

Rebound eosinophilia after treatment of hypereosinophilic syndrome and eosinophilic gastroenteritis with monoclonal anti-IL-5 antibody SCH55700

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Background: Hypereosinophilic syndrome and eosinophilic gastroenteritis with peripheral eosinophilia are characterized by sustained eosinophilia and eosinophil-mediated tissue damage. Although treatment with the humanized monoclonal anti-IL-5 antibody SCH55700 resulted in improvement of eosinophilia and clinical symptoms in 6 of 8 of patients with hypereosinophilic syndrome or eosinophilic gastroenteritis with peripheral eosinophilia for as long as 12 weeks, eosinophil counts subsequently rose above baseline levels, accompanied by an exacerbation of symptoms.

Objective: To identify the mechanism underlying this rebound eosinophilia.

Methods: Purified eosinophils from patients or normal donors were cultured with IL-5, patient serum, and/or anticytokine antibodies, and eosinophil survival was assessed by flow cytometry. Serum and intracellular cytokine levels were measured by multiplex sandwich ELISA and flow cytometry, respectively.

Results: Before treatment with SCH55700, *in vitro* eosinophil survival in media and in response to recombinant IL-5 was similar in patients and normal donors. At 1 month posttreatment, the eosinophil survival curves were unchanged in 4 of 5 patients in media and in all 5 patients in response to recombinant IL-5. Normal eosinophil survival was prolonged in cultures containing posttreatment but not pretreatment sera (pretreatment vs posttreatment, 10.74% vs 73.02% live cells; $P = .01$). This posttreatment serum effect on eosinophil survival was reversed by the addition of the monoclonal anti-IL-5 antibody TRFK5. Although increased levels of serum IL-5 were observed at 1 month compared with 2 to 3 days posttreatment in 5 of 6 patients ($P = .04$), intracellular cytokine analysis did not reveal increased production of IL-5 by peripheral blood mononuclear cells.

Conclusions: The rebound eosinophilia after SCH55700 treatment is a result of a serum factor that enhances eosinophil

survival. Reversal of this effect by the addition of antibody to IL-5 suggests that this factor may be IL-5 itself. (J Allergy Clin Immunol 2004;114:1449-55.)

Key words: Anti-IL-5, hypereosinophilic syndrome, eosinophilic gastroenteritis, monoclonal antibody, eosinophil

Hypereosinophilic syndrome (HES) is a rare hematologic disorder characterized by sustained overproduction of eosinophils in the bone marrow, eosinophilia (>1500 eosinophils/mm³ for at least 6 months) and tissue infiltration by eosinophils, resulting in end-organ damage.¹ Current therapies for HES, including corticosteroids, IFN- α , hydroxyurea,¹⁻³ and imatinib mesylate,⁴⁻⁸ are aimed at the reduction of end-organ damage by controlling peripheral blood eosinophil counts. Eosinophilic gastroenteritis (EG) is a disorder in which eosinophilic infiltration of the gastrointestinal wall results in a wide variety of gastrointestinal symptoms.^{9,10} EG can lead to significant loss of function because of chronic abdominal pain, malabsorption, and weight loss. Marked peripheral blood eosinophilia is seen in association with EG in some patients and leads to overlap in the diagnosis with HES. EG is generally responsive to corticosteroids, although high doses may be necessary. A subset of patients with HES or EG become refractory to or develop significant side effects from conventional therapy, and it was for this group that novel therapeutic approaches were sought.

SCH55700 is a humanized antihuman IL-5 mAb that incorporates the antigen recognition sites from 39D10, a rat IgG2a antibody against human IL-5, and a consensus human IgG4 (κ) constant region.¹¹ SCH55700 has been shown to bind human IL-5 with high affinity ($K_d = 20$ pmol/L) *in vitro* and to suppress tissue eosinophilia (lung and skin) in a variety of species *in vivo*.¹¹ In a pilot study of patients with persistent asthma, reduction of peripheral blood eosinophilia after SCH55700 administration was apparent within 48 hours and persisted for as long as 30 days in a dose-dependent manner (0.3 mg/kg vs 1 mg/kg) without any significant adverse effects. Although the FEV₁ of the patients with asthma improved at 24 hours after drug infusion, there were no significant changes in clinical indices of disease activity.¹²

