T Cell Mediated Pathogenesis in EAE: Molecular Mechanisms

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T cells are major initiators and mediators of disease in multiple sclerosis (MS) and in its animal model experimental autoimmune encephalomyelitis (EAE). EAE is an antigen-driven autoimmune model in which immunization against myelin autoantigens elicits strong T cell responses which initiate its pathology with CNS myelin destruction. T cells cause pathogenic events by several mechanisms; some work in a direct fashion in the CNS, such as direct cytokine-induced damage, granzyme-mediated killing, or glutamate-induced neurotoxicity, whereas most are indirect mechanisms, such as activation of other cell types like macrophages, B cells, or neutrophils. This review aims to describe and discuss the molecular effector mechanism by which T cells harm the CNS during EAE. *(Biomed J 2015;38:183-193)*

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EAE – What is it?

When seen in its entirety, the animal model experimental autoimmune encephalomyelitis (EAE) bears significant similarities to multiple sclerosis (MS) and its different varieties. Depending on the model used, EAE may develop in highly distinct forms such as acute, relapsing-remitting, and primary or even secondary progressive. Although the model was discovered and developed already in the 1930s,[1] it was only in the 1980s that it was clearly proven that T cells are the major driver of disease when mice are immunized by CNS antigen in complete Freund's adjuvant (CFA). The major evidence came from adoptive transfer experiments using T cell lines in rats and mice.[2-5] Even though many similarities are observed between MS and its animal model, major concerns still exist about the etiology and the mechanism (s) of disease development of MS. For example, the autoimmune etiology of MS is still questioned and some researches claim that infections with, for example, Epstein–Barr virus, may play an important role in MS immunopathology.[6,7] Others suggested for some subforms of MS, a primary degenerative scenario with immune-mediated damage playing a secondary role in the disease.[8-12] Also, the composition of CNS-infiltrating cell types differs between EAE and MS; for example, $CD8^+$ T cells, which may be seen as the dominating T cell population in MS histology,[13,14] are not essential for EAE.[15] Another difference might be the presence and the role of neutrophils in MS versus EAE, with EAE being dependent on neutrophils whereas MS seems to lack a clear association with neutrophils. Still, it needs to be emphasized that the pathogenesis in the induced EAE model (but not in spontaneous models) is time wise well controlled and the effect of a certain cellular population can be effectively observed and tested, whereas in MS the timing of the immune response is out of sight and can only be observed during or after acute attacks when the role of populations such as neutrophils may already be blunted.[16]

In the standard model most frequently used, EAE is induced by immunization using a water-in-oil emulsion of CFA into which the autoantigen such as myelin oligodendrocyte glycoprotein (MOG), myelin basic protein (MBP), or proteolipid protein (PLP) is mixed. CFA contains high amounts of heat-inactivated *Mycobacterium tuberculosis*. This emulsion is usually injected subcutaneously in the back skin next to the tail base. Also, pertussis toxin injections are given the same day and 2 days later intraperitoneally. EAE normally develops after about 10 days when scores with ascending paralysis usually become visible.

T cells in EAE

T cell differentiation is highly influenced and dependent on the cytokine milieu present in the draining lymph nodes

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during T cell receptor (TCR) stimulation. In infections, the type of cytokines that are induced is determined by the type of the infectious agent. Sensing of this is realized by antigen presenting cells (APCs) or also directly by the T cells via toll-like receptors (TLRs) activated by distinct pathogen-associated molecular patterns (PAMPs) produced by the invaders.[17,18] Therefore, the use of *M. tuberculosis* in CFA provides a clear bias to a specific immune response, namely, the one T cells normally initiate in tuberculosis infection to which differentiation to Th1 or Th17 cells (see below) also belongs. Thereby, the pathway by which autoimmunity is elicited, either by active induction or by creation of TCR transgenic animals, may subvert the type of immune response and the associated molecular mechanisms leading to disease. Also, the type of antigen, or rather the exact epitope used for immunization, was shown to govern the response type and also the disease pattern in EAE. Certain antigens elicit a predominant Th17 and cerebellar disease, while other antigens evoke a mixed Th1/Th17 response with typical lumbar inflammation.[19]

