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A Novel Strategy To Reduce the Immunogenicity of Biological Therapies

Jennifer Somerfield,* Grant A. Hill-Cawthorne,[†] Andrew Lin,[†] Michael S. Zandi,[†] Claire McCarthy,[†] Joanne L. Jones,[†] Michael Willcox,[‡] David Shaw,[‡] Sara A. J. Thompson,[†] Alastair S. Compston,[†] Geoff Hale,[‡] Herman Waldmann,^{§,1} and Alasdair J. Coles^{†,1}

Biological therapies, even humanized mAbs, may induce antiglobulin responses that impair efficacy. We tested a novel strategy to induce tolerance to a therapeutic mAb. Twenty patients with relapsing–remitting multiple sclerosis received an initial cycle of alemtuzumab (Campath-1H), up to 120 mg over 5 d, preceded by 500 mg SM3. This Ab differs from alemtuzumab by a single point mutation and is designed not to bind to cells. Twelve months later, they received a second cycle of alemtuzumab, up to 72 mg over 3 d. One month after that, 4 of 19 (21%) patients had detectable serum anti-alemtuzumab Abs compared with 145 of 197 (74%) patients who received two cycles of alemtuzumab without SM3 in the phase 2 CAMMS223 trial ($p < 0.001$). The efficacy and safety profile of alemtuzumab was unaffected by SM3 pretreatment. Long-lasting “high-zone” tolerance to a biological therapy may be induced by pretreatment with a high i.v. dose of a drug variant, altered to reduce target-binding. *The Journal of Immunology*, 2010, 185: 763–768.

Biological therapies induce Ab responses that may neutralize efficacy and reduce long-term usefulness, for instance, IFN- α treatment of hepatitis C (1), animal insulin for diabetes mellitus (2), human growth hormone for short stature (3), factor VIII in hemophilia (4), and IFN- β treatment of multiple sclerosis (5, 6).

The first therapeutic rat mAbs briskly induced antiglobulin responses in humans (7). Attempts to circumvent this problem included replacing much of the murine Ab with a human Ig framework, creating first “chimeric” and then “humanized” Abs. Alemtuzumab (Campath-1H) was the first therapeutic humanized Ab, in which only the complementarity-determining regions were retained from the original murine Ab, Campath-1G (8); binding to CD52, it targets human B and T lymphocytes, eosinophils, and monocytes (9) and induces long-lasting lymphopenia (10, 11). However, these unique idiotypic residues may still induce an antiglobulin response, particularly if, like alemtuzumab, they target Abs on the surface of hematopoietic cells (12). In a single-dose escalation study of alemtuzumab treatment of rheumatoid arthritis, 63% of patients developed antiglobulin responses with an observed reduction in

efficacy (13). In a phase 2 study in patients with early relapsing–remitting multiple sclerosis, alemtuzumab was given as annual cycles. Most patients’ antiglobulin responses had resolved prior to the second and third exposures. We have already reported that only 1 of 208 (0.5%) and 51 of 194 patients (26.3%) had significant alemtuzumab-binding Abs (above a prespecified threshold of 2000 U/ml) at months 12 and 24 (14). There was no indication, from these limited data, that the presence of alemtuzumab-binding Abs influenced efficacy, infusion-associated reactions, lymphocyte depletion, or repopulation. However, we anticipate that antiglobulin responses might be more prolonged after repeated subsequent alemtuzumab infusions and might therefore impact on the Abs efficacy.

Various strategies have been proposed that aim to reduce the immunogenicity of biological therapies. We sought to investigate whether long-lasting tolerance to a mAb could be induced by emulating classical experimental studies showing that high doses of soluble protein may induce “high-zone” tolerance (15) and that soluble monomeric IgG can tolerize mice to aggregated Ig (16). However, at the doses required to test this effect, most cytotoxic Abs would induce unacceptable adverse effects. For instance, even low doses of alemtuzumab may cause an infusion reaction consisting of pyrexia, tachycardia, and bronchospasm due to the release of cytokines including IFN- γ and IL-6 (17). Furthermore the “danger signal” of cytotoxicity is likely to undermine induction of tolerance. Therefore, a noncell-binding variant of alemtuzumab was constructed that could be given at a high dose. Point mutations were made in the H2 loop of the H chain, which has been shown to be critical for Ag binding (18, 19). Variant SM3, with a charge reversal (Lys⁵³ to Asp⁵³), was found in experimental animals to abrogate binding activity so that it could be given in high doses. This was nonimmunogenic and induced long-lasting tolerance to subsequent cycles of alemtuzumab in mice expressing a human CD52 transgene (19).

