

Accuracy of US Food and Drug Administration–cleared IgE antibody assays in the presence of anti-IgE (omalizumab)

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Background: Although serological IgE measurements can aid in efficacy assessment of patients with asthma on omalizumab (Xolair [Genentech, Inc., South San Francisco, Calif]; humanized IgG₁ antihuman IgE Fc), its effect on clinically used IgE assay performance is unknown.

Objective: This study investigated the hypothesis that IgE:IgG–anti-IgE immune complex formation after omalizumab administration diminishes accuracy and increases variability of IgE assays performed in diagnostic immunology laboratories.

Methods: Plasma from 4 atopic adults was incubated with omalizumab (50 or 200 molar excess to IgE) or buffer. Paired specimens were sent masked to 159 clinical laboratories in the College of American Pathologists Diagnostic Allergy Proficiency Survey (cycles SE-C-2003, SE-A-2005). Inhibitory effects produced by omalizumab on accuracy and reproducibility of US Food and Drug Administration (FDA)–cleared total and allergen-specific IgE assays were computed by using national proficiency survey data.

Results: Total serum IgE levels measured in the 2 ImmunoCAP assays were minimally reduced (2.4–9.0%) by the presence of omalizumab, whereas 5 other assays showed marked reductions from 12.5% to 67.2% ($P < .001$) that increased in proportion to total serum IgE levels. The degree of interference was not large enough to produce detectable misclassification of IgE antibody results (positive to negative) in 4 multiallergen screening assays. Of 11 FDA-cleared IgE antibody assays, only 3 (ImmunoCAP, UniCAP, Immulite-2000) generated quantitative IU/mL results that could be assessed for interference. Omalizumab produced 0.8% to 27.8% reduction in allergen-specific IgE levels across 8 allergen specificities.

Conclusion: Although as much as 62% loss in accuracy was observed in FDA-cleared human IgE assays, the ImmunoCAP system was sufficiently robust to provide accurate and

reproducible total and allergen-specific IgE antibody results in a clinical setting where therapeutic levels of omalizumab are present in serum.

Clinical implications: Accurate monitoring of total and allergen-specific IgE, together with free IgE levels, in serum from patients on omalizumab may help optimize dosing and maximize the efficacy of Xolair therapy. (*J Allergy Clin Immunol* 2006;117:759–66.)

Key words: Human, IgE, IgE antibody, omalizumab (Xolair), human IgG₁ anti-IgE, human IgG antihuman IgE autoantibodies, College of American Pathologists proficiency survey, immuno-enzymetric assay, α -Fc ϵ R1

mAbs specific for human IgE were initially proposed as a potential therapeutic for IgE-mediated allergic diseases in 1990.¹ By 2003, a recombinant humanized IgG₁ monoclonal antihuman IgE Fc (omalizumab [Xolair; Genentech, Inc., South San Francisco, Calif]) was licensed in the United States as a treatment for moderate to severe persistent allergic asthma.² By binding onto the epitope on the ϵ heavy chain that interacts with the α chain of the high-affinity IgE receptor (α -Fc ϵ R1), omalizumab interferes with IgE binding to the surface of mast cells and basophils. Using optimum dosing, there is a marked reduction in the number of IgE molecules bound to effector cells and a concomitant decrease in the cell surface density of Fc ϵ R1.^{3–5} These events ultimately lead to a reduction in allergic symptoms and a detectable 5-fold to 6-fold increase in serum IgE levels that persists well beyond discontinuation.^{2,6–8} According to Novartis, ~35,000 patients with extrinsic asthma are receiving Xolair, and this number is expected to increase with new clinical indications and markets.

One measure of effective omalizumab dosing is a reduction in the level of circulating total free IgE in serum that remains unbound with omalizumab and capable of binding Fc ϵ R1.^{5–8} The level of free IgE can be measured in serum with research enzyme immunoassays that use the α -Fc ϵ R1 as a detection reagent.^{9,10} What has remained unclear, however, is the effect that the binding of omalizumab to IgE has on the accuracy and reproducibility of clinically used total and allergen-specific IgE antibody immunoassays. Clinicians have remained concerned that they can not obtain accurate total and allergen-specific IgE antibody measurements from their

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Abbreviations used

CAP: College of American Pathologists
CV: Coefficient of variation
FDA: US Food and Drug Administration
SE: Diagnostic Allergy Proficiency Survey

clinical immunology laboratory once their patients have received Xolair. Moreover, allergists occasionally would like to assess the efficacy of the omalizumab therapy in patients who have minimal apparent clinical benefit with total and allergen-specific IgE antibody assays other than those that measure free IgE.

