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Deciphering the cellular and molecular events leading to a successful remyelination in multiple sclerosis patients

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THESE DE DOCTORAT
DE L'UNIVERSITE PIERRE ET MARIE CURIE

Ecole doctorale: Cerveau, Cognition, Comportement

Deciphering the cellular and molecular events leading to a successful remyelination in multiple sclerosis patients

Explorer et définir les évènements cellulaires et moléculaires nécessaires à une remyelinisation efficace chez les patients atteints de sclérose en plaques.

Présentée et défendue par
Charles Sanson
Pour obtenir le grade de Docteur de l'UPMC

Jury

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Abstract

Inflammation in the central nervous system leads to demyelination and neurodegeneration in patients with multiple sclerosis (MS). While the etiology of the disease is not known, genetic studies have highlighted 110 genetic variants responsible for MS susceptibility.

The processes responsible for the spectrum of disease severity are not well understood. However, the severity of MS is closely correlated with the efficacy of myelin repair, or remyelination, a process whose effectiveness is very heterogeneous among patients. Why remyelination fails or succeeds in patients remains largely unknown, mainly because this process has never been addressed in a humanized pathological context.

To tackle this question, we developed a new model combining focal demyelination in nude mice and graft of MS or healthy donor (HD) lymphocytes (LT). We were able to reproduce at least partially remyelination heterogeneity, with some patients LT inducing a defect in remyelination and some others inducing a myelin repair comparable to HD. Comparing the secretory profile of patient LT according to their repair capacities, we highlighted an heterogeneous cytokine response to the same stimulus, leading to a differential microglial activation and ultimately an efficient or inefficient differentiation of precursor cells during remyelination.

To decipher why LT from patients have differential abilities to respond to a stimulus, we hypothesized that the genetic variants known for MS susceptibility could also drive disease severity by influencing LT functions and therefore remyelination. We found that the interaction of variants associated with genes responsible for T Folicular Helper and naïve Th0 cells functions induce the secretion by patient LT of a cytokine profile leading to a pro-inflammatory activation in microglia, leading to remyelination failure.

Overall, we discovered a new role for LT in remyelination: they orchestrate MIG activation and are therefore at least partially responsible for the success or failure of the myelin repair process. Capitalizing on patients with high repair capacities to understand the cellular and molecular actors leading to successful remyelination in pathological conditions seem to be a key approach to develop therapeutical targets.

Résumé

Les processus inflammatoires médiés par le système immunitaire induisent une demyélinisation et une mort neuronale dans le système nerveux central des patients atteints de sclérose en plaques (SEP). L'étiologie exacte de la maladie est inconnue, même si la connaissance de sa composante génétique a été enrichie par la découverte de 110 variants responsable de sa prédisposition.

Les évènements responsables de l'hétérogénéité de sévérité de la SEP chez les patients sont encore mal compris. Néanmoins, la capacité de remyélinisation (un procédé endogène durant lequel la myéline détruite est reformée), elle aussi très hétérogène chez les patients, est intimement corrélée à la sévérité des symptômes. Les raisons des différences d'efficacités de remyélinisation chez les patients ne sont pas connues, principalement car cette hétérogénéité n'a jamais été étudié dans un contexte expérimental approprié.

Pour mieux comprendre ce phénomène, nous avons développé un nouveau modèle murin humanisé en combinant une démyélinisation focale chez des souris nude à une greffe de lymphocytes (LT) provenant de donneurs sains (DS) ou de patients SEP. Nos résultats ont démontré que cette greffe permet de reproduire au moins partiellement l'hétérogénéité de remyélinisation présente chez les patients : Certains LT de patients induisaient un défaut de remyélinisation, pendant que d'autres induisaient une réparation de la myéline comparable à celle induite par les LT de DS. En comparant le profil de sécrétion des patients selon leur capacité à induire un contexte favorable à la remyélinisation, nous avons mis en évidence des différences dans la sécrétion de certaines cytokines, ce qui induisaient selon le cas une activation bénéfique ou délétère des cellules microgliales ayant pour conséquence une différentiation efficace ou entravée des cellules précurseurs d'oligodendrocytes pendant la remyélinisation.

Pour comprendre pourquoi les LT de patients répondaient de façon différente à une même stimulation, nous avons émis l'hypothèque qu'une interaction entre les variants génétiques de prédisposition à la SEP pouvait également modifier la fonctionnalité des LT pendant la remyélinisation. Nous avons mis en évidence que les LT de patients ayant des variants associés à des gènes impliqués dans la fonction des lymphocytes T CD4+ folliculaires (TFH) et des

lymphocytes T naïfs (Th0) induisaient un défaut de remyélinisation lorsque greffés dans une lésion démyélinisée, et ce en dirigeant l'activation microgliale vers un phénotype délétère.

En résumé, nous avons mis en évidence un nouveau rôle pour les lymphocytes lors de la remyélinisation : ils influencent l'activation microgliale et sont donc au moins partiellement responsables de l'échec ou de la réussite de la réparation de myéline chez les patients. La façon dont ils réagissent à la démyélinisation est au moins en partie due au patrimoine génétique du patient.

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Abbreviations

- APC: Antigen presenting cell
- Arg-1: Arginase 1
- BBB: Blood brain barrier
- CC1: Adenomatous polyposis coli protein clone antibody CC1
- CCL: C-C motif chemokine ligand
- CCR: C-C motif chemokine receptor
- CD: Cluster of Differentiation
- CNPase: 2',3'-Cyclic-nucleotide 3'-phosphodiesterase
- CNS: Central nervous system
- CSF : Cerebrospinal fluid
- CXCL: C-X-C motif chemokine ligand
- CXCR: C-X-C motif chemokine receptor
- DHSs: Desoxyribonuclease I hypersensitivity sites
- EAE: Experimental auto-immune encephalomyelitis
- EBV: Epstein-Barr virus
- EDSS: Expanded disability status scale
- FDA: Food and drug association
- eQTL: Expression quantitative trait loci
- GA: Glatiramer acetate
- GalC: Galactosylcerebroside
- GALT: Gut-associated lymphoid tissue
- GFAP: Glial fibrillary acidic protein
- GWAS: Genome-wide association study
- HD: Healthy donors
- HLA: Human leukocyte antigen
- HSC: Hematopoetic stem cells
- IFN: Interferon
- IGF-1: Insulin growth factor 1
- IL: Interleukin
- IMSGC: International multiple sclerosis genetics consortium
- iNOS: Inducible nitric oxide synthase
- LT: Lymphocyte
- LPC: Lysophosphatidylcholine
- MBP: Myelin basic protein
- MHC: Major histocompatibility complex
- MIG: Microglia
- MOG: Myelin oligodendrocyte glycoprotein
- MRI: Magnetic resonance imaging
- MS: Multiple sclerosis
- MSC: Mesenchymal stem cells
- MS: Multiple sclerosis
- MSSS: Multiple sclerosis severity score
- NPC: Neural precursor cell
- NO: Nitric Oxyde
- OL: Oligodendrocyte
- OPC: Oligodendrocyte precursor cell
- P0: Protein 0
- PDGFR: Platelet-derived growth factor receptor
- PNS: Peripheral nervous system
- PP: Primary progressive
- PML: Progressive multifocal leukoencephalopathy
- ROS: Reactive oxygen species
- RR: Relapsing-remitting
- SC: Schwann cell
- SNP: Single nucleotide polymorphism
- SP: secondary progressive
- Th: T helper
- TNF: Tumor necrosis factor
- Treg : Regulatory T cells
- UV: Ultra violet radiation
- VEP: Visual evoked potential

Chapter I: Introduction

I. Multiple sclerosis : Etiology and treatments

II. Pathophysiology and Immunopathology of multiple sclerosis

III. Remyelination

IV. Aims of the project

I. Multiple sclerosis: Etiology and treatments

1. Foreword

Multiple sclerosis (MS) is an auto-immune disease leading to demyelination and neurodegeneration in the central nervous system (CNS). In this pathology, an abnormal immune response initiated by lymphocytes (LT) (Compston and Coles, 2008; Dendrou et al., 2015) is leading to a chain of events resulting in an invasion of the CNS by both innate and adaptive immune cells, causing neuroinflammation. The inflammatory attacks induce myelin destruction and oligodendrocyte (OL) death. Then, demyelinated axons degenerate causing a heterogeneous spectrum of symptoms in MS patients. The cause of disease onset is still not understood, but MS appears in patients with a genetic predisposition and who are exposed to environmental factors contributing to the triggering of the abnormal immune response.

2. Myelin

A. Structure and physiological myelination

Myelin structure and molecular composition

Myelin is a lipid-rich substance wrapping the axons of neurons. Myelin's structure results from the wrapping of successive layers of plasma membrane of myelinating cells (Figure 1). Myelin is composed by 70% of lipids and 30 % of proteins. In the CNS, myelin is formed by OL. In the peripheral nervous system (PNS), myelin is formed by Schwann Cells (SC). The PNS and the CNS myelin are fairly similar, up to a few exceptions in their protein composition (Aggarwal et al., 2011; Kursula, 2008). Therefore, specific markers exist to discriminate between the two kind of myelin: the Myelin Oligodendrocyte Glycoprotein (MOG) only expressed in the CNS myelin, whereas the Protein 0 (P0) is exclusively expressed in the PNS. Structurally, one SC only wraps one axon, whereas one OL can form myelin on up to 60 segments of axons. Myelin along the axon is not a continuous structure: it forms segments, called internodes, which have an average length of 1µm, separated by a structure called node of Ranvier where the axon is not myelinated. In the node of Ranvier, in the neuron plasma membrane, a high number of sodic and calcic voltage dependent channels are concentrated (Figure 3).

Oligodendrocyte and Schwann cells differentiation during myelination

During the myelination process occurring during development, myelinating OL are derived from the differentiation of oligodendrocytes precursor cells (OPCs). Several waves of OPC migration from ventral and dorsal domain occur during embryonic life. After migration, OPCs

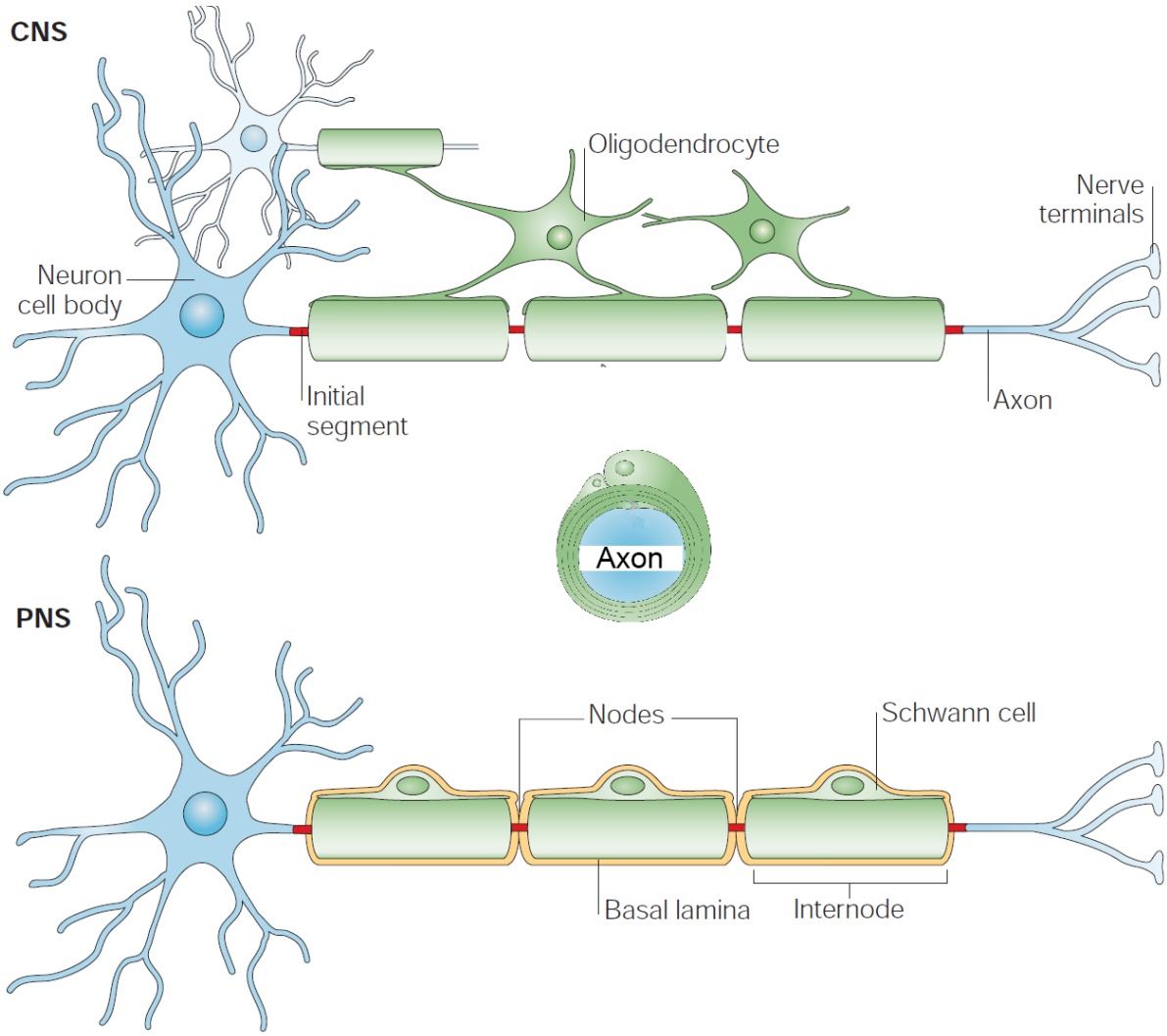

Figure 1: Representation of myelin structure and organization in the CNS and the PNS. In the CNS, myelin is formed by OL that can wrap multiple axonal segments. In the PNS, SC wrap only one axon segment. Modified from (Poliak and Peles, 2003)

differentiate into mature myelin forming OL (Bercury and Macklin, 2015). During the differentiation process, cells of the oligodendroglial lineage go through different stages that can be characterized by specific markers. For instance, OPCs express A2B5 and platelet derived receptor-α (PDGFRα), Pre-OL express O4, mature OL express galactocerebroside (GalC) and adenomatous polyposis coli clone CC1 (CC1) and myelin producing OL express myelin basic protein (MBP), 2',3'-Cyclic-nucleotide 3'-phosphodiesterase (CNPase) and myelin

oligodendrocyte glycoprotein (MOG). The immunostaining against those proteins allow the characterization of the progress of the differentiation process (Figure 2A).

SC arise from neural crest, a multipotent cell population formed in the dorsal part of the neural tube. SC precursors are formed after specification of a subpopulation of neural crest cells when they encounter and contact axons (Jessen and Mirsky, 2005). Then, SC become immature and wrap (without forming myelin) several axons. They can then form myelinating or non-myelinating SC, in part depending on the diameter of the axon they contact (Figure 2B).

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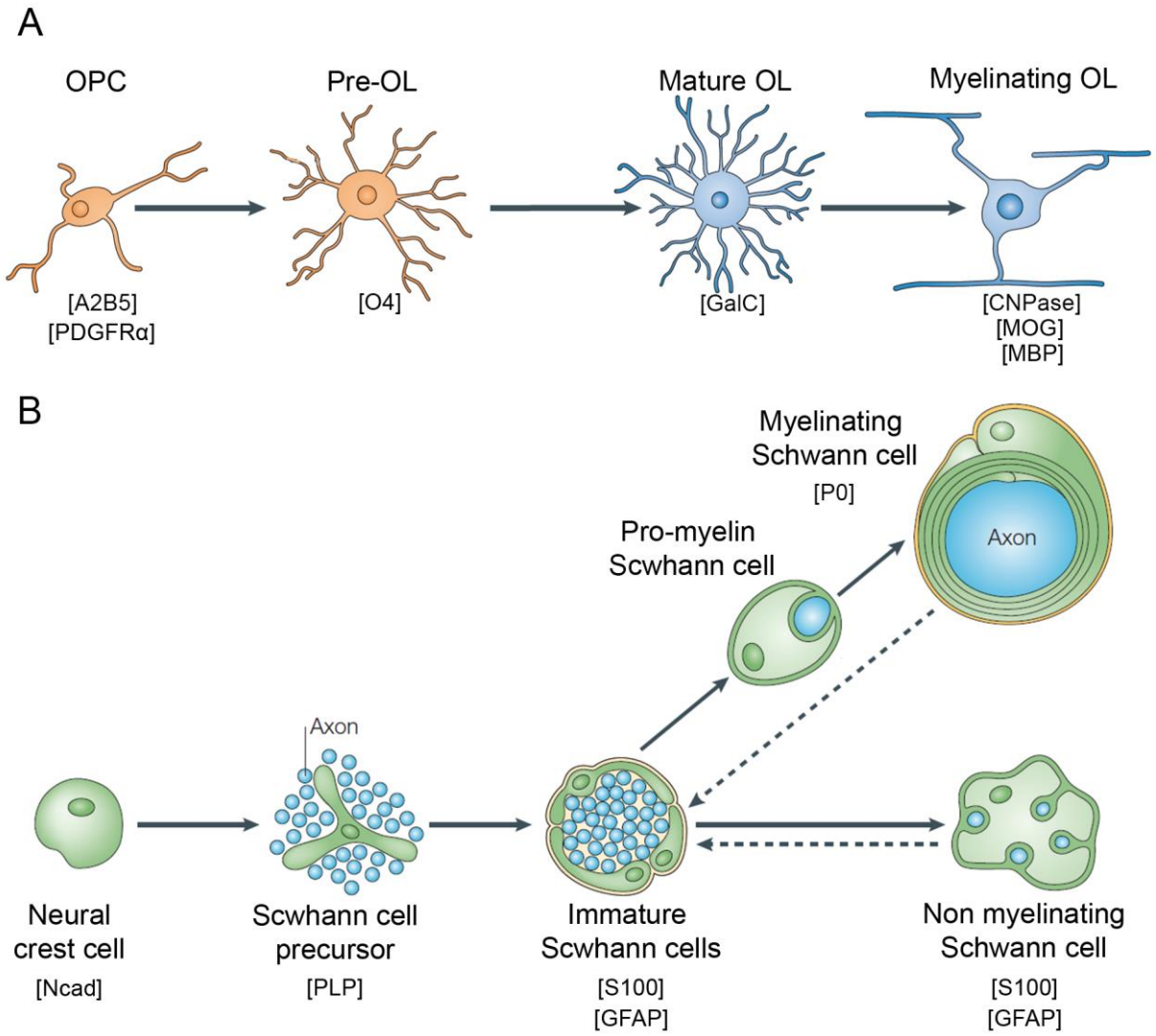

Figure 2: Representation of the OL and SC lineage progression during differentiation. The associated specific markers for each stages are indicated between brackets. Modified from (Jessen and Mirsky, 2005; Nishiyama et al., 2009).

B. Function

Fast axonal conduction

During evolution, organisms grew in size. As a result, axonal conduction speed from the CNS to the extremities of the body was one of the requirements for a fast circulation of the information in the body (Zalc et al., 2008). Myelin allowed an acceleration of the speed of conduction up to 100 times faster by two mechanisms: first, its fat-enriched composition acts like a natural insulator, reducing the capacitance of the axon membrane and therefore accelerating axonal conduction (Figure 3A). Second, the myelin is clustering the voltage-dependant channels at the Node of Ranvier leading to so called saltatory conduction: the axonal influx is going to "jump" from one Node of Ranvier to another, inducing the propagation of the depolarization only in this structure (Figure 3B) (Hartline and Colman, 2007). This way of transmitting depolarization is considerably faster than if the electric current had to pass from one channel to another all along the axon.

Metabolic support and protection of axons

The myelin sheath wrapping the axon also has other, more recently discovered, roles (Fünfschilling et al., 2012; Simons and Nave, 2016). OL provide a metabolic support to neurons by transforming glucose into lactate and pyruvate. These metabolites can be transferred from the OL to the neuron cytoplasm, and used as a source of energy by the neuron. In CNPase 1 knock-out model (in which the gene is inactivated), the myelination occurs but its structure is abnormal (Rasband et al., 2005). This hinders the metabolite exchange between OL and neurons, and leads to axonal transport defects in neurons resulting in an early death of animal due to neuroinflammation and neurodegeneration. In addition, it has been demonstrated that OL can secrete neuronal pro survival factors such as insulin growth factor-1 (IGF-1) and neurotrophins (Byravan et al., 1994; Dai et al., 2001, 2003; Wilkins et al., 2001) . Finally, myelin represents a physical barrier between the axon and the extracellular domain, protecting it from inflammatory stimuli occurring during neuroinflammation.

In summary, myelin is a multi-functional and an indispensable element of the healthy CNS.

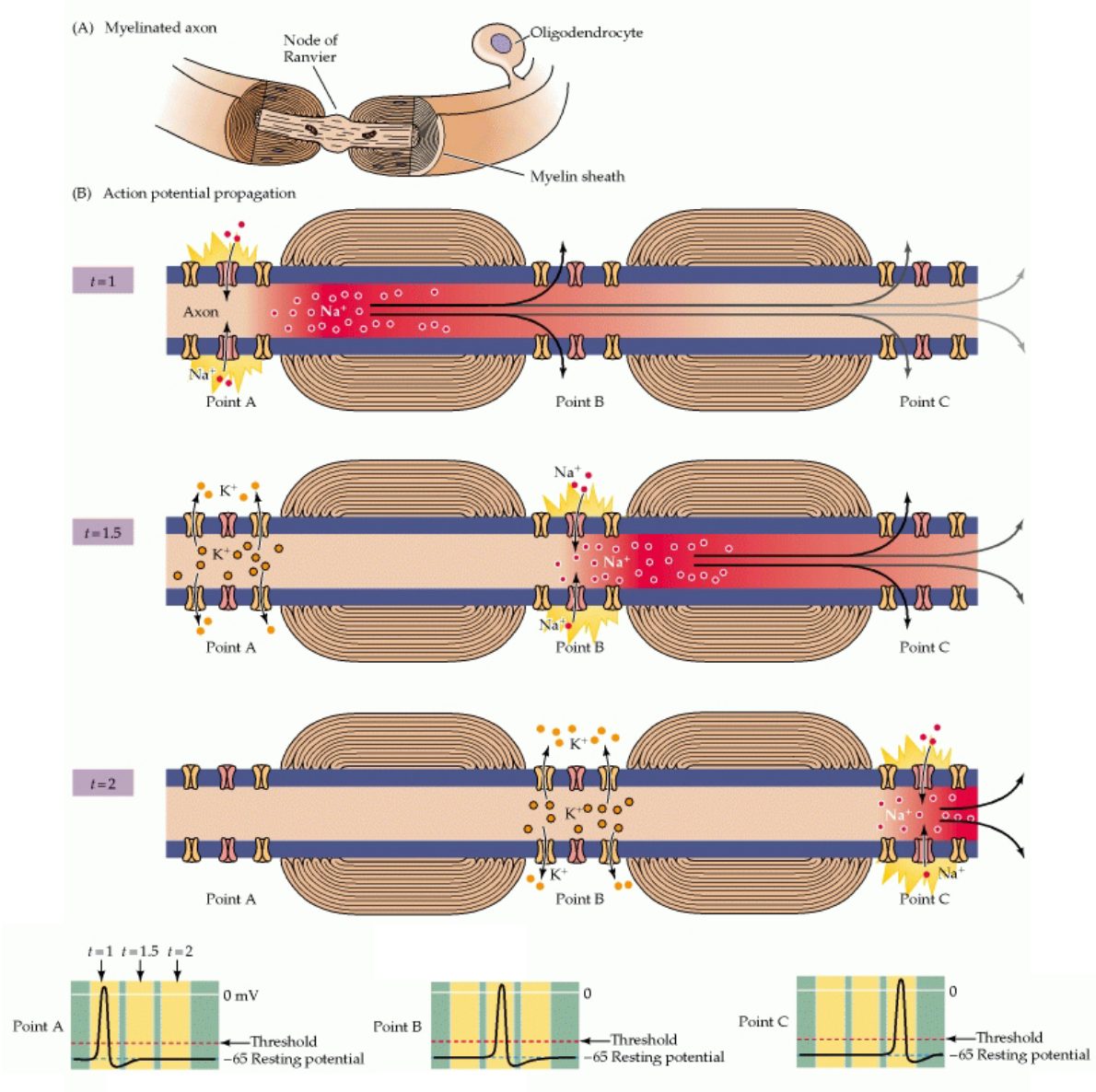

Figure 3: Molecular organization of myelin allowing fast saltatory conduction. (A) Diagram of a myelinated axon. (B) Ion current occurring during saltatory conduction. The depolarization of the axonal membrane only occurs in nodes Ranvier in which myelin is absent and voltage dependent channel are clustered resulting in an accelerated velocity of axonal conduction. The lower panel represent the changes in the axonal membrane potential during the propagation of the action potential. From (Purves et al. 2001).

