Immune monitoring of patients with multiple sclerosis

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Conflict of Interest Disclosure

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This COI Disclosure Form can be viewed at the ESCCA 2019 Conference website www.escca.eu/norway2019 - Programme section / Accreditation page

Contribution of immune cells in MS pathogenesis

CD4+ lymphocytes

- combined IFN-g- and IL-17-driven condition
- PB Th17 cells may also be indicative of relapse
- Higher proportions of total GM-CSF+, GM-CSF+/IFN-g+ and GM-CSF+/IFN-g+CD4+ T cells
- Lower proportion of Tregs

A. P. Jones et al. Clin and Exper. Immunology 2017

Hallmarks on the understanding of the role of the Th17 pathways in MS

- Increased IL-17 found in the blood and CSF of RRMS patients, especially during relapse
- IL-17-producing T cells identified in EAE
- Increased Th17 cells and IL-17 found in the brain of MS patients
- IL-17 production correlates with MRI activity
- Secukinumab (anti-IL-17A monoclonal antibody) reduces MRI lesions in a phase II clinical trial

Dos Passos GR et al. Mediators Inflamm 2016.

Role of B cells in MS

Ai-Lan Nguyen et al. Br J Pharmacology 2017
Potential B cell functions in multiple sclerosis

B cell lineage and surface marker expression

The role of cytometry lab?

Biological drugs

A substance that is made from a living organism or its products and is used in the prevention, diagnosis, or treatment of cancer and other diseases. Biological drugs include antibodies, interleukins, and vaccines. Also called biologic agent and biological agent.

- Immune check point inhibitors (PD-1, PD-L1, and CTLA-4 targets)
- Immune Cell Therapy (also called Adoptive Cell Therapy or Adoptive Immunotherapy) (TILs, CAR T cells)
- Therapeutic antibodies
- Immune-Modulating Agents
- Therapeutic Vaccines

Among the 10 top biologic drugs in USA

- **Humira** TNF blocker for Rheumatoid arthritis, plaque psoriasis, Crohn’s disease, ulcerative colitis, ankylosing spondylitis, psoriatic arthritis, polyarticular juvenile idiopathic arthritis
- Rheumatologists, gastroenterologists
- **Rituxan** (rituximab) anti CD20 for Non-Hodgkins lymphoma, chronic lymphocytic leukemia, rheumatoid arthritis, multiple sclerosis
  - Rheumatologists, hematologists, neurologists
- **Anovex interferon β** for multiple sclerosis
  - Neurologists
Response of MS patients to IFNβ

- Basal blood immune cell subsets contribute to identify MS patients with a high probability of showing an optimal response to IFN-beta.
- Percentages below 3% of CD19+CD5+ cells
- or above 2.6% of CD8+perforin+ T cells
- increased the probability of achieving no evidence of disease activity status during treatment.

Raquel Alenda et al J of Neurology 2018

**Multicenter study for the gating strategy regarding CD19+CD5+ and CD8+perforin+ cells for the study of the MS patients before IFNβ treatment**

Neslia Villarreal et al Clinica Chimica Acta 2019

**Fingolimod**

- sphingosine-1-phosphate receptor modulator,
- Immunomodulating drug
- It sequesters lymphocytes in lymph nodes, preventing them from contributing to an autoimmune reaction.

Noelia Villarrubia et al J of Neurology 2018

**Monoclonal Antibodies**

- Anti-B cell therapies

Julia Rudnicka et al Clinical Immunology 2015

<table>
<thead>
<tr>
<th>Approved Usages</th>
<th>Other Applications (Literature)</th>
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<tbody>
<tr>
<td>- Secondary Sclerotic Arthritis</td>
<td>- Severe Sarcoidosis</td>
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<td>- Autoimmune Hemolytic Anemia</td>
<td>- Sjögren’s Syndrome</td>
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<td>- Anterior Uveitis</td>
<td>- Psoriasis Arthritis</td>
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<td>- Relapsing-Remitting MS</td>
<td>- Rheumatoid Arthritis</td>
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<td>- Primary Progressive MS</td>
<td>- Inflammatory bowel disease</td>
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<td>- Relapsing-Remitting SLE</td>
<td>- Chronic obstructive pulmonary disease</td>
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<td>- T2D</td>
<td>- Polyarteritis nodosa</td>
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<tr>
<td>- Sjögren’s Syndrome</td>
<td>- Raynaud’s phenomenon</td>
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<td>- Type 1 Diabetes Mellitus</td>
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<td>- Demyelinating diseases</td>
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<td>- Ophthalmic Neuritis</td>
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<td>- Chorioretinitis</td>
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<td>- Anti-IL6 Aba Remant in Transplants</td>
<td>- Autoimmune pancreatitis syndromes</td>
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<td>- Anti-IL12 Aba Remain in Transplants</td>
<td>- Autoimmune hepatitis</td>
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**B-Cell Depletion Therapies With Anti-CD20 Continue to Expand in the Treatment of Immune-Mediated Diseases**
Examples of monoclonal antibodies

- **Rituximab** anti-CD20
  - Chimeric mouse-human IgG1 mAb that binds to the CD20 cell surface epitope on circulating B-cells
  - Although rituximab has not been approved for the treatment of MS, it can be approved for off-label use in certain countries and it is definitely in use.
  - More studies are to follow.

