21].

On the other hand, as shown in a recent paper, macrophage-selective ablation of Mecp2 alone was not sufficient to generate a RTT phenotype but instead induced obesity in mice (*i.e.* enlarged livers, as well as more abundant visceral and subcutaneous adipose tissue) [22]. This metabolic imbalance was related to a compromised sympathetic innervation of brown adipose tissue with a resulting disruption in energy expenditure [22,23]. Although no sign of inflammation was evident (*i.e.* no difference in iNOS and TNF gene expression, nor IL-1β protein levels), pre-obese mutant mice showed higher serum levels of leptin, a cytokine that can contribute to a low-grade chronic inflammation in obese individuals and already found increased in RTT patients [24,25]; however, in this case, the translation of those data to humans should be cautious since

RTT are not obese. Collectively, these findings suggest that also dysfunctional *Mecp2*-deficient macrophages could contribute to the metabolic component observed in RTT patients [26].

Unlike *Mecp2*-null mice, no difference in microglia and macrophage numbers or localization was detected in a *mecp2*-null zebrafish model that, nevertheless, showed clear signs of systemic inflammation (*i.e.* overexpression of C reactive protein, *il1b* and *il10* genes) with increased neutrophil infiltration in the gastrointestinal tract [27]. Furthermore, deficiency of Mecp2 was associated with decreased *tnfα* expression, which did not even respond to an inflammatory stimulus, suggesting a direct role of Mecp2 as an immunological modulator during zebrafish development and inflammation [27].

In addition to the innate immune system, also cells of the adaptive immune system such as lymphocytes showed some abnormalities in RTT. While no difference in lymphocyte populations or activation status was revealed by Plioplys et al. [28] in 8 RTT patients, in a later study, Fiumara et al. [29] reported different results with a reduced percentage of $CD8^+$ suppressor-cytotoxic T cells and a consequent increase in $CD4^+/CD8^+$ ratio in 20 RTT females. Of note, patients under the age of 6 years had low levels of $CD57^+$ cytotoxic natural killer (NK) cells and increased levels of soluble interleukin-2 receptor. These results could be indicative of a defective defense against foreign antigens in RTT [29]. *MECP2* mutation not only negatively affects *in vitro* growth of lymphocytes [30], but also alters the transcript levels of genes related to their immune function including, for example, CCL5 (C-C motif chemokine ligand 5) that induces lymphocytes and monocytes migration or NCR3 (natural cytotoxicity receptor□3) important for NK cell activity [31]. In addition, by maintaining the stable expression of the transcription factor Foxp3, Mecp2 has been implicated also in the regulation of the immunosuppressive activity of regulatory T (Treg) cells, an indispensable T-cell subset

responsible for peripheral tolerance and prevention of autoimmunity during inflammation or infection [32]. Indeed, mice with Treg-specific deletion of *Mecp2* gene showed spontaneous $CD4^+$ T-cell activation with increased IL-17 production, resulting in the development of autoimmunity, skin lesions and wide tissue inflammatory infiltration [32]. On the other hand, specific deletion of MeCP2 in $CD4^+$ T cells impaired their differentiation into T helper type 1 (TH1) and TH17 cells due the failure in the MeCP2-miR-124-SOCS5 axis required for the activation of signal transducer of activation (STAT) pathway [33]. Therefore, these results support the evidence of the critical role played by the epigenetic regulator MeCP2 in influencing the delicate balance between the different T-cell subsets.

As further evidence of the involvement of MeCP2 in the regulation of immune cell functions, O'Driscoll et al. [34,35] have shown that MeCP2 deficiency was associated with an enhanced NF-κB signaling in human peripheral blood mononuclear cells (PBMCs) and human monocyte line THP1. Moreover, NF-κB activation was also coupled to increased glutamate release as well as upregulated expression levels of TNFα, IL-6, and IL-3 mRNAs [4,5,35]. In line with these studies, our recent work reinforced the idea of a possible role of Mecp2 deficiency in immune dysfunction as suggested by a perturbed cytokine/chemokine profile, *i.e.* high serum levels of IL-8, IL-9, and IL-13, together with an abnormal cellular morphology observed in PBMCs isolated from RTT patients [36]. In particular, the ultrastructural abnormalities with enlarged mitochondria and disarranged cristae in RTT PBMCs could be indicative of a status of cellular hyperactivation and impaired energy metabolism. In support of these findings, a microarray study on RTT PBMCs indicated an overexpression of genes related to mitochondrial function and organization, indicating a possible altered energy requirement in the immune cells [37]. Moreover, in a recent study, RTT PBMCs showed also an upregulated expression of

