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Research paper

Immunological methods for quantifying free and total serum IgE levels in allergy patients receiving Omalizumab (Xolair) therapy

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Abstract

Omalizumab (humanized-IgG1 anti-human IgE Fc, Xolair®) complexes circulating IgE, blocking IgE binding to high affinity epsilon Fc receptors (FcεR1) on mast cells and basophils. Free (non-Omalizumab bound) IgE levels in serum are a measure of effective Omalizumab dosing. The goal of this study was to quantify free (non-Omalizumab-complexed) and total serum IgE levels in asthma patients on Xolair®. The concentration of (non-Omalizumab bound) free IgE in human serum was measured using a solid phase immunoassay (IEMA) in which IgE was captured from serum with monoclonal anti-human IgE (clone HP6061) and detected with labeled-FcεR1α. In a companion total human serum IEMA, IgE was captured from serum with the same anti-human IgE (clone HP6061) and all bound IgE was detected with labeled monoclonal anti-human IgE Fc (clone HP6029). Free and total IgE levels were quantified in pre- and 1 and 3 months post Omalizumab therapy sera from 12 allergic asthma patients. In the absence of Omalizumab, working ranges of the free and total IgE IEMAs were comparable (10–1000 kIU/l), with excellent precision, reproducibility and parallelism. Pre-Omalizumab total and free IgE levels by IEMA were highly correlated ($r^2=0.99$, $Y=0.9X+0.32$, $p<0.001$), as were total serum IgE levels by IEMA and ImmunoCAP-250 ($r^2=0.98$, $Y=1.1X-0.05$, $p<0.001$, $n=33$). In vitro reduction of free IgE (>90%) occurred at [Omalizumab:IgE] molar ratios of 2–20. Total IgE levels in 12 asthmatics increased from pre-therapy levels (52–658 kIU/l) by 1.5–5.5-fold at 1 month and 1.7–8.6 fold at 3 months of uninterrupted Omalizumab treatment. Free IgE levels fell by

Abbreviations: FcεR1α, alpha chain of the high affinity IgE Fc receptor on mast cells and basophils; BSA, bovine serum albumin; CV, coefficient of variation; FcεR1, high affinity IgE Fc receptor on mast cells and basophils; IEMA, immunoassay; IU, international unit of IgE=2.4 ng of IgE; Omalizumab (Xolair), humanized IgG1 anti-human IgE Fc; PBS, phosphate buffered saline.

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49%–97% at 1 month and 45%–98% by 3 months of Omalizumab treatment. Free and total IgE levels by IEMA aid in monitoring patients receiving Omalizumab therapy.

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1. Introduction

Human allergic disease has been managed using a combination of allergen avoidance, pharmacotherapy and immunotherapy (Naclerio, 1991; Norman, 2004). In 2003, Omalizumab, a recombinant humanized IgG1 monoclonal anti-human IgE Fc (Xolair®), was licensed for use in the United States to treat moderate to severe persistent allergic asthma. Omalizumab binds circulating IgE, blocking IgE binding to alpha chain of FcεR1 receptors and down regulating the number of FcεR1 on mast cells and basophils (Chang et al., 1990; Saini et al., 1999; Davis, 2004). Reduced cell bound IgE can result in a concomitant reduction in mediator release and reduced allergy symptoms (Holgate et al., 2004; Lin et al., 2004). The best clinical results have been observed in asthma patients receiving 0.016 mg of Omalizumab per kg per KIU/l of IgE for a minimum duration of 12 weeks (Bousquet et al., 2004).

Unfortunately, not all asthma patients receiving Omalizumab experience the desired clinical benefits of reduced usage of their rescue or controller medications, improved lung function, decrease in asthma exacerbation rates and/or enhanced quality of life. In these patients, the treating physician needs to know whether the prescribed Omalizumab dose has reduced free serum IgE levels into the therapeutic range (>95%). To assess this issue, a serological measurement of non-Omalizumab complexed “free” IgE is needed, since the level of free IgE in serum identifies patients who may have been under dosed and in whom non-complexed IgE is free to continue to arm FcεR1 on mast cells and basophils. Presently, no CLIA-88 licensed clinical laboratory measures free human IgE because documented serological assays have not been available. This report describes the development and validation of a panel of free and total serum IgE IEMAs that are specifically designed to evaluate the serum of patients on Omalizumab. Due

to the selection of uniquely matched immunochemical reagents, the assay displays the robustness required for clinical analysis of serum containing Omalizumab. Application is demonstrated by the evaluation of serum from asthma patients receiving Omalizumab therapy.

2. Materials and methods

2.1. Reagents

Pharmaceutical grade Omalizumab was purchased from Genentech (South San Francisco, CA) and reconstituted as recommended in the package insert. The 147 mg/ml stock was diluted further with phosphate buffered saline containing 1% bovine serum albumin (PBS-BSA) to prepare Omalizumab (anti-IgE) concentrations from 100 ng/ml to 1 mg/ml. These Omalizumab preparations were incubated overnight with human serum containing known quantities of IgE to prepare complexes at [IgG-anti-IgE:IgE] molar ratios from 0.22 to 9500.

