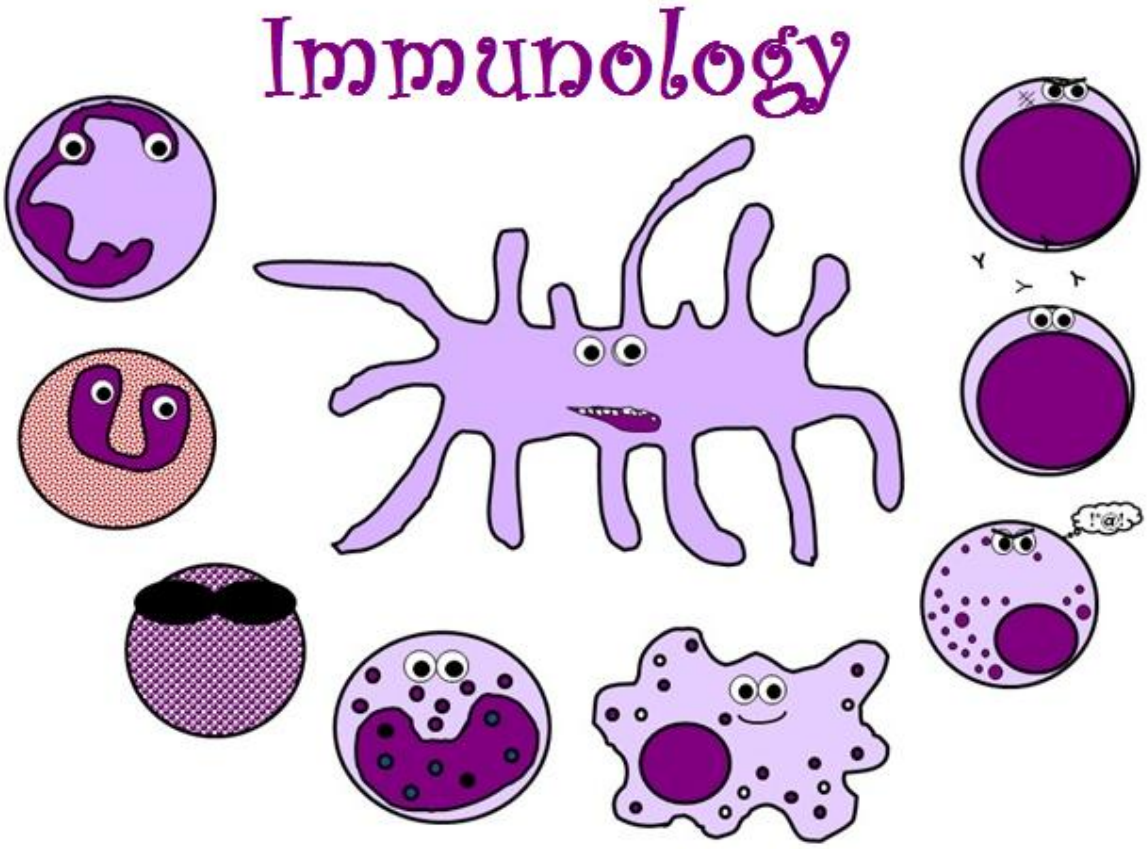

Sheet Slides

Number: 20

Subject: Multiple Sclerosis "MS"

Done by: Ayat M.Zghoul

Corrected by:

Doctor: Issa Abi Dayya

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By the name of Allah the Compassionate the Merciful

Multiple Sclerosis

An autoimmune attack on the central nervous system.

As we saw in rheumatoid arthritis (Case 36), autoimmune disease can be caused by activated effector T cells specific for self peptides. When T cells recognize self-peptide:MHC complexes and become activated, they can cause local inflammation by activating macrophages, for example, with consequent tissue damage. Another example of a T cell-mediated autoimmune disease is the neurologic disease multiple sclerosis (MS). However, unlike the involvement of autoantibodies in some types of autoimmune diseases (see Case 42), it has been difficult to prove the involvement of T cells in MS, because T cells do not cross the placenta into the fetus and experimental T-cell transfer is not allowed in humans.

Seventy years ago, an experimental model of MS was established in mice, in which the injection of myelin in adjuvant caused the development of neurologic symptoms similar to those of MS. This disease is called experimental autoimmune encephalomyelitis (EAE). The antigens in myelin that can induce EAE are myelin basic protein (MBP), proteolipid protein (PLP), and myelin oligodendrocyte glycoprotein (MOG). The disease can be transferred to syngeneic animals by cloned antigen-specific T-cell lines derived from animals with EAE (Fig. 40.1). When the recipient animals are immunized with MBP, for example, they develop active disease. T cells specific for MBP, PLP, and MOG have been found in the blood and cerebrospinal fluid (CSF) of patients with MS.

