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Int Arch Allergy Immunol. Author manuscript; available in PMC 2014 September 11.

Published in final edited form as:
Int Arch Allergy Immunol. 2013 ; 162(1): 89–93. doi:10.1159/000350486.

Immunologic Effects of Omalizumab in Children with Severe Refractory Atopic Dermatitis: A Randomized, Placebo-Controlled Clinical Trial

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Abstract

Background—Severe refractory atopic dermatitis (AD) is a chronic, debilitating condition that is associated with elevated serum immunoglobulin E (IgE) levels. Thymic stromal lymphopoietin (TSLP), thymus and activation-regulated chemokine (TARC) and OX40 ligand (OX40L) are important immunologic factors involved in the pathogenesis of AD. Omalizumab, an anti-IgE antibody indicated for use in allergic asthma, is implicated in regulating allergen presentation by dendritic cells and the T cell response during the effector phases of allergic disease. We investigated if anti-IgE therapy modulates the allergen-specific responses mediated by the TSLP pathway in young patients with severe refractory AD.

Methods—This was a randomized, double-blind, placebo-controlled study of 8 patients between the ages of 4 and 22 years (mean = 11.6 years) with severe refractory AD (clinical trials.gov NCT01678092). Serum IgE ranged from 218 to 1,890 (mean = 1,068 IU/ml). Subjects received omalizumab (n = 4) or placebo (n = 4) every 2–4 weeks over 24 weeks using a regimen extrapolated from the package insert. TSLP, TARC, OX40L and other cytokines involved in AD were measured by using cytometric bead arrays.

Results—All patients receiving omalizumab had strikingly decreased levels of TSLP, OX40L, TARC (involved in Th2 polarization) and interleukin (IL)-9 compared to placebo. In addition, there was a marked increase in IL-10, a tolerogenic cytokine, in the omalizumab-treated group. Patients on anti-IgE therapy had an improvement in clinical outcomes as measured by the SCORAD system; however, these effects were comparable to improvements in the control group.

Conclusions—Anti-IgE therapy with omalizumab decreases levels of cytokines that are involved in Th2 polarization and allergic inflammation, including TSLP, TARC and OX40L.

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Keywords

Atopic dermatitis; Immunoglobulin E; Omalizumab; Cytokine expression

Introduction

Omalizumab is a humanized monoclonal anti-immunoglobulin E (IgE) antibody that binds to the IgE molecule at the high-affinity IgE receptor (Fc epsilon RI, FcεRI) binding site and is indicated for use in allergic asthma [1]. Anti-IgE therapy significantly reduces free IgE circulating in serum [2], and reduces the expression of the FcεRI on multiple cell types, including mast cells and basophils [3]. These actions inhibit mast cell and basophil activation, thereby decreasing both the early- and late-phase allergic response. In addition to its effects on immediate hypersensitivity, anti-IgE also decreases FcεRI expression by dendritic cells (DCs) [3] and has been implicated in the regulation of T cell responses through effects on Th2 polarization.

Although historically viewed solely as a physical barrier, emerging evidence now indicates that the epithelium plays a central role in the Th2-cell sensitization process through its stimulatory effects on DCs. Atopic dermatitis (AD), or allergic eczema, is a common pediatric problem that affects approximately 10–15% of children [4] and arises due to defects in the epithelial barrier that are thought to result in excessive T cell activation. AD is associated with elevated serum levels of IgE, and recent data indicate that systemic activation of T cells may play an important role. Patients with AD have increased numbers of circulating activated T cells, and elevated serum L-selectin levels, a marker for leukocyte activation that correlates with AD disease severity [5].

