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Insights into the Mechanisms of the Therapeutic Efficacy of Alemtuzumab in Multiple Sclerosis

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Abstract

The pathogenesis of multiple sclerosis (MS) is thought to involve peripheral activation of immune cells against central nervous system (CNS) antigens and their migration across the blood–brain barrier, leading to CNS inflammation and neurodegeneration. Alemtuzumab, a humanized anti-CD52 monoclonal antibody that rapidly depletes CD52-expressing cells from the circulation, is being investigated as a new treatment option in relapsing-remitting MS (RRMS). Clinical and radiologic results indicate robust suppression of inflammation related to the depletion of T and B lymphocytes during each treatment course of alemtuzumab. Furthermore, several lines of evidence suggest that the long-term clinical effects of alemtuzumab are attributable to qualitative changes in repopulating lymphocyte subsets potentially leading to a rebalancing of the immune system. Here, we review the contribution of data from animal models, *ex vivo* human studies, and clinical trials to the understanding of the mechanisms underlying the therapeutic effect of alemtuzumab in patients with RRMS.

Keywords: Alemtuzumab; Multiple sclerosis; Mechanism of action; Efficacy; Safety

Abbreviations: Ad2: Adenovirus Vector; ADCC: Antibody-dependent Cell-mediated Cytolysis; BAFF: B-cell Activating Factor; BBB: Blood–Brain Barrier; BDNF: Brain-derived Neurotrophic Factor; CDC: Complement-dependent Cytolysis; CNS: Central Nervous System; DC: Dendritic Cell; EDSS: Expanded Disability Status Scale; HSCT: Hematopoietic Stem Cell Transplantation; IFN: Interferon; IL: Interleukin; MS: Multiple Sclerosis; NK: Natural Killer; PBMC: Peripheral Blood Mononuclear Cell; RRMS: Relapsing-remitting Multiple Sclerosis; SAD: Sustained Accumulation of Disability; SP: Secondary Progressive; Th1: T-helper 1; Treg: Regulatory T Cell

Introduction

Multiple sclerosis (MS) is a chronic inflammatory, presumably autoimmune, disease of the central nervous system (CNS) and is the most common cause of chronic neurological disability in young adults [1]. Great progress in the pharmacotherapeutic management of MS has been achieved in the past decade. However, despite established efficacy in reducing the frequency of relapses and accumulation of disability, approved therapies have not been shown to completely inhibit progression of the disease or reverse existing CNS damage or disability [1]. Here, we review the biological effects of alemtuzumab, a humanized monoclonal antibody that has been demonstrated to provide a long-term clinical benefit including a decrease in disability in relapsing-remitting MS (RRMS) patients in clinical trials. We focus on elements of the mechanism of action of alemtuzumab that are believed to account for its efficacy and safety profile.

The Role of Lymphocytes in MS Pathogenesis

The etiology of MS is unknown, but is believed to involve complex interactions among genetic background, the immune system, and environmental factors [2]. MS pathogenesis is initiated by abnormal activation and proliferation of autoreactive T cells in the peripheral circulation, followed by migration of inflammatory cells across the blood–brain barrier (BBB) leading to damage of the CNS tissue [1,3]. Multifocal inflammatory lesions, demyelination, axonal loss, ineffective myelin-axonal repair, and neurodegeneration characterize MS pathophysiology [1,2].

MS is generally considered a primarily T-cell–mediated autoimmune disease, although a role for B lymphocytes has also been recognized [2]. Whereas the normal adaptive immune system, composed of T and B lymphocytes, efficiently discriminates between self- and foreign antigens [3], autoimmune diseases are associated with impaired tolerance to self-antigens. A peripheral activation of T cells autoreactive to CNS antigens is considered a key event in the pathogenesis of MS [2,3]. Upregulation of adhesion molecules on activated T cells leads to their migration across the BBB. Matrix metalloproteinases also facilitate T-cell migration by degrading the extracellular matrix and basement membrane of the BBB [2]. Chemokines and their receptors on circulating leukocytes additionally contribute to their extravasation [4].

Within the CNS, activated T cells assume a pathogenic role, presumably aided by regulatory defects that fail to suppress autoreactive effector cells [5]. Following activation, naïve T cells are capable of differentiating into several T-cell subpopulations with different effector functions [2]. T-helper 1 (Th1) cells produce pro-inflammatory cytokines such as interferon (IFN)-γ, which activate macrophages. Th2 cells secrete anti-inflammatory cytokines such as interleukin 4 (IL-4) and are essential for eradicating extracellular pathogens. Dysregulation of the balance between Th1 and Th2 cytokines has historically been implicated in MS pathogenesis; however, many MS investigators now

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believe that Th17 cells play the most critical role in the development of the autoimmune response [5]. Th17 cells are stimulated by IL-23 and secrete pro-inflammatory cytokines such as IL-17A, IL-17F, IL-21, IL-22, and tumor necrosis factor (TNF)-α. An accumulation of Th17 cells has been described in active MS lesions compared with normal-appearing white matter (NAWM) [6], and increased percentages of IL-17A–expressing $CD4^+$ T cells were reported in the peripheral circulation of MS patients in comparison to healthy individuals [7]. Although regulatory T cells (Tregs) keep autoreactive lymphocytes under control in normal individuals, defects in the number and functional activity of Tregs have been described in MS patients [8,9]. A reduction in thymic output of Tregs has been observed in MS patients [8] as well as defects in the capacity of peripheral blood Tregs to suppress the activation of myelin-specific T cells *in vitro* [10]. A study by Tzartos et al. failed to detect Tregs in MS brain tissue, suggesting an absence of T-cell suppression in CNS MS lesions [6].