Topics bearing on this case:

- Inflammatory reactions
- Interactions of co-stimulatory molecules with their receptors
- The development of tolerance to self antigens
- Activation of self-reactive T cells
- Immunologically privileged sites
- Experimental autoimmune encephalomyelitis
- Induction of oral

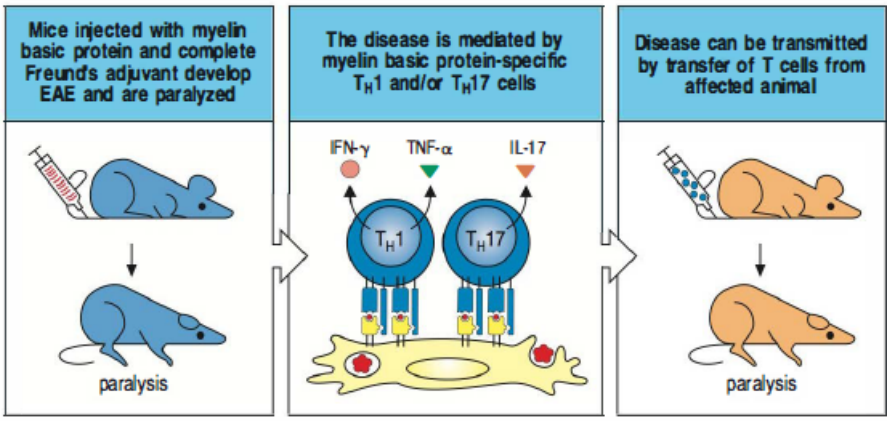


Fig. 40.1 T cells specific for myelin basic protein mediate inflammation of the brain in experimental autoimmune encephalomyelitis (EAE). This disease is produced in experimental animals by injecting them with isolated spinal cord homogenized in complete Freund's adjuvant. EAE is due to an inflammatory reaction in the brain that causes a progressive paralysis affecting first the tail and hindlimbs before progressing to forelimb paralysis and eventual death. One of the autoantigens identified in the spinal cord homogenate is myelin basic protein (MBP). Immunization with MBP alone in complete Freund's adjuvant can also cause these disease symptoms. Inflammation of the brain and paralysis are mediated by T_H1 and/or T_H17 cells specific for MBP. Cloned MBP-specific T_H1 and/or T_H17 cells can transfer symptoms of EAE to naive recipients provided that the recipients carry the correct MHC allele. In this system it has therefore proved possible to identify the peptide:MHC complex recognized by the TH clones that transfer disease.

The case of Vivie Warren: an oboist who has difficulty reading a musical score.

Mrs Vivie Warren, a 29-year-old professional oboe player, was in good health until one

The case of Vivie Warren: an oboist who has difficulty reading a musical score.

Mrs Vivie Warren, a 29-year-old professional oboe player, was in good health until one morning she noticed a loss of vision in her left eye. Her physician referred her to a neurologist, who found that her eye movement was normal and not accompanied by any pain. The visual acuity in Vivie's left eye was 20/100 and in her right eye 20/200. Her retina was normal, and a detailed neurologic examination also proved normal. The neurologist diagnosed optic neuritis (inflammation of the optic nerve). Her family history was informative, however, in that her mother had severe MS and was permanently disabled, and a magnetic resonance imaging (MRI) brain scan was ordered.

Vivie was given a 5-day course of intravenous corticosteroids, and her vision returned to normal over the next 3 weeks. The MRI scan revealed multiple lesions in the white matter of the brain under the cortex and around the ventricles (Fig. 40.2). Intravenous injection of gadolinium, a contrast agent that leaks from blood vessels in recently inflamed tissue, showed that some of the brain lesions were probably of recent origin (Fig. 40.3). The neurologist told Vivie that she had a high probability of developing MS and advised her to return for frequent neurologic examination.

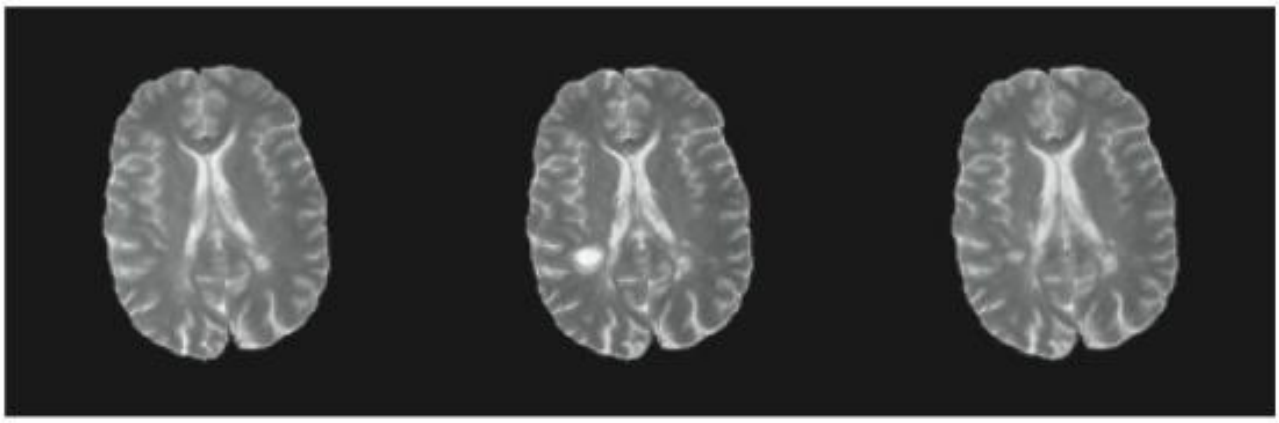


Fig. 40.2 Three-dimensional magnetic resonance images of the brain at three different time points in the course of MS. Left, early; center, during acute exacerbation; right, after therapy. The technique used causes fluid to appear white. The lateral ventricles in the middle of the brain scan and the sulci of the cerebral cortex around the edge appear white as a result of normal cerebrospinal fluid. The white spots, which are due to edema fluid and decreased myelin, are MS lesions.