A core group of cytokines drive Th2-mediated allergic inflammation in AD. Thymic stromal lymphopoietin (TSLP), an epithelial cell-derived cytokine, is induced in keratinocytes of AD skin lesions and has been shown to play an important role in the pathogenesis of AD [6, 7]. TSLP modulates polarization of DCs by increasing OX40 ligand (OX40L) DC surface expression and secretion of Th2 cell-attracting chemokines, like thymus and activation-regulated chemokine (TARC)/chemokine (C-C motif) ligand 17 (CCL17). Activated DCs expressing the costimulatory molecule OX40L interact with OX40 on the membrane of naïve T helper cells, resulting in Th2 cell proliferation and cytokine production. TSLP has also been implicated in the amplification of Th2 cytokine production by mast cells and natural killer T cells [8]. Therefore, TSLP plays a critical role in promoting Th2-mediated allergic inflammation in AD. Other cytokines involved in the pathogenesis of AD include interleukin (IL)-9, which is significantly increased in lesional skin areas of AD patients and other allergic inflammatory diseases, like asthma [9].

Several case reports investigating anti-IgE therapy in patients with AD found symptomatic improvement with omalizumab [10, 11], but none have done so in a placebo-controlled manner. To test the hypothesis that anti-IgE therapy modulates the TSLP pathway and improves clinical outcomes in patients with AD, we assessed TSLP, OX40L, TARC and other cytokines as well as several clinical measures in AD patients during a double-blind, placebo- controlled pilot study of omalizumab. We evaluated 8 young patients with severe

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refractory AD, using a higher dosing schedule of omalizumab approved by the FDA to neutralize the higher levels of IgE (see table 1).

Material and Methods

Study Design

This randomized, double-blind, placebo-controlled study was approved by the Stanford University Medical Center Investigational Review Committee and registered at clinical trials.gov (NCT01678092). Written informed consent was obtained from all study participants. On enrollment, blood samples were collected (20 ml) and prior eczema medications used by study patients were changed to medication regimens consisting mainly of cetirizine, triamcinolone ointment and pimecrolimus. Skin condition and the baseline use of medications were assessed by parents with a diary. The diary was provided to document their child's skin condition and use of medication over a 2-week period. All medications were then discontinued 1 week prior to the first dose of omalizumab/placebo in order to also determine the severity of AD in these patients off all therapy.

Omalizumab or placebo was started 4 weeks after the baseline screening visit. Patients subsequently received either omalizumab (4 patients) or placebo (4 patients) every 2–4 weeks subcutaneously over the next 24 weeks. Omalizumab was administered using a regimen extrapolated from the package insert dosages (150–375 mg every 2–4 weeks). SCORAD (Scoring Atopic Dermatitis; see table 1) and blood samples were taken before each monthly visit. Serum levels of IgE and relevant cytokines were determined as described below. In addition, routine blood counts and serum chemistries were measured for determining safety.

Cytokine and IgE Measurements

Cytokines were measured using the Th2 Flex cytometric bead array (BD Biosciences, San Jose, Calif., USA). In brief, 50 μl of the mixed capture beads and 50 μl of plasma were incubated for 1 h at room temperature. After adding 50 μl of the phycoerythrin detection reagent to the mixture and incubation for 2 h at room temperature, the beads were then washed with the wash buffer and analyzed with a BD FACSArray Bioanalyzer (BD Biosciences). The cytometric bead array data were analyzed with FCAP Array software version 1.0.1 (BD Biosciences). The minimal detection limits are 5 pg/ml. ELISA (Invitrogen, Carlsbad) was used for those factors and cytokines not within the Th2 flex set (antibodies were obtained from BD Biosciences and were used as per manufacturer's instructions). Adverse events and clinical status were monitored per NIH guidelines. Free IgE levels were determined using previously outlined methods [12] in which the serum concentration of (non-omalizumab-bound) free IgE was measured using a solid phase immunoenzymetric assay. IgE was captured from serum with monoclonal anti-human IgE (clone HP6061) and detected with labeled-FcεR1a.

