

Original article

After 6 years with Xolair; a 3-year withdrawal follow-up

Background: This study reports the clinical and immunological state of patients 3 years after a 6-year period of Xolair treatment for severe allergic asthma.

Methods: The patient's cat allergen sensitivity, measured as CD-sens, IgE and IgE- and IgG4 antibodies, was analysed and compared with asthma severity evaluated from FEV₁ and a questionnaire.

Results: Three years after treatment with Xolair was stopped, 12/18 patients reported improved or unchanged asthma compared with ongoing Xolair treatment. Most of the patients were in a stable clinical condition, 16/18 had not increased nightly asthma attacks and 14/18 little or no increase in medication. The CD-sens to cat was still significantly lower ($P < 0.02$) than untreated patients with allergic asthma and lower than expected from their serum IgE antibody levels.

Conclusion: Most of the patients in this study had, still 3 years after closing of 6 years Xolair treatment, a surprisingly mild and stable asthma. Interestingly, the observed, considerable, downregulation of basophil allergen sensitivity, CD-sens, most likely representing mast cell allergen sensitivity, contributed to the clinical results.

A. Nopp¹, S. G. O. Johansson^{1,2},
J. Adédoyin¹, J. Ankerst³,
M. Palmqvist⁴, H. Öman⁵

¹Department of Medicine, Clinical Immunology and Allergy Unit, Karolinska Institute, Stockholm;

²Department of Clinical Immunology and Transfusion Medicine, Karolinska University Hospital, Stockholm; ³Clinical Sciences, Department of Medicine, Lund; ⁴Department of Respiratory Medicine and Allergology, Sahlgrenska University Hospital, Göteborg; ⁵MIAB, Uppsala, Sweden

Key words: CD-sens; IgE antibody; omalizumab.

Anna Nopp, PhD
Clinical Immunology and Allergy Unit
Karolinska University Hospital L2:04
S-171 76 Stockholm
Sweden

Accepted for publication 8 June 2009

For several years an alternative treatment of moderate-to-severe IgE-mediated allergic asthma has been available in many countries. IgE-specific immunomodulation therapy (ESIT), refers to the administration of a humanized monoclonal anti-IgE antibody, Xolair, (omalizumab; Novartis, Basel Switzerland) which will, within days, reduce the level of monomeric serum IgE, sometimes referred to as 'free IgE', by as much as 96% followed by a 73% decrease in FcεRI expression on basophils (1, 2). As a result, by time, nasal, bronchial and skin test allergen responses get significantly reduced (3).

When to stop the treatment has become a clinical dilemma. Withdrawal of omalizumab after less than a year of treatment results in a return to pretreatment clinical state, FcεR number and serum IgE levels within months (4). However, long-term treatment, in this case 6 years, seems most favourable. Despite being selected for a severe asthma the patients were in good condition during treatment and no re-bounce effect on clinical or immunological parameters was seen during withdrawal (5).

The aim of this study was to continue to follow the long-term Xolair-treated patients, for an additional 2 years after withdrawal, with basophil allergen sensitivity, CD-sens, serological parameters and the perception of symptoms and need for drugs.

Material and methods

Subjects

The allergic patients (18 of the initial group of 22 patients participated in this study; 15 cat allergic and three mite allergic), age median 50 years, range 39–73, had received ESIT, (omalizumab) for 6 years as part of a prospective, clinical trial of severe allergic asthma which needed high doses of inhaled, but no per-oral, steroids (CIGE 0250011 followed by three extensions). Their clinical and immunological state during the first 12 months after withdrawal has been reported (5) and some data from that report will be included in this study to allow comparisons.

The patients were invited to a disease evaluation, including forced expiratory volume in one second (FEV₁), 3 years after Xolair withdrawal. Each patient was asked to fill out a questionnaire, similar to the one used for the initial selection 1998, with 10 questions about asthma symptoms, medication and emergency hospital visits. In principle, each question contained three parts 'better', 'unchanged' or 'worse' and was given 0, 2 and 4 score points respectively. In addition to CD-sens and serological parameters, the number of IgE molecules and FcεRI receptors per basophil were calculated and compared with similar, unpublished, data from a previous study, a Pilot Investigation of Xolair (PIX) (6).

