ORIGINAL ARTICLE

Passive Prophylactic Administration with a Single Dose of Anti–Fel d 1 Monoclonal Antibodies REGN1908–1909 in Cat Allergen–induced Allergic Rhinitis

A Randomized, Double-Blind, Placebo-controlled Clinical Trial

Mohamed H. Shamji[1], Iesha Singh[1], Janice A. Layhadi[1], Constance Ito[1], Angeliki Karamani[1], Lubna Kouser[1], Hanisah Sharif[1], Jiaqian Tang[1], Sava Handijiev[1], Rebecca V. Parkin[1], Stephen R. Durham[1], Ana Kostic[2], Jamie M. Orengo[2], Michelle DeVeaux[2], Mohamed Kamal[2], Neil Stahl[2], George D. Yancopoulos[2], Claire Q. Wang[2], and Allen R. Radin[2]

[1]Immunomodulation and Tolerance Group, Allergy and Clinical Immunology, Inflammation, Repair and Development, Medical Research Center–Asthma United Kingdom Centre in Allergic Mechanisms of Asthma, National Heart and Lung Institute, Imperial College London, London, United Kingdom; and [2]Regeneron Pharmaceuticals, Inc., Tarrytown, New York

ORCID IDs: 0000-0003-3425-3463 (M.H.S.); 0000-0002-2695-6508 (J.M.O.).

Abstract

Rationale: Sensitization to Fel d 1 (*Felis domesticus* allergen 1) contributes to persistent allergic rhinitis and asthma. Existing treatment options for cat allergy, including allergen immunotherapy, are only moderately effective, and allergen immunotherapy has limited use because of safety concerns.

Objectives: To explore the relationship among the pharmacokinetic, clinical, and immunological effects of anti–Fel d 1 monoclonal antibodies (REGN1908–1909) in patients after treatment.

Methods: Patients received REGN1908–1909 ($n = 36$) or a placebo ($n = 37$) in a phase 1b study. Fel d 1–induced basophil and IgE-facilitated allergen binding responses were evaluated at baseline and Days 8, 29, and 85. Cytokine and chemokine concentrations in nasal fluids were measured, and REGN1908–1909 inhibition of allergen–IgE binding in patient serum was evaluated.

Measurements and Main Results: Peak serum drug concentrations were concordant with maximal observed clinical response. The anti–Fel d 1 IgE/cat dander IgE ratio in pretreatment serum correlated with Total Nasal Symptom Score improvement. The allergen-neutralizing capacity of REGN1908–1909 was observed in serum and nasal fluid and was detected in an inhibition assay. Type 2 cytokines (IL-4, IL-5, and IL-13) and chemokines (CCL17/TARC, CCL5/RANTES [regulated upon activation, normal T-cell expressed and secreted]) in nasal fluid were inhibited in REGN1908–1909–treated patients compared with placebo ($P < 0.05$ for all); IL-13 and IL-5 concentrations correlated with Total Nasal Symptom Score improvement. *Ex vivo* assays demonstrated that REGN1908 and REGN1909 combined were more potent than each alone for inhibiting FcεRI- and FcεRII (CD23)–mediated allergic responses and subsequent T-cell activation.

Conclusions: A single, passive-dose administration of Fel d 1–neutralizing IgG antibodies improved nasal symptoms in cat-allergic patients and was underscored by suppression of FcεRI-, FcεRII-, and T-helper cell type 2–mediated allergic responses.

Clinical trial registered with www.clinicaltrials.gov (NCT02127801)

Keywords: cat allergy; Fel d 1; IgG monoclonal antibodies; immunotherapy; blocking antibodies

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Correspondence and requests for reprints should be addressed to Mohamed H. Shamji, B.Sc., M.Sc., Ph.D., Immunomodulation and Tolerance Group, Allergy and Clinical Immunology, Section of Inflammation, Repair and Development, National Heart and Lung Institute, Imperial College London, First Floor, Room 111, Sir Alexander Fleming Building, South Kensington Campus, London SW7 2AZ, UK. E-mail: m.shamji@imperial.ac.uk.

This article has a related editorial.

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At a Glance Commentary

Scientific Knowledge on the Subject: Induction of allergen-specific IgG antibodies and associated shifts from T-helper cell type 2 (Th2)- to Th1-type responses, induction of regulatory T cells, and production of allergen-neutralizing antibodies have been implicated in the observed benefits of allergen immunotherapy for cat allergy. However, such therapy requires a long duration and may be associated with the potential for severe adverse effects.

What This Study Adds to the Field: We showed that a single subcutaneous prophylactic dose of a combination of two anti–Fel d 1 (*Felis domesticus* allergen 1) monoclonal antibodies (REGN1908–1909) reduced nasal symptoms in cat-allergic patients challenged with cat allergen by suppressing FcεRI-, FcεRII-, and Th2-mediated allergic responses.

Sensitization to the major cat allergen, Fel d 1 (*Felis domesticus* allergen 1), is a main contributor to persistent allergic rhinitis with or without asthma (1). The risk of asthma-like respiratory symptoms upon cat allergen exposure increases with increasing levels of the allergen-specific IgE (sIgE) for cat dander (2).

Although cat allergy management relies on oral antihistamines and nasal corticosteroids (3–5), allergen immunotherapy (AIT) is indicated for those who do not respond to symptomatic pharmacotherapy (1). AIT involves repeated long-term subcutaneous administration of the sensitizing allergen for at least 3 years. AIT is clinically effective for allergic rhinitis and asthma (6–12), but efficacy is equivocal for cat allergy (13). Furthermore, because AIT may be associated with severe and occasionally life-threatening reactions in people with asthma (14, 15), it is contraindicated for people with moderate-to-severe asthma who have a cat-induced allergy.

The disease-modifying effects of AIT are attributed to shifts from T-helper cell type 2 (Th2) to Th1 responses, the induction of regulatory T cells, and the production of allergen-neutralizing antibodies, with the induction of allergen-specific IgG (sIgG) antibodies consistently being observed (1, 16–18). Through direct competition with IgE for allergen binding, IgG-associated blocking antibodies are believed to inhibit the allergen-induced release of inflammatory mediators from basophils and mast cells, thus preventing early-phase allergic reactions (1, 19–21). Elevation of the sIgG/sIgE ratio has been also reported to correlate with symptom improvement during AIT (1, 22).

Recently, two fully human IgG4 monoclonal antibodies (mAbs) directed against two distinct, nonoverlapping epitopes on Fel d 1 were developed using Regeneron's VelocImmune Human antibody mouse platform (REGN1908 and REGN1909) (23). Both molecules bind noncompetitively to these epitopes. A phase 1b, randomized, double-blind, placebo-controlled, proof-of-mechanism study determined that passive administration of neutralizing sIgG for Fel d 1 could inhibit cat hair extract–induced allergic nasal symptoms in patients with cat allergy. A single subcutaneous dose of the combined mAbs (REGN1908–1909; 600 mg, 1:1 ratio) in patients with cat allergy rapidly reduced total nasal symptoms by blocking the early-phase allergic response to nasal allergen challenge (NAC) with cat allergen and significantly reduced mean wheal size after skin prick testing with cat allergen within a week of initial dosing (23).