Although $CD4^+$ T cells have been historically considered the predominant mediator of neuropathology in MS, more recent studies indicate that $CD8^+$ T cells also play an important role [11-13]. Adoptive transfer of activated myelin-specific $CD8^+$ T cells induces experimental autoimmune encephalomyelitis (EAE), an animal model of MS [11]. $CD8^+$ T-cell perivascular infiltrates are common in MS plaques [12], with some $CD8^+$ T-cell clones persisting in the brain, cerebrospinal fluid, and blood for years [13]. Studies by Bitsch et al. and Medana et al. have shown that $CD8^+$ T cells may be involved in neuronal damage [4,14].

Increasing evidence supports a substantial role for B lymphocytes in the pathogenesis of MS. Post-mortem studies have demonstrated that autoantibodies recognizing myelin oligodendrocyte glycoprotein (MOG) were found in high concentrations in the CNS parenchyma of patients with chronic CNS inflammation, suggesting that B cells may participate in demyelination through local production of pathogenic antibodies [15]. Besides their role in acute demyelination, B cells may contribute to the disease progression through their antigen presentation and cytokine secretion [16,17]. The formation of ectopic B-cell follicles has been reported in the cerebral meninges of a substantial proportion of MS patients with a chronic progressive disease [18]. However, perhaps the most compelling evidence that B cells contribute to the pathogenesis of MS is that rituximab, a depleting anti-CD20 monoclonal antibody specific for B cells, decreased inflammation and reduced the number of relapses within several months of the treatment onset in patients with MS [19].

Although studies of the pathogenesis of MS have traditionally focused on the adaptive immune system, an important role for the innate immune system is also recognized. Dendritic cells (DCs) participate in both innate and adaptive immune responses [20]. Other innate immune cells, including natural killer (NK) cells, may also modify the inflammatory process in RRMS [21]. Activated microglia may also activate T cells and release cytotoxic cytokines that destroy oligodendrocytes [5].

Alemtuzumab is a humanized monoclonal antibody directed against CD52, a glycosylated, glycosylphosphatidylinositol-anchored, cell-surface protein that is expressed at high levels on T and B lymphocytes [22,23]. CD52 is also expressed at lower levels on NK cells, monocytes, DCs, macrophages, and eosinophils, with little to no expression on neutrophils, plasma cells, and bone marrow stem cells [22]. The function of CD52 is unknown, but evidence suggests it may be involved in T-cell co-stimulation [24] and migration [25]. Alemtuzumab can deplete CD52-positive cells through antibody-dependent cell-mediated cytolysis (ADCC) [22,26], complement-dependent cytolysis (CDC), and induction of apoptosis [27]. This selective cell depletion is the first step in a series of immunological changes that may contribute to the long-term benefit of alemtuzumab in MS patients.

Efficacy of Alemtuzumab in MS Clinical Trials

Based on the hypothesis that a brief course of alemtuzumab may result in depletion of lymphocytes and disrupt the inflammatory processes of MS, Coles et al. began treating MS patients with alemtuzumab in 1991 [28]. In a small exploratory clinical trial, they have demonstrated that this antibody effectively suppressed clinical activity (relapse rate) in both the RR and secondary progressive (SP) stages of MS [28]. In contrast to RRMS patients who experienced significant reductions in disability at 6 months, patients with SPMS treated with a single course of alemtuzumab did not experience a noticeable improvement in their disability. Therefore, the subsequent 3-year, CAMMS223 Phase II study (NCT00050778) [29] examined the clinical effects of alemtuzumab in previously untreated patients with early RRMS. Compared with subcutaneous (SC) IFNβ-1a 44 μg injections three times a week, two annual courses with alemtuzumab resulted in significant reductions in relapse frequency, sustained accumulation of disability (SAD), and T2 lesion burden over the 36-month study [29]. Findings of the CAMMS223 trial were confirmed in the Phase III Comparison of Alemtuzumab and Rebif® Efficacy in Multiple Sclerosis (CARE-MS) studies, in which alemtuzumab showed superior efficacy compared with SC IFNβ-1a 44 μg over 2 years in RRMS patients (Table 1) [30,31]. In CARE-MS I (NCT00530348), which enrolled treatment-naïve patients, alemtuzumab reduced the relapse rate by 55% ($p<0.0001$), but the 30% reduction in SAD was not significant ($p=0.22$) [30]. However, in CARE-MS II (NCT00548405), which enrolled patients who had experienced disease activity while on prior therapy, alemtuzumab reduced the relapse rate by 49% ($p<0.0001$) and risk of SAD by 42% ($p=0.0084$) [31]. Although mean disability in SC IFNβ-1a–treated patients continued to worsen over the course of CARE-MS II, disability in alemtuzumab-treated patients actually improved compared with baseline; the difference in mean disability between treatment groups was also statistically significant [31]. For CARE-MS II patients with Expanded Disability Status Scale (EDSS) ≥ 2, a sustained reduction in disability (SRD; defined as a decrease in EDSS by ≥ 1 point, sustained for a consecutive 6-month period) was more than twice as likely to occur in alemtuzumab-treated patients compared with SC IFNβ-1a–treated patients [31]. Consistent with the SAD data, there was no statistically significant difference between the treatment groups in CARE-MS I for either mean disability or the percentages of patients experiencing SRD [30]. Significant treatment effects of alemtuzumab compared with SC IFNβ-1a were also observed on loss of brain volume in both CARE-MS studies [30,31].

