Experimental Autoimmune Encephalomyelitis: A Model of Multiple Sclerosis

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Multiple Sclerosis: Year 1868

• Jean-Martin Charcot provides first documented description of Multiple Sclerosis (MS)
• French Neurologist and Pathologists
• Described the disease based on similar clinical observations and pathology observed by himself and other neurologists.
“On histological sections, Multiple Sclerosis lesions contain perivascular inflammation and demyelination. Plaques occur anywhere within the white matter of the central nervous system. The most frequently affected sites are optic nerves, brainstem, cerebellum and spinal cord. Lesions in these locations often correlate with clinical symptoms. In the cerebral hemispheres periventricular distribution of plaques is often seen. When plaques are adjacent to the cortex, subcortical myelinated nerves are often spared. Plaques located near the gray matter, may spread into the gray matter, including deep nuclei and the cortex. Axons are spared within the initial lesions, but are later destroyed."
Multiple Sclerosis (MS)

MS is a disease of the Central Nervous System (brain and spinal cord) and affects the myelin, the insulation around the nerves. Myelin is damaged, reducing conduction along the axons.
Multiple Sclerosis (MS)

**Incidence:** 1/1000

**Female:Male Ratio** 4:1

Historically, about 50% of MS patients become wheelchair-bound within 10 years of diagnosis.

**Disease course** varies.

**Cause:** Unknown

**Main symptoms of Multiple sclerosis**

- **Central:**
  - Fatigue
  - Cognitive impairment
  - Depression
  - Unstable mood

- **Visual:**
  - Nystagmus
  - Optic neuritis
  - Diplopia

- **Speech:**
  - Dysarthria

- **Throat:**
  - Dysphagia

- **Musculoskeletal:**
  - Weakness
  - Spasms
  - Ataxia

- **Sensation:**
  - Pain
  - Hypoesthesias
  - Paraesthesias

- **Bowel:**
  - Incontinence
  - Diarrhea or constipation

- **Urinary:**
  - Incontinence
  - Frequency or retention
Year: 1885

Louis Pasteur

Joseph Meister
Year: 1888

• First cases of paralysis associated with rabies vaccination in humans.
• Rabies in a neurotrophic virus, so initially believed to be caused by inadequate attenuation of rabies virus.
## TABLE III

*Summary of the Results of Experiment XI in Which Monkeys Received Repeated Injections of Emulsion and Extracts of Normal Rabbit Brains*

<table>
<thead>
<tr>
<th>Monkey No.</th>
<th>No. of injections of brain emulsions</th>
<th>No. of injections of brain extracts</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>26</td>
<td>5</td>
<td>9</td>
<td>Negative. Used in Experiment XII</td>
</tr>
<tr>
<td>27</td>
<td>5</td>
<td>9</td>
<td>&quot; &quot; &quot; &quot; &quot; XII</td>
</tr>
<tr>
<td>28</td>
<td>24</td>
<td>26</td>
<td>Died suddenly after 50th injection. Brain and cord negative</td>
</tr>
<tr>
<td>29</td>
<td>52</td>
<td>41</td>
<td>Negative. Used in Experiment XIII</td>
</tr>
<tr>
<td>30</td>
<td>46</td>
<td>38</td>
<td>Developed a tendency to hold chin on left shoulder, ataxia, and weakness. Sacrificed. Section showed involvement of midbrain, pons, cerebellum, and medulla, with perivascular demyelination</td>
</tr>
<tr>
<td>31</td>
<td>28</td>
<td>24</td>
<td>Developed ataxia, general weakness, and paresis of left leg and arm. Sacrificed. Section through right parietal and temporal lobes showed marked involvement of white matter with perivascular demyelination. Tract degeneration in cord</td>
</tr>
<tr>
<td>32</td>
<td>52</td>
<td>41</td>
<td>Negative. Used in Experiment XIII</td>
</tr>
<tr>
<td>33</td>
<td>52</td>
<td>41</td>
<td>&quot; &quot; &quot; &quot; &quot; XIII</td>
</tr>
</tbody>
</table>

Thomas Rivers - Virologist
Year: 1949

- EAE was induced in mice
- Mice developed a monophasic paraparesis that resolved within a couple of weeks.
- The term “Experimental Allergic Encephalomyelitis” was used to describe this disease in mice.

Year: 1953


Guinea-pig spinal cord suspended in 0.230 ml phenolized distilled water, 0.297 ml of paraffin oil, 0.053 ml Arlacel A and 1.4 mg (dry weight) of killed tubercle bacilli. The injections were made into ten sites (0.07 ml per site).

The rats of each parabiotic pair were of the same sex and, as a rule, littermates; they were matched in weight as closely as possible, usually to within 10 grams. The left side of the “donor” was joined with the right side of the normal mate rat from the ear to the base of the tail, and the peritoneal walls were exposed by blunt dissection. In the earlier experiments an incision was made through the abdominal wall of each animal from the last rib to the ileum and the resulting four edges were sutured together (20). In later experiments, a coelio-anastomosis was established by cutting through the abdominal walls and sewing them so as to form a common peritoneal cavity. In both methods, the ventral edges of the
Year: 1955

• Cases of MS were documented in the Faroe Islands for the first time.

• **Faroe Islands** – An isolated group of islands between Iceland and Norway. The islands were settled by Vikings and thus the genetic background of the islanders was similar to that of the Norwegians (high risk population).

• No known cases of MS were documented on the Faroe Islands prior to World War II.

• British troops occupied the Faroe Islands during World War II.

• It appears that the British troops brought a pathogen that triggered the onset of MS in a genetically susceptible population.

