BRIEF REPORT

Use of an Anti–Interleukin-5 Antibody in the Hypereosinophilic Syndrome with Eosinophilic Dermatitis

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The hypereosinophilic syndrome comprises a heterogeneous group of conditions characterized by hypereosinophilia and organ dysfunction caused by eosinophil-mediated tissue damage.[1,2] The highly variable response to treatment reflects the heterogeneity of the syndrome. Current therapies include corticosteroids, hydroxyurea, interferon alfa, and imatinib mesylate. Imatinib can be effective in patients with the syndrome who have normal[3] or increased[4] serum concentrations of interleukin-5. Recently, patients with hypereosinophilia and fusion of the *Fip1-like 1* gene (*FIP1L1*) and the gene that encodes platelet-derived growth factor receptor α (*PDGFRA*) were found to have a response to imatinib mesylate.[5] Such patients probably have a myeloproliferative disease or a clonal disorder that is largely independent of interleukin-5, a cytokine required for the differentiation, activation, and survival of eosinophils.[5] However, in other patients with the syndrome — namely, those who have clonal T cells and polymorphous skin lesions — interleukin-5 does seem to have a critical role.[6-9] Here we report the effect of mepolizumab, a neutralizing anti–interleukin-5 antibody,[10,11] in three patients with a hypereosinophilic syndrome and dermatologic manifestations.[12,13]

CASE REPORTS

PATIENT 1

The first patient was a 60-year-old woman who had received a diagnosis of eosinophilic dermatitis five years earlier. The skin disease initially manifested as erythematous, centrally ulcerated nodules, angioedema, and eczematous lesions, and evolved into a diffuse erythroderma with severe pruritus, swelling, and tenseness in all four limbs. The patient also had a peroneal mononeuropathy, and echocardiography showed thickening of the mitral-valve leaflets. The proportion of eosinophils in the peripheral blood ranged from 15 to 41 percent, there were numerous eosinophils in the bone marrow, and examination of skin-biopsy specimens revealed dense eosinophilic infiltration of the dermis and subcutis. The patient had no personal or family history of atopy, and no hypersensitivity reactions were detected with standard allergy tests (including 20 environmental allergens). The serum IgE level was 655 kU per liter (normal value, <100). There was no evidence of parasitic infection. There was no evidence of a clonal T-cell or B-cell population on immunophenotyping or molecular genetic analysis, and *FIP1L1–PDGFRA* gene fusion was not detected.

Initial treatment with antihistamines, dapsone, and topical corticosteroids failed to ameliorate the skin lesions, and subsequent treatment with systemic corticosteroids

(60 mg of prednisolone daily for four months) also failed to improve the skin-related symptoms and hematologic abnormalities. Treatment with 750 mg of mepolizumab by intravenous infusion was followed within one day by a decrease in the peripheral-blood eosinophil count (Fig. 1A) and in the serum level of eosinophil cationic protein (data not shown). Three days after the first infusion, the pruritus and skin lesions had completely resolved. A second infusion was given, and the patient has remained symptom-free for 17 months without corticosteroid therapy or any other additional therapy, and no adverse events have occurred.

PATIENT 2

The second patient was a 62-year-old woman with eosinophilic dermatitis who had had dyspnea, night sweats, low-grade fever, fatigue, weight loss, abdominal pain, and severe, generalized pruritus for seven months. She had no personal or family history of atopy, and no hypersensitivity reactions were detected with standard allergy tests. The serum IgE level was 224 kU per liter. Gastroscopy and colonoscopy (during corticosteroid treatment) revealed no abnormalities. There was no evidence of parasitic infection. The widespread skin lesions included pruriginous, urticarial, and eczematous lesions

