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Nonclinical Safety, Pharmacokinetics, and Pharmacodynamics of Atacicept

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Atacicept, a soluble recombinant fusion protein of the human immunoglobulin (Ig) G_1 Fc and the extracellular domain of the human transmembrane activator and calcium modulator and cyclophylin ligand interactor receptor, acts as an antagonist of both B lymphocyte stimulator and a proliferating–inducing ligand. Here we determined the nonclinical safety, pharmacokinetics and pharmacodynamics of atacicept in mice and cynomolgus monkeys. Subcutaneous atacicept treatment (twice weekly in cynomolgus monkeys, three times weekly in mice) was generally safe and well tolerated safe and well tolerated with dosing up to 10 mg/kg every other day for up to 39 weeks or up to 80 mg/kg when dosed for 4 weeks. At a dose of 1 mg/kg subcutaneous (sc) bioavailability of atacicept in mice and monkeys was 76 and 92%, with a mean serum $t_{1/2}$ of 44 and 179 h, respectively. In accord with its anticipated mechanism of action, repeated administration of atacicept decreased serum IgG concentrations up to 50%, IgM concentrations >99%, and circulating mature B-cell concentrations up to 60%. These effects were dose-related but reversible, as determined in a 25-week follow-up period. Microscopically, B cells numbers were reduced in the follicular marginal zone of the spleen and the mantle surrounding germinal centers of the lymph nodes. These data confirm the preclinical safety and the pharmacological activity of atacicept and support its clinical development.

Key Words: **autoimmune; toxicity; chronic; immunotoxicity; pharmaceuticals.**

B lymphocyte stimulator (BLyS, CD257) and a proliferating–inducing ligand (APRIL, CD256) are members of the tumor necrosis factor ligand superfamily that demonstrate specific activity toward B cells, and support B-cell proliferation, differentiation, and survival (Dillon *et al*., 2006; Mackay *et al*., 2007; Moore *et al*., 1999). BLyS transgenic mice harbor increased $B220^+$ B cells and plasma cells in the spleen and lymph nodes, and develop antidouble-stranded DNA antibodies, proteinuria, and glomerulonephritis consistent with a systemic lupus erythematosus (SLE)–like autoimmunity as they age (Gross *et al*., 2000; Khare *et al*., 2000; Mackay *et al*., 1999). APRIL transgenic mice have a slightly increased number of B cells in peripheral lymph nodes and increased serum immunoglobulin M (IgM) levels (Stein *et al*., 2002). In contrast, BLyS knockout mice have a markedly reduced mature B-cell population and decreased serum Ig levels (Gross *et al*., 2001), whereas APRIL knockout mice have normal B-cell development (Varfolomeev *et al*., 2004) and an impaired IgA class switching (Castigli *et al*., 2004). Elevated BLyS and APRIL levels have been measured in sera from patients with SLE (Koyama *et al*., 2005; Stohl, 2004) and multiple myeloma (Moreaux *et al*., 2004; Novak *et al*., 2004), and in sera and synovial fluid of patients with rheumatoid arthritis (Cheema *et al*., 2001; Nagatani *et al*., 2007; Tan *et al*., 2003). The biologic effects of BLyS and APRIL suggest that specific inhibitors could have therapeutic use in the treatment of autoimmunity and B-cell malignancies, and current clinical trials are evaluating effects in rheumatoid arthritis, SLE, multiple sclerosis, multiple myeloma, and non-Hodgkin's lymphoma.

BLyS and APRIL are produced by a variety of cell types, including cells of the myeloid lineage such as dendritic cells and macrophages, and their expression is upregulated by interferon-γ and other cytokines (Litinskiy *et al*., 2002; Nardelli *et al*., 2001). These ligands signal through two common receptors: B-cell maturation antigen and transmembrane activator and CAML interactor (TACI) (Mackay *et al*., 2003). In addition, BLyS also binds a third receptor, B-cell activation factor of the TNF family (BAFF) receptor (BAFF-R, also known as BLyS receptor 3, BR3). Binding of BLyS to the majority of B cells occurs predominantly through TACI and BAFF-R (Novak *et al*., 2004; Stohl, 2004). BLyS signal transduction occurs through nuclear factor-kappa B and JNK, and results in increased Bcl-2 and $BclX_L$ protein expression, thus attenuating apoptosis and increasing cell survival (Amanna *et al*., 2003; Baker, 2004; Mackay *et al*., 2003).

Atacicept has been engineered as a recombinant fusion protein containing the extracellular, ligand-binding portion of the human TACI receptor (with specificity for both APRIL and

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TABLE 1
Nonclinical Safety Studies of Atacicept

Study type	Species	Route	Regimen	Dose (mg/kg)	Sacrifice (weeks)		
					Interim	Terminal	Recovery
General toxicology	Mouse	sc, iv	Single dose	600, 1200	—	2	—
General toxicology	Mouse	sc	Q2D	0, 5, 20, 80	2	4	4
General toxicology	Mouse	sc	Q2D	0, 5, 20	—	4	4, 8, 16
General toxicology	Mouse	sc	Q2D	0, 0.4, 2, 10	13	26	13
General toxicology	Monkey	sc	Single dose	240	—	2	—
General toxicology	Monkey	sc	Q3D	0, 5, 20, 80	—	4	4
General toxicology	Monkey	sc	Q3D	0, 0.4, 2, 10	13	39	25
Cardiovascular safety pharmacology[a]	Monkey	sc	Single dose	0, 20, 80	—	—	—
Nervous system safety pharmacology	Mouse	sc	Single dose	0, 5, 20, 80	—	—	—
Respiratory safety pharmacology	Mouse	sc	Single dose	0, 5, 20, 80	—	—	—
PK[b]	Monkey	sc, iv	Single dose	sc: 1, 5, 15; iv: 1	—	—	—
PK	Mouse	sc, iv	Single dose	sc: 1, 5, 15; iv: 1	—	—	—

Note. Q2D, every other day; Q3D, every third day.
[a]Animals were retreated after a wash-out period as described in the Methods section.
[b]Each monkey received two doses, separated by a 2-week wash-out period.

BLyS) and a modified Fc portion of human IgG_1. Atacicept has demonstrated efficacy in a variety of murine models of autoimmunity, including a NZBW F1 murine model of spontaneous SLE (Gross *et al*., 2000) and a murine model of collagen-induced arthritis (Gross *et al*., 2001). To support subsequent clinical evaluation of atacicept in autoimmunity and B-cell malignancies, the nonclinical safety, pharmacokinetics (PK), and pharmacodynamics (PD) of atacicept were evaluated and the results are summarized herein. These studies encompassed evaluation of acute and chronic toxicity, safety pharmacology, PK, and PD. The nonclinical studies for this program were conducted in mice and cynomolgus monkeys based on evidence of therapeutic activity in mouse models of autoimmunity, demonstrable biological activity on B cells and circulating immunoglobulin consistent with the expected biological activity of BLyS/APRIL inhibition, and demonstrated drug-ligand binding to BLyS and APRIL in both of these species.

MATERIALS AND METHODS

Test Items and Formulation

All test materials were provided by Merck Serono International S.A. (Geneva, Switzerland). The manufacturing and characterization of atacicept have been previously described (Roque *et al*., 2006). Frozen aliquots of atacicept stock solution were thawed on each day of dosing and used for dose preparation. Atacicept stock was either used as provided for the 5.0 mg/kg dose or diluted in phosphate-buffered solution (PBS) as needed for dosing. Stability and recovery of the low-concentration solutions were evaluated to confirm appropriate dosing.

Animal Care and Use and Regulatory Compliance

Toxicology studies were conducted in Swiss mice (safety pharmacology; Depre Breeding Centre, France), in CD-1 (ICR) BR mice (general toxicology studies; Charles River Laboratories, Italy) and with experimentally naïve, purpose-bred, young adult to adult male and female cynomolgus monkeys (*Macaca fascicularis*) originating from China (general toxicology studies) or Mauritius (safety pharmacology). All study procedures were conducted according to a written study protocol and facility standard operating procedures, and in strict compliance with accepted animal welfare standards. Housing and protection of animals used in the studies were maintained in accordance with Directive 86/609/EEC and the Italian D.L. No. 116 of January 27, 1992. All studies were conducted in accordance with the Organization for Economic Co-operation and Development (C[97] 186 [Final]) Principles of Good Laboratory Practice, as incorporated into EEC Directives 87/18, 88/320, 1999/11, and 1999/12, and enforced by the Italian Health Authority. The nonclinical safety program was conducted in accordance with regulatory recommendations for the nonclinical safety evaluation of biotechnology-derived therapeutics (International Conference on Harmonization, 1998).

Safety Pharmacology

Evaluation of effects on respiration in unrestrained conscious mice. Respiration was monitored in mice (eight males per group) treated with a single dose of vehicle control (phosphate-buffered saline, PBS), positive control (carbamylcholine, 1 mg/kg), or atacicept (5, 20, and 80 mg/kg) by sc interscapular injection through previously implanted catheters (see Table 1). The mice were placed in a whole body plethysmograph for measurement of respiratory rate, peak inspiratory and expiratory flows, inspiration and expiration times, minute volume, tidal volume, and airway resistance (Chand *et al*., 1993), and respiration cycles were acquired (Dataquest A.R.T. v. 2.1, Data Sciences, Inc., St Paul, MN) before administration of the test item and at 1, 5, 10, 15, 30, 60, 90, 120, 180, and 240 min following administration.