For the first time in humans, we tested the strategy of tolerizing to a biological therapy by pretreatment with a high dose of a noncell-binding variant. Our principal outcome measure was the incidence of anti-alemtuzumab Abs after a second cycle of alemtuzumab. We also tested whether this strategy interfered with the efficacy of

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Abbreviations used in this paper: ITP, idiopathic thrombocytopenic purpura; MRI, magnetic resonance imaging.

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alemtuzumab on clinical and magnetic resonance imaging (MRI) outcomes, comparing with results from the previously reported CAMMS223 trial.

Materials and Methods

Patients

Twenty patients were recruited for this pilot study from August 2005 to July 2007. We included male or nonpregnant and nonlactating female patients with relapsing–remitting multiple sclerosis, Expanded Disability Status Scale (EDSS) score 0–6.0 (inclusive) at screening visit, at least three clinical episodes in the 2 y prior to study entry, and MRI brain scan compatible with the diagnosis of multiple sclerosis, who met McDonald criteria for the diagnosis of multiple sclerosis (20). Key exclusion criteria were progressive multiple sclerosis, prior alemtuzumab treatment, prior immunotherapy (except for pulsed corticosteroids and IFN- β), and the presence of a malignancy or major systemic and autoimmune disease. Patients taking IFN- β stopped that treatment at least 1 mo before study entry. All of the patients provided written informed consent (EUDRACT 2005-002305-23, CTA 12854/0008/001, and REC 05/Q0501/64). Control data for the primary outcome measure came from the 216 patients who received two doses of alemtuzumab in the CAMMS223 trial (108 each receiving the 12 or 24 mg/d doses), of which samples were available from 212 at month 1 and from 197 at month 13 (14).

Procedures

On day 1, 50 mg SM3 was administered by i.v. infusion, and patients were observed for the next 24 h for adverse effects. The following day, 450 mg was given over 4 h. The first dose of alemtuzumab was administered on day 8. Alemtuzumab was administered by daily i.v. infusion. For the first two patients, 120 mg was given over five consecutive days (24 mg/d on days 8–12 of the study), and at month 12, these patients were treated with 72 mg alemtuzumab alone over three consecutive days (24 mg/d). After a fatal case of idiopathic thrombocytopenic purpura (ITP) occurred in the phase 2 alemtuzumab trial (CAMMS223) in June 2005, dosing in this trial was temporarily suspended and then restarted (for the remaining 18 patients) at a reduced dose of 60 mg over five consecutive days (12 mg/d on days 8–12 of the study) and 36 mg over three consecutive days (12 mg/d) at month 12 (14). Data from the CAMMS223 trial suggest that these doses are equally immunogenic. All of the patients received 1 g methylprednisolone i.v. immediately before the first three doses of alemtuzumab to ameliorate the cytokine release syndrome (17). Additional medications to manage infusion-associated reactions, such as antihistamines and antipyretic agents, were used as required.

Clinical assessments

Patients were monitored in hospital during treatment with SM3 and alemtuzumab. Follow-up was at 1 mo after discharge then every 3 mo for a minimum of 24 mo with monitoring of thyroid function, blood count, and lymphocyte phenotypes. After a risk management plan for ITP was adopted within the CAMMS223 trial (14), similar procedures were put in place in this trial, including monitoring monthly platelet counts for 3 y after each alemtuzumab infusion. An unblinded treating physician was responsible for patient care, the management of adverse events, and reviewing of laboratory data. Disability, as determined by the EDSS (21), was assessed by a blinded neurologist. A relapse was defined as new neurologic symptoms attributed to multiple sclerosis, lasting >48 h with an objective change in the neurologic examination. In a subset of patients, monthly gadolinium-enhanced T1 and T2 MRI scans were performed.