Several years ago, we hypothesized that the binding of omalizumab to IgE interferes with binding of antihuman IgE reagents in US Food and Drug Administration (FDA)-cleared immunoassays and thus may lead to both falsely lower estimations of total and allergen-specific IgE (a loss of accuracy) and increased imprecision because of the dynamic nature of immune complex formation and dissociation. In a preliminary study,¹¹ IgE-omalizumab complexes were produced *in vitro* at various molar ratios and analyzed in 1 widely used FDA-cleared third-generation immunoassay for total and allergen-specific IgE. To our surprise, only a minimal reduction in measured IgE levels was detected with serum containing molar excess of omalizumab to IgE.

The current study extends these early observations and examines the effect of omalizumab on the performance of all of the clinical IgE assays currently used in diagnostic immunology laboratories. We have taken advantage of our ability to add trace volumes of omalizumab or buffer (sham control) to identical serum specimens from atopic individuals and insert these serum pairs into the College of American Pathologists (CAP) Diagnostic Allergy Proficiency (SE) Survey, which involves more than 150 clinical immunology laboratories that perform IgE assays using 20 different formats. The data obtained in the current study support the hypothesis that interference does occur; however, the extent of accuracy loss is highly assay-dependent. Although reproducibility is not compromised, the accuracy of some but not all total and allergen-specific IgE assays is markedly compromised by the presence of omalizumab in serum. Importantly, the results of the current study indicate that accurate total and allergen-specific IgE results can be obtained with selected immunoassays, even if the serum contains therapeutic levels of omalizumab.

METHODS

Reagents

The clinical grade omalizumab used to produce the human IgE:anti-IgE complexes in test sera was purchased from Genentech Inc. A vial containing 150 mg was reconstituted as recommended in the package insert and used to construct anti-IgE:IgE complexes with a 50 to 200 molar excess of omalizumab. Purified mouse monoclonal IgM antihuman IgE (HP6061P) and biotinylated mouse monoclonal IgG₁ antihuman IgE Fc (HP6029)¹² used in the free and total IgE

immunoassays, respectively, were purchased from EMD Biosciences (La Jolla, Calif). Biotinylated α -Fc ϵ R1¹³ used in the free IgE assay was purchased from Heska Corp (Ft Collins, Colo).

Specimens

The human sera used in this study were prepared for inclusion in 2003 Cycle C and 2005 Cycle A of the CAP SE Survey. The CAP SE Survey is a national proficiency program that submits 6 challenge sera per cycle, 3 cycles per year to laboratories, which have 10 days to return total serum IgE, multiallergen screen, and 5 allergen-specific IgE antibody measurements to the CAP coordinating office. Table 1 presents the total serum IgE levels (mean $\pm$ 1 SD) in both halves of each of the 4 paired specimens before addition of omalizumab or buffer.

2003 Study

Two 600-mL units of plasma were collected from a 38-year-old atopic man with a history of rhinitis and positive puncture skin tests to *Phleum pratense*, *Ambrosia artemisiifolia*, and *Felis domesticus* allergen extracts. The plasma was pooled and recalcified to remove fibrin by addition of 1 mol/L CaCl₂ and 10,000 U bovine thrombin. The serum was then split into 2 equal 600-mL volumes. One half (coded SE-99-2003) received omalizumab (200 molar excess to IgE [70 kIU/L]), and the other half (SE13-2003-sham control) received an equal volume of PBS. Both samples were incubated (2 h, 23°C; 16 h, 4°C) to ensure maximum omalizumab:IgE complex formation. They were then sent with 4 nontreated serum specimens to 128 laboratories participating in the 2003 CAP SE Survey (Cycle C). Total and allergen-specific IgE measurements were performed in each laboratory using FDA-cleared assays, and IgE data were returned to CAP for collation and statistical analysis.

2005 Study

To confirm the 2003 study observations, plasma was collected from 3 atopic individuals that contained total serum IgE levels from 90 to 800 kIU/L (Table 1). Three 600-mL units of plasma were collected at several-month intervals from ET (31-year-old woman), RG (51-year-old man), and GB (29-year-old woman). All plasma from a given subject were pooled, combined, and converted to serum as described. On the basis of the total IgE as measured with the Pharmacia ImmunoCAP-250 (Table 1), omalizumab was added to one half to achieve a 50:1 molar ratio of IgG₁-anti-IgE to human IgE (volume change, 0.008% to 0.01%). The free IgE level was reduced to 10% to 15% in the omalizumab-containing specimens by analysis in the free IgE enzyme immunoassay.¹⁰ An identical volume of PBS was added to the second half of the 3 specimens. All six 900-mL samples were incubated (2 h, 23°C; 16 h, 4°C) and then sent as SE-01-2005 to SE-05-2005 and SE-98-2005 (Table 1) to 159 participating laboratories as specimens in the 2005 CAP SE Survey (Cycle A).