Results

Patients recruited for the study ranged between 4 and 22 years of age (mean = 11.6 years), and serum IgE ranged from 218 to 1,890 (mean = 1,068 IU/ml; see table 1). All patients had

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severe AD that had failed standard therapy. Several patients had histories of hospitalization due to refractory AD, and all had markedly elevated baseline skin assessment scores (using SCORAD [13] ; see table 1). Half of the study patients (n = 4) had baseline SCORAD scores greater than 90, exemplifying the complex, refractory nature of their chronic disease. Two study patients (patients 6 and 7) in the treatment group had had prior hospitalizations for skin infections and had been placed on antibiotic prophylaxis several times in the past. In addition, most patients had concurrent asthma (n = 6) and allergic rhinitis (n = 7).

Omalizumab was well tolerated, and serious adverse events deemed related to omalizumab treatment were not observed. Patients in the omalizumab group were much younger than those in the placebo group (mean age was 7.4 vs. 15.8 years in placebo group). Study patients receiving omalizumab had significant decreases in their free serum IgE levels [12] in the first serum sample drawn on therapy (week 8). These levels dropped even further (<15 U/l) by week 24 (see table 1). This demonstrates that the treatment regimen administered to patients in the omalizumab group was sufficient to achieve almost complete binding of free serum IgE despite their high pretreatment free serum IgE levels.

Cytokines implicated in the pathogenesis of AD were examined in the plasma of participants via previously described ELISA methods. There were marked differences in TSLP [14] and TARC/CCL17 [15] between the 2 groups. TSLP was reduced by 50–75% in all study patients in the omalizumab-treated group, with no observed reduction in the placebo-treated group (see table 1). OX40L was reduced by 70–80% in the omalizumab-treated group (see table 1). TARC levels were reduced in all 4 study patients in the omalizumab-treated group (between 60 and 80% in 3 patients; see table 1) compared to the placebo-treated group. IL-9, a cytokine secreted in affected skin areas of AD patients that also potentiates IL-4-mediated IgE production from B cells [9], was also decreased in the omalizumab-treated group (by 50–75%), with increasing levels noted in the majority of patients in the placebo-treated group over the course of the study (data not shown). Serum levels of IL-10, a tolerogenic cytokine produced by T regulatory cells, was increased between 80 and 100% in omalizumab-treated, but not in the placebo-treated study patients (see table 1). Patients in the omalizumab group had SCORAD reductions of approximately 20–50%. However, a SCORAD reduction of approximately 45–80% was observed in the placebo group (see table 1).

Discussion

Anti-IgE therapy has been hypothesized to have significant immunomodulatory activity beyond its role in immediate hypersensitivity. As IgE may facilitate antigen presentation by DCs, inhibition of IgE may result in a decrease in T cell antigen-specific responses [16]. In addition, blocking serum IgE from binding and activating the FcεRI on mast cells and basophils likely results in the diminution of other components of the allergic response.

A recent study by Schroeder et al. [17] demonstrated that omalizumab treatment significantly decreased cat-allergen- specific T cell proliferation and Th2 cytokine expression by approximately 25 and 50%, respectively. This suggests that IgE facilitates allergen presentation by DCs in vivo and possibly regulates DC-dependent T cell cytokines

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during effector phases of allergic disease. A recent study by our group investigating 11 children treated with omalizumab while undergoing oral immunotherapy revealed near elimination of the CD4+ T cell response to milk within a week of treatment [18]. The rapid reduction in T cell response could have been potentiated by both the oral desensitization process as well as the effects of anti-IgE therapy. Comparable oral immunotherapy studies in peanut-allergic patients not given anti-IgE have revealed T cell changes occurring only after several months [19]. Therefore, it is likely that anti-IgE plays an important role in decreasing the antigen-specific T cell response to milk in this study.