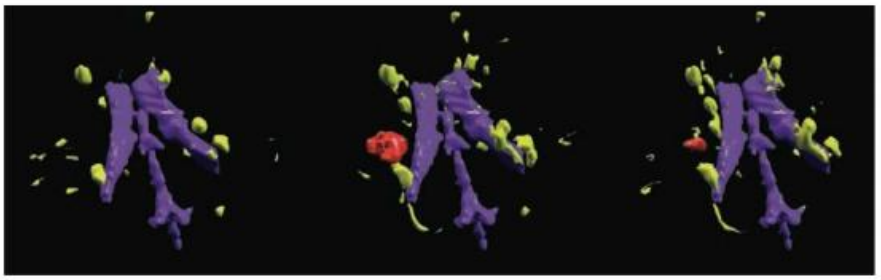


Fig. 40.3 Three computer-generated images of MRI scans of the brain shown in Fig. 40.2 at the same time points. Several levels of MRI scans have been integrated by computer. The lateral and middle ventricles appear in purple. Old MS lesions appear yellow-green. A new, gadolinium-enhanced, lesion appears orange. On the right, this lesion has diminished in size after immunosuppressive therapy.

Vivie remained well for a further 3 years and then developed weakness of the muscles on the left side of her face that were innervated by the seventh cranial nerve. A repeat MRI scan with gadolinium enhancement showed new lesions in the left middle cerebellar peduncle and in the pons. CSF was obtained by lumbar puncture. It contained 28 mg dl^{-1} protein (normal) and 8 lymphocytes ml^{-1} (normal 0–3 ml^{-1}). At this point a firm diagnosis of MS was made. Despite the normal level of protein in the CSF, the IgG content was raised. On electrophoresis, discrete bands of IgG were observed, indicating clonal expansion of restricted B-cell populations in the central nervous system (CNS). Another 5-day course of corticosteroids was administered intravenously, and Vivie's symptoms improved. Weekly intramuscular injections of interferon (IFN)-β were started to prevent progression of the disease.

Muscle weakness developing on left side; repeat MRI scan; MS diagnosed.

Vivie did well for 3 more years, after which she developed a weakness in her left leg and left hand. Her speech became slurred. She developed nystagmus (rapid uncontrolled horizontal jerking eye movements when attempting to fix the gaze on something) and ataxia (wide-based staggering gait). Vivie was given another course of corticosteroids, after which her symptoms improved, but 8 months later they recurred. The injections of IFN-β were stopped and she was put on high doses of cyclophosphamide and corticosteroids at monthly intervals. After 3 months of this therapy, the cyclophosphamide and corticosteroid injections were gradually reduced to every 12 weeks. Her neurological examination became normal and no new lesions were observed on gadolinium-enhanced MRI.

Relapse; give weekly IFN-β.

Relapse; aggressive immunosuppressive therapy started.

Multiple sclerosis.

Multiple sclerosis (MS) was first described by the great French neurologist Jean-Martin Charcot in the 1860s. It was noted at autopsy that patients who died of this disease had multiple hard (sclerotic) plaques scattered throughout the white matter of the CNS. The disease is 10 times more frequent in women than in men and is associated with HLA-DR2. Those affected have a variety of nervous symptoms, such as urinary incontinence, blindness, ataxia, muscle weakness, and paralysis of limbs. The plaques characteristic of the disease show dissolution of myelin along with infiltrates of lymphocytes and macrophages, particularly along blood vessels. The inflammatory exudate causes increased vascular permeability.

The CNS is a relatively immunologically privileged site from which antigens do not normally reach the lymphoid tissues, and so there is no negative selection of T cells with the potential to react against CNS antigens. In MS, an unknown injurious event is presumed to provoke the release of CNS antigens and their presentation to lymphocytes in the peripheral lymphoid organs. This results in the expansion of clones of autoreactive T cells and their differentiation into T_H1 cells, which home to the CNS and initiate inflammation. These T_H1 cells can be readily identified in the CNS of patients with MS.