The reference group consisted of patients ($n = 15$) with a clinical history of moderate, well controlled asthma and/or rhinoconjunctivitis and who tested positive for IgE antibodies to cat. All patients gave their informed consent to participate in the study, which was approved by the ethics committee of University of Gothenburg, Sweden.

Basophil activation

Basophil allergen threshold sensitivity analyses were performed as previously described (7, 8). CD-sens was defined as the inverted value for the allergen concentration giving a 50% of maximum CD63% upregulation multiplied by 100, and used to describe a patient's allergen-specific sensitivity. The higher the CD-sens, the higher the basophil allergen sensitivity. The intra-assay CV was 13.1% and the interassay CV 5.4% (7).

Calculation of allergen binding activity

The CD-sens from untreated whole blood was compared with the CD-sens in blood samples where the plasma had been removed by washing the cells twice with PBS before allergen stimulation. The ratio CD-sens after/before washing was calculated and termed 'allergen binding activity' (ABA) (5).

Calculation of IgE per basophils

The number of IgE molecules per basophil was calculated using an FITC-conjugated antibody to IgE when compared with a standard curve of calibration and expressed as previously described (7).

Immunoglobulin analyses

The serum concentrations of IgE (kU/l) were determined using ImmunoCAP™ Total IgE (Phadia AB, Uppsala, Sweden) and of IgE- (kU_A/l) and IgG4 (mg/l) antibodies to cat dander (e1) and house dust mite (d1) using ImmunoCAP™ Specific IgE (Phadia AB) according to the manufacturer's instructions. The relative IgE antibody concentration, i.e. the size of the IgE antibody fraction, was expressed as absolute value in percentage of IgE.

Statistical analysis

Data are presented as 'medians and interquartile range'. Paired comparisons over time within patients were performed using the nonparametric method Wilcoxon paired test and between patients and controls by using the nonparametric method Mann-Whitney *U*-test. Correlations between the different parameters were sought by calculating the Spearman's correlation coefficient. Differences were considered statistically significant at $P < 0.05$.

Results

Clinical state

During the 3 years after withdrawal, the patients were clinically stable in their asthma, and no significant changes in FEV₁ could be detected at the 3-year visit (Fig. 1). The clinical evaluation at the visit showed that eight of the 18 patients judged their asthma symptoms to be less severe than the same when they were on Xolair treatment, i.e. close to three times as many as 1 year after withdrawal (Fig. 2A). Sixteen of the 18 patients reported that their nightly asthma exacerbations had decreased or were unchanged after the Xolair withdrawal (Fig. 2B), while two reported more

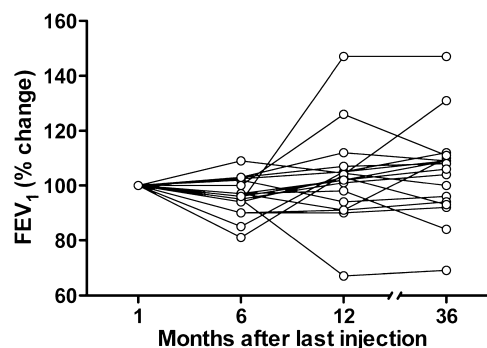


Figure 1. Measurements of the 18 patient's lung function (FEV₁) at approximately 3 years after Xolair withdrawal. The samples from each patient are linked. The results from months 1, 6 and 12 and controls have previously been published (5).

problems. Only four of the 18 patients reported an increase in asthma medication (Fig. 2C), but neither of them needed per-oral steroids.