FDA-cleared assay analyses

Each participating laboratory measured total serum IgE, a multi-allergen screen, and IgE antibody levels to 5 allergen specificities in each of the specimens using their choice of FDA-cleared commercially available clinical assays (listed in Tables II and III). The frequency of use of the different total and allergen-specific IgE assay methods among clinical laboratories in North America is reflected by the percentage of laboratories reporting data for any given method in the CAP SE Survey as presented in Table II.

Total serum IgE results were reported from 4 assays supplied by 3 manufacturers (2003) and 7 assays supplied by 5 manufacturers (2005) in quantitative units (kIU/L), all which were cross-calibrated to the World Health Organization 75/502 IgE reference (1 IU is equivalent to 2.44 ng IgE). The Dade Behring System (Deerfield,

TABLE I. Total serum IgE levels during CAP SE Survey: specimen preparation

		Total IgE, IU/mL	
		Pre-omalizumab or buffer addition	
Subject code	Randomized specimen code	Mean ± 1 SD (%CV)	Treatment*
SM	SE99-2003	69.5	Omalizumab
SM	SE13-2003	70.2	Buffer
	SE(13/99)-2003	69.9 ± 0.5 (0.7% CV)	
ET	SE01-2005	92.0	Omalizumab
ET	SE05-2005	93.1	Buffer
	(SE01/05)-2005	92.6 ± 0.8 (0.8% CV)	
RG	SE02-2005	201.0	Omalizumab
RG	SE04-2005	196.4	Buffer
	(SE02/04)-2005	198.7 ± 3.3 (1.6% CV)	
GB	SE03-2005	807.9	Omalizumab
GB	SE98-2005	805.3	Buffer
	(SE03/98)-2005	806.6 ± 1.8 (0.2% CV)	

*Pretreatment total serum IgE levels were measured with the Pharmacia ImmunoCAP System. The highlighted areas indicate the mean $\pm$ 1 SD and interspecimen CV obtained for each of the 4 specimens. These prebottling values were used as target levels of IgE from which postbottling results were compared.

III) is a nephelometric assay, whereas the remaining total IgE assays from Diagnostic Products Corp (Immulate-2000 and IRMA; Los Angeles, Calif), Pharmacia (AutoCAP, UniCAP, ImmunoCAP; Kalamazoo, Mich), Bayer (ADVIA Centaur; Leverkusen, Germany), and Beckman (Access/Access2; Fullerton, Calif) are immunometric assays using a variety of isotopic (IRMA) or nonisotopic (fluorescent or chemiluminescent) labels.

The multiallergen screen is a single qualitative IgE antibody screening assay that is used clinically to assess the atopic status of an individual. Seven assays from 3 manufacturers (SE-C-2003) and 9 assays from 4 manufacturers (SE-A-2005) were used by participants submitting qualitative dichotomous (positive or negative) multiallergen screen results.

Allergen-specific IgE antibody was measured by 11 assays from 5 manufacturers. IgE antibody results were reported either as quantitative IU_A/mL results interpolated from a total serum IgE reference curve or as arbitrary semiquantitative results categorized into classes from 0 to VI on the basis of the response data. Only IgE antibody results from the 3 assays where quantitative IU_A/mL results were reported could be evaluated for a possible reduction in accuracy caused by omalizumab.

Omalizumab molar ratio study

Three sera different from the SE survey sera were collected from atopic individuals and mixed *in vitro* with equal volumes of buffer or anti-IgE (omalizumab) to obtain anti-IgE:human IgE molar ratios ranging from 0.01 to 10,000. All of the specimens were analyzed for total and allergen-specific IgE antibodies to 8 allergen specificities in the ImmunoCAP System. The ImmunoCAP was selected to examine whether worse case (extreme) anti-IgE to IgE molar ratios interfere more effectively in the assay because it was the assay that displayed minimal interference in the CAP 2003 and 2005 proficiency surveys.