The anti-human IgE monoclonal antibodies used as capture and detection reagents were produced from clones HP6061 (mouse IgM anti-human IgE Fc) and HP6029 (mouse IgG1 anti-human IgE Fc) (Reimer, 1986), respectively. Both clones were prepared using PS-IgE myeloma as the immunogen and purchased as chromatographically purified antibody at 2 mg/ml in PBS from EMD Biosciences Corporation (La Jolla, CA). Both purified monoclonal antibodies were biotinylated using the biotin-hydroxysuccinimide ester method (EMD Biosciences, La Jolla California) and stored frozen at 1 mg/ml in PBS-BSA.

A recombinant form of the extracellular domain of the alpha chain of the human high affinity IgE receptor (FcεR1α) was purchased from Heska Corporation (Fort Collins, CO). It was produced in a Baculovirus expression system as previously described (Stedman

et al., 2001) and was subsequently purified by Q-Sepharose anion exchange chromatography and gel filtration. Purified FcεR1α was biotinylated with biotin hydrazide using the periodate oxidation method.

2.2. Human sera

The human sera that were used to construct the [IgG-anti-IgE:IgE] complexes were obtained from healthy adults with total serum IgE levels ranging from <2 to 100,000 kIU/l. Moreover, serum from 33 adults pre-Omalizumab treatment were collected for correlation studies to compare the free and total serum IgE IEMA results with total serum IgE measurements obtained in the ImmunoCAP 250 (Pharmacia, Kalamazoo, MI) (Hamilton and Adkinson, 2004). In the prospective Omalizumab treatment study, serum was collected from 12 patients with allergic asthma before Omalizumab treatment (baseline) and at 1 and 3 months during Omalizumab treatment. All patients received 0.016 mg of Omalizumab per kg of body weight per IU/ml of baseline total serum IgE for the management of their allergic asthma (Table 1). All sera were used to examine various aspects of the analytical performance of the free and total IgE IEMAs in the absence and presence of Omalizumab. Sera were collected in accordance with procedures approved by the Johns Hopkins Bayview Institutional Review Board.

2.3. Free human IgE Immunoenzymetric assay

The concentration of (non-Omalizumab bound) free IgE in human serum was measured using a solid phase IEMA in which IgE was captured from serum with anti-human IgE and detected with labeled-FcεR1α. Monoclonal murine IgM anti-human IgE Fc (Clone HP6061P) was adsorbed onto sterile flat-bottom polystyrene 96-well Bacti plates (Nalge-Nunc International, Rochester, NY) by pipetting 0.1 ml per well of a 10 µg per ml solution in PBS (pH 7.4, 16–18 h at 2–8 °C). The plates were washed once with PBS containing 0.05% Tween 20 and 0.01% thimerosal (PBS-Tween) and blocked with 300 µl per well of blocking buffer (PBS-1% BSA, 0.01% thimerosal). Following 5 buffer washes, 50 µl of blocking buffer were distributed in all wells. Then, 50 µl of each total IgE reference serum calibrators [11 two-

Table 1
Demographics and total and free serum IgE results of allergic asthmatic subjects on Omalizumab^a

Subject code	Gender	Age (years)	Weight (kg)	T=0 [Total IgE] (kIU/l)	1 Month [Total IgE] (kIU/l) (fold change ^b)	3 Months [Total IgE] (kIU/l) (fold change ^b)	T=0 [Free IgE] (kIU/l)	1 Month [Free IgE] (% reduction from baseline)	3 Months [Free IgE] (kIU/l) (% reduction from baseline)
1	M	50	85	418	1184 (+2.8)	1029 (+2.5)	433	11.4 (–97)	10.0 (–98)
2	F	51	91	34	119 (+3.5)	139 (+4.1)	40.8	20.7 (–49)	20.3 (–50)
3	M	75	66	114	631 (+5.5)	859 (+7.5)	137	18.9 (–86)	16.9 (–88)
4	M	41	84	116	534 (+4.6)	993 (+8.6)	172	19.4 (–89)	18.8 (–89)
5	F	48	68	92	425 (+4.6)	583 (+6.3)	98	19.2 (–80)	13.7 (–86)
6	M	61	97	151	658 (+4.4)	1293 (+8.6)	218	22.0 (–90)	17.1 (–92)
7	F	66	86	137	204 (+1.5)	233 (+1.7)	87	16.5 (–81)	15.3 (–82)
8	M	49	72	191	NA	478 (+2.5)	316	NA	25 (–92)
9	F	26	90	460	1409 (+3.1)	1430 (+3.1)	486	25.0 (–95)	27.0 (–94)
10	M	46	90	156	734 (+4.7)	1057 (+6.8)	195	27.0 (–86)	29.0 (–85)
11	F	50	73	52	239 (+4.6)	216 (+4.2)	58	24.0 (–59)	32.0 (–45)
12	F	59	60	587	1053 (+1.8)	1080 (+1.8)	658	18.6 (–97)	17.7 (–97)

NA=serum not available for this time point.

^a Serum specimens were collected at baseline and at 1 and 3 months post Omalizumab treatment.

