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Preclinical Safety and Pharmacology of an Anti-Human Interleukin-13 Monoclonal Antibody in Normal Macaques and in Macaques with Allergic Asthma

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Interleukin-13 (IL-13) plays a central role in chronic airway diseases, including asthma. These studies were conducted to evaluate the safety of administration of a human anti-IL-13 monoclonal antibody (mAb) to normal macaques and in macaques with allergic asthma. In addition, serum and bronchioalveolar lavage fluid were collected from allergic cynomolgus macaques in order to identify potential surrogate markers of IL-13 pharmacology that could be useful for subsequent clinical trials. In vitro studies demonstrated that the anti-IL-13 mAb inhibited the pharmacological actions of both human and cynomolgus macaque IL-13. Allergic macaques were treated systemically with 10 mg/kg anti-IL-13 mAb 1 day prior to inhaled *Ascaris suum* antigen challenge. Normal macaques were dosed intravenously with anti-IL-13 once per week for 3 weeks at doses of 10 or 50 mg/kg. Treatment of macaques with the anti-IL-13 mAb was not associated with any toxicologically significant findings. A slight treatment-related but nonadverse decrease in platelet counts was observed in both the normal and allergic macaques. In allergic macaques, the anti-IL-13 mAb treatment did not affect lung function, lung eosinophilia, or serum or BAL immunoglobulin E (IgE) concentrations but did produce a reduction in BAL and serum eotaxin concentrations ($p < .05$) at 6 h post antigen challenge. This study shows that administration of an anti-IL-13 mAb was well tolerated in both normal and allergic asthmatic macaques and that serum eotaxin concentrations may be a useful early in vivo marker for evaluating IL-13 inhibition in patients with asthma.

Keywords Allergy, Asthma, Eotaxin, Interleukin 13, Macaque

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Asthma is a chronic inflammatory lung disease that is characterized by airway hyperreactivity (AHR) in response to various stimuli (cholinergic agonists, histamine, exercise) and by mucus hypersecretion. Lung eosinophilia and increased immunoglobulin E (IgE) levels are also characteristic features of the disease. As the disease progresses, lung remodeling occurs that consists of goblet cell hyperplasia, airway smooth muscle hypertrophy, and subepithelial fibrosis (Homer and Elias 2005). Interleukin-13 (IL-13) has been shown in numerous studies to play a central role in the development of chronic airway diseases and may therefore be a suitable target for therapeutic intervention in asthma (Wynn 2003; Wills-Karp 2004).

IL-13 is a Th2-type cytokine produced by activated T cells and other cells. Functionally, IL-13 is related to IL-4 because they share the IL-4R α subunit. IL-13 binds to IL-13R α 1 with relatively low affinity but forms a high-affinity receptor signaling complex with IL-4R α 2 (Hershey 2003; Izuhara and Arima 2004). IL-13 also binds to IL-13R α 2 with high affinity and the interaction between IL-13 and IL-13R α 2 may be involved in the pathogenesis of fibrosis (Fichtner-Feigl et al. 2006). It has been reported that IL-4 is important in the initial differentiation of CD4 T cells into Th2-type cells, whereas IL-13 is important in the effector phase of allergic inflammation and is involved in mucus secretion, tissue remodeling and fibrosis (Wynn 2003).

Administration of IL-13 to mice, or sensitization of mice with ovalbumin, induces an asthma phenotype consisting of AHR, pulmonary eosinophilia, increased airway mucus production, and increased serum IgE (Wills-Karp et al. 1998). IL-13 induces the secretion of eotaxin from airway epithelial cells (Li et al. 1999) and transforms airway epithelium into a secretory phenotype (Danahay et al. 2002). Mice genetically altered to lack IL-13 show a reduced development of certain T-cell lineages, a reduction in Th2-cell cytokines, and reduced IgE production (McKenzie et al. 1998; Walter et al. 2001). These mice also

show reduced lung inflammation and reduced lung eosinophils relative to wild-type mice (Herrick et al. 2003). Inhibitors of IL-13 have been shown to reduce airway inflammation in mouse (Wills-Karp et al. 1998; Grunig et al. 1998; Kumar et al. 2004; Yang et al. 2004, 2005, Blanchard et al. 2005), guinea pig (Morse et al. 2002), sheep (Kasaian et al. 2007), and monkey (Bree et al. 2007) models of asthma.

