

Monoclonal antibodies in MS

Mechanisms of action

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ABSTRACT

The development of monoclonal antibodies (mAbs) presents an emerging, highly specific therapeutic strategy for the treatment of multiple sclerosis (MS). mAbs target selective molecules and have shown early promise, along with notable risks, in the treatment of MS and other immune-mediated diseases. The mechanism of action of the 4 mAbs under active investigation for MS (natalizumab, rituximab, alemtuzumab, and daclizumab) are reviewed, with a discussion of how mAb interaction with each target antigen may produce direct and indirect effects (proven and hypothesized) on immune cell activity, CNS-related inflammatory processes, and clinical outcomes. **NEUROLOGY 2010;74 (Suppl 1):S31-S40**

GLOSSARY

ADCC = Ab-dependent cellular cytotoxicity; **BBB** = blood-brain barrier; **CDC** = complement-dependent cytotoxicity; **CEL** = contrast-enhancing lesion; **DC** = dendritic cell; **FDA** = Food and Drug Administration; **IFN β -1a** = interferon beta-1a; **Ig** = immunoglobulin; **IL** = interleukin; **IL-2R** = interleukin-2 receptor; **ITP** = immune thrombocytopenic purpura; **mAb** = monoclonal antibody; **MoA** = mechanism of action; **MS** = multiple sclerosis; **NK** = natural killer; **PML** = progressive multifocal leukoencephalopathy; **RA** = rheumatoid arthritis; **RRMS** = relapsing-remitting multiple sclerosis; **SC** = subcutaneous; **VLA-4** = very late activating antigen-4.

INTRODUCTION Monoclonal antibodies (mAbs) offer clinicians a highly selective group of therapeutic agents that target specific molecules expressed on the cell surface. mAbs in development for multiple sclerosis (MS) currently target only molecules of the immune system. These emerging therapies have potent immunomodulatory effects, both direct and indirect. This review summarizes the known or hypothesized mechanisms of action of 4 mAbs in use or under study (with demonstrated efficacy in phase II and phase III trials) for the treatment of MS.

OVERVIEW mAbs, which have been under investigation for human therapeutic use since the mid-1980s, are inherently limited by their immunogenicity if they are of nonhuman origin. Researchers have sought to overcome these limits with the development of humanized, chimeric, and fully human mAbs. Humanized mAbs consist of >90% human components, with the balance from the original (usually murine) structure; these include natalizumab, alemtuzumab, and daclizumab.

(The nomenclature of mAbs is coded to indicate their origin, as seen in the italicized syllables.) Chimeric mAbs are ~66% human structure and ~34% murine (from the variable region of the original mouse Ab) and include rituximab and basiliximab. Fully human mAbs, such as ofatumumab and adalimumab, have a 100% human structure and may be derived from either human cells or genetically engineered mice.^{1,2}

mAbs consist of fragment antigen-binding (F_{ab}) and fragment-crystallizable (F_c) domains arranged in a rough Y shape. The F_c, or complement-binding, domains, located on the stem, modulate immune cell activity, determining whether the mAb binds to different F_c receptors expressed on immune cells or whether it activates complement. The F_{ab}, or antigen-binding, regions, found at the terminal ends of the arms, determine how the antibody will bind to the epitope of its target molecule and with what degree of affinity.

DETERMINANTS OF mAb ACTION Four main factors determine how a mAb acts on its target: the

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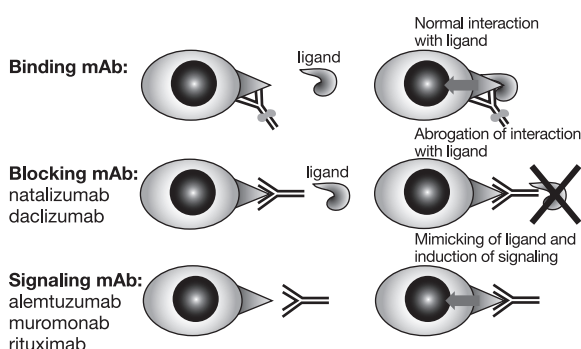
Table 1 Target molecules: Distribution and function^{9,76-78}