• The pathogen was never found.
Year: 1960

- EAE is transferred in rats by lymph node cells (Paterson PY, J. Exp. Med. 1960;111:119-136)
Year: 1984

- Relapsing Remitting EAE! Looks like MS!

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Adoptive transfer of chronic relapsing EAE in SJL/J mice by LNC primed in vivo and cultured in vitro with GPBP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type and no. of cells transferred $\times 10^{-7}$</td>
<td>Day of first attack</td>
</tr>
<tr>
<td>Expt 1*</td>
<td></td>
</tr>
<tr>
<td>LNC</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>6</td>
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<tr>
<td>6</td>
<td>7</td>
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<td>2</td>
<td>11</td>
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<tr>
<td>0.7</td>
<td>10</td>
</tr>
<tr>
<td>0.7</td>
<td>10</td>
</tr>
<tr>
<td>Expt 2‡</td>
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<td>T cells</td>
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<td>0.7</td>
<td>10</td>
</tr>
</tbody>
</table>

* Observation period was 180 days for this experiment.
† Mice were killed for histological study.
‡ LNC were depleted of B cells before the culture and of macrophages after the 4-day culture. The observation period for this experiment was 95 days.
MS Pathology
MS: Autoimmune Disease?

Actively Induced EAE

Immunize with myelin peptide in CFA

Develop EAE in 10-20 days

EAE Clinical Scores
1=limp tail
2=moderate hind limb weakness
3=severe hind limb weakness
4=hind limb paralysis
5=quadriplegia or moribund
6=death due to EAE
MS: Autoimmune Disease?

Adoptive Transfer Model of EAE

DLN = draining lymph nodes.

Lymph nodes contain lymphocytes that have become activated by the immunization (T cells, B cells, and macrophages).

Isolation of T cells and transfer of these cells is sufficient to cause EAE.

EAE is definitely an autoimmune disease mediated by T cells specific for myelin.
A New EAE Model

T cells express unique receptors on their cell surfaces that allow them to recognize different pathogens, allowing our immune system to fight many different infections very specifically.

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**Modified Model Adoptive Transfer of EAE**

- Remove spleen
- Culture splenocytes with MBP Ac1-11 (+ cytokines)
- Inject cells into wild-type mice
Identifying Therapeutic Targets

What molecules are required for T cells to become encephalitogenic?

T-bet = A transcription factor critical for a subpopulation of T cells to differentiate into effector T cells.

Therapeutic Strategy = Small interfering RNA (siRNA)
Identifying Therapeutic Targets

T-bet does not appear to be important in most immune cells. Therefore, inhibiting this molecule was unlikely to cause immune suppression.

![Graph showing mean clinical score over days post immunization for different T-bet genotypes. The x-axis represents days post immunization, ranging from 0 to 25. The y-axis represents mean clinical score, ranging from 0.0 to 3.5. The graph includes data for T-bet +/+ (n=2), T-bet +/- (n=3), and T-bet -/- (n=5). The bars indicate the variability in clinical scores over time.]}
RNA Interference


Attempted to overexpress carotenone synthase (CHS) in pigmented petunias by introducing a chimeric CHS gene to enhance color. In contrast, the petunias were white. Further analysis revealed that the mRNA for both CHS genes was being made, but levels of CHS mRNA in the cells was 50-fold lower than normal.

Virus-induced gene silencing (VIGS)
Small Interfering RNA (siRNA)

DNA → Transcription → RNA → Translation → Protein

Replication

Synthetic siRNA → Argonaute 2 → RNA induced silencing complex (RISC)

Guide strand of siRNA

mRNA complementary to siRNA

RISC with guide strand can continue to bind mRNA

AAA

RNA degradation

AAA
T-bet siRNA prevents myelin-specific T cells from transferring EAE

Transgenic mice with T cell receptor specific for MBP Ac1-11

Remove spleen

Transfect cells with T-bet siRNA

Activate with MBP Ac1-11

Non-transfected (3/5)
siRNA-NS (3/3)
siRNA-Tbet (1/6)
AS-Tbet (1/6)
AS-GATA3 (2/2)

Mean Clinical Score

Days Post Transfer
Gene Silencing by Systemic Delivery of Synthetic siRNA in Adult Mice

T-bet siRNA as a Therapy for EAE

Prevention

Days Post Immunization

Days Post Transfer

Mean Clinical Score

Mean Clinical Score
T-bet siRNA Ameliorates EAE

siRNA-NS

siRNA-Tbet
Axonal Transection in MS


• Green – nonphosphorylated neurofilaments
• Red – myelin in B and C
• Red – macrophage/microglia in D and E
Nogo-A

Nogo-A is expressed in the adult CNS. It inhibits axonal growth.

Developed Nogo-A siRNA to test in EAE.
siRNA-NogoA Treatment

Control – siRNA-NS

siRNA-NogoA
Nogo-A Expression is Suppressed in Mice treated with siRNA-NogoA

A

Mouse A

Mouse B

B

Target Area [mm^2]

Lesion | Border | Lesion | Border

Target Area

p=0.019  p=0.021

[Image of immunofluorescence images showing CD3 and Nogo-A expression in different conditions]
GAP43, a protein expressed in growing axons, is upregulated with siRNA-NogoA.
Is combining siRNA-Tbet and siRNA-NogoA effective?

![Graph showing mean clinical scores over days post immunization for different treatments: siRNA-NS, siRNA-NogoA, siRNA-Tbet, and siRNA-NogoA+Tbet. The graph indicates a comparison of these treatments over time, with arrows marking specific time points.](image-url)
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