Immunosuppressants in multiple sclerosis: the past, the present and the future

R. E. Gonsette
National Centre for Multiple Sclerosis.
Melsbroek. Belgium.

ABSTRACT. Immunosuppressants are used in multiple sclerosis (MS) since the early sixties but important methodological problems made difficult the interpretation of the results. In the seventies, several off-label studies strongly suggested that a short-term, intensive immunosuppression with cyclophosphamide were effective to reduce the number of relapses and the progression of disability. More recently, the significant benefit on clinical and radiological signs of disease activity of mitoxantrone, a better tolerated potent immunosuppressant, led to the approval of this molecule by the FDA for the treatment of breakthrough MS. Since then, many clinical trials are in progress to evaluate the efficacy and the safety of newly developed non-specific immunosuppressive agents such as mycophenolate mofetil, cladribine, isoxazole derivatives and pixantrone as well as monoclonal antibodies that target more specific immune mechanisms. Agents interfering with cell trafficking at the level of the blood brain barrier or sequestering lymphocyte in peripheral lymphoid organs are also new interesting therapeutic avenues. The development of effective and safe immunosuppressants might lead to new treatment strategies: 1. immunosuppression in patients with less aggressive secondary progressive MS; 2. breakdown of disease activity in young patients with clinical and/or radiological poor prognostic factors in order to delay the progressive phase; 3. further immunosuppression in patients previously treated with MX and re-progressing; 4. tolerogenic immunotherapy (maintenance phase) after short-term immunosuppression (induction phase) in patients with aggressive MS.

Key words: multiple sclerosis, IL-2 inhibitors, antimetabolites, cytolytic agents, monoclonal antibodies, fusion proteins, cell trafficking, tolerogenic immunosuppression.

RESUMEN. Los inmunosupresores se han utilizado en la esclerosis múltiple (EM) desde el inicio de los años sesenta, pero debido a importantes problemas metodológicos, es muy difícil la interpretación de los resultados de su uso. En los años setenta, varios estudios fueron de indicación sugirieron que la inmunosupresión intensiva con ciclofosfamida era efectiva para reducir el número de brotes y la progresión de la discapacidad. Más recientemente, el beneficio significativo de los parámetros clínicos y radiológicos de actividad de la enfermedad, con el mitoxantrona, un inmunosupresor potente mejor tolerado, llevó a la aprobación de esta molécula por la FDA para el tratamiento de la enfermedad muy activa. Desde entonces, hay numerosos ensayos clínicos en progreso, para evaluar la eficacia y seguridad de nuevos agentes inmunosupresores inespecíficos, tales como el micofenolato de mofetilo, la cladribina, los derivados isoxazólicos y la pixantrona, así como anticuerpos monoclonales que se dirigen a mecanismos inmunológicos más específicos. Los agentes que interfieren con la transmigración celular en la barrera hematocerebral o que se lesiona en los linfocitos en los órganos linfoides periféricos constituyen también nuevas e interesantes aproximaciones terapéuticas. El desarrollo de inmunosupresores efectivos y seguros podría llevar a nuevas estrategias terapéuticas: 1. inmunosupresión en pacientes con formas de EM secundariamente progresivas menos agresivas; 2. disminución de la actividad en pacientes jóvenes con factores clínicos o radiológicos de mal pronóstico, con el fin de retrasar la fase progresiva; 3. seguimiento de la inmunosupresión en pacientes tratados previamente con MX y que vuelven a progresar; 4. inmunoterapia tolerogénica (fase de mantenimiento) tras cursos cortos de inmunosupresión intensa (fase de inducción) en pacientes con EM agresiva.

Palabras clave: esclerosis múltiple, inhibidores de IL-2, antimetabolitos, agentes citotóxicos, anticuerpos monoclonales, proteínas de fusión, trasmigración celular, inmunosupresión tolerogénica.