3. Etiology

A. MS: a complex disease

Diseases can be caused by environmental factors such as viruses, microbes, parasites or toxins but can also be purely genetic, in which the disease is the consequence of a DNA mutation and in which the environment has no effect on disease triggering. The simplest form of genetic diseases is monogenic pathologies: they result from a mutation in a single gene. This mutation triggers an impairment of function in the protein coded by the gene leading to the disease. These mutations are rare, and the disease is hereditary according to Mendel's law. MS is a complex disease, in which both genetic and environmental factors are involved. It occurs in patients carrying predisposition variants and exposed to environmental factors increasing the odds of disease onset. Each of the variants is frequent in the general population and is neither sufficient nor necessary to trigger the disease.

B. Genetic predisposition

Evidence of a genetic component in MS

The simplest and definitive evidence that MS has a genetic component comes from studies of families in which there is an MS patient. MS has a familial recurrence rate of about 20% (20

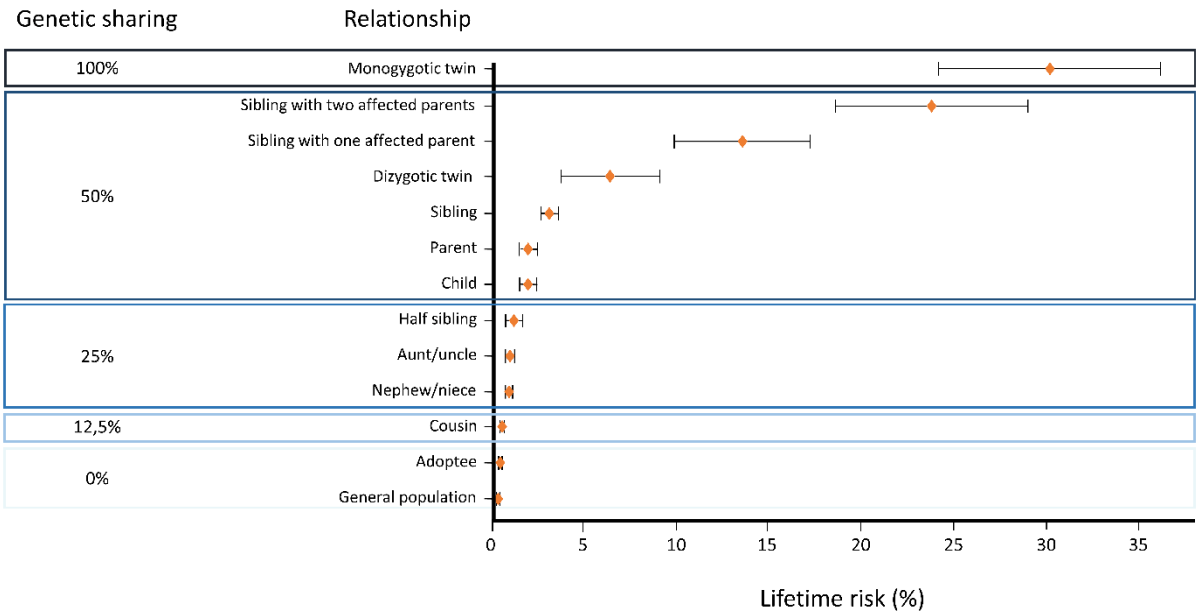

Figure 4: Risk to develop MS in family's age-adjusted recurrence risks for different relatives. These data represent pooled studies from population-based survey. Modified from (Compston and Coles, 2008)

% of MS patients have at least one affected relative). The risk for a monozygote twin to develop the disease when its twin is affected is around 30%, as compared to 5% when the twins are dizygote (Figure 4) (Compston and Coles, 2008; Hansen et al., 2005), demonstrating a genetic involvement in the probability to develop MS. However, as stated above, the genetic causes of MS explain only a part of the susceptibility.

HLA genes and MS

Human leukocyte antigen (HLA) genes are involved in the Human Major Histocompatibility Complex (MHC) located on chromosome 6. This zone of the genome is highly polymorphic, and HLA genes can be divided into two majors groups: class I HLA and class II HLA. They both encode for cell-surface glycoproteins (Strominger, 1986). Class I HLA molecules are expressed by almost all cell types and they will by default present a subset of peptides that have been degraded in the cytoplasm. If a cell presents an exogenous peptide on its class I HLA (for instance, in case of intracellular infection), it will be recognized and the cell will be killed by CD8+ T cells. Class II HLA molecules are only expressed by antigen presenting cells (APCs), which phagocytose and present peptide debris on the surface of the glycoproteins. This can be recognized by CD4+ T cells, triggering the adaptive immune response if the peptide is exogenous. HLA molecules are in this way implicated in immune surveillance and tolerance.

The presence of the allele HLA DRB1*1501 (of the HLA class II) has been known to be a risk for developing MS since the 1970s. The risk to develop MS in individuals homozygous for HLA DRB1*1501 is around 3 times higher compared to someone not carrying the risk allele. This is the genetic factor with the largest impact on the risk to develop MS. In nearly all studies of genetic predisposition, the frequency of this allele was higher in the MS population compared to the healthy controls. Other variants of HLA molecules are known to be either a risk factor (HLA DRB1*03, DRB1*08:01) or a protective factor (HLA DRB1*14:01) (Hollenbach and Oksenberg, 2015).

GWAS and Immunochip: a revolution in the genetics of MS

Until a few years ago, little progress had been made in the understanding of MS genetics. Only a few variants were discovered in addition to the HLA related ones, related to IL7R and IL2RA genes (Gregory et al., 2007; Munoz-Culla et al., 2013). The real revolution happened when genome wide association studies (GWAS) were realized. GWAS is a method screening the genome for single nucleotide polymorphisms (SNP) and evaluating their association with

disease susceptibility. Several thousands of SNPs can be analyzed at the same time. The largest GWAS study of MS was the fruit of an international collaboration between members of the International Multiple Sclerosis Consortium (IMSGC). By comparing 475 806 SNPs in the genome of 9 772 MS patients and 17 376 healthy donors (HD), the study highlighted 34 new susceptibility variants and confirmed 23 others (The international multiple sclerosis genetics Consortium (IMSGC), 2012). Shortly after, a large meta-analysis called Immunochip was performed by analyzing GWAS data from MS and other auto-immune diseases and new variants were discovered, carrying the total to 110 susceptibility variants for MS (The international multiple sclerosis genetics Consortium (IMSGC), 2013) (Figure 5) . The vast majority of the SNPs are closely associated with genes having a role in immune pathways (Sawcer et al., 2014).

Linking genotypes to MS susceptibility and severity

After the discovery of several susceptibility variants for MS, attempts were made to link the genotype of patients with their phenotype to predict disease course and severity. For instance, patients carrying the (HLA) DRB1*1501 allele show cognitive impairments due to more important neuronal degeneration (Okuda et al., 2009). A recently discovered polymorphism in the oligoadenylate synthetase 1 gene is linked to increased disease activity and relapse frequency in patients carrying the risk allele (O'Brien et al., 2010).

Several other polymorphisms have well established consequences on LT functions: A loss of function on regulatory anti-inflammatory processes is involved in MS susceptibility. For instance, Regulatory T cells (Treg) of patients carrying the risk allele of the CD226 gene showed reduced immunosuppressive capacity and therefore could contribute to a decrease of the peripheral tolerance leading to the survival and the proliferation of autoreactive T cells (Piédavent-Salomon et al., 2015). Mice carrying the risk allele also had a loss of function of Treg cells leading to an exacerbated disability score when Experimental autoimmune encephalomyelinitis (EAE), an animal model of MS, was induced. A gain of function of pro-inflammatory processes is also responsible for MS onset: One of the variants associated to the SLC9A9 gene led to a reduced expression of its mRNA in MS patient carrying this risk allele and this reduction induced an increased expression of IFN-γ by T-cells (Esposito et al., 2015). Mechanistically, a reduced expression of SLC9A9 favors differentiation into T helper 1 (Th1)

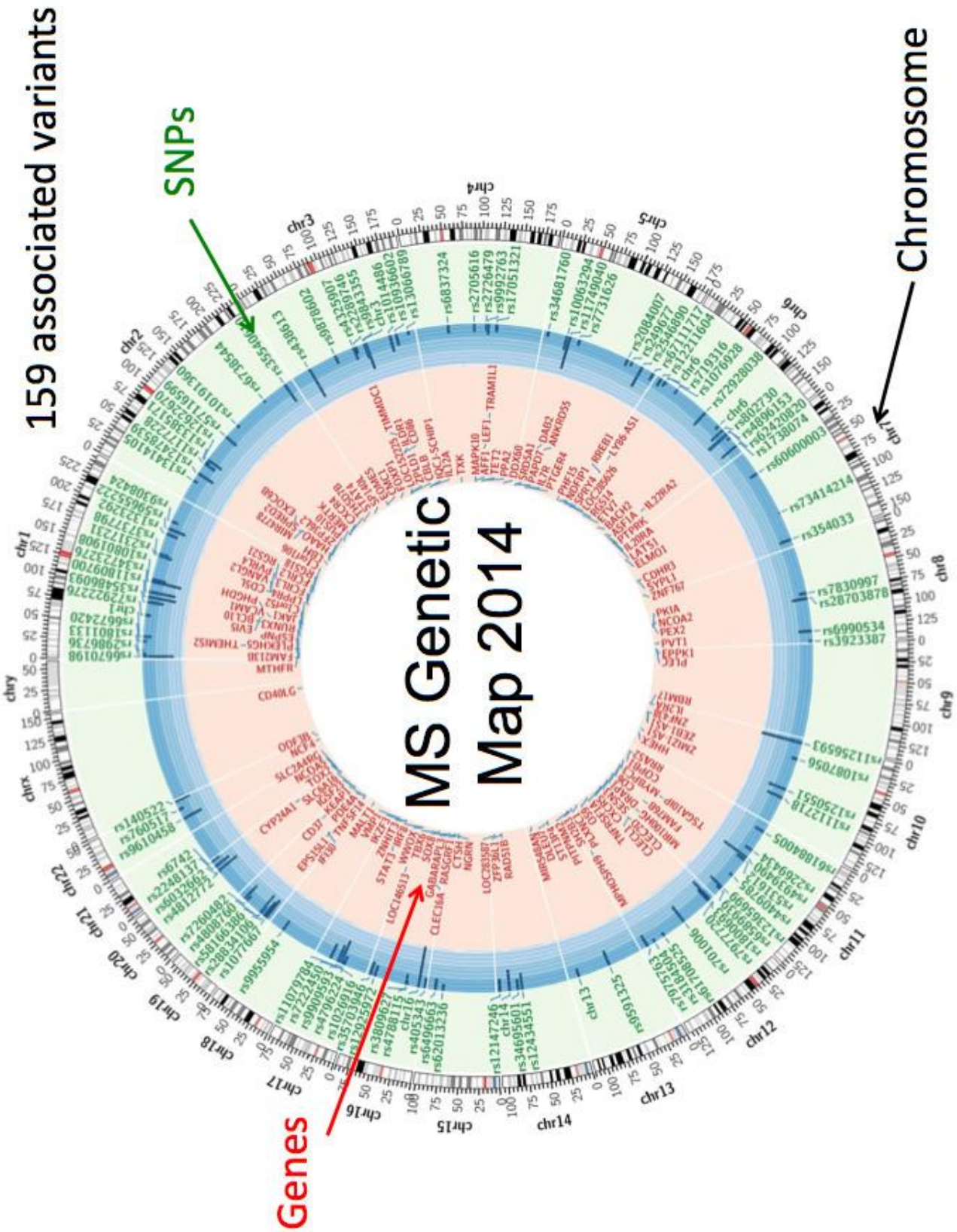

Figure 5: MS genetic maps with all known predisposition SNPs and associated genes and their location in the genome. In 2014, 110 SNPs were correlated with MS susceptibility. From P. De Jager.

Interferon-γ (IFN-γ) secreting cells among $CD4^+$ cells. Th1 cells are one of the pathogenic cell type in MS, and it is therefore likely that this variant can play a role in disease onset.

Interestingly, MS predisposition polymorphisms can also drive disease severity by influencing the myelin repair process: remyelination. In a murine model of demyelination/remyelination, a polymorphism in the epidermal growth factor gene can severely impede the myelin repair process (Bieber et al., 2010). Combined, these data indicate that the SNPs carried by patients not only predispose them to MS, but can also drive the disease evolution by worsening inflammatory attacks or preventing myelin repair. However, the genotype of patients is not yet routinely used in the clinic, and further investigation is needed to predict even partially disease evolution and severity using genomic data.

Epigenetic component of MS

Several elements argue in favor of an epigenetic component in MS: the established interaction between genes and environmental factors (smoking with HLA-DRB1*15:01 for instance (Olsson et al., 2016)) and the fact that the loci discovered for MS susceptibility only explain half of the genetic predisposition risk for MS (Zheleznyakova et al., 2017). But perhaps the most convincing evidence is the study of monozygotic twins. Despite identical genetic background, the risk of the twin of an MS patient developing MS themselves is only 30%. Therefore, another mechanism of gene expression regulation are likely to be involved and epigenetic is the most likely hypothesis (Xiang et al., 2017).

To highlight an epigenetic effect on MS susceptibility, the epigenome of twins discordant for MS have been studied. However, no differences were found in the methylation of CpG islands (the most studied epigenetic trait) of 18000 genes (Baranzini et al., 2010). However, this negative result does not exclude the epigenetic hypothesis, as several other epigenetic mechanisms exist (e.g. acetylation of histones and non-coding RNA) and these have not been studied in detail in MS. Furthermore, recent evidence argues in favor of a critical role of methylation in MHC related genes in patients with the relapsing form of MS (Maltby et al., 2015, 2017).

Multiple other putative epigenetic mechanisms have been proposed to be responsible for MS susceptibility and severity (Küçükali et al., 2015), e.g. miRNA inducing a defect in phagocytosis,

methylation of anti-inflammatory genes (i.e FoxP3) or acetylation of genes in the Th17 pathway, but further studies are needed to validate these hypothesis.

C. Environmental triggers

As previously stated, MS is a complex disease: the disease is triggered in individuals with a genetic predisposition who are exposed to environmental risk factors. Several of these risk factors are known.

North-south gradient of MS prevalence and vitamin D

There is a North-South gradient of the prevalence of MS in the world (Figure 6). Likewise, there is a strong inverse correlation between ultra violet radiation (UV) exposure and risk for MS. In other words, it is likely sun exposure decreases the risk of developing MS.

Vitamin D and its active derivative cholecalciferol and ergocalciferol, has a well described role in calcium metabolism and notably in skeleton remodeling. The main source of Vitamin D in humans is the skin which synthesizes it after exposure to UV. Therefore, there is also a North-South gradient of blood levels of Vitamin D in the world. These phenomena are only correlative and not demonstrated to be causative, but there is an accumulation of clues in the direction of low vitamin D levels as a susceptibility factor for MS (Ascherio et al., 2010; Lucas et al., 2015): Retrospective studies show that in average MS patients had lower vitamin D level in the blood before the disease onset than the general population and people that follow a vitamin D treatment have lower risk of developing MS (Duan et al., 2014; Martinelli et al., 2014). Finally, migration studies show that individuals who have moved from their country of origin to a more southern country have a lower risk of MS (Gale C.R., 1995).

Vitamin D has potent immunomodulatory effects that could explain its protective role for MS (Ascherio et al., 2010; Koch et al., 2013; Prietl et al., 2013). Notably, vitamin D has been shown to increase suppressive properties of Tregs, induce tolerogenic antigen presenting cells, reduce the invasion of macrophages in the CNS during EAE, and foster Th cell differentiation towards the Th2 phenotype which has immunomodulatory properties. In addition, vitamin D levels are lower in MS patients, and there is a correlation between low level of Vitamin D and severity of the disease. However, while vitamin D treatment ameliorated the wellbeing of patients, it did not show any promising effect on disease severity or frequency of relapses.

Other factors could contribute to the north-south gradient of MS prevalence, such as viral infections and alimentary habits. These putative causes are detailed below.

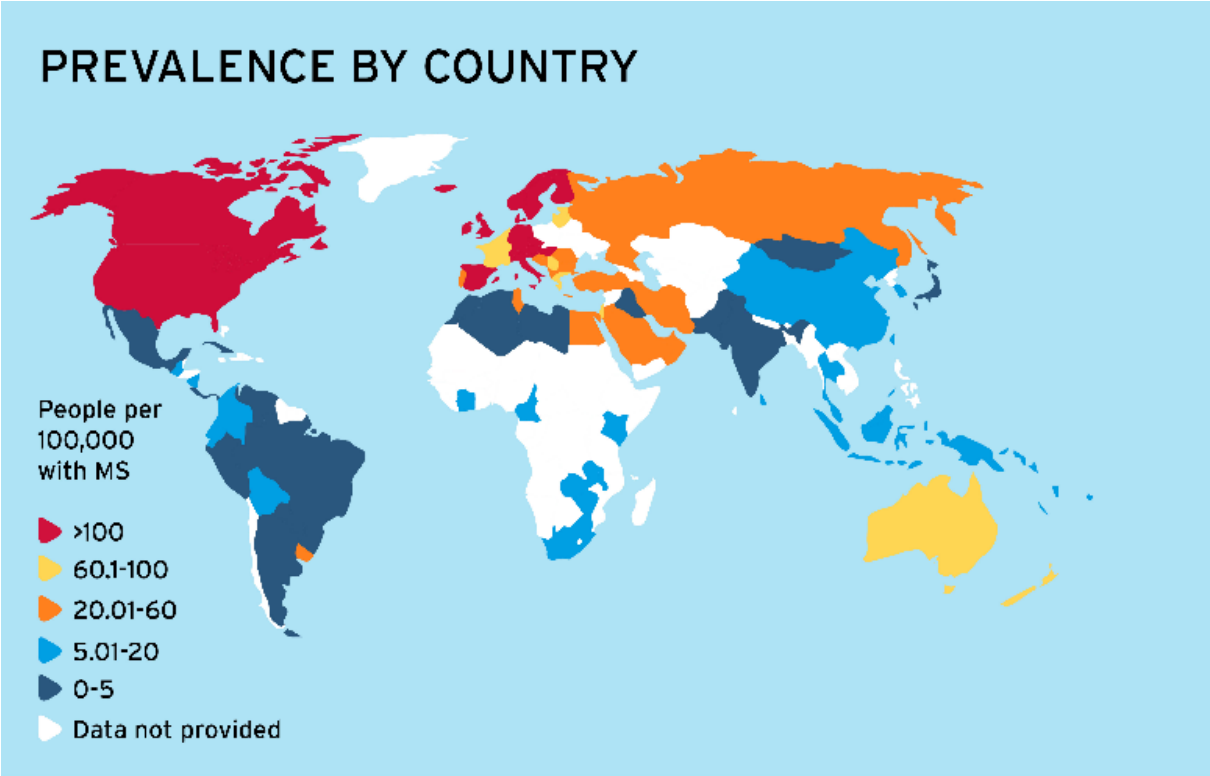

Figure 6: World map of the prevalence of MS in 2014. A clear north-south gradient can be observed. Modified from oysterhc.com

Viral infection

Infection with Herpes virus and Epstein-Barr virus (EBV) increase the risk to develop MS. The link between EBV infection and MS is well established from epidemiological studies, with an approximate 2-fold increase of the risk of developing MS in patients that developed mononucleosis during their lifetime.

The mechanism by which being infected by EBV increases the risk to develop MS is not known. The most intuitive hypothesis is molecular mimicry: if one of the antigens of the virus is very close in its molecular structure from a protein of the myelin, it could trigger the generation of Myelin-reactive T cells (Koch et al., 2013). T cells of MS patients present a cross reactivity between the myelin protein MBP and some of the EBV antigens. Once the virus is eliminated from the system, the inflammatory attack could still occur against myelin that is recognized as

similar to the pathogen. Other hypotheses could also explain the increased risk of MS induced by EBV infection, such as that myelin specific T cells could be activated by a generic inflammatory context induced by the virus, or that the B cells in the meninges, which are often affected by EBV, would secrete antibodies against EBV antigens that cross react with myelin protein, inducing myelin destruction.

Other environmental factors

Smoking has more recently been recognized as a factor leading to an increased risk of developing MS and is associated with a greater disease activity and higher disability (Healy et al., 2009; Manouchehrinia et al., 2013) . There is a dose effect of the number of cigarette smoked daily on the risk for MS (Hernán et al., 2001) but the mechanism behind this risk increase remains unclear even if some putative mechanism have been proposed. In the lung of smokers MS patients, there is an increase of T cell proliferation and an increased number of macrophages. This global immune response could be partially responsible for triggering MS (Ockinger et al., 2016). A salt-enriched diet is also a risk factor for MS, as high dose of sodium chloride leads to a more severe phenotype in EAE. Sodium chloride induces a pathogenic Th17 polarization in $CD4^+$ cells and inhibit Treg functions (Hernandez et al., 2015; Kleinewietfeld et al., 2013).

Other factors could be implicated, such as a fat-enriched diet, alcohol and coffee consumption or night work. However, the experimental proofs are insufficient (due to low numbers of cases or unreliability of the measures) and no causative proofs are established.

Microbiota

The gut-associated lymphoïd tissue (GALT) represents around 80% of the total body's immune system. Every day, because of food consumption, foreign components are constantly passing through the gut and therefore a strong immune tolerance must be put in place to avoid constant immune activation against alimentary products. Bacteria of the gut, or gut microbiota, takes advantage of this immune tolerance to colonize the intestinal tract. Because of the strong interaction between the immune system and the microbiota, a dysfunction of the immune tolerance induced by the microbiota is likely to promote the triggering of autoimmune diseases (Colpitts et al., 2017).