As part of a clinical study to assess the safety and efficacy of a single 1-mg/kg dose of SCH55700 in reducing peripheral blood eosinophilia in patients with

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Abbreviations used

- EG: Eosinophilic gastroenteritis
 EGE: Eosinophilic gastroenteritis with peripheral eosinophilia
 HES: Hypereosinophilic syndrome

HES or EG with peripheral eosinophilia (EGE), eosinophil counts and symptoms were monitored in 8 patients for as long as 3 months after drug administration. Although peripheral eosinophilia resolved and clinical symptoms improved in 6 of the 8 patients within 48 hours of drug administration, eosinophil counts subsequently rose above baseline levels in all 6 SCH55700 responders.^{13,14}

The aim of the current study was to elucidate the mechanism of the rebound eosinophilia observed after anti-IL-5 therapy. Our data suggest that anti-IL-5 therapy induced significantly increased IL-5 levels by 1 month after therapy and that this increase in serum IL-5 was responsible for the rebound eosinophilia observed in patients with either HES or EGE.

METHODS**Participants**

Eight patients with HES (n = 4) or EGE (n = 4) refractory to or intolerant of conventional therapy were enrolled in a single-dose study of SCH55700 treatment. The demographic and clinical characteristics of these study participants are summarized in Table I. Peripheral blood samples were also obtained from 8 normal volunteers. All participants were seen as part of clinical protocols that were approved by the Institutional Review Board of the National Institute of Allergy and Infectious Diseases, and informed consent was obtained from all participants. Peripheral eosinophil counts were performed in the Clinical Pathology Department at the National Institutes of Health Clinical Center using an automated cell counter.

Anti-IL-5 treatment

Patients received a single intravenous 1-mg/kg dose of the humanized monoclonal anti-IL-5 antibody SCH55700 (provided by Schering-Plough Research Institute, Kenilworth, NJ) and were followed in an in-patient setting for a minimum of 72 hours to monitor adverse effects secondary to the infusion. Follow-up visits were performed at days 7, 14, and 28 in all patients and then monthly until the eosinophil count returned to baseline for ≥ 2 months. Therapies known to affect eosinophilia, including prednisone, hydroxyurea, and IFN- α , that were initiated before SCH55700 infusion were maintained at the same dose throughout the study period. Drug levels and pharmacokinetics of SCH55700 were comparable among the patients who were and were not taking other medications.

Eosinophil purification

Eosinophils were isolated from peripheral blood by immunomagnetic negative selection using a modification of the method of Hansel et al.¹⁵ Briefly, EDTA venous blood diluted with PBS (pH 7.4) or granulocyte-enriched apheresis product were layered on Ficoll-Paque (Amersham Biosciences AB, Uppsala, Sweden) and centrifuged at 576g for 30 minutes. After removing the supernatant and PBMC layer, the granulocytes covering the red blood cell pellet were collected, and the red blood cells were lysed 2 times by hypotonic

shock with sterile endotoxin-free ice-cold distilled water. To eliminate the neutrophils, granulocytes were incubated with micro-magnetic beads coated with anti-CD16 (Miltenyi Biotech, Bergisch, Bladbach, Germany) for 40 minutes on ice before passage through the magnetic-activated separation column (Miltenyi Biotech). To remove further the minute numbers of contaminating T and B lymphocytes and monocytes, the CD16⁺ cells were incubated with additional micromagnetic beads coated with anti-CD3, anti-CD14, and anti-CD19 (Miltenyi Biotech) for 20 minutes on ice before the second passage through the column (Miltenyi Biotech). Cytospin slides of purified eosinophils were stained with Diff-Quick fixative and solutions I and II (Dade Behring, Newark, Del), and 500 cells were counted to determine purity. The purity of eosinophils was consistently >99%.

