Immune competence after alemtuzumab treatment of multiple sclerosis

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ABSTRACT

Objective: To determine the immunocompetency of patients with multiple sclerosis treated with the lymphodepleting humanized monoclonal antibody alemtuzumab.

Methods: In this pilot case-control study, we assessed immunocompetence in 24 patients after alemtuzumab treatment by measuring antibody responses to 3 vaccines (diphtheria, tetanus, and poliomyelitis vaccine, *Haemophilus influenzae* type b and meningococcal group C conjugate vaccine, and pneumococcal polysaccharide vaccine). In 20 patients, antibodies to common viruses (mumps, rubella, varicella-zoster, and Epstein-Barr virus) were measured before alemtuzumab treatment, then at 1 and 9–11 months after treatment. Results were compared with well-defined historical controls.

Results: Serum antibodies against common viruses remained detectable after treatment, and vaccine responses were normal to T-cell–dependent recall antigens (tetanus, diphtheria, and polio), a T-cell–dependent novel antigen (meningococcus C), and T-cell–independent antigens (pneumococcal). There was no evidence for a diminished response to vaccinations in 5 patients studied within 6 months of alemtuzumab treatment.

Conclusion: In this small historically controlled pilot study, we demonstrated i) retained humoral immunologic memory (in the form of antibodies against common viruses and response to recall antigens), and ii) the retained ability to mount a humoral immune response against a novel antigen after treatment with alemtuzumab.

Classification of evidence: This pilot study provides Class III evidence that patients with relapsing-remitting multiple sclerosis appear immunocompetent after treatment with alemtuzumab. *Neurology*® 2013;81:872–876

GLOSSARY

GMTR = geometric mean titer ratio; **Hib** = *Haemophilus influenzae* type b; **IgG** = immunoglobulin G; **Men C** = meningococcal type C; **MS** = multiple sclerosis; **VZV** = varicella-zoster virus.

Alemtuzumab is a potential new treatment for relapsing-remitting multiple sclerosis (MS). In a phase II trial, compared with interferon β-1a, alemtuzumab reduced the risks for relapse and sustained accumulation of disability by more than 70% at 3 years, with sustained efficacy at 5 years.[1] Two phase III trials (CARE-MS I and CARE-MS II) have confirmed its efficacy in treatment-naive patients, and established superiority over interferon β-1a in patients with disease activity despite first-line therapy.[1]

Alemtuzumab is a lymphocyte-depleting, anti-CD52 monoclonal antibody. After depletion, cell numbers recover but at varying rates: B cells recover to the lower limit of normal by 7 months, $CD8^+$ T cells by 20 months, and $CD4^+$ T cells by 35 months.[1] After alemtuzumab treatment, the B-cell compartment is composed of naive cells that have emerged from the bone marrow,[1] whereas T cells are largely memory, dominated for 6 months by those with a regulatory phenotype.[1] Despite this, infections are not a major concern; in CARE-MS II, the incidence of any infection was 77% after alemtuzumab vs 66% with interferon β-1a, and these were predominantly mild-moderate upper respiratory or urinary tract infections. In contrast,

Supplemental data at www.neurology.org

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Table 1 Characteristics of the study population at the time of vaccination

No.	24
Median age, y (range)	38.5 (23–52)
Female/male	15/9
Median disease duration, y (range)	5 (2.5–15)
Median last recorded EDSS score (range)	2.0 (0.0–6.0)
Median no. of cycles of alemtuzumab (range)	2 (1–5)
Median time from last dose of alemtuzumab, mo (range)	18 (1.5–86)
Median $CD4^+$ T-cell count, normal range 0.3–1.4 × 10^9/L (patient range)	0.32 (0.03–0.81)
Median $CD8^+$ T-cell count, normal range 0.2–0.9 × 10^9/L (patient range)	0.16 (0.01–1.03)
Median $CD19^+$ B-cell count, normal range 0.1–0.5 × 10^9/L (patient range)	0.30 (0.06–0.77)

Abbreviation: EDSS = Expanded Disability Status Scale.