The immune tolerance in the gut is a perpetually moving equilibrium between the induction of pro-inflammatory Th17 cells, essential for our defense against bacterial and fungal pathogens, and Tregs, critical for peripheral tolerance and inflammation resolution. Both of these cell types are numerous in the gut, and bacterial exposure is critical for their maturation (Kamada and Nunez, 2013). As a result, germ-free mice (mice bred in sterile conditions) that do not have a microbiota are protected against EAE because of the drastic reduction of pathogenic Th17 cells (Lee et al., 2011). The equilibrium in the gut can be broken by a pathogenic population of bacteria, or a non-pathogenic bacteria inducing a strong immune reaction. If this reaction appears in genetically predisposed patients, it could lead to the appearance of MS. In the last few years, a lot of studies have been performed to evaluate if the microbiota composition is different in MS patients. It was found that the diversity of the microbiota is reduced in a subpopulation of MS patients, and some bacteria are found enriched in these patients compared to healthy controls (Chen et al., 2016; Lee et al., 2011). Further studies are necessary to understand how the microbiota influences the disease triggering and severity.

4. Clinical description & existing treatments

A. Epidemiology and social cost

MS affects more than 2.4 million people worldwide. The average incidence is about 100/100 000 per year (0.1%) in North America and Europe, and of 2/100 000 (0.002%) in Eastern Asia and Africa. This disease is therefore not classified as a rare disease in the western world. The disease affects more often women than men (sex ratio of 2.7 women per 1 man in 2014 in France (Foulon et al., 2017)), the underlying cause remaining purely speculative.

It is the second cause of disability in young adults in Europe (Compston and Coles, 2008). Even if worldwide data on the mean age of disease onset are difficult to get (due to difference of diagnosis criteria, lack of public available data and other causes) the estimated average age at disease onset is around 30. Because of its occurrence in early adult life and its disabling symptoms, unemployment rate is high in patients. In addition to that, frequent hospitalization and frequent life-long treatment make the societal cost high in MS: 50 k€ per year and per patient in average in Europe (Kobelt et al., 2006).

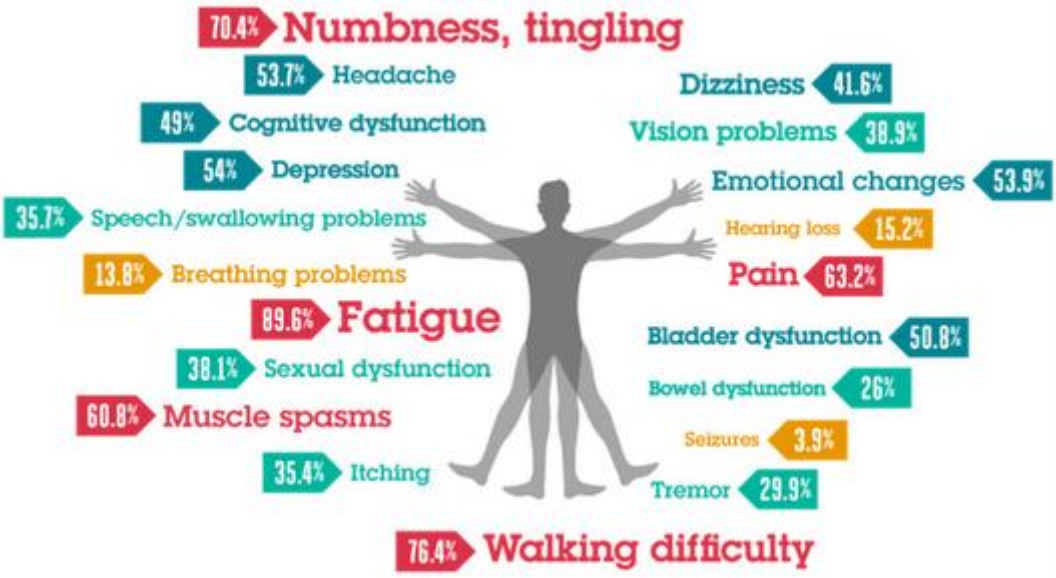

Figure 7: Frequency and spectrum of symptoms affecting MS patients. From the national multiple sclerosis society.

B. Symptoms and diagnosis

MS lesions can occur anywhere in the CNS and the symptoms of patients will depend on the function of the neurons affected by demyelination and neurodegeneration. Therefore MS patients can experience a large spectrum of symptoms (Figure 7), from loss of vision to cognitive impairment. The diagnosis of MS is certain when two lesions in the white matter, separated in time (>1month) and in space appears in the CNS. Usually, the lesions are visualized by magnetic resonance imaging (MRI) (Compston and Coles, 2008). Other diagnosis tools are available to the clinicians such as the high concentrations of IgG antibodies and/or oligoclonal bands in the cerebrospinal fluid (CSF), or measurement of evoked potential (visual, brainstem, sensory) which can be slowed down in case of demyelination (Compston and Coles, 2008). In the clinical routine, disease evolution and severity is calculated only taking the walking difficulty into account (Expanded Disability Status Scale or EDSS), or the EDSS weighted by disease duration (Multiple Sclerosis Severity Score or MSSS).

C. MS clinical forms

The pathology of MS includes three distinct clinical forms (Loma and Heyman, 2011). The most common one, affecting 70-85% of patients is the Relapsing-Remitting form (RRMS), characterized by acute relapses that can last days or weeks, during which patients experience a sudden worsening of symptoms, interspersed by remission phases that can last for several months (Figure 8A). During remission, RRMS patients partially or totally recover the neurological functions that were affected during relapses. Twenty years after diagnosis, 80-90% of RRMS patients have developed the Secondary Progressive form (SPMS) (Scalfari et al., 2010; Trojano et al., 2003), in which, after several round of relapses and remission, deterioration of neurological function is progressive and steady (Figure 8B). The third common form of MS is the Primary Progressive form (PPMS), usually the most severe form, which starts from disease onset and continues throughout the patient's life with a progressive neurological deterioration in patients, without or with very little recovery and without relapses (Figure 8C). In the progressive phase of MS, the worsening of disabilities and symptoms is the consequence of axonal loss leading to cerebral atrophy (0.6-1% a year compared to 0.1-0.3% a year in healthy controls) (Miller et al., 2002). A fourth form of MS exists, the Progressive-Relapsing form, but the fact that it is often misdiagnosed as PPMS in early stages and that it affects less than 5% of the patients, make studies to characterize this disease form extremely challenging.

The clinical evolution is largely unpredictable in patients. An accumulating number of proofs show that the clinical or radiological characteristics gathered during two years of the disease course do not have predictive value (Cree et al., 2016). Even if new experimental techniques are regularly discovered, few of them are used in a routine fashion. In addition, disease evolution often takes only the EDSS into account, which represents only one aspect of the disease. From that point of view, a new MS score must be defined, taking into account physical disabilities, cognitive impairment, MRI score over a long period of time, remyelination capacities, and vision problems, among other things. Only then can the evolution of MS really be defined and, maybe, predictable biomarkers be found.

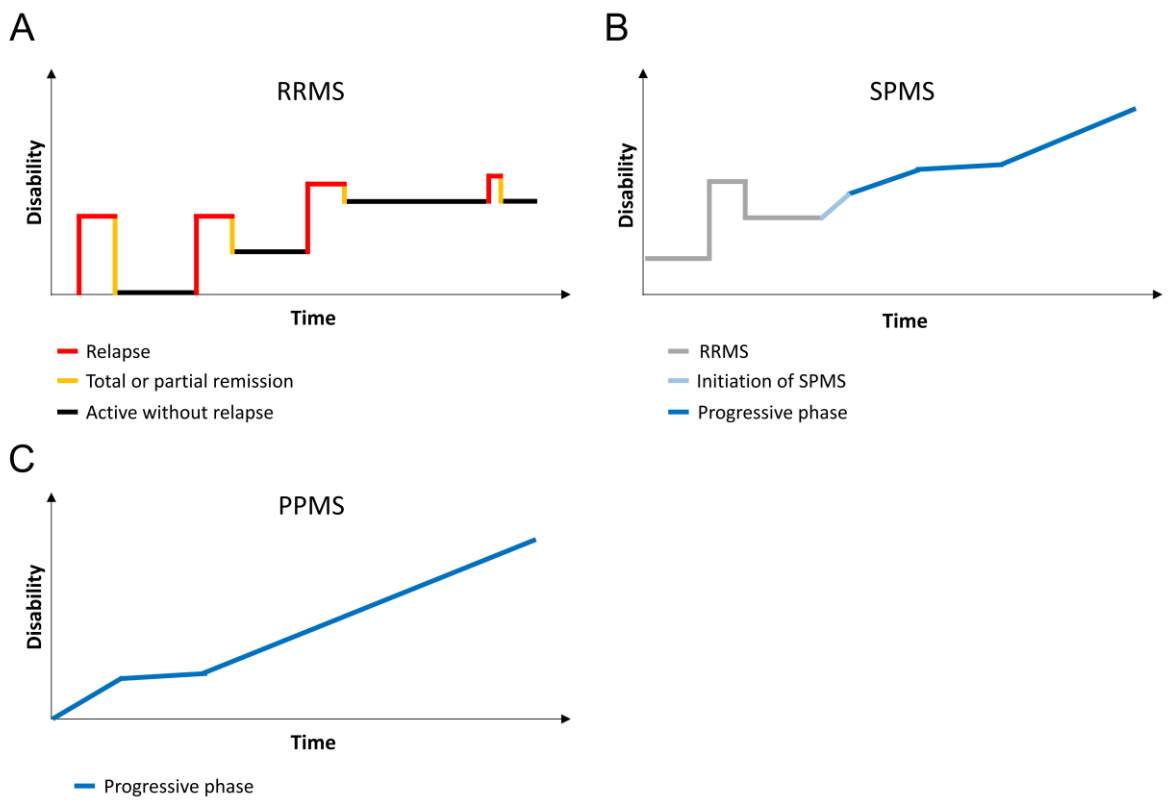

Figure 8: Representation of disability progression in the RR (A), SP (B) and PP (C) forms of MS. Modified from (Lublin et al. 2014).

D. FDA-approved treatments

Nowadays, there is no cure for MS. However, huge improvements have been made in the treatment of patients, especially patients affected with RRMS, in the last two decades. Treatments for MS can be divided in two major categories: first line medications, with moderate efficacy but limited side effect and second line medications, prescribed to patients with severe disease disease form and/or in which first line therapy has failed (Table 1). Second line medications have usually a better efficacy but can induce severe side effects. All disease modifying treatments on the market target the inflammatory component of MS, but pro-remyelinating and neuroprotective treatment are under pre-clinical and clinical trial and could revolutionize the therapeutic strategy of MS.

Treatments for RRMS

The first FDA-approved treatment was Interferon-β (IFN-β) in the early 1990s. Currently, there are several variants of IFN-β on the market, with limited severe side effects but a modest (around 1/3) reduction of relapse rate (Table 1). The mechanisms of action of this drug are not

totally understood, but administration of IFN-β reduces the production of IFN-γ and reduces antigen presentation by macrophages leading to decreased activation of myelin specific T cells (Yong et al., 1998). Like IFN-β, Glatimer acetate (GA) is an injectable drug, with a comparable efficacy on relapse rate and even rarer serious side effects. GA is a polymer of four amino acids found in the myelin protein MBP. Here again, the mechanisms of action are not totally understood but the injection leads to shift in CD4+ cells, from a Th1 pro-inflammatory to a Th2 anti-inflammatory population. However, GA did not show any effect on disability progression in randomized trials, contrary to IFN-β (Loma and Heyman, 2011) (Table 1). Another immunomodulatory drug, dimethyl fumarate, is available. It seems that this treatment acts on several deleterious events occurring during MS: it has a pro-apoptotic effect on mature T cells while promoting Th2 differentiation in naïve CD4+ cells, and reduces antigen presentation in macrophages and dendritic cells (de Jong et al., 1996; Linker and Haghikia, 2016; Treumer et al., 2003).

In the 21th century, antibody-based therapy emerged in the treatment of MS. Natalizumab seemed to be a great improvement in the treatment of RRMS: the reduction of the frequency of relapse was of 68% against placebo, twice more efficient than existing treatments and a reduced disability progression of 42 % (Table 1).. Natalizumab is a monoclonal antibody against the adhesion molecule α4-integrin, a molecule used by T cells to cross the Blood brain barrier (BBB) and induce myelin destruction. Unfortunately, Natalizumab can induce a severe lymphopenia in patients, who can therefore develop a progressive multifocal leukoencephalopathy (PML) due to the infection by the John Cunningham Virus. This pathology is extremely severe, has no treatment, is fatal in 30% of cases, and leads to severe neurological damage in 50% of the patients that survive. The frequency of patients developing PML under Natalizumab treatment is low (between 0.1% and 0.5% according to the studies), but because the risk is not negligible, patients under Natalizumab must have a very close follow-up, and this drug is only used in severe cases of MS in which the gain-risk balance is positive (Cross and Naismith, 2014; Winkelmann et al., 2013). Another antibody-based treatment, Alemtuzumab is available on the market. It targets CD52, an antigen carried by T and B cells, and leads to their depletion. But here again, due to severe side effects (infections, secondary autoimmune diseases) it is only used to treat patients with extremely severe cases of MS (Havrdova et al., 2015) (Table 1).

As listed above, several treatments targeting the immune system are available for RRMS. However, they either display limited efficacy or strong side-effects. This is one of the characteristics of treatment against auto-immune diseases: it is extremely challenging to target specifically the deleterious effects of the immune system while conserving the indispensable functions of immunity against pathogens.

Table 1: Characteristics of FDA-approved treatments for MS

Molecule	**Name** ***Company***	**Class**	**Administration**	**MS form**	**ARR relative reduction**	**EDSS relative reduction**	**Severe side effects**
Interferon β1a	Avonex ® *Biogen*	1st Line	i.m	RR	18%	37%	
Interferon β1a	Rebif ® *Merck*	1st Line	s.c	RR	32%	32%	
Interferon β1b	Extavia ® *Novartis*	1st Line	s.c	RR	34%	29% (n.s)	
Interferon β1b	Betaferon ® *Bayer*	1st Line	s.c	RR	34%	29% (n.s)	
Glatiramer Acetate	Copaxone ® *Teva*	1st Line	s.c	RR	29%	12% (n.s)	
Dimethyl Fumarate	Tecdifera ® *Biogen*	1st Line	oral	RR	53%	41%	PML Lymphopenia
Teriflunomide	Aubagio ® *Sanofi*	1st Line	oral	RR	36%	24%	Breathing problems
Fingolimod	Gylenya ® *Novartis*	2nd Line	oral	RR	55%	28%	Bradycardia Skin cancer PML
Natalizumab	Tysabri ® *Biogen*	2nd Line	i.v	RR	68%	42%	PML
Alemtuzumab	Campath ® *Sanofi*	2nd Line	i.v	RR	49% vs IFN β1a s.c	42% vs IFN β1a s.c	Secondary autoimmune diseases
Mitoxantrone	Novantrone ® *Mylan*	2nd Line	i.v	RR, SP	66%	64%	Cardiomyopathy Leukopenia
Ocrelizumab	Ocrevus ® *Roche*	1st Line	i.v	PP		24%	Infections

ARR: Annual relapsing rate, i.m: intramuscular, s.c: subcutaneous, i.v: intravenous n.s: non-significant. ARR and EDSS relative reductions are calculated vs placebo if not stated otherwise. In light blue, 1st line treatments with immunomodulatory properties. In light grey, 2nd line treatments with immunosuppressory properties.

Treatment for progressive MS

The progressive form of MS, corresponding to slow but steady neurodegeneration, is not as well understood as the inflammatory part occurring in RRMS (see II. Immunopathology of MS). More than 50 molecules have been tested in phase II or III clinical trials since the 1980s (Abdelhak et al., 2017), yet only two molecules are available on the market: mitoxantrone for SPMS and Ocrelizumab for PPMS. Mitoxantrone is a molecule originally developed to treat lymphoma. It is a DNA intercalating agent that blocks topoisomerase II DNA repair. It induces systemic immunosuppression by being cytotoxic to highly proliferating cells such as T cells and macrophages. It has a moderate efficacy on disease progression, and can have irreversible side effects like cardiomyopathy and sterility. Here again, very careful monitoring of patients is necessary. Ocrelizumab was FDA-approved in March 2017 as the first treatment against PPMS. Like Mitoxantrone, Ocrelizumab was originally developed to be used against lymphoma. It is an anti-CD20 antibody targeting B cells, a cell type that has been ignored for a long time in MS research. Ocrelizumab slowed disease progression in PPMS patients by 24%, with moderate side-effects (Montalban et al., 2016). This treatment has very limited efficacy, but was nevertheless FDA-approved because it is the first molecule demonstrated to be more efficient than placebo in PPMS.

The therapeutic situation in patients for PPMS is much worse than for RRMS patients, with very few available treatments. However, the recently discovered role of B cells in the pathology gives hope for the treatment of progressive MS, and tens of molecule are under clinical trial currently (Shirani et al., 2016).

Future treatments: remyelination therapy

In the last decade, MS research has been notably focusing on preventing the transition between the relapsing and the progressive phase of MS, characterized by a massive axonal loss. To preserve axons from degeneration, the most promising strategy is to enhance remyelination of demyelinated axons. Remyelination can fail in patients because of OPC recruitment, survival or differentiation, and some pro-remyelinating compounds are ongoing clinical trial (Plemel et al., 2017).

Future treatments: cell therapy

Cell therapy was deeply explored in the last 20 years as a potential treatment for MS. Three major approaches were or are still tried: replacing the destroyed myelin by transplanting

myelinating cells, enhancing repair and neuronal survival by grafting cells with pro-repair and neurotrophic properties and calming the inflammatory attacks using cells with immunomodulatory properties (Ben-Hur, 2011). We will focus on the latest strategy, as it is the most advanced one in terms of clinical trials.

Mesenchymal stem cell (MSC) injection is one of the most promising cell therapy in trial for MS. MSC are multipotent cells that can give rise to a wide variety of cell types (e.g. neural cells, fibroblasts, myocytes). The routine source of adult MSC in the body is the bone marrow. MSC can easily be amplified *in vitro*. Therefore, technically these cells are extremely practical as they can be obtained by a moderately invasive procedure, can be amplified easily and autologous graft can be performed. Their potential beneficial role in MS is important. They can differentiate into neurons and they have strong immunomodulatory properties. MSC can, among all the processes studied, induce the induction of Treg, inhibit Th17 response and antigen presentation (Caprnda et al., 2017; Cobo et al., 2013; Payne et al., 2013). It is interesting to note that the immunomodulatory effect of MSC is systemic: it does not only act in the CNS (Salinas Tejedor et al., 2015). They can inhibit apoptosis of neurons, induce angiogenesis, and promote neuroprotection and remyelination (Caprnda et al., 2017; Teixeira et al., 2013). In EAE, MSC treatment gave very interesting results with an important decrease of disease severity due to an impairment of inflammatory processes leading to demyelination and neuronal death (Fisher-Shoval et al., 2012; Zappia et al., 2008). MSC injected intrathecally in patients are well tolerated (Connick et al., 2011) and the preliminary results in phase I and II show globally a deceleration of disease progression in SP and PPMS patients (Meamar et al., 2016).

Another serious candidate for cell therapy is hematopoietic stem cells (HSC). HSC are multipotent cells giving rise to hematic and immune cells (macrophages, dendritic cells, LT). HSC do not have a sensible impact on neuroprotection nor remyelination. However, transplanting HSC allow to reboot the immune system, with the hope that newly formed cells will not induce an autoimmune response. They are tested in very aggressive forms of MS after ablation of all immune cells by chemotherapy or total body irradiation. HSC seem to be more efficient than MSC to slow disease progression in the preliminary results of phase I and II studies (Burt et al., 2009). However, the medical procedures involve the total suppression of the patient's immune cells, inducing a strong risk of secondary infection.

Finally, neural stem cells are extremely interesting for MS therapy as they have both systemic immunomodulatory and myelinating properties and are undergoing clinical trial for some leukodystrophies (a group of pathology affecting the myelin) and other neurodegenerative diseases (Goldman, 2016),.

II. Pathophysiology and Immunopathology of MS

1. Inflammation, demyelination and neurodegeneration

The hallmark of MS is the presence of several demyelinated plaques disseminated within the CNS. MS lesions were first characterized in the white matter but lesions can also be found in the gray matter of patients. These demyelinating plaques are the consequences of an autoimmune attack against myelin mediated by LT and invading macrophages crossing the BBB and by resident microglia (MIG) (Figure 9). The autoimmune attack occurs because of a failure of suppression of autoreactive T cells and of a dysregulation of the global inflammatory response (Compston and Coles, 2008; Dendrou et al., 2015).

A. Relapsing phase of MS

MS can be considered as a disease with two phases: The RR phase characterized by a strong inflammation and the progressive phase (including SP and PP) in which neurodegeneration occurs with a decreased presence of inflammation in the CNS.

In RRMS, relapses are triggered by a massive inflammatory attack leading to demyelination. What is causing the immune system to trigger the inflammatory storm during a relapse is not known. Chronic inflammation will induce OL death, leading to chronic demyelination. In this stage of the disease, several gadolinium positive active plaques are found, the vast majority in the white matter. Once the inflammatory storm is over, myelin repair can occur. When this process is efficient, axons do not degenerate and a normal axonal function is restored (Duncan et al., 2009). This process is believed to allow a total or a partial remission in patients . However, axonal death is already present in lesion of RRMS patients in early disease course (Hauser and Oksenberg, 2006), that could explain why the remission is sometimes not total in patients.

B. Progressive phase of MS

In the progressive phase of MS, there is a chronic and steady axonal loss correlating with disability progression in the patient. The progressive phase is characterized by neuronal death leading to brain atrophy affecting white and grey matter (Fisher et al., 2008; Jacobsen et al., 2014; Lanz et al., 2007; Losseff et al., 1996). Inflammation is less prominent in plaques.

The mechanisms leading from the inflammatory component to the neurodegenerative component are not fully understood and several hypotheses have been proposed: 1) MS is a primary neurodegenerative disorder and the inflammatory processes are not causing axonal death. 2) The inflammation and neurodegeneration are interlinked, and neurodegeneration is the result of chronic demyelination due to inflammatory processes.

The first hypothesis is supported by the fact that MRI studies show subtle changes in the white-matter of patients before the appearance of a lesion and breakdown of the BBB (Filippi et al., 1998). However, experimental and clinical data are arguing for the second hypothesis in which neurodegeneration is driven by inflammation. In EAE, neurodegeneration can be triggered by priming T cell against myelin antigen (Kornek et al., 2000a). In patients, active cortical plaques are always associated with immune cell infiltration (T and B cells) in the meninges and there is a correlation between B cell infiltration and disability progression (Kutzelnigg et al., 2005). Diffuse white matter injury associated with perivascular and parenchymal infiltration of T cells and MIG activation is also found in patients suffering from progressive MS (Prineas et al., 2001).

Globally, the view on neurodegenerative processes in progressive MS as being inflammation-independent is unlikely. There is a large amount of diffuse, perivascular and meningeal inflammation mediated by T cell, B cell and substantial MIG activation (Lassmann, 2010). These inflammatory processes are strongly correlated with neuronal death (Frischer et al., 2009).