Monoclonal Ab	Targeted molecule	Cell expression
Alemtuzumab (Campath-1H)	CD52	All T and B cells, NK cells, majority of MΦ, DCs, most granulocytes (except neutrophils), and tissue of the male reproductive system
Daclizumab (Zenapax)	CD25 (IL-2R α)	Activated T cells, some regulatory T cells (FoxP3 ⁺), CD56 ^{bright} NK cells, activated MΦ, activated DCs, and oligodendrocytes
Natalizumab (Tysabri)	CD49d (VLA-4)	All T and B cells, NK cells, majority of monocytes and MΦ, and most granulocytes (except neutrophils)
Rituximab (Rituxan)	CD20	B cells (but not plasma cells)

Ab = antibody; NK = natural killer; MΦ = macrophages; DCs = dendritic cells; IL-2R = interleukin-2 receptor; VLA-4 = very late activating antigen-4.

function and distribution of the targeted molecule; the antibody's mode of interaction with its target; its effector functions; and its efficacy in reaching the target tissues (in this setting, the CNS).

Characteristics of target molecule. Each mAb binds only to its target molecule, which is expressed on particular cells for specific functions, such as cell activation, survival, or migration. The mAbs that are currently most relevant to MS therapy, and their targeted molecules are shown in table 1. Alemtuzumab (Campath®, Genzyme, Cambridge, MA) binds to CD52, an antigen widely expressed in the immune system, including on the surface of T and B lymphocytes, natural killer (NK) cells, and a majority of monocytes and macrophages. Daclizumab (Facet Biotech, Redwood City, CA, and Biogen Idec, Cambridge, MA) targets CD25, expressed on activated T cells and some regulatory T cells, among others. The target of natalizumab (Tysabri®, Biogen Idec, Cambridge, MA, and Elan Pharmaceuticals, Dublin, Ireland) is CD49d (a subunit of adhesion molecule very late activating antigen-4 [VLA-4]), another molecule with wide expression in the immune system. CD20, expressed only on B cells, is the target of rituximab (Rituxan®, Genentech, South San Francisco, CA, and Biogen Idec, Cambridge, MA).

Figure 1 The properties of the mAb with respect to the target molecule (F_{ab} dependent)^{18,31,49,65,79}

How the mAb interacts with the target molecule. A particular mAb may interact with its target by binding, blocking, or signaling, depending on its F_{ab} activity (figure 1). Binding mAbs bind to the specific molecule but do not interfere with its function. Therefore, the physiologic ligand is still able to bind to the molecule targeted by a mAb and to signal. A binding mAb can mark a target cell for destruction through its effector functions (see below) or through conjugation of mAb to toxin (currently developed only for cancer applications). Blocking mAbs block the epitope needed for ligand interaction, preventing the target molecule from signaling but (usually) sparing the cell itself. Both natalizumab and daclizumab are blocking mAbs. Signaling mAbs mimic the ligand and induce signaling between molecules. Depending on the level of cellular activation the signaling mAb induces, treatment with it may be associated with in vivo activation of targeted cells and their release of cytokines. This cytokine release syndrome, first observed in association with muromonab (Orthoclone OKT®3, Ortho-Biotech Products, Bridgewater, NJ), an antirejection agent, is also to a lesser degree associated with the use of alemtuzumab and rituximab,³ 2 signaling mAbs in development for the treatment of MS.^{4,5}

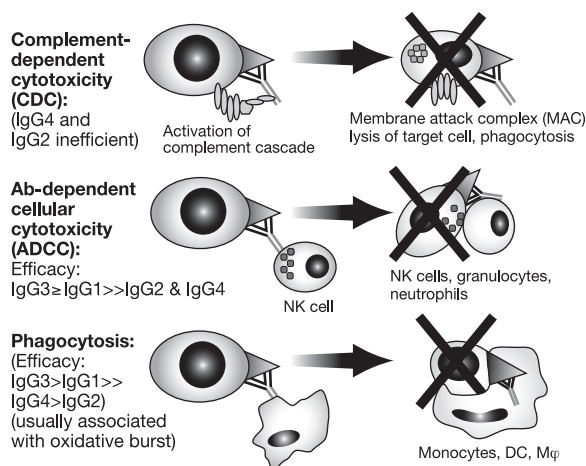
mAb effector functions. mAbs may mediate cytotoxic immune responses and destroy targeted cells. These effector functions depend on the F_c domain of the immunoglobulin (Ig) molecule, and their efficacy varies by IgG subgroup (figure 2).⁶⁻⁸