The primary objective of these studies was to evaluate the pre-clinical safety of administration of an anti-human monoclonal antibody (mAb) to normal cynomolgus macaques. A secondary objective of these studies was to identify a potential *in vivo* serum marker for IL-13 inhibitory activity in asthmatic subjects. The antibody used in these studies has previously been shown to inhibit the release of the eosinophil chemoattractant cytokine, eotaxin, from human peripheral blood monocytes *in vitro* (Syed et al. 2007). These studies extend this *in vitro* observation by demonstrating that administration of the anti-IL-13 mAb to cynomolgus macaques was well tolerated and that in the allergic asthmatic animals inhibition of IL-13 was associated with a decrease in serum eotaxin concentrations.

MATERIALS AND METHODS

All *in-life* procedures were conducted in compliance with the Animal Welfare Act, the *Guide for the Care and Use of Laboratory Animals*, and the Office of Laboratory Animal Welfare. Study protocols were reviewed prior to the initiation of the studies and approved by the Institutional Animal Care and Use Committees.

Test and Control Articles

The anti-IL-13 antibody used in these studies is a fully human IgG1 λ monoclonal antibody against human IL-13 (Centocor R&D). The limit of solubility of this antibody in saline or serum is approximately 10 mg/ml. For the multiple-dose intravenous toxicology studies, the anti-IL-13 mAb was formulated as a solution at 50 mg/ml in 10 mM sodium acetate, 200 mM sucrose, 30 mM glycine, 30 mM mannitol, 0.01% polysorbate 80 (*w/v*), pH 5.0. The 50 mg/ml solution was diluted to 4 mg/ml in 5% dextrose solution and was administered as an infusion at a rate of 20 ml/kg/h. For the single-dose safety/pharmacology studies in the monkey, allergic asthma model the anti-IL-13 mAb was formulated as a solution at a concentration of 10 mg/ml in 50 mM acetate buffer, pH 5.0. The 10 mg/ml solution was administered as a slow bolus injection. Test solutions were prepared freshly on each day of administration and were filtered with a 0.22- μ m Millex-CV filter prior to administration.

In Vitro Pharmacology of Anti-IL-13 mAb

The determination of the suitability of the cynomolgus macaque as a relevant species for pharmacology and toxicology studies was evaluated in a series of *in vitro* assays.

Receptor-Binding Assay

The potency (IC₅₀) of recombinant human and cynomolgus macaque IL-13 for binding to human IL-13R α 1 and IL-13R α 2 was evaluated in an *in vitro* receptor-binding assay using an enzyme-linked immunosorbent assay (ELISA) method.

Serial dilutions of recombinant human IL-13 (R&D Systems, Minneapolis, MN) and cynomolgus macaque IL-13 (generated at Centocor R&D) were added to human IL13R α 1-Fc or IL-13R α 2-Fc (R&D Systems) coated plates. Bound IL-13 was detected using a polyclonal biotinylated anti-IL-13 antibody, followed by streptavidin-conjugated to alkaline phosphatase. Plates were read on a fluorescence plate reader.

STAT6 Phosphorylation by Fluorescence-Activated Cell Sorting (FACS) Analysis

The ability of the anti-IL-13 mAb to neutralize the activity of human and cynomolgus macaque IL-13 was evaluated in an *in vitro* assay using the human monocytic cell line THP-1 that expresses IL-13R α 1 and IL-4R α 2 (Fichtner-Feigl et al. 2006).

THP-1 cell suspensions (TIB-202; ATCC, Manassas, VA) were mixed with serial dilutions of recombinant human or cynomolgus macaque IL-13. The ability of the cells to generate pSTAT6 in response to IL-13 stimulation was measured by FACS analysis. EC₅₀ values for pSTAT6 stimulation were determined. The ability of this signal to be inhibited by the anti-IL-13 mAb was determined by mixing serial dilutions of anti-IL-13 mAb with 0.5 ng/ml of human IL-13 or 10 ng/ml of macaque IL-13. STAT6 phosphorylation was measured by FACS analysis.

Eotaxin Production/Inhibition Assays

The ability of the anti-IL-13 mAb to neutralize human and cynomolgus monkey IL-13 stimulated eotaxin release was evaluated *in vitro* in normal human lung fibroblast (NHLF) cells.