Our placebo-controlled pilot study of omalizumab in children with severe AD demonstrated several distinct immunologic changes in key molecules known to be involved in the underlying pathogenesis of AD. We found that omalizumab effectively reduced free IgE levels, even in patients with serum IgE levels up to 1,890 IU/ml, as free serum IgE levels decreased early on in the study period. In addition, all patients receiving omalizumab had decreased levels of TSLP, TARC, OX40L and IL-9. TSLP, a potent activator of myeloid DCs that can polarize naïve CD4+ T cells towards Th2 cells [20], is expressed at high levels by the epidermal keratinocytes of AD patients [21] as well as the asthmatic bronchial epithelium [20]. OX40L, a costimulatory molecule expressed on DCs, is a critical in vivo mediator of TSLP-mediated Th2 responses and is involved in the amplification of Th2 differentiation. In vivo studies examining mice treated with OX40L-blocking antibodies showed substantial inhibition of TSLP-induced immune responses in the skin and lungs [22]. Our results suggest that preventing IgE-mediated activation may help to reduce Th2 polarization in AD. In addition, other mediators of Th2 inflammation which have been linked to the severity of AD, such as TARC and IL-9, were also found to be reduced. Conversely, IL-10, a regulatory cytokine secreted by T regulatory cells that has been implicated in mitigating allergic inflammatory responses, was increased in the omalizumab-treated group. Therefore, by blocking serum IgE from binding and activating the FcεRI on mast cells, mast cell activation could be abrogated and result in the decrease of several components involved in the allergic response.

To date, all published studies examining the clinical effects of anti-IgE therapy in patients with AD have not been placebo-controlled. These reports uniformly demonstrate a clinical improvement with anti-IgE therapy, as demonstrated by decreased pruritus and improved quality of life scores in AD patients [23]. Similar to these studies, all 4 patients in the omalizumab group had clinical improvement (SCORAD reduction of approximately 20–50%). However, the changes noted in the omalizumab treatment group were comparable to the clinical effects (SCORAD reduction of approximately 45–80%) observed in the placebo group. The high rate of response to placebo has been well documented in these types of studies and, had any of the above published studies examining the clinical effects of anti-IgE included a placebo arm, a similar rate of response may have been seen. However, given that this was a pilot study involving 8 patients in total, there were multiple additional factors that may have contributed to the lack of a clear difference in clinical outcomes with omalizumab treatment in spite of substantial reductions in the serum levels of key cytokines. First, inherent differences in patient characteristics between the omalizumab-treated and placebo-treated groups may have affected outcomes. Patients in the omalizumab group were much younger than those receiving placebo (a mean of 7.4 years in omalizumab group and 15.8

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years in placebo group). This occurred despite the use of a double-blinded randomization procedure generated by the Stanford University Hospital pharmacy. This discrepancy in age between the treatment and placebo group was likely partially due to the small study population size. As more severe clinical disease is often seen in younger patients, this may have resulted in the omalizumab-treated group having significantly more severe clinical disease. In addition, all patients had markedly complex allergic disease. This was marked by the presence of not only severe refractory AD, but other concomitant allergic diseases as well (like asthma and allergic rhinitis). These factors, combined with a small study population size, could also have contributed to a lack of difference in clinical outcomes among the 2 groups, despite significant changes in cytokines implicated in AD pathogenesis. Lastly, although our study showed that treatment with omalizumab markedly decreased many Th2 inflammatory markers, including TSLP and TARC, these levels continued to remain strikingly abnormal when compared to healthy controls (see table 1). Therefore, it is possible that a longer period of dosing is necessary for these molecular markers to further decrease to that of healthy controls, leading to observable clinical changes. In addition, more information regarding serum levels of these markers in healthy controls needs to be obtained in order to establish normative data.

In summary, given the favorable data on molecular biomarker changes observed in this placebo-controlled pilot study, we believe that these data establish a basis for undertaking further evaluation of the effects of anti-IgE in AD. A larger placebo-controlled trial examining the effects of omalizumab on antigen-specific T cell proliferation and function would allow a more comprehensive elucidation of the role of IgE in immunologic processes beyond immediate hypersensitivity.

Acknowledgments

We would like to thank the Oxnard Foundation for supporting this study.