2. Role of T cells in MS and animal models

MS is a disease of the immune system. The vast majority of predisposition polymorphism are associated with genes having functions in immune pathway and among the immune genes

affected, many are involved in T cell function (The international multiple sclerosis genetics Consortium (IMSGC), 2013). In addition, T cells are able to infiltrate the CNS, and MS patients have a higher proportion on myelin-reactive T cells in their blood. T cells are divided into two groups depending on the expression of CD4 or CD8 surface receptors. From a functional point of view, CD4+ cells are cells coordinating the adaptive immune response: they induce and drive the most adaptive immune answer to fight the pathogen encountered. Naïve CD4+ cells, or Th0 cells, can be polarized in different functional phenotypes according to the co-stimulation received during antigen presentation (Figure 10): They are activated into the Th1 pro-inflammatory phenotype in response to intracellular bacteria or protozoa and into the Th17 pro-inflammatory phenotype in case of cancer or fungus. After this inflammatory phase aiming at destroying the pathogen, Th2 cells inhibit Th1 activation. Of note, Th2 have also a role in pathogen neutralization, in case of extracellular parasites. Treg are extremely efficient at calming inflammation, by suppressing T cell proliferation. Finally, T follicular helper cells (TFH) are activated and proliferate to help B cell maturation in case of a humoral immune response. CD8+ cells (or effector T cells) are soldiers of the immune response: they kill infected cells by lysing their membrane. CD4+ cells are believed to play a critical role in MS, by inducing the immune response against myelin leading to its destruction and not correctly suppressing the inflammatory attacks. CD8+ cells are suspected to be one of the main effectors of myelin destruction (Figure 9).

A. Th1 and Th17 cells in EAE and MS

The vast majority of cellular and molecular mechanisms believed to cause myelin destruction and degeneration in MS were discovered in the murine model EAE. In EAE, the immune system is primed against myelin by injection of myelin peptides. With the addition of adjuvants to stimulate the innate immune system, this causes a violent inflammatory response against myelin leading to demyelination and neurodegeneration. EAE is the most accurate model of MS as it reproduces some of the clinical and immunological aspects of MS. CD4+ cells are the main effectors of EAE as the injection of myelin-specific CD4+ cells is enough to trigger the disease (Pettinelli and McFarlin, 1981). More precisely, Th1 and Th17 are the pathogenic cell types in EAE as they can both induce the disease (Figure 10). In EAE, these two cell types are able to cross the BBB and induce a strong neuroinflammation leading to demyelination and/or neurodegeneration. In MS, the role of Th1 and Th17 cells is more obscure even if some hints

argue for an important role in the disease. IFN-γ production (a Th1 produced cytokine) is partially associated with relapses in RRMS patients and microarrays in MS lesions showed an upregulation of the production of IL-17 (a Th17 produced cytokine) (Lock et al., 2002). Finally, treatment by GA, inducing a switch from Th1 to a Th2 immune response, reduces relapse rate in patients (Cross and Naismith, 2014).

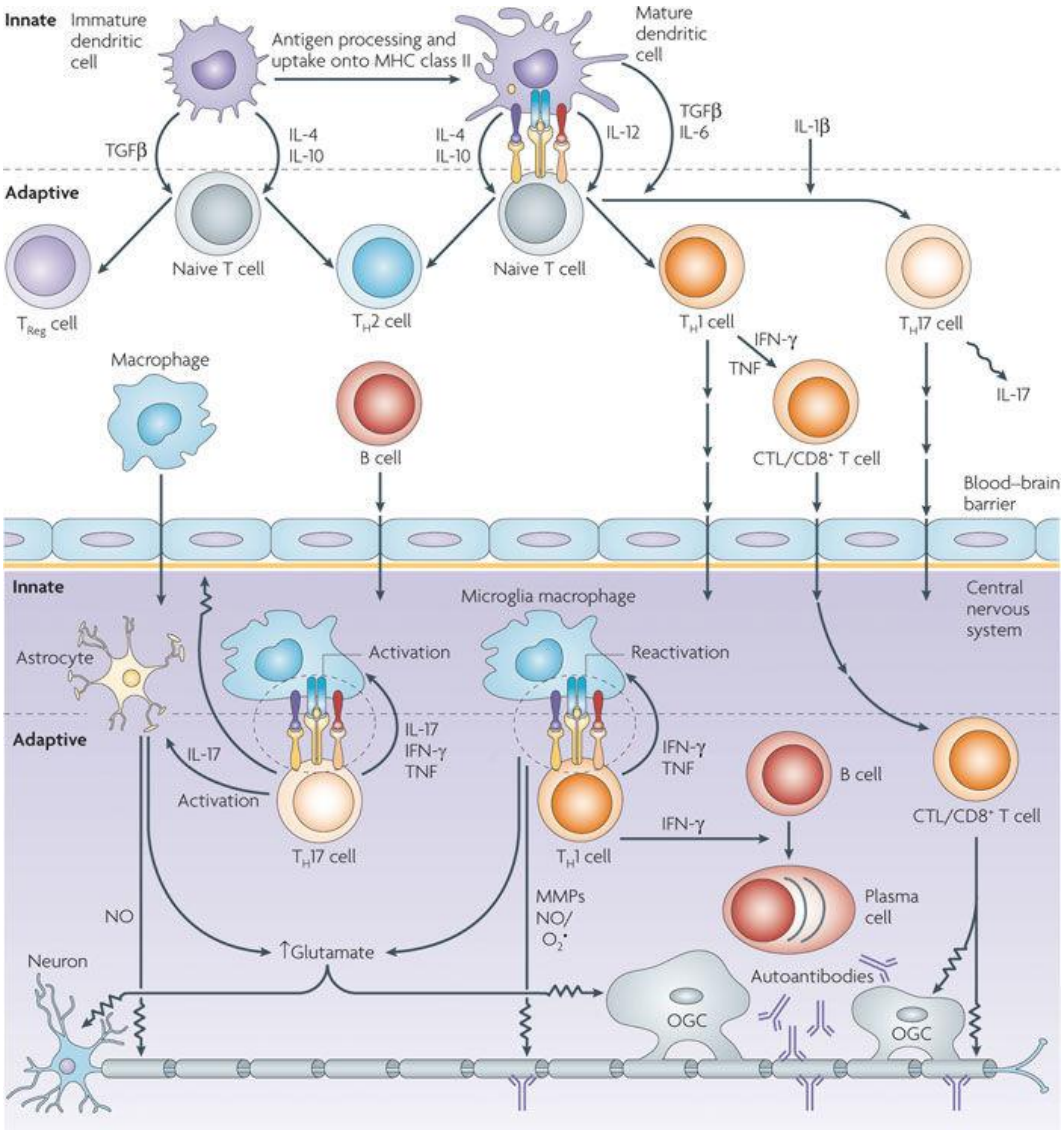

Figure 9: Simplified view of the mechanisms leading to myelin destruction in MS. A peripheral mis-activation leads to the proliferation of myelin-reactive T and B cells. The dysfunction of the peripheral tolerance induce their survival. Invading Th1, Th17 and B cells induce the first demyelination, leading to the invasion of the CNS by macrophages and to MIG activation. MIG and macrophages, in response to demyelination, induce a pro-inflammatory environment inducing a secondary myelin damage. Invading cells can be reactivated by MIG leading to the chronicity of demyelinating attacks in MS. From (Macmillan et al. 2008)

B. Th2 and Treg cells in EAE and MS

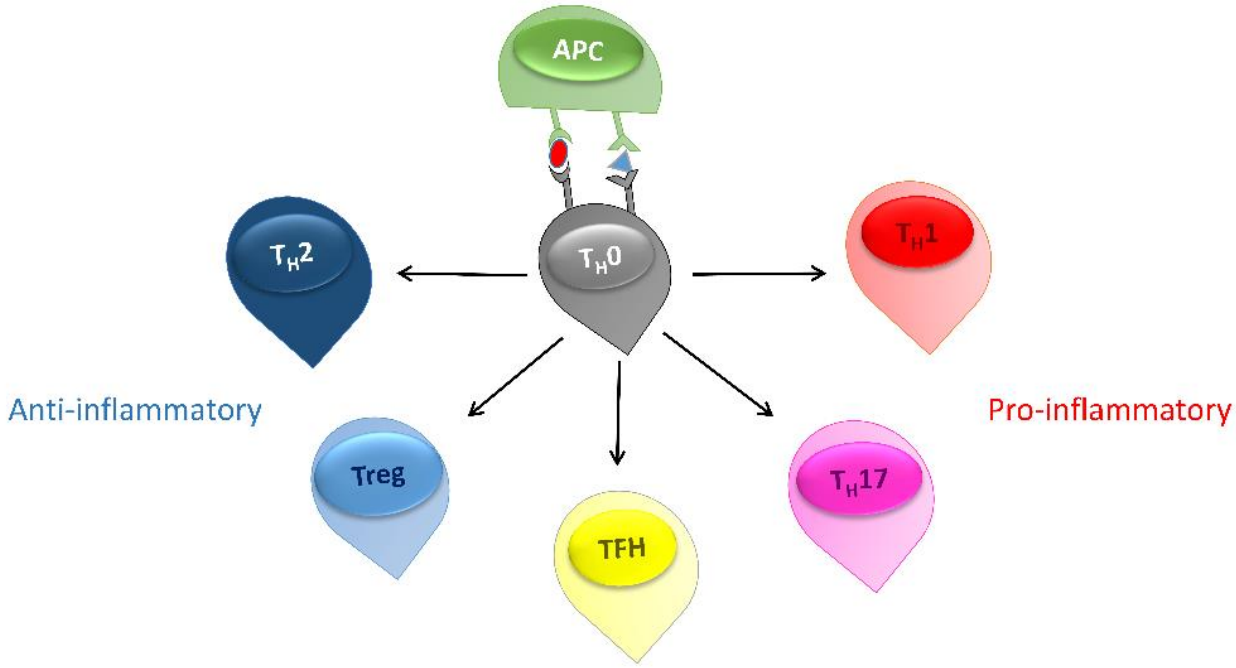

Figure 10: CD4+ T helper cells differentiation pathways. After antigen presentation and co-stimulation, naïve Th0 cells can differentiate into different phenotypes : The Th1 or Th17 pro –inflammatory phenotypes, involved in anti microbian response, The Th2 or Treg anti-inflammatory and immunoregulatory phenotypes that can regulate and calm the inflammatory response and finally, the TFH phenotype, involved in B cell maturation

The role of Th2 cells in MS and even in EAE is not fully understood. However, this cell type seems to be protective (Nagelkerken, 1998). For instance, there is an upregulation of Th2 secreted cytokines in the blood of patients in remission phase (Oreja-Guevara et al., 2012) and there is an upregulation of Th2 cells produced in the remission phase of EAE induced in SJL mice. Treg cells role, on the contrary, is better understood both in EAE and MS pathology. Transferring Treg cells after priming of the immune system against myelin is sufficient to prevent the appearance of EAE in mice, indicating a crucial role of these cells in peripheral tolerance and in preventing autoimmune reaction (Sakaguchi et al., 2008). The number of Treg cells does not seem to be decreased in MS patients (Feger et al., 2007) but some studies indicate that their suppressive capacities could be affected: In some patients, Tregs are no longer able to suppress the proliferation of myelin-specific T cells , and are also not able to proliferate efficiently to calm the inflammatory storm (Haas et al., 2005; Kumar et al., 2006;

Viglietta et al., 2004). A loss of function of Tregs could explain why auto-reactive T cells are not eliminated in MS patients, triggering the attack of the myelin.

C. CD8+ cells in EAE and MS

EAE is proven to be mainly a CD4+ cells dependent disease. However in MS, CD8+ cells seem to be pathogenic as a great number are found in MS plaques. On average, autopsies of MS patients show that there are more invading CD8+ than CD4+ cells (Hauser et al., 1986) . CD8+ cells are located at close distance from damaged axons and are going through oligoclonal expansion in the CSF, blood and brain of MS patients (Babbe et al., 2000; Jacobsen et al., 2002; Junker et al., 2007; Skulina et al., 2004). Moreover, CD8+ cells are likely to be found in cortical plaques, a location strongly associated with clinical progression in MS (Lucchinetti et al., 2011).

In animal models, MOG reactive CD8+ cells can induce severe EAE (Sun et al., 2001) with more neuronal death than if MOG reactive CD4+ cells are injected. In patients, axonal damage is correlated with the number of CD8+ but not CD4+ cells in the lesion (Bitsch et al., 2000). All these data suggest than CD8+ cells could be the main actor responsible for axonal damage in MS.

3. Role of B cells in MS and animal models

B cells are LT specialized in the humoral response: They secrete antibodies to neutralize extracellular pathogens. The role of B cells in MS has for a long time been underestimated. In the last decade, several roles in pathogenicity of these cells have been established and an FDA-approved treatment targeting B cells, efficient in PPMS, has been commercialized. B cells are found in the brain undergoing neuroinflammation. Several molecules secreted by the innate immune system are known to attract B cells into the CNS: C-C motif chemokine ligand 20 (CCL20) (Kalinowska-Łyszczarz et al., 2011), C-X-C motif chemokine ligand 13 (CXCL13) (Kowarik et al., 2012) and CCL19 (Krumbholz et al., 2007), among others (Blauth et al., 2015).

B cells from MS patients can induce demyelination by secreting IgG antibodies that target myelin *in vivo* and *in vitro* (Elliott et al., 2012) confirming the long debated hypothesis of myelin specific antibodies. Several myelin specific antibodies are now known to target MBP,

MOG, SPAG16 and other myelin components (Claes et al., 2015). One of the major discoveries of the last few years was that B cells aggregate in the CNS in areas called ectopic lymphoid follicles, located in the brain meninges, from which they induce inflammatory response (Figure 11). From these locations, B cells can induce myelin specific T cell expansion, and induce inflammation efficiently by secreting a vast panel of cytokines that leads to neurodegeneration and disability progression in the progressive phase of MS (Magliozzi et al., 2007).

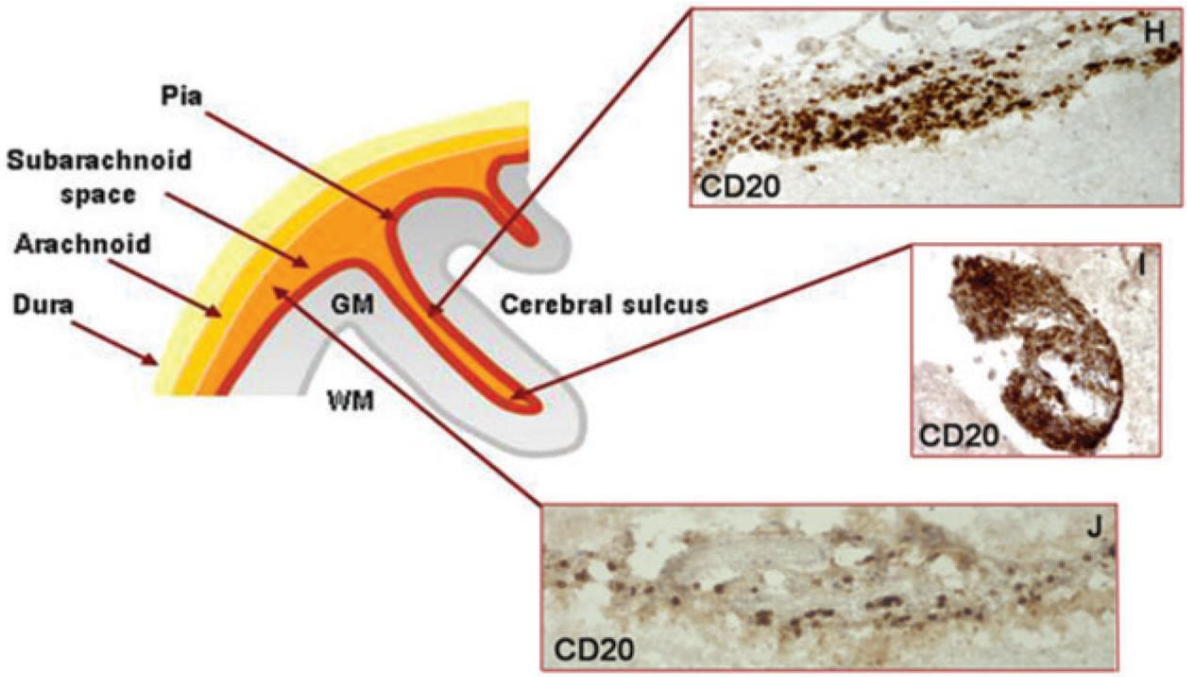

Figure 11: Representation of B-cells follicles in the meninges of patients with progressive form of MS. CD20+ B cells are infiltrating the along and in the depth of the cerebral sulci, from where they induce a strong inflammatory response leading to grey matter damage.

4. Role of Macrophages and Microglia in MS and animal models

Like T and B cells, invading macrophages and MIG are found in MS plaques suggesting a role in the physiopathology of MS. In EAE, the disease progression is linked to the infiltration of macrophages in the CNS (Ajami et al., 2011) and inhibition or depletion of macrophages generally attenuate the paralysis progression occurring in EAE (Agrawal et al., 2006; Bhasin et al., 2007; Martiney et al., 1998) .

A. Direct role on demyelination and neurodegeneration

Macrophages and MIG participate directly to the demyelination and neurodegeneration in MS: a huge number of macrophage and/or MIG secreted cytotoxic compounds or cytokines are found in active MS plaques and the surrounding tissue (Lassmann, 2014). One of the well described mechanisms of demyelination is the action of the tumor necrosis factor α (TNF-α): It is toxic at high doses for OL and induce their death (Selmaj and Raine, 1988; Zajicek et al., 1992) and this mechanism is mediated notably through cell contact mechanisms. Nitric oxide (NO), normally used for intracellular signaling can also be secreted by MIG leading to OL death *in vitro* (Merrill et al., 1993). Similarly, a lot of other cytokines are toxic to human OL *in vitro*, such as IFN-γ and Interleukin-6.

The innate immune system also plays a role in axonal loss and neurodegeneration occurring after demyelination. The CSF and the conditioned media of MS patient macrophages in late stage disease are able to induce neurotoxicity in human neurons *in vitro* (Alcazar et al., 2000; Piani and Fontana, 1994). The cytotoxic elements released by innate immune cells during the demyelination process can also induce neuronal death. Of note, reactive oxygen species, released in great quantity during neuroinflammation, can cause DNA damage inducing neurodegeneration in patients (Vladimirova et al., 1999) and antioxidant treatments efficiently suppress the appearance of EAE (Marracci et al., 2002). NO, produced by the iNOS (Nitric oxide synthetase) enzyme in macrophages and MIG, is also cytotoxic to neurons, inducing their apoptosis (Emerson and LeVine, 2000). Glutamate clearance by OL and astrocytes is crucial for neuronal survival as a high extracellular concentration in neurons can induce excitotoxicity in neurons leading to their apoptosis. In EAE like in MS the oligodendroglial death prevents glutamate clearance, and activated macrophages secrete more of this neurotransmitter in inflammatory conditions than in a physiological context (Fine et al., 1996; Hendriks et al., 2005). In addition, inflammatory cytokines prevent glutamate uptake by astrocytes. Therefore, macrophages induce directly and indirectly neuronal death by glutamate excitotoxicity by secreting large amount of glutamate and preventing its processing by OL and astrocytes. Other mechanisms have been discovered more recently involving the toxicity of macrophages secreted metalloproteases (Lu et al., 2002).

B. Role in lymphocytes recruitment, antigen presentation and lymphocyte activation

To invade the CNS, T cells, B cells and macrophages require appropriate attracting signal, a role usually attributed to C-C chemokine ligand 2 (CCL2). CCL2, produced by MIG is one of the most understood signals triggering CNS invasion by T cells. In CCL2 knock-out mice, there is a large decrease of the invasion of CNS by leukocytes during EAE leading to less severe symptoms (Kim et al., 2014) and high levels of CCL2 are found in MS plaques (McManus et al., 1998). After being activated in the periphery, invading T and B cells can also be reactivated against myelin in the CNS. To do so, they need MHC class II antigen presentation in addition to co-stimulation. During EAE, MIG increase the expression of MHC class II molecules and co stimulatory molecules such as CD86 (Olson and Miller, 2004). These data indicate that after invading the CNS, T and B cells are reactivated against myelin and proliferate, worsening the extent of the inflammatory attack. Other mechanisms implicating the innate immune system are suspected to be part of the pathophysiological process of MS: The B7-H1/PD-L1 is a molecular pathway implicating a cross-talk between macrophages and CD4+ cells. This pathway is normally an inhibitor of the maturation of CD4+ cells. In MS, there is a dysregulation of this pathway leading to an increase of the IL-17 secretion inducing a preferential maturation of myelin-reactive Th17 cells (Chastain et al., 2011).

5. MS Lesions

The histopathological landmark of MS is demyelinated lesions in the CNS called plaques. They can appear anywhere in the brain and the spinal cord and are not only located in the white matter. They can be visualized by MRI, but to determine at what stage the lesion is, post-mortem analysis or biopsy are indispensable. MS lesions can be classified according to the extent of myelin loss, inflammation, neurodegeneration, and remyelination.

A. Acute active plaques

In early stages of RRMS, acute active plaques are found frequently: they are characterized by the presence of huge inflammatory infiltrates. The infiltrates are composed in vast majority of

macrophages distributed equally all over the lesion. Activated MIG are also present throughout the lesion. In addition to macrophages, the infiltrate is composed of T cells (with a large part of CD8+ cells) and B cells, usually in close proximity to blood vessels. The amount of demyelination in acute active plaques is variable and can be measured directly by oil-red O staining or indirectly by evaluating myelin phagocytosis by macrophages (Popescu and Pirko, 2013). The heterogeneity of demyelination extent and other features led to a more complex classification of acute plaques, in which four different patterns can be found (Lucchinetti et al., 2000). Patterns I and II are very close histopathologically: myelin destruction is the consequence of a predominant T cell- and macrophage-induced inflammation. In pattern II plaques (the most frequent type of lesions), there is a strong presence of myelin specific antibody and complement activation indicating a very important role of B cells in the demyelination process. Most of the time, the infiltrates are centered on veins and venules. In pattern III, the demyelination and OL apoptosis are usually very strong and are preferentially affecting periaxonal myelin components, but only in the plaque borders (in contrary to pattern I and II). Demyelination is even affecting the white matter around the lesion. In pattern IV, a type of lesion found very rarely in patients, the OL death is not apoptotic, suggesting a mechanism of OL death independent of inflammatory attacks. The common features of acute plaques include demyelination but axonal damage can also occur.

B. Chronic plaques

Chronic plaques are most often seen in the progressive phase of MS. They can be defined as active, when demyelination is still ongoing (in this case, foamy macrophages containing myelin fragments can be seen) or inactive when demyelination is complete. Inflammation is less prominent in chronic plaques than in acute plaques and its pattern is different. In chronic plaques, T and B cells are localized in vast follicular structures from which they induce neurodegeneration (Popescu and Pirko, 2013; Wu and Alvarez, 2001). It is in chronic plaques that the axonal damage and neuronal death is mainly occurring, by several mechanisms: axonal degeneration due to lack of metabolic support by OL, oxidative stress, cytotoxicity inflammation-induced, and glutamate toxicity, among others.