arachidonate 15-lipoxygenase (ALOX15), an enzyme implicated in the oxidation of polyunsaturated fatty acids including linoleic acid and generation of bioactive lipid metabolites such as 13- and 9-hydroxyoctadecadienoic acids (13- and 9-HODEs) [38]. Indeed, ALOX15 transcript levels correlated with increased serum levels of 13-HODE in patients, confirming the involvement of PBMCs in RTT subclinical inflammation [38].

Role of non-immune cells in RTT immunological dysfunction

It is now generally accepted that also non-immune cells like fibroblasts possess immune properties and can participate in immune processes by releasing pro- and anti-inflammatory mediators such as cytokines, chemokines, growth factors and antimicrobial agents. In particular, a growing body of research suggests the involvement of fibroblasts in pathological conditions characterized by persistent chronic inflammation [39].

In line with this evidence, recently our group demonstrated an aberrant activation of NLRP3 inflammasome system in primary dermal fibroblasts isolated from RTT patients (Table 1) [40]. Inflammasomes are multi-protein complexes with a central role in innate immune responses to microbial infection and cellular damage. Nevertheless, the abnormal expression and/or activation of the inflammasomes have been associated with many different human disorders. After activation and assembly, NLRP3-ASC-caspase-1 complexes lead to the cleavage and release of interleukin-1β (IL-1β) and IL-18, proinflammatory cytokines linked to a variety of innate immune responses [41]. In our study, RTT fibroblasts exhibited a constitutively activated state of NLRP3/ASC complex associated with increased cellular levels of both the components of the inflammasome and IL-1β. Concomitantly, there was also a constitutive nuclear translocation of NF-κB p65, mediator of the priming signal of inflammasome, corroborating previous data from

human *MECP2*-deficient PBMCs and RTT mouse models (see next section for a more detailed description of NF-κB signaling in RTT) [34,35,40,42]. However, inflammasome machinery and NF-κB p65 were unable to respond to pro-inflammatory challenge in RTT fibroblasts [40]. It is likely that the redox imbalance – linked to mitochondrial dysfunction, NADPH oxidase activation as well as defective antioxidant defense – can constitute the source of cellular stress-signals that constantly stimulate inflammasome in RTT fibroblasts [40,43]. On the other hand, the absence of response to proinflammatory stimulation could be explained by the fact that the steady state in the activity of the inflammasome has already reached its plateau in RTT cells. These results obtained from patients primary dermal fibroblasts, which has been confirmed to be a good model to study this disease [44], could be extrapolated to immune cells, justifying the chronic inflammatory state and immune impairment observed in the disorder [4,5]. Indeed, an inflammasome machinery constantly activated but, at the same time inefficient, can cause collateral damage to tissues and organs, increasing the vulnerability of RTT patients to unknown endogenous factors or infections such as those clinically observed in the lower respiratory tract and intestine [45,46].

NF-κB signaling pathway in RTT

As mentioned above, there is growing evidence supporting a role for NF-κB signaling in RTT. This pleiotropic transcription factor carries out key functions not only in innate and adaptive immune responses, but also in inflammation. An increased NF-κB activity coupled with upregulated gene expression of some pro-inflammatory cytokines (*i.e.* TNFα, IL-6, and IL-3) was first demonstrated in MeCP2 deficient immune cells (*i.e.* human PBMCs and THP1 cell line) [34,35]. Recently, we also corroborated these results, showing an increased nuclear

translocation of NF-κB p65 coupled with high IL-1β levels that could be related to a constitutive activated state of NLRP3 inflammasome in primary dermal fibroblasts obtained from RTT patients (see previous section) [40]. In addition, a transcriptome profile analysis of whole blood samples from classic RTT and RTT-like patients identified in NF-κB a shared altered pathway in both subtypes of the syndrome [47]. In line with these results, a microarray study on primary cortical astrocytes from Mecp2$^{308/y}$ mice showed a dysregulated expression of some NF-κB target genes, although no significant variation in NF-κB DNA binding activity was detected [48].