The past

The concept of immunosuppression as a treatment for multiple sclerosis (MS) dates back to the sixties with the publications of Aimard et al about MS therapy with “antimitotics” and of Cendrowski about “immunosuppressants” in MS. Two hypotheses concerning MS etiopathogenesis were in direct opposition at that time. The first described MS as being caused by anergy against a viral infection and recommended treatments to correct deficient immune functions. The second, based on observations in experimental allergic encephalomyelitis (EAE), described MS as an immune-mediated inflammatory disease and suggested the use of immunosuppressive agents which had been found effective in EAE. In the course of the seventies, several clinical trials with immunosuppressants in MS were performed using cyclophosphamide (CY), azathioprine (AZA), antilymphocyte or antithymocyte globulins. The methodology of clinical trials was imperfect and treated patients served as their own control or were compared to matched untreated controls. Only one trial was a double-blind study. However, the follow-up of rather large groups of patients (~100) for several years (2-5 years) strongly suggested that CY and AZA as...
a single agent\textsuperscript{6,7} or in combination therapy\textsuperscript{3,8} had a transient beneficial effect on relapses and progression of disability in relapsing-progressive (RP) MS but was ineffective in secondary progressive (SP) patients. Since then, new classes of immunosuppressive drugs have been developed notably inhibitors of production or function of IL-2 that interfere with cell-cycle replication.

In the past decade, monoclonal antibodies (mAbs) have been generated for therapeutic applications. In contrast to other immunosuppressants, they interact with precise targets. Generated from rodents after immunization with a target molecule, they are recognized as foreign proteins. To reduce the immunogenicity, they have been “humanized” by fusion with human IgG constant region genes or by grafting the complementary determining region of the Ab into human IgG backbone. More recently, fully human mAbs have been generated by using transgenic mice carrying human Ig genes or by cloning Abs directly from the human immune repertoire\textsuperscript{9}. Fusion proteins have been created through the fusion of genes which originally coded for separate proteins. Translation of this fusion gene results in a chimeric polypeptide with functions derived from the original proteins.

Lastly, immunosuppressants with a new and unique mechanism of action have been proposed. They prevent the invasion of brain parenchyma by inflammatory cells. Two options are currently available: to block adhesion molecules expressed on endothelial cells and migrating immunocompetent cells or to sequester lymphocytes in lymphoid organs.

In this paper, we will only review approved and experimental immunosuppressants whose clinical efficacy and safety can be reliably evaluated from published data.

\textbf{The present}

Immunosuppressants can be categorized in three major groups: intracellular ligands, cell surface ligands and molecules affecting immunocompetent cell trafficking (Table I).

\textbf{1.- Intracellular ligands}

A first group of intracellular ligands interfere with the cell-cycle replication of immunocompetent cells and reduce their proliferation by inactivation of IL-2 production or function.

\textbf{1. 1.- Inhibitors of IL-2}

\textbf{1. 1. 1.- Inhibitors of IL-2 activation or production}

\textit{Cyclosporine A}, a breakthrough in organ transplantation was found very effective in EAE and raised great hopes for multiple sclerosis (MS) treatment. Unfortunately, the questionable benefit in MS did not justify the adverse reactions caused by its renal toxicity.

\textit{Tacrolimus} in association with mycophenolate mofetil is a standard treatment after liver transplantation. Two MS patients under interferon beta (IFN\textbeta) treatment experienced acute liver failure that required liver transplantation. In both cases, this post-transplant treatment regimen led to an improvement of their neurological state maintained for several years\textsuperscript{10,11}. Those observations raised the question whether tacrolimus, effective in EAE\textsuperscript{12}, might be a treatment option for MS, alone or in combination with mycophenolate mofetil. Of note that tacrolimus is currently investigated in combination with IFN\textbeta\textsuperscript{13}.

\textbf{1. 1. 2.- Inhibitor of IL-2 signal transduction}

\textit{Rapamycine} (sirolimus) induces structural changes in astrocytes and several cases of Posterior Reversible Encephalopathy (PRE) have been reported in patients treated with sirolimus after lung

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transplantation. A phase I/II trial in MS is in progress. Temsirolimus, a new formulation, has not been tested in animal models of MS. A phase II clinical trial in relapsing-remitting (RR) MS patients showed a beneficial effect on relapse rate and gadolinium (Gd) active lesions but the high risk-benefit ratio does not seem to justify further trial in MS.

1. 2.- Antimetabolites

Antimetabolites interfere with purine or pyrimidine synthesis pathways essential for DNA synthesis and cell replication.

Azathioprine (AZA) is used in MS since more than 30 years with conflicting results. More recently, it has been observed that an add-on therapy with AZA in patients refractory to IFNβ led to a reduction of the relapse rate and a decrease of the MRI total lesion load as well as of Gd active lesions. The efficacy of AZA as a single therapeutic agent was confirmed both on Gd active and new T2 lesions. Interestingly, a head to head comparison in RR MS patients concludes that more patients remain relapse free with AZA than with IFNβ. Lastly, two meta-analyses of clinical trials with AZA conclude that the safety profile of this immunosuppressant is acceptable and that less patients experience relapses. Currently, several clinical trials are in progress associating AZA with IFNβ.