Proposed Mechanisms of Action of Alemtuzumab

The effects of alemtuzumab treatment in human CD52 transgenic mice

Alemtuzumab is specific for human CD52 (huCD52) and was engineered by grafting the complementarity-determining region of a rat monoclonal antibody onto a human heavy- and light-chain immunoglobulin G1 (IgG1) framework [26,32]. Alemtuzumab does not cross-react with mouse CD52, making *in vivo* studies in wild-type mice unfeasible. Therefore, a transgenic mouse model expressing huCD52 was created on an outbred CD1 background (CD1) [22]. Histological evaluation of the resulting transgenic mouse showed that expression of

	CAMMS223 Primary analysis [29]		CAMMS223 Extension study [37]		CARE-MS I [30]		CARE-MS II [31]	
Study design	Randomized (1:1:1 alemtuzumab 12 mg IV vs alemtuzumab 24 mg IV vs SC IFNB-1a 44 µg), parallel-arm, rater-blinded, multicenter				Randomized (2:1 alemtuzumab 12 mg IV vs SC IFNB-1a 44 µg), parallel-arm, rater-blinded, multicenter		Randomized (2:1 alemtuzumab 12 mg IV vs SC IFNB-1a 44 µg), parallel-arm, rater-blinded, multicenter	
Study type	Phase II				Phase III		Phase III	
Study duration, yrs	3		5		2		2	
Patient population	Active RRMS; treatment-naïve; EDSS ≤ 3; onset ≤ 3 yrs				Active RRMS; treatment-naïve; EDSS ≤ 3; onset ≤ 5 yrs		Active RRMS; relapsing on prior therapy; EDSS ≤ 5; onset ≤ 10 yrs	
Co-primary endpoint results	**SC IFNB-1a (n=111)**	**Alemtuzumab 12 mg (n=112)**	**SC IFNB-1a (n=111)**	**Alemtuzumab 12 mg (n=112)**	**SC IFNB-1a (n=187)**	**Alemtuzumab 12 mg (n=376)**	**SC IFNB-1a (n=202)**	**Alemtuzumab 12 mg (n=426)**
ARR Relative reduction vs SC IFNB-1a	0.36	0.11 69% (*p<0.0001*)	0.35	0.12 66% (*p<0.0001*)	0.39	0.18 55% (*p<0.0001*)	0.52	0.26 49% (*p<0.0001*)
Patients with SAD, % Relative reduction vs SC IFNB-1a	24	8 75% (*p<0.001*)	30	13 69% (*p=0.0005*)	11	8 NS	21.1	12.7 42% (*p=0.0084*)
Additional clinical efficacy outcomes								
Proportion of relapse-free patients, %	52	77	41	68	59	78 (*p<0.0001*)	47	65 (*p<0.0001*)
EDSS score change from baseline	+0.46	-0.32 (*p<0.001*)	+0.46	-0.15 (*p=0.014*)	No significant treatment difference		+0.24	-0.17 (*p<0.0001*)
Proportion of patients with SRD, %	NA	NA	NA	NA	25	23 (HR=0.87) (*p=NS*)	13	29 (HR=2.57) (*p=0.0002*)
MSFC score change from baseline	NA	NA	NA	NA	+0.03	+0.11 (*p=0.011*)	-0.04	+0.08 (*p=0.0022*)

ARR: Annualized relapse rate; EDSS: Expanded Disability Status Scale; HR: Hazard ratio; IFNB: Interferon beta; IV: Intravenous; MSFC: MS Functional Composite; NA: Not available; NS: Nonsignificant; RRMS: Relapsing-remitting MS; SAD: Sustained accumulation of disability; SC: Subcutaneous; SRD: Sustained reduction in disability

Table 1: Clinical efficacy of alemtuzumab in relapsing-remitting MS.

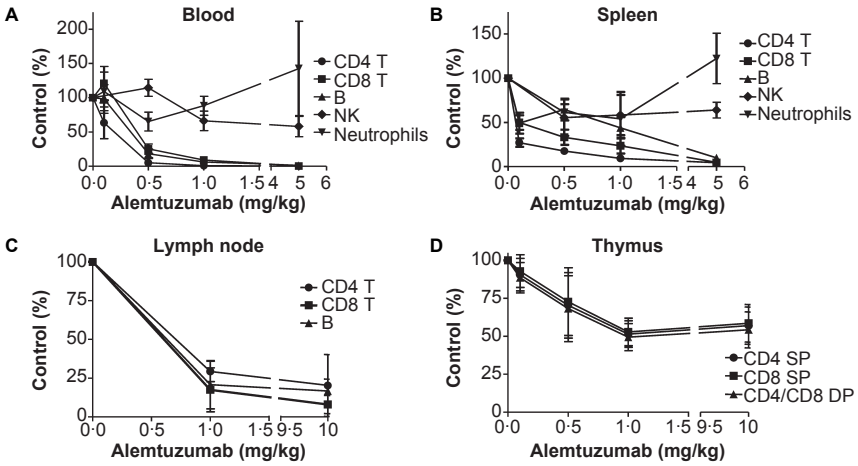

Figure 1: Immune cell depletion following alemtuzumab treatment in a human CD52-transgenic mouse model