The molecular mechanisms underlying the activated state of NF-κB in RTT could be explained by the study of Kishi et al. [42] that detected an aberrant p65/RelA signaling linked to *Irak1* overexpression in the cortex of *Mecp2*-null mice. In particular, *Mecp2* loss-of-function resulted in loss of its transcriptional repression on *Irak1* levels, a kinase involved in the Toll-like receptor (TLR) signaling pathway and NF-κB activation. Interestingly, *Mecp2*-null cortical callosal projection neurons displayed also a reduced dendritic complexity that could be rescued by the modulation of NF-κB pathway, improving also health and lifespan of mice [42].

Similarly, nuclear p65/RelA levels were upregulated in cerebellar primary neurons from *Mecp2*-null mice that also exhibited increased total p65 levels in the brain [49]. In this study, the dysregulated NF-κB signaling was linked to a cerebellum-specific upregulation of glycogen synthase kinase-3 beta (GSK-3β) activity, since its inhibition could attenuate nuclear activity of NF-κB and inflammation markers (*i.e.* IL-1, IL-4, IL-12p70, and IL-17 levels) in the cerebellar area, increasing the lifespan of the animals [49].

Taken together, these results suggest that NF-κB could play a significant role in RTT and modulation of this signaling pathway by inhibition of IRAK1 and/or GSK-3β could be a future therapeutic approach for improving patient quality of life (Figure 1).

Inflammatory mediators in biological fluids of RTT patients

Alterations of immunological biomarkers in biological fluids provide further evidence of immune dysfunction in RTT. Several studies have shown changes in circulating cytokines and chemokines in RTT patients (Table 2). Recently, Byiers et al. [50] first demonstrated the predictive value of salivary testing to assess immune-inflammatory alterations in RTT, showing increased concentrations of IL-1β, IL-6, IL-8, IL-10, GM-CSF, TNF-α and VEGF in saliva samples of affected patients. The authors also found a strong correlation between the cytokine concentration and clinical severity score, further highlighting the potential negative impact of immune deregulation in RTT clinical phenotype [50].

Previously, other two studies evaluated the cytokine expression patterns in RTT blood samples, specifically plasma and serum [36,51]. Increased levels of IL-8, IL-9, and IL-13 have been detected in serum samples of 12 RTT patients that also showed a probable activated status of PBMCs with abnormal morphology (see previous section) [36]. In particular, the increased concentrations of IL-9 and IL-13 could be indicative of a switch to Th2-related cytokines involved in immune humoral responses such as antibody production. A Th2-shifted balance with increased plasma levels of several cytokines (*i.e.* TNF-α, IL-4, IL-5, IL-6, IL-8, IL-17A, IL-33 and IL-37) was also proved in another study of 16 patients with classic RTT [51]. Contrariwise, plasma levels of IFN-??, IL-12p70, IL-22, TGF-??1, IP-10, I-TAC, and RANTES were significantly reduced in RTT compared to the control group. Cytokine changes were also

associated with a proinflammatory status, as evidenced by elevated values of erythrocyte sedimentation rate [51], a prognostic marker of overall inflammation that depends on the concentration of acute-phase response (APR) proteins circulating in the blood.