Mycophenolate Mofetil (MM) has some advantages compared with AZA: it has no potential carcinogenicity and, in addition to its immunosuppressive activity, it inhibits adhesion molecules and inducible nitric oxide synthase production. Administration of MM in patients with increasing disability despite standard treatments seems to have a beneficial effect on progression. A retrospective study in patients receiving MM as an adjunct therapy to IFNβ or glatiramer acetate or as a single agent indicates an acceptable tolerance and some benefit on progression. Recently MM has been associated with IFNβ for the treatment of RR MS. Gastrointestinal discomfort is frequent but other side effects appear acceptable. The relapse rate was reduced by 71% (relative risk reduction, RRR) and Gd active lesions were eradicated after 6 months. Interestingly, in a small group of patients with neuromyelitis optica (NMO) MM induced a significant decrease in the relapse rate after a median follow-up for 16 months even in patients who did not respond previously to AZA.

Methotrexate (MTX) was found modestly effective in reducing progression of disability in a double-blind trial and MRI data showed marginal effects on T2 lesions and Gd active lesions. A modest efficacy on progression was also suggested in a meta-analysis of published trials. A recent review of clinical studies with MTX in MS does not advocate its use as a single agent in RR or progressive MS. Combination therapy with IFNβ and MTX suggests an additional reduction in Gd active lesion by 44% and a trend toward fewer relapses. Of note that an acute MTX-induced encephalopathy has been recently reported.

Cladribine (CdA), a purine analogue resistant to adenosine deaminase, specifically accumulates in leucocytes where it incorporates in DNA, blocks cell cycle replication and induces a selective lymphopenia. It is definitely the most effective immunosuppressant among the antimetabolite family, leading to a marked and sustained depletion of CD4, CD8 and B cells as well as of CD25+ expressing cells. Methodology limitations make it difficult to validly evaluate the clinical benefit in three double-blind trials in RR and SP MS. However, radiological evaluations demonstrate a drastic reduction in the number and the volume of Gd active lesions, a mild effect on T2 lesion load and no effect on brain atrophy progression. Interestingly, CdA has been used as a rescue therapy in patients with breakthrough MS. Patients remained free of relapses and progression. However a second course of CdA was administered after 1 year from first dose in most patients because of recurrence of relapses.

Sustained immunosuppressive effects of CdA make it suitable for intermittent oral dosing but the safety profile of the oral tablet formulation remains to be established. Haematopoietic toxicity and opportunistic infections are the most frequent adverse effects. The possibility to reveal unrecognized myelodysplastic disorders as a consequence of marrow suppression cannot be neglected. Two clinical trials with the oral formulation of CdA as monotherapy or add-on therapy to IFNβ are in progress.

Ioxazole derivatives were found to reduce diapedesis and proliferation of immunocompetent cells. Teriflunomide has been investigated in a phase II trial in RR and SP MS. A reduction of the relapse rate (-32%), of the mean EDSS score (-69%) and of the median number of combined unique active lesions (-61%) was observed. Serious adverse events were noted: hepatic dysfunction, neutropenia and rhabdomyolysis. Based on animal studies, the reproductive toxicity might be a concern. Malononitrilamides are active metabolites of another isoxazole derivative (leflunomide) and seem to have a safer toxicity profile.

1. 3.- Cytolytic agents

Cytolytic agents delete immunocompetent cells participating to the inflammatory cascade when they go trough lymph nodes, peripheral blood and choroid
plexus stroma. Their clinical efficacy is thus definitely linked to their anti-inflammatory activity.