Eosinophil culture and survival assay

Purified eosinophils from patients (pretreatment, n = 8; 1 month posttreatment, n = 5) and normal controls (n = 8) were cultured (10^5 cells/200 μ L/well) in media containing RPMI-1640 (BioWhittaker, Walkersville, Md) supplemented with 10% heat-inactivated defined FCS (Hyclone, Logan, Utah), 20 mmol/L L-glutamine (BioWhittaker), 10 mmol/L HEPES (BioWhittaker), 50 μ g/mL gentamicin (Mediatech, Herndon, Va), and 1 mmol/L pyruvate (Biofluids, Gaithersburg, Md) at 37°C in 96-well flat-bottom culture plates (Corning, Corning, NY). Various concentrations (50 fg/mL [~ 1.67 pmol/L] to 5 ng/mL [~ 166.67 pmol/L]) of IL-5 (PharMingen, San Diego, Calif) were added to the culture media. All cultures were performed in triplicate. After 4 days of culture, cells were stained with annexin V and propidium iodide, and the percentage of live eosinophils (annexin V-negative and propidium iodide-negative cells) was determined by flow cytometry. In some cultures, purified eosinophils from a normal control donor were cultured with 1% heat-inactivated sera from patients before and at 1 month after treatment with SCH55700.

In vitro blocking experiment

To identify the nature of the serum factor responsible for the prolongation of eosinophil survival *in vitro*, blocking experiments were performed with anticytokine mAbs. First, the minimum cytokine concentration required to produce >50% survival of normal donor eosinophils after 4 days in culture was determined for each cytokine (IL-5, IL-3, GM-CSF, and IFN- γ). Next, normal donor eosinophils were cultured in the presence of this fixed cytokine concentration over a wide range of concentrations of the corresponding neutralizing antibody (10 ng/mL to 25 μ g/mL). Purified eosinophils from a normal donor were then incubated with 1% posttreatment serum in the presence or absence of the same range of concentrations of neutralizing anticytokine antibodies (anti-GM-CSF, anti-IFN- γ , anti-IL-3; BD Pharmingen) or isotype control antibodies (rat IgG2 α for anti-GM-CSF; mouse IgG1 for anti-IFN- γ ; rat IgG1 for anti-IL-3 and anti-IL-5; BD Pharmingen), and viability was assessed as described.

Cytokine measurement and intracellular cytokine frequency assessment

Serum levels of cytokines (IL-2, IL-3, IL-5, IL-8, IL-15, GM-CSF, IFN- γ , and TNF- α) were determined using a multiplex sandwich ELISA (Pierce Biotechnology, Rockford, Ill).

PBMC intracellular cytokine production was measured before and at 1 month posttreatment in 4 patients (2 patients with HES and 2 with EGE) by flow cytometry. Briefly, PBMCs were isolated by Ficoll diatrizoate gradient centrifugation lymphocyte separation medium; ICN Biomedicals, Aurora, Ohio). Erythrocytes were lysed using ACK lysis buffer (BioSource International, Camarillo, Calif). Cells

TABLE I. Patient characteristics

Patient number	1	2	3	4	5	6	7	8
Diagnosis	HES				EGE			
Age (y)	50	30	36	45	53	43	38	44
Sex	M	F	M	F	F	F	M	M
Concomitant therapy*	H	P	P, H, I	H	N	N	P	N
Baseline eosinophil count (per mm ³)	3630	2620	6133	3209	1579	1883	1617	819
Baseline serum IL-5† (pg/mL)	2.0	2.0	<1.95	223.0	<1.95	<1.95	7.0	<1.95
Response to treatment‡	R	R	NR	NR	R	R	R	R

*P, Prednisone; H, hydroxyurea; I, IFN-α; N, none.
†Serum IL-5 was measured by using a multiplex sandwich ELISA.
‡R, Responder; NR, nonresponder, as defined by a decrease in absolute eosinophil count of ≥30% in response to a single 1 mg/kg dose of SCH55700 and symptomatic improvement.