Absolute numbers of immune cell populations remaining at 72 hours after the administration of various intravenous doses of alemtuzumab were assessed. Results shown are the mean ± SEM of individual mice (n=5) and are expressed as the percentage of cells remaining after treatment relative to the number of cells present in vehicle-treated control mice (% Control). The organs examined included the blood (A), spleen (B), inguinal lymph nodes (C), and thymus (D). The cell populations analyzed consisted of $CD4^+$ T cells, $CD8^+$ T cells, single-positive (SP) and double-positive (DP) thymocytes, $B220^+$ B cells, $NK1.1^+$ $CD49b^+$ NK cells and $Gr\text{-}1^+$ neutrophils. (Reprinted from Figure 4 in Hu et al. [22].)

huCD52 did not affect tissue architecture compared with that of wild-type mice. Distribution of CD52 in lymphoid tissues and CD52 antigen density on different immune cell types was comparable with that seen in humans. Studies using the huCD52 transgenic mouse model have allowed assessment of lymphocyte populations in lymphoid organs beyond the peripheral blood compartment available in human studies [22].

Experiments involving administration of alemtuzumab to huCD52 transgenic mice replicated the depletion of lymphocytes observed in human studies, with near-complete depletion of T and B lymphocytes from the circulation and little effect on cells of the innate immune system (neutrophils and NK cells) [22]. Lymphocyte depletion in the mouse model appeared to be mediated primarily through ADCC by neutrophils and NK cells, and was largely independent of CDC [22].

Such a clear distinction may not occur in humans since *in vitro* studies in human cell lines have also indicated roles for CDC [33] and apoptosis induction [34] in alemtuzumab-mediated cell lysis.

The degree of lymphocyte depletion in the lymphoid organs (spleen, lymph nodes, bone marrow, and thymus) was less profound than in the peripheral circulation (Figure 1). It is possible that penetration and exposure to alemtuzumab may be reduced, or that effector mechanisms responsible for cell depletion (e.g., ADCC) may not be as abundant in lymphoid organs compared with peripheral blood. Further analysis of the T-cell populations present in the spleen after alemtuzumab treatment showed that naïve $CD4^+$ and $CD8^+$ T cells were the most susceptible to depletion, whereas central and effector memory T cells, as well as Tregs, were depleted to a lesser extent, despite equivalent levels of CD52 expression on their surfaces [35]. B lymphocytes returned to pretreatment levels most rapidly, followed by protracted recovery of $CD8^+$ and $CD4^+$ T lymphocytes [22]. The lack of effect of alemtuzumab on CD52-negative cells, including bone marrow precursors, may explain the relatively rapid recovery of B lymphocytes [22], whereas the mechanism responsible for the delayed and incomplete recovery of T lymphocytes is unknown [36].

The function of immune cells that remained following alemtuzumab treatment in huCD52 mice was also evaluated [35]. Residual T lymphocytes from alemtuzumab-treated huCD52 mice showed *in vitro* proliferation rates and cytokine production in response to anti-CD3 monoclonal antibody stimulation similar to that in vehicle-treated mice. The diversity of the T-cell receptor repertoire was also maintained post alemtuzumab treatment. In order to examine the primary T-cell responses, huCD52 mice were challenged with an adenovirus vector (Ad2) at 7, 21, or 35 days after alemtuzumab treatment. T-cell response was reduced at 7 and 21 days post alemtuzumab treatment, but was comparable to that in control animals by day 35, well before peripheral T-cell counts returned to baseline levels. The impact of alemtuzumab treatment on primary B-cell responses in huCD52 mice was also modest. Transgenic mice developed *de novo* antibody responses to immunization with a T-independent antigen 3 or 21 days post alemtuzumab treatment that were comparable with vehicle controls. Antibody response to a T-dependent antigen was attenuated 3 days after alemtuzumab treatment (the nadir of lymphocyte depletion), but was similar to vehicle-treated controls at 21 days [35]. Preserved immune responses in alemtuzumab-treated animals may be due, in part, to the activity of residual B and T lymphocytes within peripheral lymphoid organs.

Innate immune cells express only low levels of CD52 and undergo minimal and transient depletion post alemtuzumab treatment (Figure 2D). Their functionality was evaluated following alemtuzumab in a peritonitis model of inflammation employing huCD52 mice [35]. Macrophages, monocytes, and polymorphonuclear cells retained the ability to migrate to the site of inflammation, and the cellular composition of peritoneal exudate 48 hours after injection of the inflammatory agent thioglycolate was comparable with that of the vehicle-treated mice. Macrophages recruited to the site of inflammation displayed normal phagocytosis of particulates and production of cytokines. In additional studies, NK cells from alemtuzumab-treated huCD52 mice retained their ability to lyse target cells *in vitro* [35]. The limited impact of alemtuzumab treatment on the ability of innate immune cells to respond to inflammation further supports

