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REVIEW

Translational utility of experimental autoimmune encephalomyelitis: recent developments

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Abstract: Multiple sclerosis (MS) is a complex autoimmune condition with firmly established genetic and environmental components. Genome-wide association studies (GWAS) have revealed a large number of genetic polymorphisms in the vicinity of, and within, genes that associate to disease. However, the significance of these single-nucleotide polymorphisms in disease and possible mechanisms of action remain, with a few exceptions, to be established. While the animal model for MS, experimental autoimmune encephalomyelitis (EAE), has been instrumental in understanding immunity in general and mechanisms of MS disease in particular, much of the translational information gathered from the model in terms of treatment development (glatiramer acetate and natalizumab) has been extensively summarized. In this review, we would thus like to cover the work done in EAE from a GWAS perspective, highlighting the research that has addressed the role of different GWAS genes and their pathways in EAE pathogenesis. Understanding the contribution of these pathways to disease might allow for the stratification of disease subphenotypes in patients and in turn open the possibility for new and individualized treatment approaches in the future.

Keywords: autoimmunity, multiple sclerosis, risk genes, EAE, knockouts, pathways

Introduction

Multiple sclerosis (MS) is a debilitating chronic inflammatory disease of the central nervous system (CNS) characterized by autoimmune destruction of myelin and subsequent loss of neurons. The cause of disease remains unknown, but epidemiological studies have clearly established genetic factors in MS etiology.[1–3] The first genetic risk factor has been described in early 1970s and mapped to the human leukocyte antigen (HLA) complex,[4,5] which encodes numerous genes with immune functions. More recently, this strongest genetic influence was refined to HLA-DRB1*1501 that confers threefold increased risk to develop MS[5,6] and encodes molecules involved in the presentation of antigens to T-cells. With the advent of genome-wide association studies (GWAS) and large international efforts to gather sufficiently powered cohorts, more than 100 non-HLA variants have been identified to predispose for MS[5,7–9] together with multiple variants and alleles within the HLA locus itself.[5,10] The identified MS risk variants collectively indicate genetically regulated immune functions that control disease susceptibility and they have set the stage for molecular characterization of mechanisms causing MS. Nevertheless, apart from few examples,[11–13] interpretation of the causal variants is limited and their mechanisms are still largely unknown.

Experimental autoimmune encephalomyelitis (EAE) is an animal model widely used to study mechanisms of inflammation in the CNS.[14] EAE can be induced in a

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variety of species either by active immunization with CNS antigens in adjuvant or by passive transfer of CNS-specific T-cells. Although no single EAE model can recapitulate the complexity of MS, EAE has been successfully used to study mechanisms of relevance for MS and translate them into therapeutic interventions.[15] The knowledge regarding the role of MS risk genes in vivo largely comes from EAE models, primarily owing to the possibility of gene targeting in mice. In this review, we summarize the current knowledge of the mechanisms of well-established MS risk genes[5,9] (Table 1) and discuss more thoroughly those for which more abundant EAE data are available. Where possible, the genes were grouped, in the text, according to the pathways or cellular functions they fulfill. Additionally, we address in Table 1 whether data on the functional consequence of the human single-nucleotide polymorphism (SNP) is available as well as whether any clinical trials targeting these molecules are underway.

APC function and costimulation

CD86

CD86 (B7.2), together with the structurally homologous CD80 (B7.1), are important costimulatory molecules that regulate the crosstalk between antigen presenting cells (APCs) and T-cells, delivering "signal 2" for T-cell activation. They are upregulated upon APC activation in specific and distinct temporal patterns and bind to both CD28 and CTLA-4 on T-lymphocytes, leading either to enhancement or inhibition of T-cell function, respectively.[16] In EAE, because of greatly overlapping and compensatory effects between CD86 and CD80,[17] double-knock-out (KO) animals (*Cd80/Cd86*$^{-/-}$) have been used to address the role of the receptors in disease development. *Cd80/Cd86*$^{-/-}$ animals immunized with myelin oligodendrocyte glycoprotein (MOG) show an impaired induction phase of EAE. However, transfer of MOG-specific wild-type (WT) T-cells into *Cd80/Cd86*$^{-/-}$ mice, in which any defects in priming are overridden, also leads to less severe disease with eventual complete remission, while WT recipients present with chronic progressive disease. These experiments point to a role of this costimulatory pathway in the priming of the response as well as in the effectors phase.[17] Administration of antibodies against CD28[18] or CTLA-4Ig fusion protein,[19] which both block the pathway, lead to a reduction of disease severity during the effector phase both when given systemically as well as intrathecally, for the latter.[20] Abrogation of the pathway attenuates the immune response at least partly due to death in situ of encephalitogenic T-cells.[21] Because of the widespread expression of CD80/CD86 in the CNS during EAE, it is difficult to discriminate whether local APCs (microglia, dendric cells [DCs]) or infiltrating cells (monocytes) are responsible for the costimulatory events that sustain inflammation. However, even though microglia in preactive and remyelinating MS lesions do express CD86,[22] the expression levels are much lower than on classical DCs or monocyte-derived DCs as judged from EAE experiments,[23,24] suggesting the latter as crucial cells in the aforementioned restimulation events.