EAE, when induced using CFA, is, in the majority of cases, a $CD4^+$ T helper cell-mediated disease. There are some $CD8^+$ T cell-mediated EAE models (see below), though the major culprit in inducing cytotoxic T cell responses is the use of CFA as it funnels in the typical major histocompatibility class (MHC) class II presentation pathway of APCs for T cell activation. Since the discovery of distinct T helper subpopulations in the late 1980s by Mossman and Coffman,[20] EAE was thought to be a prototypical Th1 autoimmune disease due to its strong association with interferon-gamma (IFN-γ) secreting T cells found in the CNS.[21,22] Findings with transferred TCR transgenic Th2 cells eliciting an allergic form of EAE already perturbed this picture.[23] A further challenge to a Th1 view of EAE was the reports published in the 1990s showing that mice deficient for IFN-γ,[24] its receptor,[25] or the cytokine subunit interleukin (IL)-12-p35 (necessary for Th1 cell differentiation)[26,27] or the IL-12 specific receptor subunit IL-12Rβ2[28] developed normal or even stronger progression of EAE than their wild-type (WT) control animals. It was soon shown that this discrepancy was due to another form of an immune response, namely, the IL-23/Th17 axis.[29-31] It turned out that the major important immune response of T cells in EAE was not Th1 but was dominated by T helper cells producing IL-17 (IL-17A and IL-17F) after immunization. These Th17 cells are now considered the initiators of disease in the standard EAE model. Th17 cells need external IL-23 and the master transcription factors retinoid-related orphan receptor gamma t (RORγt)[32] as well as RORα .[33] These cells were later shown to switch their phenotype and co-produce IFN-γ and granulocyte macrophage-colony stimulating factor (GM-CSF) and, for a major part, also lose the expression of IL-17 in the CNS completely.[34,35] The latter finding also explains why EAE was seen initially as a Th1 disease. Many experts in this field see such multi-cytokine-producing cells as most pathogenic, especially since co-expression of the Th1 master transcription factor Tbet was shown to be mandatory for the encephalogenicity of Th17 cells.[36] The Th17 supportive cytokine IL-23 was indeed shown to induce Tbet expression in Th17 cells,[35,37] but the role of IFN-γ production by Th1/Th17 cells remains elusive. IFN-γ is a pleiotropic cytokine with potent immune-stimulating features and, on the other hand, with immune-suppressing features. These include containment of T cell expansion,[38] suppression of IL-1β induction by *M. tuberculosis* in EAE,[39] induction of T regulatory (Treg) cells,[40] and induction of multiple immunosuppressive secondary molecules such as Indoleamine 2,3-dioxygenase (IDO)[41] or Programmed death-ligand 1 (PD-L1).[42]

T helper cells – How do they do it?

As described above, the autoantigen-containing CFA emulsion needs to be taken up by endocytosis from APCs. This pathway is luminal and not cytosolic; therefore, most of the injected antigen will be loaded on MHC class II in the endolysosomal MIIC compartment. A further bias for the $CD4^+$ T cell induction by contemporary protocols (e.g., MOG peptide 35–55) is that these are long peptides which contain immunodominant epitopes defined for binding to MHC class II molecules. These peptides then stimulate autoantigen-specific T cells that escaped thymic negative selection. $CD4^+$ effector T cells that are commonly induced in EAE start with the expression of IL-17 in the lymph nodes. Some days later, already in the lymph nodes, plasticity of these Th17 cells, which is driven by IL-23,[35] initiates the multi-cytokine program with co-expression of RORγt and Tbet. Finally in the CNS, effector T cells are found to express either IL-17 or IFN-γ or GM-CSF or combinations of all these three cytokines [Figure 1].[34,35,43]

As pointed out earlier, most EAE models are primarily based on activation of encephalitogenic $CD4^+$ T helper cells. Their major mechanism is to govern the response mechanism of other cells by secreting cytokines and by direct interaction with these cells via CD40L–CD40 interaction.[44-46] Cytokines are very interesting molecules, most of them acting by distinct so-called Janus kinase (JAK)/Signal Transducer and Activator of Transcription (STAT) pathways. Although activation of the STATs can be measured by phosphostaining, the type of responses induced by cytokines is hard to predict. Most cytokines act in minute amounts, and can elicit robust and also dangerous responses. Therefore, their expression and secretion is highly regulated.