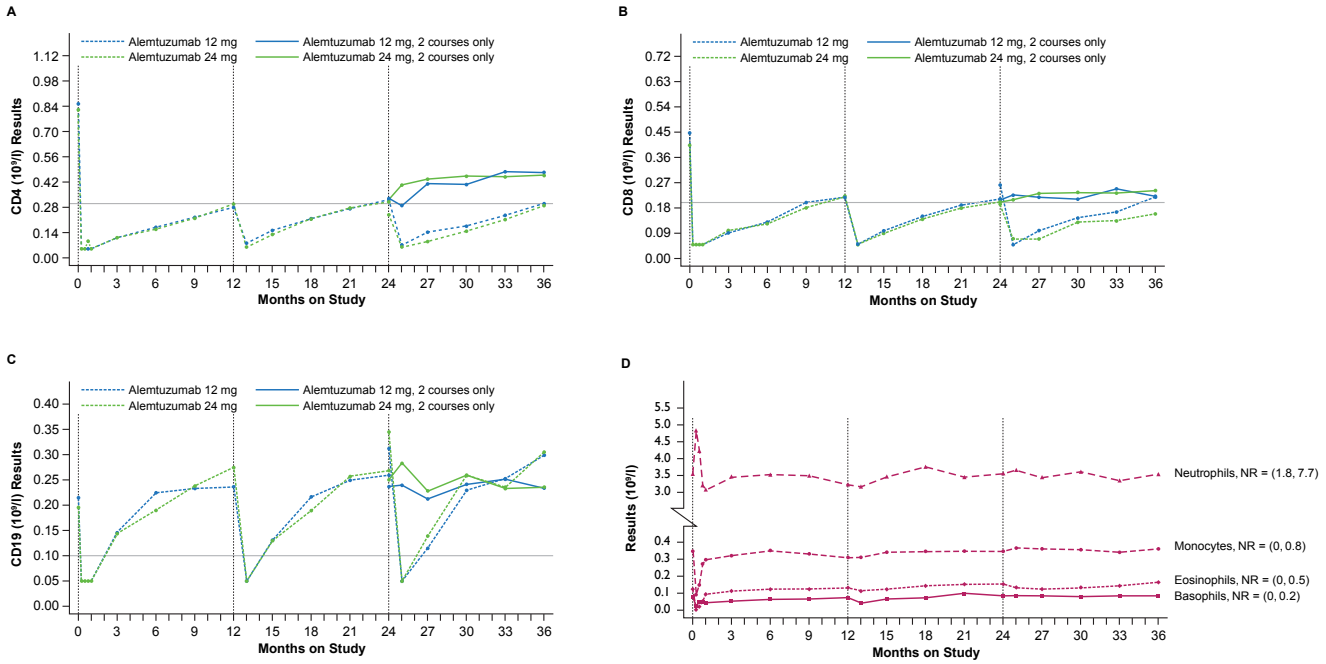

Figure 2: Effects of alemtuzumab on median lymphocyte and leukocyte counts over 36 months in the CAMMS223 Phase II study

Alemtuzumab rapidly depleted the circulating T- and B-lymphocyte subsets studied (CD4, CD8 and CD19) after each treatment course. CD4 (A) and CD8 (B) T cells reconstituted gradually after each treatment course without reaching baseline values. Median B-cell values returned to baseline and remained within normal ranges 6 months after each treatment course (C). Circulating monocyte, eosinophil, and basophil cell counts were minimally or transiently affected after each treatment course. The mean number of circulating neutrophil cells initially increased (while remaining within normal limits), but normalized within 4 weeks (D). NR=normal range; vertical reference lines represent alemtuzumab dosing; grey, horizontal lines represent the lower limit of normal. (Data from Coles et al. [36]).

a level of preserved immunocompetence, and may help explain why alemtuzumab-treated MS patients experience a low incidence of serious infections.

The 5-year follow-up of the Phase II clinical trial of alemtuzumab noted a relatively low incidence of serious infection (alemtuzumab 7%, IFNβ-1a 3%) despite lymphopenia and protracted repopulation [37], a finding that was supported by Phase III data from the CARE-MS studies [30,31]. In the Phase II 5-year follow-up, infections were predominantly (96%) mild or moderate in severity, and no infections were life-threatening or fatal. Herpes infections and superficial fungal infections were more common in alemtuzumab-treated compared with IFNβ-1a–treated patients (17% vs 4%). Infection rates were similar between patients who received two or three courses of alemtuzumab treatment [37]. These clinical observations concur with preclinical and transgenic mouse studies showing minimal and transient effects of alemtuzumab on the innate immune system (NK cells, DCs, neutrophils, and macrophages), and functional activity of cell types that are spared [22,35]. These data also suggest that immunocompetence may not be adequately reflected by simple peripheral lymphocyte counts [38]. A further, recent finding with potential relevance to limiting the occurrence of serious infections was the observation that alemtuzumab depleted circulating central memory T cells, but spared skin-resident effector memory T cells when administered to patients with cutaneous T-cell lymphomas [39].