Here, we demonstrate that REGN1908–1909 administration suppresses early- and late-phase allergic responses in patients after NAC and further elucidate the mechanism of passive administration with these mAbs for cat allergy. By exploring the correlation among pharmacokinetics, biomarkers, and clinical outcomes, we aimed to gain new mechanistic insights into how anti–Fel d 1 antibodies modulate the local allergic response to cat allergen.

Methods

Study Design

This randomized, double-blind, placebo-controlled, phase 1b trial evaluated the efficacy of REGN1908–1909 (Regeneron Pharmaceuticals) for inhibiting the allergic response after NAC. Part of this study, including outcome data on the Total Nasal Symptom Score (TNSS) and peak nasal inspiratory flow (PNIF), was previously published, as were clinical characteristics of the subjects and details of the protocol (23). This study was approved by the sponsor (Regeneron Pharmaceuticals) and Ethics Committees (Stichting Beoordeling Ethiek Biomedisch Onderzoek, The Hague, the Netherlands; Health and Disability Ethics Committees, Wellington, New Zealand; Regional Ethical Review Board in Lund, Lund, Sweden; Health Research Authority—National Research Ethics Service, Manchester, United Kingdom). All participants provided written informed consent (ClinicalTrials.gov identifier NCT02127801).

NAC

The NAC, performed as previously described (24), used increasing doses of cat hair extract (100–330, 1,000–3,300, 10,000–33,000 SQ-U/ml) applied with an Aptar Biodose device delivering 100 μl per nostril every 10 minutes for 1 hour, or until a TNSS (0–12) $\geq$7 was reached. TNSS is a composite assessment of congestion, itching, and rhinorrhea (each scored 0–3; 3 = severe), and sneezing (3 = >5 sneezes). Cat-sensitized allergic patients were eligible for enrollment if the TNSS was $\leq$2 before the screening NAC (baseline) and the peak TNSS was $\geq$7 within the first hour (early-phase response) after NAC. In addition, the nasal symptom visual analog scale (VAS) score (0–100) and PNIF (L/min) were prespecified endpoints. Patients were randomized to receive a single subcutaneous dose (as three 2-ml injections) of blinded REGN1908–1909 cocktail at 600 mg (300 mg of each mAb) or a placebo on Study Day 1 (14 d after the screening visit). NAC was conducted on Study Days 8, 29, 57, and 85 using the titration procedure performed at screening. The treatment response was measured as the reduction in the TNSS from baseline at each subsequent NAC. The primary efficacy analysis included the change in the TNSS area under the curve (AUC) over the first hour after NAC ($AUC_{0-1\ hr}$) as an early-phase response and included the change in the TNSS AUC from hours 1 to 6 after NAC ($AUC_{1-6\ hr}$) as a late-phase response.

Immunological Analysis

Isolation of peripheral blood mononuclear cells (PBMCs), nasal fluid collection, multiplex cytokine assays, allergen-induced basophil activation testing in patient whole blood, B-cell antigen presentation, T-cell proliferation assays, and allergen-induced basophil activation tests are described in the online supplement.

IgE-facilitated Allergen Binding

Sera and nasal fluids from REGN1908–1909–treated and placebo-treated

patients were used to evaluate inhibition of allergen–IgE complex binding to low-affinity B-cell receptors (CD23), as previously described (25), with quantitative analysis (FACS Canto II flow cytometry; BD Bioscience).

The optimal concentration of REGN1908–1909 for inhibiting Fel d 1 allergen and IgE binding on the ImmunoCAP platform (Thermo Fisher Scientific) was determined. REGN1908–1909 was spiked at 20 concentrations (0.146 μg/ml to 242 μg/ml) into commercially available serum samples pooled from cat-allergic subjects; 75 μg/ml was selected as the optimal interference concentration. Baseline pretreatment serum samples from 26 patients in the REGN1908–1909 treatment group who consented for future research were analyzed. All samples produced uninhibited and inhibited results above the sIgE ImmunoCAP lower limit of quantitation (0.10 kU_A/L). Inhibitory effects of REGN1908–1909 for each patient were calculated by using the percent signal inhibition: *percent signal inhibition* = ([*uninhibited sample* − *inhibited sample*]/*uninhibited sample*) × 100.

Statistical Analysis

Statistical analyses were performed using Prism version 7.0 (GraphPad Software). For *ex vivo* and *in vitro* assays, between-group and within-group comparisons were performed using the Mann-Whitney *U* test and the Wilcoxon matched-pairs, signed-rank test, respectively. The Benjamini-Hochberg correction was used to control for multiple comparisons of cytokines (26). Differences in the relationship between the cytokine AUC and the percent improvement in the TNSS AUC in REGN1908–1909 versus placebo were assessed by testing the treatment-by-cytokine interaction term in a linear model with the percent reduction in TNSS AUC as the dependent variable. An analysis of covariance model was used for nasal symptom assessment. For the basophil activation test and IgE-facilitated allergen binding (FAB) assay, the Mann-Whitney *U* test was used to compare treatment groups; *P* values <0.05 were considered to indicate statistical significance.

Results

REGN1908–1909 Improved Nasal Symptoms and PNIF

Treatment groups consisted of 36 REGN1908–1909–treated patients and 37 placebo-treated patients (*see* Table E1 in the online supplement), between whom clinical efficacy was compared at 0–1 hour after NAC to cat hair extract. Consistent with significant reductions from baseline in TNSS $AUC_{0\text{-}1hr}$ post-NAC in REGN1908-1909- versus placebo-treated patients (23), significant reductions from baseline in $AUC_{0\text{-}1hr}$ were observed for VAS and increases for PNIF on Days 8, 29, and 85 (Table E2); loss of statistical significance on Day 57 may have been due to the dropout of 2 patients on that visit day (Table E3).

In a new *post hoc* analysis, we explored the relationship between pharmacokinetics and pharmacodynamics in patients who received treatment with REGN1908–1909. Serum concentrations of REGN1908 and REGN1909 peaked at Day 8 and steadily declined to Day 85 (Figures 1A and 1B). Peak nasal symptom improvements (VAS score and PNIF) were concurrent with the peak drug concentration (Day 8) and remained statistically significant until Day 29 ($P < 0.05$ for all) (Figures 1A and 1B). On Day 85, the serum concentration of REGN1908–1909 was approximately 10 mg/L, substantially higher than sIgG4 (specific to cat dander) concentrations induced after 1 year of AIT (~1.8 μg/ml) (8), and VAS-score and PNIF improvements were still numerically greater than those from placebo (Figures 1A and 1B).

The proportions of REGN1908–1909–treated patients who had 50%, 75%, and 90% reductions in the VAS score after NAC were significantly higher than those of placebo-treated patients at Days 8 and 29 ($P < 0.05$ for all), and these proportions were numerically higher on Day 85 (Figures 1C–1E). The VAS score, an additional subjective clinical outcome measure, had not been previously reported by Orengo and colleagues (23). These data highlight the effectiveness and durability of REGN1908–1909 in blocking the early-phase allergic response after cat hair extract exposure.