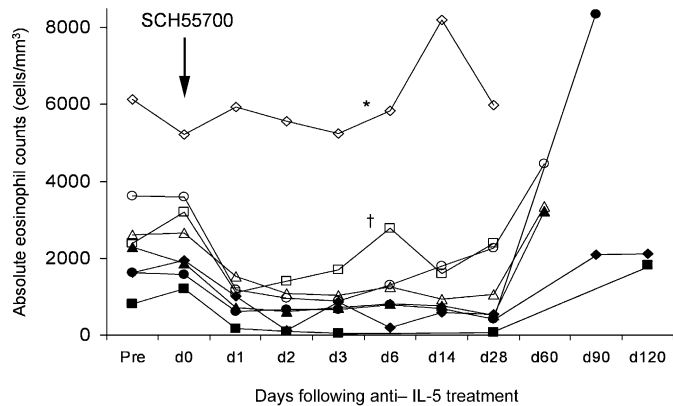


FIG 1. Rebound eosinophilia after anti-IL-5 treatment. Each line shows an individual patient’s changes in eosinophil counts. The dates plotted on the x-axis are not linear to show better the changes in eosinophil count observed during the early time points after treatment. Open symbols, patients with HES; solid, patients with EGE. *, †, 2 nonresponding patients, patients 3 and 4.

were then washed and cultured at a concentration of 2×10^6 /mL for 6 hours with or without phorbol 12-myristate 13-acetate (PMA; 50 ng/mL)/ionomycin (1 μ g/mL) in RPMI 1640 (BioWhittaker) supplemented with 10 mmol/L HEPES (BioWhittaker), 20 mmol/L glutamine (BioWhittaker), 10% heat-inactivated FCS (Harlan Bioproducts for Science, Madison, Wis), and 50 μ g/mL gentamicin (Mediatech). Monensin (2 μ mol/L) was added after the first hour of culture to block cytokine secretion. Cells were then fixed with 4% paraformaldehyde and frozen at -70°C until use. Fixed cells were thawed, permeabilized in PBS/0.1% saponin, and stained concurrently for surface and intracellular flow cytometry using the following antibodies from BD Pharmingen: fluorescein isothiocyanate-labeled antihuman CD3 and CD14; peridinin chlorophyll-*a* protein (PerCP)-labeled antihuman CD4, CD8, and CD19; phycoerythrin-labeled GM-CSF and IL-3; and allophycocyanin-labeled IL-5 and IFN- γ . Fluorescence was measured on a FACS Calibur (BD Biosciences, San José, Calif) using 50,000 gated lymphocytes, and frequencies of cells for each cytokine were analyzed. Because surface expression of CD4 is diminished in response to PMA, CD3⁺CD8[−] cells were used as a surrogate for CD3⁺CD4⁺ cells in PMA-treated cultures.

Statistical analysis

Nonparametric comparisons of group means were made using the Mann-Whitney *U* test for unpaired data and the Wilcoxon signed-rank test for paired comparisons. Data are presented as medians and

interquartile ranges for medians. A *P* value <.05 was considered statistically significant for all tests.

RESULTS

Rebound eosinophilia

Within 24 hours of SCH55700 administration, patient eosinophil counts decreased dramatically with concomitant symptomatic improvement. There was a sustained decrease in eosinophilia and symptoms in 6 patients; however, rebound eosinophilia (an increase in eosinophilia to levels greater than or equal to pretreatment levels) was observed in all 6 responders, with peak eosinophil counts occurring between 60 and 90 days posttreatment (Fig 1). Rebound eosinophilia was accompanied by a severe exacerbation of symptoms, including skin rash, mucosal ulceration, angioedema, fatigue, myalgias, and arthralgias.¹³ With respect to the 2 nonresponders, there was no effect of SCH55700 on eosinophilia or symptoms in HES patient 3 (* in Fig 1) and a transient decrease in eosinophilia but no improvement in symptoms in HES patient 4 († in Fig 1). Both patients (HES 3 and HES 4)

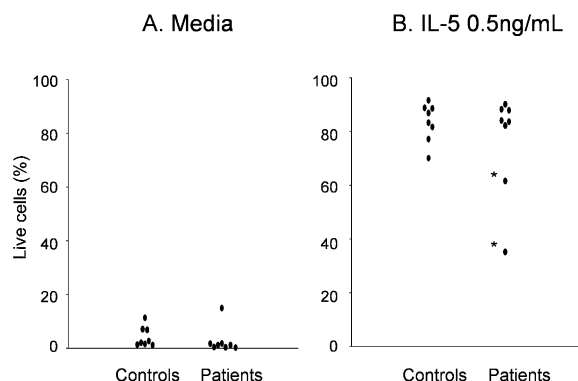


FIG 2. Normal control and patient pretreatment *in vitro* eosinophil survival. Eosinophil survival was assessed in media (**A**) or in the presence of IL-5 (**B**) for patients and controls. Results are expressed as the geometric mean values for triplicate cultures for each donor. Each symbol represents 1 individual. *Patients with the *FIP1L1/PDGFRα* fusion.

were treated with additional agents (imatinib mesylate and cyclosporine A, respectively) after the 1-month post-SCH55700 time point to control their eosinophilia and related symptoms.