**Cyclophosphamide** (CY) is used in MS as an off-label medication since several decades. The potent anti-inflammatory activity of CY explains its marked beneficial effects on relapses in RR MS\(^{38}\) and Gd active lesions\(^{39}\). Its efficacy on progression is clearly better shortly after the conversion to the progressive stage and in patients with clinical and radiological signs of inflammatory activity. Of note that administration of very high doses (200 mg/kg over 4 days) was recently found effective to halt progression in severely disabled patients\(^{40}\). However blood transfusions and granulocyte colony-stimulating factor were required to control the haematopoietic toxicity. A short-term immunosuppression with CY has been proposed as a potential therapeutic option in breakthrough MS since it induces eradication of Gd active lesions, stabilization of EDSS and, interestingly, a reduction in brain atrophy progression\(^{41,42}\). Recently, the potential serious adverse events of mitoxantrone (cardiotoxicity, acute myeloid leukaemia) have rekindled the interest for CY. The respective efficacy and short-term safety of those two immunosuppressants have been compared in aggressive MS. No statistically differences were observed concerning clinical and radiological efficacy. In two trials, CY was found to reduce brain atrophy progression. Of note that discontinuation was definitely more frequent in the CY group due to short-term side effects\(^{43-45}\).

**Treosulfan** is another alkylating agent with more potent myeloablative and immunosuppressive activity than CY, particularly concerning depletion of splenic T and B cells\(^{46}\). An induction period with four monthly administrations followed by quarterly infusions for 1 year induced a reduction in Gd active lesions and relapses. However, signs of recurrent disease activity were observed at the end of the follow-up\(^{47}\). This is not surprising as several trials have shown that to obtain a clinical benefit with CY the interval between infusions must not exceed 2 months. Side effects and adverse reactions with treosulfan do not appear to differ significantly from those of CY. A phase II trial is in progress.

**Mitoxantrone** (MX) has been approved by the FDA in 2000 for the treatment of aggressive MS. The anti-inflammatory activity of MX results from its broad immunosuppressive effects on CD4, CD8, B cells, dendritic cells, monocytes and macrophages for unusually long periods of time (several months). Patients with a marked inflammatory form of the disease respond best to MX. The optimal treatment regimen is still to be defined: 1. an induction period followed by maintenance therapy versus quarterly administration; 2. high doses (12 mg/m\(^2\)) versus lower doses (8-5 mg/m\(^2\)); 3. maintenance therapy versus a single short-term treatment (4-6 months) and re-treatment with MX delayed till recurrence of severe symptoms. The cardiotoxicity is a major dose-limiting factor for both the selection of patients and the duration of treatment. Cardiotoxicity may occur at lower doses than the recommended cumulative dose (140 mg/m\(^2\)). There is now ample support to believe that asymptomatic myocardial dysfunction may occur in MS patients without apparent pre-existing cardiac risks. Also of concern is that cardiotoxicity has been observed years after the last infusion of MX. This prompted health authorities to monitor left ventricular ejection fraction before each administration and every 6 months for 5 years after cessation of MX administration. There is now mounting evidence that severe cardiac dysfunctions can be prevented by a careful cardiac monitoring.

Another concern is the increasing number of reports about therapy-related acute leukaemia (TRAL). The exact incidence is difficult to evaluate as the occurrence of TRAL is likely subject to under-reporting. More than 25 cases have been reported so far and the most worrying observations are the high incidence (up to 3.4%) in some small series of MX treated patients\(^{48-50}\). No significant correlation has been found so far with the cumulative dose. The interval between the diagnosis and the last MX infusion varies from a few months to several years. Two types of acute leukaemia (promyeloid and myeloid) and only one case of chronic myeloid leukaemia\(^{51}\) have been reported. The incidence of TRAL is thus a major concern since definitely more MS patients treated with MX died from leukaemia than from congestive heart failure.

**Pixantrone** (PIX) is an analogue of MX designed to reduce cardiotoxicity in cancer therapy. The absence of severe cardiotoxicity has been confirmed in patients treated with PIX for non-Hodgkin’s lymphoma. Moreover, the weaker DNA constant binding, the lower stimulation of topoisomerase II-mediated DNA changes and the specificity for topoisomerase II poisoning of PIX suggest a lower risk for TRAL. In the acute and chronic models of EAE, PIX was found as effective as MX and definitely less cardiotoxic. This new molecule appears thus a promising substitute for MX. Unfortunately, the pharmaceutical company owner of the molecule does not want to invest in clinical trials with PIX for other indications than oncology. Finally the company agreed to provide the drug and a phase I/II trial in MS, sponsored by a non-profit organization, is in progress.

**Ethonafide**, an anthracene-based anticancer agent
with a low cardiotoxicity has been proposed as another substitute for MX. Immunological studies in EAE showed that ethonafide and MX have similar impact on CD4, CD8, NK, B cells and macrophages. However, the benefit on clinical deficits in EAE appears lower with ethonafide\(^6\).