Data analysis

Statistical analysis of the total and allergen-specific IgE antibody SE survey results was performed at the centralized CAP computer data processing facility. Precision was computed as the intramethod, interlaboratory coefficient of variation (CV) for each quantitative IgE assay result. Statistical analyses were not performed on dichotomous multiallergen screen and qualitative IgE antibody data

(classes, modified scoring units, arbitrary response units). For the 7 total IgE assays and 3 third-generation allergen-specific IgE antibody assays reported in quantitative IU/mL levels, effects of omalizumab on assay accuracy and reproducibility were assessed by comparing the levels of IgE measured in the absence and presence of omalizumab using the following formula: $\{([IgE_{\text{buffer}}] - [IgE_{\text{omalizumab}}]) / [IgE_{\text{buffer}}]\}$. Statistical analyses (*t* test) were performed by using SysStat 3.1 (<http://www.Systat.com>) to identify significant reductions in total or allergen-specific IgE levels that resulted from omalizumab in the test sera.

RESULTS

Total IgE

Fig 1 displays the total serum IgE results measured in the 2003 serum pair that contained 72 IU/mL of total IgE and either buffer (*solid bars*) or 200 molar excess of anti-IgE to IgE (*gray bars*). omalizumab produced a significant loss of accuracy in the measured total IgE levels, ranging from 3.8% to 65% in 4 of the 5 assays: ADVIA, Nephelometer, Immulate-2000, and UniCAP. The ImmunoCAP system showed no significant loss in accuracy, with a 2.4% reduction in measured IgE levels in the presence of omalizumab.

These observations were confirmed in the 2005 survey using 50 molar excess of omalizumab. Table II displays the total serum IgE results for 2 of the 3 serum pairs containing the highest IgE levels (mean, 218 IU/mL, *top*; 816 IU/mL, *bottom*) and displaying the greatest degrees of omalizumab interference. In the absence of omalizumab, all of the total IgE assays performed comparably well, exhibiting excellent intramethod and intermethod CVs $\leq$ 15.3% (Table II). The presence of omalizumab produced significant reductions in the detected levels of serum IgE in all of the assays, with the magnitude of the reduction increasing in proportion to the total serum IgE level (Fig 2). The Pharmacia ImmunoCAP System was the only assay that showed no statistical decrease in measured IgE in the presence of 50 molar excess of omalizumab to

TABLE II. Total serum IgE levels in the absence and presence of omalizumab

Panel A							
Subject RG-2005							
Method	N	Total IgE SE04 (buffer)	Interlaboratory CV	Total IgE SE02 (omalizumab*)	Interlaboratory CV	% Reduction	Anti-IgE vs buffer P**
Bayer	17	230.1	8.7%	108.4	6.4%	52.9%	<.001
Avida Centaur							
Beckman	7	221.0	8.1%	192.3	5.1%	13.0%	.003
Access/Access2							
Dade Behring	7	254.3	4.6%	203.0	5.0%	20.2%	<.001
Nephelometer							
Diagnostic Products Corp	26	210.4	6.2%	129.2	6.8%	38.6%	<.001
Immulin-2000							
Diagnostic Products Corp	5	211.2	7.0%	121.0	14.5%	42.7%	<.001
IRMA							
Pharmacia	27	201.3	8.1%	197.4	6.2%	1.9%	.327
ImmunoCAP System							
Pharmacia	70	200.1	7.4%	194.5	7.2%	2.8%	.023
UniCAP System							
All methods	159	218.3 ± 19.0 8.7% CV					
Panel B							
Subject GB-2005							
Method	N	Total IgE SE98 (buffer)	Interlaboratory CV	Total IgE SE03 (omalizumab)	Interlaboratory CV	% Reduction	Anti-IgE vs buffer P**
Bayer	17	840.1	11.5%	275.2	5.2%	67.2%	<.001
Avida Centaur							
Beckman	7	864.7	9.5%	607.0	10.2%	29.8%	<.001
Access/Access2							
Dade Behring	7	1040.4	3.9%	728.0	4.1%	30.0%	<.001
Nephelometer							
Diagnostic Products Corp	26	734.0	8.1%	354.0	6.3%	51.8%	<.001
Immulin-2000							
Diagnostic Products Corp	5	622.2	15.3%	327.0	37.0%	47.4%	<.001
IRMA							
Pharmacia	27	792.0	10.9%	730.7	9.1%	7.7%	.005
CAP/AutoCAP							
Pharmacia	70	816.2	7.9%	742.7	7.6%	9.0%	<.001
UniCAP System							
All methods	159	815.7 ± 118.4 14.5% CV					

*Fifty molar excess of omalizumab to IgE was added to the SE02-2005 specimen.

