Original Paper

International Archives of Allergy and Immunology

Int Arch Allergy Immunol 2006;141:290–294
DOI: 10.1159/000095299

Received: January 31, 2006
Accepted after revision: April 20, 2006
Published online: August 23, 2006

No Effect of Anti-Interleukin-5 Therapy (Mepolizumab) on the Atopy Patch Test in Atopic Dermatitis Patients

J.M. Oldhoff[a] U. Darsow[b, c] T. Werfel[d] I.C. Bihari[a] K. Katzer[b, c] J. Laifaoui[b, c] S. Plötz[b, c] A. Kapp[d] E.F. Knol[a] C.A.F.M. Bruijnzeel-Koomen[a] J. Ring[b, c] M.S. de Bruin-Weller[a]

[a]Department of Dermatology and Allergology, UMC Utrecht, Utrecht, The Netherlands; [b]Department of Dermatology and Allergy Biederstein, and [c]Division of Environmental Dermatology and Allergy GSF, Technical University Munich, Munich, and [d]Department of Dermatology and Allergology, Hannover Medical University, Hannover, Germany

Key Words
Anti-interleukin-5 antibody · Atopic dermatitis · Atopy patch test · Eosinophils · Mepolizumab · Skin biopsies

Abstract
Background: The atopy patch test (APT) is an in vivo model to study the induction of eczema by inhalant allergens in atopic dermatitis (AD) patients. Mepolizumab is a monoclonal antibody to interleukin-5, which reduces peripheral blood eosinophils. Previously, we reported that mepolizumab treatment did not result in clinical improvement in AD. The current study investigates the effect of mepolizumab therapy on the APT in the same patients. ***Methods:*** Mepolizumab treatment was given at days 0 and 7 in a double-blind placebo-controlled design. The APT was applied at days –2, 0, 14 and 28. Clinical evaluation of each APT was conducted 48 h after application at days 0, 2, 16 and 30. Skin biopsies were taken at days 0, 2 and 16 for eosinophil counts. ***Results:*** The mepolizumab-treated group showed no significant reduction in macroscopic outcome of the APT. Tissue eosinophils were reduced in the mepolizumab-treated group at day 16 compared with placebo; however, this was not significant. ***Conclusion:*** Mepolizumab therapy cannot prevent the eczematous reaction induced by the APT. Furthermore, the influx of tissue eosinophil numbers in the APT is not significantly inhibited after mepolizumab treatment compared with placebo, despite a significant reduction in peripheral blood eosinophils.

Introduction

Atopic dermatitis (AD, atopic eczema) is a chronic, relapsing, itchy inflammatory disease of the skin. T cells, dendritic cells and eosinophils are thought to play a major role in the pathogenesis of the disease [1, 2].

Eosinophils are typically present in peripheral blood and tissue of patients with allergic diseases, such as AD, and eosinophils have been ascribed an important role in its pathogenesis [3, 4]. Interleukin (IL)-5 is the key cyto-

J.M.O. and U.D. contributed equally to this study.

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1018–2438/06/1413–0290$23.50/0

Accessible online at:
www.karger.com/iaa

Correspondence to: Dr. J.M. Oldhoff
Department of Dermatology/Allergology, UMC Utrecht
Heidelberglaan 100, G02.124
NL–3584 CX Utrecht (The Netherlands)
Tel. +31 30 250 7388, Fax +31 30 250 5404, E-Mail j.m.oldhoff@umcutrecht.nl

kine in eosinophil differentiation and growth in the bone marrow [5] and stimulates the release of eosinophils from the bone marrow into the peripheral circulation [6]. Moreover, IL-5 renders eosinophils more sensitive for stimuli, a process called priming [7]. For these reasons, IL-5 has been a target in the development of new therapeutic strategies for atopic diseases. Recently, we have reported a clinical study on the effect of mepolizumab therapy in atopic dermatitis patients [8]. In these patients, a significant decrease in peripheral blood eosinophils was recorded, but only a nonsignificant tendency to clinical improvement was reached. This suggests that peripheral reduction in eosinophils has no effect on existing lesions. In the same study population, we now test if eosinophil depletion might prevent the development of new eczema lesions. The atopy patch test (APT) is a frequently used in vivo model to study the mechanisms involved in the induction of eczema by inhalant allergens [9]. Furthermore, the APT is a useful tool to study the therapeutic modulation of AD [10, 11]. The cellular infiltrate during the APT reaction consists of T cells, dendritic cells, macrophages and eosinophils [10, 12–14]. The influx of eosinophils starts already 6–8 h after patch testing, and the maximum number of eosinophils is present after 24–48 h. Part of the eosinophils are activated, and depositions of eosinophil-derived proteins, such as major basic protein and eosinophil cationic protein, are present in the dermis [12]. Due to this observation, an important role has been ascribed to eosinophils in the reaction mechanism of the APT. The aim of the current study is to investigate the effect of mepolizumab therapy on the induction of new eczema lesions using the APT as a model.