References

1. Berger W, Gupta N, McAlary M, Fowler-Taylor A. Evaluation of long-term safety of the anti-IgE antibody, omalizumab, in children with allergic asthma. Ann Allergy Asthma Immunol. 2003; 91:182–188. [PubMed: 12952113]
2. MacGlashan DW Jr, Bochner BS, Adelman DC, Jardieu PM, Togias A, McKenzie-White J, Sterbinsky SA, Hamilton RG, Lichtenstein LM. Down-regulation of Fc(epsilon)RI expression on human basophils during in vivo treatment of atopic patients with anti-IgE antibody. J Immunol. 1997; 158:1438–1445. [PubMed: 9013989]
3. Prussin C, Griffith DT, Boesel KM, Lin H, Foster B, Casale TB. Omalizumab treatment downregulates dendritic cell FcepsilonRI expression. J Allergy Clin Immunol. 2003; 112:1147–1154. [PubMed: 14657874]
4. Kemp AS. Atopic eczema: Its social and financial costs. J Paediatr Child Health. 1999; 35:229–231. [PubMed: 10404440]
5. Hirata T, Furie BC, Furie B. P-, e-, and l-selectin mediate migration of activated CD8+ T lymphocytes into inflamed skin. J Immunol. 2002; 169:4307–4313. [PubMed: 12370362]
6. Soumelis V, Reche PA, Kanzler H, Yuan W, Edward G, Homey B, Gilliet M, Ho S, Antonenko S, Lauerma A, Smith K, Gorman D, Zurawski S, Abrams J, Menon S, McClanahan T, de Waal-Malefyt Rd R, Bazan F, Kastelein RA, Liu YJ. Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. Nat Immunol. 2002; 3:673–680. [PubMed: 12055625]