Pretreatment Concentration of Serum Anti–Cat Dander IgE and Anti–Fel d 1 IgE Correlated with TNSS Improvement

It was hypothesized that sIgG ameliorates the allergic response via direct competition with sIgE for allergen binding. To understand how the relative ratio of anti–Fel d 1 IgG4 mAbs and serum sIgE may impact the treatment response, correlation analyses were conducted between the baseline sIgE concentrations and the TNSS response. Anti–cat dander IgE inversely correlated with the percent improvement in the TNSS on Days 29 ($r = -0.58$; $P < 0.001$), 57 ($r = -0.54$; $P = 0.001$), and 85 ($r = -0.50$; $P = 0.002$) (Figure 2A). Similarly, anti–Fel d 1 IgE inversely correlated with the percent improvement in the TNSS on Day 29 ($r = -0.45$; $P = 0.007$) but did not correlate with the percent improvement on Days 57 or 85 (Figure 2B). These correlations indicate that the greater the sIgG versus sIgE ratio, the more effective REGN1908–1909 may be in competing with endogenous sIgE in patient serum. Furthermore, direct correlations were observed between the anti–Fel d 1 IgE/anti–cat hair dander IgE ratio and percentage reduction in the TNSS $AUC_{0-1\ hr}$ (Figure 2C) on Days 29 ($r = 0.36$, $P = 0.03$), 57 ($r = 0.57$, $P < 0.001$), and 85 ($r = 0.63$, $P < 0.001$), suggesting greater benefits with REGN1908–1909 if the predominant drivers of allergic symptoms are anti–Fel d 1 IgEs rather than IgEs against cat allergens Fel d 2, 4, 7, and 8. Improvement in the TNSS $AUC_{0-1\ hr}$ from baseline on Day 8 directly correlated with REGN1908–1909 inhibition of Fel d 1 binding to endogenous IgE ($r = 0.48$; $P = 0.015$) (Figure 2D). It should be noted that Fel d 1 sIgE readout appears to be higher than cat dander IgE in some subjects because of higher binding affinity and accessibility of the recombinant Fel d 1 peptide to serum IgE on the ImmunoCAP assay platform relative to the crude cat dander extract. This set of correlation analyses may be further expanded in future studies for the development of assays that may predict the patient response.

REGN1908–1909 Attenuates Fel d 1–induced Basophil Responsiveness

In *ex vivo* assays, Fel d 1 elicited concentration-dependent increases in basophil activation ($CD63^+CRTH2^+$ basophils) from whole blood of REGN1908–1909–treated patients ($n = 4$) and placebo-treated patients ($n = 5$) from a single site. No difference in response was observed at baseline between treatment groups, possibly because of the small sample. A rightward shift of the response curve to allergen stimulation was observed in REGN1908–1909–treated patients versus placebo-treated patients at Day 29 (Figure E1), suggesting a trend for REGN1908–1909 suppression of FcεRI-mediated allergic responses.

REGN1908–1909 Treatment Inhibited CD23-mediated Proallergic Responses

Using FAB assays, we observed significant reductions in the relative binding of allergen–IgE complexes to CD23 on B cells in the sera of REGN1908–1909–treated

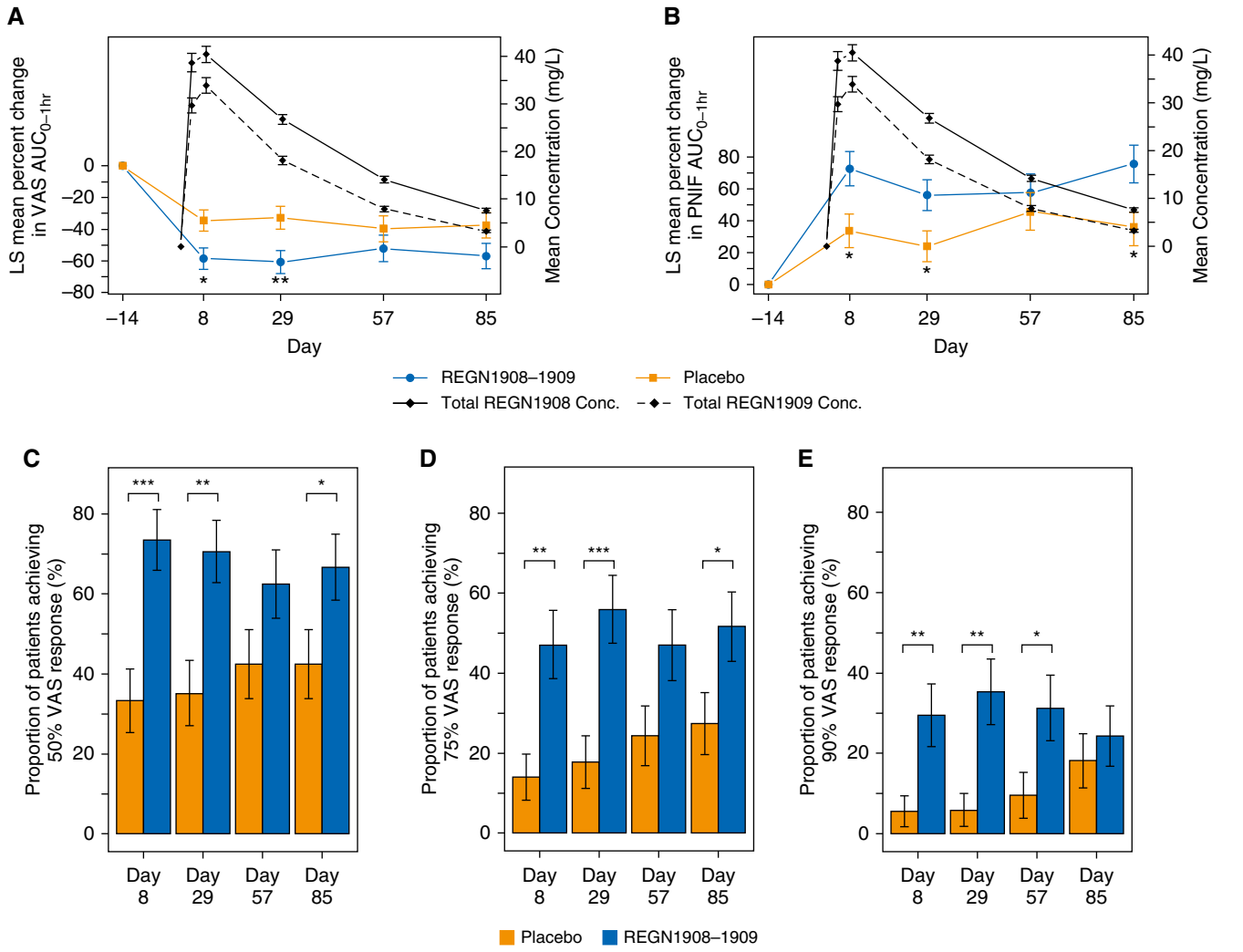

Figure 1. Peak blood concentrations of two anti–Fel d 1 (*Felis domesticus* allergen 1) monoclonal antibodies (REGN1908 and REGN1909) were concordant with the maximal observed clinical response, supporting a direct pharmacodynamic effect. Concentrations of REGN1908 and REGN1909 in the serum of REGN1908–1909–treated patients. (*A*) Visual analog scale (VAS) score and (*B*) peak nasal inspiratory flow (PNIF) were also measured in REGN1908–1909–treated patients and placebo-treated patients at Day −14 (screening), Day 8, Day 29, Day 57, and Day 85. Analyses are based on analysis of covariance with treatment as a factor and baseline as a covariate. The proportion of patients who responded to REGN1908–1909 treatment also supports a pharmacodynamic effect. Nasal symptom VAS scores were reported by REGN1908–1909–treated patients and placebo-treated patients on Day 8, Day 29, Day 57, and Day 85 and were analyzed using a test for proportion with Yate's correction. Data are presented as proportions of patients who had a reduction in their VAS score by (*C*) 50%, (*D*) 75%, and (*E*) 90%. Time points were treated as factors and Day −14 was treated as a covariate. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$. $AUC_{0–1\ hr}$ = area under the curve over the first hour after nasal allergen challenge; Conc. = concentration; S = least squares.

patients in response to 0.03 μg/ml Fel d 1 at Day 8 compared with baseline ($P < 0.001$) that persisted to Day 85 (all, $P < 0.001$) (Figure 3). In addition, REGN1909–1908 reached local nasal tissue, with sufficient concentrations in nasal fluid to induce a significant reduction in binding of allergen–IgE complexes to B cells *in vitro* on Day 8 compared with baseline ($P < 0.001$) (Figure 3). This inhibitory effect persisted at Days 29 ($P = 0.0042$) and 57 ($P = 0.0311$) but was not detected at Day 85. No inhibition of binding was observed with placebo treatment.