Materials and Methods

Study Design

The clinical study of anti-IL-5 (mepolizumab) in AD has been described previously [8]. In brief, 43 patients with moderate to severe AD were recruited for a multi-center randomized, placebo-controlled parallel group study of which 20 received mepolizumab. Local and systemic treatment was discontinued at least 14 days before inclusion.

At screening (day –2), an APT was performed. A positive reaction was an inclusion criterion for this study. At day 0, the APT (48 h after patch testing) was evaluated and a biopsy was taken. One patient who took part in the clinical study has been included, despite of lacking a positive APT. This patient was excluded for analysis of the current study. Patients were randomized to receive either a dose of 750 mg of humanized (IgG-k) monoclonal antibody to IL-5 intravenously (mepolizumab) or placebo intravenously. Immediately after dosing, a second APT with the same allergen (day 0) was performed. At day 2, patients returned for peripheral blood analysis, APT evaluation (48 h) and an APT biopsy. At day 7, patients received a second dose of 750 mg mepolizumab or placebo. At day 14, patients returned for blood analysis and a third APT with subsequent reading of the APT and biopsy at day 16. Patients did not use any other treatment during the study period, except for nonmedicated emollients and bath oil as needed. Topical hydrocortisone acetate 1% was allowed for eczema lesions on the face. Nonresponders to therapy received fluticasone propionate cream 0.05% once daily as rescue medication from day 16 on. At day 28, all patients returned for a safety follow-up visit and a fourth APT was performed which was read at day 30 (48 h) (fig. 1).

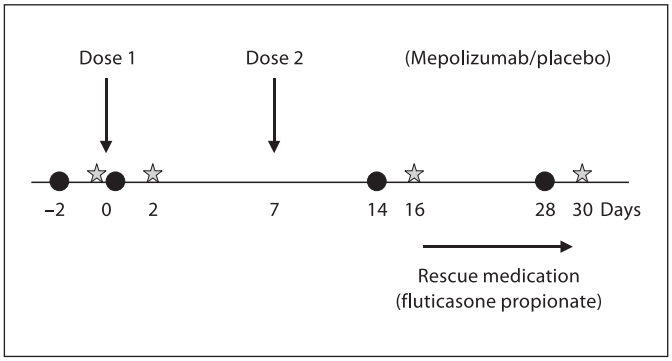

Fig. 1. Study design. ● = APT application; ☆ = APT reading and biopsy, except on day 30; APT reading without biopsy.

An automatic hematology analyzer (Beckman Coulter Gens, Paris, France) was used to measure peripheral blood eosinophils in a central laboratory (Laboratory Dr. Spranger, Ingolstadt, Germany). All participants gave their informed consent, and this study was approved by the local medical ethical committee which follows the protocol of the Declaration of Helsinki.

Atopy Patch Test

APTs were performed as described before [15] with extracts containing 200 index of reactivity (IR)/g of house dust mite or grass pollen or cat in a petrolatum vehicle (Stallergènes, Antony, France) and applied with large Finn chambers (diameter = 12 mm; Epitest Oy, Finland). The allergenic potency was translated as an IR and was 200 IR/ml for all allergens. The major allergen of house dust mite, *Dermatophagoides pteronyssinus* (Der p), consisted of 40 μg/ml Der p 1 and 8 μg/ml Der p 2. The major allergen of grass pollen consisted of 14 μg/ml Phl p 5, and the major allergen of cat consisted of 90 μg/ml Fel d 1. Petrolatum was applied as a negative control in all subjects. No tape stripping was done before allergen application, and the site of application was clinically uninvolved skin of the back. The patch test reactions were read after 48 h. Patch test reactions were recorded as positive when at least erythema and induration occurred and no reaction was observed in the control patch: 1+, erythema with slight induration; 2+, marked erythema and induration, papules; 3+, marked erythema and induration, papules and vesicles.