Mechanistic observations in patients with MS

Anti-inflammatory effects: In an early study, a single course of alemtuzumab in 16 patients with RRMS resulted in robust depletion of peripheral lymphocytes and monocytes [40]. Monocytes returned to pretreatment levels within the first month, whereas B cells recovered fully within 3 months and slightly superseded baseline numbers for the remainder of the 1-year follow-up. T-cell subsets were much slower to recover, with $CD8^+$ and $CD4^+$ T cells reaching 55.4% and 32.9% of pretreatment values, respectively, by 12 months. Similar patterns of T-cell depletion and repopulation were observed in the 36-month CAMMS223 study (Figures 2A and 2B) [36]. Long-term T-cell repopulation data from a 5-year follow-up of the CAMMS223 study demonstrated that nearly all alemtuzumab-treated patients returned to the lower limit of normal (LLN) range (estimated median time to LLN: 11 months for $CD8^+$, 12 months for $CD4^+$) [37]. Still, these findings suggested that repopulation of T cells following alemtuzumab treatment may result in a new, lower baseline level. Delayed $CD4^+$ T-cell recovery has been observed after hematopoietic stem cell transplantation (HSCT) for rheumatoid arthritis and has been linked to poor memory T-cell expansion and low levels of IL-7 [41]. However, in a study by Cox et al., levels of IL-7, which converts naïve and effector T cells to a memory T-cell phenotype, were increased an average of five-fold following alemtuzumab treatment, progressively decreasing throughout the 12-month study, but remaining significantly above pretreatment levels [40]. Furthermore, $CD4^+$ memory cells ($CD4^+CD45RO^+$) dominated the depleted T-cell pool during the first 3 months following alemtuzumab. A subset of memory T cells, Tregs (i.e., $CD4^+CD25^{high}$), were significantly over-represented for the first 6 months after alemtuzumab treatment, even though the proportion of Tregs to total $CD4^+$ T cells was similar in MS patients and healthy controls prior to treatment. The over-representation of Tregs in the repopulating T-cell pool, first described by Cox et al., has subsequently been corroborated (Figure 3) in the large Phase III CARE-MS I study and suggests that strictly quantitative effects on circulating lymphocytes may oversimplify the impact of alemtuzumab on the immune system [42]. Changes in the composition and activity of T-cell subsets during immune repopulation following alemtuzumab-induced lymphopenia may also contribute to the observed long-lasting suppression of disease activity. For example, characterization of T-cell subsets and *ex vivo* cytokine production post alemtuzumab treatment showed not only preferential expansion of Tregs within the $CD4^+$ lymphocytes, but also a significant expansion of $CD4^+$ Th2 cells and a sustained increase in the production of the immunoregulatory cytokines TGFβ-1 and IL-10 along with a decrease in the percentages of $CD4^+$ Th1 and Th17 cells. IFNβ-1a control treatment also induced a significant increase in the percentage of IL-10–producing $CD4^+$ and $CD8^+$ T cells, and significant decreases in the percentages of Th1 and Th17 cells. However, the increase in the percentage of the IL-10–secreting $CD4^+$ T cells in IFNβ-1a–treated patients was significantly lower than that in the alemtuzumab-treated patients [43].

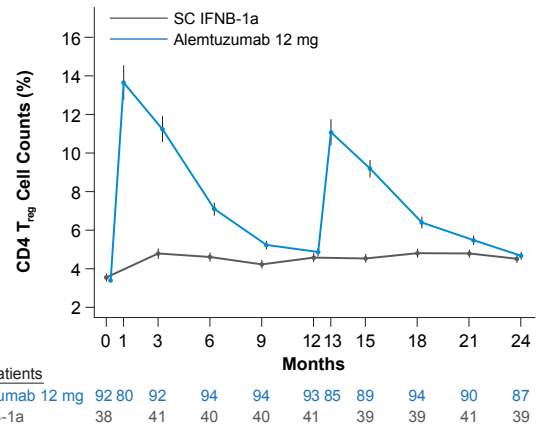

Figure 3: Proportion of $CD4^+$ T cells with a regulatory phenotype

The percentage of T lymphocytes with a regulatory T-cell (Treg) phenotype was elevated following alemtuzumab treatment (12 mg; 2 annual courses of IV infusions) in treatment-naïve, relapsing-remitting MS patients (CARE-MS I; unpublished data). The proportion of Treg cells gradually returned toward baseline levels, but remained elevated at Month 12. (Data from Hartung et al. [42]).

Jones et al. also used *ex vivo* patient samples to study changes in T-cell proliferation and apoptosis as potential mechanisms underlying the durability of alemtuzumab's effect in MS patients [44]. Although T-cell proliferation was similar in healthy controls and untreated MS patients, T cells from untreated MS patients had a four-fold greater survival compared with those from healthy controls, and were resistant to both passive and Fas-mediated apoptosis. The resistance to apoptosis in untreated MS patients correlated with reduced levels of caspase mRNA expression [44]. These observations corroborated previous reports and suggested that reduced T-cell death may be an inherent feature of untreated MS [45,46]. Alemtuzumab treatment resulted in a significantly higher proliferative index and significantly greater T-cell apoptosis (and caspase mRNA expression) in both $CD4^+$ and $CD8^+$ cell populations. Increased T-lymphocyte apoptosis persisted for at least 18 months after treatment with alemtuzumab [44]. Sustained high levels of apoptosis may explain why alemtuzumab-induced T-cell lymphopenia in MS patients lasts longer than expected based on its lack of effect on hematologic precursors [44].

A subsequent study by Thompson et al. focused on B-cell reconstitution following alemtuzumab in 78 patients with RRMS who were participants in Phase II trials [47]. As in previous studies, the initial depletion of monocytes, B cells, and T cells was followed by