TRAF3

TRAF3 is part of the TNF receptor-associated factor family and is an adapter protein. It is a potent inhibitor of different signaling pathways including CD154 (CD40L), toll-like receptors (TLR), and IL-17R.[25–27] TRAF3 can negatively regulate IL-17 signaling; *Traf3* transgenic mice, which express significantly higher levels of TRAF3, have reduced EAE score and later onset. Accordingly, *Traf3* knock down mice have exacerbated disease.[27] Peli-1, which promotes degradation of TRAF3, is abundantly expressed in microglia. *Peli-1*-deficient mice, in which levels of TRAF3 remain high, have reduced EAE as well, in spite of normal peripheral T-cell activation.[28] This reduction in EAE is due to an impaired response of microglia to inflammatory stimuli. In summary, TRAF3 is a negative regulator of signaling pathway in multiple cell types, affecting both peripheral as well as CNS immune activation stages.

TNFSF14

TNFSF14 encodes for LIGHT, a newly identified costimulatory ligand expressed on DCs, T-cells, natural killer (NK) cells, monocytes, and granulocytes.[29] LIGHT binds to three receptors, DcR3, herpes virus entry mediator (HVEM), and lymphotoxin b receptor (LTbR), and drives increased T-cell proliferation and Th1 cytokine expression.

LIGHT has been shown, in one study, to be an important factor for the recovery phase of EAE.[29] *Light*-deficient C57BL/6 mice develop a more severe EAE after immunization with $MOG_{35–55}$ peptide compared to WT mice. While KO mice have more activated microglia/macrophages in the CNS, $CD4^+$ T-cells from lymph nodes draining the immunization site exhibit lower IFNγ and IL-17 production. The paradoxical effect of disease exacerbation in *Light*-deficient mice in spite of lower Th1/Th17 effector functions is explained by adoptive transfer of encephalitogenic T-cells into KO mice, showing that LIGHT is not essential for disease induction but plays a major role in limiting disease progression and tissue damage by controlling activated macrophages/microglia in the CNS during inflammation.[29]

Table 1 MS risk genes for which EAE data are available

Chromosome: SNP	CG name	CG function	Cells expressing CG	CG role in EAE	Clinical trials targeting CG[a]	Functional consequence of the SNP
Chr1: rs11581062a	*VCAM1*	Endothelial ligand for VLA-4 (integrin α4β1)	Endothelium	VCAM1 is the ligand for α4β1 integrin (VLA-4), important for migration of activated lymphocytes into the CNS	Natalizumab (Tysabri®, Biogen Idec, Weston, MA, USA), which blocks the α4β1 integrin, is currently in use as a therapeutic drug in MS[141]	–
Chr2: rs9967792	*STAT4*	Signal transducer; promotes Th1 differentiation in $CD4^+$ T-cells	Primarily $CD4^+$ T-cells	KO mice are completely resistant to EAE[64]	–	–
Chr3: rs2371108	*EOMES*	Transcription factor; regulates differentiation of $CD8^+$ T-cells	Widespread during development	*T-bet*/*Eomes* double-KO leads to a Tc17 phenotype in $CD8^+$ T-cells[142]	–	–
Chr3: rs4679081	*CCR4* (CD194)	Chemokine receptor; binds CCL2, CCL4, CCL5, CCL17, and CCL22	Widespread in the immune system; high expression in Th2 and Tregs cells	KO mice develop less severe EAE, with later onset and lower score[143,144]	Mogamulizumab (a humanized antibody against CCR4) is currently in use against lymphoma; Phase I trial against asthma terminated NCT01514981	–
Chr 3: rs2028597/ rs12487066	*CBLB*	E3 ubiquitin-protein ligase, regulates immune receptor signaling	Widespread, with prominence in immune tissue	KO mice have increased incidence and severity[62]	–	The risk allele at rs12487066 confers lower CBLB expression in $CD4^+$ T-cells and defines carriers as worse IFNβ responders[145]
Chr3: rs2255214/ rs9282641	*CD86*	Costimulatory molecule	APCs	*Cd80*/*Cd86*$^{-/-}$ double KOs are fully protected due to impaired priming[17]	Abatacept (a fusion protein of IgG1-Fc with the extracellular domain of CTLA-4 that blocks CD86) is currently in use for RA. Phase II trial for MS completed NCT01116427	–
Chr3: rs1014486	*IL12A* (p35)	Subunit (p35) of IL-12; promotes Th1 immune responses	APCs	KO mice have unchanged or slightly worse disease[72,73]	–	–
Chr4: rs7665090/ rs228614	*NFKB1*	Transcription factor; controls many processes such as inflammation, immunity, cell differentiation, cell growth, tumorigenesis, and apoptosis	Widespread	KO mice show attenuated EAE incidence, clinical score and CNS inflammation[115]	Agents that target the pathway are under trial; Curcumin (Phase II) for MS NCT01514370 Bortezomib in trial for RA, SLE, and MG 2013-005362-19	The disease predisposing variant at rs228614 positively correlates with a reduction in spinal cord area[146]

(*Continued*)

Table 1 (*Continued*)