Behavioral Irwin test and effect on body temperature in mice. Effects on behavior and body temperature were measured in mice (eight males per group) following a single dose of vehicle control (PBS), positive control (chlorpromazine, 10 mg/kg), or atacicept (5, 20, and 80 mg/kg) by sc interscapular injection (see Table 1). The mice were observed according to a standardized observation battery to detect neurobehavioral, neurovegetative, or psychotropic signs or neurotoxic effects (Irwin, 1968). Body temperature was measured via rectal probe (Physitemp Model Ret-2, Type 2, Physitemp Instruments, Inc., Clifton, NJ). The Irwin scores and measurements of body temperature were performed before administration of the test item and at 1, 3, 6, 8, 24, and 48 h following administration.

Evaluation of effects on blood pressure, heart rate, and electrocardiogram in conscious cynomolgus monkeys. Cardiovascular safety evaluations were conducted in cynomolgus monkeys (three males and three females) fitted with telemetric transmitters for measurement of blood pressure (mean, systolic, and diastolic), heart rate, and electrocardiography via six leads (leads I, II, III, aVL, aVR, aVF) in conscious unrestrained monkeys. The monkeys were serially treated with vehicle control (PBS) and atacicept (20 and 80 mg/kg, see Table 1). A wash-out period of 48 h between the vehicle and the 20 mg/kg dose was followed by 1 week between the two doses of atacicept; no toxicokinetic analyses were conducted on these animals and exposure was estimated based on results from other studies. Telemetric measurements of blood pressure, heart rate, and electrocardiography (lead II) started at least 24 h before administration and were continued for 24 h (20 mg/kg atacicept) or 48 h (vehicle and 80 mg/kg atacicept). The effects of atacicept were determined by comparison against control monkeys (administered vehicle alone) and using the monkey's own basal values. The effects of atacicept and the method-control substance on the QT interval were analyzed by a method that takes into account the QT/RR relationship according to Fridericia's formula (Fridericia, 1920), Van de Water's formula (Van de Water *et al*., 1989) and Bazett's formula (Bazett, 1920).

General Toxicology, Pharmacokinetics, and Pharmacodynamics

The toxicity, PK, and PD of atacicept in mice and cynomolgus monkeys were evaluated following both single and repeated dose administration, as summarized in Table 1. General toxicology studies of atacicept sc injection (5−10 ml/kg) in mice were conducted after a single dose, and after repeated dosing every other day (Q2D) for 2, 4, and 26 weeks. As a general rule, three to six animals per sex were evaluated for toxicological and PK endpoints at each sampling interval, and animals were 6 weeks old at study initiation. General toxicology studies of atacicept sc injection (interscapular or medial thigh, 1−3 ml/kg) in cynomolgus monkeys were conducted after a single dose and after repeated dosing every third day (Q3D) for 4, 13, and 39 weeks. With the exception of the high dose tolerability study, which was conducted with one male and one female, toxicology and PK studies involved three to five male and female animals per dose group and animals were generally 3−6 years old at study initiation. Because B-cell recovery was incomplete after a 4-week dose-free period in both species, subsequent studies were conducted to evaluate the recovery kinetics of B cells after longer dose-free periods. Evaluations conducted in these species generally included clinical signs, food consumption, body weight, serum chemistry, hematology, urinalysis, flow cytometric immunophenotyping of peripheral blood, serum Ig (IgG and IgM), ophthalmology, gross pathology, organ weights, and microscopic pathology. In addition, serum samples were collected for evaluation of serum atacicept concentrations and anti-atacicept antibody formation. Additional analyses conducted only in cynomolgus monkeys included evaluation of plasma coagulation and electrocardiography.

Clinical observations. Mortality observations were recorded twice daily, and clinical observations of physical appearance and behavior were recorded daily during the predose, dosing, and dose-free recovery phases of the studies. Body weight measurements were recorded once weekly (monkeys) or twice weekly (mice), and food consumption (estimated in monkeys) was recorded weekly during the predose, dosing, and dose-free recovery phases of the studies.

Electrocardiography. Electrocardiogram (ECG) tracings were recorded on monkeys in general toxicology studies according to Bailey's hexaxial lead system by a three-channel electrocardiograph (Bosch EKG 506 D). Heart rate, PQ interval, QRS complex, and QT interval lengths were determined from DII tracings; QTc was calculated according to Bazett's formula. For the recordings, the monkeys were lightly anesthetized with 8 mg/kg Zoletil 100 (Virbac, Carros, France; tiletamine and zolazepam 1:1) administered by intramuscular injection. During the ECG recordings, the monkeys were kept in right lateral recumbency. ECG tracings were recorded at various time points before dosing, during dosing, and at the end of the treatment and recovery periods.

Ophthalmology. Ophthalmologic examinations were performed in mice or monkeys by macroscopic examination of the conjunctiva, cornea, anterior chamber, iris, lens, vitreous body, and fundus by means of an indirect ophthalmoscope (Omega 100, Heine Optotechnik, Herrsching, Germany) after instillation of 0.5% tropicamide (Visumidriatic fenilefrina, Visufarma, Italy) in both eyes. Ophthalmology examinations were conducted during the predose, dosing, and recovery phases of the studies.

Clinical pathology. Blood and urine samples were collected periodically for clinical pathology analyses during the predose (cynomolgus monkeys) and postdose (mice and cynomolgus monkeys) periods. For example, for the chronic toxicity study in cynomolgus monkeys, clinical pathology analyses were conducted predose on days −13/−11 and −5/−7; during dosing on days 42, 78, or 81, 171, and 270 or 273; and during recovery on days 312 or 325 and 442 or 448.

Comprehensive hematology analyses were conducted on tripotassium ethylenediamine tetra-acetate (K_3EDTA)-anticoagulated whole blood using a CELL-DYN 3500 (Abbott Diagnostics Division, Santa Clara, CA) for cynomolgus monkeys and AcT5diff (Beckman Coulter Inc., Miami, FL) for mice for the following parameters: erythrocyte count, hemoglobin concentration, hematocrit, total and differential leukocyte counts, mean cell volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and platelet count. Serum chemistry was analyzed using an AU400 (Olympus Diagnostic GmbH, Hamburg, Germany) for both species for the following parameters: glucose, total cholesterol, triglycerides, urea, creatinine, aspartate aminotransferase, alanine aminotransferase (ALT), alkaline phosphatase, gamma-glutamyl transferase, total bilirubin, total protein, sodium, potassium, chloride, calcium, and inorganic phosphorus. Serum protein electrophoresis of monkey sera was conducted using an SAS-3 electrophoresis analyzer (Helena Biosciences, Gateshead, UK) to determine albumin, globulin, alpha1-, alpha2-, beta-, and gamma-globulin fractions. Coagulation parameters were evaluated on sodium citrate-treated plasma samples collected from cynomolgus monkeys using an ACL 7000 IL coagulometer (Instrumentation Laboratory SpA, Milan, Italy) to determine the activated partial thromboplastin time and prothrombin time. Urinalysis was performed on urine collected during a 2-h (mice) or 16-h (cynomolgus monkeys) post-treatment period. Excreted urine volume was recorded, and urine samples were analyzed using a Super Aution SA4250 (Menarini Diagnostics, Firenze, Italy) for the following parameters: specific gravity, color, turbidity, pH, glucose, protein, bilirubin, urobilinogen, blood, and ketones.

Flow cytometry. Changes in peripheral blood lymphocytes were evaluated by direct immunofluorescence using flow cytometric immunophenotyping in both species. K_3EDTA-anticoagulated whole blood was incubated in 12 × 75 mm polystyrene round-bottom tubes with Fc Block (1:100 final dilution; BD Biosciences, San Jose, CA). Samples were mixed and incubated for 15 min at 4°C with a working antibody combination (1:40 final dilution of each antibody) consisting of CD20/IgD/CD3 for monkeys or B220/IgD/CD5 for mice (all antibodies were from BD Biosciences except for the anti-human IgD mAb from Biosource (Carlsbad, CA)). The red blood cells were lysed with FACS Lysing Solution (BD Biosciences) and fluorescent microspheres (Flow Count, Beckman Coulter, Fullerton, CA) were added to each sample tube prior to sample acquisition. Samples were acquired and gated results were evaluated to determine the absolute concentration of total B cells (CD20+ in monkeys, B220+ in mice), IgD+ and IgD− B cells, and total T cells in monkey (CD3+) and mouse (CD5+) peripheral blood samples. Immunophenotyping analysis was conducted on a FACSCalibur flow cytometer using CellQuest software (BD Biosciences). Determinations were conducted at various time points before dosing, during dosing, and at the end of the treatment and recovery periods. For example, flow cytometric analyses were conducted predose (week −3); during dosing on weeks 7, 13, 21, 24, 29, and 33; and during recovery on weeks 38 and 43 in the chronic toxicity study conducted in cynomolgus monkeys.