While this classification is complete, it is complex and difficult to apply in a clinical routine. A simpler classification, aiming to unify all the data analyzed for comparability has been proposed recently (Kuhlmann et al., 2017). This new classification incorporates the majority of the histopathological elements used in previous classification. By analyzing the presence and the nature of infiltrates and of demyelination, lesions are defined as active, mixed active/inactive and inactive with our without demyelination.

III. Remyelination

1. Forewords

Remyelination is one of the few regenerative processes of the nervous system. In remyelination, OPCs proliferate, migrate toward the lesion and differentiate into new myelin-forming OL to wrap demyelinated axons. It is very efficient in the healthy CNS, it restores fast axonal conduction and allows functional recovery. The several remyelination steps are under the control of the inflammatory environment, and reinforcing its efficacy in MS patients is one of the most promising therapies to treat MS.

2. Histological description and clinical relevance

A. Evidence of remyelination in the human brain

Existence of remyelination was demonstrated in the PNS prior to the CNS. Two major arguments (reviewed in (Hommes, 1980)) led scientists in the 1980s to conclude that remyelination could also occur in the spinal cord and the brain: the appearance of myelin on fibers that were demyelinated and the fact that this myelin was short and thin (and therefore different from developmental myelin). Remyelination can be observed post-mortem in the CNS of MS patients: Using luxol fast blue, a dye staining myelin, the areas where the myelin is destroyed or has been repaired (shadow plaques) are observable (Figure 12A, (Prineas et al., 1984)). The post-mortem analysis in patients allowed measurement of the efficacy of the phenomenon, by measuring the number of lesions that undergo remyelination at a certain point. However, with this analysis, one cannot be sure that the efficacy of the process observed at the moment of death is representative of the efficacy of remyelination in the disease. In other words, *in vivo* longitudinal studies for evaluating the extent of the repair process would be more accurate to assess remyelination in patients.

Numerous imagery techniques are used to evaluate the brain compartment of MS patients (Filippi et al., 2012) but until recently, none of them can label myelin specifically. A longitudinal study was published recently: using a compound labelling myelin specifically ([^{11}C]PiB) and the combination of multiple MRI and PET-scans (Figure 12B-B'), they provided new insight about remyelination efficacy in MS patients (Bodini et al., 2016).

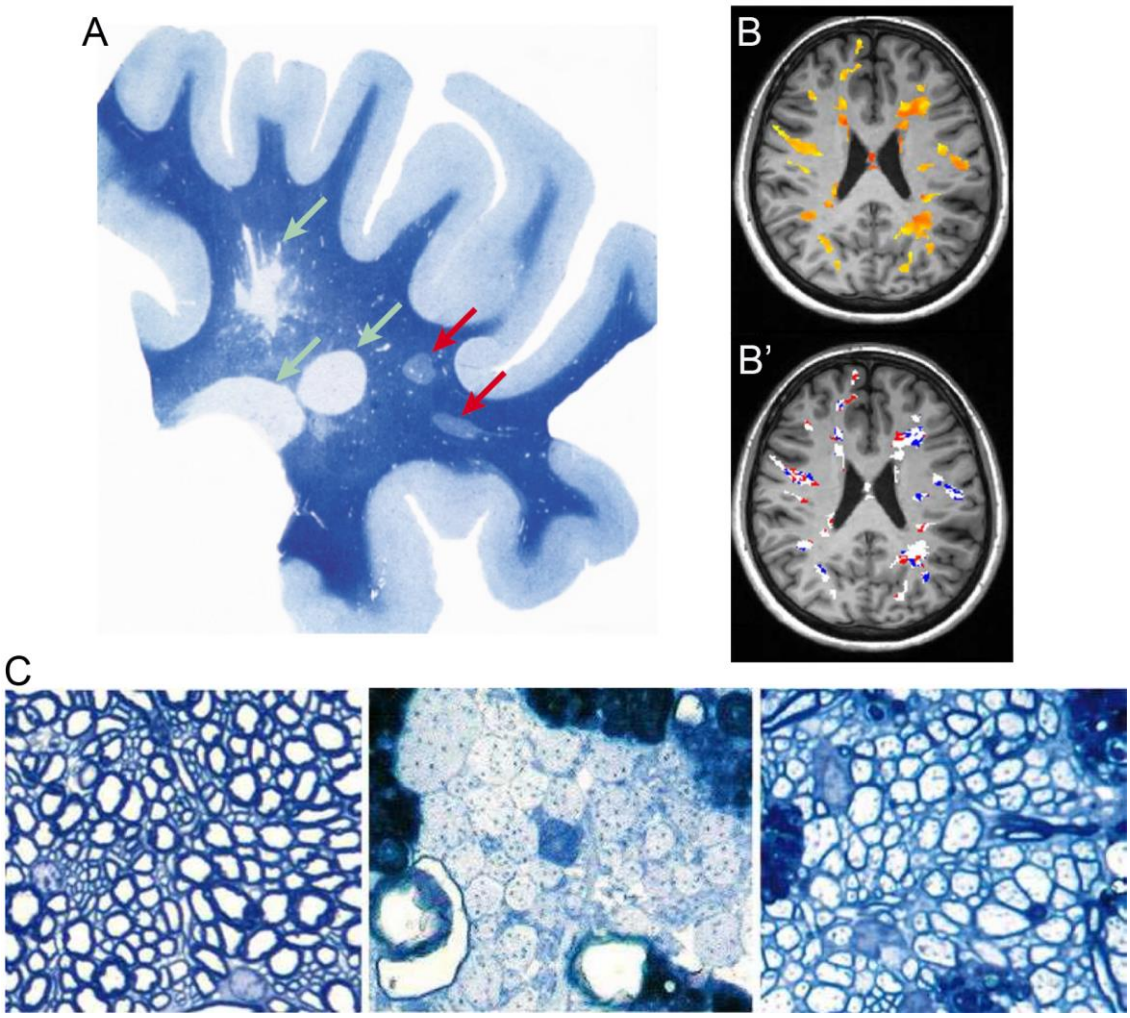

Figure 12: Remyelination is occurring in the CNS of MS patients and can be studied using focal demyelination animal models. In Patients, remyelination can be visualized on brain slices. After luxol fast blue staining, demyelinated area (green arrows) and area with ongoing remyelination (red arrows) can be noticed (A). From the Wolfe Medical Publications Ltd, 1989. Remyelination can also be visualized by PET-SCAN and the specific marker [^{11}C]PiB. Here is represented the myelin baseline content of a patient (B), the demyelinated (red) and remyelinated area (blue) following a longitudinal imaging session three month after (B'). From Bodini et al. 2016. Remyelination can also be studied in animal models (C): here are shown axons in the rat cerebellum before demyelination (left panel), following the injection of the demyelinating agent ethidium bromide (center panel), and remyelination of axons four weeks post demyelination (right panel). Myelin was stained using luxol fast blue. From (Franklin, 2002)

B. Remyelination in animal models

To decipher the mechanisms of MS and of remyelination, animal models are extremely useful.

Remyelination in EAE

The most accurate model of MS is EAE. In this model, an auto-immune attack against myelin is triggered. EAE is a great model of CNS inflammation and neurodegeneration. However, it is not very well suited for remyelination for several reasons (Ransohoff, 2012; Tanaka and Yoshida, 2014): lesions are disseminated stochastically in time and in space, it is therefore hard to know where and when to look to observe the process. In addition, demyelination and remyelination occur at the same time, making it difficult to determine which process is ongoing when observed. Finally, axonal damage and neurodegeneration occur rapidly in EAE, making the remyelination process rather limited.

Toxic models of demyelination and remyelination

Two majors models of toxic demyelination are extensively used nowadays: Cuprizone feeding and lysolecithin/lysophosphatidylcholine (LPC) or ethidium bromide induced focal demyelination (Figure 12C). Cuprizone is a copper chelator which, when added to the food or water of mice, is toxic for OL: they die within 4-6 weeks of treatment and a severe demyelination is observed in the corpus callosum and in the hippocampus (Ransohoff, 2012). Cuprizone lesions induce glial activation, like in MS, and the lesions observed in this model resemble type II MS lesions. The principal setback of the cuprizone model is that demyelination and remyelination occurs at the same time, making difficult to study the kinetics of myelin repair (Kipp et al., 2009).

To study remyelination, the micro-injection of a detergent can also be performed. This is the case for LPC or ethidium bromide focal demyelination in which a small volume of the compound is injected in the spinal cord or the corpus callosum of mice, rats or cats. This causes a demyelination at the site of injection by solubilization of the myelin by the detergent. This model has been used with great success to determine the cellular and molecular of remyelination. In this model, remyelination is spontaneous and extremely efficient (Jeffery and Blakemore, 1995). The kinetics of the repair process are well defined. After two days, the demyelination is complete. After 7-8 days, OPCs have proliferated and migrated to the lesion.

The lesion is infiltrated by a large number of macrophages from the periphery, as well as T and B cells. After 21 days, OPC have differentiated and remyelination is complete (Figure 13C).

C. Remyelination, neuroprotection and disease severity

The first evidence that remyelination is a beneficial mechanism for recovery was the discovery that this process restores a fast saltatory conduction in remyelinated neurons (Smith et al., 1979). This restauration of a proper neural influx is the consequence of sodium channel clustering at regular intervals along the axons occurring even before the formation of the new myelin sheath (Coman et al., 2006; Freeman et al., 2015). In addition, locomotor symptoms induced by focal demyelination in cat spinal cord are rescued by remyelination (Jeffery and Blakemore, 1997) and this data was further confirmed in a more severe model of demyelination (Duncan et al., 2009).

Remyelination is beneficial in animal models but also in MS patients: in shadow plaques of post mortem tissue, damaged axons are rare (comparable to normal appearing white matter) compared to chronic lesions (Kornek et al., 2000b) indicating that remyelination protects from neurodegeneration. The mechanisms by which remyelination in patients prevents neurodegeneration are not fully understood, however the trophic support to neurons provided by OL and the fact that myelin physically protects against inflammation are believed to be the two major mechanisms of promoting axonal survive. An additional proof that remyelination allows functional recovery for at least locomotor functions is that the remyelination capacity in patients is inversely correlated to the MS severity score (evaluating walking capacities of patients) (Bodini et al., 2016).

All these data indicate that reinforcing remyelination in patients is a promising strategy to treat MS.

3. Mechanisms of remyelination

A. *Role of oligodendrocyte precursor cells*

Oligodendrocyte precursor cells: an adult precursor population

Adult OPCs are an abundant cell population in the brain, representing around 5 % of the total CNS cell population. A large amount of OPCs generated during development do not differentiate and give rise to the stock of adult OPCs. OPCs are distributed roughly equally throughout the CNS, they can therefore respond to an injury happening everywhere in the CNS. *In vivo*, adult OPCs have a complex morphology, with a multi arborized cell membrane that does not fit the usual representation of the embryonic bipolar cell when observed *in vitro*. Adult OPCs are different from developmental OPCs from a transcriptomic point of view and in physiological conditions, and their role in physiological conditions is starting to be unraveled, with studies highlighting their role in synaptic growth , synaptic plasticity and motor skill learning (Nishiyama et al., 2009; Polito and Reynolds, 2005; Xiao et al., 2016).

Recruitment of OPCs in response to demyelination

In physiological conditions, OPCs are a quiescent and rarely dividing cell population. After a demyelinating injury, OPCs are activated and exit their resting state in order to execute the different steps leading to forming new myelin. After sensing inflammatory stimuli, OPCs will go through morphological change (Levine and Reynolds, 1999) and a deep change in their gene expression profile: They start to express genes that are well known in OL development such as OLIG2, NKX2.2, MYT1 among others (Fancy et al., 2004a; Franklin and ffrench-Constant, 2008; Vana et al., 2007). Due to mitogenic and chemoattractant factors, OPCs will go through the recruitment phase: They commence proliferation and migrate towards the demyelinated area in response notably to PDGF and FGF-2 (Zhao et al., 2005). Only OPCs located at a small distance from the injury will be able to be recruited to the lesion (Franklin et al., 1997).

In animal models of focal demyelination, OPC recruitment happens in the first week post remyelination, as the maximum number of precursor cells is reached at day 7 post injury (Remington et al., 2007). These first steps are crucial for the success of remyelination as OPCs need to be in close proximity to axons to reform myelin and in sufficient number to ensure a full repair of destroyed myelin in the area.

OPC recruitment is dependent on the inflammatory micro-environment. It is therefore extremely important for a successful repair process that the inflammatory response is well controlled in time and intensity.

OPC differentiation in remyelination

Once OPCs reach the lesion, the final step of remyelination is reforming myelin around naked axons. To do so, it is believed (even if not fully demonstrated) that OPCs go through the same stage of differentiation as for developmental myelination: when they receive pro-differentiation clues, OPCs extend their processes to contact axons and wrap them to form new myelin. In animal models, this process takes up to three weeks to be completed (Jeffery and Blakemore, 1995).

The newly formed myelin is not exactly identical to the myelin formed during development. The internodes are on average shorter and the myelin itself is thinner. The mechanisms underlying this difference in the structure of myelin are not known, but the differences argue for a different mechanism in axon myelination and remyelination. Even though the myelin is thinner, the newly formed myelin is still sufficient to reestablish normal axonal conduction, to allow functional recovery and slow/prevent neurodegeneration (Duncan et al., 2009; Liebetanz and Merkler, 2006; Mei et al., 2016; Schultz et al., 2017).

Are OPCs the only source of remyelinating cells in the CNS?

Several studies have already suggested that mature oligodendrocytes targeted in MS do not participate in remyelination and this was finally well demonstrated by the use of inducible myelin-CreER mouse lines in which only mature OL are labelled: Labelled mature OL did not show any production of new myelin, nor did they proliferate or migrate to the demyelinated area (Crawford et al., 2016). On the other hand, OPCs are present in the demyelinating lesion before the appearance of new myelin and they give rise to OL forming new myelin as demonstrated by tracking experiments (Gensert and Goldman, 1997).

At least two other cell types can participate in reforming myelin: Neural precursor cells (NPCs) and SC. NPCs of the subventricular zone can also give rise to remyelinating OL in animal models and in MS (Nait-Oumesmar et al., 2008), however their global contribution compared to adult OPC seems modest and limited to the corpus callosum.

In numerous MS animal models, SC can enter the CNS and form new myelin. It is particularly the case in the spinal cord, where the PNS is in close contact with the CNS. However, some other areas like the cerebellum, the optic nerve and the brain stem can also be remyelinated by SC (Duncan and Hoffman, 1997; Itoyama et al., 1983; Zujovic et al., 2008). In a subset of MS patients, SC remyelination can be extensive in the spinal cord, and was even more important than OPC remyelination in a cohort of Japanese patients (Itoyama et al., 1983). In the spinal cord, SC remyelinate the center of the lesion, whereas OPCs remyelinate the border. While the equilibrium between SC and OPC remyelination seems to be astrocyte driven (Monteiro De Castro et al., 2015), the mechanism implicated in SC remyelination is not yet fully described. While some studies by lineage tracking suggested that remyelinating SC are derived from CNS glial precursors (Zawadzka et al., 2010), other studies have shown that a large amount of SC participating in remyelination are derived from the periphery (Oudega and Xu, 2006). In addition, it is still unknown if SC derived from the periphery are dedifferentiating to migrate and form myelin or if they are derived from a pool of PNS stem cells.

B. Role of inflammation

Inflammation is a two-edged sword in MS – it induces myelin destruction, but without inflammatory stimuli remyelination would fail. Inflammation is notably induced by cells of the innate immune system: resident MIG and invading macrophages from the periphery. Macrophages and MIG coordinate remyelination after being activated: they phagocytose myelin debris and induce OPC recruitment and differentiation.

Macrophages and MIG activation

After an injury in the CNS, resident MIG and invading macrophages will acquire a functional phenotype to respond to the injury in a process called “activation” or “polarization”. The activation profile was first believed to be dichotomous: in response to pro-inflammatory stimuli like IFN-γ or LPS, MIG and macrophages will be “classically activated” or “M1” and display pro inflammatory properties, while in response to anti-inflammatory stimuli like IL-4, MIG and macrophages will be “alternatively activated” or “M2” and display anti-inflammatory properties (Martinez et al., 2008). This very simple dual vision of innate immune system was challenged with the discovery of novel activation phenotypes: The M2b phenotype (in

response to IL-1β), which has immunoregulatory properties, and M2c (in response to IL-10), which has immunoregulatory and pro-regenerative capacities. The M2 phenotype in response to IL-4 is now called M2a (Martinez and Gordon, 2014; Murray et al., 2014a). Moreover, a large transcriptomic study showed that human macrophages have at least nine different phenotypes of activation in response to various stimuli (Xue et al., 2014a).

The M1/M2 is still used for convenience and because they are the only phenotypes of activation with a thoroughly described functional description. In remyelination, MIG and macrophages acquire an M1 pro-inflammatory phenotype that participates to the phagocytosis of myelin debris and induces recruitment of OPCs towards the lesion, before a switch toward the M2 phenotype that will allow OPC differentiation.

Phagocytosis of myelin debris

Myelin is formed by several layers of plasma membrane wrapping around axons. After a demyelinating injury, a lot of myelin debris is produced. Clearance of myelin debris is the first and a crucial step to induce myelin repair because myelin debris itself interferes with the maturation process of OPCs. *In vitro*, exposing OPCs to CNS myelin leads to a blockage of their differentiation (Robinson and Miller, 1999), and injection of exogenous myelin debris in a focal demyelination model leads to impediment of myelin repair due here again to a blockage of OPC differentiation and not recruitment (Kotter, 2006). The exact molecular mechanism of the effect of myelin is not totally unraveled. Clearing myelin debris in the CNS is one of the roles of the innate immune system : in mice with macrophages lacking CCR2, a receptor indispensable for phagocytosis function, remyelination was impeded by over-accumulation of myelin debris (Ruckh et al., 2012). In the same way, in mice with phagocytosis-deficient MIG, remyelination is impaired (Lampron et al., 2015). These two simple experiments showed that the two components (resident and invading) of the innate immune are required for phagocytosis of myelin debris and therefore giving a proper start to myelin repair (Figure 13B). In MS patients post-mortem tissue, remyelinated shadow plaques are most of the time surrounded by large number of macrophages positive for myelin staining indicating that efficient phagocytosis is correlated with efficient remyelination (Prineas et al., 1993), even if this correlation has not been proven causative in humans.

Recruitment of OPCs

MIG are extremely vigilant cells which actively sense their environment. When a demyelinating injury occurs, MIG will recruit monocytes from the circulation to perform phagocytosis of myelin debris. Both of these cell types will then induce OPC recruitment (proliferation and migration towards the lesion) (Figure 13C). The factors inducing these two steps are surprisingly not very well know. Among the few factors discovered, PDGF and FGF-2 (secreted by astrocytes and MIG) were demonstrated to induce OPC proliferation. In TNF receptor 1 knock-out mice, there is a huge decrease in OPC proliferation after demyelination, demonstrating a critical role for this MIG/macrophage-secreted cytokine in remyelination (Arnett et al., 2001). The conditioned media of M1 macrophages induces proliferation and migration of OPCs *in vitro*, and by specific depletion of M1 macrophages *in vivo*, proliferation of OPCs was impeded (Miron et al., 2013). These data indicate a crucial role of MIG/macrophages for OPC recruitment but further investigation is needed to highlight the panel of secreted factors acting on OPCs.

OPC differentiation

There has been an intense debate about the need of external factors for OPCs to differentiate and reform myelin. In physiological conditions, OPCs myelinate axons by two mechanisms: one termed “by default” is an intrinsic property of OPCs to wrap small caliber axons, and another which is dependent of electrical activity of axons (Sherman and Brophy, 2005). In remyelination however, several elements make axon wrapping more challenging: the axon can be injured, detrimental inflammation can still be present, and myelin debris and other extracellular elements detrimental for the myelin repair process can still be present. Inflammation is the element that will trigger and accelerate OPC differentiation to make remyelination as efficient as possible.

After a specific time, there is a switch in the phenotype of activation in innate immune cells, when they become M2 and release immunomodulatory and pro-regenerative factors (Figure 13D). Several factors secreted by anti-inflammatory M2 MIG or macrophages are known to induce OPC differentiation (Patel and Klein, 2011; Yong and Rivest, 2009; Zhang et al., 2011). Of note, IGF-1 increases OPC differentiation and myelin production *in vitro* and administration of IGF-1 decreases area of demyelination and increases the number of remyelinated axons in EAE (Yao et al., 1995). The Leukemia Inhibitory Factor (LIF) is both protective against OL death

and also increases remyelination by promoting OPC differentiation (Levy et al., 2015). Activin-A, another MIG/macrophage-secreted factor enhances OPC differentiation *in vitro* and remyelination *in vivo* (Miron et al., 2013).

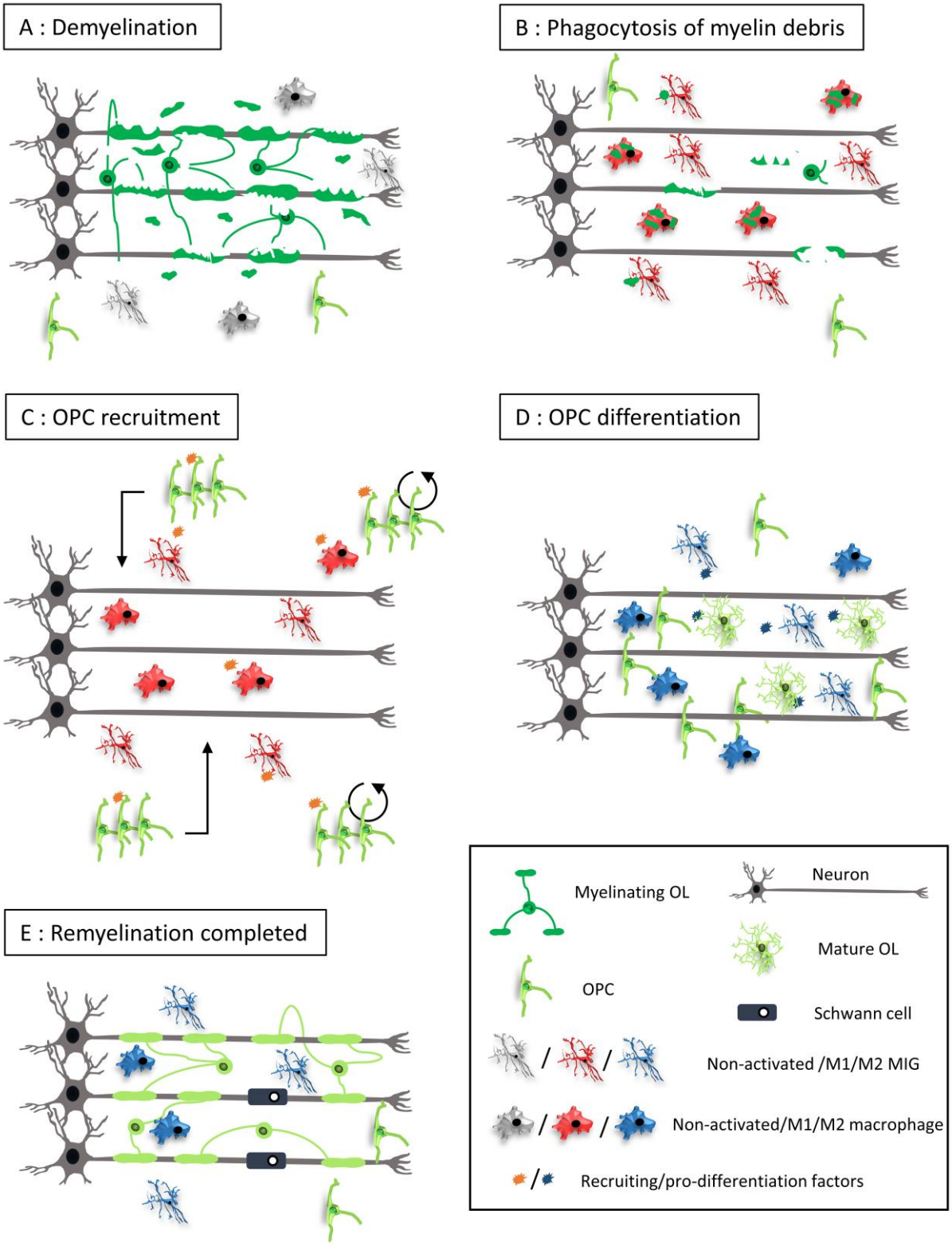

Figure 13: Sequential event in remyelination. After a demyelination injury (A), MIG and macrophages areactivated toward a pro-inflammatory M1 phenotype. Both cell types will phagocytose myelin debris even if some experimental evidence suggests a better efficacy of macrophages for this process (B). M1 macrophages and MIG will then secrete proliferation and migration factors that will trigger OPC recruitment toward the lesion (C). A switch to an M2 anti-inflammatory status occurs in MIG and macrophages , triggering the secretion of pro-differentiation and trophic factors leading to the start of the differentiation process in OPC (D) and finally to a complete remyelination (E).