Basophil allergen sensitivity, CD-sens

At the 3-year follow-up, four of the 15 cat allergic patients had a CD-sens ≤ 0.4 , i.e. below the low quartile of the asthma controls. The CD-sens to cat had increased in eight of the 15 patients and remained the same in four of the 15 or decreased in three of the 15 patients compared with the 1-year sample. However, the CD-sens (1.3; 0.5–4.4) was still significantly ($P < 0.02$) lower than that of the controls (6.8; 2.9–14.1) (Fig. 3). The CD-sens of the 3 mite allergic patients followed the same pattern as that of the cat allergic patients and they had only weakly positive CD-sens at the 3-year sampling. One patient had developed a remarkable cat sensitivity during the last 2 years; CD-sens had increased from 0 to 57.1, i.e. close to 10 times the mean of the controls.

There was a higher median CD-sens in the patient groups reporting worse asthma, need for more medication and in those reporting increase in nightly asthma (Table 1) when compared with those reporting improvements or unchanged conditions. This became even more clear when the cat allergic patients were classified by their clinician as having controlled, partially controlled or uncontrolled asthma (Table 2); the group with disease under control had a much lower CD-sens and symptom score than the others, while the ABA och IgG4 antibody concentrations were slightly higher. However, no difference was seen for IgE antibody values.

Serological parameters

To study whether ESIT treatment has an effect on serological mechanisms ABA was calculated for cat allergy. After 3 years, there was no significant difference

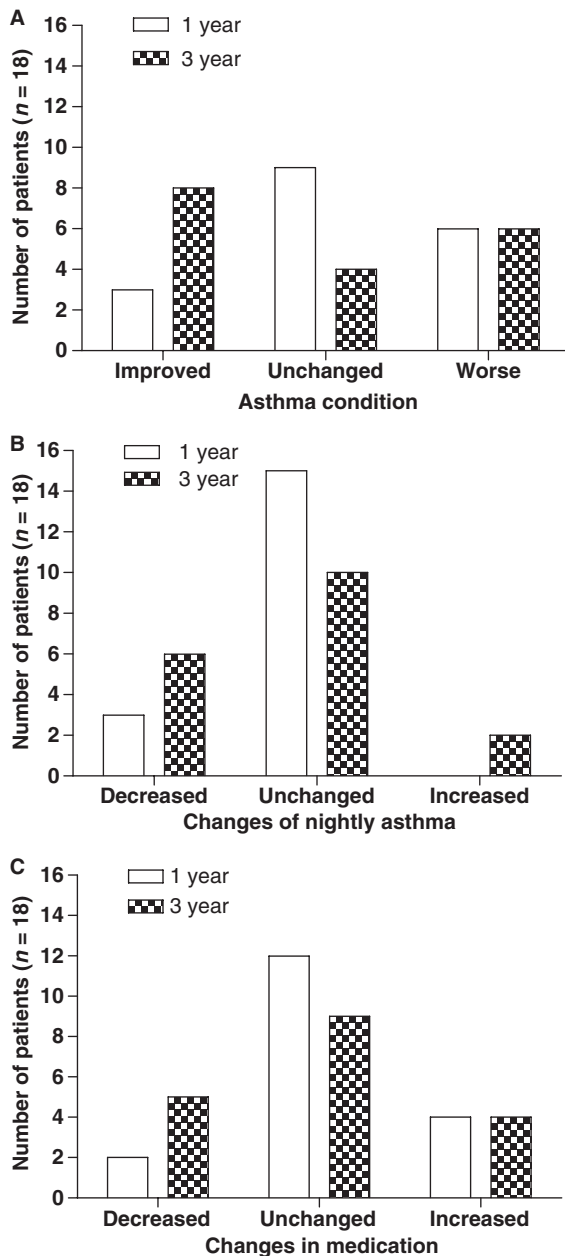


Figure 2. A clinical evaluation was performed approximately 3 years after the end of ESIT treatment and compared to similar, published, data at 12 months (5). The patients were invited to answer a small questionnaire: 'Since you stopped the Xolair treatment 3 years ago (A) How do you feel that your asthma has been? (B) Has your nightly asthma symptoms changed? (C) Has your asthma medication changed?'.

in ABA between patients and the controls. Similarly, the levels of IgG4 antibodies to cat, which were the highest before Xolair treatment started, remained almost the same during the 3 years follow-up. No significant correlation was found between ABA and serum levels of IgG4 antibodies to cat neither in the patients nor in the controls.