a rapid recovery of monocytes and B cells, but a protracted recovery period for $CD8^+$ and $CD4^+$ T cells. Following their return to baseline, B cells continued to rise, reaching 165% of baseline by 12 months. Similar enrichment of B cells was observed in the CAMMS223 Phase II study (Figure 2C) [36]. Soon after alemtuzumab treatment, the B-cell pool was dominated by cells with a transitional-type 1 (T1) phenotype (representing recent bone marrow emigrants; $CD19^+/CD23^-/CD27^-$). By 3 months, a subset of B cells with a phenotype intermediate between T1 and memory B cells (defined as "mature naïve", $CD19^+/CD23^+/CD27^-$) dominated the B-cell pool, and continued to dominate until the second course of alemtuzumab at 12 months. Conversely, memory B cells ($CD27^+$) were significantly reduced compared with baseline at 1 month and remained significantly suppressed, reaching only 25% of baseline at 12 months. Serum levels of B-cell activating factor (BAFF), which mediates the survival of peripheral immature B cells, increased three-fold over baseline at 1 month and remained significantly elevated at all subsequent time points. The surge in serum levels of BAFF coincided with the dominance of the intermediate $CD19^+/CD23^+/CD27^-$ phenotype in the reconstituting B-cell pool [47]. The authors suggested that lymphocyte repopulation following alemtuzumab treatment resulted in a B-cell pool skewed toward immature phenotypes, driven by increased serum levels of BAFF, and that prolonged memory B-cell lymphopenia may contribute to the efficacy of alemtuzumab in MS [47]. Thus, the long-term control of disease by alemtuzumab in RRMS patients may involve sustained alteration in the distributions of B- and T-cell subsets.

Neuroprotection: The notion of "neuroprotection" — a loosely defined entity — implies that a treatment leads to preservation of neural elements that would otherwise sustain injury in the absence of such treatment. Neuroprotective effects may also help explain the long-term benefits of alemtuzumab observed in MS patients. Alemtuzumab-mediated neuroprotection may involve both the elimination of damaging inflammation through lymphocyte depletion and the stimulation of neurotrophins production. Although it is difficult to distinguish between the contributions of these two components, evidence suggests that the early depletion of T cells may be followed by production of neurotrophins by lymphocytes. Jones et al. reported that peripheral blood mononuclear cells (PBMCs) isolated from alemtuzumab-treated patients in Phase II clinical studies produced increased concentrations of brain-derived neurotrophic factor (BDNF), platelet-derived growth factor (PDGF), and ciliary neurotrophic factor when stimulated with myelin antigens *in vitro* [48]. These increases were detectable at 6 months post-treatment, and further increased through 12 months post-treatment. Alemtuzumab treatment also led to decreased secretion of IL-17 and IL-2 in response to myelin antigen—changes that may reduce T-cell–mediated inflammation. *In vitro* results demonstrated that PBMC-conditioned media derived from alemtuzumab-treated patients promoted survival of neurons and oligodendrocyte precursors, enhanced oligodendrocyte differentiation, and increased axonal length compared with controls [48]. Although it remains to be determined how neurotrophin-secreting immune cells might penetrate the CNS, there is evidence from post-mortem studies that peripheral immune cells secreting BDNF (mainly macrophages/microglia and T cells) are capable of entering the CNS and producing neurotrophic factors [49]. Increases in BDNF (but not ciliary neurotrophic factor or PDGF) have been reported in the serum and cerebrospinal fluid following treatment with IFNβ [50] and in the serum of patients treated with laquinimod [51] and glatiramer acetate [52]. Although additional studies will be needed to demonstrate whether improvements in disability correlate with increased growth factor production, these data suggest that the impact of alemtuzumab might extend beyond the anti-inflammatory effects.

Safety and Tolerability of Alemtuzumab

In Phase II and III studies, the most frequently reported adverse events (AE) following alemtuzumab treatment were infusion-associated reactions (IARs) [29-31]. Other notable safety observations included the development of secondary autoimmunity and infections of predominantly mild to moderate severity [27,30,31,48,53].

IARs were defined in alemtuzumab studies as any AE with reported onset during or within 24 hours after alemtuzumab infusion; the majority were mild to moderate in severity and were effectively managed by premedication with methylprednisolone, antipyretics, and antihistamines. The proportion of patients with IARs was higher during the first course of alemtuzumab than the second and, within each treatment course, the greatest numbers of IARs occurred on the first day of infusion [54].

The most common autoimmune event was thyroid disease [29-31], with an incidence of 30% in alemtuzumab-treated patients followed up to 5 years compared with 4% for those treated with IFNβ-1a [37]. Studies have shown that autoimmune diseases, particularly Graves' disease [55], are increased among family members of patients with MS, and in MS patients compared with non-MS patients [56], although at a substantially lower prevalence than that seen with alemtuzumab in MS. Autoimmune thyroid disease has not been associated with the use of alemtuzumab in patients with B-chronic lymphocytic leukemia (B-CLL), perhaps due to the higher dosages of alemtuzumab used in treatment of B-CLL and/or the nature of the patient population whose immune system is disrupted by leukemic cells and who often receive additional treatment with immunosuppressive cytotoxic agents. Interestingly, autoimmunity has not been associated with alemtuzumab use in more than 600 patients treated for other disorders [32,57]. However, "reconstitution autoimmunity" has been observed in other clinical contexts involving homeostatic responses to lymphopenia, including HSCT and antiretroviral treatment of HIV [58]. In the face of lymphopenia, non-depleted T cells undergo compensatory expansion termed "homeostatic proliferation" [44]. Jones et al. have speculated that the rapidly expanding T cells acquire a memory cell phenotype characterized by reduced dependence on co-stimulation, an ability to respond to lower doses of antigen compared with naïve cells, and secretion of inflammatory cytokines on restimulation (which can further promote breakdown of self-tolerance) [44].