Monocyte derived macrophages and MIG: different name, same function?

Macrophages derived from circulating monocytes and MIG are often both denominated as macrophages and were for a long time considered to have the same origin and functions in physiological and pathological conditions. This confusion persisted because the two cell population express the same markers (Iba-1, CD68, F4/80). It was therefore virtually impossible to study independently one cell population from another. However, it is well demonstrated now that these two cells types have different embryonic origins and that their function might be different during myelin repair: Experiments of fate mapping using transgenic lines demonstrated clearly that MIG arise from progenitors in the yolk sac (an annex embryonic structure) (Ginhoux et al., 2010) whereas monocytes derived macrophages are generated by hematopoiesis in the bone-marrow.

The first argument that led to speculation that monocyte derived macrophages and MIG have different functions was the transcriptomic analysis of these cells that showed that, in physiological conditions, even if they express a large number of identical genes, there are genes expressed specifically in each cell type (Hickman et al., 2013). In EAE mice, the transcriptional profile also differs between MIG and macrophages: macrophages are more activated and proliferate more in the early acute phase of the disease whereas MIG have a more resting phenotype. These data could indicate that macrophages are the major pathogenic actor in the early step of EAE (Vainchtein et al., 2014). MIG, however, upregulate MHC II genes and the co-stimulating molecules CD80, CD86 and CD40, arguing for a role in the reactivation of the invading adaptive immune cells (Lewis et al., 2014). In EAE, it seems that MIG have better phagocytic capacities than macrophages (even if the two cell types are both essential) as demonstrated by the upregulation of the expression of phagocytosis genes and functional studies (Lewis et al., 2014; Yamasaki et al., 2014) and the secretion of growth

factors by MIG seems to be critical for OPC differentiation. Finally, MIG have better immunosuppressive capacities. Still, further studies are needed to decipher more precisely the differences of action of MIG versus monocyte-derived macrophages in remyelination.

Use of the M1/M2 nomenclature

The bipolar view of classically (M1) and alternatively (M2) macrophages and MIG activation has been challenged in the last few years. Originally, this nomenclature was used to fit the Th1/Th2 dual view in immune response elicited by CD4+ cells. *In vitro*, macrophages stimulated with Th1 secreted IFN-γ would secrete cytotoxic NO, characteristic of the M1 activation, whereas an IL-4 stimulation elicited by Th2 cells would induce the secretion of trophic polyamine in macrophages, defining the M2 activation (Mills et al., 2000). The term was next exported to MIG, believed to have the same activation profile in response to the same stimuli. From there, markers expressed by M1 cells and M2 cells were used to define macrophages activation in pathological conditions.

This nomenclature raises several problems. The first one is that the M1 state if defined now as being the result of exposition to IFN-γ, Lipopolysaccharide or both, even though the transcriptomic responses induced in macrophages are different (Martinez et al., 2006). This differences in the transcriptional response could indicate distinct role in physiological and pathological conditions. The same shortcut exists for the M2a phenotype, defined as IL-4 or IL-13 activated macrophages even though the resulting signature is close, but not identical (Scotton et al., 2005). Overall, macrophages with a different response to distinct stimuli are classified as a same activation profile.

Secondly, when studied *in vitro*, macrophage activation is not limited to M1 and M2 activation. A large study on human macrophage showed the existence of at least 9 unique transcriptomic signature in response to 29 different stimuli arguing for a spectrum model of activation in macrophages (Xue et al., 2014b).

Another issue with this classification is the use of the term M1 and M2 *in vivo*. They simply not exist *in situ* as the stimulation received by macrophages are never just one cytokine. Macrophage activation *in vivo* is a complex process, involving cytokine signaling, cell adhesion, cell-cell interactions … In pathological study, the term M1 and M2 are used when the innate

immune cells analyzed express express *in vivo* the same marker than an M1 macrophage. However, a simple common marker does not imply the same functional role.

The M1/M2 paradigm, despite all its weaknesses, is useful to get an overview of an extremely complex process. In the case of remyelination, we chose to keep it as it seems that there is a well characterized functional role of macrophages expressing M1 and M2 markers in the process (Miron and Franklin, 2014).

The established M1/M2 framework and the uses of associated markers allowed major discoveries in the study of macrophages in neurodegenerative processes: In MS, several molecules with high potential therapeutical applications have been discovered by studying the secretion panel of macrophages positive for M2 markers. For instance, Activin-A can induce OPC differentiation during remyelination (Miron et al., 2013).

Overall, macrophages *in vivo* do not have a binary choice during activation, they acquire a mixed subset of phenotypes that if probably continuous. A new nomenclature was proposed by a group of macrophages specialist and propose to define macrophages subset according to the stimulation used to polarize them (i.e. M(IL-4) for IL-4 stimulated macrophages) (Murray et al., 2014b). This solves the problem of the large number of stimulation defining the M1 or the M2 stage but does not clarify how we should define macrophages positive for M1 or M2 markers *in vivo*.

Role of the adaptive immune system.

The role of the adaptive immune system in the myelin repair process remains less understood compared to the innate immune system. However, some clues indicate a critical action of LT in remyelination. More than a decade ago, it was discovered than T cells play a role in remyelination. In mice deficient for T and B cells, or mice depleted specifically of CD4+ or CD8+, the myelin repair process was less efficient in a model of focal demyelination, indicating that these cells are necessary for remyelination (Bieber et al., 2003). One of the putative mechanisms is that T cells are able to induce OPC proliferation, as it was demonstrated in a mouse model (Hvilsted Nielsen et al., 2011a).

4. Remyelination heterogeneity: causes of remyelination failure

A. In animal models

In animal models of demyelination/remyelination and of MS, the major known factor leading to remyelination failure is aging. After a focal demyelination was induced in rats, both OPC recruitment (proliferation and migration to the lesion) and differentiation were impeded in aged animals compared to younger controls (Sim et al., 2002) leading to a decrease in remyelination efficacy (Shields et al., 1999).

The cause for OPC recruitment failure is still under debate: experimentally, repeated demyelination episodes induced by cuprizone treatment, mimicking a long disease course, led to a decrease of the number of OPCs at the lesion and therefore a diminished remyelination efficacy. The underlying mechanism is however not known and several hypotheses can be formulated: exhaustion of OPC stock, or diminished capacity of OPCs to proliferate and/or migrate. However, several studies do not argue for this OPC recruitment failure hypothesis, as repeated rounds of demyelination do not disturb remyelination in a focal demyelination model (Levine and Reynolds, 1999; Penderis et al., 2003).

The effect of aging on OPC differentiation has been more extensively described and replicated. In aged animals, the induction of critical signaling pathways and transcription factors for OL differentiation is delayed (Fancy et al., 2004b) and therefore the maturation of OPC becomes less efficient. Here again, it seems to depend on inflammation. In aged animals, macrophage function is altered. First, phagocytosis of myelin debris is less efficient in aged animals, leading to OPC differentiation failure. This poor remyelination is rescued in aged animals when they share blood circulation, and therefore innate immune cells, with young animals (Ruckh et al., 2012). In the same experiment of parabiosis, OPC proliferation is increased suggesting that the rescue of remyelination is not only due to the rescue of phagocytic capacities but also in other macrophage functions. In fact, the cytokine secretion panel of macrophage changes over time (Zhao et al., 2006). Because cytokines secreted by macrophages and MIG drive OPC behavior during myelin repair, this data could explain why OPCs do not get the right signal at the right time to enter recruitment and/or differentiation. The differential cytokine secretion panel could be explained by a misactivation of old macrophages. Another experiment of heterochronic parabiosis model demonstrated that young macrophages are more activated

into an M2 phenotype in the final stage of remyelination leading to a proper OPC maturation (Miron et al., 2013).

All these data demonstrate that in animal models, aging is affecting the efficacy of remyelination due to a less efficient inflammatory response.

B. In MS patients

Is aging the principal cause of remyelination failure in patients?

As several repair processes and more generally biological processes show decreasing efficacy with age, and because the efficacy of remyelination decreases in animal models, it would be expected to find the same results in patients: patients with long lasting disease should have less efficient remyelination. The classical view in the field is that remyelination is efficient in early disease stages, where the inflammatory component is strong and that, with time, the neurodegenerative stage of the disease takes over, leading to chronic plaques in which remyelination is absent. However, the reality seems to be more complex than that.

The first clues that can contradict this theory come from the analysis of post mortem tissues of MS patients. In a study of two patients with long (21 and 22 years) disease course, analysis of remyelination revealed that 73% of lesions were at least partially remyelinated (Patani et al., 2007). Even if the number of patients analyzed in this study is very low, it demonstrates that the idea that myelin repair does not occur after a long disease course is at least partially wrong. Furthermore, a wider study on 51 patients showed a positive correlation between age of patients and remyelination (Patrikios et al., 2006). This result seriously challenges the idea that aging is the cause of remyelination failure in MS patients.

One might argue that analysis of post mortem tissue is a 'snapshot' of a specific time and does not reflect the global capacity of a patient to induce myelin repair over time. Recently, new techniques combining PET-scan and MRI allowed for the first time to visualized remyelination in patients by imagery (Bodini et al., 2016). Using a specific marker of myelin ([^{11}C]PiB), two scans were realized at 1-4 month of interval in 20 patients. The new [^{11}C]PiB positive voxels appearing on the latest scan images reflected the newly formed myelin, and a remyelination index was calculated. Interestingly, no correlation between the age or disease duration and the remyelination index was found.

All these data suggest that neither age nor disease is the principal factor of remyelination failure in patients.

What is causing remyelination failure in some patients?

The three studies mentioned above (Bodini et al., 2016; Patani et al., 2007; Patrikios et al., 2006) and some others (Bruck et al., 2003; Strijbis et al., 2017) led to a consensus in the field: Remyelination is heterogeneous in MS patients. There is an individual capacity to induce myelin repair. Some patients have this process working efficiently during all their lifetime, and this is correlated to a lower severity score (Bodini et al., 2016), while for some other patients, the repair process fails (Figure 14). Multiple histological studies have been performed to decipher the cellular mechanisms of remyelination failure in some patients. One of the causes investigated was, like in animal models, a failure of OPC recruitment and/or a depletion of the OPC stock after repeated rounds of demyelination/remyelination. This has been notably investigated in post mortem tissues of patients and, globally, OPCs are always present in demyelinated lesions, even if the number of OPCs can change with the clinical features of patients. OPCs are present in a considerable number in chronically demyelinated lesions (Chang et al., 2002; Wolswijk, 1998a) and even more so in early active lesions (Chang et al., 2000; Kuhlmann et al., 2008). All these data concur to disprove the recruitment and/or depletion theory: in MS patients, the OPC stock is never depleted and these cells can be recruited to the lesion.

While OPCs are present in the lesion, multiple pieces of evidence demonstrate a failure of OPC differentiation. In chronic and active lesions, there is a strong inter-individual capacity in the number of differentiating OPCs (Kuhlmann et al., 2008; Wolswijk, 1998b). Several markers of maturation were used in these studies, and it seems that the differentiation block is not homogeneous between patients. In some, cells are blocked in early steps of maturation whereas in another subgroup of patients, cells express markers of mature OLs but do not form myelin (Chang et al., 2002).

Because it has been demonstrated recently that the innate immune system is driving remyelination is MS, a few studies on this have been conducted on biopsies and/or post mortem tissues. M2 MIG seems to be more present in early active lesions than in chronic lesions (Miron et al., 2013) and the activation status of innate immune cells seems to depend on the lesion type (Peferoen et al., 2015).

However, a recent study challenged the results of former groups, as most of the markers used to discriminate between M1 and M2 macrophages/MIG were co-expressed by these cells indicating that either the status of activation of these cells is more complex than the dual M1/M2 view or that the markers used previously were not representative of each activation status (Vogel et al., 2013). Therefore, more advanced techniques (transcriptomic and

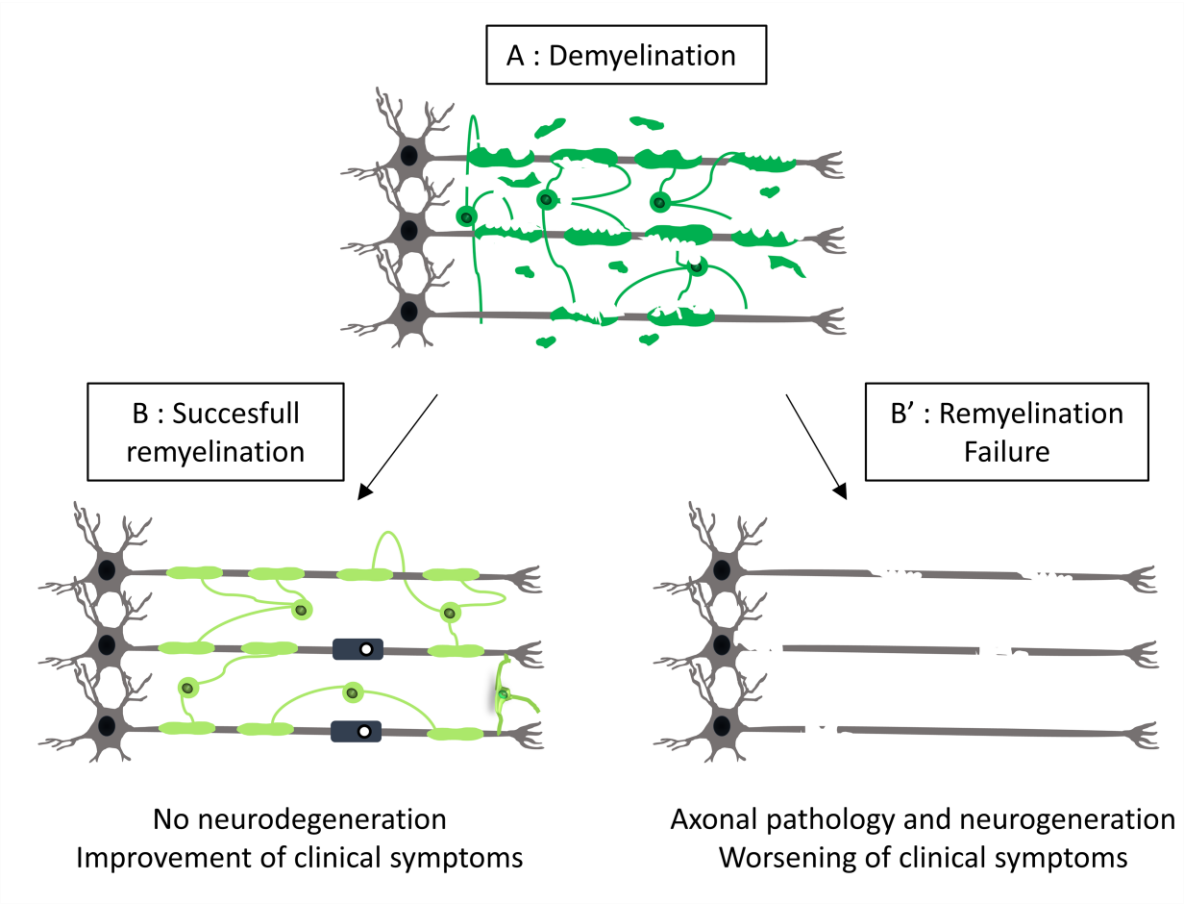

Figure 14: Fate of remyelination and consequences on clinical course of patients. After a demyelinating injury (A), the endogenous myelin repair process can either succeed (B) or fail (B') Remyelination can fail for different reasons: Poor myelin debris phagocytosis, failure of OPC recruitment, failure of OPC differentiation. In patients, the latest hypothesis is the most documented and demonstrated.

proteomic) are needed to define more precisely the activation status of the innate immune system in MS lesions and explain why OPC differentiation can fail in some patients.

Globally, remyelination fails in some patients due to a deleterious micro-environment that will prevent OPC to reform new myelin.

IV. Aims of the project

Even if some substantial progresses have been made in the treatment of MS, there is still no cure nor both very efficient and safe disease modifying treatment. This is partly explained by the view of inflammation as only a detrimental actor in the pathophysiological process. As the demyelination was cause by inflammatory attacks, it was logical to focus research effort on neutralizing the immune system. In the last decade however, inflammation was demonstrated to be beneficial and indispensable for remyelination. There is therefore a beneficial inflammatory process that should not be targeted, but on the contrary, reinforced to provide remyelination and neuroprotection.

Understanding the cellular and molecular events leading to a successful remyelination is critical to develop innovative treatment to foster this mechanisms. The huge majority of studies studying remyelination have been performed using only murine models and were not able to reproduce nor explain the spectrum of remyelination efficacy found in patients. In MS, even if the use of those models allowed tremendous progress on understanding the pathophysiological mechanisms at stake, the use of patient cells in humanized model could bring some more precise insight on the cellular and molecular events leading to a successful remyelination.

LT invade the CNS and induce myelin destruction. To do so, they induce a pro-inflammatory environment that will cause a chain reaction and induce a large immune response. By being major actor of the induction of an inflammatory micro-environment in the CNS, LT could play a role a remyelination. Therefore, using a humanized model, we tackled first the following questions:

1) **Do human LT from patients or HD influence the remyelination process ? If that is the case, what are the molecular and cellular events, influenced by LT, leading to a succesful remyelination ? Those questions will be tackled in chapter 2.**

In chapter 2, we discovered that the secretory profile of LT upon stimulation can vary considerably from an individual to another leading to a chain of event inducing remyelination failure or success. In the second part of the project, we tried in a preliminary study to decipher what is causing this inter-individual variation by studying the effect of susceptibility SNP concentrated in a specific LT pathway on remyelination.

2) **In patients, do SNPs concentrated in one Th cell pathway can influence remyelination outcome? Can one SNP or a combination of SNP be predictive of the success of remyelination ? Those questions will be tackled in chapter 3.**

Chapter 2: Evaluation of the role of lymphocytes in remyelination and defining the molecular basis for an efficient myelin repair in patients.

I. Introduction
II. Article 1 and contribution
III. Supplementary unpublished results
IV. Patent

I. Introduction

After destruction of the myelin in MS, remyelination is triggered. From experimental data, this system is mainly mediated by the innate immune system and its state of activation to realize the different steps of the process.

As it was shown in vivo and in vitro (Butovsky et al., 2006; Miron et al., 2013; Zhang et al., 2014), LT secreted cytokines can influence the state of activation of macrophages and MIG. However, this data use only mouse cells that make difficult to evaluate the consequences of a cross talk between LT and innate immune cells during remyelination. Moreover, remyelination has not being extensively studied using models including pathological MS cells from patients.

To tackle those questions, we created a novel model in vivo model by grafting MS or HD LT into a focally demyelinated lesion in the spinal cord of nude mice. We also developed new in vitro protocols to evaluate the influence of LT on innate immune cells. Finally, we analyzed the heterogeneity of remyelination in patients by defining the molecular landscape necessary for a successful myelin repair.

II. Article 1 and contribution

Adaptive human immunity drives remyelination in a mouse model of demyelination

M. El Behi [1#], **C. Sanson[1#],** C. Bachelin [1], L. Guillot-Noël [1], J. Fransson [1], B. Stankoff [1,2], E. Maillart [3], N. Sarrazin [1], V. Guillemot [1] , H. Abdi [4], I. Cournu-Rebeix [1†], B. Fontaine [1-3†], V. Zujovic [1†,*].
(2017) *Brain* 140:967-980.

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#: Co-first authors / †: Co-last authors

Figure 1: Participation in surgeries, perfusions, tissue processing. Main contributor of Immunostainings, quantification, statistical tests and figure drawing.

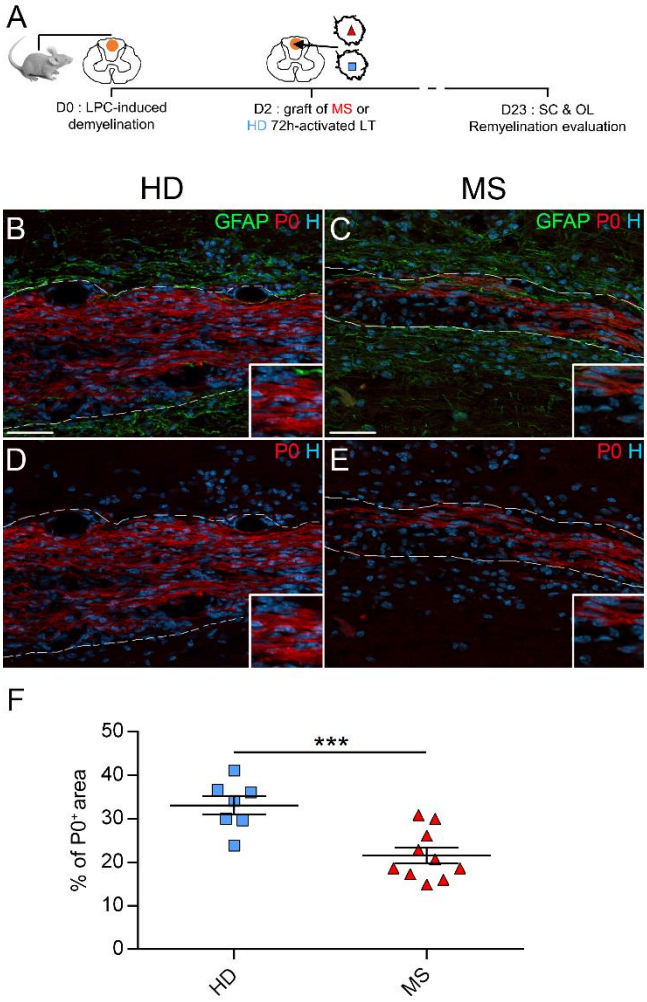
Figure 2-3-5-7-S1-S2-S3-S4-S5: main contributor.

Figure 4: Participation in statistical analysis, heatmap realization and figure drawing.