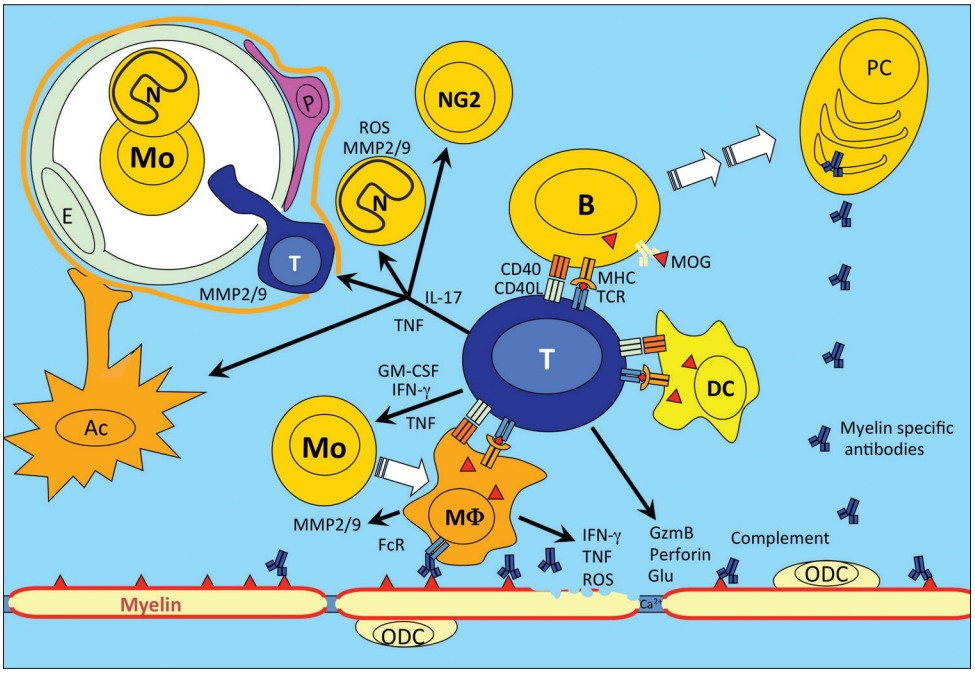

Figure 1: T cell effector mechanisms in EAE. T cells enter the CNS via postcapillary venules and have to cross the blood brain barrier, composed of endothelial cells, the basement membrane, and the parenchymal basement membrane of the glia limitans.[137] They often accumulate in the perivascular space between the two basement membranes. Penetration of the parenchymal basement membrane is facilitated by matrix metalloproteases (MMP2 and MMP9) secreted by T cells, macrophages, and neutrophils. T cells in the CNS are reactivated by macrophages, DCs, and B cells presenting myelin autoantigens and secrete, among others, the cytokines IL-17, IFN-γ, TNF and GM-CSF. IL-17 induces secondary cytokines, chemokines and MMPs, which help in the breakdown of BBB and in the attraction of monocytes and neutrophils. GM-CSF (together with G-CSF) has long-distance effects on neutrophil mobilization, but possibly also has direct influences on inflammatory monocytes and their capacity to polarize T helper cell differentiation. It probably works additionally by inhibiting Treg function via IL-6 induction in myeloid cells. Cytokines secreted by T cells also influence astrocytes and oligodendrocyte precursor cells (NG2 cells) in their differentiation and in their proliferation capacity.[138] IFN-γ and TNF may have direct toxic effects on ODC, but most of all stimulate myeloid effector cells such as inflammatory monocytes, macrophages, and neutrophils. Stimulation of these cells leads to damage of myelin by the secreted reactive oxygen species followed by myelin attack and ingestion by macrophages. Macrophages are also activated by antibodies bound to myelin via FC receptors and by the complement products activated by these antibodies. Myelin-specific antibodies may be released from antibodysecreting plasma cells or plasma blasts originating from myelin-specific B cells activated by T cells in the CNS or in peripheral lymph nodes. Th17 cells damage axons also directly by secretion of glutamate, whereas cytotoxic $CD8^+$ T cells secrete perforin, granzymes, and IFN-γ to directly attack ODCs. Abbreviations: E: Endothelial cell; P: Pericyte; T: T cell; B: B cell; PC: Plasma cell; Mo: Monocyte; Ac: Astrocyte; N: Neutrophil, MΦ: Macrophage; ODC: Myelin forming oligodendrocyte; DC: Dendritic cell; ROS: Reactive oxygen species.