Lymphocytes and other blood cells do not normally cross the blood–brain barrier. If tissue becomes inflamed, however, activated CD4 T cells autoreactive for a brain antigen and expressing α_4:β_1 integrin, which binds vascular cell adhesion molecules (VCAM) on the surface of activated venule endothelium, can migrate out of the blood into the brain. There they reencounter their specific autoantigen presented by MHC class II molecules on microglial cells and produce pro-inflammatory cytokines such as IFN-γ (Fig. 40.4). Microglia are phagocytic macrophage-like cells of the innate immune system resident in the CNS and, like macrophages, can act as antigen-presenting cells. Inflammation causes increased vascular permeability, and the site becomes heavily infiltrated by activated macrophages and T_H1 cells, which produce pro-inflammatory cytokines that exacerbate the inflammation, resulting in the further recruitment of T cells, B cells, macrophages, and dendritic cells to the site of the lesion. Autoreactive B cells produce autoantibodies against myelin antigens with help from T cells. Activated mast cells release histamine, contributing to the inflammation. In some way that is not yet fully understood, these combined activities lead to demyelination and interference with neuronal function.

Mice deficient in IFN-γ are not protected from the development of EAE, suggesting that additional T_H cells may drive CNS inflammation. T_H17 cells are a recently identified helper T-cell population that can be induced from memory T cells by a combination of cytokines including IL-6, IL-21, and transforming growth factor-β (TGF-β) and are sustained by IL-23. Although T_H17 cells do not seem to mediate inflammation in all contexts, studies in mouse EAE and human MS reveal a pathologic role for T_H17 cells and their associated cytokine, IL-17. Adoptive transfer of MOG-specific T_H1 and T_H17 cells differentiated *in vitro* induces EAE with a distinct histologic appearance, indicating that several effector T-cell types drive autoimmunity in MS. Autoreactive T_H1 cells with specificity for MS-associated myelin antigens can be found in healthy patients without MS. Investigators searching for the suppressive mechanisms that inhibit potentially autoreactive T cells in healthy individuals have focused on regulatory T cells (T_{reg} cells), a subset of naturally occurring CD4 $CD25^+$ T cells that promote peripheral tolerance and inhibit autoimmunity in multiple organs. Although patients with MS have normal numbers of T_{reg} cells, the cells have a decreased ability to suppress autoreactive T cells *in vitro*. Future research will seek to further investigate the regulation of T_H-cell subtypes and the interplay of autoreactive and suppressive T cells in MS.

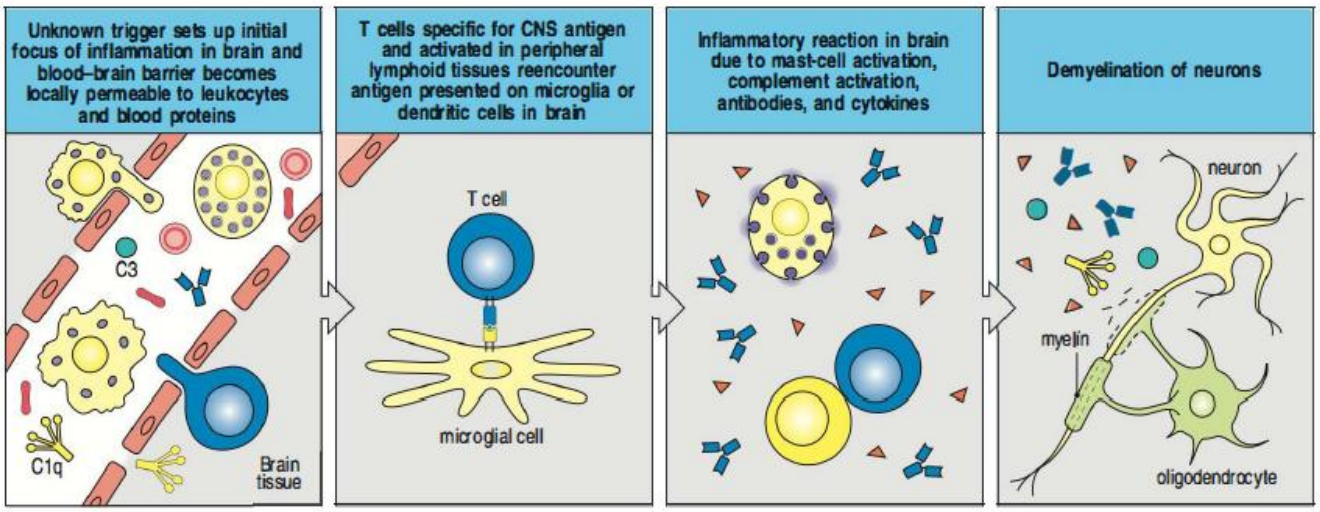


Fig. 40.4 The pathogenesis of multiple sclerosis. At sites of inflammation, activated T cells autoreactive for brain antigens can cross the blood–brain barrier and enter the brain, where they reencounter their antigens on microglial cells and secrete cytokines such as IFN-γ and IL-17. The production of T-cell and macrophage cytokines exacerbates the inflammation and induces a further influx of blood cells (including macrophages, dendritic cells, and B cells) and blood proteins (such as complement) into the affected site. Mast cells also become activated. The individual roles of these components in demyelination and loss of neuronal function are still not well understood.

In mice, feeding with MBP before immunization with MBP in adjuvant protects against the development of EAE. MBP-specific T cells can be identified in the brain of protected mice, but they secrete TGF-β rather than IFN-γ and fail to initiate an inflammatory reaction. Furthermore, adoptive transfer of T cells from mice fed MBP can confer protection against EAE. Therapeutic attempts to treat patients with MS by oral MBP have not been successful, however. This suggests that therapeutic interventions that induce oral tolerance may not be effective in already established disease.