^b The fold change in the total serum IgE levels as measured by IEMA in the pre-Omalizumab therapy versus 1 and 3 months post Omalizumab total serum IgE levels, e.g., subject 1 had a 2.8-fold increase in total serum IgE after 1 month of Omalizumab treatment and a decrease of 92.8% in free IgE level from time 0 (baseline).

fold dilutions of the 2000 kIU/l calibrator from 1000 to 1 kIU/l] and total serum IgE high, medium and low control sera (Pharmacia, Kalamazoo, MI) and test sera were pipetted into their respective wells. If serum had to be diluted to insure the IgE level in the specimen was within the working range of the assay, a serum from a non-atopic blood donor (IgE level <2 kIU/l) was used as the diluent. Plates were incubated 2 h at 37 °C and then without any buffer wash, biotinylated-FcεR1α was pipetted into all wells (3 µg per ml, 0.05 ml/well). The serum–biotin–FcεR1α mixture was incubated 1 h at 37 °C. Following 5 PBS-Tween washes, avidin-horseradish peroxidase (Sigma Chemical Company, St. Louis, MO) was pipetted into each well (1 µg per ml, 0.1 ml/well) and the plate was incubated for 1 h at 37 °C. Following this final incubation, the plates were washed 5 times with PBS-Tween and ABTS substrate, containing 1 µl of H₂O₂ per ml of ABTS, was pipetted into each well (0.1 ml/well). The enzymatic reaction was stopped with 0.1 ml of 1 M sodium azide per well when the top point of the reference curve reached an optical density of 1.5 to 2.0. Each plate was read in an ELISA plate reader (MR4000, Dynatech Technologies, Maclean, VA) at 405 nm.

Optical density of the IgE calibrators was plotted versus IgE concentration and test serum optical density values were interpolated into free IgE concentrations in kIU/l from the calibration curve. Acceptance of each assay was based on the low (16–28 kIU/l), medium (69–117 kIU/l) and high (300–500) total serum IgE controls being in their target range and the intra-assay CV for each specimen being <15%. The interpolated IgE concentrations in the free IgE IEMA were deemed to be “free” IgE levels because they were able to bind labeled-FcεR1α (Casale et al., 1997). Intra-assay variation of the free IgE IEMA was assessed by precision profile analysis in which 180 duplicate were performed in 5 different assays with sera containing IgE levels across the working range of the assay (10 to 1000 kIU/l). Inter-assay variation was assessed by analyzing sera containing IgE levels across the working range of the assay in multiple assays performed on separate days. Parallelism (linearity) was assessed by computing the inter-dilutional coefficients of variation of specimens analyzed in the same assay at multiple dilutions.

2.4. Total serum IgE immunoenzymetric assay

Total serum IgE levels in the test sera were measured using a previously reported monoclonal antibody-based IEMA (Hamilton and Adkinson, 1992) and the ImmunoCAP 250 (Pharmacia, Kalamazoo, MI). The total serum IgE ImmunoCAP is an FDA-cleared solid phase immunometric assay in which anti-IgE that is attached to a CAP matrix binds IgE from serum. Following a wash step, labeled anti-human IgE is then added to quantitatively detect bound IgE. The total serum IgE IEMA involved the identical procedure described for the free serum IgE IEMA, except that the plate was washed between the serum incubation and biotin-anti-human IgE addition, and bound IgE was detected with biotinylated monoclonal murine IgG1 anti-human IgE Fc (clone HP6029) at 1 µg per ml (0.1 ml/well). The monoclonal antibody pair (clones HP6061 for capture and HP6029-biotin for detection) are reported to bind to different determinants on the Fc region of the human epsilon heavy chain (Hamilton and Adkinson, 1992). Neither antibody impedes the binding of IgE to the FcεR1α on basophils and both activate histamine release in sensitized basophils. The total serum IgE IEMA in this study was calibrated using commercially-available human IgE standards (Pharmacia, Kalamazoo, MI) that were traceable to the World Health Organization IgE Standard. Since baseline IgE levels will be measured in clinical laboratories using one of several clinical assays such as the ImmunoCAP, we chose to compare the total serum IEMA results obtained in this study to those generated in the ImmunoCAP-250 with the same sera.

2.5. Omalizumab (anti-IgE):IgE complex preparation in varying molar ratios

The performance of the free IgE IEMA was evaluated using a matrix of [human IgG1 anti-human IgE to human IgE] complexes with molar ratios spanning the therapeutically reported range (1 to 20) (Casale et al., 1997). In the present study, human sera containing IgE levels from 111.4 to 480 kIU/l were mixed with equal volumes of buffer (0 control) or 1 of 5 ten-fold concentrations of Omalizumab (100 ng/ml to 1 mg/ml). The final IgE concentration in the absence of Omalizumab (buffer control condition) ranged from 55.7 to 240 kIU/l. Omalizumab to human IgE molar ratios

ranged from 0.22 to 9500, adjusting for the different molecular weights of IgG and IgE. The [sera:buffer] or [serum:Omalizumab] mixtures were incubated at least 2 h at room temperature to allow complex formation prior to their analysis in the free and total IgE IEMAs.

2.6. Data and statistical analyses

All IEMA data were computed using a spline curve-fitting algorithm in a Johns Hopkins University DAPI Laboratory Immunoassay Data Processing program. Precision profile analysis involving assessment of precision of the dose as a function of the measured dose across the working range of the assay was performed as previously described (Ekins, 1991). Coefficients of variation (CV) of replicate data and linear regression analysis to assess relationships between the free and total serum IgE levels of the various assays were performed using commercial software (Microsoft Excel and SPSS).