Other assessments

Serum was assayed for alemtuzumab-binding Abs using a validated bridging ELISA performed by BioAnaLab, which has a lower threshold for detection of 444 ng/ml of a monoclonal anti-alemtuzumab reference standard (22). Additional assays for alemtuzumab-binding Ab isotypes were developed in-house. Anti-alemtuzumab IgG titers were assessed by ELISA. Microtiter high binding ELISA plates (Greiner Bio One, Paris, France) were coated with 10 μ g/ml rat Ab, Campath-1G, in PBS plus 0.05% sodium azide overnight at 4°C. Plates were blocked with 2% albumin BSA (A1470; Sigma-Aldrich, St. Louis, MO) in PBS for 2 h before being washed four times in PBS plus 0.05% Tween 20 (P1379; Sigma-Aldrich). Test serum samples were added to the plates at 50 μ l per well in a dilution of 1:2 in PBS. Samples were incubated for 1 h at 20°C. The plates were washed as above between each step and followed sequentially by 2.5 μ g/ml biotinylated mouse anti-human IgG clone 8a4 (Immunotech, Luminy, France) and

1:2000 extravidin–peroxidase (E2886; Sigma-Aldrich) in PBS, each incubated for 1 h at 20°C. Bound enzyme was detected by the addition of *o*-phenylenediamine (P8287; Sigma-Aldrich), and absorbance was read at 450 nm on a kinetic protocol with readings every 60 s for 20 min using a Bio-Rad 680 Microplate Reader (Hercules, CA). Readings were displayed using Bio-Rad Microplate Manager 5.2 software as maximum velocity of absorbance (mOD/min), because no standard exists for anti-alemtuzumab IgG.

A similar protocol was used for the anti-alemtuzumab IgM assay with the following differences. Microtiter plates were coated with 8 μ g/ml anti-IgM Ab (ab36088; AbCam, Cambridge, MA) in 15 mM Na₂CO₃ and 35 mM NaHCO₃ overnight at 4°C. Blocking buffer was 0.5% casein (C4765; Sigma-Aldrich) in PBS with a 1 h incubation. Detecting Ab was 2 μ g/ml biotinylated Campath-1H in PBS followed by 1:2000 extravidin–peroxidase in PBS. Bound enzyme was detected using tetramethylbenzidine substrate (T0440; Sigma-Aldrich), the reaction being stopped with 2 M H₂SO₄ after 20 min. Absorbance was read at 450 nm on an end point protocol. No standard could be used; therefore, samples and controls were run on the same plate, allowing comparative ODs to be recorded. For both assays, a negative control (serum from a healthy control) and a positive control (serum from N.C., a patient who had received multiple alemtuzumab infusions for a non-multiple-sclerosis indication) were used.

Concentrations of SM3 in patient sera were measured by sandwich immunoassay using a Gyrolab (Upsalla, Sweden) xP instrument. Biotin-labeled rat anti-Campath idotype mAb (YID13.9) was captured to a Gyrolab Bioaffy 200 disc at a concentration of 74 μ g/ml in Gyrolab Rxxip A assay diluent. Test samples and SM3 calibrators over the concentration range of 39–50,000 ng/ml prepared in normal serum were diluted to 10% with Gyrolab Rxxip H assay diluent and applied to the affinity matrix. Binding of SM3 was detected with an Alexa Fluor 647-labeled goat anti-human IgG1 Ab at a concentration of 3 nM. Fluorescence output was measured at a photomultiplier setting of 1%, and the concentration of SM3 was determined by interpolation on the standard curve.

ELISAs were performed to quantify levels of IFN- γ , TNF- α , and IL6 (DuoSet DY285, DY210, and DY206, respectively; R&D Systems, Minneapolis, MN) on serum samples from patients at baseline, 2, 4, and 6 h after SM3 infusion on days 1 and 2 and at baseline on day 3 according to the manufacturer's instructions.