Serum immunoglobulin (IgG, IgM, IgA). Monkey sera were evaluated to determine total IgA, IgG, and IgM concentration by an immuno-turbidimetric method (SYNCHRON CX Clinical System, Beckman Coulter). Whereas IgA

serum concentration was only evaluated in a 39-week monkey toxicity study, IgG and IgM serum concentrations were evaluated in all safety studies conducted in monkeys. Total IgG and IgM levels were determined in serum samples by enzyme-linked immunosorbent assay (ELISA) methods that allow the relative quantification of IgG or IgM present in test samples using a commercially available purified mouse IgG or IgM as a standard material (Jackson ImmunoResearch Europe Ltd, Suffolk, UK, and BD Biosciences). Microtiter plates were coated with a polyclonal antibody directed against the Fc portion of the IgG or IgM (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, MD) and incubated overnight at approximately 4°C. Plates were blocked with Super Block reagent (Pierce, Rockford, IL). Standards and samples were added to wells in duplicate and incubated for 1 h at room temperature while shaken. Horseradish peroxidase (HRP)–conjugated goat anti-mouse IgG or IgM (Jackson ImmunoResearch Europe, Ltd) were added and incubated for 1 h at room temperature while shaken. Tetramethylbenzidine substrate (BioFX Laboratories, Owings Mills, MD) was added and the subsequent reaction was quantitated at 450 nm with a Victor 1420 reader (Perkin Elmer, Waltham, MA). Serum Ig concentrations were calculated using a four-parameter standard curve. Determinations were conducted at various time points before dosing, during dosing, and at the end of the treatment and recovery periods. For example, analyses of serum total immunoglobulin were conducted predose (week −3); during dosing on weeks 7, 13, 21, 24, 29, and 33; and during recovery on weeks 38 and 43 in the chronic toxicity study conducted in cynomolgus monkeys.

Serum atacicept concentration. Serum atacicept concentrations in both species were evaluated using a quantitative sandwich ELISA method. Microtiter plates were coated with a monoclonal antibody specific for atacicept and incubated overnight at approximately 4°C. Plates were blocked with Super Block reagent (Pierce). Standards and samples were added to wells in duplicate and incubated (1 h, 37°C). Biotinylated anti-atacicept monoclonal antibody was added and incubated on a shaker plate (1 h, 37°C). NeutrAvidine-HRP (Pierce) and a chemiluminescent substrate (Super Signal Pico, Pierce) were added and the subsequent signal detected at 425 nm using a Victor 1420 reader (Perkin Elmer). The standard curve was fitted using quadratic regression (four-parameter logistic curve). Determinations were conducted at various time points as described under section "Pharmacokinetics."

Anti-atacicept antibodies. The presence of anti-atacicept binding antibodies in sera was evaluated by an ELISA method in both species. The method used streptavidin-coated microplates (Thermo Labsystems, Franklin, MA) and was based on the formation of a sandwich between biotin-atacicept conjugate, anti-atacicept antibody, and HRP-atacicept conjugate. Results were expressed as the ratio between optical densities for the postdose sample and for a blank monkey/mouse serum (considered as nonspecific binding). Pooled sera spiked with a goat-anti-human TACI polyclonal antibody (R&D Systems, Minneapolis, MN) were used as positive control samples. Determinations were conducted at various time points before dosing, during dosing, and at the end of the treatment and recovery periods. For example, sera was collected for analysis of anti-atacicept antibodies predose (week −1); during dosing on weeks 4, 8, 12, 16, 20, 25, 29, and 33; and during recovery on weeks 38 and 43 during the chronic toxicity study in cynomolgus monkeys.

Anatomic pathology. The animals were euthanized by exsanguination while under deep anesthesia induced with sodium pentothal. A complete gross necropsy was performed and included collection of macroscopic observations, organ weight measurements, and a comprehensive collection of tissue samples for microscopic examination. Tissues were preserved in neutral buffered 10% formalin, routinely processed and embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined by light microscopy by a board-certified veterinary pathologist. The eyes of both species and the testes and epididymides of monkeys were fixed in modified Davidson's solution and processed with the other tissues. The testes and epididymides of mice were fixed for at least 24 h in Bouin's fixative, transferred to 50% alcohol, and subsequently processed with the other tissues.

Immunohistochemistry. Immunohistochemical analysis for B and T cells was performed at the end of dosing (week 4) and recovery (week 8) in the spleen and mesenteric lymph nodes of mice and in the spleen of cynomolgus monkeys treated for 4 weeks with vehicle or atacicept. In these studies, immunohistochemical detection of B cells was conducted using an anti-CD45R/B220 monoclonal antibody (clone RA3-6B2; BD Biosciences) in mice and an anti-CD20 monoclonal antibody (clone L26; Dako Italia SpA, Milan, Italy) in monkeys. Immunohistochemical detection of T cells was conducted using an anti-CD3 polyclonal antibody (Sigma, St Louis, MO) in mice and an anti-CD3 monoclonal antibody (clone PC3/188A, Dako Italia SpA) in monkeys. In the 39-week study in monkeys, bone marrow, spleen, and mesenteric lymph nodes were evaluated at the end of dosing (week 39) and recovery (week 54) for B-cells (anti-CD20, L26 clone; Dako), IgA (goat anti-monkey IgA, α-chain-specific clone; Tebu-bio, Milan, Italy), IgG (goat anti-monkey IgG, γ-chain-specific clone; Tebu-bio) and IgM (goat anti-monkey IgM, μ-chain-specific clone; Tebu-bio). In addition, plasma cells (anti-CD138, DL-101 clone; Tebu-bio) were evaluated in bone marrow. An evaluation of optimal antibody titrations was conducted to minimize non-specific staining.

Following deparaffination and rehydration, the primary antibodies to the specific targets were applied and incubated. For all procedures, biotin-conjugated primary or secondary antibodies were subsequently incubated and stained with streptavidin-HRP. Detection signals were based on the final conversion of diaminobenzidine as a substrate.

Pharmacokinetics. The PK of atacicept was estimated following a single intravenous (iv) or sc dose (PK studies) and following repeated sc doses (toxicokinetic studies) in both mice and monkeys (see Table 1). In PK studies, samples were collected at 0 (predose), 0.083 (iv only), 0.25, 0.5, 1, 2, 4, 6 (sc only), 8, 16, 24, 72, 120, 168, and 336 h after dose administration. In toxicokinetic studies, samples were collected in mice at 2, 6, and 48 h following the first treatment and at similar times on the day of sacrifice as shown in Table 1. In monkey studies, samples for toxicokinetic evaluation were collected at the time of the first dose, in weeks 2 and 4 during the 4-week study, and generally in weeks 6, 12, 26, and 39 during the 39-week study. Once in each of those weeks, blood was drawn predose and at 2-, 6-, 24-, 48-, and 72-h postdose. Atacicept serum levels were determined according to the above-described method.

Noncompartmental PK parameters were estimated using WinNonLin software (v. 3.1, Pharsight Corporation, Mountain View, CA). Composite serum versus time profiles were derived for mice and individual serum versus time profiles were derived for monkeys. Peak concentration after dosing (C_{max}) and time-to-peak concentration after dosing (T_{max}) were the values estimated. The area under the serum concentration versus time curve (AUC) was calculated using the log-linear trapezoidal rule (i.e., linear up to the C_{max}, logarithmic after the C_{max}). The terminal half-life ($t_{1/2}$) was calculated by linear regression of the terminal (log-linear) portion of the concentration versus time curve. The total clearance (CL) was calculated as dose/AUC and the volume of distribution at steady state (Vss) was calculated as CL × mean residence time. Absolute sc bioavailability (F) was calculated as the ratio of the sc dose-normalized AUC to the iv AUC.

Statistical analyses. In the repeated-dose toxicity studies, numerical data from dosed animals were compared with those from the control group. Group variances were compared using Bartlett's test. For data with homogenous variances across all groups, a one-way ANOVA was performed. If significant differences ($p \leq 0.05$) were indicated by the ANOVA, a test for differences between the control and treatment groups was conducted using Dunnett's test. When data had nonhomogeneous variance, a Kruskal-Wallis nonparametric ANOVA analysis was conducted, and a Mann-Whitney "U" test was conducted if warranted. In the safety pharmacology studies, analysis of variance for repeated measurements was performed with a Newman-Keuls test ($p \leq 0.05$) versus controls.

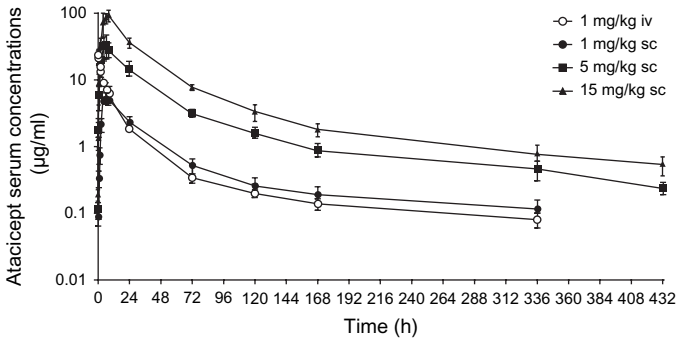

FIG. 1. Atacicept PK profile in cynomolgus monkeys after a single dose. Three monkeys per group were treated with a single iv (1 mg/kg) or sc (1, 5, or 15 mg/kg) injection of atacicept. Data presented as mean serum atacicept concentration (µg/ml) ± SD.