Chromosome: SNP	CG name	CG function	Cells expressing CG	CG role in EAE	Clinical trials targeting CG[a]	Functional consequence of the SNP
Chr5: rs6881706	*IL7R* (CD127)	Subunit of the receptors for IL-7 and TSLP	Immune cells of the lymphoid lineage	KO mice, administration of recombinant IL-7 or of neutralizing antibodies to IL-7 show that IL-7 signaling is necessary for disease[77–79]	Phase Ib trial of anti-IL-7RA (RN168) for RRMS NCT02045732	–
Chr5: rs6880778	*PTGER4* (EP4)	Receptor for prostaglandin E2	Widespread	KO mice have reduced severity of disease; EP4 antagonists reduce disease if given before onset but EP4 agonists reduce disease if given after onset[124,125]	Phase II trial of EP4 agonist for ulcerative colitis (terminated) NCT00296556	–
Chr5: rs756699	*TCF7* (TCF-1)	Transcription factor; important for the differentiation of T-cells	Hematopoietic cells	KO mice as well as wild-type mice adoptively transferred with KO T-cells have more severe disease[147]	–	–
Chr5: rs2546890	*IL12B* (p40)	Common subunit (p40) of IL-12 and IL-23. Also exists as a monomer or homodimer	APCs	KO mice have less severe disease;[72,75] neutralizing antibodies to IL-12p40 homodimer results in less severe disease; recombinant homodimer administration results in more severe disease[76]	Phase II trials of anti-IL-12p40 for RRMS NCT00207727 NCT00086671	–
Chr6: rs17066096	*IL22RA2* (IL-22BP)	Soluble antagonist molecule for IL-22	APCs	KO mice have less severe disease during chronic phase[148]	–	–
Chr6: rs67297943	*TNFAIP3* (A20)	Ubiquitin editing enzyme; negatively regulates NF-κB activity	Widespread	Systemic lentiviral knockdown as well as conditional KO in either neuroectodermal cells or in astrocytes results in more severe disease[149,150]	–	
Chr7: rs1843938	*CARD11* (CARMA1)	Membrane associated scaffold protein; contributes to activation of NF-κB	Hematopoietic cells	KO mice are completely resistant to EAE[119]	–	–
Chr7: rs706015	*SKAP2* (SKAP-HOM)	Cytosolic adaptor molecule; involved in leukocyte adhesion	Ubiquitous	KO mice have less severe EAE[151]	–	–
Chr10: rs2104286	*IL2RA* (CD25)	Subunit of the high affinity receptor for IL-2	High expression in activated T-cells and Tregs	Administration of IL-2 results in less severe disease and administration of soluble CD25 results in more severe disease[84,85,87]	Anti-CD25 (daclizumab) has been tested in several Phase III clinical trials and is a potential new treatment for MS. A Phase II trial of low dose IL-2 for RRMS is planned NCT02424396	–

Chr11: rs523604	*CXCR5* (CD185)	Chemokine receptor; binds CXCL13	High expression in B-cells and TFH cells; transient expression in activated T-cells	KO not addressed; blocking of the ligand CXCL13 ameliorates EAE[152,153]	–	–
Chr12: rs1800693	*TNFRSF1A*	Major receptor for TNF	Widespread	KO mice are resistant to EAE;[132] TNFR1-selective antagonist and antibody-mediated inhibitor ameliorates EAE[130,131]	Blocking TNF induces onset or excarbation of disease[126–128]	TNFRSF1A SNP rs1800693 directs the expression of a soluble receptor variant, which mirrors the blocking TNF treatment;[12] the same SNP leads to higher response to TNF stimulation[129]
Chr14: rs4903324	*BATF*	Transcription factor; regulates Th17 differentiation	Hematopoietic cells	KO mice are resistant to EAE;[154] *Egr-2* deficient mice (a negative regulator of BATF) have exacerbated EAE[155]	–	–
Chr14: rs12148050	*TRAF3*	Adapter protein; negative regulator of several immune pathways	Hematopoietic cells	*Traf3* tg mice have reduced EAE;[27] *Traf3* knock down mice have increased EAE score;[27] *Peli-1* deficient mice have reduced EAE (Peli-1 promotes TRAF3 degradation)[28]	–	–
Chr16: rs35929052	*IRF8*	Transcription factor; important for myeloid cell differentiation	B-cells, DCs, and macrophages	KO mice are resistant to EAE; IRF8 Lys-M-cre mice have less severe EAE[156]	–	IRF8 rs17445836G risk variant is associated with low-type I IFN levels[157]
Chr17: rs4796791	*STAT3*	Transcription factor; promotes Th17 cell differentiation	Widespread	Conditional KO mice in the T-cell compartment are resistant in EAE[91,92]	Several STAT3 blocking agents are in clinical trials for different cancers	–
Chr18: rs7238078	*MALT1*	Caspase-like cysteine protease; participates in the activation of NF-κB together with CARMA1 and BCL10	Hematopoietic cells	KO mice are resistant to EAE;[120,121] pharmacological inhibition ameliorates EAE[123]	–	–
Chr19: rs1077667	*TNFSF14* (LIGHT)	Costimulatory ligand; controls APC activation	DCs, T-cells, NK cells, monocytes, and granulocytes	KO mice develop more severe EAE[29]	–	–
Chr19: rs34536443	*TYK2*	Transcription factor; promotes Th1 cell differentiation	Widespread	KO mice are resistant in EAE;[89] Mice that carry a G→A missense mutation in *TYK2* are resistant in EAE[90]	Phase I trial of a selective inhibitor (PF-06263276) NCT01981681	The protective allele at rs34536443 favors a Th2 cytokine secretion profile from in vitro stimulated patient-derived T-cells[11]

(*Continued*)

Table 1 (*Continued*)