In line with these data, a proteomic study confirmed the occurrence of a subclinical persistent inflammation in RTT, revealing an altered abundance of APR proteins in plasma of patients in pseudo-autistic phase (stage II) (*e.g.* upregulation of positive APR proteins, such as alpha-1-antitrypsin and serum amyloid A-1 protein, and downregulation of negative APR proteins, including apolipoprotein A1 and retinol-binding protein 4) [52]. Similar results with changed expression of some APR proteins (*e.g.* alpha-1-antitrypsin and apolipoprotein A1) were also obtained by examining the plasma proteome of symptomatic Mecp2-308 female mice [53], thus making them an excellent model for future research in understanding the underlying molecular mechanisms of the persistent low-grade inflammation in RTT. In a different RTT mouse model, *i.e. Mecp2*-null mice, the increased plasma levels of the APR protein "serum amyloid P component" could be indicative of a severe systemic inflammatory response associated with inflammatory lung injury due to aspiration pneumonia [54]. Since also RTT patients have shown some evidence of an underlying lung disease associated with greater vulnerability to lower respiratory tract infections [45], it is possible to hypothesize that this condition may contribute, at least in part, to their systemic inflammatory status.

As previously mentioned, recently our group found increased levels of HODEs, oxidized derivatives of linoleic acid, in serum samples from 42 patients with classic syndrome. In particular, the high concentrations of 13-HODE may depend on the upregulation of ALOX15 transcript levels in RTT PBMCs [38]. It is known that ALOX15 is strongly upregulated by IL-4 and IL-13, the levels of which are also increased in RTT [36,51,55]. By contrast, 9-HODE can

be generated by multiple enzymes and non-enzymatic oxidation of linoleic acid under oxidative stress, a condition well established in RTT [4,5]. Acting through the GPR132 receptor, 9-HODE promotes proinflammatory events such as the release of IL-6 and IL-8, which are increased in RTT [36,51]. On the other hand, 13-HODE is a ligand for PPARγ and displays anti-inflammatory and pro-resolving functions. Therefore, since HODEs show both pro- and anti-inflammatory properties [56], these results suggest that the immune/inflammatory responses in RTT could be more complex than expected.

A further aspect to be considered in RTT immune dysregulation is the involvement of *MECP2* gene polymorphism as a candidate risk factor for autoimmune conditions such as systemic sclerosis, juvenile idiopathic arthritis and systemic lupus erythematosus [33]. In this context, a possible relationship between RTT and autoimmunity was demonstrated by an early study showing an increase in serum levels of brain-directed autoantibodies specifically targeting the neurotrophin nerve growth factor, the loss of which could affect the development and maintenance of nerve cells in both the peripheral and CNS [57]. Furthermore, a later work found low concentrations of folate in the cerebrospinal fluid of RTT patients living in Northwest Europe that could be explained by the presence of serum autoantibodies against the folate receptor [58]. Finally, a study from Papini and coworkers [59] observed in RTT patients a significant increase in serum IgM autoantibodies directed against N-glycosylated targets which, by influencing protein N-glycosylation rate, could play a pathogenic role in the disorder.

Despite these results, other studies failed to demonstrate autoimmune processes in RTT, reporting no differences in the levels of anti-thyroid antibodies such as anti-thyroglobulin and anti-thyroid-stimulating hormone receptor autoantibodies [60]. Nevertheless, the fact that humoral immunity is aberrant in RTT is clearly beyond doubt. For example, RTT patients

showed high serum levels of IgA antibodies against gluten, gliadin and casein proteins, a phenomenon that could probably be linked to an increase in the uptake of some proteins from a damaged intestinal epithelium [61]. Confirming this hypothesis, alterations in intestinal morphology and microbiota have been demonstrated in *Mecp2*-null mice and RTT patients, respectively [46,62]. In particular, *Mecp2*-null mice showed abnormalities in the colon similar to those detected in colitis including colon and crypt shortening together with an aberrant expression of membrane proteins involved in electrolytes absorption on intestinal epithelial cells [62]. Moreover, a recent study suggested that the lower microbiota diversity with enrichment in pro-inflammatory species detected in the gut of RTT patients could correlate with a more susceptibility to developing intestinal inflammatory conditions able to alter the intestinal barrier permeability [46]. It's worth mentioning at this point that an altered gut microbiota in RTT could act not only locally but also systemically, influencing immunity in other tissues and organs including the brain. In this context, future studies in RTT animal models could shed a light on the role of the crosstalk between intestinal microbiota and immune function on the gut–brain axis.