Questions.

1 *Oligoclonal immunoglobulins were found in Mrs Warren's central nervous system. How do you explain this?*

2 *Mrs Warren was treated with corticosteroids, cyclophosphamide, and IFN-β. What was the aim of this therapy?*

3 *An attempt has been made to treat MS patients with IFN-γ. Can you predict what the outcome was and why?*

4 *What is the rationale behind feeding MBP to mice to prevent EAE?*

5 *Can you predict whether EAE can be induced in CD28 knockout mice?*

Answer 1

The oligoclonality of the immunoglobulins in the cerebrospinal fluid reflects the activation of a limited number of B-cell clones that have gained entry into the central nervous system after the breakdown of the blood–brain barrier. Only those B cells that recognize antigen via their surface immunoglobulin receptor and receive a stimulatory signal from an activated T cell will proceed to synthesize and secrete immunoglobulins.

Answer 2

Corticosteroids and cyclophosphamide (a powerful cytotoxic drug) inhibit T-cell proliferation and thus interfere with the secretion of cytokines that drive the inflammation and further T-cell activation. The mechanism of action of IFN-β is not known. More recently, a monoclonal antibody, natalizumab (Tysabri), that targets the α_4 integrin subunit has been reapproved in the United States for a restricted subset of patients with MS, after being withdrawn from the market in 2005 because three patients developed progressive multifocal leukoencephalopathy due to the JC virus. This drug is aimed at blocking the movement of leukocytes from the blood into sites of inflammation.

Answer 3

The patients got markedly worse. IFN-γ upregulates the expression of MHC class II molecules and thus enhances antigen presentation. In addition, it drives the differentiation of T_H1 cells, which are involved in the pathogenesis of MS.

Answer 4

Proteins eaten as part of food have long been known not to elicit routine immune responses. The reason seems to be that there are antigen-specific mechanisms in the gut for suppressing peripheral immune responses to antigens delivered by mouth. One is that when T cells in gut-associated lymphoid tissues are presented with orally delivered protein antigens in the absence of an infection, a lack of co-stimulatory signals induces the T cells to become anergic. Another involves the development of regulatory T cells (see Case 18), which can actively suppress antigen-specific responses after rechallenge with antigen. Such cells produce cytokines, including interleukin-4 (IL-4), IL-10, and TGF-β, which inhibit the development of T_H1 responses and are associated with low levels of antibody and virtually absent inflammatory T-cell responses. However, attempts to treat MS in humans by feeding the MBP antigen have proved unsuccessful.

Answer 5

EAE cannot be induced in mice lacking CD28. CD28 on T cells is the receptor for the B7 co-stimulatory molecules, which are essential for the activation of naive antigen-specific T cells, including T cells that recognize MBP. In contrast, mice in which the cell-surface protein CTLA-4, another receptor for B7 molecules, has been knocked out develop EAE more readily than their normal littermates. This is because CTLA-4 binds B7 molecules about 20 times more strongly than does CD28, and normally delivers an inhibitory signal to the activated T cell. In CTLA-4 knockout mice, this inhibitory signal is missing and so the T cells are more readily activated.

Doctor's Notes ...

- Multiple sclerosis " MS " is **Type 4 hypersensitivity disease** that **measns it's not dependent on the antibodies** , but when we do some tests we notice that there is increase in the IgG?! but what we mean by antibody *dependent that if there are no antibodies then the diseae wouldn't occur* ,in our case **" MS " , the disease occur when there are auto reactive T cells !**
- It's T cell-mediated autoimmune disease **; TH1** cells attacking the myelin basic protein (**MBP**) in the CNS .
- Remember that , Asthma , Anaphylactic shock and allergies are mostly **TH2 mediated response.**
- SO , both TH1 and TH2 can be good and harmful . *In other words think of them as absolutely necessary (in normal cases) and as any other components in abnormal situations they are pathogenic* ; TH1 is important in leishmaniasis and cancer but it's bad in case of MS . TH2 is needed in the parasitic infections but it's bad in other cases " mentioned above " .