RESULTS

PK of Atacicept in Mice and Monkeys

Single-dose PK. Single-dose atacicept PK were evaluated in cynomolgus monkeys (using a cross-over study design) and mice, as summarized in Table 1 (Figs. 1 and 2). In the first period, three monkeys (group 1) received atacicept as a single iv dose (1 mg/kg) while three other monkeys (group 2) received atacicept as a single sc dose (1 mg/kg). After a 2-week wash-out (period 2), animals in both groups received atacicept as a single sc dose (group 1, 5 mg/kg; group 2, 15 mg/kg).

In mice, after a single 1 mg/kg iv injection, atacicept was eliminated from the body with an elimination half-life of 44 h and the AUC_{INF} was 100 h x µg/ml. A sc injection of 1 mg/kg atacicept was associated with an AUC_{INF} of 76.6 h x µg/ml, resulting in an estimated 77% F. Whereas the high sc dose increased 15-fold relative to the low dose, the AUC_{INF} increased only eightfold, suggesting a loss of dose proportionality at the high dose; as a result, the estimated bioavailability (F) decreased to 0.42 at the high dose.

The elimination half-life of atacicept in monkeys was 179 h and the AUC_{INF} was 215 h × µg/ml following a single 1 mg/kg iv injection. The AUC_{INF} was dose proportional following sc administration. The bioavailability of sc atacicept was 0.92, 1.02, and 0.81 following 1, 5, and 15 mg/kg doses, respectively. This suggests nearly complete absorption of atacicept by this route of administration. Low levels of atacicept were found in the predose samples for all six monkeys from period 2. This was anticipated because the cross-over design of the study only allowed for two half-lives during the wash-out period before the second dose administration. However, only an estimated 2% of the AUC_{INF} from the previous dose in period 1 overlapped with the AUC_{INF} in period 2.

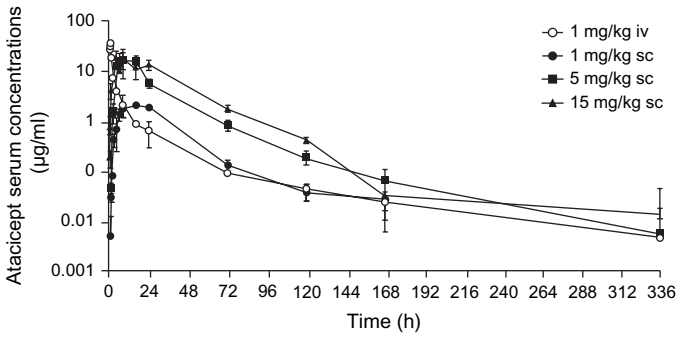

FIG. 2. Atacicept PK profile in mice after a single dose. Seventy mice per group were treated with a single iv (1 mg/kg) or sc (1, 5, or 15 mg/kg) injection of atacicept. Data presented as mean serum atacicept concentration (µg/ml) ± SD.

Overall, atacicept was well absorbed when sc administered, with bioavailability after a single dose approximately equal to 100% in both species at doses ranging from 1 to 15 mg/kg (except at 15 mg/kg in mice, where it was lower than expected). T_{max} was 4–16 h in mice and 6–8 h in monkeys, with $t_{1/2}$ calculated to be approximately 40–50 h in mice and 140–190 h in monkeys.

Repeat-dose PK and antibody determinations in CD-1 mice. Following a single sc dose of atacicept (0.4, 2, or 10 mg/kg), measures of exposure (C_{max} and $AUC_{2–48}$) increased in an approximately dose-proportional fashion (Tables 2 and 3). However, there were notable changes in C_{max} and $AUC_{2–48}$ between the first dose and after 13 or 26 weeks of dosing. In the 0.4 dose group, exposure was decreased at week 13 compared with day 1 (Table 2). In the 0.4 mg/kg dose groups, exposure was increased at week 13 compared with day 1. After 26 weeks of dosing, exposure was decreased in both the 0.4 and 2 mg/kg dose groups and increased in the 10 mg/kg dose group compared with day 1. These differential changes in exposure between the dose levels resulted in a loss of proportionality in exposure between the dose groups.

The PK of atacicept after repeated administration of higher doses was evaluated in the 4-week toxicology study only (Table 3). Following a single sc dose of atacicept (5, 20, or 80 mg/kg), the measures of exposure (C_{max} and $AUC_{2–48}$) increased in an approximately dose-proportional fashion (Table 3). After repeated dosing, exposure decreased when compared with day 1, the decrease being more evident for the 80 mg/kg dose group, resulting in a loss of proportionality at the highest dose tested.

In the 26-week toxicology study, binding antibodies were measurable starting at week 4 in approximately half of the mice at the 0.4 mg/kg dose, and in approximately a quarter of the mice at the 2 mg/kg dose. No anti-atacicept binding antibodies were observed in the 10 mg/kg dose group, whereas the antibody response persisted through 13 weeks of recovery in the 0.4 and 2 mg/kg dose groups. These results suggest that the relationship between dose and incidence rate of anti-atacicept antibody-positive mice was not attributable to interference in the antibody assay. Antibody responses developed in the dose groups where decreased exposure was observed, suggesting that the decrease in mean atacicept exposure could be due to

TABLE 2
C_{max} and AUC in Mice and Monkeys after 26 or 39 Weeks of Repeated Subcutaneous Dosing

Species	Week	C_{max} (μg/ml)			AUCtau[a] (h × μg/ml)		
		0.4 mg/kg	2 mg/kg	10 mg/kg	0.4 mg/kg	2 mg/kg	10 mg/kg
Mouse	Day 1	0.218	1.88	4.71	7.18	52.3	153
	Week 13	0.141	3.32	10.2	2.71	95.8	318
	Week 26	0.059	1.51	7.36	2.33	57.2	248
Monkey	Day 1	1.06	5.62	32.3	30.8	152	900
	Week 6/7	1.10	9.94	35.8	45.2	429	1500
	Week 13	1.36	10.8	49.8	60.2	426	1940
	Week 26	0.87	9.09	34.6	27.1	365	1550
	Week 39	1.12	6.89	39.0	34.4	291	1770

Note. C_{max}, peak concentration after dosing; AUC, area under curve.
[a]tau, 48 h in mouse and 72 h in monkey.

the presence of anti-atacicept binding antibodies in some mice. Mice that were positive for anti-atacicept binding antibodies showed higher IgG and IgM serum levels compared with concurrently treated mice, which suggested that development of anti-human atacicept antibodies in this species interfered with the biological activity of atacicept. Gender differences in PK were not observed at any dose tested in mice.

Repeated-dose PK and antibody determinations in cynomolgus monkeys. Following a single sc dose of 0.4, 2, or 10 mg/kg atacicept to cynomolgus monkeys, measures of exposure (C_{max} and $AUC_{0–72}$) increased in an approximately dose-proportional fashion. As expected, based on the half-life of atacicept, accumulation was observed with dosing Q3D, and exposure at week 13 was approximately double that of Day 1 (Table 2). At week 26, a decrease in the mean exposure was observed at the 0.4 mg/kg dose. However, this reduced exposure was no longer observed by week 39. In monkeys that were followed through the recovery period after 13 weeks (high dose group only) or 26 weeks of dosing, atacicept serum levels slowly decreased and were quantifiable up to 168–250 h after the last dose in the low dose group and up to 672 h in the middle and high dose groups. From week 6/7 onwards, the atacicept serum levels decreased in some monkeys. This decrease correlated with the development of an anti-atacicept antibody detected in one to two monkeys per group. The incidence rate of antibody-positive monkeys tended to stay the same or decrease during the recovery, suggesting low drug interference in the antibody assay.

Similarly to mice, the PK of atacicept after repeated administration of higher doses in monkeys was evaluated in the 4-week toxicology study only (Table 3). Following a single sc dose of atacicept (5, 20, or 80 mg/kg), the $AUC_{0–72}$ increased in an approximately dose-proportional fashion whereas the C_{max} increased with the dose but not in a proportional way. Accumulation was expected and observed after 4 weeks of thrice-weekly dosing, even if at the highest dose group, 80 mg/kg, the increase was less than expected from the dose increase, resulting in a loss of proportionality.

TABLE 3
C_{max} and AUC in Mice and Monkeys after 4 Weeks of Repeated Subcutaneous Dosing

Species	Week	C_{max} (μg/ml)			AUCtau[a] (h × μg/ml)		
		5 mg/kg	20 mg/kg	80 mg/kg	5 mg/kg	20 mg/kg	80 mg/kg
Mouse	Day 1	6.71	26.4	155	207	863	3950
	Week 2	2.80	14.9	173	89.5	393	4250
	Week 4	4.08	17.4	50.7	126	482	1310
Monkey	Day 1	29.9	209	1080	957	4210	24200
	Week 2	40.1	242	1880	1360	6200	35900
	Week 4	55.3	326	931	2340	11,200	28100

Note. C_{max}, peak concentration after dosing; AUC, area under curve.
[a]tau, 48 h in mouse and 72 h in monkey.