3. Results

3.1. Free IgE IEMA

In preliminary experiments, various combinations and concentrations of the available human IgE specific immunochemical reagents were evaluated for their utility in the free IgE IEMA. Monoclonal anti-human IgE (clones HP6029 and HP6061) and Fc ϵ R1 α were used in different combinations either as a purified reagent at 2.5, 5, 10, 20 μ g per ml on the plate to capture IgE or as a biotinylated human IgE detection reagent at 0.5, 1, 2 and 4 μ g per ml. To mimic a previously reported free IgE assay configuration (Casale et al., 1997), Fc ϵ R1 α was initially insolubilized on plastic plates and used in combination with either clone of biotinylated anti-human IgE monoclonal detection antibody. However, purified Fc ϵ R1 α displayed poor IgE reactivity when adsorbed directly onto a plastic surface. The optimal free IgE IEMA configuration was anti-human IgE (clone HP6061) adsorbed on the microtiter plate at 10 μ g per ml to capture IgE from serum and biotinylated Fc ϵ R1 α at 3 μ g per ml to detect bound IgE that was free of Omalizumab binding. Elimination of the wash step between the addition of serum and the biotinylated-

Fc ϵ R1 α minimized in vitro dissociation of the pre-formed [IgG anti-IgE:IgE] complexes and maximized analytical sensitivity of the free IgE IEMA (data not shown).

The assay in its optimal configuration displayed a working range of 10 to 1000 kIU/l in the absence of exogenously administered Omalizumab (Fig. 1). Precision profile analysis demonstrated acceptable precision with intra-assay coefficients of variation <15% over the working range of the assay (Fig. 2). Reproducibility was demonstrated with inter-assay coefficients of variation <18%. Parallelism (linearity) was shown with inter-dilutional CVs <20% (illustrated in Fig. 1).

Regression analysis demonstrated that the free serum IgE levels in 33 pre-Omalizumab treatment sera were statistically correlated with the total serum IgE in the same sera as measured in the IEMA and ImmunoCAP 250 (Fig. 3). Fig. 4 displays the effect of increasing concentrations of Omalizumab on the percentage reduction in free serum IgE. The concentration of free IgE detected in the IEMA decreased as the anti-human IgE concentration increased from 100 ng/ml to 1 mg/ml. Free IgE decreases >90% occurred at in vitro-formed [IgG1-anti-IgE:IgE] molar ratios of 2 to 20. Total serum IgE IEMA results in the absence and presence of varying concentrations of Omalizumab were equivalent, indicating that Omalizumab levels as high as 1 mg/ml did not detectably interfere in the total serum IgE IEMA (Fig. 5).

3.1.1. Free and total IgE measurements in patients on Omalizumab

Table 1 presents the demographics and free and total IgE serological results at baseline and 1 and 3 months following continuous Omalizumab therapy in 12 subjects with asthma. The patient group included 6 females and 6 males with ages ranging from 41 to 75. Baseline (pre-Omalizumab treatment) total serum IgE and free IgE levels as measured by IEMA agreed well with each other. The total serum IgE increased from 1.5 to 8.6 times baseline levels depending on the individual and the time interval after initiation of Omalizumab treatment (Table 1). In contrast, levels of free IgE (unbound with Omalizumab) decreased in all patients, but to varying degrees. Fig. 6 displays the total and free IgE levels at the baseline, 1- and 3-month time points in two patients (subjects 1 and 2)

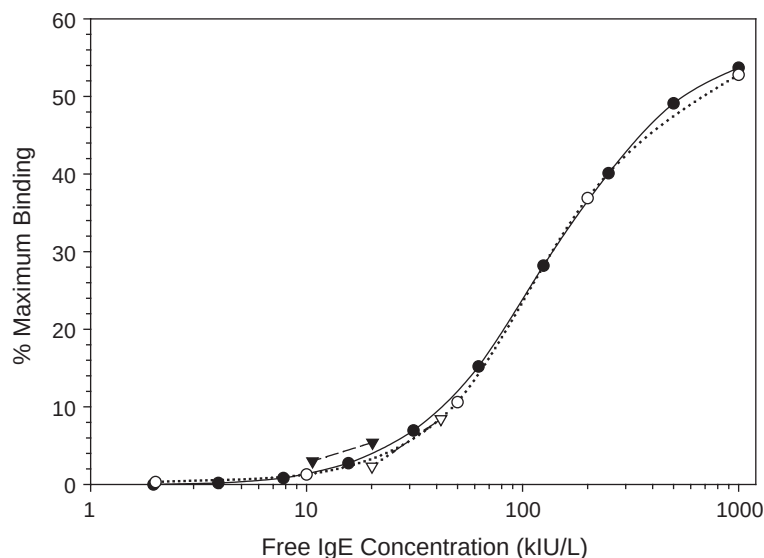


Fig. 1. The free IgE IEMA 5 point calibration curve (open circles: 1000, 200, 50, 20, 2 kIU/l) and 10 point calibration curve (closed circles: 1000, 500, 250, 125, 62.5, 31.25, 15.625, 7.813, 3.906, 1.953 kIU/l) that were analyzed in the same assay. The assay demonstrates parallelism as shown by results of the two dilutions of low IgE control (closed triangle; inter-dilutional %CV=3.7%) and a test serum (open triangle, inter-dilutional %CV=3.0) that contained free IgE levels close to the analytical sensitivity of the assay.

who displayed among the highest and lowest decreases in free IgE following Omalizumab administration. Subject 1 achieved a 97% and 98% reduction in free IgE levels at 1 and 3 months with a 2.5–

2.8-fold increase in total IgE levels over the same time period. In contrast, subject 2 achieved only a 49% (1 month) and 50% (3 month) reduction in the free IgE level from a baseline free IgE of 41 kIU/l. Since