Inhibitory activity of REGN1908–1909 in serum (REGN1908–1909, $n = 27$; placebo, $n = 33$) was associated with the reduction of the TNSS at Days 8, 29, and 85 (Figure E2A); reduction of the VAS score was also observed at these time points (Table E2). This inhibitory effect was consistent with concentrations of REGN1908 and REGN1909 in serum from all time points except Day 57. At Day 85, low serum concentrations of REGN1908 and REGN1909 showed inhibitory effects (Figure E2B). Moreover, among all patients, binding of allergen–IgE complexes to B cells inversely correlated with both TNSS and VAS scores at Days 8, 29, and 85 (Table E3).

REGN1908–1909 Suppressed Local Nasal Fluid Type 2 Cytokines and Chemokines

To capture the late-phase allergic response that typically peaks at 6 hours after NAC, nasal fluid samples were collected from 0 to 8 hours after

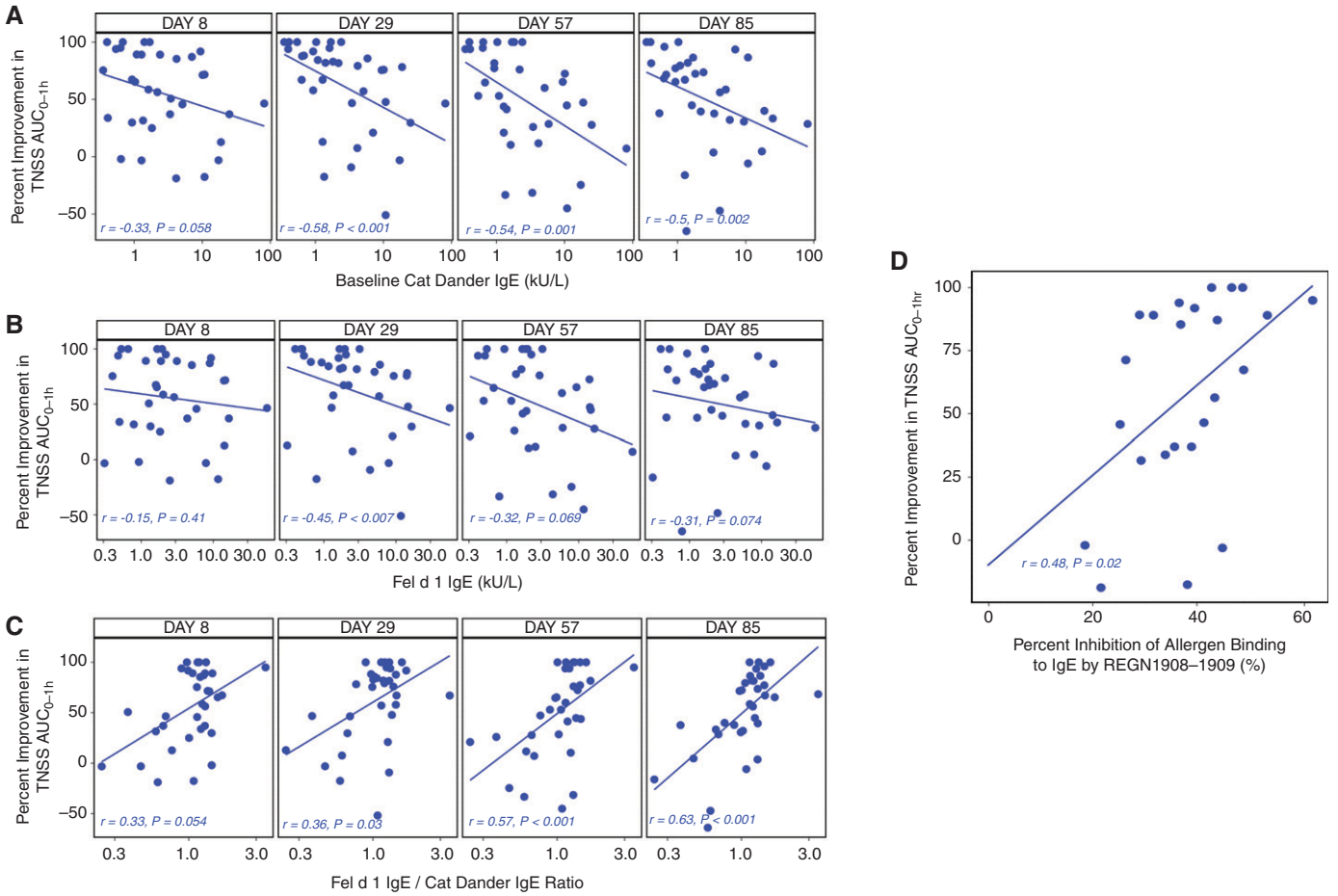

Figure 2. Significant relationships were observed between anti–Fel d 1 (*Felis domesticus* allergen 1)/anti–cat dander IgE and the Total Nasal Symptom Score (TNSS) $AUC_{0–1\ hr}$ response. (*A*) Anti–cat dander IgE is inversely correlated with percentage improvement in TNSS (Spearman's correlation). (*B*) Anti–Fel d 1 IgE inversely correlated with percentage improvement in TNSS (Spearman's correlation). (*C*) The anti–Fel d 1 IgE/anti–cat dander IgE ratio directly correlated with percentage improvement in TNSS (Spearman's correlation). (*D*) Percentage improvement in TNSS at Day 8 correlated with percentage inhibition of Fel d 1 binding to endogenous IgE by anti–Fel d 1 monoclonal antibodies (REGN1908–1909) (Pearson's correlation). $AUC_{0–1\ hr}$ = area under the curve over the first hour after nasal allergen challenge.

NAC (REGN1908–1909, $n = 34$; placebo, $n = 36$). Because of missing samples at 8 hours, only results from 0–6 hours were analyzed for this report. At Day 8, significant inhibition in the induction of the type 2 cytokines IL-4, IL-5, and IL-13 were observed in REGN1908–1909–treated patients versus placebo-treated patients at 6 hours after NAC (all, $P < 0.05$) (Figures 4A–4C). Levels of CCL17/TARC, a chemoattractant for type-2 inflammatory cells, and CCL5/RANTES (regulated upon activation, normal T-cell expressed and secreted) were also lower at 6 hours in REGN1908–1909–treated patients than in placebo-treated patients ($P < 0.05$ for both) (Figures 4D and 4E). Earlier time points did not appear to be significant for these cytokines (Figure E3). Concentrations of eotaxin, IL-9, IL-10, IL-12p40, IL-12p70, IL-17A, IL17E/IL-25, IL-27, IL-33, ECP, and MDC were not significantly different between treatment groups, nor were they different after treatment relative to baseline concentrations (data not shown).