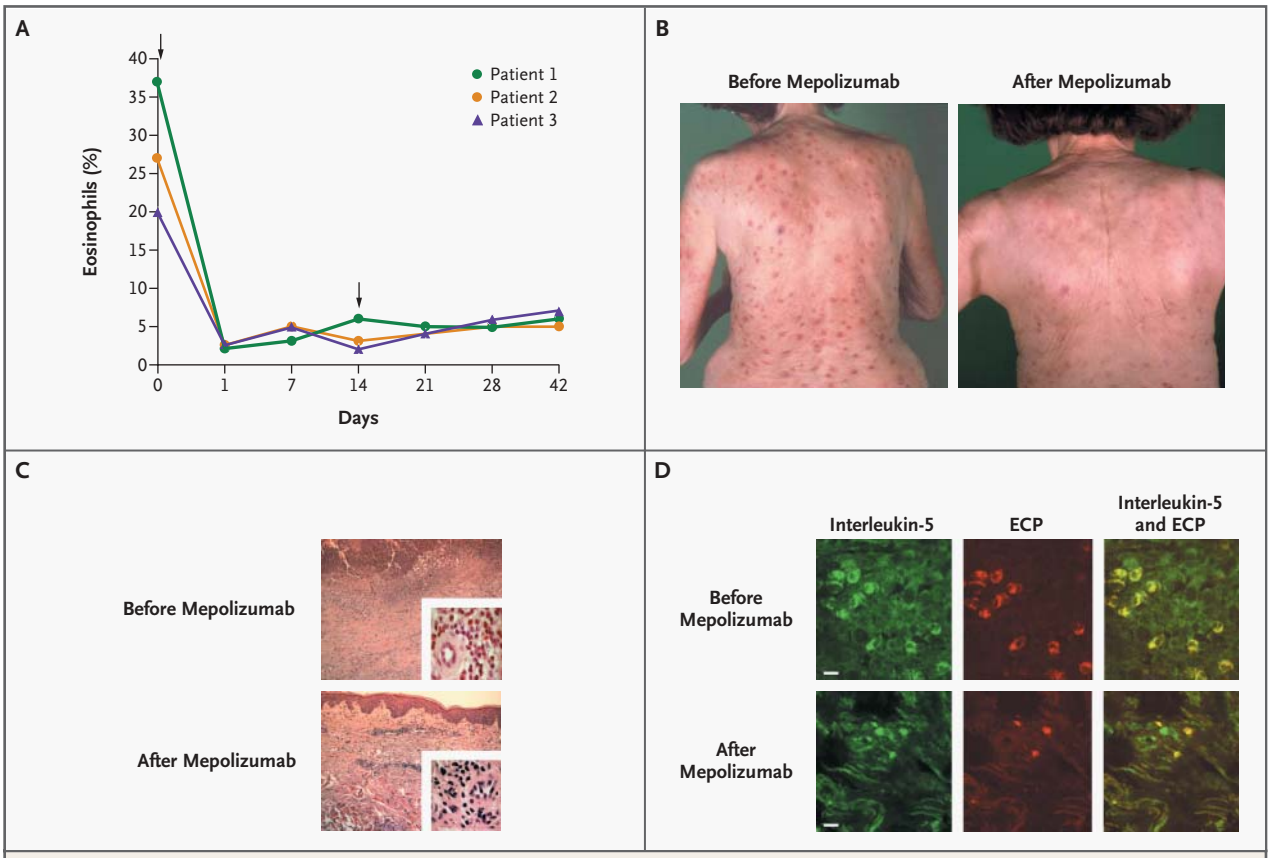

Figure 1. The Effects of Mepolizumab Treatment.

Panel A shows the effect of mepolizumab infusions (arrows) on the percentage of peripheral-blood eosinophils in each of the three patients. Panel B shows the clinical response on day 21, after two mepolizumab infusions, in Patient 2. Panels C and D show the reduction in the number of eosinophils in the skin before and on day 21 after the start of mepolizumab therapy in Patient 2. Before therapy with mepolizumab, hematoxylin-and-eosin–stained skin-biopsy specimens (Panel C, ×100) contained inflammatory-cell infiltrates largely consisting of eosinophils and lymphocytes. After therapy, the number of inflammatory cells had decreased, and no eosinophils were detected. The insets show the same specimens at a higher magnification (×1000). Double immunofluorescence staining with anti–interleukin-5 and anti–eosinophil cationic protein (ECP) antibodies (Panel D) showed that most of the infiltrating eosinophils expressed interleukin-5 before therapy with mepolizumab. After therapy, fewer eosinophils were detected, although they did contain interleukin-5. The scale bars represent 10 μm.

as well as erythematous, centrally ulcerated nodules. Skin and bone marrow biopsy specimens contained numerous eosinophils. There was no evidence of a clonal T-cell or B-cell population or of *FIP1L1–PDGFRA* gene fusion.