***In vitro* eosinophil survival**

To assess the effect of anti-IL-5 therapy on eosinophil survival, purified eosinophils from normal donors and from patients before treatment with SCH55700 were cultured in the presence or absence of IL-5 and assessed for viability after 4 days of culture. The median percentages of live eosinophils from normal donors and patients did not differ either in media (controls vs patients, 2.97% [1.49-11.24] vs 0.97% [0.35-14.89]) or in response to IL-5 (controls vs patients, 83.2% [80.50-91.57] vs 73.8% [77.05-90.12]; **Fig 2, A and B**). Two patients with HES (patients 1 and 3) with the myeloproliferative variant of HES⁴ showed decreased eosinophil survival in response to IL-5 (* in **Fig 2, B**).

Eosinophil survival was compared between eosinophils obtained before and at 1 month post-SCH55700 treatment from the 5 patients for whom eosinophils were available from both time points (**Fig 3**). Eosinophil survival was comparable at the 2 time points in media in 4 patients (patients 1, 2, 3, and 8) and in the presence of IL-5 in all 5 patients. Although the eosinophils from 1 patient with EGE (patient 6) did show increased survival in media (62.49%) at 1 month posttreatment, the response to IL-5 was unchanged (90.12% vs 87.68%). These results suggest that increased responsiveness of eosinophils to IL-5 is not observed after anti-IL-5 administration.

Posttreatment sera prolong survival of normal eosinophils

When normal eosinophils were cultured in media containing 1% patient sera, eosinophil survival was prolonged at day 4 in cultures containing sera obtained 1 month posttreatment compared with those containing pretreatment sera in all 8 patients regardless of their

clinical diagnosis (HES or EGE; pretreatment vs posttreatment, median, 10.74% [6.08-18.17] vs 73.02% [59.01-79.22] of live cells; $P = .01$, Wilcoxon signed rank test; **Fig 4**). Of interest, this posttreatment serum effect on prolonged eosinophil survival was reversed by the addition of the rat monoclonal anti-IL-5 antibody TRFK5 in a concentration-dependent manner (**Fig 5, B**), similar to that observed in control experiments by using TRFK5 to block the effect of recombinant IL-5 on eosinophil survival (**Fig 5, A**) but not by anti-GM-CSF, anti-IFN- γ , or anti-IL-3 (data not shown).

Serum cytokine measurements

Among the serum cytokines measured (IL-2, IL-3, IL-5, IL-8, IL-15, GM-CSF, IFN- γ , and TNF- α), IL-5 was the only cytokine for which a significant change was observed after anti-IL-5 therapy. Serum IL-5 levels were available for 6 patients at all 3 time points (pretreatment, 2-3 days posttreatment, and 1 month posttreatment). Before treatment, 1 patient with HES (patient 4) had markedly elevated serum IL-5 levels (223 pg/mL), and 2 patients with HES (patients 1 and 2) had low but detectable levels of serum IL-5 (2 pg/mL). Serum IL-5 levels fell by 2 to 3 days posttreatment in all 3 patients (patients 1, 2, and 4) with detectable pretreatment levels, although this was most dramatic in the patient with the highest levels at baseline (patient 4; **Fig 6, A**). When posttreatment serum IL-5 levels were compared, increased levels were observed at 1 month posttreatment compared with levels 2 to 3 days posttreatment in 5 of 6 patients tested ($P = .04$; **Fig 6, B**). Levels remained undetectable at all time points in HES patient 3 (**Fig 6, A and B**). There was no correlation between serum IL-5 levels and the eosinophil survival-enhancing activity of serum.