Adherent NHLF cells (Cambrex, East Rutherford, NJ) were treated with serial dilutions of either human or cynomolgus macaque recombinant IL-13 and human eotaxin production was measured using a commercially available ELISA kit (Biosource, Calsbad, CA). EC₅₀ values for IL-13-stimulated eotaxin production were determined. For the anti-IL-13 mAb inhibition assay, serial dilutions of the anti-IL-13 mAb were mixed with either 10 ng/ml of recombinant human IL-13 or 150 ng/ml of recombinant cynomolgus macaque IL-13.

Safety of Repeated Intravenous Administration of Anti-IL-13 mAb to Normal Healthy Cynomolgus Macaques

Male and female cynomolgus macaques were dosed once a week for 3 weeks via intravenous infusion with control article (5% dextrose solution, five males and five females) or anti-IL-13 mAb 10 mg/kg (three males and three females) or 50 mg/kg (five males and five females). Moribundity/mortality checks were performed twice daily (AM and PM). Clinical observations were

performed at least once daily. A physical examination, including a record of general condition, rectal body temperature, respiratory rate, heart rate, and capillary refill time, was performed for each animal prior to initial treatment, approximately 1 to 3 h following the final treatment in week 3, and prior to the day 47 necropsy. Ophthalmic examinations, including macroscopic examinations of the anterior portion of the eye, the optic media, and the ocular fundus, were performed prior to initial treatment and during weeks 3 and 7. Body weights were recorded at least twice prior to initial treatment (including day -1), approximately weekly thereafter, and on the day prior to necropsy. Food consumption was qualitatively measured daily. Electrocardiogram (ECG) tracings (10 s each) were obtained prior to initial treatment and during week 3 (after last dose) and week 7 using 12 leads and a chart speed of 50 mm/s. Indirect blood pressure and heart rate were measured for all animals prior to initial treatment and during weeks 3 and 7. Urine samples were collected via cystocentesis prior to necropsy for routine urinalysis using the Clinitek 200+ and an appropriate test strip. Blood samples were collected for hematology and were analyzed using a Bayer ADVIA 120 hematology analyzer. Blood samples collected for coagulation parameters were processed for plasma, and plasma was analyzed for prothrombin time (PT), activated partial thromboplastin time, and fibrinogen with an MLA Eclera 1400C coagulation analyzer. Blood samples were processed for serum and clinical chemistry parameters were determined using a Boehringer Mannheim Hitachi 717 chemistry analyzer. Animals were euthanized on day 19 (three per sex per group) or day 47 (two per sex per group) in groups 1 and 3 in accordance with accepted American Veterinary Medical Association guidelines.

A comprehensive necropsy, defined as the macroscopic examination of the external surface of the body, all orifices, and the cranial, thoracic, abdominal and pelvic cavities and their contents, was performed on all animals. Organs and tissues were dissected free and fixed in 10% neutral-buffered formalin. Tissues were trimmed, embedded, and sectioned. Slides were stained with hematoxylin and eosin and examined microscopically.

Safety and Pharmacology of Anti-IL-13 mAb Treatment in Macaques with Allergic Asthma

Eight cynomolgus macaques (*macaca fascicularis*), which were from a colony of animals, that had been shown to demonstrate a positive bronchoconstrictor response to inhaled *Ascaris suum* (*A. suum*) antigen were selected for this study. The study consisted of two sessions (sessions 1 and 2), which were separated by 3 weeks. Saline (1 ml/kg) was administered on day -1 of session 1 in order to establish a control response to *A. suum* antigen challenge. Animals were anesthetized with propofol during antigen challenge, which was administered via intermittent positive pressure breathing with a ventilator and in-line ultrasonic nebulizer. Animals were monitored for signs of distress

throughout the challenge period. Anti-IL-13 mAb (10 mg/kg) was administered on day -1 (24 h prior to antigen challenge) of session 2 to four animals by subcutaneous injection and to four animals by intravenous injection. On day 1 of each session, a single dose of *A. suum* antigen was administered in 15 breaths at optimal response dose via aerosol inhalation in order to induce the asthmatic response. The optimal response dose was defined as the dose that historically has resulted in >40% increase in lung resistance (R_L) and >35% decrease in dynamic compliance (C_{DYN}).