Antihistamines, dapsone, and topical corticosteroids were given without effect, and systemic corticosteroid therapy (60 mg of prednisolone daily for four months) also failed to improve the patient's condition. The proportion of eosinophils in the peripheral blood was 11 to 27 percent throughout the period of treatment with prednisolone. Within 24 hours after treatment with 750 mg of intravenous mepolizumab, however, there was a dramatic decrease in the peripheral-blood eosinophil count (Fig. 1A) and in the serum levels of eosinophil cationic protein (data not shown). After a second infusion of mepolizumab, two weeks later, the patient's clinical condition began to improve. Three weeks after the initiation of antibody treatment, the patient was almost free of pruritus and other symptoms (Fig. 1B); the fever, fatigue, and abdominal pain had also regressed considerably.

Five weeks after the second dose of mepolizumab had been administered, the patient's clinical condition began to deteriorate, and the peripheral-blood eosinophil count increased. A third dose of mepolizumab led to an immediate reduction in the eosinophil count, regression of the skin lesions, and lessening of the pruritus. The patient received 750 mg of mepolizumab monthly for an additional seven months and was almost symptom-free with this therapy. No side effects have been observed.

We were forced to stop treatment with mepolizumab because we were unable to obtain an additional supply. Since then, the patient's skin lesions and pruritus have again worsened. Currently, the patient is being treated with corticosteroids and imatinib mesylate, with only marginal success.

PATIENT 3

The third patient was an 82-year-old woman who had had severe pruritus and eczematous and prurigenous skin lesions for one year. There was no other organ involvement, and there was no personal or family history of atopy; multiple allergy tests showed no hypersensitivity reactions. The serum IgE level was 710 kU per liter. There was no evidence of parasitic infection. Examination of skin and bone marrow–biopsy specimens showed dense eosinophilic infiltration. Immunophenotyping revealed an abnormal CD8+ T-cell subpopulation with decreased expression of CD5 and CD6, suggesting a clonal T-cell disease.[7] However, there was no direct evidence of a clonal T-cell receptor rearrangement, of a cutaneous T-cell lymphoma, or of *FIP1L1–PDGFRA* fusion.

Systemic corticosteroid treatment (60 mg of prednisolone daily for eight weeks) had no effect on the symptoms and did not control the eosinophilia; the proportion of eosinophils in the peripheral blood remained within the range of 16 to 37 percent during therapy. The patient was treated twice with 750 mg of mepolizumab, with the second dose given 14 days after the first. The eosinophil count (Fig. 1A) and serum levels of eosinophil cationic protein (data not shown) decreased within one day after the first infusion. The skin lesions regressed and the pruritus decreased within two weeks after the initiation of therapy.

Five weeks after the second infusion, however, the pruritus worsened and the eosinophil count increased. A third dose of mepolizumab was followed by an immediate drop in the peripheral-blood eosinophil count and by regression of the skin lesions and pruritus. Deterioration occurred seven weeks after the third dose of mepolizumab had been given. The patient then received 750 mg of mepolizumab monthly for five more months. Because of the improvement in her condition, treatment was stopped, and she has remained almost symptom-free. No side effects have been observed.

METHODS

CLINICAL FEATURES

The hypereosinophilic syndrome was defined as a peripheral-blood eosinophil count of 1500 per cubic millimeter persisting for at least six months in the absence of infectious, allergic, vasculitic, rheumatic, or malignant causes of eosinophilia. All three patients had symptomatic disease that was deemed to require cytoreductive treatment because of the failure of systemic corticosteroid therapy. They underwent a base-line bone marrow biopsy with cytogenetic analysis. There was no evidence of a clonal cytogenetic abnormality, of bone marrow hyperplasia, of an excess of circulating or marrow blasts, or of dysplastic changes in cells of noneosinophilic lineages. Since evidence of eosinophil-mediated inflammation was confined to the skin, all three patients received the diagnosis of eosinophilic dermatitis.[12,13]

TREATMENT

Between May 2002 and March 2003, the three patients were treated with the humanized anti–interleukin-5 antibody mepolizumab (GlaxoSmithKline), and the clinical and histologic courses of the disease were assessed. Mepolizumab was administered intravenously at a dose of 750 mg, diluted in 150 ml of 0.9 percent sodium chloride solution. The study was approved by the local ethics committee, and written informed consent was obtained from each patient before treatment.