The percent improvement in the TNSS $AUC_{0–1\ hr}$ inversely correlated with the nasal cytokine level $AUC_{1–6\ hr}$ for IL-13 ($P = 0.005$) and IL-5 ($r = -0.59$; $P < 0.001$) (Figure E4) in REGN1908–1909–treated patients, and, for IL-5, this relationship was significantly different for REGN1908–1909–treated patients and placebo-treated patients (test of interaction, $P < 0.05$), suggesting that amelioration of the early-phase allergic response with blocking of sIgE by sIgG may also inhibit mediators of the late-phase response. In addition, the percent improvement in the TNSS $AUC_{1–6\ hr}$ trended toward an inverse correlation with the nasal cytokine level $AUC_{1–6\ hr}$ for IL-13 ($r = -0.34$; $P = 0.08$) and IL-5 ($r = -0.38$; $P = 0.053$) (Figure E4).

REGN1908, REGN1909, and REGN1908–1909 Inhibited FcRI-mediated Basophil Responsiveness *Ex Vivo*

In separate cohorts of cat-allergic and nonatopic control donors that did not receive treatment (Table E4), the proportion of activated basophils ($CD63^+$) was elevated in cat-allergic donors compared with nonatopic control donors when stimulated *ex vivo* with Fel d 1 (3 ng/ml) ($P < 0.05$) (Figures 5A and 5B).

Allergen-induced basophil histamine release was evaluated by quantitative flow cytometry using fluorochrome-labeled DAO (diamine oxidase) (20). *In vitro* expression of $CD63^+$ and histamine release was lower in cat-

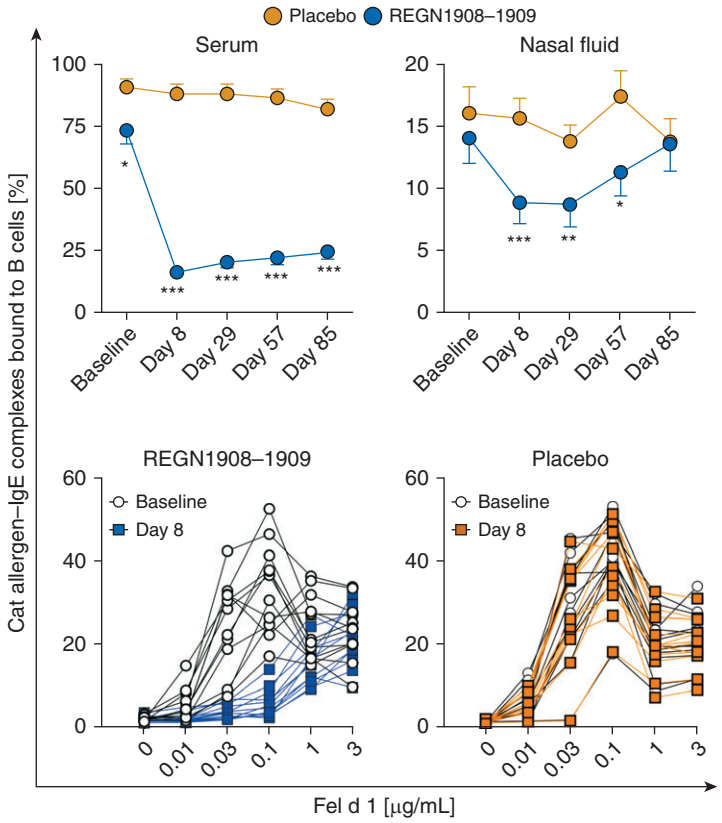

Figure 3. Single subcutaneous injection of a combination of anti–Fel d 1 (*Felis domesticus* allergen 1) monoclonal antibodies (REGN1908–1909) but not placebo suppressed cat allergen–induced, FcεRII (CD23)–mediated proallergic responses. Inhibition of CD23-mediated allergen–IgE binding to B cells was measured in the serum (REGN1908–1909, $n = 27$; placebo, $n = 33$; top left panel) and nasal fluid (REGN1908–1909, $n = 37$; placebo, $n = 36$; top right panel) at baseline, Day 8, Day 29, Day 57, and Day 85. The effect of inhibitory activity in the sera of REGN1908–1909–treated patients or placebo-treated patients (both, $n = 12$; bottom panels) at increasing concentrations of Fel d 1 allergen at baseline and Day 8 is shown. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ using Mann-Whitney *U* test.

allergic donors in the presence of REGN1908, REGN1909, and REGN1908–1909 (10 ng/ml) compared with a control mAb (Figure 5C). Moreover, the proportion of $CD63^{+}CRTH2^{+}$ and $CD203c^{Hi}CRTH2^{+}$ basophils preincubated with REGN1908–1909 was significantly lower when stimulated with Fel d 1 at 1, 3, and 10 ng/ml ($P < 0.05$ for all) compared with a control mAb (Figure 5D). The proportion of $DAO^{-}CD63^{+}$ and $DAO^{-}CD203c^{Hi}$ basophils was significantly lower in the presence of REGN1908 and REGN1909 when stimulated with 3, 10, 33, and 100 ng/ml of Fel d 1 compared with the control mAb (all, $P < 0.05$) (Figure 5E). Using Erk phosphorylation and $CD63^{+}$ upregulation as a proximal readout of basophil activation, we previously showed that REGN1908–1909 prevented greater IgE engagement than each individual antibody alone (23). Here, we show that although each mAb inhibited basophil activation and histamine release, with REGN1908 being more potent than REGN1909, the effects were greater with REGN1908–1909 than with each mAb alone.

REGN1908, REGN1909, and REGN1908–1909 Inhibited CD23-mediated FAB to B Cells (FAB Assay)

Allergen–IgE complexes bound to B cells in a Fel d 1 dose–dependent manner only in sera from cat-allergic donors (Figures 6A and 6B). However, when sera from cat-allergic donors were preincubated with REGN1908–1909, or each mAb alone, binding was inhibited in a dose-dependent manner. REGN1908–1909 had the highest potency (half maximal inhibitory concentration [IC_{50}] = 10.47 ± 1.14 μg/ml [$3.50 \pm 0.38 \times 10^{-8}$ M for each antibody]) relative to REGN1908 (IC_{50} = 33.84 ± 5.39 μg/ml [$22.56 \pm 3.59 \times 10^{-8}$ M] and REGN1909 (IC_{50} = 304.4 ± 52.95 μg/ml [$202.93 \pm 35.30 \times 10^{-8}$ M]) (Figure 6C). No inhibition was observed after preincubation with control mAb. These data support REGN1908–1909 as being more effective than each antibody alone in inhibiting allergic responses.