Intracellular cytokine flow-cytometry analysis

Frequencies of cytokine-producing PBMCs (T cells, B cells, and monocytes) were assessed by intracellular flow cytometry before and at 1 month posttreatment with SCH55700 in 4 patients (2 with HES and 2 with EGE). Frequencies of CD3⁺ cells producing IL-5 spontaneously or in response to PMA/ionomycin were extremely low (<2.5%) in all patients before SCH55700 treatment. Furthermore, there was no significant change in the frequencies of IL-5-producing cells at 1 month posttreatment either unstimulated or PMA/ionomycin-stimulated. Indeed, the median values for CD3⁺CD8⁺ unstimulated cells were 0.00 vs 0.00 (pretreatment vs posttreatment) and for PMA/ionomycin-stimulated cells, 0.03 vs 0.15 ($P > .35$ for both comparisons). Similarly, for the CD3⁺CD4⁺ cells, the unstimulated median frequencies were 1.06 vs 0.00 ($P = .89$), and for the stimulated cells, 0.39 vs 0.60 ($P = .77$). No significant change in the frequencies of GM-CSF-producing, IL-3-producing, and IFN- γ -producing T cells, B cells, or monocytes (both unstimulated and stimulated) was observed after SCH55700 treatment ($P > .2$ for all comparisons).

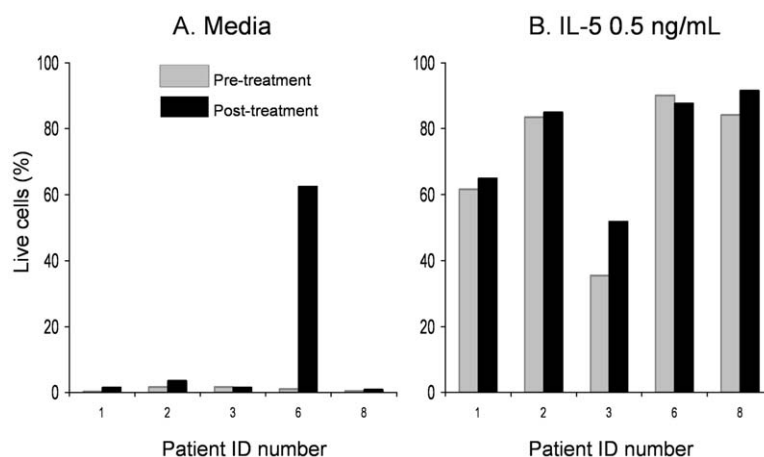


FIG 3. *In vitro* eosinophil survival responses before and after treatment with anti-IL-5. Eosinophil survival was assessed before and at 1 month posttreatment in media (**A**) and in the presence of IL-5 (**B**). Results are expressed as the geometric mean values for triplicate cultures for each donor. Pretreatment and posttreatment responses are indicated by gray and black bars, respectively.

DISCUSSION

Rebound eosinophilia was observed in all 6 subjects with HES and EGE who responded to SCH55700 with a sustained drop in eosinophil count and was accompanied by recurrence or exacerbation of symptoms in 75% (6/8) of subjects. This rebound appeared to be independent of the underlying cause of the eosinophilia, because it was seen in subjects with either *FIP1L1/PDGFR*A-positive or *FIP1L1/PDGFR*A-negative HES¹³ and in subjects with EGE.¹⁴ A similar rebound eosinophilia was observed in a pilot double-blind, randomized study of SCH55700 in human asthma after a single dose of 0.3 mg/kg, with a doubling of mean baseline eosinophil counts by day 90 posttreatment.¹² Rebound was not seen with higher-dose therapy (1 mg/kg), suggesting that the relationship between IL-5 and anti-IL-5 levels may be crucial in determining when and whether rebound eosinophilia occurs. Although an increase in eosinophil count above baseline was not specifically described in a recent study of anti-IL-5 antibody (mepolizumab) treatment of 3 patients with HES and eosinophilic dermatitis,¹⁶ additional antibody was given in 2 of the 3 patients for worsening eosinophil count and symptoms. However, differences in the antibodies (mepolizumab vs SCH55700), dosing (750 mg/dose of mepolizumab vs 1 mg/kg of SCH55700), patient populations, and study designs between the mepolizumab study and the current report make direct comparisons difficult.