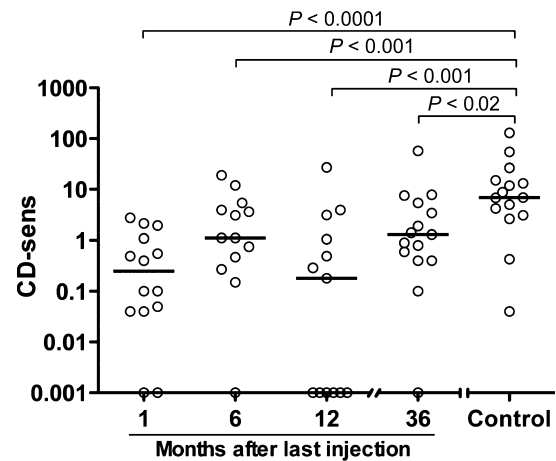


Figure 3. The CD-sens was measured in samples from all cat allergic patients 3 years after withdrawal of Xolair and was found to be significantly lower ($P < 0.02$) than that of the controls. At each sample, the median is presented as a horizontal line. The results from months 1, 6 and 12 and controls have previously been published (5).

IgE on basophils and in serum

In the samples taken before start of ESIT, the absolute levels of IgE antibodies to cat were rather low (3.0; 1.7–6.9 kU_A/l) (5), but not significantly different from the controls (3.9; 2.0–7.2 kU_A/l). At 3 years, the levels (2.8; 1.9–4.2 kU_A/l) were the same as before the treatment. Similarly, the size of the fraction of IgE antibodies to cat, i.e. the percentage of IgE antibodies of IgE, was not significantly different before (4.0; 2.6–10.2) (5) or 3 years after (3.8; 1.5–8.5) Xolair withdrawal when compared with the controls (4.4; 1.9–8.2). However, before eight of the 15 had an IgE antibodies fraction $> 3.8\%$.

When comparing the ratios of the number of IgE molecules over FcεRI receptors per basophil among the ESIT-treated cat patients at 3 years with allergic patients of the PIX study (6) having a low or high percentage of IgE antibodies to cat, significantly ($P < 0.001$) higher ratios were seen (Table 3). The IgE antibody fraction of the ESIT patients was significantly ($P < 0.001$) larger than that of those with the low, but not significantly different to that of those with the large, IgE antibody fraction. However, most surprisingly, the latter group had a more than 10 times higher median CD-sens to cat, a difference that was highly significant ($P < 0.001$).

Discussion

Only one study has reported the effect on clinical and immunological parameters of withdrawal of anti-IgE, Xolair, after long-term treatment (5). In 1998, 22 patients from Sweden were enrolled in the international omalizumab trial of severe allergic asthma, CIGE 0250011, followed by three extensions. Because of the

Table 1. CD-sens at 3 years after Xolair withdrawal in patients grouped according to reported symptoms or need of medication

	Asthma condition		Nightly asthma		Changes in medication	
	Improved or unchanged	Worse	Decreased or unchanged	Increased	Decreased or unchanged	Increased
No. patients	10	5	13	2	12	3
Median CD-sens	0.9	1.9	0.9	4.6	0.9	1.4

Table 2. The 15 cat allergic patients were divided on clinical basis in those with controlled (10 patients) and partially or uncontrolled (5 patients) asthma

Symptom group	Symptom score	CD-sens	% IgE antibody	IgE kU/l	Allergen binding activity	IgG4 antibody mg/l
Controlled	5	0.7	4.4	80	2.9	0.7
Partially or uncontrolled	16	1.9	5.1	67	1.8	0.3

For each group is given medians for each parameter.