Figure 6: Participation in the design of the bioinformatics analysis. Main contributor for the *in vitro* validation of CCL19.

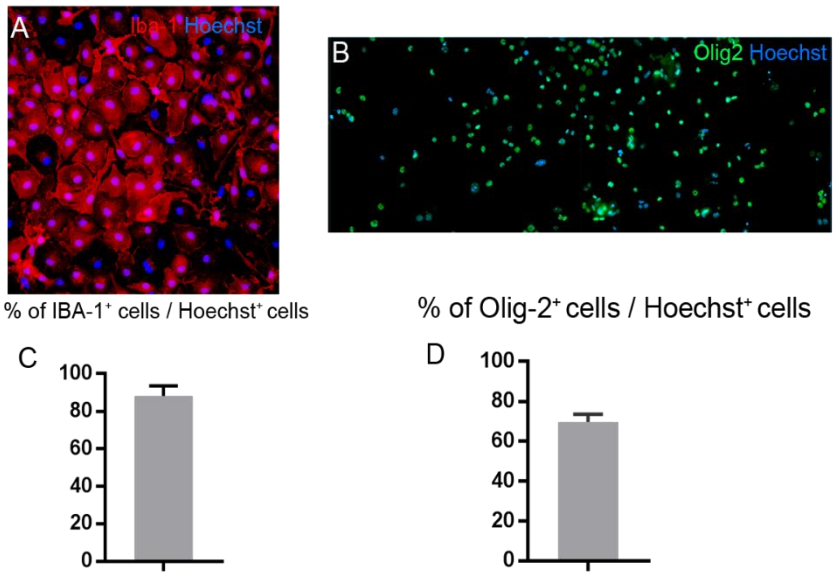
III. Supplementary unpublished results

To evaluate the global effect on remyelination, the influence of MS LT on SC remyelination has also been evaluated using a specific marker of PNS myelin. Our results show a similar results than for OL remyelination as MS LT impede the process compare to HD LT (supplementary Figure 6).



Supplementary figure 6: MS patient LT impede SC remyelination. Schematic of the remyelination essay after LT graft (A). 48h after chemically induced demyelination in the dorsal spinal cord of nude mice, HD (B, D) or MS patient LT (C, E) were grafted. After 21 days, SC remyelination was evidenced by PO immunostaining (B-E) within the lesion delimitated by GFAP (white dotted line). The percentage of $P0^+$ area in the $GFAP^-$ lesion (F) were calculated in the HD (n=7) and MS (n=10) conditions. *p<0.05, *** p<0,001. Healthy donors vs MS patients. Student t-test. Scale bar : 50µm.

To eliminate the hypothesis of another cell type having an indirect role on MIG activation or OPC differentiation (see figure 2 and 3 of the publication), the purity of the primary culture of those two cell types was evaluated (supplementary Figure 7). The protocol that we used to isolate mouse or rat MIG and OPCs from mixed glial cells culture in commonly used, with minor variations, among researchers in the field (Butovsky, 2006; Foote and Blakemore, 2005; Miron et al., 2013) and several protocol paper detail very precisely the different steps to isolate MIG or OPCs from rodent brains (O'Meara et al., 2011; Tamashiro et al., 2012). Using this technique, we obtained a proportion pure cells of 88,1 +/- 5,5% for MIG and 69,7 +/- 9,2 % for OPCs. These values are of the same order of magnitude of what is obtained by other teams using this protocol.



Supplementary figure 7: To validate purity of the MIG and of the OPC culture, cells were stained respectively with Iba-1, a general marker of MIG, or with Olig-2 a marker of the OL lineage. Cells were also counterstained with Hoechst to reveal the nucleus of all the cells present in the cultures. The % of Iba-1$^+$ or of Olig-2$^+$ cells were calculated. n= 6 independent experiments.

IV. Patent

Europaisches Patentamt
European Patent Office
Office européen des brevets

PROGNOSIS OF DEMYELINATING DISEASES PATIENTS AND TREATMENT THEREOF

Application number: EP16194192

Submitted: 17 October 2016

FIELD OF INVENTION

The present invention relates to the field of demyelinating diseases, to methods for prognosing demyelinating diseases and to methods for treating thereof.

ABSTRACT

The present invention relates to an *in vitro* method for the prognostic of a subject affected by a demyelinating disease comprising detecting and quantifying the level of at least one of CCL19, IL-15, ENA78 and/or TRAIL in a biological fluid from the subject. The invention also relates to the treatment of a subject affected by a demyelinating disease comprising the use of at least one modulator of CCL19, IL-15, ENA78 and/or TRAIL.

INVENTORS (contribution)

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El Behi Mohamed (20%)
Reibex Isabelle (20%)

SUMMARY

The present invention relates to an in vitro method for the prognostic of a subject affected by a demyelinating disease comprising detecting and quantifying the level of at least one of CCL19, IL-15, ENA78 and/or TRAIL in a biological fluid from the subject.

In one embodiment, the *in vitro* method further comprises detecting and quantifying the level of at least one of LIF and SDF-1.

In one embodiment, the level of the at least one of CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL allows classification of the subject in a group having a low remyelination profile or in a group having a high remyelination profile.

In one embodiment, the measured levels of CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL is/are compared to reference values. Preferably, said reference values correspond respectively to the median levels of CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL in a group of patients with a demyelinating disease.

The present invention also relates to a composition for use in the treatment of a demyelinating disease comprising at least one modulator of CCL19, IL-15, ENA78 and/or TRAIL.

In one embodiment, the composition for use in the treatment of a demyelinating disease further comprises at least one modulator of LIF and/or SDF-1.

In one embodiment, the at least one modulator of CCL19, IL-15, ENA78, TRAIL, LIF and/or SDF-1 is an antibody or fragment or mimetic thereof, an aptamer, a small molecule, a peptide mimetic, a siRNA, an asRNA, an antagonist, an agonist or an inverse agonist.

In one embodiment, the composition for use in the treatment of a demyelinating disease comprises at least one inhibitor of CCL19, ENA78 and/or SDF-1 and/or at least one 25 activator of IL-15, LIF and/or TRAIL.

In another embodiment, the composition for use in the treatment of a demyelinating disease comprises at least one antagonist or at least one inverse agonist of CCR7, CXCR2 and/or CXCR4; and/or at least one agonist of IL-15R, LIFR, TRAIL-RI and/or TRAILRII.

In one embodiment, the composition is for use in the treatment of a demyelinating disease selected from the group comprising inflammatory demyelinating disorders, multiple sclerosis,

acute disseminated encephalomyelitis, transverse myelitis, acute transverse myelitis, Guillain-Barre syndrome, brainstem encephalitis, optic neuritis, neuromyelitis optica, leukodystrophy, adrenoleukodystrophy, adrenomyeloma neuropathy, idiopathic inflammatory demyelinating disease, central pontine myelinolysis, optic neuritis, aquaporin 4 antibody-negative neuromyelitis optica, progressive multifocal leukoencephalopathy, periventricular leukomalacia, Vitamin B12 deficiency, Wernicke's encephalopathy, osmotic demyelination syndrome, Leigh's disease.

In one embodiment, the modulator is to be administered in a subject having a low remyelination profile.

CLAIMS

1. An *in vitro* method for the prognostic of a subject affected by a demyelinating disease comprising detecting and quantifying the level of at least one of CCL19, IL-15, ENA78 and/or TRAIL in a biological fluid from the subject.

2. The *in vitro* method according to claim **1**, further comprising detecting and quantifying the level of at least one of LIF and SDF-1.

3. The *in vitro* method according to claim **1** or **2**, wherein the level of at least one of CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL allows classification of the subject in a group having a low remyelination profile or in a group having a high remyelination profile.

4. The *in vitro* method according to any one of claims **1** to **3**, wherein the measured levels of CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL is/are compared to reference values, preferably said reference values correspond respectively to the median levels of CCL19, IL-15, ENA78, LIF, SDF-1, and/or TRAIL in a group of patients with a demyelinating disease.

5. A composition for use in the treatment of a demyelinating disease comprising at least one modulator of CCL19, IL-15, ENA78 and/or TRAIL.

6. The composition for use according to claim **5**, further comprising at least one modulator of LIF and/or SDF-1.

7. The composition for use according to any one of claims **5** to **6**, wherein said modulator is an antibody or fragment or mimetic thereof, an aptamer, a small molecule, a peptide mimetic, a siRNA, an asRNA, an antagonist, an agonist or an inverse agonist.

The composition for use according to any one of claims **5** to **7**, comprising at least one inhibitor of CCL19, ENA78 and/or SDF-1 and/or at least one activator of IL15, LIF and/or TRAIL

Chapter 3: Linking MS susceptibility variants to remyelination capacity

Article 2 and contribution

In this preliminary study, the consequences of genetic variants in MS patient LT on remyelination was evaluated.

Chapter 4: Discussion and conclusion

I. Role of lymphocytes in remyelination

1. Experimental evidence

LT were not originally thought to participate in myelin repair, whereas several investigations demonstrated a major role of the innate immune system. However, being present during remyelination, especially in acute plaques (Popescu and Pirko, 2013), LT could potentially influence the outcome of the process.

The involvement of LT in remyelination did not attract much attention until the early 2000's. Using knock-out mice or antibody depletion, it was demonstrated that T cells are necessary an efficient remyelination (Bieber et al., 2003): in B6-Rag1^{tm1Mom} mice, lacking B and T cells, the density of remyelinated axons 35 days after focal demyelination of the spinal cord was reduced three-fold. In mice lacking CD4+ (CD4^{tm1Mak} knock-out model or depletion induced by anti-CD4 antibody) or CD8+ cells (CD8^{tm1Mak} or depletion induced by an anti-CD8 antibody), remyelination was also strongly impeded. This pioneer study used a focal demyelination model, in which the mice LT are not myelin-primed and therefore non pathological. Mechanistically, these results could be partially explained by recent publications showing a direct regenerative role of Tregs in remyelination: First, there is a Tregs induction at the initiation of remyelination (Plaisted et al., 2016). In addition, in a knock-out mouse for Foxp3, in which Tregs are absent, remyelination was impeded and rescued by the graft of exogenous wild type Tregs (Dombrowski et al., 2015). Tregs were shown to have a direct pro-differentiation effect on OPCs *in vitro* mediated by the secretion of CCN3. Even if the well-known immunomodulatory role of Tregs cannot be excluded to have played a role in the rescue of remyeliation, Tregs seem to have a direct regenerative role on promoting OPC maturation. Interestingly, it was also shown that myelin-specific T cells can induce a proliferation of OPC after axonal damage through an unknown mechanism (Hvilsted Nielsen et al., 2011b).

Other studies have pointed out a deleterious role of LT in remyelination: using only human cells, it was shown that the supernatant of Th1 and Th17 cells, pathogenic in MS, have direct cytotoxic effects and indirect differentiation blocking effects on OPCs *in vitro* (Moore et al., 2015). The molecular actors of this cytotoxic effect were not revealed in the study, but the fact that the supernatant alone has an effect indicates a cytokine-mediated mechanism. *In vivo*, the graft of myelin-reactive Th17 cells in the cuprizone model delayed remyelination by inducing a pro-inflammatory phenotype in macrophages (Baxi et al., 2015).

Overall, pathogenic T cells (Th1 and Th17), are, by inducing a pro-inflammatory cytotoxic environment are directly and indirectly inhibiting remyelination whereas T cells with immunoregulatory phenotypes are fostering remyelination by calming the inflammation storm and promoting OPC differentiation.

2. Cellular mechanisms involved

The accumulation of experimental evidence seems to point toward a prominent indirect action of LT on OPCs. In the study of Bieber et al., they already suggested that LT could act on MIG and macrophages to influence remyelination.

The cross-talk between adaptive and innate immune cells is well established in the immune response: antigen-presenting cells, mostly MIG and macrophages, can trigger lymphocyte activation and proliferation at the initiation of the adaptive immune response. Moreover, MIG are able to reactivate LT once they cross the BBB in multiple sclerosis, inducing chronic demyelination.

In remyelination, it is likely that this cross-talk goes both ways and that LT could influence MIG and macrophage activation: First, it was demonstrated two diametric opposite phenotypes of macrophage activation, driven by LT secreted cytokines IFN-γ and IL-4, have distinct roles in oligodendrogenesis and neurogenesis (Butovsky et al., 2006). *In vitro*, it was shown that the supernatant of Th1 cells could drive an M1 response in MIG (Prajeeth et al., 2014) Moreover, in our studies, we clearly demonstrated by immunohistochemistry and qPCR that MS LT are able to influence MIG activation toward a pro-inflammatory M1 phenotype (El-Behi *et al.*, 2011, Sanson et al. in preparation). Finally, a predominant M1 phenotype was found in

macrophages after Th17 grafts in a cuprizone model compared to the non-grafted animal arguing for a cross talk between LT and innate immune cells as the cause for remyelination failure (Baxi et al., 2015). An induction of an M1 phenotype in MIG and macrophages could also explain why myelin reactive T cells induce OPC proliferation *in vivo* (Hvilsted Nielsen et al., 2011b), as the M1 phenotype is known to induce this effect on OPCs (Butovsky et al., 2006; Miron et al., 2013).

Direct mechanisms of LT on OPCs are also likely to influence remyelination even if less experimental proof exists: as stated above, Tregs can induce OPC differentiation and Th1 cells are cytotoxic for OPCs. However, these results must be taken with cautions as the direct effect was demonstrated *in vitro.* In our first study, we did not find any direct effect of MS LT on OPC differentiation (Behi et al., 2017). However, we also demonstrated a strong inter individual heterogeneity of the effect of MS LT on remyelination, making any conclusions difficult since the effect on remyelination is the results of pro and anti-differentiation signals from LT.

The results of the study arguing for a beneficial role versus the ones arguing for a detrimental role of LT in remyelination are not necessarily contradictory. Inflammation in MS is a two-edged sword inducing both myelin destruction and repair. Remyelination is a mechanism requiring both pro- and anti-inflammatory stimuli to be completed, and these stimuli must occur sequentially to ensure that remyelination to be completed. Therefore, if the pro-inflammatory micro-environment is exacerbated like when Th17 cells are grafted in a cuprizone model (Baxi et al., 2015), this will prevent OPC differentiation as the M1 phenotype induced by Th17 cells can inhibit the maturation process. It is thus likely that pro-inflammatory LT can induce a strong pro-inflammatory response, preventing the regeneration steps to be performed in MS whereas anti-inflammatory LT drives an anti-inflammatory environment that do not inhibit the early steps but foster the later steps of remyelination.

Overall, LT can influence remyelination by direct effects on OPCs and by driving MIG and macrophage activation.

3. Modeling LT role in remyelination

Advantages of our model

Our studies are the only ones using MS patient LT to study the role of these cells in remyelination. Interestingly, the graft of these cells in a demyelinated lesion of the spinal cord was sufficient to induce a heterogeneous pattern of remyelination, with LT from some patients inducing a remyelination level comparable to HD levels, and those from other patients inducing a less efficient myelin repair process. Several studies have tried to understand why remyelination sometimes fails, but as said previously (see Chapter 1, III.4), the remyelination heterogeneity in patients was never properly modeled. . To understand why it fails in some patients, a new model was needed and we proposed a new experimental paradigm combining the advantages of focal demyelination, in which the timing and the cellular and molecular events of myelin repair are well known, and human LT from MS patients and HD, allowing to better model of remyelination in a pathological context.

A second advantage of our model is that the mouse strain (RjOrl:NMRI-Foxn1$^{nu/}$Foxn1^{nu}) in which LT were grafted is athymic, and therefore does not have any T cells, thus the specific effect observed is most likely due to grafted LT. Nude mice do however have B and natural killer cells, and we can therefore not completely rule out the hypothesis that human grafted LT could influence the murine adaptive immune system and eventual consequences on remyelination.

An advantage of using LPC-induced demyelination is that the myelin destruction event happens only before (not during nor after) remyelination, making it easy to separate the events due to demyelination and the ones due to remyelination.

The major issue with rodent remyelination models (such as cuprizone, LPC) is that myelin repair in these models is spontaneous and extremely efficient. Therefore what can be observed in experimental conditions is only acceleration or a deceleration of remyelination. Therefore, these models are not perfectly suited for pharmacological studies as the putative effect induced by a tested compound is an acceleration of remyelination and not an overcome of remyelination failure: in other words, the tested compound might change the kinetics of remyelination but its capacity to induce myelin repair in a inhibitory micro-environement cannot be evaluated. In our model, however, we have a remyelination failure caused by LT

from patients with low repair capacity. This model would therefore be more suited to highlight the effect of a compound that could overcome failure of myelin repair.

Interestingly, our model reproduces most of the characteristic of an active lesion of patients with short disease course analyzed by biopsy: we found a similar density of LT (Behi et al., 2017; Kuhlmann et al., 2002), and LT are localized preferentially near blood vessels.

Drawbacks of the model

Our model also presents some drawbacks: grafting human LT in a mouse will not account for immune activation that is cell contact dependent as the human TCR and the mouse MHC are not compatible. Thus, a part of the cross-talk between adaptive and innate immune cells that putatively happens during remyelination is not represented in our model. In addition, the graft of human cells in a mouse does not allow a perfect cytokine communication, even though the vast majority of cytokine pathway are common between mice and human.

When the graft was performed, it contained a mixed population of T and B cells. Therefore we cannot conclude which cell type influence the most the remyelination process. It would be necessary to sort LT through FACS before grafting to test which LT subset is influencing MIG activation and OPC behavior.

The only unquestionable way to demonstrate that our model reproduces remyelination heterogeneity in patients would be to graft the LT of patients whose remyelination capacities are known, such as the patients from the study evaluating remyelination by PET imaging (Bodini et al., 2016).

We established a major role of LT in driving remyelination but we cannot exclude that the individual capacities of patients to instruct remyelination could also be the consequence of other cellular actors and molecular mechanisms that are independent from any LT involvement or that the LT sampled in the blood of patients are not a representative population of the LT invading the CNS in those patients.

II. Enhancing endogenous remyelination: acting directly on OPCs

1. Rational

Preventing neurodegeneration is now the most interesting strategy to cure MS as axonal death is the cause of irreversible disability. There is an accumulating amount of evidence showing that remyelination is beneficial both in animal models (Duncan et al., 2009; Slowik et al., 2015; Smith et al., 1979) and MS patients (Bodini et al., 2016; Bramow et al., 2010; Kornek et al., 2000a) by restoring loss of function and providing neuroprotection. In the last decade, a vast number of studies therefore aimed to to enhance the endogenous myelin repair process in order to find new treatments for MS.

As detailed in chapter 1 III., remyelination failure in patients is most likely the consequence of OPC differentiation failure due to a deleterious micro-environment and the presence of OPC differentiation inhibitors, such as cytokines, chemokines, growth factors, microRNAs, myelin debris, and extracellular matrix proteins (Franklin, 2002; Franklin and Ffrench-Constant, 2008; Patel and Klein, 2011). Therefore, huge efforts have been made to discover molecules directly enhancing OPC differentiation. Several screenings have been performed, using hundreds of already FDA approved treatments (Najm et al., 2015) or using thousands of compounds (Deshmukh et al., 2013; Mei et al., 2014), highlighting several candidates enhancing OPC differentiation *in vitro* and promoting remyelination *in vivo* (Figure 16). Several of them are under clinical trial (Plemel et al., 2017).

Clemastine is one of the first pro-remyelinating agents that have gone through clinical trial. It was discovered after screening using an elegant technique utilizing micropillars around which OPC can differentiate and form myelin (Mei et al., 2014). While the first results of clemastine were promising, the secondary endpoint of the phase II clinical trial (NCT02040298) showed a mild enhancement of visual evoked potential (VEP, a value measuring the speed of conduction through the visual pathway, often slowed down in MS patients because of demyelination of the optic nerve), but no improvement in EDSS and a worsening of fatigue in patients receiving the treatment.

An anti-LINGO-1 antibody treatment was also tested in a clinical trial. LINGO-1 is a transmembrane signaling protein expressed by OPCs inhibiting their differentiation (Jepson et al., 2012). Anti-LINGO-1 treatment was shown to have beneficial effects on EAE and to promote remyelination (Mi et al., 2007, 2009). The results of the clinical trial were globally disappointing at first as the treatment did not reach the first end-point (VEP-latency) but a significant difference was found at a second end-point (Cadavid et al., 2017). Another clinical trial is ongoing, with an augmented number of patients to demonstrate the potential beneficial effect of anti-LINGO-1 treatment.

2. Limitations

A. Choosing the right MS population to demonstrate the efficacy of a pro-remyelination treatment

Theoretically, patients with RRMS and who are at an early stage in the disease course seem to be the ideal cohort, as their axons are not yet undergoing extensive neurodegeneration and are therefore available for remyelination. The best window of action for a pro-remyelinating treatment is still debated: If the treatment is given too early after a relapse, OPC recruitment will not have occurred, and therefore giving a pro-differentiating drug would not favor remyelination. If it is too late after a relapse, at that moment the inflammatory process would have calmed down, and their beneficial effect could be lost.

Patients with progressive forms of MS would theoretically be less sensitive to pro-remyelinating drugs, as a massive axonal degeneration would have started, making remyelination less extended.

Patients with progressive forms of MS would theoretically less sensitive to pro-remyelinating drugs, as a massive axonal degeneration would have started, making remyelination less extensive.

B. The dual role of inflammation

Promoting OPC differentiation in MS patients could potentially enhance remyelination in patients. However, one of the major flaws of this strategy is that inflammation would not be modulated.