In addition to thyroid autoimmunity, immune thrombocytopenia (ITP) was observed in 1% of patients receiving alemtuzumab 12 mg compared with no SC IFNβ-1a–treated patients in each of the CARE-MS Phase III studies, and typically responded to conventional therapies [30,31]. Unlike other forms of drug-induced ITP, which normally occur within days of exposure to the therapy, alemtuzumab-associated ITP has a delayed onset [59]; cases in the CARE-MS studies typically occurred several months after receipt of alemtuzumab [30,31]. Three cases of Goodpasture's disease (anti-glomerular basement membrane antibody-mediated disease) have been also reported following treatment with alemtuzumab (one patient in a controlled clinical study [60] and two patients treated outside of clinical trials [61]) in the past 10 years.

Although the mechanisms responsible for secondary autoimmunity in alemtuzumab-treated patients are unknown, it may be relevant that all are predominantly antibody-mediated. This phenomenon is under active investigation.

Studies conducted to characterize alemtuzumab-induced autoimmunity have produced some insight into the immunological mechanisms underlying alemtuzumab's activity. Although an association between lymphopenia and secondary autoimmunity is recognized, the majority of lymphopenic patients do not develop autoimmune complications. Krupica et al. suggested a two-hit model in which lymphopenia, combined with a secondary insult, is required to induce autoimmunity [38]. Jones et al. reported that patients who developed autoimmunity generally had lower serum levels of IL-7 compared with patients who did not develop autoimmunity [44,62,63]. Additionally, secondary autoimmunity in patients treated with alemtuzumab correlated with greater T-cell apoptosis and cell cycling compared with patients who did not develop autoimmunity. Furthermore, antibody-mediated autoimmunity has been associated with elevated levels of BAFF [47], which are observed during B-cell recovery following alemtuzumab treatment. Other cytokines may also contribute to the development of secondary autoimmunity and are being examined.

Conclusions

Alemtuzumab is an emerging therapy for RRMS that acts by selectively targeting cells expressing the CD52 antigen, resulting in the depletion of both circulating T and B lymphocytes, with less profound lymphocyte depletion in lymphoid organs and largely preserved levels of cell types involved in innate immunity. In spite of the marked peripheral lymphopenia that follows alemtuzumab treatment, few serious infections have occurred in MS patients receiving alemtuzumab. Mouse studies suggest that this may be explained by the maintenance of a level of immune competence characterized by largely unaffected innate immunity and serum immunoglobulins, and functionality of remaining lymphocytes with a transient decrease in the ability to respond to novel antigens.

Significant reductions in relapse and magnetic resonance imaging activity are consistent with alemtuzumab's ability to suppress inflammation in the CNS due to lymphocyte depletion, whereas other clinical effects may relate to the capacity of alemtuzumab to alter the immune system during the repopulation process. Lymphocyte repopulation after alemtuzumab treatment features an early recovery of B cells followed by a delayed recovery of T-cell subsets. Driven by elevated levels of BAFF, B cells recover within approximately 3 months and then exceed pretreatment levels. The repopulating B-cell pool is skewed toward immature B-cell phenotypes with a slow recovery of B-memory cells. Prolonged memory B-cell lymphopenia may contribute to the durable efficacy of alemtuzumab observed in long-term follow-up. Early T-cell recovery is characterized by an increase in the proportion of memory T cells with a regulatory phenotype, followed by a gradual return of $CD8^+$ and $CD4^+$ T cells. Although the regulatory function of Tregs following alemtuzumab treatment has not been formally evaluated in MS patients, their enrichment in the recovering $CD4^+$ pool may contribute to the persistent control of inflammatory processes in MS. Changes in the proportions of Th2/Th17/Th1 subsets and the cytokines that they produce may also act to "reshape" the immune response post alemtuzumab treatment.

In addition, repopulating T cells display rapid cell cycling (i.e., increased proliferation and apoptosis) that may in part be driven by IL-21. A sustained increase in T-cell apoptosis may explain the delay in recovery of T-cell populations following alemtuzumab despite preservation of hematologic precursors and a half-life of circulating alemtuzumab of only 6 days. Rapid cycling of T cells may also increase the likelihood of secondary autoimmunity.

The most compelling data suggesting that alemtuzumab's effects extend beyond inhibition of inflammation involve the long-term control of disease despite lymphocyte repopulation. Although the reduction of progression of disability may relate to anti-inflammatory effects, a sustained decrease in disability — maintained for 4 years after the last alemtuzumab treatment in the majority of patients — suggests that alemtuzumab's mechanism may involve a more extensive immunomodulation that potentially creates a rebalanced immune system, leading to a reduction in MS disease activity. Neuroprotective effects may also play a role, but require additional study.

Conflicts of Interest

Dr. MS Freedman has received research funding from Genzyme and Bayer Healthcare and consultant fees from Actelion, Bayer, Biogen Idec, Genzyme, Glycominds, Merck Serono, Novartis, Opexa, Sanofi-Aventis and Teva Canada Innovation. Dr. Silva Markovic-Plese received research funding from Genzyme Inc., Serono EMD, Biogen Idec, honoraria for consulting from Genzyme Inc and Serono EMD. Dr. Johanne Kaplan is an employee of Genzyme, a Sanofi Company.

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Search Strategy and Selection Criteria

References for this review were identified through searches of PubMed with the search terms "alemtuzumab" and "multiple sclerosis" or "lymphocyte" or "CD52" or "regulatory" from 1998 until May 2012. Articles were also identified from the authors' own libraries and references therein. Only papers published in English were reviewed. The final reference list was generated based on relevance to the broad scope of this review.

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