Complement-dependent cytotoxicity. In this scenario, the F_c receptor activates the complement system, forming a membrane attack complex that may destroy the target cells. In theory, both IgG1 and IgG3 should activate complement-dependent cytotoxicity (CDC); on the other hand, human IgG2 binds complement inefficiently, and IgG4 does not activate complement at all, making these IgG subtypes unlikely to provoke CDC.⁶

Ab-dependent cellular cytotoxicity. In this process, the mAb binds to F_cγ receptors on the surface of potentially cytotoxic immune cells such as NK cells, monocytes, and macrophages, triggering their degranulation and destruction of the target cell.^{8,9}

Phagocytosis. Targeted cells may be engulfed and destroyed, and their proteins were used for antigen presentation if the mAb binds to immune cells capable of phagocytosis, such as macrophages, monocytes, dendritic cells (DCs), and neutrophils.^{8,9} For both Ab-dependent cellular cytotoxicity (ADCC) and phagocytosis, the IgG subtypes vary in F_c receptor binding affinity, with IgG3 generally demonstrating the

Figure 2 The effector function of mAb (F_c dependent)^{7,8}



NK = natural killer; DCs = dendritic cells; Mφ = macrophages.

highest affinity, followed by IgG1 with intermediate affinity, IgG4 with low affinity, and IgG2 with the lowest affinity.⁶⁻⁸

Although mAbs exert direct effects only on cells that express their target molecule, mAb therapy may cause more widespread immune perturbation than would be expected solely from their direct effects on targeted cells. Examples of these secondary effects, which usually take longer to occur, include the functional inhibition of T cells after B-cell depletion by rituximab^{10,11}; increased levels of FoxP3⁺ T-reg cells after immune reconstitution post alemtuzumab¹² and rituximab¹³ therapy; and upregulation of CD56^{bright} NK cells by daclizumab¹⁴ and rituximab.¹⁵ Based on this foundation, what follows is a synopsis of the known or theorized mechanisms of action of the mAbs under study or in current use for therapy of MS.

Access of mAb to CNS tissue. mAbs are large molecules and therefore need to be transported across the intact blood-brain barrier (BBB) by limited active transport mechanisms. It has been documented that only ~0.1% of systemically administered mAbs reach the intrathecal compartment.^{16,17} This proportion is probably significantly higher in patients with gross disruption of the

BBB, such as patients with MS with contrast-enhancing lesions (CELs) on brain MRI. The positive therapeutic results in patients with relapsing-remitting MS (RRMS) with CELs indicate either that a sufficient amount of mAb reaches the CNS compartment in these patients or that the therapeutic benefit required only systemic effects.

NATALIZUMAB Adhesion molecules mediate the entry of immune cells into the CNS. Natalizumab, a humanized antibody with an IgG4 framework, is a selective adhesion molecule inhibitor whose target is CD49, the α_4 subunit of the VLA-4 receptor (table 2). VLA-4 interacts with vascular cell adhesion molecule-1 for immune cells to migrate across the BBB. Natalizumab binds to α_4 -integrin expressed on the surface of activated T cells and other mononuclear leukocytes, where it prevents adhesion between the endothelial cell and the immune cell. This action inhibits the migration of leukocytes into CNS tissue.^{9,18,19}

Direct effects. Two phase III clinical trials have shown natalizumab to effectively inhibit brain inflammatory activity as measured on MRI.^{20,21} High efficacy of natalizumab in blocking the migration of immune cells into the CNS is likely the most important early therapeutic mechanism of action (MoA) for this mAb in MS.²² However, the same MoA also underlies, or at least contributes to, the 1 problematic effect of natalizumab treatment: decreased CNS immunosurveillance,²³ especially as it relates to the risk of progressive multifocal leukoencephalopathy (PML). The risk of PML with natalizumab therapy has been estimated at ~1/1,000 patients treated for a mean of 17.9 months.²⁴ Natalizumab treatment has been shown to result in a prolonged decrease of lymphocytes in CSF, up to 6 months after therapy has been stopped.²⁵ The explanation for this prolonged effect may reside in poor antigen presentation in the CNS, because long-term natalizumab treatment is associated with significantly decreased frequency of DCs in the CNS perivascular spaces.¹⁹ Nevertheless, it remains controversial whether lack of CNS immune surveillance is solely responsible

Table 2 Mechanisms of action of natalizumab^{19,22,80}

Direct effects	Indirect effects	Consequences (therapeutic and side effects)
Inhibition of migration of immune cells into CNS	Stabilization of BBB	Probably most important early therapeutic MoA decreased CNS immunosurveillance
Inhibition of APC turnover in the CNS	Decreased antigen presentation in the CNS	Possibly MoA that underlies prolonged efficacy, but also further limits CNS immunosurveillance
Mobilization of lymphoid (especially pre-B) and lymphoid/myeloid (CD34 ⁺) precursors from the BM		Likely contributes to risk of PML

BBB = blood-brain barrier; MoA = mechanism of action; APC = antigen-presenting cell; PML = progressive multifocal leukoencephalopathy; BM = bone marrow.