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7. Corrigan CJ, Jayaratnam A, Wang Y, Liu Y, de Waal Malefyt R, Meng Q, Kay AB, Phipps S, Lee TH, Ying S. Early production of thymic stromal lymphopoietin precedes infiltration of dendritic cells expressing its receptor in allergen-induced late-phase cutaneous responses in atopic subjects. Allergy. 2009; 64:1014–1022. [PubMed: 19187393]
8. Liu YJ, Soumelis V, Watanabe N, Ito T, Wang YH, Malefyt ReW, Omori M, Zhou B, Ziegler SF. TSLP: An epithelial cell cytokine that regulates T cell differentiation by conditioning dendritic cell maturation. Annu Rev Immunol. 2007; 25:193–219. [PubMed: 17129180]
9. Sismanopoulos N, Delivanis DA, Alysandratos KD, Angelidou A, Vasiadi M, Therianou A, Theoharides TC. IL-9 induces VEGF secretion from human mast cells and IL-9/IL-9 receptor genes are overexpressed in atopic dermatitis. PLoS One. 2012; 7:e33271. [PubMed: 22413008]
10. Belloni B, Ziai M, Lim A, Lemercier B, Sbornik M, Weidinger S, Andres C, Schnopp C, Ring J, Hein R, Ollert M, Mempel M. Low-dose anti-IgE therapy in patients with atopic eczema with high serum IgE levels. J Allergy Clin Immunol. 2007; 120:1223–1225. [PubMed: 17936892]
11. Velling P, Skowasch D, Pabst S, Jansen E, Tuleta I, Grohé C. Improvement of quality of life in patients with concomitant allergic asthma and atopic dermatitis: one year follow-up of omalizumab therapy. Eur J Med Res. 2011; 16:407–410. [PubMed: 22024441]
12. Hamilton RG, Marcotte GV, Saini SS. Immunological methods for quantifying free and total serum IgE levels in allergy patients receiving omalizumab (Xolair) therapy. J Immunol Methods. 2005; 303:81–91. [PubMed: 16045925]
13. Severity scoring of atopic dermatitis: The SCORAD index. Consensus report of the European Task Force on atopic dermatitis. Dermatology. 1993; 186:23–31. [PubMed: 8435513]
14. Ebner S, Nguyen VA, Forstner M, Wang YH, Wolfram D, Liu YJ, Romani N. Thymic stromal lymphopoietin converts human epidermal Langerhans cells into antigen-presenting cells that induce proallergic T cells. J Allergy Clin Immunol. 2007; 119:982–990. [PubMed: 17320941]
15. Kakinuma T, Nakamura K, Wakugawa M, Mitsui H, Tada Y, Saeki H, Torii H, Asahina A, Onai N, Matsushima K, Tamaki K. Thymus and activation-regulated chemokine in atopic dermatitis: serum thymus and activation- regulated chemokine level is closely related with disease activity. J Allergy Clin Immunol. 2001; 107:535–541. [PubMed: 11240957]
16. Holgate S, Smith N, Massanari M, Jimenez P. Effects of omalizumab on markers of inflammation in patients with allergic asthma. Allergy. 2009; 64:1728–1736. [PubMed: 19839977]
17. Schroeder JT, Bieneman AP, Chichester KL, Hamilton RG, Xiao H, Saini SS, Liu MC. Decreases in human dendritic cell-dependent T(h)2-like responses after acute in vivo IgE neutralization. J Allergy Clin Immunol. 2010; 125:896–901. e896. [PubMed: 20132969]
18. Bedoret D, Singh AK, Shaw V, Hoyte EG, Hamilton R, Dekruyff RH, Schneider LC, Nadeau KC, Umetsu DT. Changes in antigen-specific T-cell number and function during oral desensitization in cow's milk allergy enabled with omalizumab. Mucosal Immunol. 2012; 5:267–276. [PubMed: 22318492]
19. Varshney P, Jones SM, Scurlock AM, Perry TT, Kemper A, Steele P, Hiegel A, Kamilaris J, Carlisle S, Yue X, Kulis M, Pons L, Vickery B, Burks AW. A randomized controlled study of peanut oral immunotherapy: clinical desensitization and modulation of the allergic response. J Allergy Clin Immunol. 2011; 127:654–660. [PubMed: 21377034]
20. Liu YJ. Thymic stromal lymphopoietin: Master switch for allergic inflammation. J Exp Med. 2006; 203:269–273. [PubMed: 16432252]
21. Yoo J, Omori M, Gyarmati D, Zhou B, Aye T, Brewer A, Comeau MR, Campbell DJ, Ziegler SF. Spontaneous atopic dermatitis in mice expressing an inducible thymic stromal lymphopoietin transgene specifically in the skin. J Exp Med. 2005; 202:541–549. [PubMed: 16103410]
22. Seshasayee D, Lee WP, Zhou M, Shu J, Suto E, Zhang J, Diehl L, Austin CD, Meng YG, Tan M, Bullens SL, Seeber S, Fuentes ME, Labrijn AF, Graus YM, Miller LA, Schelegle ES, Hyde DM, Wu LC, Hymowitz SG, Martin F. In vivo blockade of OX40 ligand inhibits thymic stromal lymphopoietin-driven atopic inflammation. J Clin Invest. 2007; 117:3868–3878. [PubMed: 18060034]
23. Barrios JL, Paradis L, Desroches A. Efficacy of omalizumab therapy in a case of severe atopic dermatitis. Allergy Asthma Clin Immunol. 2011; 7(suppl 2):A33.

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Table 1

Serum markers and SCORAD

Patient	Total IgE, IU/ml	Free IgE, IU/ml		TSLP, ng/ml		OX40L, ng/ml		TARC, pg/ml		IL-10, pg/ml		SCORAD	
	pre	pre	post	pre	post	pre	post	pre	post	pre	post	pre	post
Placebo													
1	1,784	1,690	1,356	250	260	690	690	300	330	120	110	94	16
2	438	282	265	320	340	730	770	320	300	110	100	93	<5
3	1,840	1,783	1,867	370	410	830	850	390	450	150	140	>99	56
4	218	95	204	290	300	740	770	310	320	140	140	37	15
Omalizumab													
5	375	269	10	370	110	710	200	280	70	130	240	80	65
6	1,326	912	12	280	100	750	210	250	200	100	210	>99	83
7	1,890	1,874	9	360	90	770	140	340	90	120	280	45	37
8	672	355	8	310	150	670	190	330	70	110	260	70	34
Healthy control	908			30		50		30		460			