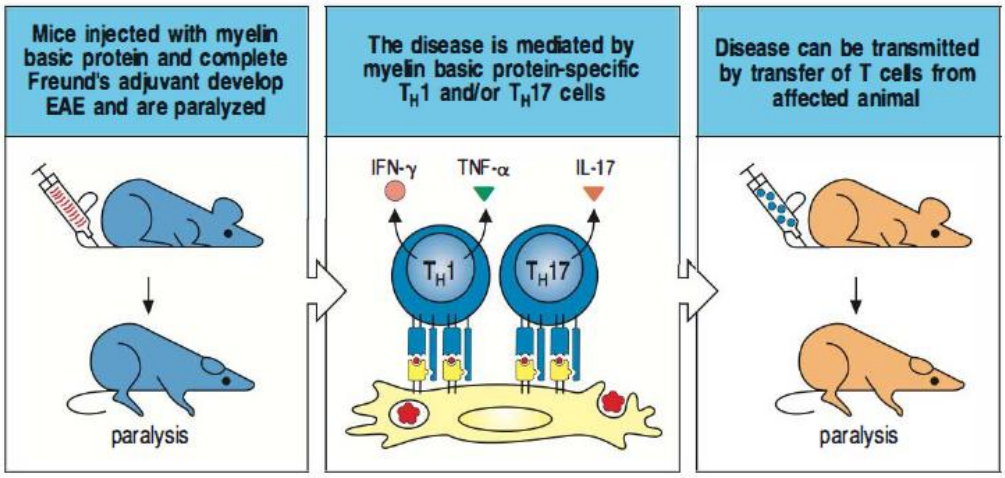
- Experimental autoimmune encephalomyelitis (**EAE**) :
  - Inject the mice with the myelin basic protein (MBP) { the target of the disease } in the **adjuvant** causes a progressive paralysis affecting first the tail and hind limbs before progressing to forelimb paralysis and eventual death.
  - Inflammation of the brain and paralysis are mediated by T H 1(Mainly) and/or T H 1 7 cells specific for MBP . { TH1 and TH17 are pro inflammatory and secret several cytokines that contribute in the inflammation : INF-gamma , TNF-alpha and IL-17 } that produce effect mimicking the MS in those mice .

What's the Adjuvant ?!

When the antigen **isn't strongly immunogenic or even not immunogenic at all** then to have a good immune response we mix these weak immunogenic antigens with adjuvant **to rise their immunogenicity** then the immune system would exist a strong immune response , that why we use adjuvant in **vaccinations** .
In other words the adjuvant is a *substance which enhances the body's immune response to an antigen.*

  - To prove that MS is Type 4 hypersensitivity and Igs don't play a critical role in , they do **Adoptive T cell Transfer :**
    - We bring two **syngeneic mice with similar genetic** background.
    - The **1st one already has EAE** then we isolate the lymphocytes then isolate **the specific T cells (specific for MBP)** .
    - Then inject these specific T cells in **the 2nd mouse { does not have EAE** } , this mice would **develop EAE mimicking the MS** .
    - That proves >> MS is a T cells mediated auto immune disease { **T cells alone are sufficient to result in the MS** } .

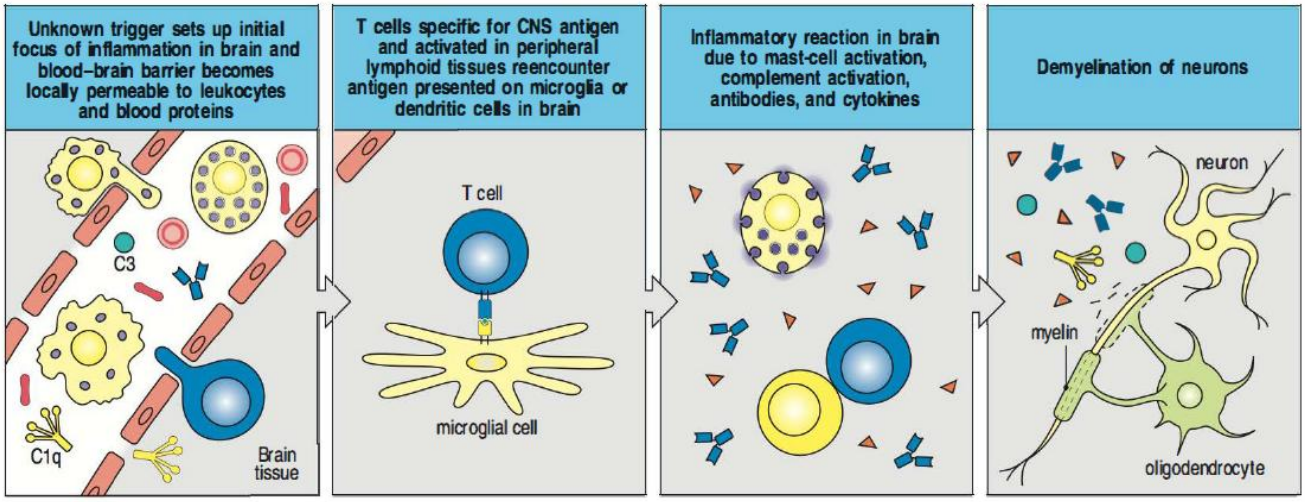
- EAE is very important because **it's the only way to study MS** ; it's really difficult to study MS on Human as we noticed in the case of the Vivie Warren , **the progression of the disease is very slow** , we need 8 years or more to study one case ! ,,, then we use the mice in this study for many reasons : short life span , fast breed then we case study the MS in shorter time !



❖ The mode of Action :

It's mostly **the breakage of Blood Brain Barrier** { tight barrier that won't allow many of the immune cells to enter in } . When these T cells enter **the immune reaction** take place , that will also cause complement activation , antibodies and cytokines >> **Demyelination** occurs .