Gender differences in PK were not observed at any dose tested in monkeys.

Safety Pharmacology

A single sc injection of up to 80 mg/kg atacicept caused no demonstrable effect on respiratory function, cardiovascular function, or body temperature. In contrast, administration of a single sc injection of 1 mg/kg carbamylcholine as a positive control agent in the respiratory safety pharmacology study induced a bronchoconstrictor effect typical of this agent (Lai *et al*., 2004; Okazawa *et al*., 1999). Whereas a single sc injection of 5 or 20 mg/kg atacicept had no demonstrable effect on neurobehavioral endpoints, a trend toward hyperalertness and concomitantly increased spontaneous locomotor activity was observed after administration of 80 mg/kg atacicept; this trend reached statistical significance at 1-h postdosing. Clinical observations of mice and cynomolgus monkeys following repeated administration of up to 80 mg/kg atacicept in the course of general toxicity studies did not reveal alterations in alertness or behavior, suggesting that this may have been an incidental finding. Administration of a single intrascapular sc injection of 10 mg/kg chlorpromazine as a positive control agent in the neurobehavioral safety pharmacology study was associated with the expected signs of depressed activity, neurovegetation, and hypothermia in treated mice (Irwin, 1968; Laverty and Taylor, 1969; Mattsson *et al*., 1996). The sc injection of 20 and 80 mg/kg atacicept in conscious cynomolgus monkeys did not induce any change in arterial blood pressure, heart rate or ECG recordings. No adverse clinical signs were observed during the study at any dose level. An absence of effect on these cardiology endpoints was confirmed in repeated-dose general toxicity studies conduced with up to 80 mg/kg atacicept.

General Safety in Mice and Cynomolgus Monkeys

Single dose toxicity studies in mice and cynomolgus monkeys. A single iv or sc injection of up to 1200 mg/kg atacicept in mice did not induce appreciable general or local abnormalities, and no changes in hematology, serum chemistry, or gross pathology examinations were found at the end of the 14-day observation period. Similarly, no appreciable general or local clinical abnormalities were seen in cynomolgus monkeys after a single sc injection of 240 mg/kg atacicept during the 14-day observation period, including changes in body weight, food consumption, ophthalmology, electrocardiography, heart rate, hematology, serum chemistry, or plasma coagulation.

Repeated-dose toxicity studies in mice. Atacicept was well tolerated in mice when administered Q2D as repeated sc injections of 5, 20, and 80 mg/kg for 2 or 4 weeks, or 0.4, 2, and 10 mg/kg for up to 26 weeks. Because similar findings were observed across shorter- and longer-term studies of atacicept in mice, results are presented below for mice treated for 26 weeks.

No untoward clinical signs or remarkable clinical pathology findings were associated with atacicept administration (see Table 2, Supplement). Observed effects were consistent with the expected pharmacological activity of atacicept in reducing the survival of Ig-secreting B cells, including dose-related decreases in total and mature B-cell concentrations in peripheral blood (Table 3, Supplement) and decreased total serum Ig (IgG, IgM) concentrations (Table 4, Supplement) and total protein (Table 2, Supplement), without apparent effects on peripheral T cell concentrations. Because of blood volume limitations in mice, it was not possible to follow the effect of treatment over time in the same mouse, and relative differences were estimated against results obtained in a baseline (pretreatment) cohort of mice. At the end of 26 weeks of treatment with 2 or 10 mg/kg atacicept, total B cells (B220+ lymphocytes) were approximately 71–82% lower than baseline (compared with a 28% decrease in vehicle-treated mice), and IgD+ B cells were approximately 79–87% lower than baseline (compared with a 32% decrease in vehicle-treated mice). An associated decrease in total serum IgG (59–85% decrease) and IgM (> 99% decrease, to below the assay detection limit) was observed at the end of treatment among animals dosed with 2 and 10 mg/kg atacicept, respectively, compared with baseline mice. In contrast, both IgG and IgM increased, by 466 and 248%, respectively, among vehicle-treated mice at the end of treatment compared with baseline. Serum Ig concentrations recovered over the course of the 12-week dose-free period and were generally similar to results in vehicle-treated control mice; a similar trend toward recovery in peripheral blood B-cell populations was apparent over the 12-week dose-free period, although the total and IgD+ B-cell populations remained lower than in baseline or vehicle-treated mice. An observed increase in total lymphocytes among atacicept-treated mice as assessed by hematology analysis (Table 2, Supplement) is not explained by observed effects on T or B cells as assessed by flow cytometry, and may represent an artifact of sampling different mice for these endpoints. The flow cytometric and serum immunoglobulin analyses demonstrate that administration of atacicept specifically reduces B220+ and IgD+ B cells and concomitantly reduces IgG and IgM serum levels.

Histopathology analyses demonstrated a dose-related lymphocyte reduction in lymph node cortices (data not shown) and in the marginal zone of the spleen (Fig. 3). Immunohistochemistry confirmed a dose-related reduction of B cells (anti-CD45R/B220) in the spleen and mesenteric lymph nodes, whereas no changes in the T cell population (anti-CD3) were observed (data not shown). A dose-related sc inflammation with eosinophilia was observed at the injection site in some mice. Following a postdosing recovery period of 4 weeks in mice treated with atacicept for up to 4 weeks or 13 weeks in mice treated for up to 26 weeks, there was nearly complete or complete resolution of histopathology findings.

Repeated-dose toxicity studies in monkeys. Atacicept was well tolerated in cynomolgus monkeys when administered

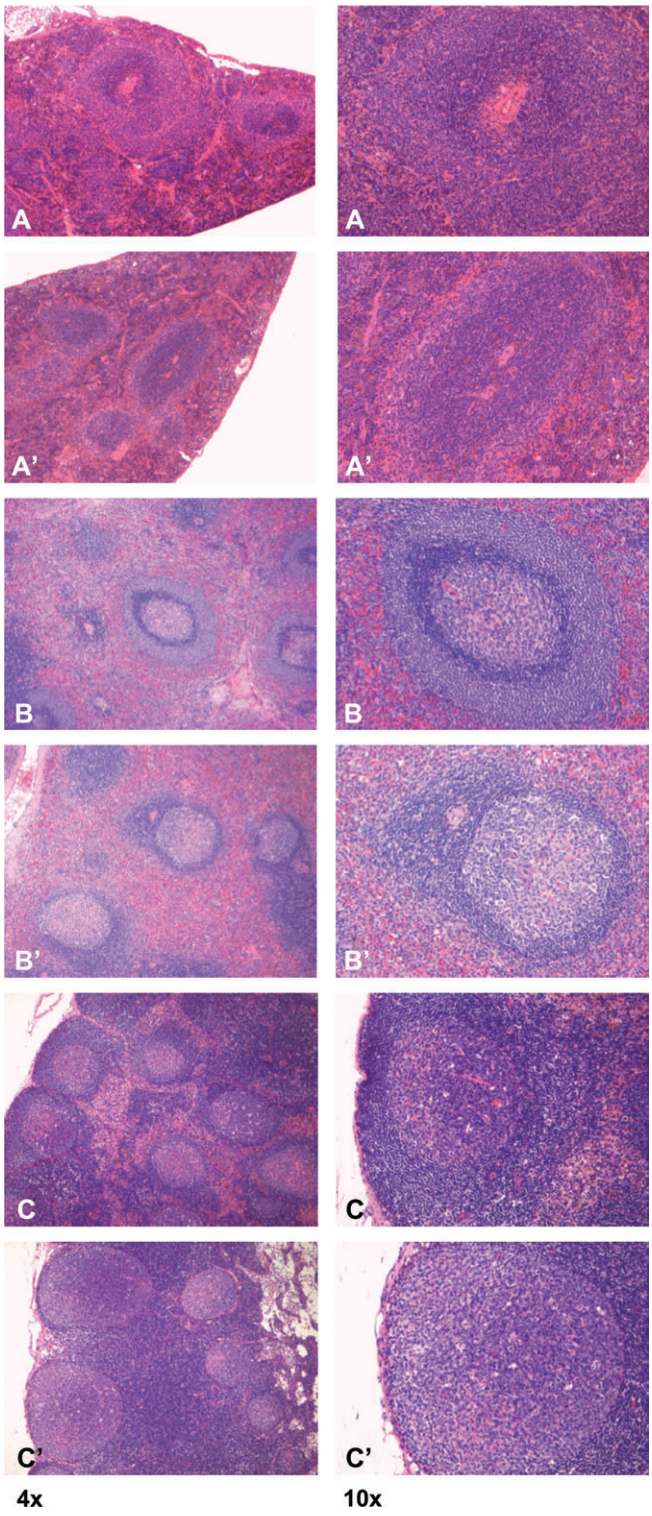

FIG. 3. Photomicrographs of the normal mouse spleen (A), and cynomolgus monkey spleen (B) and lymph node (C) stained with hematoxylin and eosin. Lymphocyte depletion was observed in the splenic follicular marginal zone after treatment with atacicept in mice (A′) and cynomolgus monkeys (B′), and in the corona (mantle) of the germinal centers of the lymph node of cynomolgus monkeys (C′). In these studies, 10 mg/kg atacicept was administered via sc injection to mice (three times weekly, 26 weeks) or cynomolgus monkeys (twice weekly, 39 weeks).