Conclusions

It is evident that immune/inflammatory dysfunctions in RTT are not only clinical manifestations and alterations of their parameters nor biomarkers but rather can contribute to the pathophysiology of this syndrome. From the studies described in this review, it is clear that *MECP2* mutation, resulting in the impairment of a myriad of signaling pathways and cellular processes, affects the immune system and, *via* aberrant immune and non-immune cells, triggers atypical phenomena of humoral and cell-mediated immunity in different tissues and organs with

both local and systemic manifestations. Therefore, it is plausible that alterations of the immune system, starting from critical prenatal and postnatal developmental windows, could participate in the onset of the clinical symptoms of RTT and, then, in its deteriorating course. Within this context, the imbalance of redox homeostasis, a phenomenon widely documented in RTT, could exacerbate the derangement of immune/inflammatory processes, fueling the production of proinflammatory mediators. The establishment of the crosstalk between a persistent low-grade inflammatory response and oxidative stress leads to a vicious circle of chronic disturbance known as oxinflammation that has been shown to be a pathophysiological mechanism involved in this pathology [5,5,38,40].

A future challenge in the treatment of RTT, for which there is currently no cure, could be the development and identification of new therapeutic agents able to modulate the compromised immune response and, therefore, preventing the interplay of oxidative and inflammatory pathways. In this regard, some interesting results come from some studies evaluating the effects of omega-3 polyunsaturated fatty acids (ω-3 PUFAs) supplementation on RTT immune function. Dietary supplementation with ω-3 PUFAs for 12 months in a total of 24 RTT patients was able to partially rescue the altered plasma proteome related to APR (see previous section) [63]. In an another study, a beneficial modulatory effects of ω-3 PUFAs on the altered profile of plasma cytokines in RTT was observed associated with improved redox homeostasis and inflammatory status [51]. The beneficial impact of ω-3 PUFAs dietary intake is attributed mainly to their bioactive metabolites such as resolvins and protectins generated *in vivo* by cyclooxygenases, lipoxygenases, and cytochrome P450 monooxygenases. In this context, the decreased serum levels of HODEs detected by our group in ω-3 PUFAs-supplemented RTT patients respect to the not supplemented group could indicate a shift from the production of ω-6 metabolites toward

competitive and proresolving lipid mediators derived by ω-3 PUFAs [38]. Future investigations could help to better clarify the molecular mechanisms involved in the action of ω-3 PUFAs in RTT, supporting their use in this rare disorder that still do not have any effective treatment options.

Figure legends

Figure 1. Proposed mechanisms of dysregulated NF-κB pathway in Rett syndrome. In *Mecp2*-null mice, loss of Mecp2 function is associated with upregulated expression of *Irak1*, which is involved in NF-κB activation [42]. Inhibition of increased activity of GSK-3β in *Mecp2*-null mice can attenuate NF-κB activation [49]. Increased nuclear translocation of NF-κB can indirectly influence the expression of inflammasome components in primary dermal fibroblasts of RTT patients [40]. Dashed lines indicate indirect actions, while solid lines indicate direct actions. IRAK1, interleukin 1 receptor associated kinase 1; TLR, toll-like receptor; IL, interleukin; IL-1R, interleukin-1 receptor; MeCP2, methyl-CpG binding protein 2; GSK-3β, glycogen synthase kinase-3 beta; TNF, tumor necrosis factor.

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Table 1. Cellular specific inflammatory responses in RTT.