*** ***very important note*** : the antigens of the CNS are **not available in plenty in the lymphoid tissue and thymus** } then the **–ve selection of the T cells is limited** , that result **in releasing many of auto Tcells in the circulation** , then what can protect us from the MS ?! **the perpheral tolerance mechanisms** do that thankfully ☺ , the most important mechanism is the **T regulatory cells** { who have defect T reg will go on mostly to develop MS }



The case of Vivie Warren :

- ✓ Age of 29 years >>> **the disease is not limited to the elderly it can occur at any age !**
- ✓ oboe player , then she couldn't be able to see the notes properly ,, notice that onset of disease is visual >> **sudden loss of vision in one eye { In Myasthenia gravis , dropping of eye and diplopia { double vision } }**
- ✓ when there is **vision loss** we have to think of two things :
    1. **visual issues** directly related to : the strength of vision , the pressure of the eye , corneal and retinal structures and so on >> it's a job of ophthalmologist
    2. **neurological issues** >> taking a family history especially ask of **MS** , then do brain **MRI and gadolinium** …
- ✓ what is **Gadolinium** pathway ?!
    - when we inject the patient intravenously with **gadolinium**, it will go to the **sites of inflammation** which are **new lesions** and stain them with **red** color . the **old** lesions won't be stained then they appear as **yellow**..
    - the importance of gadolinium :
        - ➢ to show us the **new lesions and where is the active inflammation** >>> more accurate !
        - ➢ It helps to **see the response to treatment** , when we give the patient corticosteroid the lesion would shrink .

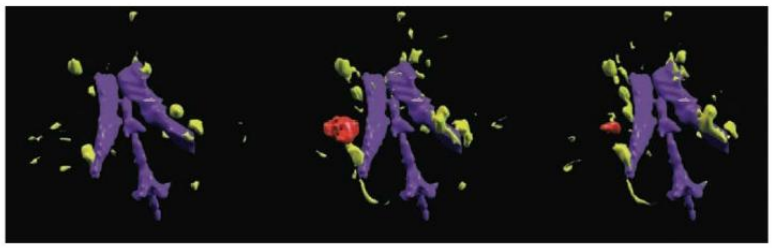


Fig. 40.3 Three computer-generated images of MRI scans of the brain shown in Fig. 40.2 at the same time points. Several levels of MRI scans have been integrated by computer. The lateral and middle ventricles appear in purple. Old MS lesions appear yellow-green. A new, gadolinium-enhanced, lesion appears orange. On the right, this lesion has diminished in size after immunosuppressive therapy.

- ✓ Treatment for 1st attack >> **Corticosteroid " immunosuppressant** ">>> her vision return to normal and the lesion become smaller .
- ✓ **3 years later** she developed weakness of the muscles on the left side of her face that were innervated by the seventh cranial nerve**.>>> the progression of the disease is slow** .
- ✓ Now the **lumbar puncture** is must >> no body want to try it but it's necessary in this case
- ✓ We also do **Protein Electrophoresis (PEP)** for both serum and CSF { **Cerebrospinal fluid} but in multiple myelomas only for serum ! { to be discussed in more details later on in the sheet }**

- ✓ We also do **CSF analysis** { the proteins , lymphocytes , Immunoglobulin level >>> are they normal or abnormal ?! } in this case it's found **that there increase in the IgG level and the lymphocytes** .

- ✓ Treatment for the 2nd attack >>> **corticosteroid again and INF-Beta** {{{ **<u>notice that we don't use INF-gamma because it is mainly involved in the pathogenesis of the MS , if I give it to the patient I just worsen his state !!! }}} **very important</u>**

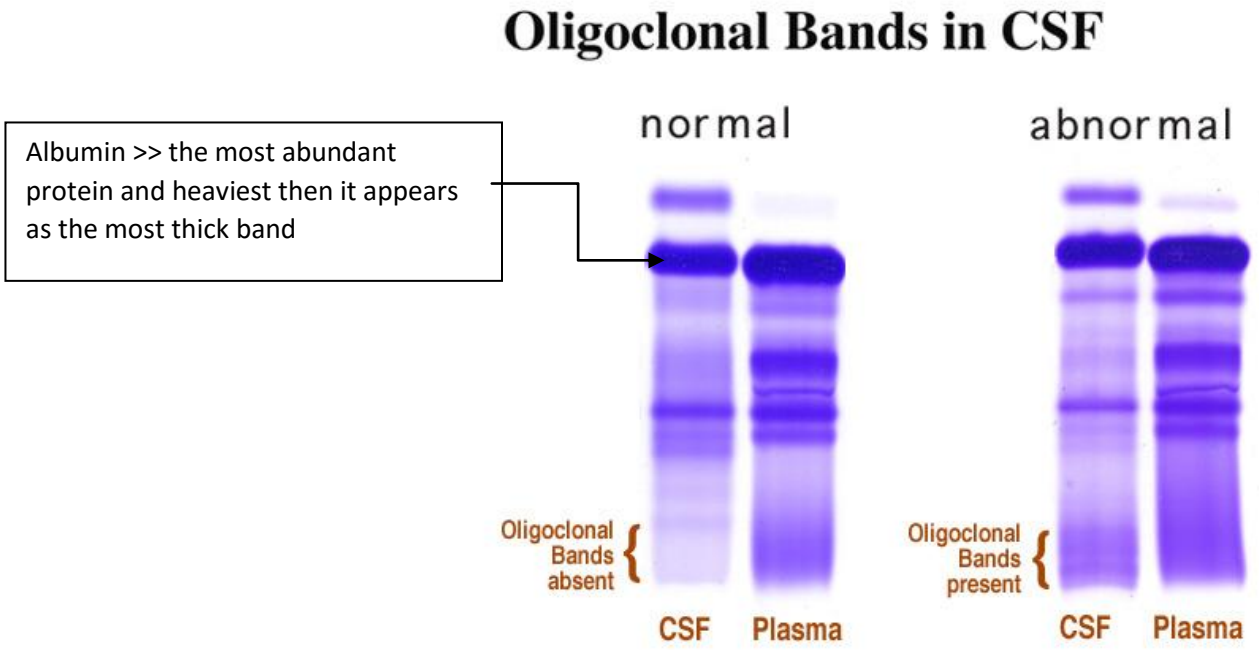
- ✓ The inherence is **related to MHC** , specially is associated with **HLA-DR2**. It's found that who have this HLA type *are more able to present the components of myelin based proteins then MS* .