Table 3 Mechanisms of action of rituximab^{9,36,41,44,47,81}

Direct effects	Indirect effects	Consequences (therapeutic and side effects)
Depletion of B cells (by ADCC and CDC)	Inhibition of function of F _{cγ} R-bearing cells such as macrophages ("immune-decoy hypothesis")	Probably most important early therapeutic MoA
	Lack of B-cell help/loss of APC function for T-cell-mediated immune responses	Possibly a contributing MoA
	Prolonged depletion of memory B cells may lead to lack of turnover of long-lived plasma cells	Possible effect on Ab-mediated immunity
	Early repopulation of B cells by increased egress of pre-B and naïve B cells from the BM	May be beneficial for MS (naïve B cells tend to produce anti-inflammatory cytokines like IL-10) but may also contribute to risk of PML

ADCC = Ab-dependent cellular cytotoxicity; CDC = complement-dependent cytotoxicity; MoA = mechanism of action; APC = antigen-presenting cell; Ab = antibody; BM = bone marrow; MS = multiple sclerosis; IL = interleukin; PML = progressive multifocal leukoencephalopathy.

for the increased incidence of PML in patients treated with natalizumab. Other supporting mechanisms may involve the increased release of B-cell precursors from the bone marrow into the blood observed in natalizumab-treated individuals, because bone marrow B cells are known to harbor JC virus.²⁶ Accelerating the clearance of mAbs from the body through plasma exchange has been proposed as 1 way to restore immune function in the CNS in the treatment of rare but serious complications such as PML.²⁷

Clinical trial evidence. The detailed analysis of the outcomes of 2 major phase III clinical trials of natalizumab for MS suggests that this mAb may be most effective for treatment of highly inflammatory early MS and less effective in later stages of the disease, characterized more by neurodegenerative than by inflammatory processes.

- In the SENTINEL trial, 1,196 patients with RRMS who experienced relapse despite at least a year of interferon beta-1a (IFNβ-1a) were randomized to combined treatment with natalizumab and IFNβ-1a (Avonex®, Biogen Idec) or to continued therapy with IFNβ-1a alone.²¹ Combination therapy resulted in a 24% reduction in the relative risk of sustained disability progression compared with IFNβ-1a alone ($p = 0.02$).
- In the AFFIRM trial, monotherapy with natalizumab was compared with placebo for RRMS in a total of 942 patients, 627 of whom received the mAb. After 2 years, natalizumab reduced the risk of sustained disability progression by 42% (almost twice the effect on this end point as in the mAb-treated group in SENTINEL) and reduced the rate of clinical relapse at 1 year by 68% (both $p = 0.001$).²⁰

There is evidence that the pathophysiologic mechanisms of MS evolve over the course of the disease, with inflammation characterizing the early relapsing-remitting stage and neurodegeneration more important in later stages.²⁸ The disparate results of these 2 trials of

natalizumab may reflect this progression; the population in SENTINEL was somewhat older than that in AFFIRM (38.9 years vs 36 years) and had a longer duration of disease (7 years vs 5 years). Detailed post hoc analysis of patient subgroups in both trials fully supports the notion that natalizumab (and likely all agents targeting the inflammatory component of MS) may be most effective in younger patients with early MS and in highly active disease, when inflammation is the dominant process.²⁹

RITUXIMAB Although investigations into MS pathophysiology have focused mainly on T cells, growing evidence suggests a key role for B lymphocytes as a potential therapeutic target. Rituximab is a chimeric murine/human IgG1_κ mAb that targets the CD20 antigen, which is expressed only on mature-B lymphocytes and not on Ab-producing plasma cells. The primary MoA of rituximab, at least early in therapy, is a complete but transient depletion of B cells. Although rituximab has some intrinsic cytotoxic activity toward B cells in vitro, it is believed that its main in vivo B-cell-depleting activity involves ADCC³⁰ and, to a lesser extent, CDC (table 3).^{31,32} To date, rituximab has been approved by the Food and Drug Administration (FDA) for use in rheumatoid arthritis (RA) and B-cell lymphomas. However, several published studies of rituximab in MS^{10,32-35} indicate that this B-cell-targeting therapy may be highly effective in inhibiting MS-related inflammation, perhaps leading us to critically reconsider our belief that MS is a predominantly T-cell-mediated autoimmune process.