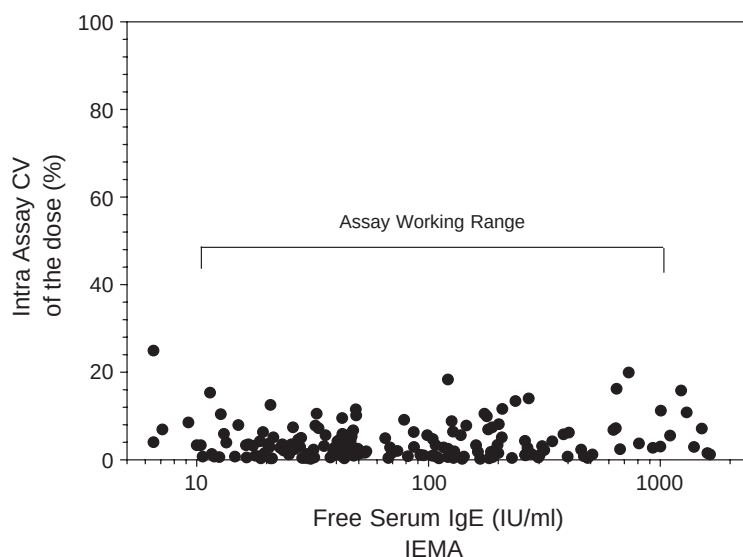


Fig. 2. Precision profile analysis of the free serum IgE IEMA. The intra-assay coefficient of the variation (CV) of the interpolated dose (Y-axis) is plotted against the dose (X-axis). The working range of the assay is defined from 10 kIU/l (twice the negative control binding) to 1000 kIU/l. Over the working range of the IEMA, intra-assay precision is <15% ($n=180$ duplicate measurements collected from 6 IEMAs).

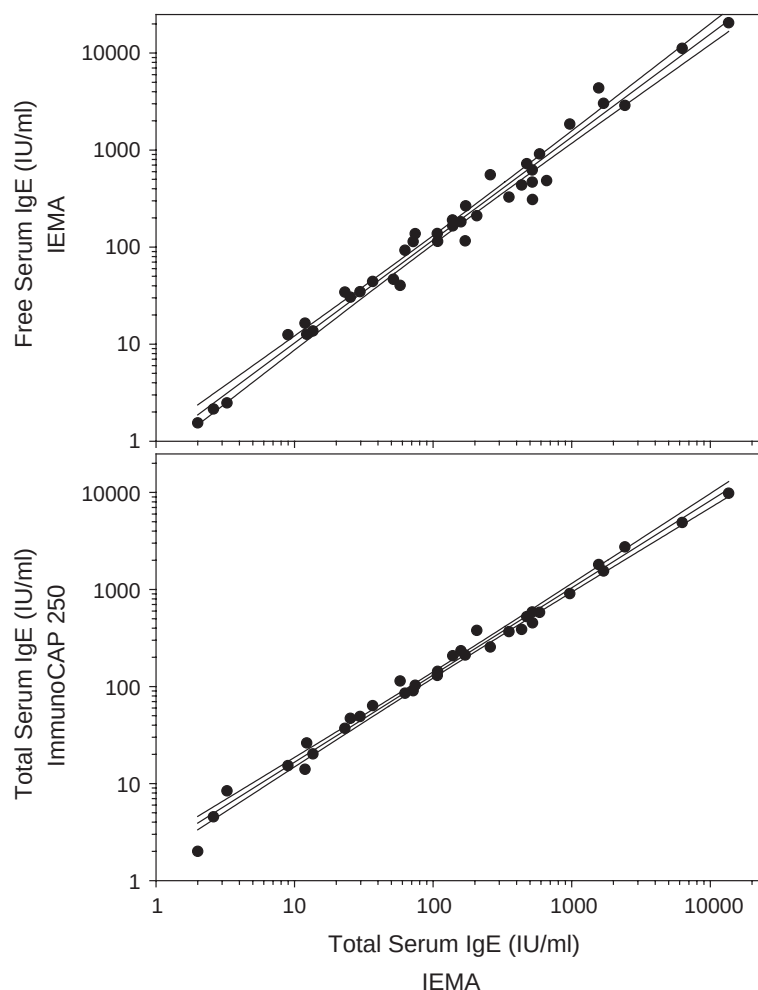


Fig. 3. *Top panel*: regression analysis of free serum IgE (Y-axis) versus total serum IgE (X-axis) levels as measured by IEMA in serum from 33 adults with a spectrum of total serum IgE levels prior to Omalizumab administration [$r^2=0.98$, $Y=1.06X-0.05$, $n=33$]. *Bottom panel*: regression analysis of the total serum IgE as measured in the same sera in the Pharmacia ImmunoCAP 250 (Y-axis) and IEMA (X-axis; bottom panel) [$r^2=0.99$, $Y=0.90X+0.32$, $n=33$].

minimal changes in free IgE were observed between the 1- and 3-month serum specimen, a 1-month post-treatment serum specimen was considered satisfactory for monitoring changes in free IgE levels in patients receiving Omalizumab treatment.