For each session, clinical observations were recorded daily for each animal and body weights were recorded prior to each antigen challenge. Pulmonary function values (R_L and C_{DYN}) were monitored for 10 min prior to antigen challenge and for at least 30 min following antigen challenge. Bronchioalveolar lavage (BAL) samples for determination of cell number, morphology, and differential were collected prior to antigen challenge and at 6 and approximately 24 h following each challenge. BAL supernatant was analyzed for eotaxin and IL-13 concentrations. Blood samples for hematology were collected for each session on day -1 (prior to dosing) and on days 1 (prior to antigen challenge), 2, and 8. Blood samples for analysis of serum anti-IL-13 mAb concentrations and IgE concentrations were collected on day -1 (prior to dosing) and on days 1 (prior to antigen challenge and 6 h following challenge), 2, 4, 6, 8, and 15. Additional aliquots from the 6h serum sample were processed for IL-13 and eotaxin in addition to IgE. Eotaxin and IL-13 concentrations were determined using commercially available ELISA kits. IgE levels were determined using a method developed by Charles River Laboratories. For IgE determinations; plates were coated with a mouse anti-human IgE antibody (Zymed/Invitrogen) and blocked with bovine serum albumin (BSA). Standards (0 to 250 ng/ml), samples (diluted fourfold in Tris-buffered saline [TBS] containing BSA) and control sera were incubated at room temperature for 30 min and detection was achieved using a horseradish peroxidase (HRP)-conjugated human monoclonal anti-IgE (Fitzgerald Industries) and tetramethyl benzidine (TMB). Color development was stopped with sulfuric acid after approximately 17 min and absorbance was read at 450 nm. Standards were fit using a four-parameter logistic model; interpolated sample values were reported as ng/ml of IgE.

Statistical Analysis

In the asthmatic macaques, differences between parameters measured during the session 1 saline treatment period and parameters measured during the session 2 anti-IL-13 mAb treatment were analyzed using a paired *t* test. In the normal macaques, differences between the pretreatment values and the post-treatment values were evaluated by one-way analysis of variance for the control and 50 mg/kg groups and by paired *t* test for the 10 mg/kg group. Differences between saline-treated normal animals and anti-IL-13 mAb-treated normal animals

were evaluated using a one-way analysis of variance. *p* values of <.05 were considered significant.

RESULTS

In Vitro Pharmacology of Anti-IL-13 mAb

Both macaque and human recombinant IL-13 exhibited saturable binding to human IL-13R α 2-Fc and human IL-12R α 1-Fc. The IC₅₀ values for both macaque and human IL-13 binding to human IL-13R α 2 (1.4 and 9.5 ng/ml, respectively) were lower than the IC₅₀ values for binding to IL-13R α 1 (195 and 64 ng/ml, respectively).

Stimulation of the human monocytic cell line, THP-1, with serial dilutions of recombinant human or cynomolgus macaque IL-13 demonstrated dose-dependent phosphorylation of STAT6 with EC₅₀ values of 0.75 and 10 ng/ml, respectively. Serial dilutions of the anti-IL-13 mAb inhibited 0.5 ng/ml of recombinant human IL-13 or 10 ng/ml of recombinant cynomolgus macaque IL-13 (selected to produce similar levels of STAT6 phosphorylation) with EC₅₀ values of 1 and 3 ng/ml, respectively.

Both human and cynomolgus macaque IL-13-stimulated normal human fibroblast cells to secrete eotaxin in a dose dependent manner with EC₅₀ values of 7.5 and 144 ng/ml, respectively. Serial dilutions of the anti-IL-13 mAb inhibited 10 ng/ml of recombinant human IL-13 and 150 ng/ml of recombinant cynomolgus macaque IL-13 (selected to produce similar levels of eotaxin release) with EC₅₀ values of 40 and 67 ng/ml, respectively.

Safety of Repeated Intravenous Administration of Anti-IL-13 mAb to Normal Cynomolgus Macaques

Treatment of normal cynomolgus macaques with anti-IL-13 mAb at weekly doses of 10 and 50 mg/kg was well tolerated and there were no signs of overt toxicity.

There were no abnormal clinical observations, physical,

ophthalmic, or electrocardiographic findings, or changes in body weight, food consumption, blood pressure, or heart rate measurements associated with anti-IL-13 mAb administration. Blood coagulation, serum chemistry, urinalysis, and organ weights were unaffected by anti-IL-13 mAb administration.