Statistical analysis

The primary outcome measure was the proportion of patients with detectable binding anti-alemtuzumab Abs in serum at 1 mo after the second alemtuzumab cycle, compared using a two-tailed Fisher's exact test to the same time point in patients receiving alemtuzumab alone in the CAMMS223 trial. Differences in the mean concentration of serum anti-alemtuzumab Abs at both time points were assessed by an unpaired two-tailed Student *t* test.

Results

Twenty patients (Table I) with relapsing–remitting multiple sclerosis were recruited to the study, of whom 11 were treatment naive, 8 had received IFN- β , and 1 had previously had plasma exchange. All met criteria for disease activity irrespective of treatment status. Twenty patients received SM3 on days 1–2 followed by alemtuzumab, consisting of daily infusions on days 8–12. One patient withdrew from the trial due to perceived lack of efficacy; the remaining 19 underwent a second cycle of alemtuzumab at month 12 (3 d of daily infusions).

Pharmacokinetics of SM3

The pharmacokinetic profile of SM3 was estimated from measurements of SM3 concentrations between day 6 and day 363 in 104

Table I. Baseline characteristics of patients

Demographics	<i>n</i>	20
Age at disease onset (y)	Mean ($\pm$ SD) Range	32 (7.04) 16–44
	Percentage female (no.)	65 (13)
Baseline disability	Mean EDSS ($\pm$ SD) Range	3.1 (1.57) 0–6
Time since first episode (mo)	Mean ($\pm$ SD) Range	43 (32) 14–133
Prior drug therapy	No (%)	9 (45)

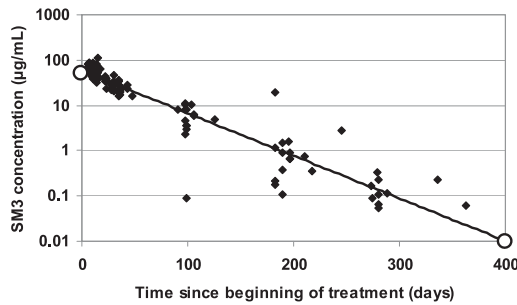


FIGURE 1. Pharmacokinetics of SM3. Concentrations of SM3 were measured by sandwich immunoassay in a Gyrolab instrument. Samples were analyzed at irregular times between 6 and 363 d from 18 patients. The log-transformed concentrations were fitted to a straight line by linear regression.

samples from 18 of the 20 patients (Fig. 1). The assay relied on the capture of SM3 to an anti-idiotypic Ab raised against alemtuzumab and therefore was able to detect both SM3 and alemtuzumab equally well. For the purpose of this preliminary pharmacokinetic analysis, the contribution of alemtuzumab to the measured concentrations was ignored because the dose was so relatively small

compared with SM3. The measured concentrations fitted a single exponential curve with a half-life of 32.1 d and an estimated concentration at time 0 of 58.0 µg/ml, corresponding to a volume of distribution of ~8.6 L. The estimated mean concentration of SM3 at 1 mo was 30.3 µg/ml and at 13 mo was 0.01 µg/ml.

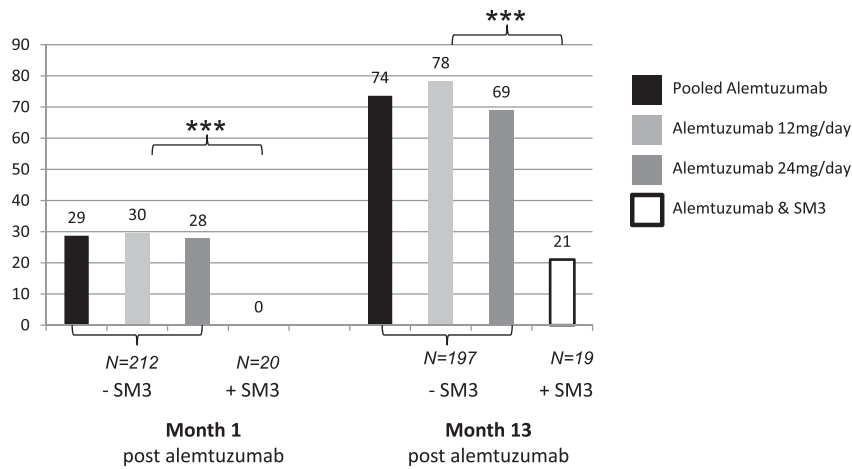
Efficacy of SM3 as tolerogen for alemtuzumab