Early infiltration – Th17 cells

T cell infiltration into the CNS occurs around day 8 or 9, and these T cells mostly express IL-17.[47] The IL-17 family is composed of six members, IL-17A–F,[48] that belong to the cysteine knot family. They are structurally unrelated to other cytokines, but have distant similarities to neurotrophins.[49] IL-17A, F, and C signal by a unique pathway, utilizing NF-κB activator 1 (ACT1)/tumor necrosis factor receptor associated factor (TRAF) 6,[50,51] with nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) playing an important role as the downstream pathway.[50,52] Of the IL-17 family, IL-17A and IL-17F are the most highly related with about 50% amino acid sequence identity.[53] They are thought to mediate highly similar responses in target tissues. IL-17C is secreted directly by the epithelial tissue cells[54] and signals very similar to IL-17A/F.[54,55] The cytokines IL-17A and IL-17F are secreted by activated αβ $CD4^+$ T cells (Th17),[56] γδ T cells,[57] natural killer (NK) T cells,[58] lymphoid tissue inducer cells,[59] and innate lymphoid cells type 3 (ILC3) subsets.[60] Furthermore, IL-17 was shown to be also expressed by mast cells[61] and neutrophils.[62-64] IL-7A/F acts mostly on the tissue cells by signaling via the heterodimeric IL-17 receptor composed of IL-17RA[65] and IL-17RC.[66,67] The action of IL-17 is manifold, as it was shown to induce the secretion of matrix metalloproteases (MMP1, MMP3, MMP9, MMP12, and MMP13) and cytokines like GM-CSF, IL-6, and a bunch of chemokines (CCL2, CCL7, CCL20, CXCL1, CXCL2, and CXCL5) by local tissue cells. This cocktail is thought to prepare the tissue and attract further T cells and myeloid

cell populations into the developing CNS lesion. IL-17 probably also acts directly on the blood–brain barrier (BBB) integrity by induction of reactive oxygen species (ROS) in endothelial cells.[68] IL-17 was also shown to induce downstream mediators of angiogenesis in rheumatoid arthritis and tumor models and in psoriasis.[69-71] Lack of IL-17 signaling hindered efficient erythema formation, which is a typical feature of imiquimod-induced dermatitis, a mouse model for psoriasis.[72] Some of its downstream cytokines, such as granulocyte-colony stimulating factor (G-CSF) and GM-CSF, also have long-distance effects like neutrophil mobilization, as also observed in EAE. Therefore, a major role of IL-17, at least in mice, is to attract the neutrophils into the CNS and activate the latter.[73] This probably occurs rather indirectly by secondary cytokines and chemokines induced by IL-17A together with tumor necrosis factor (TNF) or other cytokines such as IL-1β or IL-22. Recently, it was shown that the neutrophils express the IL-17RA and IL-17RC chains[62] and can react directly with IL-17 in an autocrine manner. Neutrophils enter the CNS early in disease.[74,75] Depletion of neutrophils or blockade of CXCR2, the main chemokine receptor for CXCL1/2 on the neutrophils, inhibited BBB breakdown and EAE manifestation, induced either actively or passively via transfer of myelin-specific T cells.[76,77] The actual role neutrophils play in the CNS is not yet clarified, but they were shown to contribute to destruction of the BBB.[78] Furthermore, it was recently shown that neutrophils play an important part in maturation of local APCs in the CNS.[79] Other target cells of IL-17 in the CNS may be the NG2-expressing oligodendrocyte precursor cells. Deletion of the IL-17 signaling molecule ACT1 in these cells was shown to have a major impact on EAE.[80] Since ACT1 may be activated by other pathways also, these experiments did not prove that NG2 cells are, indeed, the targets of IL-17 in EAE. Chemokines induced by IL-17 can also attract other cell types such as monocytes and other T cells to perpetuate the initial inflammation. IL-17 acts mostly in synergy with TNF or with IL-22, both cytokines, which are also expressed by infiltrating Th17 cells in $MOG_{35–55}$–induced EAE. IL-22, which is co-produced not only by Th17 but also by Th1 cells, does not seem to play a major role in EAE, as the knockout mice did not show a change in EAE course compared to WT controls.[81] There might be some hint though that its function may rather be protective in EAE and MS, as the mice deficient for the IL-22 scavenger protein IL-22BP develop lower EAE than WT controls.[82] Interestingly, variants of IL-22BP were independently described as risk factor in MS and EAE.[83,84]