Administration of anti-IL-13 mAb to normal macaques was associated with a statistically significant reduction in serum platelets at the 50 mg/kg dose level (Table 1). The magnitude of the decrease in the platelets was 28% when compared to the saline control treatment group and 27% when compared to the pretreatment values. An 11% reduction in platelets was also evident in the 10 mg/kg treatment group when compared to pretreatment values. There was no reduction in platelets following saline treatment. These changes were not considered to be of toxicological significance because the platelet counts were within normal limits in all animals.

Histopathological examination of a comprehensive panel of tissues showed no signs of toxicity. A minimal, nonadverse, increase in immature cells of the megakaryocytic cell series in bone marrow was observed. Bone marrow alterations were not present following a 32-day treatment-free period (day 47)

Safety and Pharmacology of Anti-IL-13 mAb Treatment in Macaques with Allergic Asthma

Inhalation challenge with *A. suum* antigen resulted in an 86% $\pm$ 16% increase in lung resistance and a 60% $\pm$ 6% decrease in dynamic compliance. The antigen-induced increase in lung resistance 1 day after anti-IL-13 mAb treatment was 71% $\pm$ 10% and the decrease in dynamic compliance was 60% $\pm$ 6%. The apparent reduction in lung resistance did not attain statistical significance (*p* = .1907).

Inhalation challenge with *A. suum* antigen resulted in an increase in the number of eosinophils in the BAL at 6 h and 1 day after antigen challenge (Table 2). Macrophage counts did not change in response to *A. suum* challenge and neutrophils were only slightly elevated in a few animals (results not shown). The increase in BAL eosinophils was not inhibited by anti-IL-13 mAb treatment.

TABLE 1
Mean $\pm$ SEM (range) of platelet counts ($\times 10^3/\mu$ l) in normal cynomolgus macaques treated with anti-IL-13 mAb and challenged with *A. suum* antigen. Platelet counts were not affected by inhalation of antigen or by anti-IL-13 mAb treatment (Table 2).

	Pretreatment	Post treatment (day 19)	Recovery (day 47)	<i>p</i> value versus pretreatment
Saline control	391 $\pm$ 20 (316–487) (<i>n</i> = 10)	424 $\pm$ 34 (299–656) (<i>n</i> = 10)	399 $\pm$ 37 (299–477) (<i>n</i> = 4)	.6874
Anti-IL-13 mAb 10 mg/kg	443 $\pm$ 29 (377–534) (<i>n</i> = 6)	379 $\pm$ 28 (289–472) (<i>n</i> = 6)	<i>ND</i>	.0061
Anti-IL-13 mAb 50 mg/kg	429 $\pm$ 12 (376–520) (<i>n</i> = 10)	308 $\pm$ 15** (254–391) (<i>n</i> = 10)	416 $\pm$ 39 (361–529) (<i>n</i> = 4)	<.0001

***p* < .01 versus saline control.

TABLE 2
Eosinophils in BAL fluid and blood from allergic macaques challenged with *Ascaris suum*

	BAL eosinophils ($\times 10^3/\mu\text{l}$)		Blood eosinophils ($\times 10^3/\mu\text{l}$)	
	Saline session 1	Treatment session 2	Saline session 1	Treatment session 2
Prior to mAb (day -1)	—	—	0.53 ± 0.09	0.61 ± 0.16
Prior to <i>A. suum</i> (day 1)	1.18 ± 0.62	6.84 ± 4.56	0.43 ± 0.11	0.86 ± 0.20
6 hours after <i>A. suum</i> (day 1)	6.75 ± 2.04	9.61 ± 2.94	—	—
1 day after <i>A. suum</i> (day 2)	16.84 ± 4.74	15.59 ± 5.38	0.52 ± 0.08	0.61 ± 0.11
1 week after <i>A. suum</i> (day 8)	—	—	0.74 ± 0.22	0.73 ± 0.19

Inhalation of *A. suum* antigen resulted in an elevation of BAL IgE and eotaxin concentrations at 6 h post challenge (Figure 1). By 24 h post challenge, the BAL eotaxin concentrations returned to close to the prechallenge levels, whereas the IgE concentrations remained elevated. The single-inhalation challenge with *A. suum* in this model did not result in an increase in serum IgE concentrations (results not shown).