LABORATORY ASSESSMENTS

Differential blood counts were obtained by standard techniques. Surface molecules on peripheral-blood lymphocytes were measured by flow cytometry.[7] To identify dominant (clonal) rearrangements of the T-cell receptor within the lymphocyte population, Southern blot analysis (of the Vβ chain) and a polymerase-chain-reaction (PCR) assay (of the Vγ chain) were performed according to standard protocols. Eosinophilic cationic protein, interleukin-5, thymus- and activation-regulated chemokine, and eotaxin levels were measured in serum with the use of immunoassays, according to the recommendations of the manufacturers. Peripheral-blood mononuclear cells were isolated and stimulated with 10 μg of phytohemagglutinin per milliliter for 24 hours, and cytokine levels were measured in culture supernatants (Th1/Th2 Cytometric Bead Array assay, Pharm-

Table 1. Hematologic Data and Levels of Cytokines and Serum Mediators before (Day 1) and 21 Days after the Initiation of Mepolizumab Treatment.

Cell or Substance	Patient 1		Patient 2		Patient 3	
	Day 1	Day 21	Day 1	Day 21	Day 1	Day 21
Blood cells						
Leukocytes (per mm^3)	7200	5900	4300	3000	10300	7500
Eosinophils (per mm^3)	1586	395	1118	30	3811	300
CD3+ T cells (%)	65	69	73	73	80	80
CD3+CD4+ T cells (%)	29	29	53	54	28	31
CD3+CD8+ T cells (%)	33	40	18	17	46	48
Ratio of CD4+ to CD8+ T cells	0.9	0.7	2.9	3.2	0.6	0.6
CD19+ B cells (%)	11	10	13	9	7	6
CD3–CD16+CD56+ natural killer cells (%)	6	7	6	4	7	7
Cytokines on stimulation with phytohemagglutinin (pg/ml)*						
Interleukin-2	763	1007	2561	2660	234	263
Interleukin-4	477	48	310	295	19	39
Interleukin-5	607	58	1011	119	58	78
Interleukin-10	837	242	657	417	53	53
Interleukin-13	974	110	1012	190	294	119
Interferon-γ	5599	6134	3943	4597	4388	4050
Tumor necrosis factor α	1209	1568	890	1359	1434	1926
Serum mediators†						
Eosinophil cationic protein (μg/ml)	120	18	69	4	84	4
Interleukin-5 (pg/ml)	16.2	<2.0	11.2	<2.0	15.4	<2.0
Thymus- and activation-regulated chemokine (pg/ml)	3250	565	3196	826	3248	1812
Eotaxin (pg/ml)	240	136	231	80	103	79

* Normal levels are as follows: interleukin-2, 600 to 3000 pg per milliliter; interleukin-4, 50 to 200 pg per milliliter; interleukin-5, 50 to 150 pg per milliliter; interleukin-10, 500 to 1000 pg per milliliter; interleukin-13, 50 to 300 pg per milliliter; interferon-γ, 3000 to 10,000 pg per milliliter; and tumor necrosis factor α, 1000 to 3000 pg per milliliter.

† Normal levels are as follows: eosinophil cationic protein, less than 15 μg per milliliter; interleukin-5, less than 2 pg per milliliter; thymus- and activation-regulated chemokine, 50 to 800 pg per milliliter; and eotaxin, 40 to 100 pg per milliliter.

Table 2. Skin-Infiltrating Eosinophils and Lymphocytes before (Day 1) and 21 Days after the Initiation of Mepolizumab Treatment.

Method and Finding	Patient 1		Patient 2		Patient 3	
	Day 1	Day 21	Day 1	Day 21	Day 1	Day 21
Hematoxylin and eosin						
Eosinophils (per 1000 skin cells)	245	0	136	0	238	3
Immunofluorescence						
Positive for eosinophil cationic protein (cells/mm²)	70	34	140	19	80	10
Positive for eosinophil cationic protein and interleukin-5 (%)	50	88	85	100	75	100
CD4+ cells (per mm²)	40	40	30	10	24	5
CD4+ and positive for interleukin-5 (%)	25	0	25	100	42	60
CD8+ cells (per mm²)	20	8	25	10	20	10
CD8+ and positive for interleukin-5 (%)	50	0	40	100	50	0

ingen, Becton Dickinson Biosciences). *FIP1L1–PDGFRA* fusions were sought by polymerase-chain-reaction assays[5] with the use of messenger RNA (mRNA) from purified eosinophil populations and DNA from blood leukocytes obtained before the initiation of antibody therapy. In these assays, we used mRNA and DNA from human eosinophilic leukemic cells (the EoL-1 cell line, Deutsche Sammlung von Mikroorganismen und Zellkulturen), which carry the interstitial chromosomal deletion leading to the *FIP1L1–PDGFRA* fusion,[14] as positive controls.