4. Discussion

An estimated 50 million people have allergic disease in America, with approximately 17 million suffering from asthma. Since allergic asthma is an IgE-

mediated disease involving the release of vasoactive mediators following the exposure to airborne allergens, reduction of IgE levels on effector cells should have beneficial effects. Unlike other forms of therapy that either treat the symptoms with drugs or modify the immune response following specific allergen immunotherapy, Omalizumab reacts with the binding site on the epsilon heavy chain and effectively blocks the binding of IgE to high affinity cellular receptors on effector cells. The consequence of this complex formation in vivo is a reduction in IgE bound to mast cells and basophils, a concomitant down regulation of

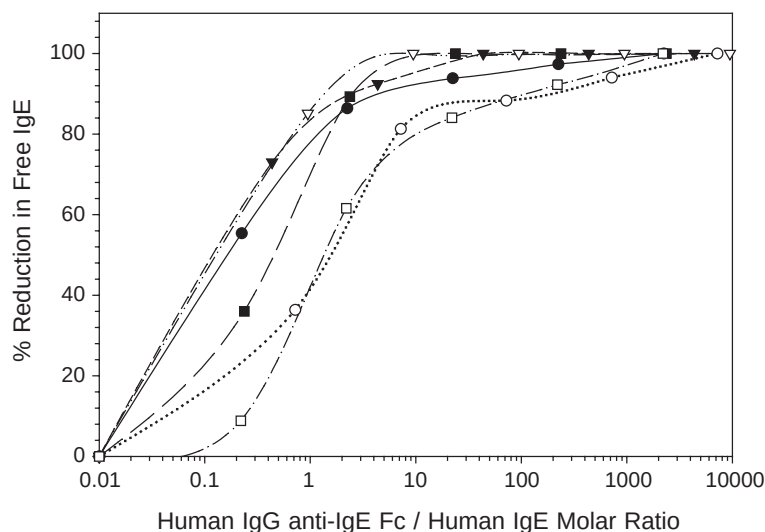


Fig. 4. Inhibition curves displaying the percentage reduction in free human IgE in 6 sera with IgE concentrations ranging from 55.7 to 240 kIU/l that had been incubated with increasing concentrations of Omalizumab to produce [humanized IgG1 anti-human IgE Fc] to [human IgE] molar ratios from 0.22 to 9500. Reductions of $\geq 90\%$ were observed at anti-IgE to IgE ratios from 2 to 200, indicating heterogeneity. The IgE concentrations of the sera in the absence of Omalizumab were 55.7 kIU/l (open triangles), 121.2 kIU/l (closed triangles), 236 kIU/l (closed circles), 222 kIU/l (closed squares), 73.3 kIU/l (open circles) and 240 kIU/l (open boxes).

Fc ϵ R1 receptor numbers on these cells and a reduction in the release of mediators following allergen exposure (Saini et al., 1999; Lin et al., 2004; Soler et al., 2001).

Once administered, maximal decreases in free circulating IgE have been reported when in vivo [IgG1-anti-IgE:IgE] molar ratios in blood of 10 to 20 are achieved (Casale et al., 1997). Because the efficacy of

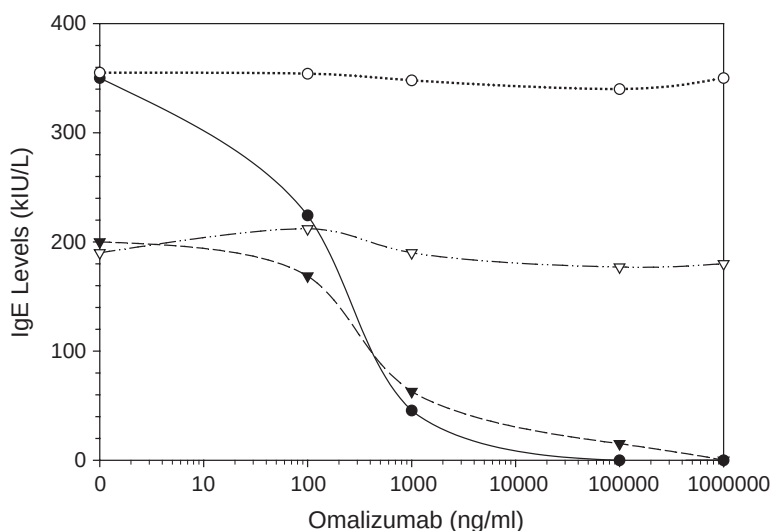


Fig. 5. Free (close symbols) and total (open symbols) serum IgE levels (kIU/L) measured in the free and total serum IgE IEMAs (Y-axis), respectively, for two sera (circles and triangles) in the absence and presence of Omalizumab from 100 ng/ml to 1 mg/ml (X-axis). No significant change in the total serum IgE levels were detected, in spite essentially complete decreases in free IgE levels which indicates that Omalizumab binding to IgE does not interfere in the total serum IgE IEMA.

