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Overexpression of methyl-CpG-binding protein 2 and autoimmunity: Evidence from *MECP2* duplication syndrome, lupus, *MECP2* transgenic and *Mecp2* deficient mice

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Abstract

Methyl-CpG-binding protein 2 (MeCP2) is a key transcriptional regulator that can induce either silencing or activation of target genes. Genetic polymorphisms in the *MECP2*/*IRAK1* locus have been associated with increased susceptibility to multiple autoimmune diseases such as lupus, primary Sjogren's syndrome, and more recently rheumatoid arthritis. Data from our group suggest that the disease risk variant in this locus is associated with gain of MeCP2 function. Recent findings indicate that *MECP2* duplication in human results in defective T helper cell type 1 (T_H1) response and IFN-γ production. Herein, we discuss the data from children with *MECP2* duplication, human lupus, and from the human *MECP2* transgenic and *Mecp2* deficient mice to support a link between *MECP2* overexpression and autoimmunity. We also provide findings from an *Mecp2* deficient mouse that independently support a role for MeCP2 in the immune response and specifically in IFN-γ expression.

Keywords

MeCP2; *MECP2* duplication; lupus; autoimmunity; T_H1 response

Abnormal DNA methylation, particularly in T cells, plays an important role in the pathogenesis of systemic lupus erythematosus, a multi-system autoimmune disease characterized by the production of autoantibodies to nuclear antigens (1, 2). We have previously identified, replicated, and confirmed that genetic variants within *MECP2* increase the susceptibility to lupus (3-5). While *MECP2* expression was shown to be reduced in total PBMCs in the presence of the lupus risk variant (6), recent data suggest that the lupus-associated variant in *MECP2* is associated with increased *MECP2* isoform 2 mRNA expression in stimulated but not unstimulated T cells (7), and that the human *MECP2* transgenic mice develop antinuclear antibodies (7). The lupus risk variant in *MECP2* is also associated with significant DNA hypomethylation in multiple interferon-regulated genes in stimulated T cells (7), supporting our earlier studies that demonstrated increased expression of interferon-regulated genes in lymphoblastoid cell lines in lupus patients with the *MECP2* risk variant (4).

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Competing interests: None.

A recent study by Yang and colleagues identified a defect in T_H1 response and the production of IFN-γ is children with a neurodevelopmental disease caused by *MECP2* duplication (8). These data were also supported by findings from a transgenic mouse that overexpresses human *MECP2* (8). We have studied female mice homozygous for a targeted truncating mutation in *Mecp2* (B6.129S-*Mecp2*^{tm1Hzo}/J) crossed onto C57BL/6 background. These mice were developed by inserting a stop codon after codon 308 in *Mecp2* (9). Splenic mouse T cells were isolated at 12 weeks of age by magnetic beads via indirect labeling (Milteny) and T cell stimulation was achieved using PMA and ionomycin. RNA was extracted from stimulated T cells from *Mecp2* deficient and C57BL/6 age- and sex-matched control mice. Gene expression experiments in five mice from each strain were performed using Illumina expression arrays. We compared mRNA expression profiles between T cells from *Mecp2* deficient mice and C57BL/6 controls and detected a number of upregulated and downregulated transcripts (Table 1). Importantly, stimulated T cells from *Mecp2* deficient mice showed increased expression of IFN-γ mRNA transcript. This is consistent with and supports the findings of Yang et al that *MECP2* duplication is associated with reduced IFN-γ response (8). In addition, we observed significant overexpression of chemokine (C-C motif) ligand 5 (*Ccl5*) and colony stimulating factor 2 (*Csf2*) in *Mecp2* deficient mice. CCL5 (also known as RANTES) is involved in T cell differentiation and particularly the activation and function of T_H1 immune response (10). CCL5 deficiency results in increased susceptibility to infections, notably pneumonia (11), which is observed in increased frequency in children with *MECP2* duplication (8). Indeed, CCL5 blockade impairs IFN-γ production and enhances the expression of the immunosuppressive cytokine IL-10 by T cells (10). CCL5 also plays an important role in suppressing viral infections (12). Csf2 (GM-CSF) plays an important role in granulocytes and macrophage differentiation and function. Interestingly, the expression of IFN-γ and Csf2 is regulated by DNA methylation (13, 14), and MeCP2 is known to mediate DNA methylation-induced transcriptional regulation and to recruit DNA methyltransferase 1 for the maintenance of methylation during DNA synthesis (15).