REGN1908, REGN1909, and REGN1908–1909 Inhibited CD23-mediated IgE-facilitated Allergen Presentation and Th2 Cell Expansion

IgE-bound allergens can be presented to T cells via high-affinity FcεRI (IgE receptors) on monocytes, macrophages, and dendritic cells and via a low-affinity IgE receptor (CD23) after uptake by B cells (27). We thus evaluated the ability of the antibodies to inhibit IgE-facilitated allergen presentation and $CD4^{+}$ T-cell activation *in vitro* (22, 23). $CD19^{+}$ B cells from cat-allergic donors and nonatopic control donors were incubated with Fel d 1 at 0.01, 0.03, and 0.1 μg/ml and with sera containing a high concentration of sIgE for Fel d 1 (>100 kU_A/L) in the presence of REGN1908, REGN1909, and REGN1908–1909 before coculturing with $CD4^{+}$ T cells. The proliferation of $CD4^{+}CD25^{-}$ T cells was observed in cat-allergic donors but not in nonatopic control donors (Figure 6D) and was inhibited in the presence of REGN1908, REGN1909, and REGN1908–1909 versus a control mAb (all $P < 0.01$) (Figure 6E). Furthermore, concentrations of IL-13, IL-17, and IL-23 in culture supernatants were significantly reduced in the presence of REGN1908, REGN1909, and REGN1908–1909 versus the control mAb (all $P < 0.05$) (Figures 6F–6H); IL-23 drives IL-17. These data highlight the ability of REGN1908, REGN1909, and REGN1908–1909 to inhibit $CD4^{+}$ T cell–mediated proallergic responses.

Discussion

Challenges associated with AIT highlight the need for safer alternatives. A hallmark of successful AIT is the induction of sIgG, which putatively competes directly with IgE for allergen binding. Inhibition of the allergic response by IgG may also be mediated by costimulation of the inhibitory IgG receptor FcεRIIb that can negatively regulate FcεRI signaling and inhibit effector-cell activation (28, 29). These postulated beneficial roles of IgG-

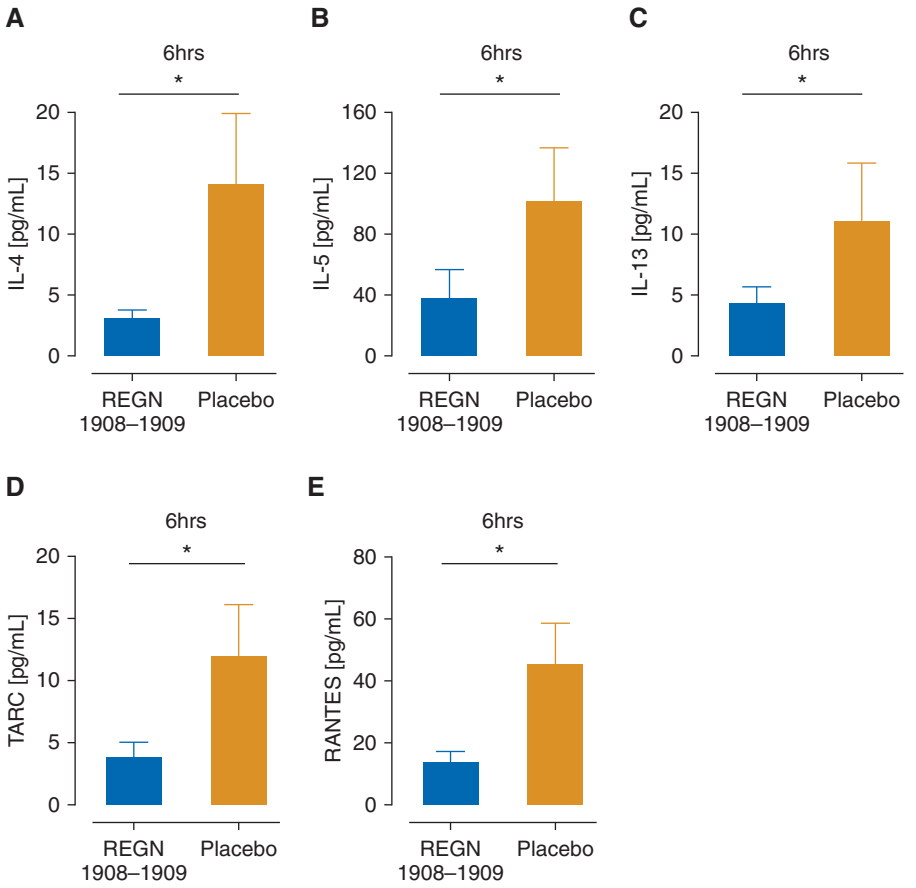

Figure 4. Single-injection of combined anti–Fel d 1 (*Felis domesticus* allergen 1) monoclonal antibodies (REGN1908–1909) inhibited type 2 cytokines and chemokines at a 6-hour time point. Levels of (*A*) IL-4, (*B*) IL-5, (*C*) IL-13, (*D*) TARC, and (*E*) RANTES (regulated upon activation, normal T-cell expressed and secreted) were measured in nasal fluid samples (Day 8) after 6 hours of intranasal cat allergen challenge. Samples were collected from REGN1908–1909– (n = 37) and placebo-treated (n = 36) patients. Data are shown as the mean ± SEM. *$P < 0.05$ using Mann-Whitney U test with Benjamini-Hochberg correction for multiple comparisons.

blocking antibodies led to the development of passive administration with sIgG mAbs as a potential new treatment for allergies.

In a clinical study, two fully human mAbs (REGN1908 and REGN1909) directed against Fel d 1 blocked the acute allergic response after intranasal exposure to cat allergen, as measured by the TNSS (23). Here, we demonstrate that a single subcutaneous dose of the combined antibodies (REGN1908–1909) rapidly suppressed nasal symptoms compared with placebo in adult patients with cat-allergic rhinitis after NAC with cat hair extract within a week of administration. This suppression is in contrast with the slow onset of efficacy for traditional AIT, which typically occurs between 6 and 12 months after treatment initiation. NAC has been shown to correlate with outcomes during immunotherapy studies (both subcutaneous and sublingual) for allergic rhinitis (24). Therefore, our observations suggest the clinical effectiveness of REGN1908–1909. To further understand the mechanisms underlying symptom improvements, biomarker analyses were performed, including basophil sensitivity to allergen stimulation, secretion of type-2 inflammatory cytokines and chemokines in nasal fluid, and FAB assay.

In this study, most of the study patients were polysensitized to other environmental allergens; only one patient in each treatment group was found to be monosensitized to cat allergen. Such polysensitization is consistent with what has been reported in other studies of cat allergenicity (30, 31). Despite being polysensitized, treatment with REGN1908–1909 benefited the majority of cat-allergic patients, but the degree of symptom improvement after nasal provocation varied. Responder analyses showed that patients who achieved greater symptom improvement after treatment had a higher baseline ratio of serum anti–Fel d 1 IgE/anti–cat dander IgE concentrations. Furthermore, inhibition assays showed that percentage inhibition of anti–Fel d 1 IgE by REGN1908–1909 in pretreatment patient serum correlated with TNSS improvements on Day 8. These data indicate that treatment with REGN1908–1909 may be most efficacious when Fel d 1 sIgE is driving allergic symptoms and when REGN1908–1909 effectively competes with endogenous IgE for binding epitopes on Fel d 1 allergen.

Using the FAB assay, we demonstrated that serum and nasal fluid from patients treated with REGN1908–1909 inhibited allergen–IgE complex formation and binding to B cells. The decrease in allergen–IgE complex formation and binding to B cells paralleled rapid clinical improvements (TNSS and VAS score); this is in contrast to AIT, which requires treatment for at least a year (20, 32).