**The significance of the reductions resulting from omalizumab interference were assessed by *t* test.

IgE at total IgE levels as high as 218 IU/mL (Table II, A). As a percentage of the uninhibited serum control, the UniCAP and ImmunoCAP were the only 2 assays whose omalizumab induced reduction in total serum IgE remained below 10% throughout the range of IgE levels investigated (72-816 IU/mL). This level of interference may be considered negligible because it is contained within the intermethod variation (Table II).

Because the goal of this study was to identify assays whose performance would be minimally affected by the presence of omalizumab, we further investigated the ImmunoCAP system because it along with its sister assay (UniCAP) was the only total serum assay to exhibit negligible interference. Three sera with total serum IgE levels of 80 to 680 IU/mL and omalizumab:IgE molar ratios from 0.01 to 10,000 were analyzed in the ImmunoCAP assay. Although minor changes were observed in the

measured IgE levels over the wide range of anti-IgE:IgE ratios, the total serum IgE levels in the specimens at the 3 IgE levels remained essentially unchanged (*P* = NS; Fig 3).

Multiallergen screen

Because the multiallergen screen assays are semiquantitative methods, essentially complete inhibition by omalizumab would be required to see a misclassification change from positive to negative. In this study, there was essentially 100% concordance between the multiallergen screen results obtained with the 4 proficiency survey specimens containing either buffer or omalizumab. This indicates that the extent of inhibition in any of the 4 multiallergen specific IgE assays evaluated in the CAP Surveys was not sufficient to cause a measurable misclassification from positive to negative with the specimens analyzed (data not shown).

TABLE III. Percentage of omalizumab-induced reduction of allergen-specific IgE as measured in third-generation assays

Allergen specificity	Fold molar excess of omalizumab*	ImmunoCAP System % reduction	UniCAP % reduction	Immulite-2000 % reduction
Common ragweed (<i>Ambrosia artemisiifolia</i> -W1)	200	8.4	9.7	ND
Common ragweed (<i>Ambrosia artemisiifolia</i> -W1)	50	10.2	11.8	10.1
Mugwort (<i>Artemisia vulgaris</i> - W6)	200	23.6	24.5	ND
Bermuda grass (<i>Cynodon dactylon</i> -G2)	50	0.8	3.4	19.7
Timothy grass (<i>Phleum pratense</i> -G6)	50	8.9	10.0	13.1
Cat dander/epithelium (<i>Felis domesticus</i> -E1)	50	27.8	26.0	10.4
Dog dander (<i>Canis familiaris</i> -E5)	50	15.8	17.9	10.2
Peanut (<i>Arachis hypogaea</i> -F13)	50	6.9	2.4	21.6
Honeybee venom (<i>Apis mellifera</i> -I1)	200	12.6	10.1	ND
Mean (reduction)		12.8	12.9	14.1
SD (reduction)		8.4	8.4	5.1
Interallergen CV		66.1%	65%	36.1
N		9	9	6

*Omalizumab-induced reduction (%) in allergen-specific IgE for 3 allergen specificities tested in the 2003 CAP SE Survey at 200 molar excess and 6 allergen specificities tested in the 2005 CAP SE Survey at 50 molar excess of omalizumab to IgE.