Q3D as repeated sc injections of 5, 20, and 80 mg/kg for 4 weeks or 0.4, 2, and 10 mg/kg for up to 39 weeks. As with mice, similar findings were observed across shorter- and longer-term studies of atacicept in monkeys; thus results are presented below for monkeys treated for 39 weeks. A slight elevation in serum ALT concentrations was observed among two monkeys treated with 10 mg/kg atacicept for 36 weeks, which was not observed in other treatment groups or at the end of the recovery period (Table 2, Supplement). No histological evidence of hepatocellular injury was observed in these monkeys, and this finding has not been observed in other studies. In addition to these findings, sc inflammation (mainly perivascular mononuclear and eosinophilic cell infiltrates) was observed histologically at the injection site and was considered to be related to the local presence of the administered exogenous protein.

Observed effects in monkeys were consistent with the expected pharmacological activity of atacicept in reducing survival of Ig-secreting B cells, including dose-related decreases in total, IgD+, and IgD− B-cell concentrations in peripheral blood without apparent effects on peripheral T-cell concentrations (Fig. 4 Table 3, Supplement, except IgD− B cells, which are not shown), decreased total lymphocytes (Table 2, Supplement), and decreased total serum Ig concentrations (IgG, IgM: Fig. 4 and Table 4, Supplement; IgA: data not shown) and total protein (Table 2, Supplement). At the end of the 39-week dosing period, total B cells were reduced by approximately 50% and IgD+ B cells were reduced by approximately 60% among monkeys treated with 2 and

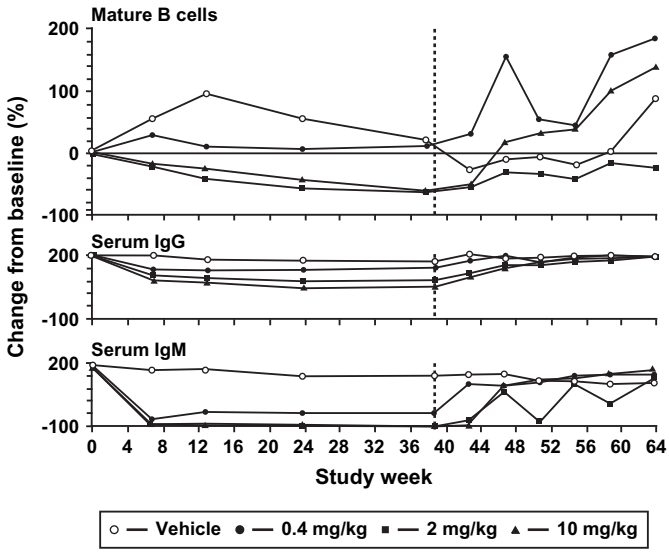

FIG. 4. Mean percentage change over time in baseline mature B cells (IgD+/CD20+) and serum IgG and serum IgM concentrations in cynomolgus monkeys treated twice weekly with vehicle or atacicept (0.4, 2, 10 mg/kg) via sc injection for 39 weeks ($n = 10$) followed by a 25-week dose-free period ($n = 4$). Summary flow cytometric and serum Ig concentrations in mice and cynomolgus monkeys over time are provided in Tables 4 and 5.

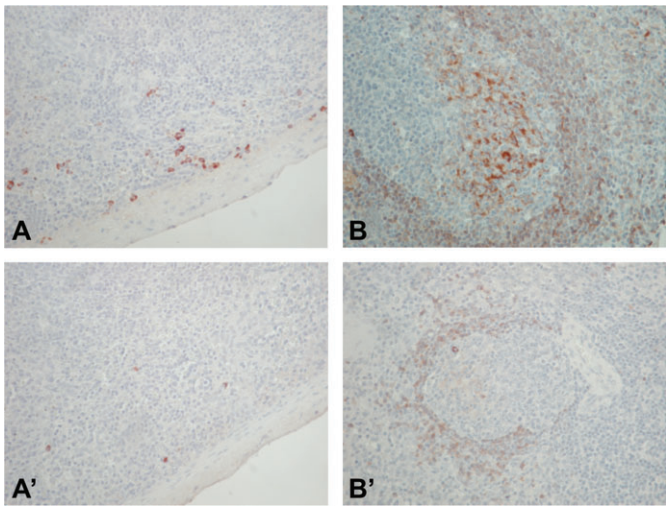

FIG. 5. Photomicrographs (×10) of the normal cynomolgus monkey spleen stained with hematoxylin and eosin and either anti-IgG (A) or anti-IgM (B). Depletion of Ig-positive lymphocytes was observed in the spleen after treatment with atacicept in cynomolgus monkeys, as identified by anti-IgG (A′) or anti-IgM (B′) immunohistochemistry. Atacicept (10 mg/kg) was administered twice weekly via sc injection to cynomolgus monkeys for 39 weeks.

10 mg/kg atacicept when compared with their own predose baseline samples. The decrease in peripheral B cells was associated with decreases in total serum IgG (39–49%) and IgM concentrations (> 99%, to below the assay detection limit) in monkeys at the end of treatment compared with their own predose baseline samples (Fig. 4 and Table 4, Supplement). Histopathology findings were limited to reversible lymphocyte reduction in the follicular marginal zone of the spleen and the corona (mantle) surrounding germinal centers of the lymph nodes (Fig. 3). Immunohistochemical analysis demonstrated reduced B cells (CD20) at all doses in the spleen but not mesenteric lymph nodes or bone marrow, with no detectable effect on plasma cells (CD138) or T cells (CD3) in these organs (data not shown). In addition, decreases in cells expressing IgG in the spleen (Fig. 5) and bone marrow (data not shown), IgA in the bone marrow (data not shown), and IgM in the spleen (Fig. 5) and mesenteric lymph nodes (data not shown) were observed after 39 weeks of dosing.

The atacicept-related effects on the immune system, including reduced B cells in peripheral blood and lymphoid tissues and reduced serum Ig concentrations (Fig. 4 and Tables 3 and 4, Supplement), were reversible with termination of treatment, and peripheral B-cell and serum total Ig concentrations were generally similar to (or higher than) baseline levels by the end of the 25-week dose-free period. We concluded from this data that administration of atacicept in both mice and monkeys reduced the number of B cells in peripheral blood and lymphoid tissues as well as circulating IgM and IgG concentrations. Given that dose-responsive changes in peripheral and tissue B cells and total immunoglobulin concentrations were consistent with the expected pharmacological activity of this molecule and that there were no untoward adverse effects attributable to atacicept under the conditions tested, a no adverse effect level was established at the highest tested dose (i.e., 80 mg/kg in 4-week studies and 10 mg/kg in chronic studies) in both mice and cynomolgus monkeys.

DISCUSSION

We describe in this report the preclinical safety and pharmacology of atacicept. Atacicept was well tolerated in both mice and cynomolgus monkeys over a broad dose range and for extensive periods of time without obvious organ toxicity without evidence of infection. Administration of atacicept reduced B cells and IgM and IgG serum levels consistent with the expected pharmacological activity of atacicept.

The lack of treatment-related findings indicating infections further corroborate our previous observation that atacicept does not impact on host resistance to influenza virus infection (Roque *et al*., 2006). In this model, no effect on viral clearance or survival was observed following atacicept treatment, despite reduction in influenza-specific Ig production and peripheral B-cell populations. Similarly, pretreatment of mice with atacicept did not alter clearance of a subsequent bacterial infection (*Streptococcus pneumoniae*, Type 14; Heffernan *et al*., 2008). These data collectively suggest that sufficient compensatory capacity to clear opportunistic infection persists in atacicept-treated animals. A mechanistic explanation could be that BLyS and APRIL are not required for mouse memory B-cell survival and function (Benson *et al*., 2008). This could be extrapolated to patients. Interestingly, SLE patients treated with atacicept had protective anti-tetanus antibody post-treatment levels (Dall'Era *et al*., 2007). This preliminary data suggest that after BLyS and APRIL blockade, a patient's memory B-cell compartment might persist and be poised to protect against previously encountered pathogens.

In both species, the immunological effects of atacicept administration were consistent with suppression of Ig-secreting B cells, without notable effects on other white blood cells, including T cells. Observed effects included reduced mature B cells in peripheral blood and lymphoid tissues, reduced serum Ig concentrations, and a decrease in Ig-producing cells in lymphoid tissues. These effects of atacicept support a specific activity of BLyS and APRIL in supporting B cell proliferation, differentiation, and survival as previously described (Dillon *et al*., 2006; Mackay *et al*., 2007). In addition, the immunomodulatory effect of atacicept was reversible, as evidenced by recovery of mature B cells and Ig concentrations within 2–3 months of treatment cessation in both mice and cynomolgus monkeys.