Table 2 Diphtheria, tetanus, and poliomyelitis vaccine, Hib and Men C conjugate vaccine, and pneumococcal polysaccharide vaccine responses after alemtuzumab treatment

Diphtheria, tetanus, and poliomyelitis vaccine (n = 22)				
	No. (%) seroprotected prevaccine	No. (%) seroprotected postvaccine	GMTR (±90% CI)	GMTR from literature controls
Diphtheria	22 (100)	22 (100)	2.6 (±1.2)	2.2[a] (2.0–2.5)
Tetanus	22 (100)	22 (100)	Not done[b]	
Polio 1	21 (95)	22 (100)	3.5 (±22)	7.3[a] (5.9–9.0)
Polio 2	21 (95)	21 (95)	5.0 (±7.5)	10.0[a] (8.4–11.9)
Polio 3	17 (77)	21 (95)	16.5 (±15.6)	17.1[a] (13.6–21.4)
Hib and Men C conjugate vaccine (n = 23)				
	No. (%) seroprotected prevaccine	No. (%) seroprotected postvaccine	No. (%) seroconversion 4-fold antibody increase	% Seroconversion from literature controls
Men C	3 (13)	21 (91)	19 (83)	97.6–100[c]
Hib	17 (74)	23 (100)	18/19 (95)[d]	82–90[e]
Pneumococcal polysaccharide vaccine (n = 21)				
	No. (%) seroconversion 2-fold antibody increase	% Seroconversion from literature controls	GMTR (90% CI)	GMTR from literature controls
Serotype 3	11/15 (73)[f]	35–47[g]	2.6 (1.7–4.0)	1.8–2.0[g]
Serotype 8	19/20 (95)[f]	81–85[g]	11.6 (6.0–17.3)	5.86–6.66[g]

Abbreviations: CI = confidence interval; GMTR = geometric mean of individual post-/pre-vaccination titers; Hib = *Haemophilus influenzae* type b; Men C = meningococcal type C.
[a] From Laroche et al.[7]
[b] Tetanus GMTR could not be calculated because the majority of patients had antibody levels above the upper detection limits of the assay.
[c] Control data from references 2 and 4.
[d] Hib seroconversion rate calculated from 19 patients because the remaining patients had antibodies above the upper detection limit of the assay.
[e] Control data from references 3 and 10.
[f] Serotype 3 and 8 seroconversion rates calculated from 15 and 20 patients, respectively, as the remaining patients had antibody titers above the upper detection limit of the assays.
[g] Control data from reference 8.

the dominant safety concern is new autoimmune disease, with 30% of patients developing autoimmune thyroid disease, and 1% immune thrombocytopenia.[1]

The objective of this pilot study was to investigate how alemtuzumab affects immunologic memory, in the form of antibodies against common viruses and responses to recall vaccinations, and the ability to mount a humoral immune response against a novel antigen.

METHODS

Standard protocol approvals, registrations, and patient consents. This study was approved by Royal Free Hospital and Medical School Research Ethics Committee (REC number: 09/H0720/64) and received clinical trial authorization from the Medicines and Healthcare Products Regulatory Agency (EudraCT 2009-011523-31). Twenty-four patients with relapsing-remitting MS, who had received alemtuzumab in any approved trial, gave written informed consent. For trial entry criteria, see appendix e-1 on the *Neurology*® Web site at www.neurology.org.

Serum samples and vaccines. Serum samples taken before, 1, and 9–11 months after alemtuzumab treatment were analyzed for antibodies to measles, mumps, rubella, varicella-zoster virus (VZV), and Epstein-Barr virus using standard assays (see appendix e-1). Participants were offered 3 vaccines:

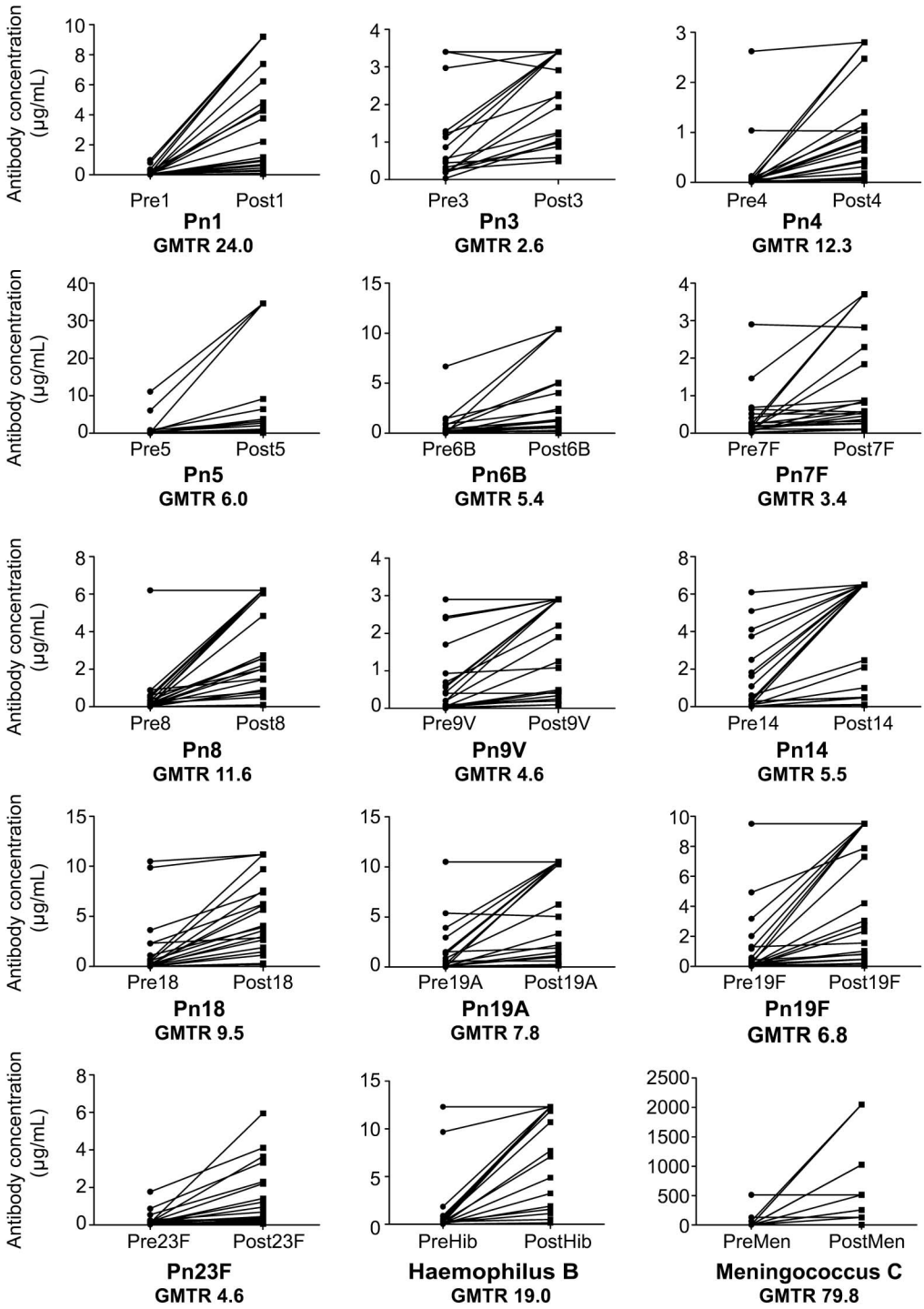
1. Pneumococcal polysaccharide vaccine (Pneumovax II; Sanofi Pasteur MSD, Maidenhead, UK), containing polysaccharides from 23 types of *Streptococcus pneumoniae* bacteria, acting as T-cell–independent recall antigens
2. Diphtheria, tetanus, and poliomyelitis vaccine (Revaxis; Sanofi Pasteur MSD), containing tetanus toxoid, diphtheria toxoid, and inactivated polio viruses 1, 2, and 3, as T-cell–dependent recall antigens
3. *Haemophilus influenzae* type b (Hib) and meningococcal group C (Men C) conjugate vaccine (Menitorix; GlaxoSmithKline, Uxbridge, UK) containing Men C polysaccharides, a T-cell–dependent novel antigen.

Immunoglobulin G (IgG) levels were measured before and 4 weeks after vaccination (see appendix e-1).