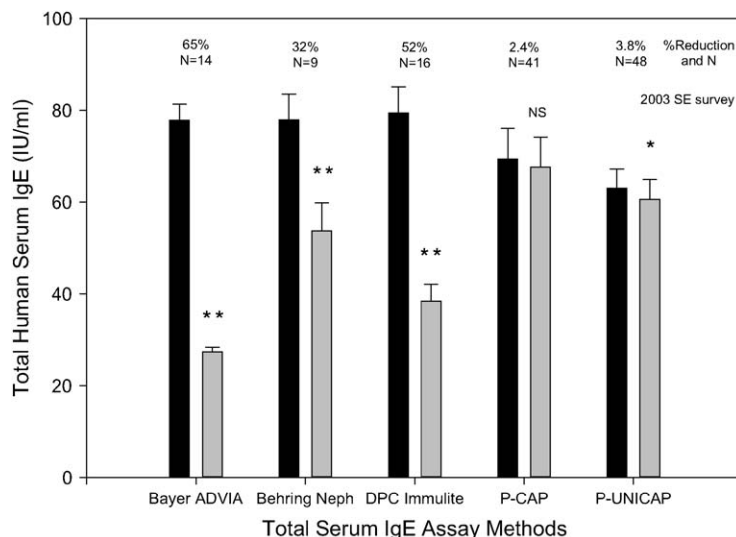


FIG 1. Total serum IgE levels (IU/mL) obtained with 5 FDA-total serum IgE assays after analysis of specimens SE13 (sham buffer control, black bars) and SE99 (200 molar excess of anti-IgE to IgE, gray bars) in the CAP SE-2003 Survey (Cycle C). Statistical differences (*t* test) in the levels of total serum IgE measured in the absence and presence of Omalizumab are indicated by the following symbols: **P* < .01; ***P* ≤ .001). SE, CAP SE Survey; NS, not significant; Neph, nephelometry; DPC, Diagnostic Products Corporation; P-CAP, Pharmacia ImmunoCAP System; P-UNICAP, Pharmacia UniCAP System.

Allergen-specific IgE antibody

Of the 11 allergen-specific IgE assays evaluated, only 3 in the CAP surveys (ImmunoCAP, UniCAP, and Immulite-2000) generated quantitative IU/mL results that could be subjected to statistical analysis. Table III

presents the percentage of omalizumab-induced reductions in allergen-specific IgE levels as measured with these 3 quantitative IgE antibody assays in the CAP SE surveys. The 3 assays displayed a comparable overall mean omalizumab-induced percentage reduction of 12.8% to 14.1% when IgE antibody was measured to 8 allergen

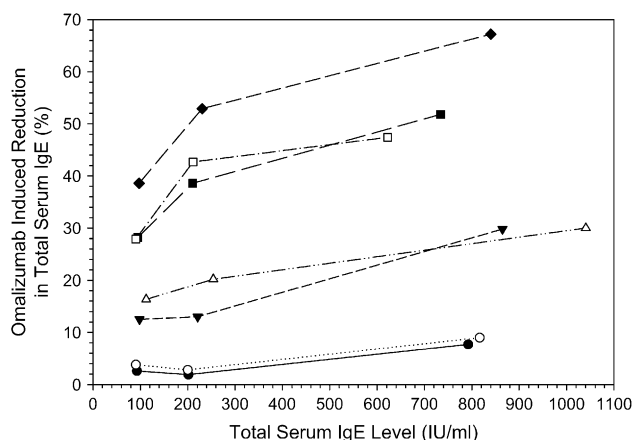


FIG 2. Percentage reduction in total serum IgE levels obtained with 7 FDA-cleared immunoassays after the analysis of 3 pairs of specimens (buffer vs 50 molar excess of anti-IgE:IgE; SE-2005 Survey, Cycle A). The assays presented are as follows: *closed circles*, Pharmacia ImmunoCAP System; *open circles*, Pharmacia UniCAP System; *open triangles*, Dade Behring Nephelometer; *closed triangles*, Beckman Access/Access2; *open squares*, Diagnostic Products Corp, IRMA; *closed squares*, Diagnostic Products Corp, Immulite-2000; *closed diamonds*, Bayer Avida Centaur.

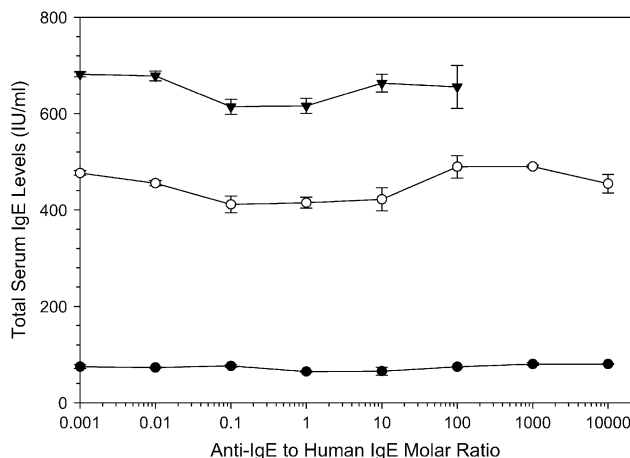


FIG 3. Total serum IgE Levels (mean $\pm$ 1 SD) measured in the Pharmacia ImmunoCAP System with sera from 3 atopic individuals (80 IU/mL, 475 IU/mL, 681 IU/mL) containing anti-IgE (omalizumab) to IgE molar ratio ranging from 0.01 to 10,000 or buffer (0.001 condition).