The primary outcome was met. The use of SM3 before the first dose of alemtuzumab reduced the proportion of patients with detectable (>444 U/ml) serum anti-alemtuzumab Abs after two cycles of alemtuzumab by 72% when compared with data from the CAMMS223 trial, using the same bridging ELISA run in a quasi-quantitative format, using a reference monoclonal anti-alemtuzumab Ab as a calibrator to allow relative estimates of Ab concentrations (Fig. 2A, 74 versus 21%, $p < 0.0001$). There was no statistically significant difference in the proportion of patients developing anti-alemtuzumab Abs in response to the two alemtuzumab doses (Fig. 2A).

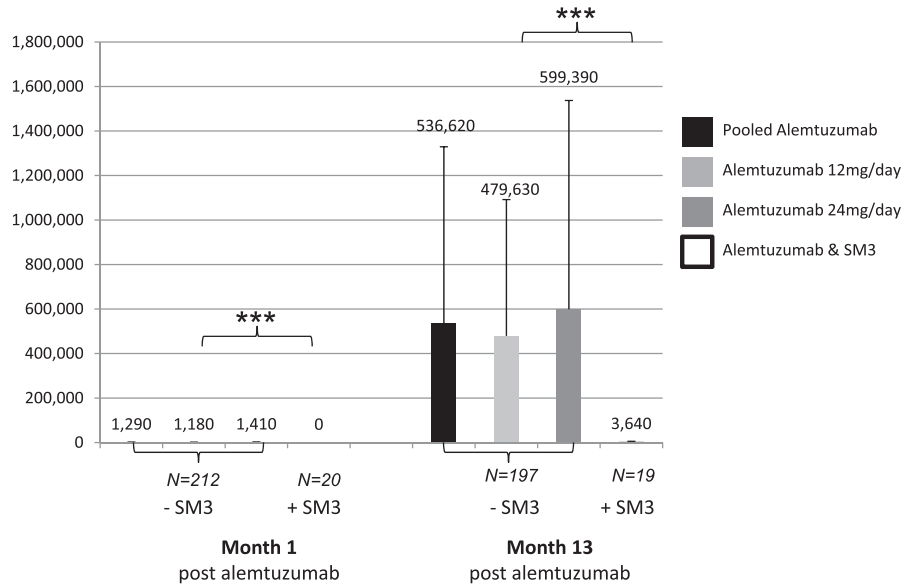
Secondary outcome measures were also met. The mean concentration of detectable anti-alemtuzumab Abs at month 13 was >100-fold lower in the SM3 group (mean 3640 U/ml) compared with the CAMMS223 group (536,600 U/ml) (Fig. 2B, $p < 0.0001$). As expected, there was a significant increase in both percentage of

FIGURE 2. Efficacy outcome measures. *A*, Percentage of patients receiving SM3 (+SM3) that had detectable (>444 U/ml) anti-alemtuzumab Abs at month 1 and month 13 (1 mo after first and second alemtuzumab treatments, respectively) compared with patients in the CAMMS223 trial (–SM3) who were on either 12 or 24 mg/d doses of alemtuzumab (with pooled data charted as well). *B*, Mean anti-alemtuzumab Ab concentrations at month 1 and month 13 of SM3 patients (+SM3) and those from the CAMMS223 trial (–SM3). Error bars indicate SDs. In both outcome measures, there was a significant difference between the CAMMS223 and SM3 groups at both time points (*** $p < 0.0001$). There was also a significant difference between the mean Ab concentrations at month 1 and month 13, within both the CAMMS223 and the SM3 groups ($p < 0.0001$, not asterisked).

A Percentage of patients with detectable anti-alemtuzumab antibodies



B Mean concentration of anti-alemtuzumab antibodies (U/ml)



patients with detectable anti-alemtuzumab Abs and Ab concentration between the first and second cycles of alemtuzumab within both groups (all $p < 0.0001$). There was no statistically significant difference in the concentration of anti-alemtuzumab Abs between the two alemtuzumab doses (Fig. 2B).