Biopsy Specimens

Punch biopsy specimens (3 mm) were taken from the APT sites after 48 h. Two percent xylocaine was used as local anesthesia. Biopsies were immediately fixed in 4% formalin and stored in small vials. Subsequently, biopsies were embedded in paraffin using an automatic tissue processor (Histokinette). Paraffin-embedded tissue was stored at room temperature until further handling. Sections (5 μm) were cut from the paraffin-embedded skin tissue and mounted on 3-aminopropyl tri-ethoxy silane-coated glass slides (Sigma-Aldrich, Deisenhofen, Germany).

Histochemical Staining

Histochemical staining was done with Congo red. The slides were stained in hematoxylin (Merck, Darmstadt, Germany) for 10 s and then incubated for 5 min in tap water under flow. Subsequently, the slides were stained in the solution containing 0.5% Congo red (Fluka AG, Buchs, Switzerland) in ethanol/0.1 M glycin (1:1) for 20 min. The slides were then rinsed in 70% ethanol until the background became clear [16].

Immunohistochemical/Cytochemical Staining

Monoclonal antibodies against eosinophilic cationic protein in eosinophils (EG2, Pharmacia, Uppsala, Sweden) were employed for immunohistochemical/cytochemical staining.

Slides were incubated with 1:50 diluted EG2 in 1% BSA in PBS, and subsequently, Powervision Poly AP-anti-mouse immunoglobulin (Immunologic, Duiven, the Netherlands) was added and incubated for 30 min.

Alkaline phosphatase activity was demonstrated using naphtol-ASMX phosphate (Sigma) as substrate and new fuchsine solution (Merck) as chromogen resulting in blue staining. Subsequently, the slides were stained with hematoxylin (Merck) for 10 s and rinsed in tap water for 5 min [16, 17].

Quantification of Staining

Skin sections stained with EG2 and Congo red were examined by light microscopy at ×400 magnification by a blinded observer. Only cells with a visible nucleus and positive EG2 or Congo red staining were counted. Congo red staining was used as control in cases of large eosinophil influx to discriminate between eosinophilic granulocytes and just eosinophilic deposits. Before evaluation, sections were compared with the isotype control-stained counter sections. Positive cells in the dermis were counted in three different sections of 1–1.5 mm^2 per section and calculated as cells per square millimeter. In fields containing sweat ducts and hair shafts, only intervening dermal regions were counted. The dermal and epidermal compartments were examined separately.

One out of every 5 analyses was controlled by a second independent observer. The mean interobserver coefficient of variation was approximately 10%.

Statistical Analysis

Statistical analysis was performed using the program SPSS for Windows (version 10.0.5, 1999). Placebo and treatment groups were compared considering the outcome of clinical and histological APT reaction on the different time points. The different allergens used in the APT were analyzed together as one group. The Wilcoxon signed rank test was used for within-group paired comparisons. For between-group comparisons, intragroup p values and the δ values of the effect of mepolizumab and placebo were compared using the nonparametric Mann-Whitney test for independent samples in the case of ordinal values and in the case of categorical data. Subgroup analyses of APT results per allergen were calculated using the one-sided Fisher's exact test. A p value <0.05 was considered significant.

Table 1. Baseline patient characteristics and study parameters at day 0

	Mepolizumab (n = 20)	Placebo (n = 22)
Age, years		
Mean	32	27
Range	19–57	18–42
Sex, M:F	10:10	10:12
SCORAD		
Mean	36.3	32.4
Range	20.8–54.5	18.5–45.4
Blood eosinophils/mm^3		
Mean	521	636
Range	123–1,251	74–1,810
APT		
Mean	1.7	1.7
Range	1–3	1–3
Eosinophils/mm^2		
Mean	87	74
Range	0–450	0–314

SCORAD = Scoring atopic dermatitis. APT score: 0 = negative reaction; 1 = erythema, induration; 2 = erythema, induration and papules; 3 = erythema, induration, papules and vesicles.

Results

Baseline characteristics (day 0) were the same in the mepolizumab-treated group compared with the placebo group (table 1). In the placebo group, 1 female patient was excluded from this study, because she was previously included despite a negative APT.

Peripheral blood eosinophils were significantly reduced in the mepolizumab-treated group from day 2 until the end of the study (table 2).

Rescue therapy from day 16 to day 30 was given in 8/18 patients in the mepolizumab-treated group and in 15/22 in the placebo-treated group.