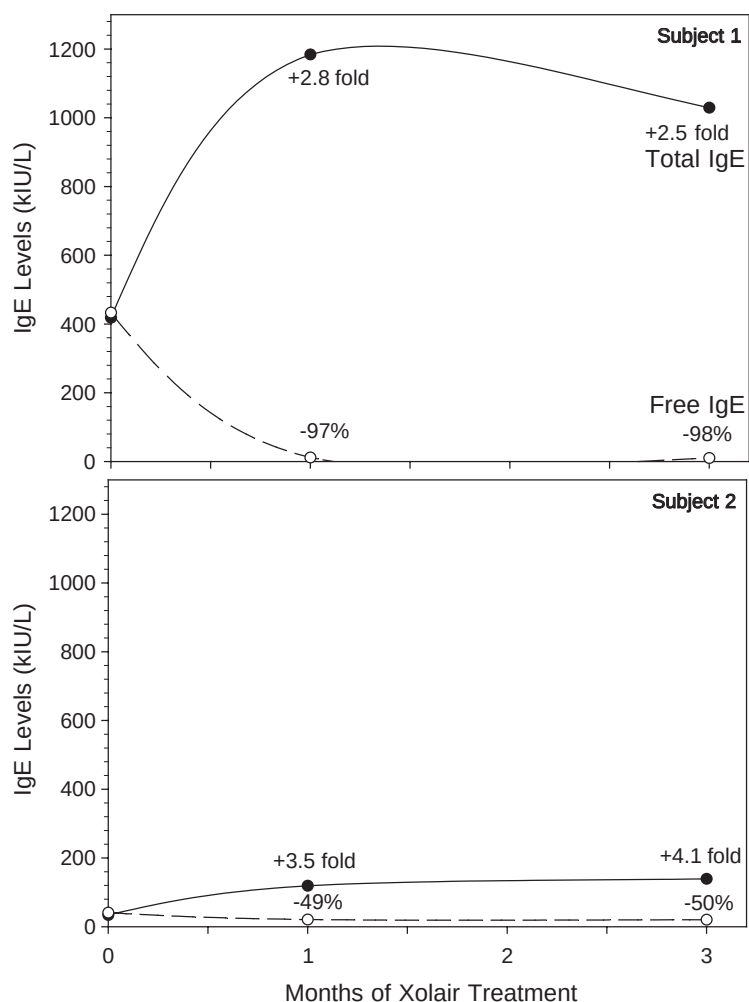


Fig. 6. Changes in the total (closed circles-solid lines) and free (open circles-dashed lines) IgE for subject 1 (top panel) and subject 2 (bottom panel) as described in Table 1 at baseline, 1 and 3 months post Omalizumab treatment. Subject 1 displayed a 98% decrease in free IgE with increases in measured total serum IgE of 2.5- to 2.8-fold. In contrast, subject 2 displayed a 50% decrease in free IgE after 3 months of Omalizumab therapy with 3.5- to 4.1-fold increases in total circulating IgE.

Omalizumab involves IgE-anti-IgE complex formation and Omalizumab interference of IgE binding onto FcεR1, the level of free (uncomplexed) IgE can be viewed as one indicator of the effectiveness of Omalizumab administration. During the development of Omalizumab, a free IgE enzyme immunoassay was developed using a human IgE receptor alpha chain-IgG chimera (Haak-Frendscho et al., 1993). This chimeric reagent when insolubilized on plastic surfaces of microtiter plate wells bound “free” IgE that was not complexed with Omalizumab. While the human FcεR1α-IgG chimera appears to be a useful reagent

for immunoassay measurement of free IgE, it is not available for use by clinical laboratories. As an alternative, a recombinant form of the extracellular domain of the alpha chain of the FcεR1 has been produced in a Baculovirus expression system that appears to be a useful alternative (Stedman et al., 2001). However, when purified FcεR1α is insolubilized on plastic surfaces, it appears to lose its reactivity for IgE, most likely due to steric hindrance. For this reason, we chose the reciprocal assay configuration of capturing IgE with a solid phase anti-IgE and then detecting bound free IgE with biotin labeled-FcεR1α. This

assay design has its strengths and limitations. It is able to capture all IgE (whether free or Omalizumab-bound) which means that sera with high total serum IgE levels can require that multiple dilutions be analyzed to insure that the measurement falls in the working range of the assay. Since baseline IgE levels for candidate Omalizumab patients are typically 30 to 700 kIU/l, this is not a serious concern because these levels are generally within the working range of both the free and total serum IgE IEMAs. However, analysis of sera from patients already receiving Omalizumab may have to be diluted since the measured total serum IgE does increase five- to six-fold due to the longer biological half-life of the complexes. The increases in level of total circulating IgE reported (Casale et al., 1997) and observed in this study following Xolair administration have been ascribed to the molar excess concentrations of anti-IgE that produce and retain small anti-IgE:IgE complexes in circulation. This increases the biological half-life of IgE in serum. One additional advantage of the IEMAs' design is that total serum IgE can be monitored even in a serum that contains Omalizumab since binding of the humanized IgG1 anti-IgE to IgE does not appear to interfere in the measurement (Fig. 5).

The free IgE level in serum when analyzed in tandem with the total IgE provides a snapshot into the status of IgE levels in patients on Omalizumab. Since not all patients on Omalizumab experience the same discernable symptom relief following Omalizumab treatment (Bousquet et al., 2004; Soler et al., 2001; Busse et al., 2001), the availability of a free IgE measurement may be a useful analytical measurement for verifying that a sufficient dose was administered and that the concentration of the Omalizumab is not simply the cause of an apparent lack of drug efficacy. In this study, 2 of the 12 patients on Omalizumab exhibited only a 50% reduction in free IgE despite an appropriate dosing based on the subject's weight and baseline total serum IgE level (subjects 2 and 11, Table 1). Both of these patients had among the lowest baseline total serum IgE levels. In vitro studies with total IgE levels in this range demonstrate some heterogeneity in the amount of anti-IgE required to reduce free IgE levels (Fig. 4). Whether this is a result of the blocking of Omalizumab binding to IgE by naturally-occurring IgG anti-IgE antibodies (Lichtenstein et al., 1992; Ritter et al., 1991) is unclear and

may need further investigation. Sustained increases in total serum IgE were observed even in these two subjects, suggesting that Omalizumab was complexing with their circulating IgE.