No anti-alemtuzumab Abs could be detected in the SM3 group at 1 mo after the first cycle of alemtuzumab; a significant reduction compared with the CAMMS223 group ($p = 0.0024$). However, considering the high concentration of SM3 still present at this time (30.3 $\mu\text{g/ml}$), it is likely that anti-alemtuzumab Abs could not have been detected because they would have been bound to the SM3 and most likely cleared from circulation.

We devised isotype-specific anti-alemtuzumab ELISAs to determine whether the antiglobulin responses seen in four SM3 patients were primary or secondary. No anti-alemtuzumab IgM was detected at month 1 or 13 (Fig. 3A). However, low levels of anti-alemtuzumab IgG were detected at month 1, which increased significantly by month 13 (Fig. 3B).

Out of the four patients who developed Abs to alemtuzumab at month 13, one had received IFN- β 1a before taking part in this trial; one had had plasma exchange; and two were immunotherapy-naïve.

There was no indication that SM3 pretreatment interfered with the efficacy of alemtuzumab either to deplete its target cells or to reduce disease activity in multiple sclerosis. Depletion and reconstitution of

lymphocytes after the first and second cycle of alemtuzumab were equivalent to those seen in patients who did not receive SM3 pretreatment; for instance, at month 12, the mean CD4, CD8, and CD19 counts were 25.6, 36.8, and 128.7% of baseline. The on-study mean annualized relapse rate was 0.08 ($\pm$ SD), and the mean change in disability over 24 mo was an improvement by 1–2 EDSS points; both results are comparable to previous trials of alemtuzumab in multiple sclerosis (10, 14). The mean numbers of new gadolinium-enhancing lesions formed on monthly MRI scans performed for 6 mo after the first and second alemtuzumab cycles were 0.38 and 0.5, respectively, which is also comparable to that seen with previous use of alemtuzumab (23, 24).

Safety and tolerability

Most (80%) patients experienced grade 1 infusion-associated reactions (Table II) associated with SM3 administration. These were milder than those seen when 12 mg alemtuzumab was administered, with methylprednisolone premedication, in the phase 2 trial of alemtuzumab (14). A total of 500 mg SM3 did reduce the total lymphocyte count to 36% of baseline (range 19–53%) by day 8 (prior to alemtuzumab), and this was accompanied by a rise in C-reactive protein up to 30 mg/ml by day 3. Eleven of 17 (65%) patients had detectable serum IL-6, maximal 6 h after starting the day 2 SM3 infusion. In 4 or 17 (24%) patients, there was

A Mean IgM anti-alemtuzumab titres (+ SD)

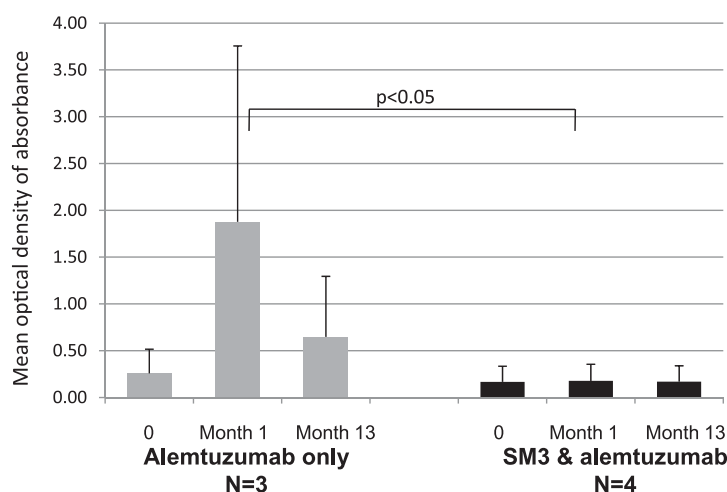


FIGURE 3. Isotype of anti-alemtuzumab Abs. ELISA measurement of (A) IgM and (B) IgG anti-alemtuzumab Abs in three representative patients treated with alemtuzumab alone (from the CAMMS223 trial) and the four patients from the SM3 trial who developed anti-alemtuzumab Ab responses at month 13. Units are based on the OD of the ELISAs.