There are numerous potential explanations for the rebound eosinophilia observed in our study. SCH55700 could have led to an increase in eosinophil responsiveness to eosinophilopoietic cytokines through modulation of receptor expression. It has been shown previously that IL-5 induces downmodulation of IL-5R α surface expression on eosinophils.¹⁷ Because the addition of anti-IL-5 *in vitro* can prevent this downregulation,¹⁸ it is theoretically

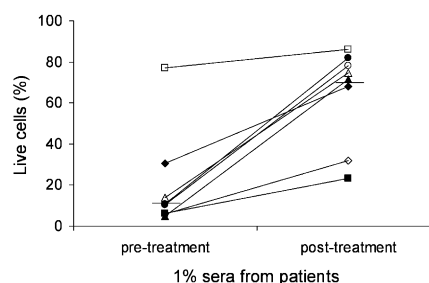


FIG 4. Prolongation of normal eosinophil survival by posttreatment patients' sera. Normal eosinophils were incubated with 1% sera from patients with HES (open symbols) or EGE (solid symbols) before and at 1 month posttreatment, and survival was assessed by flow cytometry. The horizontal bars are the median values. $P = .01$, Wilcoxon signed-rank test.

possible that the combination of increased IL-5R α receptor expression and increased IL-5 levels could have led to a transient increase in eosinophilia. To address this possibility, we examined eosinophil survival in response to various concentrations of IL-5 before and after treatment with SCH55700. Although less direct than quantification of surface expression levels of IL-5R α by flow cytometry, this assay provides a more biologically relevant assessment of eosinophil responsiveness to IL-5. Eosinophil survival in response to IL-5 was not increased posttreatment (Fig 3, B), suggesting that increased or altered surface expression of IL-5R α did not play a significant role in the rebound eosinophilia observed after treatment with SCH55700.

Potentiation of IL-5 signaling by mAbs to IL-5 has been described *in vitro*¹⁹ and could account for increased eosinophilia after the administration of SCH55700. Although the timing of the observed rebound eosinophilia seemed inconsistent with this hypothesis, IL-5-mediated

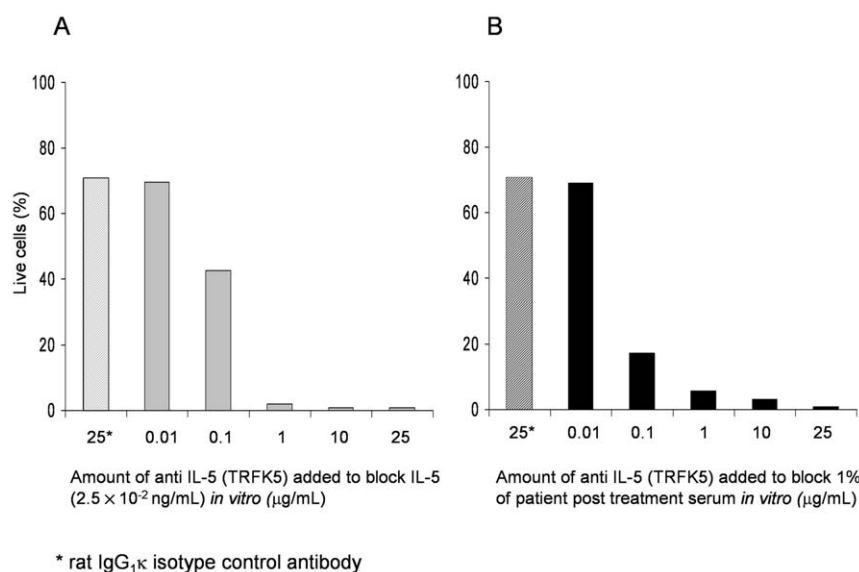


FIG 5. Reversal of the prolongation of normal eosinophil survival by posttreatment serum by the rat monoclonal antihuman IL-5 antibody TRFK5. Eosinophil survival was assessed in the presence of 1% posttreatment serum from a patient with HES and increasing concentrations of TRFK5 or rat IgG1 κ isotype control antibody (**B**). Similar data are shown using a fixed concentration of recombinant IL-5 to prolong survival (**A**).