- **Ocrelizumab** anti-CD20
  - Recombinant humanized IgG1 antibody that binds to a different but overlapping epitope compared with rituximab
  - Approval 2017??

- **Ofatumumab** anti-CD20
  - Totally human IgG1 antibody that binds to a different but overlapping epitope compared with rituximab
  - Approval expected to be complete by July 2019

B cell stages of differentiation - MoAbs action

MS patient under Rituximab treatment follow-up
ON TOTAL MEMORY

ON TOTAL NAIVE

TRANSITIONAL DEFINITION

PLASMA BLASTS

Patient 1
Multiple sclerosis
No anti-CD20 treatment
Patient 2
Multiple sclerosis
No anti-CD20 treatment

Patient 3
Multiple sclerosis
anti-CD20 treatment

Patient 4
Multiple sclerosis
After stopping anti-CD20 treatment

Patient 4
Multiple sclerosis
After stopping anti-CD20 treatment
Phenotypic and functional characterization of lymph node (LN) B cells after a single Rituximab dose (no B cells in PB)


Examples of monoclonal antibodies

- Natalizumab for MS
  - humanized IgG4 mAb
  - directed against the α4 subunit of the α4β1 and α4β7 integrins
  - prevents migration of leukocytes through the blood-brain barrier
  - modulating leukocyte recruitment and activation in the CNS
  - Increase of pre-B cells in PB (Krumholz et al., 2008; Saraste et al., 2016)
  - Approved 2004 / suspended 2005 (PML) by infection of oligodendrocytes by the John Cunningham Virus (JCV) / approved 2006 FDA, EU under follow-up

- Natalizumab treatment selectively increased the effector memory T-cell pool but not the activation state of T-cells in the blood
  - Lars Bornsen et al. PLOS one 2012

- Long term follow up of PB lymphocyte subsets after Natalizumab treatment

Examples of monoclonal antibodies

- Alemtuzumab for MS
  - humanized IgG1 mAb
  - directed against CD52
  - rapid and profound depletion of CD52+ cells by three mechanisms:
    - antibody dependent cell-mediated cytotoxicity (ADCC)
    - complement dependent cytotoxicity (CDC) and
    - induction of apoptosis (Freedman et al., 2013; Buck et al., 2015), with ADCC being the most likely predominant mechanism (König et al., 2014; Lycke, 2015).
  - This is by repopulation of peripheral T- and B-lymphocytes with an alteration in the number, proportions and functions of certain lymphocyte subsets, such as increased regulatory T-cell subsets and memory T-cells (Hartung et al., 2015; Mila, 2016).
  - predominance of immature and, later, naive-memory B-cell subsets
  - Approved 2013, 2014
Neutropenia with

- CD3-CD(16 + 56+): 47%
- CD3 + CD8+ T cells increased compared to baseline
- No B cells, very low CD3+CD4+ cells
- On 70th day 500/μl ANC, treatment discontinued
- Neutropenia resolved
- LGL (50% decrease) but % of NK cells high (48%), CD3+CD8+ % normal
- Immune derived neutropenia due to the MoAb treatment
- MS remission

CD52 expression on innate lymphoid and myeloid cells

Alemtuzumab-induced changes in the dendritic cell compartment
Alemtuzumab-induced changes in monocytes

Catharina C. Gross et al
Neurol Neuroimmunol Neuroinflamm 2016

Alemtuzumab-induced changes in the innate lymphoid cell (ILC) compartment
6 months after alemtuzumab treatment, specific DC subsets are reduced, while CD56bright NK cells expanded in patients with MS

Could it lead to autoimmunity?

Catharina C. Gross et al
Neurol Neuroimmunol Neuroinflamm 2016

Examples of monoclonal antibodies
• Daclizumab for MS
• humanized IgG1 mAb
• directed against CD25a
• expansion of immunoregulatory CD56 bright natural killer (NK) cells, which can utilize IL-2 via their low-affinity IL-2 receptor (Knier et al., 2014).
• Approved 2014 FDA, EU

Memory B Cells are Major Targets for Effective Immunotherapy in Relapsing Multiple Sclerosis
Therapies targeting CD4+ cells seem to decrease memory B cells as well.

Active DMD in MS physically or functionally deplete memory B cell activity.

David Baker et al
Ebiomedicine 2017

MS patient follow up

David Baker et al
Ebiomedicine 2017
Other populations to be studied

- Th17 cells
- Tregs
- Proinflammatory CD20+ T cells
- Myeloid and DCs

Examples of monoclonal antibodies

- Humira TNF blocker
- Associated with lymphomas

Many thanks to

- Serafeim Karathanos