Successful long-term AIT is accompanied by a shift from a Th2 response toward a Th1 response (33–36). Our findings of cytokine and chemokine levels in the nasal fluid after cat allergen NAC confirmed the reduction in Th2 responses whereby prophylactically treated REGN1908–1909 patients had reduced type 2 cytokine (IL-4, IL-5, and IL-13) concentrations compared with placebo-treated patients. IL-13 has been shown to induce eosinophil recruitment into the lung (37, 38). Although we were not able to evaluate nasal eosinophils directly, the observed IL-5 and IL-13 reduction may indicate eosinophil recruitment suppression in the target organ. Despite the reported reduction in Th2 responses, we were unable to detect changes in type 1 cytokine levels. In addition, compared with placebo-treated patients, REGN1908–1909–treated patients were characterized by reductions in the type 2 inflammatory markers RANTES and TARC, which are associated with clinical desensitization during AIT. Although TARC is elevated in the serum of patients with atopic dermatitis (39) and asthma (40, 41), RANTES is elevated in skin (42) and nasal biopsy specimens obtained after allergen challenge (43). Moreover, mRNA and protein levels of RANTES were reduced in PBMCs after rush venom immunotherapy to a level comparable with that of healthy patients (44). It has also been reported that cells expressing TARC, eotaxin, and

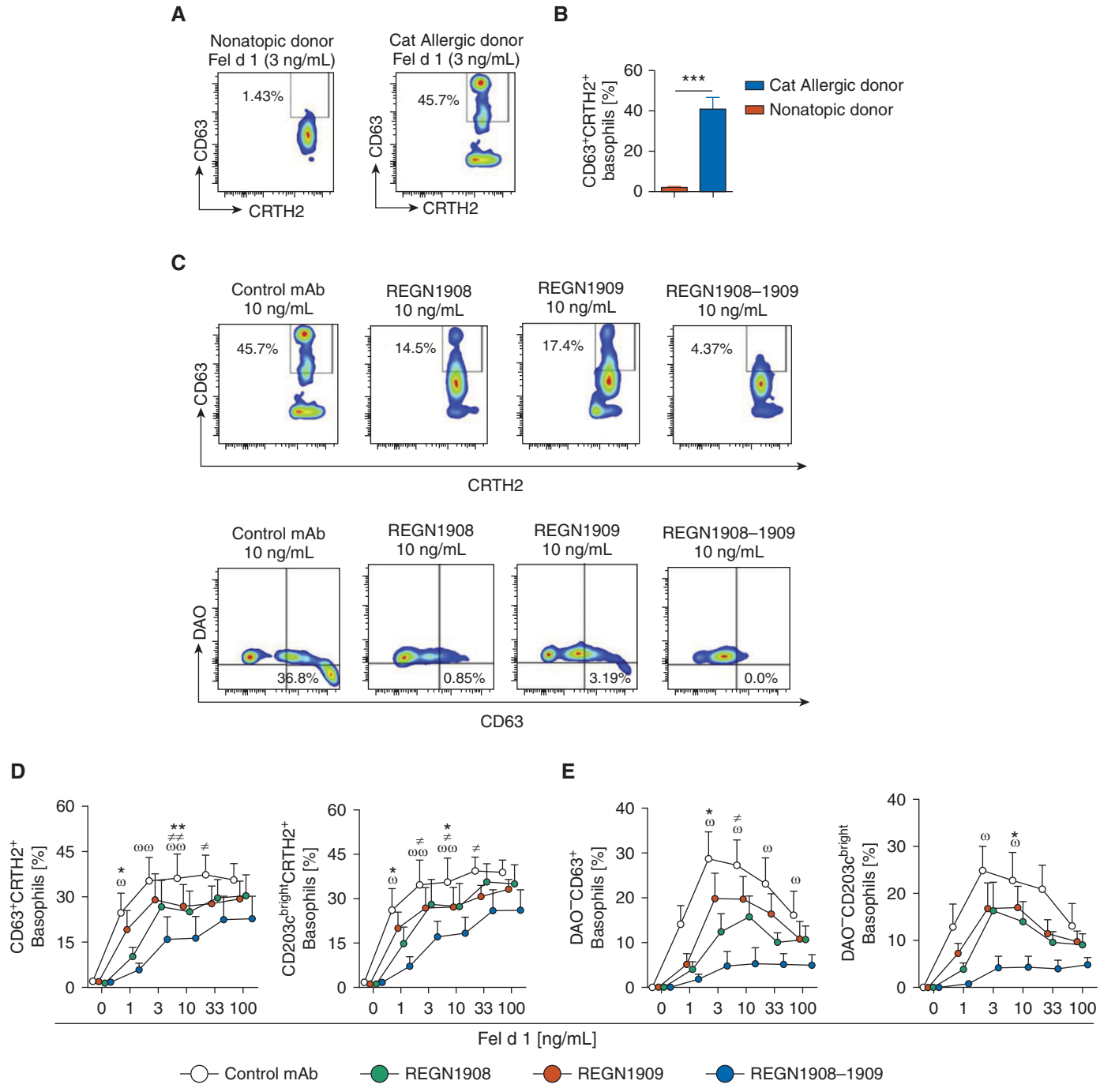

Figure 5. Anti–Fel d 1 (*Felis domesticus* allergen 1) monoclonal antibodies (mAbs) (REGN1908, REGN1909, and REGN1908–1909) inhibited Fel d 1–induced basophil activation and histamine release *in vitro*. The whole blood was stimulated with increasing concentrations of Fel d 1 (0, 1, 3, 10, 33, 100 ng/ml; 100 ng/ml Fel d 1 is equivalent to 5.56×10^{-9} M). (*A*) Representative fluorescence-activated cell sorter plot analysis of $CD63^+CRTH2^+$ basophils in cat-allergic donors and nonatopic control donors at 3 ng/ml Fel d 1. (*B*) Proportion of $CD63^+CRTH2^+$ basophils in cat-allergic donors ($n = 8$) and nonatopic control donors ($n = 9$) in response to Fel d 1 at 3 ng/ml. $^{***}P < 0.001$. (*C*) Representative fluorescence-activated cell sorter plots of $CD63^+CRTH2^+$ and DAO^-CD63^+ basophils in cat-allergic donors after stimulation with 3 ng/ml Fel d 1 in the presence of either REGN1908, REGN1909, REGN1908–1909 or control mAbs at 10 ng/ml. Proportions of (*D*) $CD63^+CRTH2^+$ and $CD203c^{Hi}CRTH2^+$, and (*E*) DAO^-CD63^+ and $DAO^-CD203c^{Hi}$ were quantified in cat-allergic donors by flow cytometry. Data are shown as the mean ± SEM. For *D–E*, $^*P < 0.05$ and $^{**}P < 0.01$ for REGN1908 versus control mAbs, $^{\neq}P < 0.05$ and $^{\neq\neq}P < 0.01$ for REGN1909 versus control mAbs; and $^{\omega}P < 0.05$ and $^{\omega\omega}P < 0.01$ for REGN1908–1909 versus control mAbs. All *P* values were calculated using the Mann-Whitney *U* test. DAO = diamine oxidase.