B Mean IgG anti-alemtuzumab titres (+SD)

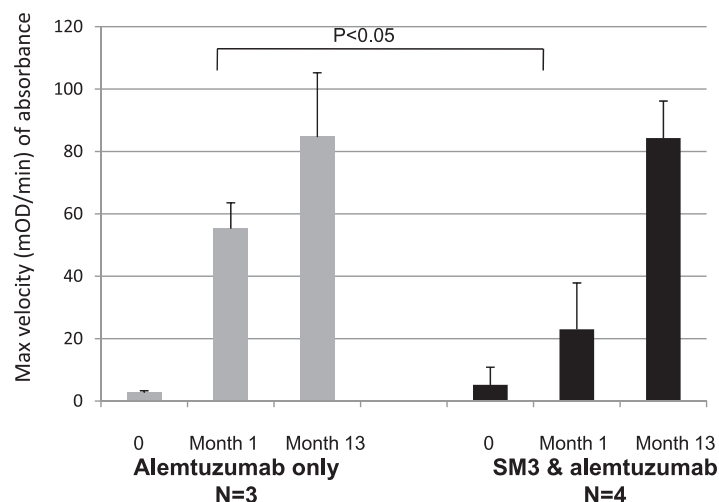


Table II. Adverse events in the SM3 trial versus the CAMMS223 trial

	SM3 n (%)	CAMMS223 (%)
Adverse events		
Serious adverse events	2 (10)	23.1
Infusion-associated reactions		
Any event	16 (80)	98.6
Serious adverse event	0	1.4
Events		
Pyrexia	15 (75)	37.5
Chills	9 (45)	17.1
Headache	8 (40)	59.3
Fatigue	2 (10)	27.8
Musculoskeletal discomfort	1 (5)	9.7
Dyspnoea	1 (0.5)	13
Infectious adverse events		
Any event	19 (95)	65.7
Serious adverse event	0 (0)	4.2
Infection-associated event		
Upper respiratory tract infection	10 (50)	47.7
Urinary tract infection	1 (5)	11.6
Varicella	4 (20)	5.6
Oral candidiasis	1 (5)	<5
Gastroenteritis	2 (10)	<5
Sinusitis	1 (5)	<5
Fungal skin infection	1 (5)	<5
Gum infection	1 (5)	<5
Nonspecific viral illness with	1 (5)	<5
Vomiting		
Otitis externa	1 (5)	<5
Tooth abscess	1 (5)	<5
Autoimmune-associated events		
Hemolytic anemia	1 (5)	0
Thyroid-associated events	2 (10)	23
Other events		
Fatigue	2 (10)	30.6
Seizure	1 (5)	<10
Pyrexia	1 (5)	11.1
Flare of asthma	1 (5)	<10
Headache	1 (5)	26.9
Skin rash	1 (5)	24.5
Carpel tunnel	1 (5)	<10
Breast fibroadenoma	1 (5)	<10
Castleman's disease	1 (5)	0

detectable serum TNF- α , but serum IFN- γ was undetectable throughout. Overall, the cytokine release was substantially less than that seen in studies of alemtuzumab administered alone (17).

There were two serious adverse events. One patient developed autoimmune hemolytic anemia at 19 mo. She was symptomatic for a few days but responded rapidly to oral corticosteroids, requiring treatment for 4 mo but now well off medication for 6 mo to date. A second patient developed Castleman's disease 31 mo after the first cycle of alemtuzumab. She presented with fever, abdominal pain, and lymphadenopathy, a lymph node biopsy showing polyclonal expansion of the TCR and B cell Ig. Tests were negative for HIV and human herpes virus 8. She received treatment with chemotherapy with cyclophosphamide, hydroxydaunorubicin (doxorubicin), Oncovin (vincristine), prednisone/prednisolone, and rituximab and has been in remission for over 12 mo to date.