Atacicept demonstrated low immunogenicity, especially in monkeys. In mice, the presence of binding antibodies was associated with a reduction of the biological activity of atacicept on IgG and IgM serum levels. In monkeys the

presence of binding antibodies was associated with decreased serum levels of atacicept. Observation of a decreased incidence of animals developing anti-drug antibodies with greater atacicept dose is likely the result of the ability of atacicept to suppress antibody responses and could be predicted for an agent that mediates B-cell inhibition; given the timing of the blood draws during the recovery period it is unlikely that the low antibody response could be attributed to assay interference by circulating atacicept.

The effects of atacicept in cynomolgus monkeys share some basic PD properties observed with other BLyS antagonists, including BR3-Fc (Vugmeyster *et al*., 2006) and belimumab (Halpern *et al*., 2006). BR3-Fc is a recombinant protein containing the extracellular domain of the human BR3 (BAFF-R) fused with a human IgG Fc domain. Belimumab is a recombinant fully human IgG_1 monoclonal antibody targeting BLyS. Both of these molecules neutralize BLyS, but not APRIL.

Similarly to atacicept, BR3-Fc and belimumab were well tolerated in safety studies conducted in nonhuman primates. Treatment with either agent was associated with reversible decreases in CD20+ B cells in peripheral blood and lymphoid tissues, without notable effects on T cells or leukocytes as assessed by flow cytometry, immunohistochemistry, and hematology analysis. In addition, both BR3-Fc and atacicept reduce marginal zone B cells in spleen and lymph nodes.

Atacicept treatment reduced serum IgG concentrations to approximately 50% of baseline and could deplete IgM to undetectable levels in both mice and monkeys. In contrast, total serum Ig levels were not markedly affected by belimumab (Halpern *et al*., 2006), and the effects of BR3-Fc on total serum Ig concentrations were not reported, although a mild, statistically significant decrease in the IgG response to repeated tetanus immunization was observed (Vugmeyster *et al*., 2006). Because atacicept targets both BLyS and APRIL, whereas BR3-Fc and belimumab target only BLyS, these data suggest a possible role for APRIL and/or the combination of BLyS and APRIL in the maintenance of serum Ig concentrations. Indeed, several recent studies in mice have demonstrated that BLyS inhibition alone with BAFFR-Ig treatment does not significantly inhibit the survival of normal or malignant (multiple myeloma) plasma cells, whereas inhibition of both BLyS and APRIL with atacicept significantly reduces Ig-secreting plasma cells without affecting memory B cells (Benson *et al*., 2008; Ramanujam *et al*., 2006; Yaccoby *et al*., 2008). Comparison of results from clinical trials of BLyS-only inhibitors, such as belimumab and BR3-Fc, to those from atacicept trials will ultimately determine whether neutralizing APRIL and BLyS together has greater therapeutic benefit than neutralizing BLyS alone.

In conclusion, atacicept was well tolerated in mice and cynomolgus monkeys, with findings limited to local inflammation at the site of atacicept administration and specific decreases in B lymphocytes and immunoglobulins in tissues and in peripheral blood, which recovered in a treatment-free follow-up period. These nonclinical studies, which evaluated administered dose levels 3- to 90-fold higher (on a body weight basis) than doses administered in Phase 1 clinical studies, supported the safe administration of atacicept in healthy volunteers and patients with SLE, rheumatoid arthritis, or B-cell malignancies (Ansell *et al*., 2008; Dall'Era *et al*., 2007; Munafo *et al*., 2007; Tak *et al*., 2008).

SUPPLEMENTARY DATA

Supplementary data are available online at http://toxsci.oxfordjournals.org/.

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TABLE 1 (Supplemental)

Pharmacokinetic Parameters in Mice and Monkeys, Single Dose

Species	Route	Dose (mg/kg)	T_{max} (h)	C_{max} (µg/ml)	AUC (h*µg/ml)	$t_{1/2}$ (h)	CL (ml/h/kg)	Vz (ml/kg)	Vss (ml/kg)	F
Mouse	iv	1	–	–	100	44	9.99	638	169	–
	sc	1	16	2.09	76.6	45	–	–	–	0.76
	sc	5	8	16.9	444	53	–	–	–	0.89
	sc	15	4	18.1	631	37	–	–	–	0.42
Monkey	iv	1	-	-	215	179	4.30	1090	382	-
	sc	1	6	5.14	185	192	–	–	–	0.92
	sc	5	6	32.4	1140	137	–	–	–	1.02
	sc	15	8	87.2	2750	153	–	–	–	0.81

AUC, area under curve; CL, total clearance; C_{max}, peak concentration after dosing; F, bioavailability; $t_{1/2}$, terminal half life; T_{max}, time to peak concentration; Vss, volume of distribution at steady state; Vz terminal exponential volume of distribution.

Table 2 (Supplement): Selected clinical pathology data (mean ± SE (n)) from cynomolgus monkeys and CD-1 mice at the end of the dosing and recovery periods[1]

		Cynomolgus monkeys		Mouse	
		Atacicept		Atacicept	
		0 mg/kg	10 mg/kg	0 mg/kg	10 mg/kg
Total Protein (g/dL)[2]	End of Dosing	7.3 ± 0.08 (9)	**6.6 ± 0.13 (10)**	5.3 ± 0.06 (20)	**5.0 ± 0.08 (20)**
	End of Recovery	7.3 ± 0.17 (4)	6.9 ± 0.12 (4)	5.5 ± 0.06 (19)	5.4 ± 0.07 (20)
Glucose (mg/dL)[2]	End of Dosing	89.7 ± 5.03 (9)	70.2 ± 3.97 (10)	171.1 ± 8.54 (20)	149.7 ± 10.55 (20)
	End of Recovery	63.0 ± 3.24 (4)	64.3 ± 5.95 (4)	152.5 ± 8.05 (19)	133.6 ± 10.55 (20)
ALT (U/L)[2]	End of Dosing	55.7 ± 4.62 (9)	**131.0 ± 47.38 (10)**	35.5 ± 3.52 (20)	43.0 ± 3.84 (20)
	End of Recovery	37.8 ± 8.75 (4)	66.3 ± 12.31 (4)	32.9 ± 2.21 (18)	32.0 ± 2.72 (20)
Cholesterol (mg/dL)[2]	End of Dosing	137.7 ± 6.38 (9)	133.8 ± 9.26 (10)	102.6 ± 5.43 (20)	95.3 ± 6.88 (20)
	End of Recovery	121.8 ± 14.94 (4)	129.25 ± 14.84 (4)	114.6 ± 8.61 (17)	85.4 ± 9.67 (17)
Triglycerides (mg/dL)[2]	End of Dosing	45.0 ± 5.01 (9)	47.2 ± 2.75 (10)	86.9 ± 7.00 (20)	75.6 ± 7.93 (20)
	End of Recovery	44.3 ± 3.69 (4)	43.8 ± 6.07 (4)	84.8 ± 8.98 (17)	56.3 ± 8.20 (17)
Bilirubin (mg/dL)[2]	End of Dosing	0.26 ± 0.02 (9)	0.23 ± 0.02 (10)	0.17 ± 0.01 (20)	0.21 ± 0.05 (20)
	End of Recovery	0.21 ± 0.03 (4)	0.22 ± 0.04 (4)	0.26 ± 0.04 (17)	0.25 ± 0.02 (17)
Creatinine (mg/dL)[2]	End of Dosing	0.88 ± 0.03 (9)	0.84 ± 0.03 (10)	0.39 ± 0.01 (20)	0.35 ± 0.01 (20)
	End of Recovery	0.91± 0.06	0.91± 0.05 (4)	0.36 ± 0.01 (19)	0.35 ± 0.01 (20)
Calcium (mg/dL)[2]	End of Dosing	8.69 ± 0.39 (9)	8.68 ± 0.34 (10)	7.63 ± 0.05 (20)	7.71 ± 0.06 (20)
	End of Recovery	10.08 ± 0.13 (4)	9.55 ± 0.11 (4)	6.55 ± 0.07 (17)	5.31 ± 0.47 (20)
Erythrocytes ($x10^6/\mu L$)[3]	End of Dosing	5.34 ± 0.15 (9)	5.59 ± 0.11 (10)	8.64± 0.17 (20)	8.70 ± 0.21 (20)
	End of Recovery	5.32 ± 0.16 (4)	5.52 ± 0.08 (4)	8.28 ± 0.14 (19)	8.18 ± 0.27 (20)
Lymphocytes ($x10^3/\mu L$)[3]	End of Dosing	7.77 ± 0.79 (9)	**5.69 ± 0.43 (10)**	0.62 ± 0.07 (20)	**1.07 ± 0.36 (20)**
	End of Recovery	7.09 ± 0.67 (4)	**5.29 ± 0.40 (4)**	0.80 ± 0.10 (19)	**1.20 ± 0.33 (20)**
Neutrophils ($x10^3/\mu L$)[3]	End of Dosing	2.91 ± 0.43 (9)	3.35 ± 0.59 (10)	0.08 ± 0.01 (20)	0.11 ± 0.02 (20)
	End of Recovery	3.14 ± 0.77 (4)	3.13 ± 0.28 (4)	0.09 ± 0.02 (19)	0.09 ± 0.01 (20)
Platelets ($x10^3/\mu L$)[3]	End of Dosing	353.3 ± 18.16 (9)	382.9 ± 27.88 (10)	698.8 ± 25.0 (20)	704.2 ± 38.1 (20)
	End of Recovery	338.8 ± 38.99 (4)	358.5 ± 44.71 (4)	760.3 ± 28.1 (19)	716.4 ± 53.9 (20)

[1] End of dosing measurements occurred during Week 27 for CD-1 mice and Week 36 for cynomolgus monkeys. End of recovery measurements occurred during Week 40 for CD-1 mice and Week 64 for cynomolgus monkeys. [2] Clinical chemistry measurements obtained using sera as described in Materials and Methods. [3] Hematology measurements obtained using EDTA-treated whole blood as described in Materials and Methods.