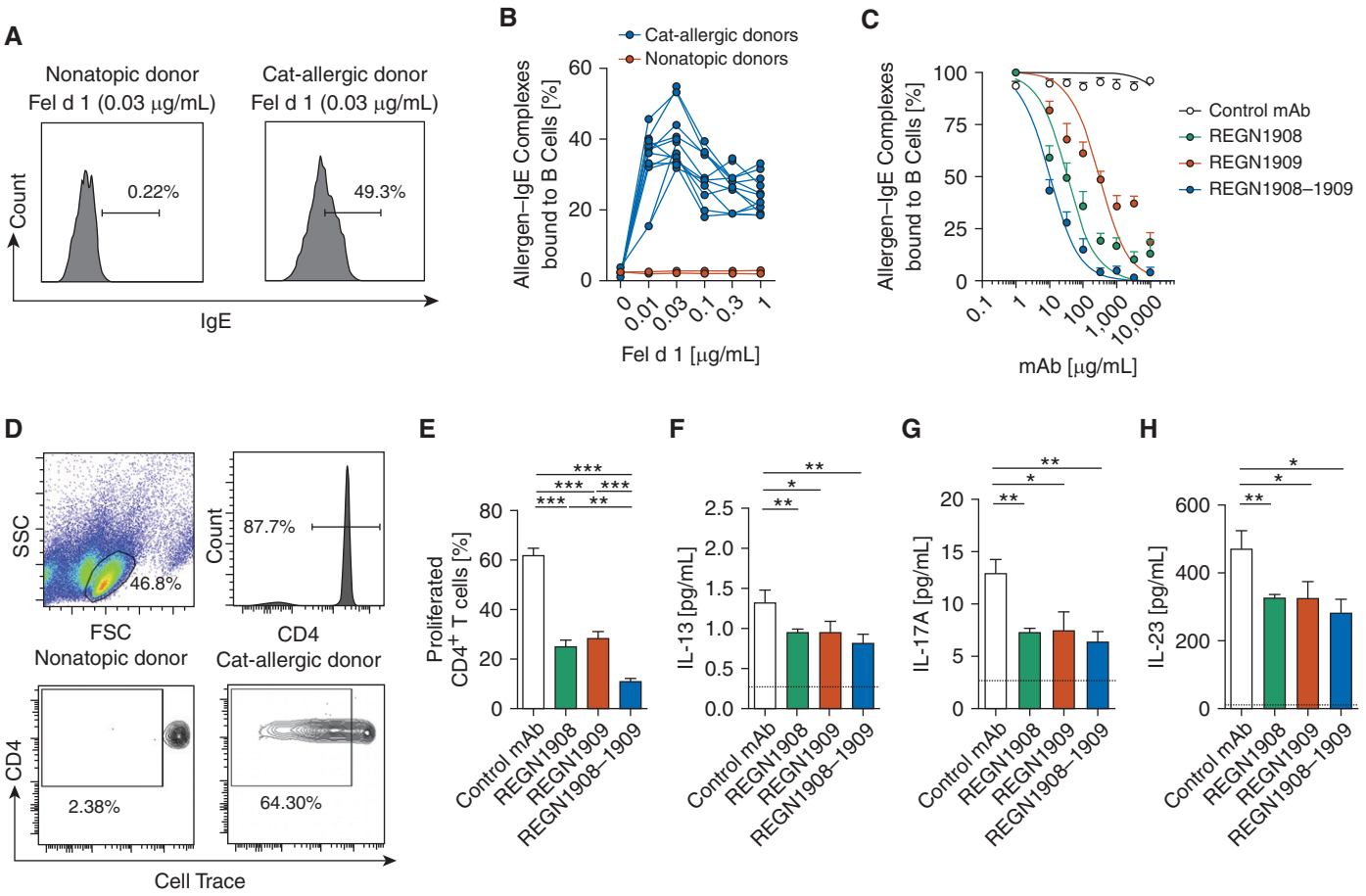

Figure 6. Inhibition of FcεRII (CD23)–mediated IgE-facilitated allergen binding to B cells and presentation to T cells by anti–Fel d 1 (*Felis domesticus* allergen 1) monoclonal antibodies (mAbs) (REGN1908, REGN1909, and REGN1908–1909). (*A*) Representative fluorescence-activated cell sorter plots of allergen–IgE complexes binding to CD23 on B cells from cat-allergic donors and nonatopic control donors. (*B*) Sera from cat-allergic (n = 12) and nonatopic control donors (n = 9) were incubated with increasing concentrations of Fel d 1 at 37°C to allow the formation of allergen–IgE complexes. Binding of cat allergen–IgE complexes to B cells was quantified by flow cytometry. (*C*) Half-maximal inhibitory concentration graph representing the inhibition of cat allergen–IgE complexes binding to B cells in cat-allergic donors. Data are represented as a normalized value of 100% of maximal binding response at 0.03 μg/ml Fel d 1. (*D*) Flow cytometry analysis showing Fel d 1–induced proliferated $CD4^+$ T cells in cat-allergic and nonatopic control donors. (*E*) The effect of REGN1908, REGN1909, REGN1908–1909, and control mAbs on proliferated $CD4^+$ T cells was evaluated in cat-allergic donors (n = 7). Levels of (*F*) IL-13, (*G*) IL-17A, and (*H*) IL-23 in cell culture supernatants were measured by using a MagPix Luminex assay. Data are shown as the mean ± SEM. $^*P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ using Mann-Whitney U test. FSC = forward scatter; SSC = side scatter.

RANTES in the nasal mucosa are reduced after 3-year birch immunotherapy (45).

A previous *in vitro* study linked increased sIgE in the serum of allergic patients with increased allergen-driven T-cell proliferation (46). After long-term grass AIT, patient sera inhibited IgE-facilitated allergen presentation by B cells to allergen-specific T cells, resulting in reductions in T-cell proliferation and cytokines and chemokines (47). Our study showed that the addition of REGN1908 and REGN1909, alone or in combination, to PBMCs from cat-allergic donors inhibited IgE-facilitated allergen presentation and subsequent $CD4^+$ T-cell activation. Furthermore, REGN1908, REGN1909, and REGN1908–1909 suppressed allergen-specific T-cell proliferation, suggesting blocking activity similar to that of sera induced by chronic AIT. The T cell–modulating effect of REGN1908–1909 is consistent with improvement of the Th2-mediated late-phase response, as shown by post-NAC reductions of type 2 cytokines and chemokines in nasal fluid.

In summary, we further explored the outcomes of a previous study showing a single subcutaneous prophylactic dose of the combination of two anti–Fel d 1 mAbs (REGN1908–1909) improved nasal symptoms induced by cat allergen in the NAC model in polysensitized patients with allergic rhinitis. One week after passive administration, we detected rapid immunological changes that would typically be observed only after 1 year of AIT. These changes indicate attenuation of the allergic response involving multiple types of effector cells, including basophils, B lymphocytes, and T lymphocytes. The significant reductions in the TNSS, VAS score, and PNIF, as well as the previously reported wheal size reduction from skin prick testing on Day 85, may indicate a potentially long-term treatment effect of

REGN1908–1909. Such an effect needs to be further confirmed in future studies with a larger sample size. It is important to note that REGN1908–1909 neutralizes only Fel d 1 but no other minor allergen components (Fel d 2, 4, 7, or 8). We demonstrated that solely blocking Fel d 1 provided substantial symptom improvement in the majority of cat-allergic patients. These findings can help design future clinical studies of novel allergen-neutralizing antibodies targeting other dominant allergen components. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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