Chromosome: SNP	CG name	CG function	Cells expressing CG	CG role in EAE	Clinical trials targeting CG[a]	Functional consequence of the SNP
Chr19: rs11554159	*IFI30* (GILT)	Lysosomal thiol reductase; involved in MHC class II Ag presentation and MHC class I cross-presentation	APCs	KO mice are resistant upon MOG_{35-55} immunization but susceptible upon MOG protein immunization[31]	–	–
Chr20: rs4810485	*CD40*	Costimulatory molecule; mediates B-cell activation and Ig production as well as DC activation and survival	B-cells, macrophages, DCs, monocytes, astrocytes, endothelial cells	KO mice are resistant in EAE;[36,37] Treatment with anti-CD40 or anti-CD40L antibodies inhibits EAE[35,36,42–51,53–58,158]	Several ongoing trials with agonists and antagonists of the pathway; Phase IIa trial of the anti-CD40 monoclonal antibody ASKP1240 in renal transplantation is ongoing NCT01780844 A trial with the anti-CD40L antibody BG9588 to treat lupus nephritis has been completed NCT00001789	–
Chr20: rs6062314	*TNFRSF6B* (DcR3)	Immunomodulator; neutralizes the effect of TNF family members	Lymphoid and myeloid cells	Intrathecal administration of DcR3 reduced EAE[159]	–	–
Chr20: rs2248359	*CYP24A1* (1,25-hydroxyvitamin D-1 alpha hydroxylase)	Hydroxylates and inactivates 1,25-dihydroxyvitamin D_3	Primarily kidneys; in the immune system expressed in macrophages, DCs, $CD4^+$ T-cells, and B-cells	Lovastatin ameliorates EAE possibly through inhibition of *Cyp24a1* expression in Th1/Th17 cells[137]	–	–
Chr21: rs2283792	*MAPK1* (ERK2, p38)	Signal transducer; induces cell growth, differentiation and development	Widespread	IFN-β-1a inhibits EAE possibly through upregulation of MAPK1 and 2 phosphorylation[160]	A large number of trials under way with different p38 inhibitors in RA, asthma, and other conditions	–

Notes: [a]Data on clinical trials was gathered from https://clinicaltrials.gov/ and https://www.clinicaltrialsregister.eu/.

Abbreviations: MS, multiple sclerosis; SNP, single-nucleotide polymorphism; EAE, experimental autoimmune encephalomyelitis; CG, candidate gene; CNS, central nervous system; APC, antigen presenting cell; TSLP, thymic stromal lymphopoietin; MOG, myelin oligodendrocyte glycoprotein; RRMS, relapsing-remitting MS; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; MG: myasthenia gravis; KO, knock-out; TFH: follicular helper T.

IFI30

IFI30 encodes for GILT, an enzyme that functions in MHC class II-restricted antigen processing and MHC class I-restricted cross-presentation.[30] GILT may alter the character of immune responses and affect central tolerance.

Gilt KO mice are resistant to EAE induced with $MOG_{35–55}$ as they fail to induce a proper antigen-specific $CD4^+$ T-cell response.[31] KO mice immunized with whole MOG protein are, however, susceptible to EAE. However, while T-cells from WT mice respond to $MOG_{35–55}$, T-cells from KO animals proliferate against a different array of peptides. Furthermore, *Gilt* KO mice develop a disease characterized by antibody-mediated effects, indicating a switch in the pathogenic mechanism due to peptide repertoire change. The role of GILT as an endosomal reductant has also been shown by Burrows et al[32] by using RTL550-CYS-MOG, a recombinant TCR (T-cell receptor) ligand (RTL) bearing cysteine-tethered antigenic peptides, to treat EAE. RTL550-CYS-MOG inhibits EAE in WT mice but not in *Gilt* KO mice, since RTLs must be endocytosed and presented by MHC class II and since GILT is required to liberate these cysteine-tethered peptide ligands in late endocytic compartments.

CD40

CD40 is a costimulatory molecule on APCs. The interaction of CD40 with its ligand CD40L (CD154), expressed on activated T-cells, influences a variety of immune functions including B-cell activation and Ig production, and DC survival.[33]

It has been shown that CD40 and CD40L expression in inflammatory cells infiltrating the CNS of mice is significantly increased during acute EAE and relapses, and decreased during remission.[34] Furthermore, perivascular infiltrates of mononuclear cells have abundant expression of CD40 and CD40L in the CNS of marmoset monkeys with demyelinating EAE.[35]

Experiments using *Cd40* KO mice have shown the importance of CD40-CD40L pathway in EAE development and Th polarization.[36,37] *Cd40* KO mice are resistant to EAE development, fail to drive Th17 differentiation, and exhibit reduced IL-6 production by DCs. Furthermore, mice that receive $Cd40^{-/-}$ DC cells prior to EAE induction exhibit an impaired ability to prime a MOG-specific IL-17 response even though their ability to induce IFN-γ production is similar to mice injected with control DCs.[38] The administration of $Cd40^{-/-}$ DC loaded with MOG prior to standard MOG immunization also prevents the onset of EAE.[39] Treatment of mice with EAE with bone marrow-derived dendritic cells (BMDC) transduced with lentiviral vectors encoding CD40 shRNA results in significant decrease of EAE compared to mice treated with BMDCs transduced with control vectors.[40] EAE inhibition is even more profound when mice are injected with BMDCs cotransduced with shRNA to both CD40 and the IL-23 p19 subunit, leading to further dampening of the Th17 response. Ablation of signaling by deletion of the ligand, CD40L, using *Cd40l* KO mice that carry a myelin basic protein (MBP)-specific transgenic TCR also leads to EAE resistance and lack of CNS inflammation.[41]