Although IL-17 bears all these important functions, it must be pointed out that IL-17 is not absolutely mandatory for active EAE induction, as IL-17RA[85] and IL-17RC knockout animals still develop some residual disease[66] (and our unpublished data) and mice which lack IL-17F and are treated with neutralizing antibody against IL-17A remain susceptible to EAE induction.[86] Further indication for this is that mice lacking RORγt are only partially resistant to EAE,[32,33] as some mice even develop a very strong pathology with a delayed kinetic (our unpublished data). Furthermore, small interfering RNA (siRNA)-mediated[87] or pharmacological inhibition of RORγt up to now, in most cases, failed to fully inhibit EAE, showing rather a shift in disease onset[88-90] (and our unpublished observations). In summary, the major function of IL-17 may be to allow initial entry of myeloid cells. In the absence of IL-17, other cytokines or T cell populations may suffice to induce a delayed form of the disease, though with a lower incidence.

What are IL-23 and GM-CSF doing?

The enigma originated by the findings that T cells n either needed IL-17 nor IFN-γ to induce EAE, leaving the question about the effector mechanism of T cells to induce pathology. It was known that the cytokine which instructs T cells and cannot be replaced in EAE induction is IL-23. But which T cell effector mechanism downstream of IL-23 is the important one? In 2011, two groups independently reported that GM-CSF secreted by T cells is a cytokine induced by IL-23 in T cells and is the irre placeable cytokine of T cells in EAE.[43,91] Although the proof for whether the major task of IL-23 is to induce GM-CSF in T cells is still lacking, namely, transgenic overexpression of GM-CSF specifically in T cells of mice which are deficient for IL-23, these findings were a major step forward and may lead to new treatment options in the future. The question of how GM-CSF, indeed, performs its task is still an enigma. It seems not to influence the number of inflammatory dendritic cells (DCs) in the CNS in EAE,[92,93] but may rather influence the polarization capacity of monocyte-derived DCs for T cells to differentiate into Th17 cells in EAE. This was recently shown by depletion of C-C chemokine receptor type 2 (CCR 2)-positive monocyte-derived DCs.[93] Together with the earlier reports, this would fall back again on the IL-17 cytokine. Therefore, probably other mechanisms of IL-23, besides GM-CSF and IL-17 induction, may also play a role in EAE. Alternatively, GM-CSF itself may work by inhibition of Tregs via induction of IL-6[94] (another irreplaceable cytokine in EAE).[95] Finally, GM-CSF may play different roles during the peripheral priming phase and in the effector phase in the CNS.

T cells activate macrophages in EAE

Macrophages play several roles in the pathogenesis of EAE. First, they act by destroying the parenchymal basement membrane formed by astrocytes in the BBB through secretion of matrix metalloproteases (MMP2 and MMP9)[96] which allow leukocytes to leave the perivascular space and

infiltrate the CNS parenchyma. Second, macrophages damage myelin efficiently by ROS generation.[97,98] In EAE and MS lesions, macrophages have been observed to take up large parts of myelin.[99] T cells do activate this process by direct interaction with the macrophages and by the cytokines IFN-γ and TNF. Macrophage expression of Fc receptors may also be important in mediating the pathogenic effects by autoantibodies in the CNS in EAE. For a long time, it was not really clear whether microglia cells in the CNS become activated in EAE and perform the same duties as macrophages differentiating from infiltrating monocytes. Recently, the group of Ransohoff demonstrated by serial block-face scanning electron microscopy that monocyte-derived macrophages are the cells initiating demyelination at the nodes of Ranvier, whereas microglia were rather found as cells clearing the debris.[100]