specificities. The specific IgE ImmunoCAP and UniCAP percentage reductions of 12.8 ± 8.4 and 12.9 ± 8.4 (mean $\pm$ 1 SD), respectively, did not appear to be different from the omalizumab reductions observed in their sister total IgE assays (maximum 7.6% to 9.1%; Table II). However, statistical analysis showed that the mean omalizumab-induced specific IgE assay reductions in the ImmunoCAP and UniCAP were in fact statistically higher ($P < .001$) than those seen in their respective total IgE assays. Moreover, no significant inhibition in allergen-specific IgE binding could be detected with the ImmunoCAP until the anti-IgE:IgE molar ratio exceeded 10 (Fig 4). At molar ratios above 10, the allergen-specific IgE antibody levels progressively declined, with the greatest reduction occurring in the serum with the lowest total serum IgE levels (32.5% inhibition at 1.42 IU/mL of IgE antidog dander [closed circles] vs 9.5% inhibition at 25.25 IU/mL of

IgE anticat dander [open triangles]; Fig 4). In contrast with the ImmunoCAP and UniCAP, the mean observed omalizumab-induced percentage reduction in specific IgE as measured in the Immulite-2000 ($14.1\% \pm 5.1\%$; $n = 6$; mean $\pm$ 1 SD; Table III) was statistically lower than interference observed in the Immulite-2000 total serum IgE assay (as much as 51.8%; $n = 26$; $P < .001$; Table II).

DISCUSSION

Historically, human allergic disease has been managed through a combination of allergen avoidance, pharmacotherapy, and immunotherapy. In 2003, the FDA licensed a recombinant, humanized IgG₁ monoclonal antihuman IgE Fc (omalizumab) for the treatment of moderate to severe persistent allergic asthma.² Subcutaneous administration

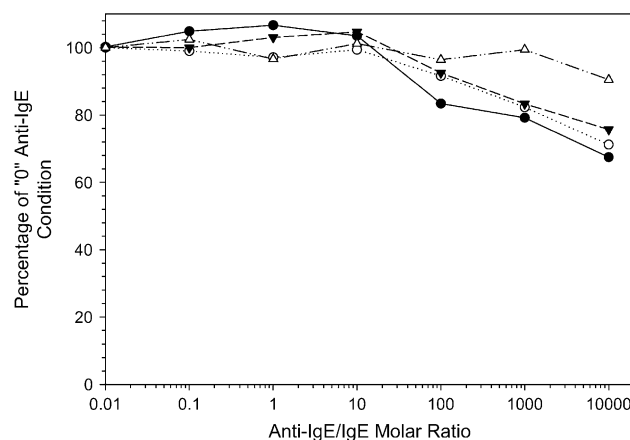


FIG 4. The percentage of inhibition of allergen-specific IgE detected in the ImmunoCAP System using sera containing differing molar ratios (0.01 to 10,000) of anti-IgE (Xolair) to human IgE for 4 allergen specificities: closed circles, dog dander, *Canis familiaris*, 1.42 IU/mL; open circles, peanut, 4.13 IU/mL; closed inverted triangles, *Dermatophagoides pteronyssinus*, 17.58 IU/mL; open triangles, cat dander, *Felis domesticus*, 25.25 IU/mL.

of omalizumab blocks the binding of IgE to high-affinity IgE receptors on mast cells and basophils receptors by attaching to the epitope on the Fc region of the ϵ heavy chain that binds the Fc ϵ R1.^{5,6} The binding of omalizumab to IgE *in vivo* reduces both the number of IgE molecules and Fc ϵ R1 receptors on the surface of the effector cells. Both effects lead to a reduction in mediator release and an anticipated reduction in allergy symptoms.^{3,6,7,14} Optimal doses of 0.016 mg omalizumab per kilogram per kIU/L IgE for a minimum duration of 12 weeks typically achieve omalizumab to IgE molar ratios from 10 to 50.

Despite this major advance in allergy treatment, the effect of omalizumab on the reproducibility and accuracy of clinically used total and allergen-specific IgE antibody assays has remained unknown. The current study investigated the potential interference of omalizumab in clinically used total and allergen-specific IgE assays by inserting masked omalizumab-containing specimens into the national proficiency survey operated by the CAP. The strength of this study stems from the analysis of masked omalizumab containing sera by ~150 diagnostic immunology laboratories that use all available FDA-cleared clinical assays. Two challenges were spaced several years apart to insure that several newly developed third-generation autoanalyzers were included in the evaluation. The study controlled for potential preanalytic variations in specimen handling and storage conditions by analyzing the specimens under actual clinical laboratory testing conditions. Excellent within-method interlaboratory variation of total and allergen-specific IgE results with sera containing omalizumab was generally <15%. This indicates that if preanalytical conditions differ between laboratories, they probably contribute only minimally to the variability observed in the extent of omalizumab interference.