In conclusion, this report describes a panel of IEMAs with acceptable performance characteristics that allow the monitoring of changes in the levels of circulating free serum IgE that is not saturated with Omalizumab and total serum IgE in the presence of Omalizumab. Analysis of the free and total IgE levels in paired pre- and 1 month post Omalizumab treatment sera may aid physicians in confirming adequate dosing and monitoring immunological changes resulting from adjustments in the dosing schedule of patients receiving Omalizumab who do not experience the anticipated clinical benefit.

References

- Bousquet, J., Wenzel, S., Holgate, S., Lumry, W., Freeman, P., Fox, H., 2004. Predicting response to Omalizumab, an anti-IgE antibody, in patients with allergic asthma. *Chest* 125, 1378.
- Busse, W., Corren, J., Lanier, B.Q., McAlary, M., Fowler-Taylor, A., Chioppa, G.D., 2001. Omalizumab, anti-IgE recombinant humanized monoclonal antibody, for the treatment of severe allergic asthma. *J. Allergy Clin. Immunol.* 108, 184.
- Casale, T.B., Bernstein, I.L., Busse, W.W., LaForce, C.F., Tinkelman, D.G., Stoltz, R.R., Dockhorn, R.J., 1997. Use of an anti-IgE humanized monoclonal antibody in ragweed induced allergic rhinitis. *J. Allergy Clin. Immunol.* 100, 110.
- Chang, T.W., Davis, F.M., Sun, N.C., Sun, C.R.Y., MacGlashan, D.W., Hamilton, R.G., 1990. Monoclonal antibodies specific for human IgE producing B-cells: a potential therapeutic for IgE mediated allergic diseases. *Biotechnology* 9, 122.
- Davis, L.A., 2004. Omalizumab: a novel therapy for allergic asthma. *Ann. Pharmacother.* 38, 1236.
- Ekins, R., 1991. Competitive, non-competitive and multi-analyte microspot immunoassays. In: Butler, J.E. (Ed.), *Immunochemistry of Solid Phase Immunoassay*. CRC Press, Boca Raton, FL, p. 105.
- Haak-Frendscho, M., Ridgeway, J., Shields, R., Robbins, K., Gorman, C., Jardieu, P., 1993. Human IgE receptor alpha-chain IgG chimera blocks passive cutaneous anaphylaxis reaction in vivo. *J. Immunol.* 151, 351.
- Hamilton, R.G., Adkinson Jr., N.F., 1992. Measurement of total serum immunoglobulin E and allergen-specific immunoglobulin E antibody. In: Rose, N., de Macario, E.C., Fahey, J.L., Friedman, H., Penn, G. (Eds.), *Manual of Clinical Immunology*, 5th ed. American Society for Microbiology, Washington, DC, p. 689.
- Hamilton, R.G., Adkinson Jr., N.F., 2004. In vitro assays for IgE mediated sensitivities (Review). *J. Allergy Clin. Immunol.* 114, 213.

- Holgate, S.T., Chuchalin, A.G., Hebert, J., Lotvall, J., Persson, G.B., Chung, K.F., Bousquet, J., 2004. Efficacy and safety of a recombinant anti-immunoglobulin E antibody (Omalizumab) in severe allergic asthma. *Clin. Exp. Allergy* 34, 632.
- Lichtenstein, L.M., Kagey Sobotka, A., White, J.M., Hamilton, R.G., 1992. Anti-human IgG causes basophil histamine release by acting on IgG-IgE complexes bound to IgE receptors. *J. Immunol.* 148, 3929.
- Lin, H., Boesel, K.M., Griffith, D.T., Prussin, C., Foster, B., Romero, F.A., Townley, R., Casale, T.B., 2004. Omalizumab rapidly decreases nasal allergic response and FcεRI on basophils. *J. Allergy Clin. Immunol.* 113, 297.
- Naclerio, R., 1991. Allergic rhinitis [review]. *N. Engl. J. Med.* 325, 860.
- Norman, P.S., 2004. Immunotherapy: 1999–2004. *J. Allergy Clin. Immunol.* 113, 1013.
- Reimer, C.B., 1986. Five hybridomas secreting monoclonal antibodies against human IgE. *Monoclon. Antib. News* 4, 2.
- Ritter, C., Battig, M., Kraemer, R., Stadler, B.M., 1991. IgE hidden in immune complexes with anti-IgE autoantibodies in children with asthma. *J. Allergy Clin. Immunol.* 88, 793.
- Saini, S.S., MacGlashan, D.W., Sterbinsky, S.A., Togias, A., Adelman, D.C., Lichtenstein, L.M., Bochner, B.S., 1999. Down-regulation of human basophil IgE and Fc epsilon RI alpha surface densities and mediator release by anti-IgE infusions is reversible in vitro and in vivo. *J. Immunol.* 162, 5624.
- Soler, M., Matz, J., Townley, R., Buhl, R., O'Brien, J., Fox, H., 2001. The anti-IgE antibody Omalizumab reduces exacerbations and steroid requirement in severe allergic asthma. *Eur. Respir. J.* 18, 254.
- Stedman, K., Lee, K., Hunter, S., Rivoire, B., McCall, C., Wassom, D., 2001. Measurement of canine IgE using the alpha chain of the human high affinity IgE receptor. *Vet. Immunol. Immunopathol.* 78, 349.