Direct effects. The depletion of B cells by rituximab is rapid (within 2 weeks); it lasts for ~6 months and even longer for CD27⁺ memory B cells.³⁶ B cells are also depleted in secondary lymphoid tissues but with slower kinetics.^{37,38} Because of the lack of expression of CD20 on plasma cells, rituximab depletes only those autoantibodies that are produced by memory B cells but not those that are produced by plasma cells. Consequently, most of the protective Abs against in-

fectious agents and vaccines are not affected by rituximab therapy.³⁹ Similarly, rituximab therapy does not cause disappearance of CSF oligoclonal bands or normalization of the IgG index.¹⁰

Possible indirect effects. The action of rituximab extends beyond B-cell depletion and suppression of autoantibody synthesis. Many studies have documented inhibition of innate immune responses and adaptive T-cell responses associated with the use of rituximab in treatment of human autoimmune diseases^{38,40}; however, the mechanisms for these observations are insufficiently understood. According to a proposed “immune complex decoy hypothesis,” the rapid formation of a large number of immune complexes of rituximab-bound B cells may exhaust the functional activity of macrophages and other cells expressing FcγR, redirecting them from foci of chronic inflammation and leading to their functional inhibition.⁴¹ Indeed, rituximab therapy has also been associated with reductions in various global biomarkers of inflammation, such as interleukin (IL)-6 and C-reactive protein.⁹

Rituximab therapy has been shown to reduce T cells in the CSF of patients with MS¹⁰ and in the inflamed synovia of patients with RA,⁴² possibly due to lack of B-cell help for T-cell-mediated immune responses, such as antigen presentation and production of proinflammatory cytokines. Longitudinal data on the effect of long-term (i.e., repeated) B-cell depletion on IgG levels of specific antibodies are not available. However, the current model of long-term human B-cell memory (i.e., humoral immunity) indicates that “long-lived” plasma cells have a lifespan of only several months and therefore need to be constantly replenished by antigen-independent homeostatic activation of memory B cells.⁴³ This suggests that long-term depletion of memory B cells (e.g., by repeated doses of rituximab) should lead to an eventual decrease in humoral immunity, reflected by a decline in IgG levels.

Early repopulation of peripheral B cells by increased egress of pre-B and naïve-B cells from bone marrow might contribute to the therapeutic benefit of rituximab, because naïve B cells produce antiinflammatory cytokines,⁴⁴ but, as mentioned earlier in discussion of natalizumab treatment, it may also contribute to the risk of PML.

Clinical trial evidence. Rituximab demonstrated a rapid and sustained improvement in disease activity (fewer relapses, Gd-enhancing lesions, new T2 lesions, and T2 lesion volumes) in a 72-week open-labeled phase 1 trial of patients with RRMS.³⁴ A comparable early and robust effect was reported in a 48-week, double-blinded, phase II trial of 104 pa-

tients with RRMS.³² In this trial, rituximab treatment (1,000 mg IV on days 1 and 15; N = 69) was associated with a highly significant reduction in CELs on brain MRI at weeks 12, 16, 20, and 24 postinfusion (80.3% vs 51.4% of patients were lesion free, and the mean number of lesions was 91% lower; $p < 0.001$) compared with placebo (N = 35). Although the level of efficacy on CELs may be overestimated in this trial due to uneven distribution of CELs in the placebo and active-treatment groups at baseline, there is little doubt that rituximab is highly effective in suppressing BBB disruption in MS.

Given its apparent mediation of the inflammatory cascade, rituximab, similar to natalizumab, might be expected to show its greatest efficacy in early-stage, inflammatory MS. A large, multicenter, phase II trial of rituximab in primary-progressive MS⁴⁵ failed to reach the primary efficacy end point, but it confirmed that in patients with active CNS inflammation, rituximab significantly decreases CELs.⁴⁶ Although >11 cases of PML have been reported after rituximab therapy in patients with PML-prone systemic lupus erythematosus, only a single case of PML has so far been identified in a patient with RA, and no case has been associated with MS therapy.⁴⁷ Only future exposure of a large number of patients who are not prone to development of PML to rituximab therapy will define the exact risk of PML associated with this mAb.