Macroscopic APT Results

At baseline (day 0), macroscopic APT results were the same in both treatment groups. At days 2, 16 and 30, no statistically significant difference was demonstrated in

Table 2. Results

Parameter	Mepolizumab day 0	Placebo day 0	Mepolizumab day 2	Placebo day 2	Mepolizumab day 16	Placebo day 16	Mepolizumab day 30	Placebo day 30
Blood eosinophils/mm^3	521 ± 79	636 ± 84	144 ± 28[a]	530 ± 98	203 ± 54[a]	660 ± 82	92 ± 17[a]	473 ± 51
APT score (0–3)	1.7 ± 0.1	1.7 ± 0.2	1.5 ± 0.2, NS	1 ± 0.1	1.2 ± 0.2, NS	1.3 ± 0.2	0.9 ± 0.2	1.2 ± 0.2
Eosinophils/mm^2	87 ± 30	74 ± 20	120 ± 49, NS	75.8 ± 45	31 ± 13, NS	115 ± 55	–	–

Data are given as means ± SEM. APT score: 0 = negative reaction; 1 = erythema, induration; 2 = erythema, induration and papules; 3 = erythema, induration, papules and vesicles; NS = nonsignificant difference compared with placebo.

[a] Significant decrease compared with placebo.

the δ APT macroscopic change between the two treatment groups (table 2).

Subgroup analysis concerning the different allergens did not result in a significant decrease in the clinical outcome of the APT reaction at days 2, 16 and 30 (data not shown).

Microscopic APT Results

At baseline, no significant differences were found considering the number of eosinophils between the two treatment groups. No significant reduction was reached in the δ of tissue eosinophils between the two treatment groups at days 2 and 16 (table 2). At day 2, a nonsignificant increase was observed in the mepolizumab-treated group concerning tissue eosinophils. A subsequent large decrease in the number of tissue eosinophils was present at day 16 in the same group. No statistic significance was found between day 16 and day 0.

Discussion

We show that mepolizumab therapy does not result in a significant decrease in clinical APT reaction. Furthermore, no significant decrease in eosinophils is reached in the APT, whereas the peripheral blood eosinophil number was significantly decreased by mepolizumab therapy.

The first APT is applied directly after the first mepolizumab dose on day 0. Lack of decrease in tissue eosinophils at this time point can be explained by the short time period between dosing and APT application. Normally, already 6–8 h after application, an influx of tissue eosinophils is seen in the APT [12].

The second APT on day 16 did show the expected reduction in tissue eosinophils in the mepolizumab group compared with placebo. However, this reduction reached no significant difference compared with day 0. This is probably due to the large variability between individuals in both treatment groups. Four patients in the mepolizumab group were partly responsible for this large variation, showing massive increases in eosinophils. Exclusion of these patients from analysis did not change the statistical significance of our results (data not shown).

Previously, Flood-Page et al. [18] observed a significant decrease in tissue eosinophil counts of bronchoalveolar lavage, bone marrow and bronchial mucosa after mepolizumab therapy, given 3 times 750 mg intravenously to asthma patients with an interval of 4 weeks. This decrease in tissue eosinophils was smaller compared with the decrease in peripheral blood eosinophils. In this study, no clinical improvement was reached after allergen challenge. Our study confirms the smaller decrease in tissue eosinophils compared with the decrease in peripheral blood eosinophils. This suggests that mepolizumab has the ability to influence tissue eosinophils, although less than in peripheral blood. The limitation of our study design is that no information can be given on the long-term effect of mepolizumab on tissue eosinophils, since rescue medication (fluticasone propionate cream 0.05%) was used by most patients from day 16 on, because clinical effect was lacking [8].

Two cooperating factors can be responsible for the persistent influx of eosinophils in the APT after mepolizumab therapy. First, peripheral blood eosinophils are effectively reduced, but not completely depleted. The remaining eosinophils can still induce a positive APT reaction, since eosinophil influx in the APT is not related to the number of blood eosinophils per se.

Second, the remaining eosinophils can still migrate to the tissue by chemokines other than IL-5, such as eotaxin. Eotaxin is also able to attract eosinophils from peripheral blood into the tissue by interaction with the CC chemokine receptor 3 present on eosinophils [19].

In conclusion, the induction of an eczematous response by the APT reaction is not decreased by mepolizumab. Furthermore, no reduction in tissue eosinophil number was observed. Due to this incomplete decrease in eosinophils by the treatment, no definitive proof can be delivered whether eosinophils are important or not for the induction of the APT. Only a nonsignificant decrease in tissue eosinophils was seen at day 16. Therefore, future studies should focus on prolonged mepolizumab therapy or a combination therapy with the CC chemokine receptor 3 antagonist.