TABLE 3 (Supplemental)

Flow Cytometric Immunophenotyping of Peripheral Blood Lymphocytes of Cynomolgus Monkeys and CD-1 Mice Prior to Dosing (Baseline), at the End of Dosing, and at the End of the Recovery Period

		Total B cells (cells/μl)[a]			Mature B cells (cells/μl)[b]			T cells (cells/μl)[c]		
Cynomolgus monkeys		*Baseline*	*Week 38*	*Week 64*	*Baseline*	*Week 38*	*Week 64*	*Baseline*	*Week 38*	*Week 64*
Vehicle	GeoMean (95% CI mean), n	793 (533, 1179) 10	903 (689, 1183) 10	1105 (390, 3133) 4	347 (208, 580) 10	417 (306, 570) 10	653 (240, 1781) 4	3700 (2692, 5086) 10	4120 (3595, 4722) 10	5504 (4166, 7271) 4
	Baseline difference (%)	–	13.9	**89.6****	–	20.1	**116.1***	–	11.3	39.4
Atacicept 0.4 mg/kg	GeoMean (95% CI mean), n	854 (645, 1132) 10	742 (492, 1119) 10	1843 (889, 3821) 4	249 (27, 2261) 10	277 (48, 1589) 10	706 (45, 10970) 4	3920 (1862, 8256) 10	4977 (2609, 9494) 10	5504 (3154, 9606) 4
	Baseline difference (%)	–	-13.2	**87.4***	–	11.4	**189.7***	–	**26.9***	39.4
Atacicept 2 mg/kg	GeoMean (95% CI mean), n	858 (685, 1075) 10	397 (281, 562) 10	636 (273, 1478) 4	347 (262, 461) 10	137 (92, 203) 10	264 (81, 865) 4	4086 (2948, 5663) 10	4349 (2990, 6326) 10	3919 (872, 17617) 4
	Baseline difference (%)	–	**-53.7*****	-12.7	–	**-60.7*****	-7.3	–	6.4	-7.3
Atacicept 10 mg/kg	GeoMean (95% CI mean), n	766 (562, 1043) 10	385 (265, 560) 10	1204 (809, 1790) 4	319 (209, 486) 10	132 (81, 213) 10	763 (472, 1235) 4	3698 (2906, 4705) 10	3730 (2812, 4948) 10	4462 (2805, 7098) 4
	Baseline difference (%)	–	**-49.8****	54.1	–	**-58.7****	**152.6***	–	0.9	**40.4***
CD-1 mice		*Baseline*	*Week 27*	*Week 38*	*Baseline*	*Week 27*	*Week 38*	*Baseline*	*Week 27*	*Week 38*
Vehicle	GeoMean (95% CI mean), n	276 (206, 371) 20	198 (110, 357) 8	180 (104, 314) 8	274 (207, 364) 20	186 (100, 346) 8	170 (96, 302) 8	576 (482, 687) 20	277 (187, 411) 8	279 (212, 368) 8

	Baseline difference (%)	–	-28.4	-34.7	–	-32.1	-37.9	–	**-51.9****	**-51.5*****
Atacicept 0.4 mg/kg	GeoMean (95% CI mean), n	–	205 (21, 1983) 8	324 (16, 6386) 7	–	181 (13, 2517) 8	282 (14, 5601) 7	–	438 (189, 1061) 8	407 (114, 1455) 7
	Baseline difference (%)	–	-25.6	17.4	–	-34.1	3.0	–	-24.0	-29.3
Atacicept 2 mg/kg	GeoMean (95% CI mean), n	–	81 (34, 189) 8	176 (55, 569) 6	–	59 (21, 162) 8	150 (52, 436) 6	–	431 (238, 781) 8	357 (221, 578) 6
	Baseline difference (%)	–	**-70.8****	-36.2	–	**-78.5*****	-45.2	–	-25.1	-37.9
Atacicept 10 mg/kg	GeoMean (95% CI mean), n	–	50 (25, 99) 8	126 (51, 313) 8	–	35 (15, 82) 8	120 (54, 270) 8	–	546 (315, 948) 8	252 (157, 406) 8
	Baseline difference (%)	–	**-81.8*****	-54.2	–	**-87.2*****	-56.1	–	-5.1	**-56.2*****

GeoMean: geometric mean; 95% CI mean: 95% confidence interval around the mean; n: number of animals per group.

*p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001.

[a]Total B cells defined within a lymphocyte (forward versus side-scatter) gate as B220+ cells for mice and CD20+ cells for cynomolgus monkeys.

[b]Mature B cells defined within a lymphocyte (forward versus side-scatter) gate as IgD+/B220+ cells for mice and IgD+/CD20+ cells for cynomolgus monkeys.

[c]Total T cells defined within a lymphocyte (forward versus side-scatter) gate as CD5+ cells for mice and CD3+ cells for cynomolgus monkeys.

TABLE 4 (Supplemental)

Serum Total IgG and IgM Concentration in Cynomolgus Monkeys and CD-1 Mice Prior to Dosing (Baseline), at the End of Dosing, and at the End of the Recovery Period

		IgG (mg/dl)			IgM (mg/dl)		
Cynomolgus monkeys		*Baseline*	*Week 39*	*Week 64*	*Baseline*	*Week 39*	*Week 64*
Vehicle	GeoMean (95% CI mean), n	1040 (942, 1150) 10	933 (868, 1004) 10	1059 (784, 1431) 4	69 (49, 97) 10	58 (45, 74) 10	46 (22, 97) 4
	Baseline difference (%)	–	**-10.3****	3.6	–	-16.6	-27.6
Atacicept 0.4 mg/kg	GeoMean (95% CI mean), n	1018 (919, 1127) 10	827 (733, 934) 10	1005 (886, 1141) 4	84 (62, 114) 10	20 (3, 134) 10	65 (40, 104) 4
	Baseline difference (%)	–	**-18.7*****	-3.5	–	-76.7	**-16***
Atacicept 2 mg/kg	GeoMean (95% CI mean), n	1107 (976, 1257) 10	673 (555, 816) 10	1143 (740, 1763) 4	62 (53, 72) 10	0 (0, 1) 10	48 (26, 87) 4
	Baseline difference (%)	–	**-39.3*****	-2.9	–	**-99.9*****	-22.5
Atacicept 10 mg/kg	GeoMean (95% CI mean), n	937 (878, 1000) 10	474 (361, 623) 10	898 (713, 1131) 4	79 (59, 105) 10	0 (0, 5) 10	69 (34, 139) 4
	Baseline difference (%)	–	**-49.4*****	-2.3	–	**-99.7****	-6.5
CD-1 mice		*Baseline*	*Week 27*	*Week 38*	*Baseline*	*Week 27*	*Week 38*
Vehicle	GeoMean (95% CI mean), n	35.7 (28.8, 44.2) 20	202.3 (145.5, 281.4) 8	144.1 (98.2, 211.4) 8	1.5 (0.77, 3.4) 20	5.2 (2.9, 9.5) 8	6.9 (4.5, 10.4) 8

	Baseline difference (%)	–	**466.28*****	**303.31*****	–	248.03	**356.99***
Atacicept 0.4 mg/kg	GeoMean (95% CI mean), n	–	97.6 (45.3, 210.2) 8	11.5 (71.6, 184.4) 8	–	1.1 (0.1, 12.6) 8	4.7 (1.9, 11.8) 8
	Baseline difference (%)	–	**173.27****	**221.61*****	–	-27.24	213.71
Atacicept 2 mg/kg	GeoMean (95% CI mean), n	–	14.7 (5.8, 37.0) 8	83.3 (40.1, 173.2) 8	–	0 (0, 0) 8	2.6 (1.9, 3.6) 8
	Baseline difference (%)	–	**-58.9***	**133.27****	–	**-100*****	73.48
Atacicept 10 mg/kg	GeoMean (95% CI mean), n	–	5.3 (2.8, 10.4) 8	39.0 (22.6, 67.1) 8	–	0 (0, 0) 8	2.8 (1.9, 4.0) 8
	Baseline difference (%)	–	**-85.05*****	9.05	–	**-100*****	83.5

GeoMean: geometric mean; 95% CI mean: 95% confidence interval around the mean; n: number of animals per group.

*p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001.