ALEMTUZUMAB Alemtuzumab is a humanized IgG1_κ mAb, currently approved by the FDA for the treatment of B-cell chronic lymphocytic leukemia. Its target is CD52, a glycoprotein expressed widely throughout the immune system on T and B lymphocytes, NK cells, DCs, most monocytes and macrophages, and some granulocytes (but not neutrophils).⁴⁸ It is also expressed in tissues of the male reproductive system. Investigation of alemtuzumab for use in MS by a team at the University of Cambridge, UK, suggests potentially impressive efficacy, offset by significant risks.

Direct and indirect effects. Alemtuzumab produces swift and almost complete depletion of CD52-bearing cells (table 4). This is probably the most important early therapeutic MoA of the mAb. Although alemtuzumab was shown to induce caspase-independent apoptosis in B-lymphoid cell lines *in vitro*,⁴⁹ it is unclear to what extent is this direct cytotoxicity relevant for its *in vivo* effect.⁵⁰ Because alemtuzumab effectively depletes cells that mediate ADCC (such as NK cells),⁵¹ it is likely that CDC represents the main effector mechanism of alemtuzumab-mediated depletion of immune cells *in vivo*.⁵²

Table 4 Mechanisms of action of alemtuzumab^{9,48,54,82}

Direct effects	Indirect effects	Consequences (therapeutic and side effects)
Depletion of CD52-bearing cells (T and B, NK cells, monocytes and MΦ, DCs, some granulocytes)	Stabilization of BBB	Probably most important early-therapeutic MoA decreased CNS immunosurveillance theoretically immunosuppressive but only modest rate of infectious complications observed so far
	Repopulation with skewed repertoire: early B-cell repopulation, late T-cell repopulation; CD25 ^{high} T cells, and Caspase3 ⁺ T cells	Theoretical benefits for long-term efficacy but may also underlie Ab-mediated autoimmune complications

NK, natural killer; MΦ, macrophages; DCs, dendritic cells; BBB, blood-brain barrier; MoA, mechanism of action; Ab = antibody.

The depletion of immune cells is associated with dramatic decrease in CELs, indicating stabilization of BBB.^{53,54} Profound lymphocyte depletion in theory should cause immunosuppression and decreased CNS immunosurveillance, although rates of infectious complications in clinical trials of MS have not been very high.^{54,55} After alemtuzumab therapy, immune-cell repopulation takes place, although with a skewed repertoire: early B-cell repopulation is followed by enrichment of CD25^{high} T cells and possibly caspase 3⁺ T cells during later T-cell repopulation.^{9,54} In theory, these repertoire changes induced by alemtuzumab may improve long-term efficacy (repopulation by naïve B cells and FoxP3⁺ T-regs may provide a tolerance-enhancing environment for T-cell immunity), but it could also underlie development of Ab-mediated autoimmune complications, such as Ab-mediated thyroid disorders, immune thrombocytopenic purpura (ITP), and Goodpasture syndrome, reported in association with alemtuzumab treatment.^{53,54,56} The development of these autoimmune complications have been now linked by very elegant work to high levels of IL-21.⁵⁷

Clinical trial evidence. Based on its apparent MoA, alemtuzumab would be expected to confer clinical benefit in early, inflammatory MS, and clinical trial data clearly support this. Early clinical experience with alemtuzumab demonstrated that despite its profound effect on inhibition of formation of focal inflammatory lesions measured by CEL on MRI, alemtuzumab did not halt the progression of disability and development of brain atrophy in the secondary-progressive stage of the disease.⁵⁵ In striking comparison, when applied to patients with very early MS with little accumulated disability but high level of clinical activity, alemtuzumab therapy stabilized disease progression.⁵³ This prompted initiation of a large, 36-month, phase II trial comparing 2 IV doses of alemtuzumab (12 mg/d or 24 mg/d in annual cycles) to subcutaneously (SC) administered IFNβ-1a (44 μg 3 times/wk).⁵⁴ This study was single blinded (i.e., patients were aware of their treatment allocation, but the evaluating physician was blinded). Alemtuzumab significantly reduced the rate of sustained accumulation of disability in compari-