2.- Cell surface ligands

2.1.- Monoclonal antibodies

Alemtuzumab (anti-CD52, campath-1H) has a marked anti-inflammatory activity and appears the most powerful mAb in MS. It rapidly produces a profound (over years) lymphopenia affecting T and B cells as well as monocytes. A first trial in SP MS suppressed relapses but did not prevent disability progression\(^5\). A recent trial in early RR MS not only demonstrates relapse eradication but also disability improvement in most patients\(^6\). The likely reason is that a very early drastic suppression of the inflammatory environment reduces the production of microglial toxic factors (oxidative stress and excitotoxicity) associated with inflammation and initiating the degenerative cascade. The principal adverse effects of alemtuzumab are the development of autoimmunity: Grave’s disease, idiopathic thrombocytopenia and Goodpasture’s disease.

Rituximab (anti-CD20, rituxan) produces a rapid, complete and sustained depletion of B cells. B lymphocytes certainly play an important role in MS as non-professional antigen-presenting cell and when humoral immunity predominates such as in Devic’s neuromyelitis optica (NMO). Rituximab significantly reduced relapses in patients with NMO\(^5\)-\(^7\). More recently, rituximab has been used in RR MS and a marked decrease in exacerbation rate as well as in Gd-active lesions was noted\(^8\)-\(^9\). Interestingly rituximab produces a depletion of T lymphocytes and reduces their reactivity to MOG confirming the capital role of B cells as Ag presenting cells\(^7\). A trial in PP MS is in progress\(^10\).

Rituximab causes several potential adverse reactions and of particular concern is the report by the FDA about 21 patients treated with rituximab for haematological malignancies who developed progressive multifocal encephalopathy (PML). Daclizumab (anti-CD 25, zenapax) inhibits the expansion of CD4 cells by blocking the binding of IL-2 to its receptor. There is only a modest depletion of CD4 and CD8 cells. Of particular interest is the expansion of CD56\(^{bright}\) NK cells considered as regulatory cells. The increase in CD56\(^{bright}\) NK cells correlated significantly with the reduction in Gd-active lesions\(^8\). Due to the role of these cells to control virus infections it is postulated that reactivation of JC virus and PML are very unlikely during daclizumab therapy\(^9\). Data from clinical trials with daclizumab available so far do not provide evidence-based information concerning its efficacy in MS\(^6\)-\(^8\).

2. 2.- Fusion proteins

Fusion proteins have been generated interacting with TNFα pathways or the costimulatory molecule CTLA4. Lenercept (a TNFα-receptor p55-IgG) and etanercept (a TNFα-P75-IgG) have been tested in RR MS. It soon appeared that exacerbations were more frequent and disability more severe in treated patients\(^6\). Interestingly, administration of an anti TNFα mAb increased MRI activity in MS patients\(^6\). In contrast, administration of an inhibitor of TNFα synthesis did not increase clinical and radiological disease activity and modestly improved disability\(^6\). A complete silencing of TNFα pathways with mAbs or fusion proteins appears clearly detrimental whereas a reduction in TNFα production might be beneficial. This reflects the complex roles of this cytokine according to TNFα environmental levels and pathological processes timing\(^6\). Other intriguing observations are several cases of CNS demyelination recently reviewed in patients treated with anti-TNFα drugs for rheumatoid arthritis or autoimmune inflammatory diseases\(^20\).

Another fusion protein CTLA4-Ig (abatacept) interferes with the two CD80/86 molecules of the costimulatory system. CD80/86 interact with both CD28 that activates lymphocytes and CTLA4 that inhibits lymphocyte activation. The balance between the booster (CD28) and the brake (CTLA4) is complex and not yet completely elucidated. Since CTLA4-Ig binds to B80/86 with a 100 higher affinity than does CD28, CD80/86 are almost completely blocked after CTLA4-Ig administration and do not interact with CD28. CTLA4-Ig is effective in EAE but a phase III trial in MS was abandoned because of severe vascular complications. Of note that a “super-agonistic” mAb against CD28 (TGN1412) was found very effective in EAE and induced a selective proliferation of T regulatory cells. Unfortunately, this mitogenic mAb activates T cells even in the absence of T-cell receptor stimulation and led to indiscriminate attacks of several organs and catastrophic adverse reactions in a phase I trial\(^7\). Manipulations of the costimulatory molecule pathways should be considered with extreme caution.