- ✓ Again**, 3 years later** , She developed **nystagmus** (rapid uncontrolled horizontal jerking eye movements when attempting to fix the gaze on something) { see this video https://www.youtube.com/watch?v=hby75sZSAvg } , **ataxia** (wide-based staggering gait) and **sturring** (unable to speak properly).

- ✓ Treatment for the 3rd attack **>>> strong corticosteroid therapy and cyclophosphamide** >>> it's really aggressive disease , notice we use **cyclophosphamide which is cytotoxic drug used in cancer therapy to kill those autoreative T cells .**

- ✓ How do the drugs given to the patient with MS work ?
    1. **corticosteroid** therapy >>> **immunosuppressant**
    2. **cyclophosphamide** >>>**cytotoxic** drug used to treat **cancer** .
    3. **INF-beta >> unknown pathway !**

- ✓ ***Now back to study the Protein Electrophoresis in MS , in more details*** >>



- ✓ # Remember : the arrangement of proteins the electrophoresis : Albumin , alph1Globulins , alph2 Globulins , beta Globulins then gamma Globulins .

- ✓ *Protein Electrophoresis is used mainly to diagnose Multiple myelomas ; we see the M spike in the*

- ✓ **Normally** the **gamma Globulins fraction is smeary " Not Bands ".** why ?!
  because there are **polyclonal antibodies** { with different isotypes , glycosylation, molecular weight } ,,, **seeing a smear is a good thing** ; if you see band then Monoclonal Gammopathy >> Multiple Myeloma or monoclonal gammopathy for unkowon cause " MGUS " { may or may not progress into Multiple myeloma } .
- ✓ **In MS ,** we compare between **the serum electrophoresis and CSF** ; in **normal** situation in the **CSF** the gamma fraction is **clear** "**no oligoclonal bands**" but in **MS** patients the gamma fraction has **oligoclonal bands** " NOT SMEARY " this give s suggestion **that immune cell infiltrate the CSF** then those T cells **activate other immune cells** including **some B cells** which when activated they produce **special kinds of immunoglobilins not found in the serum** { These B cells are only active in the CSF not in the serum **, that's why they only appear in CSF** } that help in the diagnosis of MS .

- ✓ The **Diagnosis** of MS :
  Depending on the clinical symptoms and lab studies on the CSF "its components of proteins , Igs , lymphocyes " , PEP , MRI and gadolinium.
  It's not an easy disease to diagnose unless you are a Good Doctor !

- ✓ In immunology , when you **fed the antigen** " taking it **orally** "then the antigen actually can cause **tolerization** why ?!
  - ➢ because the gut try to produce tolerization to most thing enter to , **logic** ?!
    imagine that your body produce immune response to anything you eat :O !!

  - ➢ some antigens that develop systemic diseases or disease in other tissues like MS in the myelin sheat can be given orally to mice *these mice don't develop MS ,,,the tolerization mechanism will spread to the lymphatic organs then suppress the immune response that caused the auto immunity* >> this trial is **Successful** in Mice but not Human ☹!

- You can think of it as a **transient vaccination for autoimmune diseases** :O ,,
  { ***transient*** *because the tolerization isn't something genetic so they have to take these antigens continuously specially for high risk patients* } but **that is unfortunately not applied on human beings for an unkown reason !**

- ✓ **EAE cannot be induced in mice lacking CD28** >>> CD28 important for **Costimulation** by binding to the costimulatory molecule on B cells " b7 ", then if *there is no CD28 then no costimulation ,no activatiom then No disease !*

- ✓ Mice **which lack CTIA-4 develop EAE more readily** >>> ***CTLA 4 is important in the supression of the T cells*** *>> then if it's removed T cells won't be suppressed and more active T cells >>> worsen the state in case of MS ☹*

Sorry for any mistake ^^

Shout out to **my awesome squad <3**

Ayat M.Zghoul