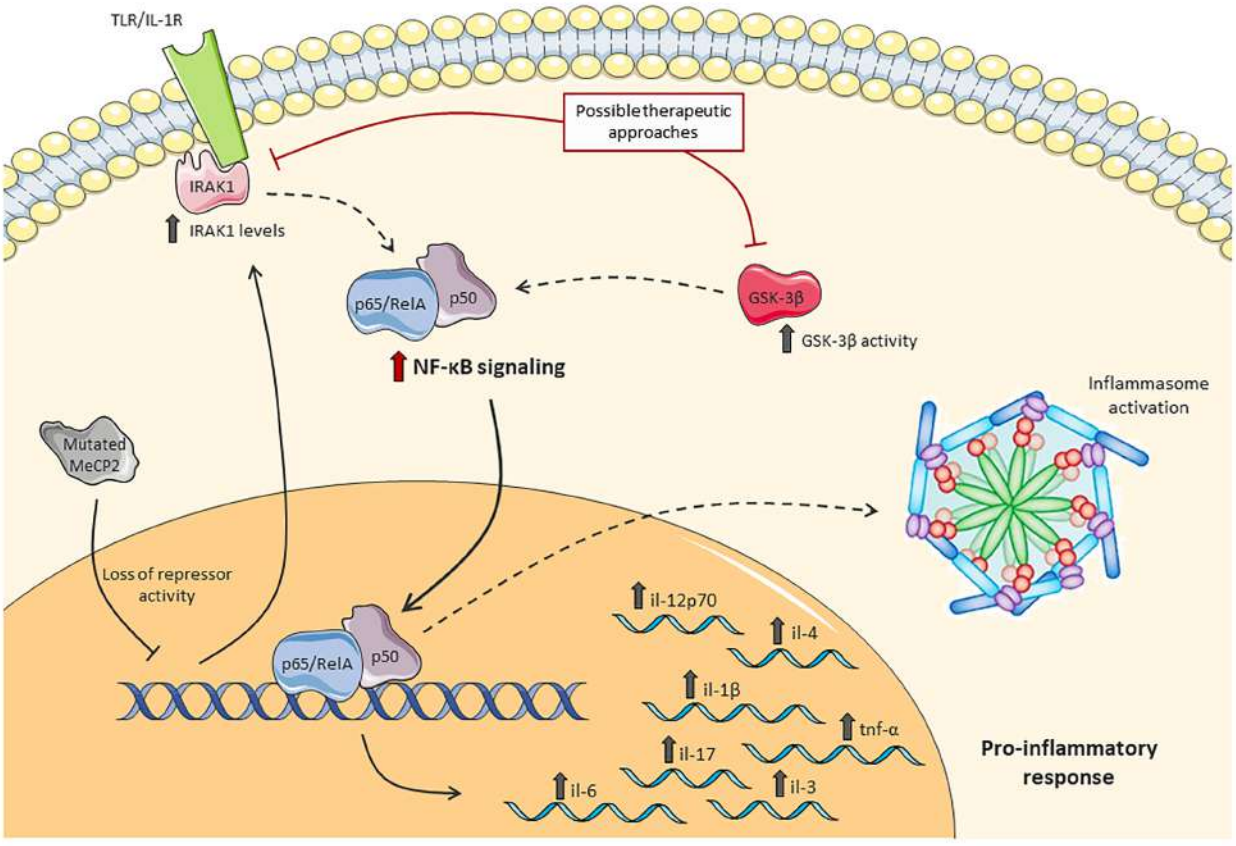
	In vitro and *in vivo* models/sources	Cytokine/chemokine/inflammatory protein expression	Cellular/tissue effects	References
Microglia	Mecp2-null mice	↑ glutamate release		[9]
		↑ tnf-α mRNA		[14]
		Dysregulated expression of genes related to innate immunity		[15]
		↑ TNF-α release		[16]
		↑ CXCL1 release		[16]
		↑ IL-10 release		[16]
		↓ IL-1β release		[16]
		No changes in IL-6 release		[16]
		No changes in INF-γ release		[16]
		No changes in IL-12p70 release		[16]
	Mecp2-null zebrafish		No changes	[27]
Peripheral immune cells	Mecp2-null mice		Loss of resident monocytes	[14]
			Loss of peripheral macrophages with disease progression	[14]
	Mecp2-null zebrafish	↑ C reactive protein mRNA	No changes in macrophages;	[27]
		↑ il-1b mRNA	↑ neutrophil infiltration	[27]
		↑ il-10 mRNA		[27]
		↓ tnf-α mRNA		[27]
	Human PBMC		No changes in lymphocytes	[28]
			↓ $CD8^+$ suppressor-cytotoxic T cells	[29]
			↓ $CD57^+$ cytotoxic natural killer cells	[29]
		↑ NF-κB signaling		[34,35]
		↑ glutamate release		[34]
		↑ tnf-α mRNA		[35]
		↑ il-6 mRNA		[35]
		↑ il-3 mRNA		[35]
			Abnormal cellular morphology with mitochondrial ultrastructural alterations	[36]
		↑ il-8 mRNA		[36]
		↑ il-13 mRNA		[36]
		↑ il-15 mRNA		[36]
		↓ il-9 mRNA		[36]
		↑ alox15 mRNA		[38]
Non-immune cells	Human fibroblasts	↑ activation of NLRP3/ASC inflammasome		[40]
		↑ NF-κB signaling		[40]
	Gut microbiota of patients		↓ microbiota diversity	[46]
			↑ pro-inflammatory microbial species	[46]