3.- Cell trafficking

Natalizumab (Tysabri) is a mAb against the α4β1 and α4β7 integrins expressed on leukocytes and reacts with the VCAM-1 receptor present on endothelial cells. This reaction prevents transmi-
Integration of certain immunocompetent cells through the BBB and induces a mild lymphocytosis. Very effective in EAE, natalizumab was evaluated in two clinical trials against placebo and in combined therapy with IFNβ. It was also evaluated as add-on therapy to glatiramer acetate. Clinical trials against placebo showed a clinically and statistically significant benefit on MRI activity (-80/90%), clinical exacerbation frequency (-50/70%) and to some extent on progression of disability. Anaphylactic reactions occurred in ~4% of patients and the therapeutic activity was neutralized by Abs developed for natalizumab in 6%. Two patients enrolled in the combination therapy with IFNβ developed PML. After a temporary suspension, natalizumab was reintroduced under a restricted distribution program (Tysabri Outreach Unified Commitment to Health-TOUCH) as a monotherapy in breakthrough MS and as a first-line treatment in aggressive MS. Recommendations for patient selection and monitoring have been proposed.

Alpha4-integrin blockade is definitely effective in MS patients. However, the potential emergence of PML after tysabri administration led to refocus on alpha4-integrin antagonists with greater safety such as orally administered small molecules. In addition to a lower cost and a more convenient dosing regimen, those new molecules do not induce neutralizing Abs and can be selectively directed against α4β1 or α4β7. It has been suggested that neutralization of α4β1 would be a risk factor for PML by diminishing immunosurveillance but on the other hand selective antagonists of α4β1 appear the most effective in MS. The most advanced molecule in development is CDP323 a phenylalanine enamide directed against both α4β1 and α4β7 integrins. A phase II trial is in progress.

FTY720 (Fingolimod) is a superagonist of the sphingosine-1-phosphate-1 receptor expressed on thymocytes and lymphocytes, causing internalization and neutralization of this receptor and subsequent impossibility for these cells to egress from secondary lymphoid organs. In contrast to tysabri, fingolimod produces a depletion of circulating lymphocytes by about 50%. Importantly, circulating B cells are markedly depleted (by 90%), CD8 are less depleted than CD4 and innate immune cells (NK, monocytes) are not affected. Fingolimod is very effective in EAE and it has been demonstrated with MRI that the transmigration of lymphocytes and macrophages is strongly reduced. In phase II trial in RR MS fingolimod produced a reduction in the relapse rate by ~50% and in Gd+ active lesions ~54% after 6 months. During the extension period, patients on placebo were randomized to low or high doses of fingolimod. The benefit on relapse rate at 12 months reached 70%. An evidence-based evaluation of the effects of this new agent on progression is difficult since the follow-up period against placebo does not exceed 6 months. During this short period of time, no difference between the placebo and treated group was noted for the mean EDSS scores. Of note that the number of progressing patients (treatment failure: EDSS + 1 point) was reduced by 50% in the low dose and by 25% in the high dose groups. An exploratory relapse rate maintenance analysis suggests a fast onset of action of fingolimod and a sustained effect over 24 months. The safety profile seems particularly good. One case of RPE with residual homonymous haemianopsia has been reported with the high dose.

It would be too dogmatic to attribute the beneficial effects of fingolimod to the restriction of T-cell trafficking resulting from lymphocyte sequestration and strengthening of the endothelial barrier. Fingolimod has pleiotropic effects. It readily enters the brain and interferes with S1P1, S1P3 and S1P5 receptors present on glial and neuronal cells. The effects of fingolimod on these receptors that play a role in astrocyte proliferation, oligodendrocyte differentiation and survival as well as in neurite outgrowth, may explain in part the favourable impact of this new molecule on MS pathomechanisms.

The Future

Immunosuppressive effects of immunosuppressants are clearly linked to their anti-inflammatory activity and inflammation is a hallmark of MS disease pathology. Recently, neurodegenerative processes have been proposed as the primary cause of MS at least in a subset of patients. In this hypothesis however, massive local oligodendrocyte apop-
tosis is surrounded by highly activated microglia and at a later stage demyelination releases neuro-Ags inducing secondary autoimmune responses and inflammatory cell recruitment. Even in this subset of MS patients, immunosuppressants might thus be effective on early microglial activation and on secondary inflammatory processes (Figure 1). On the other hand, the cellular components of inflammation definitely predominate during the RR phase and initiate degenerative mechanisms that already participate to brain lesions. During the SP phase, cellular infiltration clearly abates and the humoral inflammatory components are compartmentalized in meningeal B cell follicle-like aggregates. At that moment, degenerative processes clearly prevail and are mediated by oxidative stress and excitotoxicity (Figure 2).