Prior to *A. suum* challenge, BAL concentrations of IL-13 were below the limit of detection of the ELISA (Figure 2). At 6 h after the *A. suum* challenge, there was an increase in BAL concentrations of IL-13 and also detectable concentrations of IL-13 in the serum. The BAL concentrations decreased towards baseline by 24 h post challenge. Following anti-IL-13 mAb treatment, there was a tendency towards increased detection of IL-13 in both the BAL and the serum.

A single administration of anti-IL-13 mAb produced a significant reduction in the serum concentrations of eotaxin at 6 h post dose (Figure 3). The mean BAL eotaxin concentration was also numerically reduced in the IL-13-treated macaques versus controls but this difference did not attain statistical significance.

Treatment of asthmatic macaques with anti-IL-13 mAb produced a slight but statistically significant reduction in circulating platelets (Table 3).

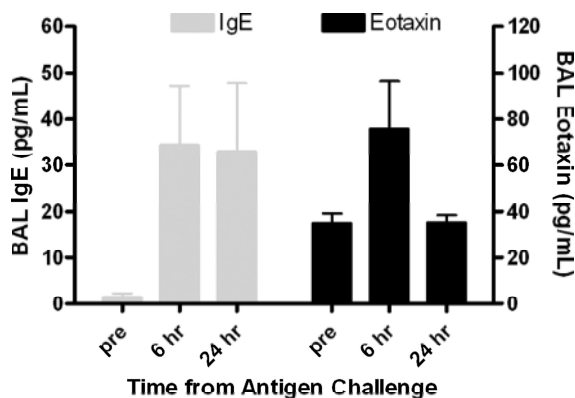


FIGURE 1

Effects of inhaled *A. suum* on BAL fluid IgE and eotaxin concentrations. Each bar represents the mean $\pm$ SEM of data from eight cynomolgus macaques.

DISCUSSION

These studies show that administration of an anti-human IL-13 mAb at doses of 10 and 50 mg/kg for one to three doses was well tolerated in normal and asthmatic cynomolgus macaques. The cynomolgus macaque was selected as a suitable species for evaluation of the preclinical safety and pharmacology of the anti-human IL-13 mAb based upon the in vitro studies that demonstrated that the anti-human IL-13 mAb inhibited the binding of both human and cynomolgus IL-13 to the IL-13R α 1 and IL-13R α 2 receptors and inhibited human and cynomolgus macaque IL-13-mediated STAT6 phosphorylation and eotaxin production.

The only treatment-related effect observed in the preclinical safety study in the normal macaques was a significant reduction in platelets. This effect was also observed in the asthmatic animals and was apparent as early as one day after the first dose. Even though there was a consistent decrease in platelets in all anti-IL-13 mAb-treated animals, the platelet counts did not fall outside of the historical control range of 240,000 to 630,000 per

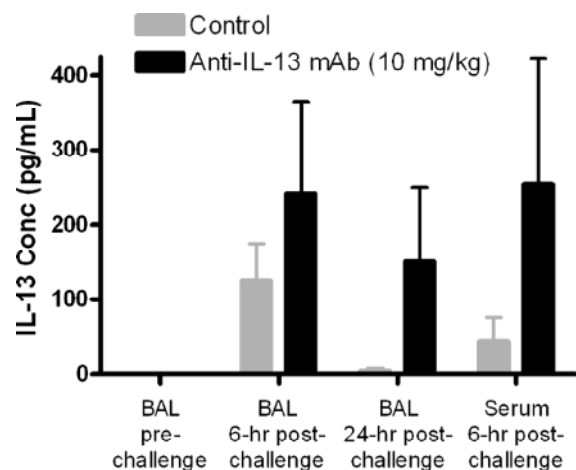


FIGURE 2

Serum IL-13 and BAL concentrations in asthmatic monkeys measured before and after *A. suum* challenge and before and after anti-IL-13 mAb treatment.

Each bar represents the mean $\pm$ SEM of data from eight cynomolgus macaques.