T cells in spontaneous EAE models

As described above, the standard model of EAE is induced by peptide or protein immunization. Some TCR transgenic mouse strains bearing TCRs for specific myelin autoantigens develop spontaneous demyelinating disease. This was first found by Goverman *et al.* under animal housing conditions which were less microbial controlled than the standard specific pathogen free (SPF) conditions.[101] Also, other TCR transgenic strains develop spontaneous EAE, but mostly with high incidence only in the recombination-activating gene (RAG) knockout background,[102] presumably due to lack of efficient Treg development in these animals.[103] One strain, the RR mouse on the SJL/J background, also developed EAE with high incidence in normal housing condition without crossing to the RAG knockout background.[104] TCR transgenic T cells in these animals activate MOG-recognizing B cells of the endogenous repertoire very early in life. These mice, therefore, develop EAE with high MOG-specific antibody titers which are complement fixing. Early depletion of B cells in these animals prevented plasma cell development and antibody titer formation, and ultimately EAE pathogenesis. This model is dependent on B cells and pathogenic antibodies, whereas another spontaneous model of EAE with Devic-like disease, in which 2D2 mice[105] were crossed with BCR transgenic IgH^{MOG} mice[106] (both recognizing MOG epitopes), has been observed not to depend on antibodies.[105,107-109] Here, B cells probably act rather as efficient APCs and cytokine producers for pathogenic T cell development, reminiscent of the situation in MS where depletion of B cells by Rituximab (an anti-CD20 monoclonal antibody) also shows a beneficial effect independent of the plasma antibody levels found.[110] Molecular interactions in these B cell-antibody dependent models probably occur via TCR/MHCII and CD40L–CD40, as well as by the cytokines produced from T cells. In the RR model, intra-CNS antibody secretion, presumably by plasma cells, can be observed. This shows similarities to the follicular structures described in the meninges of MS patients.[111] Despite the lack of CFA usage in these spontaneous models, T cells in the CNS express IL-17 and/or IFN-γ, similar to that in the induced EAE models. Nevertheless, one has to keep in mind that the TCRs used in these models descend from T cells of mice previously immunized against myelin antigens.

Direct mechanisms of damage by T helper cells?

One of the rare direct mechanisms through which T helper cells act in EAE pathology was recently demonstrated.[112] Siffrin *et al.* showed that specifically Th17 cells form immunosynapses with axons, leading to fast axonal damage. This pathway of neurotoxicity was unique as it was independent of MHCII–TCR interactions with the neurons. It was shown that Th17 cells elicited neurotoxicity by secretion of glutamate and that calcium flux in axons associated with Th17 cells was followed by axonal degeneration. The pathway of axonal damage and recognition of neurons remains to be discovered.

T helper cells can probably also harm the CNS cells directly by their cytokines, as it was shown in culture that both IFN-γ and TNF can directly kill oligodendrocytes (ODCs).[113-116] Since ODCs, the myelin-producing cells of the CNS, do not express or upregulate MHC class II in inflammation, the damage by cytokines in the CNS could occur as a side effect. This occurs when T cells are activated by monocyte-derived macrophages/DCs in the CNS which present myelin antigen on MHC class II molecules. *In vivo*, the role of direct damage by cytokines versus indirect damage is hard to distinguish since the same cytokines also activate macrophages. The usage of conditional cytokine receptor knockout mice should demonstrate inasmuch their ligands have direct effects on ODCs or neurons *in vivo*.

$CD8^+$ T cells in EAE?

$CD8^+$ T cells are equipped with a very efficient cytotoxic killing mechanism containing perforin and granzymes, which they use to kill virus-infected cells. Granzymes are serine proteases, which are released into the cytosol of target cells with the help of perforin, a Ca^{2+}-dependent pore-forming protein.[117,118] Inside the cytosol, the granzymes induce apoptosis by different mechanisms.[119,120] Cytotoxic T cells are very interesting cell types in EAE since MHC class I expression can be found on activated ODCs. Therefore, $CD8^+$ T cells are able to directly recognize myelin peptides presented by ODCs and kill the latter. Furthermore, $CD8^+$ T cells may be important players in MS.[121,122]