Discussion

We show for the first time in humans and in agreement with animal data (19) that immunogenic cell-binding Abs can be rendered tolerogenic by changing residues essential for Ag binding. Specifically, SM3 is identical to alemtuzumab in a monomeric form except for a single mutation, which significantly reduces its binding to the target Ag, CD52. We calculated that the $t_{1/2}$ of SM3 is 32.1 d. This is a little longer than that derived from the classical study of IgG1 in humans (mean 21 d from six individuals, range 15–30 d) (25) and

significantly longer than that of alemtuzumab, which shows an initial rapid clearance that depends on the lymphocyte load and a terminal $t_{1/2}$ between 7 and 21 d (26). This may suggest that alemtuzumab is cleared in part through binding to CD52. Infusion of high-dose SM3 reduced the percentage of patients with a detectable antiglobulin response to a second cycle of alemtuzumab administered 12 mo later from 74–21% ($p < 0.0001$); furthermore, the concentration of anti-alemtuzumab Abs at this time point was reduced >300-fold ($p < 0.0001$). We do not yet know how long this tolerance, partial for some patients and complete for most, will persist; responses to any future alemtuzumab administration will clarify this. Nonetheless, this proof of concept suggests one strategy for the prevention of antiglobulins against biological therapies intended to be given long term. In this small cohort, SM3 has not interfered with the efficacy of alemtuzumab as a treatment for treating multiple sclerosis over 2 y.

The strategy for reducing the immunogenicity of alemtuzumab in this study depends on two immune mechanisms: high-zone tolerance and the reduction of danger signals. High-zone tolerance was first demonstrated by s.c. injection of very high doses of BSA; subsequent Ag re-exposure with adjuvant did not result in an immune response (15). In the "Bonn protocol," high doses of factor VIII treatment induce tolerance in 87% of patients with hemophilia A who have already developed Abs to factor VIII (27), perhaps by inducing CD4⁺CD25⁺ regulatory T cells in peripheral lymphoid organs (28). Other postulated mechanisms for high-zone tolerance are inhibition of B cell Ag presentation (29) or cathepsin-induced apoptosis of T cells (30). Inflammatory mediators or "danger signal" may provoke immunogenicity (28), so, for instance, concomitant infection reduces the efficacy of the Bonn protocol (31). Drugs, such as alemtuzumab, that lyse hematopoietic cells, appear to create adjuvant activity for themselves and are consequently particularly immunogenic (12). Although SM3 was designed to be "nonbinding," it did partially deplete lymphocytes and induce some cytokine release, so there was limited danger signal at the time that alemtuzumab was subsequently given. In retrospect, this limited cell binding was evident in the original animal experiments on SM3 (19). This may explain why anti-alemtuzumab Abs were generated in four of the SM3 patients in response to the second cycle of alemtuzumab. Isotype studies suggested that this was a secondary IgG response, implying that the patients had initiated a primary response against the first cycle but below the sensitivity of our assays. Perhaps a completely nonbinding variant of alemtuzumab would be yet more successful in inducing tolerance.

Overall, the number of adverse events appeared consistent with prior use of alemtuzumab alone. We observed two serious adverse events in this study, neither of which has been previously reported after alemtuzumab. However, the autoimmune hemolytic anemia seen here is congruent with a range of Ab-mediated autoimmune diseases seen after alemtuzumab, especially thyroid disease and rarely ITP (14). Castleman's disease is a rare lymphoproliferative disorder normally caused by human herpes virus 8.

This study has shown that high doses of a monomeric, non-binding mAb minimize immunogenicity to subsequent exposure of the therapeutic analogue. Larger studies are necessary to confirm the efficacy and safety of this approach, which has the potential to be applied to many biological therapies where immunogenicity impacts on long-term efficacy.

Acknowledgments

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Disclosures

A.J.C. has received consulting fees, lecture fees, and grant support from Genzyme. A.S.C. has received consulting fees, lecture fees, and grant support from Genzyme and lecture fees from Bayer Schering Pharma on behalf of himself and the University of Cambridge. J.L.J. has received consulting fees and lecture fees from Bayer Schering Pharma. H.W. is an inventor of alemtuzumab Abs and is entitled to royalties on sales of the product. All of the royalties received are donated to scientific research funds. G.H. also receives royalties on sales of alemtuzumab and donates them to charity.

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