Table 3. CD-sens to cat, the size of the cat IgE antibody fraction (%IgE-ab to cat), the ratio FcεRI on basophils over serum IgE concentration and the ratio IgE over FcεRI on basophils in the present ESIT material at 3 years and in the patients with a low (29 patients) and high (32 patients) percentage of IgE antibodies of the Pilot Investigation of Xolair (PIX) before the study (6)

	Present material	PIX groups	
		Low %IgE-ab	High %IgE-ab
CD-sens	1.3	1.0	16.4
%IgE-ab to cat	4.9	0.5	6.2
FcεRI/IgE kU/l	320	144	286
IgE/FcεRI	2.4	1.9	1.8

good effect, they were allowed to continue the treatment. When Xolair became available by prescription in Sweden it was decided that before a decision was taken on prescribing the drug, the ongoing treatment should be closed and Xolair withdrawn. This decision presented a unique opportunity to monitor clinical and immunological parameters during the withdrawal phase.

Of the 22 patients, 18 accepted to participate in the withdrawal study. It was decided to use the basophil allergen threshold sensitivity, CD-sens, as measure of the patient's allergen sensitivity. CD-sens (7) correlates well with clinical tests for allergen sensitivity like skin prick test titration (8), nasal allergen provocation (8) and bronchial allergen provocation (B Dahlén, A Nopp, SGO Johansson, unpublished data) and has a small intra/interassay variation (8). Thus, CD-sens is useful for monitoring the efficacy of different treatments, e.g. ESIT with omalizumab (1) or allergen-specific immunotherapy (9).

This follow-up study 3 years after the last Xolair dosage was aimed at duplicating the 1-year study (5) to allow comparisons. CD-sens was analysed to both cat and mite but only cat CD-sens was used in the evaluations. The reason is that CD-sens of different allergens cannot generally be compared because of inadequate allergen extract standardization and, in addition, the number of mite allergic patients as a group was too small. As expected, the patients with a high symptom score had a high CD-sens. The median CD-sens of all 15 cat IgE-sensitized patients was still significantly ($P < 0.02$) lower at 3 years than that of cat allergic patients in general. Four of the 15 cat allergic patients had still a CD-sens ≤ 0.4 , i.e. below the low quartile of the controls, compared with eight at the 1-year control.

Only one patient seemed to have developed a significant IgE-sensitization to cat during the last 2 years. At the 1-year study his basophils did not respond to cat allergen but at 3 years his CD-sens was very high, 57.1, i.e. close to 10 times the mean of the allergic controls. He did have quite high concentrations of IgE antibodies to cat in 1998 before Xolair treatment started, 11.1 kU_A/l corresponding to 7.7% of 'total IgE', which is about twice as high as the low border of the 'high IgE antibody percentage' group in the PIX study (6). Consequently, with our current knowledge, the given dose of Xolair was too low to be effective (6). At the 1 year follow-up, the IgE antibody concentration had decreased to 2.5 kU_A/l or 6.0 %. During the last 2 years, his IgE-sensitization to cat increased and in the 3-year sample he had reached an IgE antibody level of 2.8 kU_A/l which now represented as much as 14% of the IgE. However, he had quite high levels of IgG4 antibodies to cat, 14.5 mg/l, which might explain a rather mild and stable asthma, although ABA was rather low, 4.0.

The clinical evaluation was based on a physical examination including spirometry with particular emphasis on FEV₁. Even 3 years after Xolair withdrawal most patients were surprisingly stable in their asthma and only one patient had a higher than 20% drop in FEV₁ compared with the same when he was on Xolair treatment, i.e. the same number as 1 year after withdrawal.

As in the previous follow-up study, the clinical investigation included a symptom questionnaire, simplified compared with that of the initial study in 1999, but

identical to that of the 1-year follow-up. The median symptom score was more than three times as high in the partially controlled/uncontrolled group when compared with the controlled group. Of the 18 cat or mite allergic patients, 12 reported that their asthma had improved or not changed during the 3 years they had been without Xolair compared with period when they were taking the drug. An increase in the nightly asthma exacerbations was reported by two out of 18 patients, a slight increase from the 1-year follow-up study, where no patients reported an increase. However, six of the 18 patients reported less or no nightly attacks when compared with only three of the 18 patients after 1 year. No further change in medication was seen among the patients after 3 years when compared with that after 1 year.