Given the potent anti-inflammatory activity of immunosuppressants they were found the most effective during the initial inflammatory phase of MS. In contrast, they have only a modest clinical efficacy during the progressive phase when humoral components are trapped in meninges and when degenerative processes predominate. Immunosuppressants have thus a role early after disease onset to block inflammatory processes and to prevent early axonal loss due to oxidative stress and excitotoxicity initiated by inflammation. Early administration of immunosuppressants would also prevent the development of mechanisms potentially responsible for disease progression such as the formation of B cell follicle-like aggregates that are clearly associated with frequent, early inflammation.

Some molecules such as rituximab have exquisitely selective immunosuppressive properties affecting B cell lineages only. They might be more appropriate when humoral immune mechanisms definitely prevail in certain types of MS (NMO).

The role of immunosuppressants after the blockade of acute inflammatory processes is less evident. They have been used to maintain the benefit of a short-term intensive immunosuppression, but their administration could be a double-edge sword. Indeed, there is ample support today to demonstrate that the development of intrinsic self-defence and regulatory mechanisms terminate the acute inflammatory phase and that they might be compromised by long-term immunosuppression. In organ transplantation indeed, it has been clearly demonstrated that chronic exposure to most immunosuppressants adversely affects adaptive immunoregulation exerted by regulatory T cells (Treg). Those observations led to the concept of “tolerogenic immunosuppression” based on a short-term intensive immunosuppression to prevent early graft rejection, followed by low dose, infrequent maintenance immunosuppressive monotherapy to spare the development of natural tolerance\(^4\). One may thus wonder if a maintenance therapy with tolerogenic immunosuppression would not be more pertinent in MS to maintain a tolerance state. Concerning the choice of the immunosuppressant it is interesting to note that Treg are depleted by cyclosporine A, tacrolimus and AZA and that they are spared by CY and MX whereas alemtuzumab and fingolimod favour Treg emergence.

### Table II

<table>
<thead>
<tr>
<th>Treatment regimen</th>
<th>% patients at 2 y EDSS + 1 Placebo</th>
<th>% patients at 2 y EDSS + 1 Treated</th>
<th>ARR</th>
<th>RRR</th>
<th>NNT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitoxantrone Q3 months</td>
<td>0.37</td>
<td>0.07</td>
<td>0.30</td>
<td>0.81</td>
<td>3</td>
</tr>
<tr>
<td>Tysabri Monthly</td>
<td>0.29</td>
<td>0.17</td>
<td>0.12</td>
<td>0.42</td>
<td>8</td>
</tr>
<tr>
<td>Fingolimod (FU 6 m) Daily</td>
<td>0.20</td>
<td>0.10</td>
<td>0.10</td>
<td>0.50</td>
<td>10</td>
</tr>
</tbody>
</table>

ARR: absolute risk reduction; RRR: relative risk reduction; NNT: number needed to treat (round up).
Our current clinical experience has demonstrated that potent immunosuppressants are capable to block disease activity during the inflammatory stage and that when we hit hard and early, disability progression can be significantly delayed. Unfortunately there is an inverse correlation between efficacy and toxicity and currently intensive immunosuppression only concerns patients with breakthrough MS. Cytolytic agents (CY and MX) with a broad impact on the immunocompetent cells critically involved in MS pathomechanisms, definitely remain the most effective despite the development of recent molecules (Table II). New cytolytic agents with an improved efficacy and a markedly better safety are in the pipeline of research laboratories and were already found effective in EAE. Their development might lead to new applications of immunosuppressants in MS: 1. immunosuppression in patients with less aggressive secondary progressive MS; 2. breakdown of disease activity in the early phase of RR MS (2-3 year evolution) in young patients with clinical and/or radiological poor prognostic factors in order to delay the progressive phase; 3. further immunosuppression in patients previously treated with MX and re-progressing; 4. tolerogenic immunotherapy (maintenance phase) after short-term immunosuppression (induction phase) in patients with aggressive MS.

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