Treatment with anti-CD40L monoclonal antibody (mAb) concomitantly to myelin antigen immunization completely prevents EAE development. When anti-CD40L mAb is administered after EAE onset and before peak of the disease, it significantly reduces EAE symptoms.[35,36,42] When anti-CD40L mAb is administered during EAE remission, it prevents further clinical relapses.[43] In addition, several reports have shown the short- and long-term inhibition of EAE in different EAE animal models using anti-CD40L treatments, suggesting that CD40-CD40L interactions may play a role in the ability of encephalitogenic T-cell to interact with APCs in the CNS and increase Th effector functions.[44–51] Treatment with a combination of anti-CD40L Ab and CTLA4Ig confers additive protection against EAE and is associated with complete absence of inflammatory cell infiltrates in the CNS.[52] These observations have been further corroborated by studies in marmoset monkeys and mice that show that antibodies that block CD40 inhibit EAE[53–57] and suppress magnetic resonance imaging-detectable inflammation and enlargement of brain lesions.[58] Finally, Ichikawa and Williams[59] have shown that activation of the CD40-CD40L pathway is sufficient to overcome tolerance against self-antigens.[59] In this study, myelin-reactive T-cells from tolerized donors are converted into pathogenic effector cells upon reactivation of specific lymph node cells with anti-CD40 agonists and are able to proliferate, secrete cytokines, and induce passive EAE in SJL mice.

TCR signaling

CBLB

CBLB is an E3 ubiquitin-protein ligase, which negatively regulates TCR, B-cell receptor (BCR), and FCεR1 signal transduction pathways, playing an important role in peripheral tolerance maintenance. In naïve T-cells, it inhibits VAV1 activation upon TCR engagement, but not other pathways such as Zap-70 and Lck, Ras/MAPK, PLC-γ1, or $Ca2^+$ mobilization. In this way, CBLB imposes a requirement for CD28 costimulation for proliferation and IL-2

production, heightening the activation threshold for T-cells.[60] An additional observation linking CBLB to tolerance induction is that CBLB expression in T-cells is controlled by CD28 and CTLA-4. CD28 costimulation induces CBLB ubiquitination and proteasomal degradation, while CTLA-4-B7 interaction induces *Cblb* expression.[60] Independent of the aforementioned mechanism, CBLB has also been shown to control the generation of peripheral inducible Treg cells in response to TGFβ signaling.[61] The control of tolerance at multiple levels is revealed in *Cblb*$^{-/-}$ mice immunized with MBP, which show a higher incidence and higher EAE score than their WT counterparts.[62] These animals also present T-cell abnormalities in lymph node trafficking patterns, with increased expression of $S1P_1$ on T-cells, which do however not impact their sensitivity to FTY720 (fingolimod) treatment.[63]

Cytokine signaling and Th phenotype

STAT4

STAT4 is a transcription factor essential for CD4$^+$ T-cell differentiation to the Th1 phenotype. CD4$^+$ T-cells respond to the cytokines IL-27 and IL-12 through STAT1 and STAT4 phosphorylation, respectively, leading to subsequent nuclear translocation, where they induce IFNγ production and expression of the master transcriptional regulator T-bet.

Both Th1 and Th17 T-cells can induce EAE and appear implicated in MS. However, while mice deficient in *IL-12*, *STAT1*, and *IFNγ* not only still develop EAE, but often present with exacerbated disease in the case of the latter; animals knocked out for *Stat4*[64] and *T-bet* are resistant to EAE.[65] Deletion of *T-bet* specifically on CD4$^+$ T-cells does not abrogate encephalitogenicity,[66] implying expression in other cells as essential, and leaves STAT4 as a major player in disease establishment. Additionally, the regulation of immunity by STAT4 goes beyond mere induction of gene transcription in that STAT4 can promote active epigenetic marks.[67] Recently, a study has shown that STAT4 is essential for the induction of GM-CSF secretion in both Th1 and Th17 by binding directly to the *Csf2* promoter.[68] Since GM-CSF is the only T-cell effector cytokine shown to date to be absolutely essential for EAE induction,[69,70] the results of this study come to resolve the conundrum.

While no data on SNP influence on expression or splicing of STAT4 is available, one study has addressed the role of an alternative isoform of STAT4 (STAT4β), which lacks 44 amino acids in the C-terminus, in the development of EAE. Transgenic expression of either STAT4α or STAT4β isoforms exclusively leads to reduced EAE in STAT4α expressing animals and exacerbated disease in STAT4β expressing mice as compared to controls. STAT4β expression drives increased levels of both IFNγ and IL-17 within cellular infiltrates in the CNS of immunized animals.[71]

IL12B (p40)

IL12B codes for IL-12p40 that together with IL12A (IL-12p35) and IL-23p19 forms IL-12 and IL-23 heterodimers, respectively. IL-12 and IL-23 are secreted primarily by APCs and influence the differentiation of T-cells into a Th1 or a Th17 phenotype, respectively. Both *Il-12p40* and *Il-12p35* KO mice fail to produce IL-12 heterodimer and lymph node cells from these mice show deficiencies in primary IFNγ-responses. However, IL-12p40 deficiency renders mice completely resistant to MOG-induced EAE, whereas *Il-12p35* KOs have unaltered or more severe disease compared to wild types.[72,73] It was later shown that IL-12p40 is essential for EAE as a component of IL-23 rather than of IL-12.[74] Bone marrow chimeras revealed that full disease is dependent on IL-12p40 being expressed by CNS resident cells.[75] IL-12p40 also has the capacity to homodimerize, and administration of neutralizing antibodies to this homodimer results in less severe EAE in SJL/J mice, while treating mice with recombinant IL-12p40 homodimer gives more severe disease.[76]