The group of ESIT-treated patients had a slightly, but not significantly, smaller median cat IgE antibody fraction compared with the PIX patients with a large percentage of IgE antibodies (6). However, most remarkably, the latter group had a more than 10 times higher median CD-sens to cat and this difference was highly significant. This difference is even more remarkable considering that one patient in the ESIT group had a very high, 57.1, CD-sens. A possible explanation is that the basophils of the ESIT-treated patients still 3 years after the withdrawal did not respond well to allergen stimulation despite having the same ratio of IgE to FcεRI on their basophils and FcεRI to serum IgE as non-ESIT-

treated patients with similar degree of IgE-sensitization. It seems as if long-term treatment with Xolair 'turns off' the allergen triggering of the basophils. As many of these patients seem to have received too low dosages of Xolair in relation to the size of their IgE antibody fraction (6), this clinically beneficial downregulation of the basophil allergen triggering might be possible to reach even after a shorter period of treatment than 6 years.

In summary, most of the patients in this study had, still 3 years after closing of 6-year Xolair treatment, a surprisingly mild and stable asthma. Obviously, even such a short period as 6 years can be enough for the immune system to take control of the IgE activities of an atopic individual. The symptom cover by Xolair is probably of help allowing more intense exposure to the offending allergens probably contributing to the clinical results, to a downregulation of basophil allergen sensitivity CD-sens, and presumably also mast allergen cell sensitivity. Thus, under protection of Xolair many patients might more quickly than otherwise 'grow out' of their allergy.

Acknowledgments

We would like to thank Novartis for contributing with funds to this follow-up study, Ingegerd Ågren-Andersson for performing the ImmunoCAP® analyses and Åse Olerud for helping with the basophil analyses.

References

1. Fick RB, Jardieu PM. IgE and anti-IgE therapy in asthma and allergic disease. New York: Marcel Dekker, Inc., 2002.
2. MacGlashan DW Jr, Bochner BS, Adelman DC, Jardieu PM, Togias A, McKenzie-White J et al. Down-regulation of Fc(epsilon)RI expression in human basophils during in vivo treatment of atopic patients with anti-IgE antibody. *J Immunol* 1997;**158**: 1438–1445.
3. Casale TB, Condemi J, LaForce C, Nayak A, Rowe M, Watrous M et al. Effect of omalizumab on symptoms of seasonal allergic rhinitis: a randomized controlled trial. *JAMA* 2001;**286**:2956–2967.
4. Saini SS, MacGlashan DW Jr, Sterninsky SA, Tigias AK, Adelman DC, Lichtenstein LM et al. Downregulation of human basophil IgE and FcεRIα surface densities and mediator release by anti-IgE-infusion is reversible in vitro and in vivo. *J Immunol* 1999;**162**:5624–5630.
5. Nopp A, Johansson SGO, Ankerst J, Palmqvist M, Öman H. CD-sens and clinical changes during withdrawal of Xolair after 6 years of treatment. *Allergy* 2007;**62**:1175–1181.
6. Johansson SGO, Nopp A, Öman H, Ankerst J, Cardell LO, Grönneberg R et al. The size of the disease relevant IgE antibody fraction in relation to "total-IgE" predicts the efficacy of anti-IgE (Xolair) treatment. *Allergy* 2009;**64**:1472–1477.
7. Johansson SGO, Nopp A, van Hage M, Olofsson N, Lundahl J, Wehlin L et al. Passive IgE-sensitization by blood transfusion. *Allergy* 2005;**60**:1192–1199.
8. Nopp A, Johansson SGO, Ankerst J, Bylin G, Cardell LO, Grönneberg R et al. Basophil allergen threshold sensitivity. A useful approach to anti-IgE treatment efficacy evaluation. *Allergy* 2006;**61**:298–302.
9. Nopp A, Cardell LO, Johansson SGO, Öman H. CD-sens: a biological measure of immunological changes stimulated by ASIT. *Allergy* 2009;**64**:811–814.