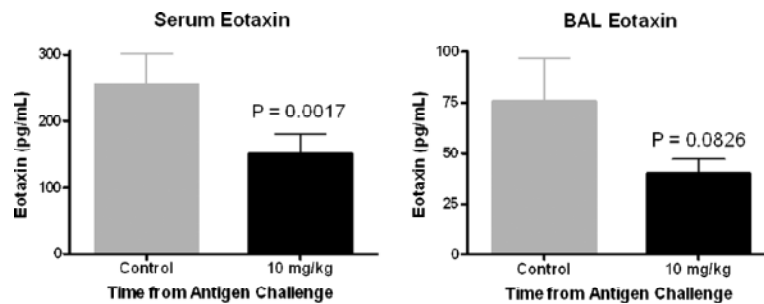


FIGURE 3

Effects of anti-IL-13 mAb treatment on BAL and serum eotaxin concentrations at 6 h after inhaled *A. suum* challenge. Each bar represents the mean $\pm$ SEM of data from eight animals.

μ l for cynomolgus macaques. No animals were considered to be thrombocytopenic, which for patients is defined as a platelet count below 150,000 cells per μ l. There was no evidence of a progressive decrease in platelet counts with continued dosing because the animals that received three doses had similar decreases in platelet counts to the animals receiving a single dose. The platelet counts showed partial recovery by 1 week post dose in the asthmatic animals dosed at 10 mg/kg and by 4 weeks after the last dose in normal animals dosed with three weekly 50 mg/kg doses. At the time of the terminal necropsy (4 days after the last treatment in normal macaques), a slight increase in megakaryocytes was observed histologically in the bone marrow. This effect was also not considered to be of toxicological significance and is likely a secondary response to the transient decrease in platelets.

The reason for the slight decrease in platelets is not apparent from these studies. A few published studies have suggested that IL-13 may play a role in megakaryocyte maturation. Messenger RNA for IL-13 has been shown to be present in megakaryocyte cell lines, suggesting a possible autocrine or paracrine action on platelet formation (Soslau et al. 1997). In vitro studies have also shown that IL-13 stimulates the proliferation of megakaryocytes (Xi et al. 1995). It is therefore possible that IL-13 may have a

minor role in the regulation of platelet production in macaques. Evaluation of the effects on platelets with other IL-13 antagonists will be of value in determining whether the reduction in platelets is related to endogenous IL-13 inhibition or is a nonspecific effect of this particular antibody.

The in vivo pharmacology of the anti-IL-13 mAb was evaluated in a macaque model of asthma. The macaque model of allergic asthma shows many similarities to humans both in terms of respiratory physiology and inflammatory mechanisms and is an acceptable model for predicting human responses (Coffman and Hessel 2005). The macaque model has the additional advantage in that many human therapeutic proteins show pharmacological activity in non-human primates but not in rodents. This is the case for the anti-IL-13 mAb used in these studies, which was shown to effectively neutralize the effects of cynomolgus macaque IL-13 but not rodent IL-13. Therefore, in order to evaluate the safety and efficacy of this mAb, a nonhuman primate model was required.

The inhalation of *A. suum* in the allergic asthmatic macaques was associated with an immediate, transient increase in lung resistance and decrease in dynamic compliance. By 6 h post challenge, there were increases in lung IL-13, eotaxin, and IgE and by 24 h post challenge, there was a marked lung eosinophilia. The administration of a single dose of anti-IL-13 mAb, 1 day

TABLE 3

Mean $\pm$ SEM (range) of platelet counts ($\times 10^3/\mu$ L) in allergic cynomolgus macaques treated with a single dose of anti-IL-13 mAb and challenged with *Ascaris suum*

	Prechallenge, pretreatment	Prechallenge, 1 day post treatment	1 day post challenge	1 week post challenge
Saline control	363 $\pm$ 18 (317–470) (n = 8)	333 $\pm$ 14 (297–422) (n = 8)	352 $\pm$ 26 (263–512) (n = 8)	407 $\pm$ 22 (324–530) (n = 8)
Anti-IL-13 mAb 10 mg/kg	398 $\pm$ 29 (321–560) (n = 8)	271 $\pm$ 26 (168–404) (n = 8)	283 $\pm$ 25 (177–403) (n = 8)	340 $\pm$ 21 (268–465) (n = 8)
p value ^a	—	.0207	.0048	.0025

^aVersus saline control period.