IL7R

IL7R codes for the IL-7 receptor α chain (IL-7Rα), which together with the common γ chain, forms the receptor for IL-7. IL-7Rα is also part of the receptor for thymic stromal lymphopoietin (TSLP). IL-7 is important for the survival and differentiation of cells of the lymphoid lineage such as B-, T-, and NK cells. *IL-7rα*$^{-/-}$ mice have a marked reduction in incidence of MOG-induced EAE. Interestingly, available data point toward a sex difference with low incidence in females, while males are completely resistant and have barely any priming of T-cells toward the CNS antigen.[77] Treating MOG-induced EAE in mice with recombinant IL-7 exacerbates disease and treatment with a blocking antibody to IL-7Rα ameliorates disease, both when given before onset or at peak of disease.[78] The antibody treatment reduces primarily the number of peripheral T-cells, whereas B- and NK cells are relatively spared, which also correlates to a lower expression of IL-7Rα on these cells. Among the T-cells, naïve and effector T-cells are the most affected, whereas central memory T-cells are largely spared. The treatment also results in an increase in absolute numbers of MOG-specific Foxp3$^+$

Tregs in the lymph nodes. Another study later confirmed the effect on EAE using a KO mouse model in which the IL-7Rα is still present in the thymus to avoid disturbing the development of a functional immune system.[79] These mice are also protected, although to a lesser extent than full KOs. Bone marrow chimeras revealed that EAE pathology is dependent on IL-7Rα expression on both hematopoietic and nonhematopoietic cells and that IL-7Rα is expressed in the CNS by oligodendrocytes and astrocytes. The effects seen in EAE after manipulating IL-7Rα could also be due to it being part of the receptor complex for TSLP. *Tslp*$^{-/-}$ mice, however, have a seemingly normal lymphocyte distribution in the naïve state, and there is no effect on EAE onset or progression. One of the MS-associated SNPs in the locus (rs6897932) has been shown to promote expression of an alternatively spliced soluble variant, thus increasing the ratio of soluble to membrane bound forms of IL-7Rα.[80] This soluble form binds to IL-7 and potentiates its activity.[81]

IL2RA

The IL-2 receptor alpha chain (*IL2RA),* also known as CD25, is a part of the high-affinity receptor complex for IL-2, which can be expressed on both hematopoietic as well as nonhematopoietic cells. High expression is found on Foxp3^{+} Treg and transiently on activated effector T-cells. IL-2 is important for the expansion of T-cells during an immune response, but it also influences their differentiation. As a result of the strict IL-2 dependency of Tregs, *Il2ra* KO mice spontaneously develop a progressive lymphoproliferative disorder[82] and have therefore not been a useful tool to study the role of this gene in EAE. It has, however, been shown in a model of spontaneous EAE that transfer of *Il2ra* KO T-cells results in little or no protection, whereas WT or *Il2* KO T-cells do. Thus, protection from disease by Tregs requires IL-2 signaling, but is not mediated by autocrine IL-2 production.[83] Similarly, injection of IL-2 coupled to a nonneutralizing antibody to increase the half-life results in an increase in Treg numbers and resistance to EAE.[84] In combination with rapamycin, this treatment also reduces severity of ongoing EAE. IL-2 treatment experiments point to the protective effect being associated to an expansion of NK cells in the periphery and in the CNS. Moreover, using a human variant of IL-2/anti-IL-2 antibody complex, a defective CD56^{+} NK cell compartment from MS patients was restored in a human/mouse chimera model.[85] Soluble CD25 (sCD25) is elevated in MS patients compared to control, and there is a positive correlation with disease severity and progression.[86] Treating mouse EAE with sCD25 exacerbates disease and increases Th17 responses.[87] This is consistent with the aforementioned studies as sCD25 acts as a decoy receptor for IL-2. IL-2 was recently shown to be a potent inducer of GM-CSF, a cytokine crucial for the development of EAE. An MS-associated polymorphism in *IL2RA* (rs2104286) gene specifically increases the frequency of GM-CSF-producing Th cells from risk allele carriers as compared to Th cells from control individuals.[13] Daclizumab is an antibody directed toward CD25 that has shown efficacy in several Phase III clinical trials for relapsing-remitting MS (RRMS) and is a potential new treatment.

TYK2 (Tyrosine Kinase 2) and STAT3

The Janus Kinase/Signal Transducer and Activator of Transcription (JAK/STAT) signaling pathway is the predominant signal transduction cascade in innate and adaptive immunity.[88] TYK2 is a member of the JAK/STAT signaling pathway and contributes mainly to the IL-12-induced Th1 cell differentiation.[88] STAT3 functions mainly as a signaling molecule and transcription factor for Th17 cell differentiation.[88] Dysregulation of the JAK/STAT pathway contributes to numerous autoimmune diseases, including MS/EAE.