son with SC IFNβ-1a (9.0% vs 29.2%, hazard ratio 0.29, $p < 0.001$). In fact, disability scores improved in alemtuzumab-treated patients by 0.39 points on the Expanded Disability Status Scale, whereas scores worsened by 0.38 points for patients treated with SC IFNβ-1a. These robust clinical efficacy data were fully supported by MRI data (CELs, T2 lesion load, and development of brain atrophy). Even though the higher alemtuzumab dose seemingly performed better on virtually all measured parameters, the difference between the 2 mAb doses did not reach statistical significance. Alemtuzumab dosing in this trial was suspended after 3 patients developed ITP, 1 of whom eventually developed fatal intracerebral hemorrhage. Documented adverse reactions to alemtuzumab include an infusion reaction believed to be mediated by cytokine release from lysed lymphocytes. Other adverse effects relate to Ab-mediated autoimmunity, particularly Grave's disease,⁵⁸ ITP,⁵⁴ and Goodpasture syndrome.⁵⁶ Finally, patients on alemtuzumab experienced a significantly higher rate of infections than SC IFNβ-1a-treated patients (66% vs 47%). Development of melanoma has been described after alemtuzumab therapy,⁵⁹ and only future studies can determine the exact risk/benefit ratio of alemtuzumab treatment for chronic autoimmune disease such as MS. Two such large phase III trials of alemtuzumab are ongoing: in treatment-naïve MS patients (CARE-MSSM I) and in patients with inadequate response to current immunomodulatory therapies (CARE-MSSM II).

DACLIZUMAB Daclizumab, widely used to prevent rejection after allogeneic tissue transplantation, is a promising new therapeutic possibility for MS. Its complex MoA, which has yet to be fully elucidated, has so far demonstrated several intriguing and unexpected features. This humanized IgG1 mAb blocks CD25, the IL-2 binding epitope (Tac epitope) of the α-chain of the IL-2 receptor (IL-2R).

The rationale for the development of daclizumab was to curb proliferation of effector T cells by blocking the formation of high-affinity IL-2R.^{60,61} CD25 is found only at low levels in resting human T cells (except for FoxP3⁺ T-regulatory cells, which express

high levels of CD25) but is significantly upregulated on activated T cells, permitting them to receive a high-affinity IL-2 signal. IL-2 is mostly produced by activated T cells (and to a lesser degree by activated DCs and monocytes/macrophages),⁶² and it was widely believed to drive expansion of activated T cells and their development of effector functions. However, this assumption, which was based on the use of IL-2 for expansion of T cells in vitro, has been questioned after IL-2-deficient or CD25-deficient transgenic animals developed generalized lymphoproliferation rather than immunodeficiency.⁶³ The current view is that IL-2 plays an important role both in regulating expansion of lymphocytes and in mediating their contraction, through the process of reactivation-induced cell death and cytokine-withdrawal cell death.⁶⁴

Direct and indirect effects. In contrast to rituximab and alemtuzumab treatment, daclizumab therapy does not lead to immediate deletion of CD25-bearing cells, despite its shared IgG1 structure.¹⁴ Why this mAb is nondepleting in vivo remains unclear; its nondepletion may be caused by low expression levels of CD25 and by the ability of cells both to downregulate and to shed this molecule in soluble form. Nevertheless, it is clear that the downmodulation/shedding of CD25 during daclizumab therapy is incomplete (~40%), and many T cells survive in the circulation with daclizumab bound to their cell surfaces.¹⁴ Although daclizumab clearly inhibits IL-2 signaling on T cells in vitro,⁶⁵ it has relatively little effect on polyclonally stimulated T cells ex vivo.¹⁴ Instead, daclizumab therapy in MS leads to profound expansion of the regulatory CD56^{bright} NK cells.^{14,66} These regulatory CD56^{bright} NK cells are present in lymph nodes,⁶⁷ where they are in a position to influence T-cell priming through early production of cytokines (table 5) and can kill immature DCs.⁶⁸ However, these cells also migrate to inflammatory lesions and participate in termination of the immune response by killing autologous activated T cells.¹⁴ It

remains unclear whether expansion and activation of CD56^{bright} NK cells represent the only MoA of daclizumab in MS, but a strong correlation of expansion of CD56^{bright} NK cells with clinical and MRI response to daclizumab^{14,66} indicates that this unexpected MoA is indeed important in vivo. Daclizumab also inhibits survival of CD25 FoxP3⁺ T-regulatory cells,^{69,70} an effect that may underlie some of its observed side effects, such as development of skin rashes.⁷¹