Skin-biopsy specimens were obtained immediately before the first infusion of mepolizumab and again 21 days later (7 days after the second dose of mepolizumab). Sections stained with hematoxylin and eosin were evaluated by three investigators. The biopsy specimens were also studied with the use of immunofluorescent methods and the following monoclonal mouse antibodies: anti–eosinophil cationic protein (Pharmacia), anti-CD4 and anti-CD8 (both Serotec), a rabbit anti–interleukin-5 antibody (Santa Cruz Biotechnology), and appropriate control antibodies. Antibody binding was detected by fluorescein-conjugated second antibodies (Jackson ImmunoResearch Laboratories). Slides were analyzed independently by each of three investigators using confocal laser scanning microscopy (LSM 510, Carl Zeiss).

RESULTS

In contrast to the patients' absolute and relative numbers of blood eosinophils (Table 1 and Fig. 1A, respectively), total numbers of leukocytes did not change after mepolizumab administration. The distribution of lymphocyte subpopulations, including the ratio of CD4+ to CD8+ T cells, and the distribution of T-cell activation markers also were not affected by treatment with mepolizumab (Table 1). However, mitogen-stimulated lymphocytes from Patients 1 and 2 produced small amounts of interleukin-4, interleukin-5, interleukin-10, and interleukin-13 (cytokines made by type 2 helper T [Th2] cells) and large amounts of interleukin-2, interferon-γ, and tumor necrosis factor α (cytokines made by type 1 helper T cells) after treatment (Table 1). The possibility that the predominance of Th2 cells in eosinophilic dermatitis was reversed by the antibody is supported by the finding of decreased serum levels of thymus- and activation-regulated chemokine, a product of activated Th2 cells,[8] after therapy. Levels of interleukin-5, eotaxin, and eosinophil cationic protein also declined dramatically after antibody administration (Table 1).

Intravenous infusions of mepolizumab were followed by a sharp decrease in the numbers of eosinophils in the skin. No eosinophils were seen in hematoxylin-and-eosin–stained skin-biopsy specimens three weeks after antibody treatment, but they were still detectable on immunofluorescence staining (Table 2 and Fig. 1C and 1D). Many of the infiltrating CD4+ and CD8+ lymphocytes expressed interleukin-5. The numbers of skin-infiltrating lymphocytes were reduced in all three patients in association with mepolizumab administration (Table 2).

DISCUSSION

Mepolizumab effectively controlled eosinophilic dermatitis in the three patients treated with this antibody. Within 24 hours after the initial treatment, eosinophil counts dropped to the normal range, and serum levels of eosinophil cationic protein became normal during the next two days. In addition, pruriginous, eczematous, and urticarial skin lesions regressed and pruritus lessened after the first infusion. No systemic or local side effects were observed, and neither leukopenia nor changes in the distribution of lymphocyte subpopulations were detected, suggesting that mepolizumab does not cause general immunosuppression.

Anti–interleukin-5 antibody has been ineffective in treating mild allergic asthma,[10] and consequently, the role of eosinophils as inflammatory effector cells in that condition has been questioned. It was recently suggested that one reason for the lack of efficacy of mepolizumab in patients with asthma is that it does not eliminate eosinophils from the allergic inflammatory site.[11]

In our study of biopsy specimens that had been stained with hematoxylin and eosin, we failed to find eosinophils in sites of previous skin lesions after treatment, but immunofluorescence staining, which is a more sensitive technique, revealed that the antibody treatment did not completely eliminate eosinophils from the dermis. Since almost all the remaining eosinophils expressed interleukin-5, it is possible that they did not undergo apoptosis because of autocrine stimulation by interleukin-5.[15]

In two of the three patients, treatment with mepolizumab was associated with decreased production of Th2 cytokines in vitro by mitogen-stimulated T cells. Similarly, T cells from mice deficient in interleukin-5 and eotaxin, in which tissue eosinophilia cannot develop, have a defect in interleukin-13 production.[16] These data suggest that eosinophils can modulate T-cell function, including differentiation and cytokine production.

Our study suggests that patients with eosinophilic dermatitis and elevated blood levels of interleukin-5 can benefit from treatment with anti–interleukin-5 antibody. Although we found no evidence of the presence of clonal T cells or eosinophilic leukemia, it remains uncertain whether the increased interleukin-5 levels reflect a reactive process or a primary malignant process. It is also not known whether the presence of a *FIP1L1–PDGFRA* fusion[5] precludes a response to antibody treatment. Long-term studies of mepolizumab for the treatment of the hypereosinophilic syndrome are warranted.

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