Tyk2$^{-/-}$ C57BL/6 mice are resistant in $MOG_{35–55}$-induced EAE with complete lack of inflammation in the CNS.[89] Adoptively transferred *Tyk2*$^{-/-}$ pathogenic CD4^{+} T-cells fail to induce EAE in WT animals pointing to a role for TYK2 in T-cells, rather than in APCs or target tissue cells, for the phenotype. B10.D1-*H2*^{q}/SgJ (*Tyk2*^{A}) mice that carry a 2538 G→A missense mutation in *Tyk2* gene are also resistant in $MOG_{79–96}$-induced EAE compared to B10.Q/Ai (*Tyk2*^{G}) mice.[90] Ex vivo restimulation of splenocytes and lymph node cells from B10.D1 (*Tyk2*^{A}) leads to lower IFNγ, IL-6, and RANTES production and a trend for lower IL-17 compared to B10.Q. Since *Tyk2*^{A} mutation impairs the IL-12R and the IL-23R pathways, the authors speculate that EAE resistance of B10.D1 (*Tyk2*^{A}) mice might be due to their inability to upregulate encephalitogenic levels of IFNγ and IL-17 on T-cells (via IL-12R and IL-23R pathways respectively). On the other hand, conditional deletion of *Stat3* in the T-cell compartment renders animals resistant to EAE, highlighting the importance of STAT3 in Th17 differentiation during EAE development.[91,92]

Different treatments such as COX-2 inhibitors, 1,25-dihydroxyvitamin D3, COP-1, lovastatin, and AZD1480 ameliorate EAE symptoms, CNS inflammation, and demyelination.[93–97] In addition, several herbal compounds such as quercetin, curcumin, berberine, embelin, cornel iridoid glycoside, and plumbagin have been shown to dampen

EAE.[98–104] All the aforementioned treatments dampen Th1 and Th17 differentiation through inhibition of JAK/STAT pathway.

Suppressors of cytokine signaling proteins (SOCS) inhibit JAK/STAT, inhibit JAK/STAT signaling by various mechanisms.[105] SOCS3 inhibits STAT3 activation and cytokine signaling in macrophages/microglia.[106] Mice with conditional KO of *Socs3* in myeloid cells develop atypical EAE compared to control mice.[107,108] Adoptive transfer of SOCS-3 transduced DCs significantly suppresses EAE and associates with impaired IL-23/STAT3 and IL-12/STAT4 signaling and further decreases Th17 and Th1 differentiation and increases Th2 induction.[109]

Additionally, Glia maturation factor (GMF),[110] miR-20b,[111] miR-125a,[112] and the organotellurium compound AS101[113] modulate EAE by directly affecting the function or transcriptional levels of STAT3.

NF-κB signaling

NFKB1 (p50)

NF-κB is a generic name for a protein complex of five protein subunits, NF-κB1 (p50), NF-κB2 (p52), RelA, RelB, and c-Rel, that act as either homo- or heterodimers, functioning primarily as transcription factors for cytokine production and cell survival. Being quite central to all immune processes, the involvement of NF-κB proteins with EAE and MS is expected as a surrogate for immune activation in most cell types. Additionally, NF-κB is constitutively active in neurons and expressed in all glial cell types, being crucial for nervous system plasticity, learning, and memory.[114] While deletions in immune system cells generally lead to reduced inflammation during EAE, CNS-restricted expression ablation has revealed both neuroprotective or detrimental roles, depending on the type of insult. Specifically for EAE, general NF-κB pathway inhibition did not modify disease progression when targeted on either neurons or oligodendrocytes, while targeting of astrocytes and microglia led to reduced inflammation.[114]

While effects on EAE have, through selective deletion of one of the five subunits or additional regulatory proteins, upstream or downstream of the activation cascade been thoroughly documented and give partially overlapping results (for an extensive review refer to Mc Guire et al[114]). In specific, NFKB1 (p50) is part of the canonical NF-κB pathway that is triggered by activation of receptors such as TNFR1, TLRs, IL-1R, TCR, and BCR. Deletion of *NFKB1* in mice attenuates EAE incidence, clinical score, and CNS inflammation due at least in part to a reduction in T-cell activation (both Th1 and Th2).[115] Target-tissue-specific effects are also evidenced by reovirus infection experiments, in which $p50^{-/-}$ mice fare better with reduced CNS apoptosis.[116] A similar effect can be observed in ischemia induction, in which damage is significantly reduced in $p50^{-/-}$ mice.[117]

While limited information is available for humans on *NFKB1* specifically, GWAS results have implicated other players in the NF-κB cascade, such as *TNFRSF1A* (TNFR1), *CARMA1* (*CARD11*), *MALT1*, *BCL10*, *PLEKHG5*, and *TNFAIP3* as MS-susceptibility loci.[9]