Although *MECP2* and the adjacent gene *IRAK1* are in strong linkage disequilibrium, we did not detect any change in *IRAK1* expression with the lupus-associated variant in this locus in stimulated T cells (7). It was previously suspected that the susceptibility to infection in patients with *MECP2* duplication may be related to *IRAK1*, which is also frequently duplicated in *MECP2* duplication syndrome. Indeed, *IRAK1* plays an important role in both the innate and the adaptive immune response. The recent data by Yang and colleague, however, shed light on this question and indicate that the immune defects observed in these patients are explained by overexpression of *MECP2* and are independent of *IRAK1* duplication (8).

Lupus is a disease characterized by B cell activation and antinuclear antibody production, suggesting an important role for T_H2 T cell differentiation in the disease pathogenesis. Upon stimulation, the percentage of IFN-γ-expressing CD4+ T cells in PBMCs is significantly lower in lupus patients compared to healthy controls (16), similar to children with *MECP2* duplication (8). Nonetheless, T_H1 effector cells and IFN-γ production within target tissue play an important role in organ damage in lupus, particularly in the onset and progression of

glomerulonephritis (17). Indeed, autoantibodies against ribosomal P, which are associated with lupus nephritis, have been shown to induce IFN-γ production (18).

Yang et al reported an attenuated IgG response after boost vaccination in some children with *MECP2* duplication, consistent with previous reports, and also consistent with recent findings in lupus patients that showed attenuated response to influenza vaccination in a subset of lupus patients (19). The presence of immature neutrophils, and IgA deficiency are other immunological features common to both *MECP2* duplication and lupus (20, 21). There is a significant higher incidence of a variety of autoimmune diseases in patients with IgA deficiency and shared genetic susceptibility loci between IgA deficiency and autoimmunity (21).

Total circulating B cells are often reduced in lupus patients, as has been also reported in a subset of patients with *MECP2* duplication (8, 22). However, while lupus patients often demonstrate an expanded memory B cell subset, memory B cells were reduced in patients with *MECP2* duplication (8, 22). Other notable differences include increased naïve CD4+ T cells and reduced antigen-experienced CD4+ T cells in patients with *MECP2* duplication, which is in contrast to lupus. Lupus patients are characterized by the presence of autoreactive T cells and loss of T cells tolerance, resulting in expansion of the antigen-experienced CD4+ T cell compartment, accumulation of terminally differentiated memory T cells, and a decrease of naïve CD4+ T cells over the course of the disease (23, 24).

In summary, we provide evidence to further support a role for MeCP2 in T_H1 differentiation. We discuss data that suggest that *MECP2* duplication might be associated with increased susceptibility to autoimmunity and highlight some similarities in the immune dysfunction observed in patients with *MECP2* duplication syndrome and lupus. Future studies to explore the occurrence and spectrum of autoimmunity in *MECP2* overexpression and to understand the functional role of *MECP2* variants and overexpression upon lupus susceptibility are warranted.

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Table 1

Differentially expressed transcripts (≥1.5-fold) in stimulated splenic T cells between *Mecp2* deficient mice and wild-type controls.

		Geometric mean			
Gene	**Transcript**	***Mecp2*-deficient**	**Wild-type**	**Ratio**	**Parametric *P* value**
4833405L16Rik	NM_177197	212.19	123.52	1.72	6.50E-05
A130020K16Rik	AK037473	130.52	191.96	0.68	4.11E-05
Ccl5	NM_013653	146.96	95.99	1.53	1.60E-06
Csf2	NM_009969	93.48	59.15	1.58	9.70E-06
D6Mit97	-	45.40	29.03	1.56	5.27E-05
Fundc2	NM_026126	29.53	57.35	0.52	< 1E-07
Ifng	NM_008337	1795.11	902.63	1.99	3.70E-06
Igk-C	XM_132633	374.80	125.48	2.99	1.27E-05
Igk-C	XM_132633	599.98	205.59	2.92	5.12E-05
Igk-V1	XM_355776	76.94	42.08	1.83	1.61E-04
Lgals3	NM_010705	146.01	80.51	1.81	3.17E-05
LOC207685	XM_358779	55.81	32.57	1.71	1.92E-05
LOC232065	XM_132611	62.99	35.41	1.78	8.46E-05
LOC243423	XM_144770	48.93	29.04	1.68	9.52E-05
LOC381774	XM_355772	66.07	27.26	2.42	1.04E-04
LOC384419	XM_357637	43.25	28.74	1.50	2.37E-04
Mecp2	NM_010788	26.85	60.34	0.44	< 1E-07