Control data and definition of response. Hib and Men C seroconversion and seroprotection rates were compared with control data from other conjugate vaccines.[2–6] Seroconversion was defined as a 4-fold increase in antibody level after vaccination and seroprotection as the minimum antibody level required to protect against disease.[7] Seroprotection and geometric mean titer ratio (GMTR; defined as the geometric mean of individual post-/pre-vaccination titers) control data were available for diphtheria, tetanus, and poliomyelitis vaccine.[7] Response to pneumococcal polysaccharide vaccine was assessed against 2 standards. First, we compared GMTRs and ability to make a 2-fold antibody response to serotypes 3 and 8 against published pneumococcal polysaccharide vaccine control data[8] (good data do not exist for the other serotypes). Second, we compared our patients' response against an expert consensus[9] that a normal response is either a postimmunization titer of 1.3 μg/mL or ≥4-fold increase over baseline titer in ≥70% of serotypes (i.e., ≥9 of the 13 tested).

Patients were defined as "responders" to all 3 vaccines if they developed the following: diphtheria, tetanus, and poliomyelitis seroprotection, Hib and Men C seroprotection and seroconversion, and a normal pneumococcal polysaccharide vaccine response (expert consensus definition). A χ^2 test was performed to determine whether

Figure Serum IgG antibody responses to pneumococcal polysaccharide vaccine and Hib and Men C conjugate vaccine



Immunoglobulin G (IgG) titers for pneumococcal serotypes (Pn), *Haemophilus influenzae* type b (Hib), and meningococcal type C (Men C) before and at 4 weeks postvaccination. The geometric mean titer ratio (GMTR; geometric mean of individual post-/pre-vaccination titers) is shown below. Where prevaccination titers were above the upper detection limit of the assay, the patient was excluded from analysis.

patients within 6 months of treatment were as likely to be "responders" as those vaccinated more than 6 months after alemtuzumab.

RESULTS Twenty-four participants had received alemtuzumab between 1.5 and 86 months previously (table 1). At the time of vaccination, the median $CD8^+$ T-cell count was low at 0.16×10^9/L (normal range 0.2–0.9 $\times$ 10^9/L); median $CD4^+$ T-cell and $CD19^+$ B-cell counts were normal. Patients treated within 6 months of alemtuzumab had low $CD4^+$ and

CD8+ T-cell counts: 0.1 × 10^9/L (normal range 0.3–1.4 × 10^9/L) and 0.03 × 10^9/L, vs 0.4 × 10^9/L and 0.2 × 10^9/L, respectively, in patients treated after 6 months.

Serum was available from 20 patients before treatment, then at 1 month and 9–11 months after alemtuzumab; all samples contained protective IgG levels to measles, rubella, and varicella. Patients had protective IgG levels to Epstein-Barr viral capsid antigen and Epstein-Barr virus nuclear antigen at all time points, except one patient whose prealemtuzumab sample contained Epstein-Barr virus nuclear antigen but not viral capsid antigen IgG. Before alemtuzumab, 17 of 20 (85%) had anti-mumps IgG; after alemtuzumab, 19 of 20 were positive, without disease in the 2 who had seroconverted.

Patients were offered all 3 vaccines. A few elected to have only 1 or 2; 21 received pneumococcal polysaccharide vaccine, 22 diphtheria, tetanus, and poliomyelitis vaccine, and 23 received Hib and Men C conjugate vaccine. The vaccines were well tolerated. Seroprotection to Hib before Hib and Men C conjugate vaccine was high (74%) and increased to 100% postvaccination, giving a seroconversion rate of 95% in assessable patients (table 2 and figure) equivalent to historical controls (100% seroprotection, 82%–90% seroconversion).[3,10] Seroprotection against the novel antigen Men C was low before vaccination, which induced 91% seroprotection and seroconversion in 19 patients (82.6%) (table 2 and figure), similar to rates after other conjugated Men C vaccines.[2,4–6]

All patients given diphtheria, tetanus, and poliomyelitis vaccine had seroprotective levels of antibodies to tetanus and diphtheria before and after vaccination, and nearly all were seroprotected against polio (table 2). High prevaccination antibody titers precluded assessment of seroconversion, but GMTRs indicate that responses to diphtheria, tetanus, and poliomyelitis vaccine antigens after alemtuzumab were equivalent to controls (table 2).

The serum antibody changes to pneumococcal polysaccharide vaccine antigens are illustrated in the figure. Two-fold responses to pneumococcal 3 and 8 serotypes after alemtuzumab were similar to published rates (table 2).[8] Eighteen of the 21 patients mounted a normal pneumococcal polysaccharide vaccine response following the expert consensus definition (see methods).