MALT1-BCL10-CARD

Triggering of antigen receptors on the surface of lymphocytes leads to the initiation of signaling pathways that regulate the activation, proliferation, and survival. One of the major pathways leads to NF-κB activation and translocation to the nucleus, where it acts as a transcriptional regulator. The so-called classical pathway of activation, in response to antigen receptors, requires the signaling molecule MALT1 and its binding partners BCL10 and CARMA1 (CARD11),[118] all three associated to MS, as well as NF-κB p50 (NFKB1) (see "NFKB1 (p50)" section).[9] While no studies have addressed the role of BCL10 directly on EAE, *Carma1* KO animals are completely protected from EAE apparently due to a strong inhibition of Th17 differentiation.[119] Similarly, $Malt1^{-/-}$ mice immunized with $MOG_{35–55}$ do not develop EAE in spite of abundant lymphocytic infiltration into the CNS. Loss of *Malt1* leads to reduced IL-17 and GM-CSF secretion from infiltrating T-cells, which fail to further recruit myeloid cells and sustain neuroinflammation, while no impact on Th17 lineage-related transcription factors or Th1 differentiation can be observed.[120] This is, however, inconclusive, since another study reveals impairment in lymphocyte activation already in the periphery under a similar EAE induction protocol.[121] Transgenic mice expressing a catalytically inactive form of MALT1, which conserves its scaffolding function, also present a strong defect in lymphocyte activation and protection from EAE.[122] Surprisingly, ablation of catalytic activity leads to an impairment in Treg cell generation and spontaneous autoimmune gastritis, which was not seen in complete KOs in the same study. Lastly, treatment of EAE in mice with the reversible MALT1 inhibitor mepazine either prophylactically or after onset of symptoms ameliorates disease.[123]

Other pathways

PTGER4 (EP4)

PTGER4 codes for EP4, which is one of the four receptors for prostaglandin E2 (PGE2). PGE2 is produced by cyclooxygenase-2 and has both pro- and anti-inflammatory

effects. Lipidomic analysis of the arachidonic acid cascade in the spinal cord of mice with EAE shows that the PGE2 pathway is favored over other eicosanoids and that the expression of the PGE2 receptors EP1, EP2, and EP4 correlates with clinical symptoms.[124] The same study also revealed that daily administration of EP4 antagonist ONO-AE3-208 before EAE onset suppresses MOG-induced EAE, likely due to reduced T-cell proliferation as well as diminished IFNγ and IL-17 expression. Ablation of all the eight prostaglandin receptors individually revealed that only *Ep4* KOs present with a significant effect, leading to decreased disease severity.[125] Inhibition of EAE is also achieved by treating mice with an EP4 antagonist during the priming phase. Paradoxically, treatment with an EP4 agonist starting at onset of disease reduces disease severity. Agonists for EP1, EP2, and EP3 have no effect.

TNFRSF1A

TNFR1, encoded by the *TNFRSF1A* gene, is the major receptor for TNF. As a pleiotropic cytokine, the role of TNF is not clearly understood and seems to have both pathogenic and protective functions in neuroinflammation. Blocking TNF in a clinical trial for MS resulted in an exacerbation of symptoms, while concomitantly, treatment of other autoimmune diseases with anti-TNF resulted in cases of neuroinflammation.[126–128] Analysis of MS GWAS data in conjunction with the 1,000 Genomes Project data implicates SNP rs1800693 as the causal variant in the *TNFRSF1A* region, leading to the production of a soluble TNFR1 in MS patients carrying the predisposing genotype.[13,129] This soluble TNF receptor acts in the same manner as the blocking treatment and could, therefore, promote neuroinflammation. TNF also has a second receptor, TNFR2, which can be inducibly expressed in endothelium and immune cells. TNFR2 has a protective effect in EAE since *Tnfr2* deficient mice have exacerbated disease while *Tnfr1* KO animals or mice treated with TNFR1 antagonists are protected.[130–134] Taken together, both human and mouse data would point to blocking of TNFR1 rather than TNF itself as a target for a potential therapy for MS.

CYP24A1 (1,25-hydroxyvitamin D-1 alpha hydroxylase)

CYP24A1 encodes for 1,25-hydroxyvitamin D-1 alpha hydroxylase, an enzyme that inactivates 1,25-dihydroxyvitamin D_3 through hydroxylation and thus regulates its levels.[135]

Female B10.PL mice fed with a diet with or without vitamin D_3 prior to MBP immunization have significantly less clinical and immunological signs of EAE compared to ovariectomized females or intact or castrated males.[136] One hypothesis for the higher levels of 1,25-dihydroxyvitamin D_3 and less *Cyp24a1* transcripts in vitamin-D-fed female mice is that an ovarian hormone inhibits *Cyp24a1* gene expression in the spinal cord, which in turn causes 1,25-dihydroxyvitamin D_3 accumulation leading to inflammation resolution before severe EAE develops.

Lovastatin treatment provides protection in EAE mice through inhibition of *Cyp24a1* gene expression in Th1/Th17 cells that may allow the accumulation of 1,25-dihydroxyvitamin D_3 in the peripheral lymphoid organs and spinal cord.[137]

Conclusion

The complexity and the heterogeneity of human MS together with inaccessibility of the target organ and events that occur prior to disease diagnosis necessitate studies in experimental models. With the tremendous progress in MS genetics, it is likely that EAE will continue to have a central role in functional in vivo complementation of human studies, especially in combination with multiple omics from human tissues that can guide the hypothesis about the nature of the causal variants.[138,139] Numerous conditional knockout mice, which enable precise gene targeting in specific cell types when crossed with appropriate Cre lines (sometimes even in an inducible manner), have already been developed by the international Knockout Mouse Project. This conventional approach can now be complemented with the latest cutting-edge technology using the CRISPR-Cas9.[140] In this way, multiple genes can be targeted simultaneously, which is likely more suitable for MS pathologies that are caused by subtle changes in genes that converge to shared pathways rather than variations in single genes. Such strategies might give rise to novel models with characteristics that mimic better certain MS pathologies, making them further adapted for translational research.

Disclosure

The authors report no conflicts of interest in this work.

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