prior to allergen challenge, did not affect the immediate transient changes in lung function, the lung eosinophilia, or IgE concentrations. The antigen challenge protocol employed in this study, which used a single antigen challenge, was not sufficient to induce an increase in serum IgE concentrations. The lack of a treatment effect on lung resistance and lung IgE concentrations is not unexpected because a number of studies conducted in mice have shown that many of the acute changes that occur following antigen challenge may not be entirely dependent upon IL-13, whereas the chronic changes that occur in asthma, such as mucus production and lung remodeling, are IL-13 dependent (Wills-Karp et al. 1998; Yang et al. 2004, 2005; Leigh et al. 2004). Because mucus production and lung remodeling can occur by mechanisms independent of eosinophils and/or IgE, a lack of inhibition of eosinophilia or total IgE in this acute monkey model cannot be interpreted as a lack of predictive value for the treatment of chronic asthma.

In a more chronic model of allergic asthma in cynomolgus macaques, treatment with an anti-human IL-13 IgG4 monoclonal antibody (CAT-354; 30 mg/kg intravenously every 4 days) was shown to produce a significant reduction in histamine induced AHR and a reduction in serum IgE (May et al. 2005; Monk et al. 2005). In those studies, a double *A. suum* antigen challenge was administered prior to a histamine provocation, which was sufficient to produce an elevation of serum IgE concentrations. In another published macaque study, a reduction in BAL eosinophil and neutrophil numbers was observed following treated with an anti-human IL-13 mAb (Bree et al. 2007). In that study, the macaques were given a segmental lung challenge with *A. suum* rather than by inhalation, which produced a profound localized airway inflammation that was associated with a significant neutrophilia in addition to the eosinophilia. Collectively, the animal disease models show that inhibitors of IL-13, including anti-human IL-13 mAbs, can inhibit various key parameters involved in the pathogenesis of asthma but that the parameters that are affected are dependent upon the type of model that is utilized.

One potential limitation of the current study is that the effects of vehicle treatment and anti-IL-13 treatment were evaluated serially in the same monkeys with a 3-week rest period between antigen challenges. Potential carryover of inflammatory effects from the vehicle treatment period to the anti-IL-13 treatment period cannot therefore be fully excluded. This is particularly relevant for the lung eosinophil counts because previous studies have shown that the eosinophils responses in *A. suum*-challenged macaques increases between one and three antigen challenges when administered biweekly (Young et al. 1999). In the current study, the BAL eosinophil numbers were greater prior to the second antigen challenge (anti-IL-13 treatment period) versus the first antigen challenge (vehicle treatment period), suggesting that BAL eosinophil number may not have fully returned to baseline by the time of the second challenge. This may have masked a potential treatment related effect on eosinophil numbers.

Serum IL-13 concentrations measured following anti-IL-13 mAb treatment tended to be greater than in the saline-treated animals. This is most likely due to the detection of macaque IL-13 complexed to the anti-human IL-13 mAb because the IL-13 ELISA assay cannot differentiate between free IL-13, and IL-13 bound to mAb. This phenomenon has been described for other anti-cytokine monoclonal antibodies in macaques (Martin et al. 2004). However, the possibility that the increased concentrations of IL-13 detected during the second phase of the study (anti-IL-13 treatment phase) represent a heightened inflammatory response following the second allergen challenge cannot be completely ruled out.

Despite the potential limitations of this macaque asthma model, this study did demonstrate that treatment of asthmatic macaques with a single dose of anti-human IL-13 mAb was sufficient to result in a significant reduction in the allergen-induced serum eotaxin concentrations. There was also a similar reduction in BAL fluid eotaxin concentrations but this decrease did not attain statistical significance, probably because of the greater degree of variability in the BAL eotaxin data versus the serum eotaxin data. These studies demonstrate that the anti-human IL-13 mAb shows in vitro and in vivo pharmacological activity in cynomolgus macaques and that measurement of serum eotaxin concentrations in patients may be a sensitive early surrogate marker for assessing the pharmacological effectiveness of IL-13 inhibition in vivo.

In summary, these studies have shown that inhibition of IL-13 using an anti-IL-13 mAb is safe and is effective at inhibiting eotaxin concentrations in a macaque model of asthma. Serum eotaxin concentrations may therefore prove to be a useful in vivo marker for evaluating the biological activity of IL-13 inhibition in patients with asthma.

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