Five patients received all 3 vaccines within 6 months of alemtuzumab treatment. Of these, 2 were "responders" (see Methods). This was not significantly different from 12 "responders" of 15 patients vaccinated more than 6 months after alemtuzumab treatment, although the number of patients vaccinated within 6 months was small and more patients would be needed to confirm this finding.

DISCUSSION This pilot study demonstrates that despite prolonged alterations in circulating B and T lymphocytes after alemtuzumab treatment of MS, immunologic memory to common viruses (in the form of IgG titers) and responses to vaccinations (T-cell–dependent and –independent recall antigens and a novel antigen) appear normal. These data are consistent with the lack of opportunistic infections after alemtuzumab treatment. It should be noted that although the vaccines studied elicit an antibody response either with or without T-cell help, we have only analyzed the humoral response to vaccination and not T-cell responses. This study is limited by the small number of patients studied and also the wide range of time between alemtuzumab treatment and vaccination.

This study is timely as alemtuzumab may enter clinical practice as a treatment for relapsing-remitting

Comment: Assessing humoral immunocompetence after alemtuzumab treatment in MS

Alemtuzumab, a humanized monoclonal antibody, depletes CD52+ circulating leukocytes. In humans, this includes most mononuclear leukocytes. Exciting results from 2 phase III trials established the superiority of alemtuzumab in reducing multiple sclerosis (MS) relapse rate compared with high-dose interferon-β.[1] Increased mild to moderate infections occurred in both trials in the alemtuzumab compared to interferon-β arms. Also noteworthy, secondary autoimmune diseases develop in a sizeable proportion of alemtuzumab-treated patients.

The infrequent annual dosing cycles of alemtuzumab underscore its very long biological half-life. In prior studies, repletion of CD52+ cells varied by cell type. Of 36 patients with MS who received alemtuzumab between 1991 and 1997, median time to recover to lower limit of normal was 35 months for CD4+ T cells, 20 months for CD8+ T cells, and 8 months for B cells. Despite the long follow-up time, CD8 and CD4 counts returned to baseline in fewer than 50%.[2] Though few serious infections have been observed in clinical trials, the low circulating T cells and long duration of effects have raised concerns regarding long-term safety.

McCarthy et al.[3] examined preservation of humoral immunity to common viruses and responses to vaccinations in 24 patients treated with alemtuzumab. Humoral responses to 3 common vaccines were normal compared to literature controls in patients vaccinated several months after alemtuzumab. However, vaccination within 6 months of treatment resulted in a smaller proportion of responders. As pointed out by the authors, T-cell responses to vaccination were not analyzed. In 20 of 24 participants, antibody responses to common viruses were measured before and after alemtuzumab, with persistence of serum antibodies in the 9-11 months after treatment. The present results, providing guarded reassurance of the safety of alemtuzumab, are timely as the US Food and Drug Administration and European Medicines Agency are currently evaluating it for possible approval.

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MS in the near future: phase III studies have shown alemtuzumab to be the most effective treatment to date and the drug has been submitted to the US Food and Drug Administration and European Medicines Agency for approval.

While the data from this pilot study suggest that patients with MS are immunocompetent after treatment with alemtuzumab, we offer 3 notes of caution. First, we have not tested immune responses against live vaccines and advise against their use on precautionary grounds. Second, infections may occur in the presence of "seroprotective" antibodies; one patient after alemtuzumab treatment developed VZV meningitis, despite protective IgG levels. The patient had been treated 5 months before VZV exposure and was profoundly T-cell lymphopenic. Third, the only patient vaccinated within 2 months of alemtuzumab treatment had a poor response to several vaccines, suggesting that immunization very early after alemtuzumab may not be effective.

AUTHOR CONTRIBUTIONS

Dr. Claire L. McCarthy designed and conducted the study, analyzed data in the study, interpreted data in the study, and drafted and revised the manuscript. Dr. Orla Tuohy conducted the study and revised the manuscript. Dr. D. Alastair S. Compston revised the manuscript. Dr. Dinakantha S. Kumararatne analyzed data in the study, interpreted data in the study, and revised the manuscript. Dr. Alasdair J. Coles designed the study, analyzed data in the study, interpreted data in the study, and revised the manuscript. Dr. Joanne L. Jones analyzed data in the study, interpreted data in the study, and revised the manuscript.

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DISCLOSURE

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