As described above, the standard models of EAE induction, which use antigens emulsified in CFA,[123] seem to be largely independent of $CD8^+$ T cells. In line with this were findings with perforin knockout mice, which showed a higher disease score than controls.[124,125] This pointed rather to a regulatory role of the perforin–granzyme pathway, maybe as part of Tregs or of $CD8^+$ suppressor T cells in EAE. Nevertheless, some reports showed that myelin-specific $CD8^+$ T cells, indeed, are able to induce EAE including demyelination. First, Joan Goverman's group described the MBP-derived peptide $MBP_{79–87}$ presented by the MHC class I molecule K^k, which was able to induce $CD8^+$ T cells in MBP-deficient C3H-shiverer mice.[126] *In vitro*-activated $CD8^+$ T cell clones against this epitope, which were isolated from immunized WT C3H mice, were able to transfer EAE with severe demyelination with a majority of lesions in brain parenchyma.[127] Although signs of major cytotoxic damage were visible in this model, the role of perforin or granzymes was not investigated; but a role for IFN-γ was demonstrated in this model. Another group also reported that MOG-specific $CD8^+$ T cells, *in vitro*-expanded from mice immunized against $MOG_{35–55}$, are able to transfer EAE with demyelination.[128] The mechanism of pathology by these transferred $CD8^+$ T cells was not investigated. Apparently, $MOG_{35–55}$ contains nested epitopes, $MOG_{37–46}$ and $MOG_{44–54}$, which can be presented by the MHC class I molecule D^b and are able to stimulate $CD8^+$ T cells.[129,130] Apparently, these cells do not seem to play an important role in primary immunizations, but can grow out from *in vitro* cultures under IL-2 stimulation. More recently, Huber *et al.* described the presence of $CD8^+$ T cells expressing IL-17A, so-called Tc17 cells, in lymph nodes and in the inflamed CNS of EAE animals immunized with $MOG_{37–55}$.[131] These cells were later shown to play a supportive function for Th17 cells in EAE.[132] In their system, Tc17 cells needed to express IL-17A to render transferred $CD4^+$ T cells pathogenic. Surprisingly, pathogenicity of the transferred $CD4^+$ T cells was independent of their own expression of IL-17A.

In addition, several CD8-based TCR transgenic models for CNS inflammation were developed by different groups. Two groups created mice expressing neo-antigens in ODCs. Na *et al.* expressed ovalbumin (OVA) cytosolically in ODCs and found a very early fulminant demyelinating CNS inflammation in double-transgenic animals co-expressing the OT-1 transgenic TCR.[133,134] Also, this group did not investigate inasmuch the perforin–granzyme pathway was implicated in disease. Like in the C3H MBP system by Goverman's group, they found that IFN-γ played an important role in pathogenicity, probably via activation of ODCs through upregulation of MHC class I and co-stimulatory molecules. The group of Lennart Mars crossed hemagglutinin (HA)-specific TCR transgenic CL4-TCR mice with mice expressing HA in ODCs.[135] In this model, a role for perforin–granzyme mediated killing of ODCs was suggested, as $CD8^+$ T cells containing polarized granzyme-filled vesicles in direct contact with ODCs were found. Recently, Cabarrocas *et al.* reported a new $CD8^+$ TCR transgenic mouse (BG1 TCR) recognizing the astrocyte-specific glial fibrillary acidic protein (GFAP) peptide 264–274 presented by K^b. Upon activation of these adoptively transferred cells by infection with recombinant GFAP-expressing viruses, EAE scores developed with infiltrating $CD8^+$ T cells expressing high levels of granzyme B and IFN-γ.[136]

Although several $CD8^+$ T cell-mediated EAE models have been developed, the detailed molecular mechanisms of pathology by these cells have not yet been clarified. Open questions include the role of perforin and whether granzymes and specifically which granzymes play a role in pathology and which proteins in ODCs or neurons are targets of the proteases in $CD8^+$ T cell-mediated EAE. Though the common molecule needed, as opposed to EAE mediated by $CD4^+$ T cells, is IFN-γ Its major function may be to upregulate MHC class I on target tissue cells for recognition of cytotoxic T cells.

Conclusion

Very distinct EAE models were developed in the past years. These models show different characteristics in disease phenotype, histopathology, and their cellular and molecular players, and thereby do reflect distinct features of MS. T cells in these different models come with different flavors and may use different molecules to interact and communicate with other cells and to direct pathological events. Therefore, certain molecular pathways are important in one model, but negligible or redundant in the other. Major non-redundant cytokines in basically all the models are IL-23, GM-CSF, and IL-6, whereas the role of IL-17 and IFN-γ may be pleiotropic and model-specific.

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