TNF, tumor necrosis factor; CXCL1, C-X-C motif chemokine ligand 1; IL, interleukin; IFN, interferon; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; alox15, arachidonate 15-Lipoxygenase; NLRP3, NOD-, LRR- and pyrin domain-containing protein 3; ASC, apoptosis-associated speck-like protein containing a CARD

Table 2. Evidence of dysregulated immune markers in RTT biological fluids.

Cytokine/chemokine/ inflammatory protein expression	Peripheral biological fluids	References
Altered APR proteins	Human plasma, plasma from Mecp2-308 mice, plasma from Mecp2-null mice	[52, 53, 54]
↑ 13-HODE	Human serum	[38]
↑ GM-CSF	Human saliva	[50]
↑ IgA against gluten, gliadin and casein	Human serum	[61]
↑ IgM AAB against N(Glc)	Human serum	[59]
↑ brain-directed AAB against NGF	Human serum	[57]
↑ IL-1β	Human saliva	[50]
↑ IL-4	Human plasma	[51]
↑ IL-5	Human plasma	[51]
↑ IL-6	Human plasma, human saliva	[50, 51]
↑ IL-8	Human serum, human plasma, human saliva	[36, 50, 51]
↑ IL-9	Human serum	[36]
↑ IL-10	Human saliva	[50]
↓ IL-12p70	Human plasma	[51]
↑ IL-13	Human serum	[36]
↑ IL-17A	Human plasma	[51]
↓ IL-22	Human plasma	[51]
↑ IL-33	Human plasma	[51]
↑ IL-37	Human plasma	[51]
↑ soluble IL-2 receptor	Human serum	[29]
↑ TNF-α	Human plasma, human saliva	[50, 51]
↑ VEGF	Human saliva	[50]
↓ IFN- γ	Human plasma	[51]
↓ IP-10	Human plasma	[51]
↓ I-TAC	Human plasma	[51]
↓ RANTES	Human plasma	[51]
↓ TGF-β1	Human plasma	[51]

APR, acute-phase response proteins; HODE, hydroxyoctadecadienoic acid; GM-CSF, granulocyte-macrophage colony-stimulating factor; AAB, autoantibodies; N(Glc), N-glucosylation; IL, interleukin; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor; IFN, interferon; IP-10, interferon gamma-induced protein 10 (alias CXCL10); I-TAC, Interferon-inducible T-cell alpha chemoattractant (alias CXCL11); RANTES, regulated on activation, normal T cell expressed and secreted (alias CCL5); TGF, transforming growth factor.


TLR/IL-1R
Possible therapeutic approaches
IRAK1
IRAK1 levels
p65/RelA
p50
GSK-3β
GSK-3β activity
NF-κB signaling
Inflammasome activation
Mutated MeCP2
Loss of repressor activity
p65/RelA
p50
il-12p70
il-4
il-1β
tnf-α
il-17
il-6
il-3
Pro-inflammatory response

Highlights

- Loss of MeCP2 function has profound impact on RTT immune system.
- Dysfunctional immune responses can participate in onset and progression of RTT.
- Targeting aberrant immune function as a therapeutic strategy for RTT management.