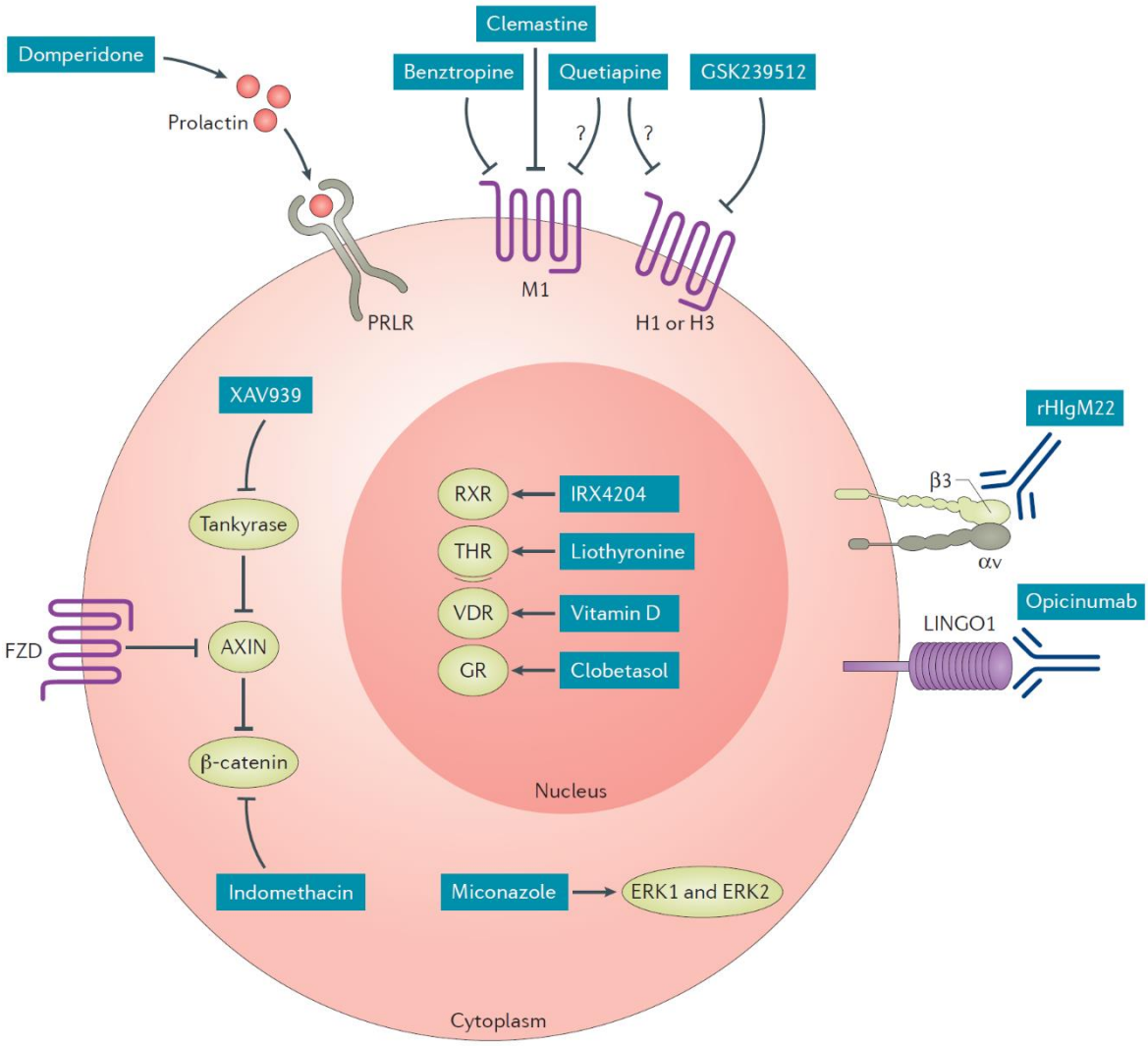


Figure 16: Pro-remyelinating compounds under clinical trial and their putative mechanisms of action. Some of them target acetylcholine or histamine receptors like benzotropine, clemastine, quetiapine and GSK239512 and induce OPC differentiation. Other intracellular receptor can be targeted by Clobetasol, Vitamine D, Liothyronine or IRX4204 to promote survival, differentiation or proliferation of OPCs. Finally, monoclonal antibodies inducing OPC differentiation are being tested (LINGO1, rHigM22). From (Plemel et al., 2017).

Even if OPC differentiation is boosted by a pro-remyelinating drug, it is not known how much this will counteract the presence of a deleterious micro-environment induced by inflammatory processes. For instance, in the presence of inhibitory molecules, molecules known to induce OPC differentiation have only a limited beneficial effect (Keough et al., 2016). One could argue that these pro-remyelinating treatments acting directly on OPCs could be given in addition to

an anti-inflammatory drug. This strategy would raise several issues: inflammation is necessary for remyelination and for its first steps: proliferation and migration. Even if many experimental observations argue for a defect of OPC differentiation as the major cause of remyelination failure in patients (Chang et al., 2002; Kuhlmann et al., 2008), some plaques analyzed post-mortem show few or no OPCs around the lesion (Boyd et al., 2013). Promoting OPC differentiation in chronic plaques in which OPC recruitment did not occur would be inefficient. Moreover, the potential deleterious interactions between drugs would have to be taken into account.

C. The stage of OPC differentiation failure may be different between patients

In patients, OPC differentiation can be blocked at different time-point of the process. The different markers of OPC maturation show that this process can be blocked early on in some patients, or at a pre-myelinating stage (Chang et al., 2002; Kuhlmann et al., 2008; Wolswijk, 1998a). We have data concurring with this observation as the differentiation block in patients with low repair capacities (Behi et al., 2017) could occur at early, late of final stage of differentiation (Figure 17) . Even if the mechanism of action of molecule promoting OPC differentiation are not fully known, they can be efficient only at certain time points of the maturation process and be inactive at others, making the drug inefficient for some patients.

In other words, this approach does not take into account the heterogeneity of remyelination efficacy in patients.

D. Lack of appropriate models for validation

The major flaw of OPC pro-differentiation molecules in the way they were validated: The different molecules tested were validated using toxic demyelination, in which there is not a failure of remyelination or were validated using EAE. EAE is not an accurate model of demyelination (Behan and Chaudhuri, 2014), and as neuronal death is occurring very rapidly, the experimental evidences demonstrating a pro-remyelinating effect of a molecule as the cause for a diminished clinical score has to be taken with caution.

As mentioned earlier, models of toxic demyelination can only prove an acceleration of remyelination and not rescue a rescue of myelin repair failure.

Moreover, as human and murine OPCs do not have exactly the same biological pathways activated during differentiation, therefore candidate molecule must be tested on human OPCs prior further investigations. For instance, clemastine was only tested and validated using murine models.

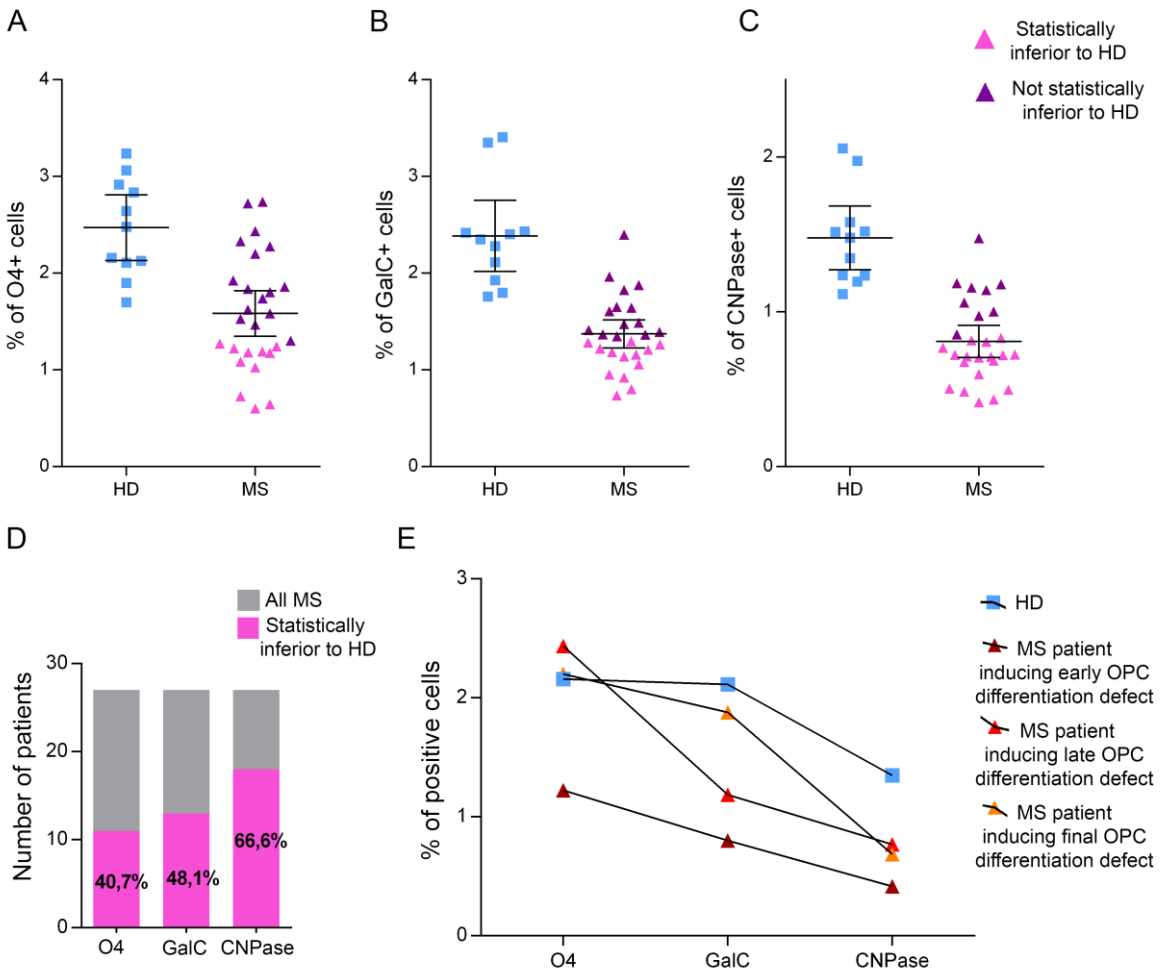
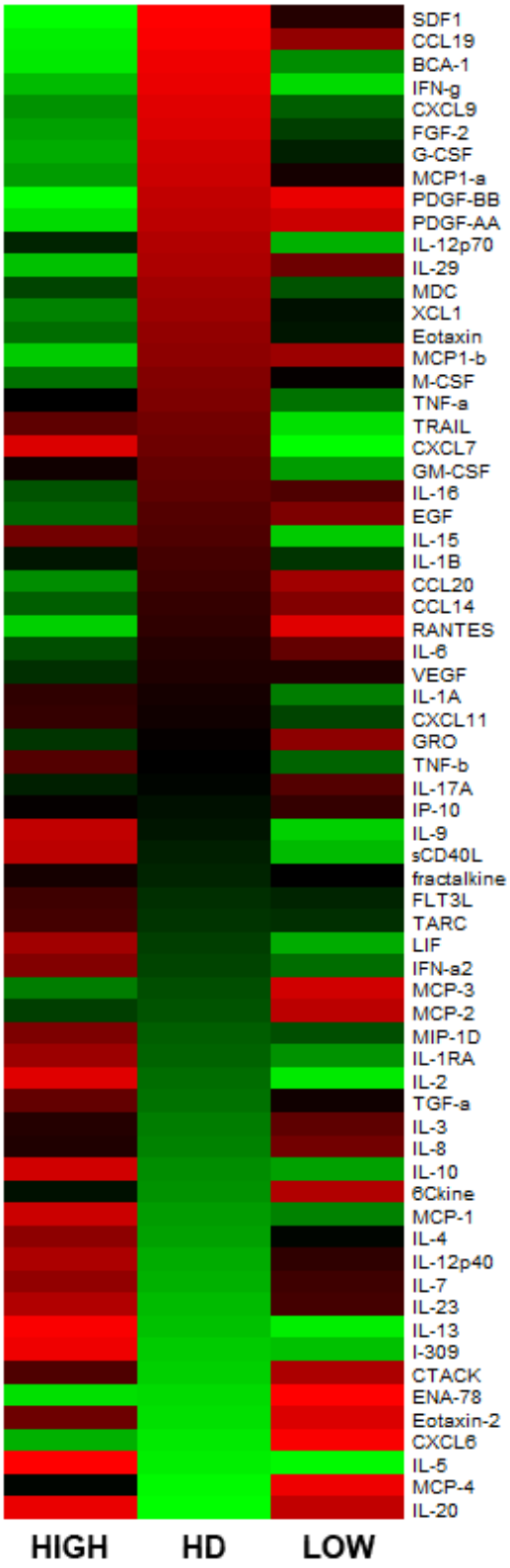


Figure 17: Representation heterogeneity of OPC differentiation blocking step in MS patients. The conditioned media of MIG pre-exposed to HD (n=12) or MS (n=27) LT supernatant was put on a primary culture of OPCs. The state of maturation was evaluated 72h later using 3 chronologically expressed markers: O4 (A), GalC (B) and CNPase (C). In A, B and C, the patients inducing a level of expression of one of the marker statistically inferior (2 standard deviation) are represented in pink, the one inducing a level of expression not statistically inferior are represented in purple. The percentage of patients inducing Low level of O4, GalC or CNPase compared to the total MS population is represented in D. Representative examples of patients inducing an early (O4 stage), a late (GalC stage) or a final differentiation defect (CNPase stage) compared to HD are represented in E.

Globally, we propose instead a more personalized strategy that take into account inter-individual variation: by exploring the cause of remyelination heterogeneity we would be able to propose a customized treatment approach. To do so, we propose to focus on patients having high remyelination capacities to define the molecular and cellular events leading to a successful myelin repair.

III. Deciphering patient's remyelination heterogeneity to determine the prerequisite for efficient myelin repair in MS



1. Involvement of LT in heterogeneity.

Like most of the pathophysiological features of MS, remyelination extent is highly variable from one patient to another, leading to more or less severe symptoms (Bodini et al., 2016; Patani et al., 2007; Patrikios et al., 2006)

Our data demonstrate that LT could be at least partially responsible for inducing this heterogeneity (Behi et al., 2017). Following a simple stimulation with antigen-presenting mimicking beads, MS patient LT had a distinctly different secretory profile compared to HD, and strong inter-individual differences between patients with high repair capacities compared to patients with a low one were also highlighted (Figure 18). This secretory profile could potentially be responsible for setting the micro-environment in a lesion and notably drive MIG activation, leading to successful or failed remyelination.

Interestingly, we found several cytokines correlated with either high or low repair capacities. Knowing that remyelination inversely correlates with disease severity, the level of these cytokines could potentially be used as biomarkers to predict disease evolution. Of course, a lot of experimental

Figure 18: Heatmap representation of the LT cytokine secretion pattern of patients with high repair capacities, low repair capacities and HD. After in vitro activation, the level of expression for 72 cytokines by HD, MS HIGH or MS low patients was evaluated by luminex. Mean values for each tested cytokine were calculated. A heatmap was generated with a color coding representing in green the values lower than, in red the values higher than and in grey values close to the mean for each cytokine in HD and multiple sclerosis HIGH and LOW conditions.

validation and replication of our data would be needed before a clinical use but this could be a promising noninvasive technique as the measurement of the levels of cytokines secreted by patient LT only requires a blood sample. Of note, a few biomarkers are promising in MS: The level of neurofilament, released after axonal damage, dosed in the CSF of patients, can predict severity progression and initiation of the SP phase in a small cohort of patients (Salzer et al., 2010). GFAP and MBP could also be used to a certain extent to predict disease evolution (Housley et al., 2015a).

2. Capitalizing on patients with high repair capacities to develop innovative therapeutic targets

The ideal treatment would have both immunomodulatory and pro-remyelinating effects. That is potentially the case of targeting CCL19. High level of CCL19 were produced by patients inducing low OPC differentiation (Behi et al., 2017) and this cytokine is found in MS actives and inactive plaques (Krumbholz et al., 2007). This cytokine was driving MIG activation towards a pro-inflammatory phenotype leading to inhibition of OPC differentiation (Behi et al., 2017). Using a compound that would either inhibit CCL19 or its receptor CCR7 could potentially restore the pro versus anti-inflammatory balance in MIG leading to a successful remyelination. Another interesting feature of CCL19 is that this cytokine is known to be involved in B and T cell migration and homeostasis (Förster et al., 2008). For instance, ectopic expression of CCR7 leads to the creation of tertiary lymphoid structures, similar to the ones found in the meninges of some patients with progressive MS. Inhibiting the receptor could also theoretically be beneficial by limiting B migration to the brain. Inhibiting the creation of ectopic follicle in the brain would prevent grey matter damage induced by B cells.

On the other hand, CCR7 is also critical for proper Tregs function, as CCR7$^{-/-}$ mouse Tregs are unable to migrate to lymph nodes and suppress the proliferation of antigen-specific T cells

(Schneider et al., 2007). It is not known if this mechanism is CCL19 dependent (CCR7 has at least one known other ligand, CCL21), but in order to develop a drug targeting CCR7, one must be careful not to target Tregs. Of course, in order to deeper explore the potential beneficial effect of CCL19 in MS, a further testing is necessary: We must determine whether an anti-CCL19 antibody is enough to restore a beneficial MIG activation and OPC differentiation, and test if a treatment targeting CCL19 can foster remyelination *in vivo*.

Intriguingly, CCL19 was also highly expressed by HD LT. This result argues that the molecular chain of events leading to a successful remyelination are specific to pathological conditions. A molecular actor having a beneficial role in physiological conditions can be deleterious in a pathological context. Therefore, we argue for a new approach in myelin repair research by focusing on patients with high remyelination capacities to find new therapeutic targets.

3. Genetic variants as the root cause

A. Rational of the genetic stratification

The questions left unresolved by our first study is why, between patients and HD and inbetween patients, are LT responding differently to a same stimulus. In other words, their secretion pattern can be extremely different in response to the same stimulation. As we studied a global LT population, one might argue that this differential response is just the consequence of an over-representation of a particular cell type. However, from a patient to another, the cell count for each of LT subtype evaluated (B cell, Th1, Th17, Treg) was similar. Moreover, the number of cells for each cell type was not different between HD and MS patients. This is concurring with the literature as it is consensual that MS patients display no or very minor changes of the proportions of LT subtypes in their blood compared to HD (Legroux and Arbour, 2015)

Therefore, it is more likely that the differences in LT response are the consequences of inner characteristics of LT that could consequently induce an abnormal activation. Genetic variants responsible for MS susceptibility influencing the capacity of LT to respond to a stimuli is the most likely hypothesis. The variants are located mostly in regulatory DNA (Sawcer et al., 2014) and can influence LT response by modifying the expression of key inflammatory genes. (Housley et al., 2015b).

The cellular events occurring during myelin repair are finely regulated mechanisms alternating between pro-inflammatory and anti-inflammatory micro-environment. If this inflammatory balance is disrupted, that could lead to remyelination failure. Since, Th cells activation can both lead to pro-inflammatory (Th1, Th17) and anti-inflammatory phenotypes (Th2, Treg), we hypothesized that genetic variants accumulated in genes involved in a specific Th phenotype could modify the global inflammatory balance leading to a modification of remyelination outcome.

We had access to a cohort of French MS patients for whom we had access to complete clinical data (clinical score, disease form, years of evolution, treatment etc) and their genotyping profile for all the MS susceptibility SNPs. We decided to focus on the SNPs associated with genes having a role in Th differentiation pathways. We took advantage of the model developed during the previous study to analyze the potential effect of SNPs concentrated in a specific immune pathway on remyelination.

Interestingly, it was the LT of the patients who have a strong MSGB in variants concentrated in pathways responsible for antigen presenting/naïve Th0 cells and TFH cells that were inducing remyelination failure. This suggests that the first step of naïve Th0 cells activation is critical for the success of the process. If this first step of the adaptive immune system is disturbing the pro/anti-inflammatory balance, this could lead to a systematic remyelination failure. Our data suggest also that a dysregulation of TFH cells differentiation might lead to LT detrimental effect on remyelination. TFH cells are a cell type indispensable for the generation and selection of high-affinity memory B cells and plasma cells in germinal centers and are suspected to help the formation of ectopic lymphoid structure in the CNS of MS patients (Crotty, 2014). However, their role is myelin repair is totally unknown.

B. Perspectives and expected outcomes

Additional experiments to complete the study

As the effect of a SNP can be tenuous, we need very accurate biological readouts covering all steps of myelin repair to identify where the SNPs on LT could act. We therefore plan to complete our preliminary data for the 2nd article to increase the numbers of biological read outs for a more thorough multivariated analysis.

We will indeed maximize the chances to spot any changes in the cellular of molecular landscape occurring during myelin repair by evaluating LT secretory pattern. An RNAseq analysis is also scheduled to decipher even more precisely the effect of the SNP on LT transcriptomics. MIG secretory pattern will also be assessed by Luminex to identify the molecular interplay between MIG and OPC. We will also pursue our analysis in vitro by quantifying OPC differentiation/proliferation/migration in response to MIG conditioned media pre-exposed to LT supernatant.

Compiling all the results, completing the ones presented in article 2, a bioinformatics analysis will be performed. The successful *in vivo* remyelination or the proper OPC proliferation/migration/differentiation will be set as an end-point value and the genetic, cellular and molecular elements needed will be analyzed. The analysis will be run both ways: the first way to see if there is a correlation between our "Th cell based" genetic stratification and patients LT effect on inflammation and remyelination, the second way by evaluating what SNPs or association of SNPs are correlated to a high or low repair capacity.

We will be therefore able to pinpoint any subtle change in the cellular and molecular actors involved, and its consequences on myelin repair.

Expected outcomes

Several outcomes with potential clinical will be obtained:

i) Correlation between the genetic profile of patients and remyelination capacity

If a SNP or a combination of SNPs is strongly correlated with remyelination failure. We would then validate this SNP in vitro. We would use the CRISPR technique, that is used to study biological consequences of a genetic variant on LT functionality and validated (Simeonov et al., 2017). If this SNP or this combination of SNPs is validated biologically, it

could be tested as a biomarker predictive of remyelination capacity and/or disease evolution.

ii) Highlights of customized therapeutical targets

The genetic background is likely to induce remyelination failure as different steps. In other words, LT of patients with low repair capacities are inducing a failure of myelin repair at a step depending on the combination of genetic variants carried by an individual.

Ideally, we would detect and validate molecule responsible of a change in the cellular or molecular landscape occurring during myelin repair in the context of a specific genetic background and develop a customized treatment approach.

C. Limitations

In this study we focused on the SNPs responsible for MS susceptibility. However, as the mechanisms driving MS triggering and disease evolution are believed to be different, it is likely that other genetic factors could drive disease evolution. It has been demonstrated for instance that variants independent of MS susceptibility can drive an heterogeneous immune response in a cohort of HD (Li et al., 2016).

The SNPs kept for the genetic stratification were chosen because they have a role in Th pathways. However, several other SNP could influence remyelination: For instance, a genetic variant is associated with a gene involved in OPC biology and is therefore likely to influence the myelin repair process independently from Th cells (Scott-Hewitt et al., 2017). More globally, the principal weakness of the approach is to consider only SNPs involved in LT functions. Even if it has been demonstrated that SNPs involved in MS susceptibility have consequences on LT (Farh et al., 2014; Maurano et al., 2012; Sawcer et al., 2014; Simeonov et al., 2017), susceptibility SNPs can also influence macrophages functions (Raj et al., 2014) and potentially others cell types involved in remyelination, like MIG and astrocytes (Domingues et al., 2016; Hammond et al., 2014), in which the potential functional consequences of the genetic variants have not been studied.

We also did not take into account the role of environmental factors that could have a role in modulating directly or via epigenetic mechanism, the immune response and the remyelination process.

More globally, even if we highlighted two genetic subgroups having LT inducing poor remyelination, we could also notice that patients having LT inducing high remyelination were coming from different subgroups. Therefore, we believe that implementing the stratification strategy by studying the effect of the presence of SNP on MS patient's macrophages could unravel new reasons why remyelination fails or succeed.

IV. Conclusion

The era of therapeutics having pro-remyelinating and/or neuroprotective effects is at its start. As the end goal of the treatment is changing from suppressing inflammation to promote repair, a shift in the routinely used model in MS research is absolutely necessary. In the quest of understanding remyelination failure in some patients, we provided a novel tool. By grafting MS patients LT in a demyelinated lesion, we reproduced at least partially the heterogeneity of myelin repair efficacy, providing new insights into the role of adaptive immune cells in this regenerative process.

In a follow-up study, we took advantage of our model to gather clues on understanding how the genetic background of a patient can influence the functionality of his immune cells during remyelination.

MS is a complex disease in which a lot of parameters can influence disease severity and evolution. Therefore, personalized therapy is probably the only approach that will lead to a cure.

Having the proper tools to understand patients' heterogeneity in their pathophysiological mechanisms should be the focus of MS research, and we provided a tool that unveiled a small part of the reasons of this variability.

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