Clinical trial evidence. Three small, open-labeled, baseline-to-treatment phase II trials established daclizumab as a potential therapy for MS.^{66,72-74} In 2 of these, patients with RRMS with incomplete clinical and MRI responses to IFN β received add-on therapy with daclizumab. Daclizumab was well tolerated and was associated with a reduction (>70%) in new CELs and also with stabilization or improvement in disability scores.⁷²⁻⁷⁴ The third trial addressed the question of synergy between IFN β and daclizumab and demonstrated that while daclizumab monotherapy is sufficient for inhibition of MS disease activity in the majority of patients with MS, IFN β + daclizumab combination therapy or, alternatively, higher dose daclizumab monotherapy may be required for inhibition of disease activity in very active patients.⁶⁶ This study demonstrated stabilization and also long-term improvement of neurologic disability in treated patients, similar to the stabilization observed in the alemtuzumab trial.⁵⁴ Nevertheless, because of the open nature of the trial, these data need to be interpreted cautiously and require confirmation in a blinded trial. The data from these small open-labeled trials were reproduced in a double-blinded, placebo-controlled trial of daclizumab vs placebo in patients with RRMS failing IFN β therapy (CHOICE study). (Results were presented at a recent meeting⁷⁵ but remain unpublished so far.) This study evaluated 2 doses of daclizumab: 1 mg/kg or 2 mg/kg administered SC every 2 weeks for a total of 24 weeks vs placebo. The patients receiving the

Table 5 Mechanisms of action of daclizumab^{14,69-71}

Direct effects	Indirect effects	Consequences (therapeutic and side effects)
Inhibition of IL-2 signaling by high-affinity IL-2R	Expansion of CD56 ^{bright} NK cells → killing of activated T cells	Probably most important therapeutic MoA
Apparently nondepleting, despite IgG1 structure		Prolonged duration of NK cell expansion may lead to lymphadenopathy but may provide some protection against viral infections
Mild inhibition of T-cell proliferation		Possibly some degree of immunosuppression
Inhibition of survival of CD25-expressing FoxP3 ⁺ T-reg cells		Likely risk factor for development of skin lesions/autoimmune complications?

IL, interleukin; IL-2R, interleukin-2 receptor; NK, natural killer; MoA, mechanism of action; Ig = immunoglobulin; T reg = T regulatory.

higher dose (which is close in bioavailability to 1 mg/kg IV every 4 weeks, the dosage used in previous open-labeled trials) experienced 72% reduction in CELs compared with patients on placebo. The drug is generally well tolerated, with the most common side effects identified in open-labeled studies being skin rashes and lymphadenopathy.⁷¹⁻⁷³ One case of CNS vasculitis was observed outside of the NIH trial, which developed on interruption of daclizumab therapy and was assessed as likely to be related to treatment (unpublished data). Therefore, the full side-effect profile of this therapy will have to be determined from large multicenter phase III trials.

CONCLUSIONS Ongoing research continues to expand our understanding of the complex and varied MoAs of various mAbs targeting the immune system for treatment of MS. Although mAbs target highly specific molecules, their in vivo MoA is remarkably pleiotropic, thanks to (often unpredicted) indirect effects. It is rather surprising that mAbs with such divergent targets as B cells (rituximab) or CD25-expressing cells (daclizumab) have demonstrated similar robust therapeutic efficacy in a single disease. This should make us reconsider our views of the complex interactions between different immune cells that underlie the development of the immune responses in humans in general and in MS in particular. Because of their large size, immunogenicity, and requirement for parenteral administration, binding mAbs may not be ideal long-term therapeutics in a chronic disease such as MS, as oral therapies are coming to the market. However, mAbs may be used as specific “probes” that help to identify therapeutic targets, and their development may be followed by searches for small molecules that explore identical MoAs. The prime example of this is the current development of small molecular antagonists of VLA-4 as the second generation of therapeutics after successful natalizumab therapy.

All mAbs reviewed in this article seem most likely to provide benefit in the earlier, inflammatory stages of MS, before the natural course of the disease evolves into a neurodegenerative phase of irreversible axonal loss. To what degree such early-aggressive treatment prevents the development of clinical disability and of the progressive stages of MS remains to be determined. Promising results in clinically and MRI-documented disease progression must be balanced against the still-unfolding picture of risk for these powerful and highly specific immunomodulating agents. Further study of the basic molecular activity of mAbs and their effects in clinical trials should provide more data to guide

the safest and most effective use of mAb therapy in MS.

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