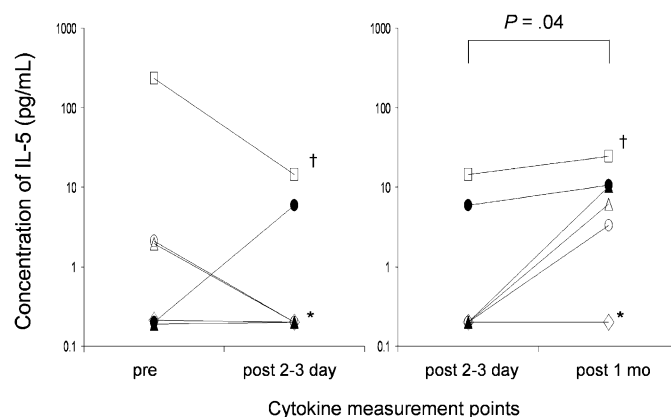


FIG 6. Changes in serum IL-5 levels. Serum IL-5 levels are compared before and at 2 to 3 days posttreatment (**A**) and at 2 to 3 days and 1 month posttreatment (**B**) for 6 patients with HES (open) or EGE (solid). *, †, 2 nonresponding patients. $P = .04$ for comparison between 2-day to 3-day posttreatment and 1-month posttreatment serum IL-5 levels.

eosinophil survival was assessed in the presence of various concentrations of TRFK5, a murine antihuman IL-5 antibody that shares antibody specificity with SCH55700. As expected, TRFK5 blocked (and did not potentiate) IL-5-induced eosinophil survival.

The formation of neutralizing antibodies against SCH55700 could also lead to rapid clearance of the drug and subsequent rebound eosinophilia, although such antibodies were not detected in preclinical studies. Serum levels of SCH55700 remained detectable throughout the study period and rose with each additional dose in patients who later received multiple doses, inconsistent with this

hypothesis.¹³ Of note, this is consistent with the data from preclinical and human asthma studies in which neutralizing anti-SCH55700 antibodies could not be demonstrated.¹²

Finally, alterations in serum cytokine or chemokine levels after SCH55700 treatment might be responsible for the rebound eosinophilia. Of the serum cytokine levels examined, only IL-5 levels correlated with the observed rebound eosinophilia. This relationship was confirmed by eosinophil survival studies that demonstrated that the prolongation of eosinophil survival observed in the presence of posttreatment sera was reversed by adding

anti-IL-5 antibody *in vitro*. The presence of increased serum levels of IL-5 can be explained by at least 2 mechanisms: increased cellular production of IL-5 and/or decreased clearance of biologically active cytokine/antibody complexes. Several cells in the bone marrow and peripheral blood—including eosinophils, mast cells, and CD4⁺ T cells—can produce significant amounts of IL-5.²⁰⁻²² Despite increased serum levels of IL-5 in 5 of 6 patients at 1 month after treatment with SCH55700, the numbers of IL-5 producing PBMCs detected by intracellular flow cytometry were very low and did not increase posttreatment. Increased IL-5 production by other cell types, including eosinophils and their progenitors, remains a possibility that could not be explored because of the lack of cells for study. Anti-IL-5 antibody has been reported to have a preferential effect on later-stage eosinophil progenitors in the bone marrow, causing a transient maturational arrest in the bone marrow.²³ Although accumulation of eosinophil progenitors was not observed in our study,¹³ the timing of the bone marrow examinations may not have been optimal to detect these changes.

Prolongation of the *in vivo* effects of cytokines by soluble cytokine/anticytokine complexes has been described *in vivo* in a murine model and depends on the molar ratio of the cytokine to its antibody.²⁴ This effect is thought to result primarily from decreased clearance of biologically active cytokine/anticytokine complexes. Although we were unable to explore this phenomenon *in vivo*, addition of soluble cytokine/anticytokine complexes over a wide range of molar ratios to normal eosinophil cultures was unable to sustain eosinophil survival *in vitro* (data not shown).

In conclusion, the rebound eosinophilia seen after SCH55700 treatment in patients with HES and EGE does not appear to be a result of a fundamental change in the eosinophils themselves but rather of a transient increase in serum IL-5 levels. The cellular source of this increased IL-5 and the mechanism by which it occurs need to be clarified to assess better the effect of rebound eosinophilia on the utility of anti-IL-5 antibody treatment for eosinophil-mediated disorders.

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