Acknowledgements

The authors wish to thank the following persons/institutions for their successful contribution to the study: J. vd Woude and B. Sukkel of the Department of Dermatology and Allergology in Utrecht, the Netherlands; M. Lahfa, F. Suarez and L. Dubertret, Department of Dermatology, Hôpital Saint Louis, Paris, France; C. Buzenet and A. Taïeb, Department of Dermatology, Hôpital St-André, Bordeaux, France; H.J. Wilkins, CDMA, GSK, USA; M. Herdman, MIGU, GSK, UK, and E. Glazenburg, Medical Department, GSK, the Netherlands. This study was funded in part by Glaxo Smith Kline Pharmaceuticals.

References

1 Leung DY, Bieber T: Atopic dermatitis. Lancet 2003;361:151–160.
2 Ruzicka T, Ring J, Przybilla B: Handbook of Atopic Eczema. Berlin, Springer, 1991.
3 Ring J, Darsow U, Jakob T, Behrendt H: Eczema and dermatitis. ACI International 2005;17:19–25.
4 Leiferman KM: A role for eosinophils in atopic dermatitis. J Am Acad Dermatol 2001; 45(suppl 1):S21–S24.
5 Sanderson CJ: Interleukin-5, eosinophils, and disease. Blood 1992;79:3101–3109.
6 Collins PD, Marleau S, Griffiths-Johnson DA, Jose PJ, Williams TJ: Cooperation between interleukin-5 and the chemokine eotaxin to induce eosinophil accumulation in vivo. J Exp Med 1995;182:1169–1174.
7 Warringa RA, Mengelers HJ, Kuijper PH, Raaijmakers JA, Bruijnzeel PL, Koenderman L: In vivo priming of platelet-activating factor-induced eosinophil chemotaxis in allergic asthmatic individuals. Blood 1992;79: 1836–1841.
8 Oldhoff JM, Darsow U, Werfel T, Katzer K, Wulf A, Laifaoui J, et al: Anti-IL-5 recombinant humanized monoclonal antibody (mepolizumab) for the treatment of atopic dermatitis. Allergy 2005;60:693–696.
9 Ring J, Darsow U, Gfesser M, Vieluf D: The 'atopy patch test' in evaluating the role of aeroallergens in atopic eczema. Int Arch Allergy Immunol 1997;113:379–383.
10 Bruin-Weller MS, Knol EF, Bruijnzeel-Koomen CA: Atopy patch testing – a diagnostic tool? Allergy 1999;54:784–791.
11 Langeveld-Wildschut EG, Riedl H, Thepen T, Bihari IC, Bruijnzeel PLB, Bruijnzeel-Koomen CAFM: Modulation of the atopy patch test reaction by topical corticosteroids and tar. J Allergy Clin Immunol 2000;106: 737–743.
12 Bruynzeel-Koomen CA, Van Wichen DF, Spry CJ, Venge P, Bruynzeel PL: Active participation of eosinophils in patch test reactions to inhalant allergens in patients with atopic dermatitis. Br J Dermatol 1988;118: 229–238.
13 Thepen T, Langeveld-Wildschut EG, Bihari IC, Van Wichen DF, Van Reijsen FC, Mudde GC, et al: Biphasic response against aeroallergen in atopic dermatitis showing a switch from an initial TH2 response to a TH1 response in situ: an immunocytochemical study. J Allergy Clin Immunol 1996;97:828–837.
14 Walker C, Kagi MK, Ingold P, Braun P, Blaser K, Bruijnzeel-Koomen CA, et al: Atopic dermatitis: correlation of peripheral blood T cell activation, eosinophilia and serum factors with clinical severity. Clin Exp Allergy 1993; 23:145–153.
15 Darsow U, Laifaoui J, Kerschenlohr K, Wollenberg A, Przybilla B, Wuthrich B, et al: The prevalence of positive reactions in the atopy patch test with aeroallergens and food allergens in subjects with atopic eczema: a European multicenter study. Allergy 2004;59: 1318–1325.
16 Ying S, Meng Q, Smith SJ, Larché M, Robinson DS, Kay AB: Methods for identifying human eosinophils in blood and tissue. ACI International 2002;14:64–71.
17 Tai PC, Spry CJ, Peterson C, Venge P, Olsson I: Monoclonal antibodies distinguish between storage and secreted forms of eosinophil cationic protein. Nature 1984;309:182–184.
18 Flood-Page PT, Menzies-Gow AN, Kay AB, Robinson DS: Eosinophil's role remains uncertain as anti-interleukin-5 only partially depletes numbers in asthmatic airway. Am J Respir Crit Care Med 2003;167:199–204.
19 Elsner J, Forssmann U: Another renaissance of eosinophils in allergic diseases. ACI International 2002;14:151–155.