Our working hypothesis was that omalizumab would interfere in anti-IgE reagent binding to IgE and diminish the accuracy of FDA-cleared IgE assays. We inferred this

from earlier work involving naturally occurring human IgG-anti-IgE autoantibodies.¹⁵⁻¹⁹ In these studies, human IgE specific autoantibodies were identified primarily among the IgG isotype. They were partially blocked by endogenous IgE and circulated in a partially immune complexed state at significantly higher levels in the serum of individuals with allergic disorders compared with nonallergic blood donors. The presence of both anaphylactogenic and nonhistamine releasing IgG anti-IgE autoantibodies in serum is now rarely disputed. The common belief is that clinically used IgE assays are minimally affected by these naturally occurring IgG anti-IgE antibodies because they are of relatively low affinity for IgE, and IgE assays use highly avid polyclonal or monoclonal anti-IgE reagents that should minimize their interference. However, the robustness of current clinical IgE assays has never been directly verified prospectively with sera containing variable amounts and specificities of naturally occurring antihuman IgE. In fact, several groups have shown the ability of naturally occurring IgG anti-IgE autoantibodies to interfere in quantitative IgE antibody serologic and cellular assays.^{20,21} It would thus be reasonable to expect that exogenous humanized IgG anti-IgE such as omalizumab would also interfere in IgE assays.

The CAP Survey data reported for the test sera in this study both support and negate the working hypothesis. Although most total serum IgE assays exhibited unacceptably large interference by omalizumab, the Pharmacia CAP system (ImmunoCAP and UniCAP) displayed minimal interference < 10% at 50 to 200 molar excess of omalizumab. Because this magnitude of omalizumab-induced accuracy loss is in a generally accepted range for interlaboratory variation (<15%), it can be assumed that "accurate" measurements of total serum IgE can be obtained with the ImmunoCAP assays when analyzing sera containing therapeutic levels of omalizumab. The IgE antibody results were interesting because significantly greater interference of $12.8\% \pm 8.4\%$ was observed with the Pharmacia allergen-specific IgE antibody assays than

was observed with the Pharmacia total IgE assays (7.6% to 9.1%). The converse was true for the Immulite-2000 assay. In both assay systems, the observed extent of omalizumab interference varied with the specificity of the allergen and the level of specific IgE antibody in the serum.

Theoretically, one might expect that the degree of omalizumab interference in the total and allergen-specific IgE assays from a given manufacturer should be comparable. This might be especially true for the Pharmacia total and specific IgE assays that use the same anti-IgE conjugate in both assays. However, all is not equal. The conjugate in the 2 assays is used at different concentrations, which effects the extent to which it binds to IgE on the basis of law of mass action considerations. Moreover, the total and specific IgE assays have quite different chemistries.²² In the total IgE assay, IgE is bound by a capture and detection antibody of different specificities for the Fc portion of IgE, whereas in the allergen-specific IgE assay, IgE binds to different molecular sizes of allergenic protein. Moreover, each allergen preparation used in the specific IgE antibody assay contains proteins that vary in size, charge, and rigidity.²³ This means that the extent of allergen-induced steric hindrance of the ϵ site recognized by omalizumab may vary with different allergen specificities. Finally, the level of specific IgE antibody in relation to the total IgE (specific to total IgE ratio) varies with each allergen specificity within a given serum, and this may also influence the extent of omalizumab interference. Because omalizumab is in dynamic equilibrium with free IgE and IgE bound to assay reagents (anti-IgE or allergen), an expected degree of interference is difficult to predict.

These results have implications for patients with allergy receiving Xolair. The serology work-up for a Xolair patient generally begins with a pretreatment total serum IgE level to both determine whether the IgE level in the indicated range of 30 to 700 IU/mL and compute the appropriate Xolair dose. Once omalizumab has been administered, data from this current study indicate that it is possible to monitor accurately both total and specific IgE antibody levels with the Pharmacia ImmunoCAP assays. These measurements together with a free total IgE level may be most useful for patients who do not appear to exhibit clinical benefit from their Xolair treatment. A repeat total and allergen-specific IgE measurement may be useful along with the free IgE measurement in determining whether the omalizumab dose has been optimized for that patient. As clinical indications for omalizumab expand, there will be increased likelihood that sera containing omalizumab will inadvertently be sent to clinical laboratories for further IgE testing. In these cases, it is important that both laboratory personnel and the ordering clinician understand what effect omalizumab may have on the performance of the actual IgE assays used to analyze their Xolair patients' serum.

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