

Rabbit F(ab')₂ antihuman IgE is a universal skin test reagent in the evaluation of skin mast cell degranulation in vivo

Michael E. Weiss, MD, Patricia Trent, BA, Robert Fisher, MD,
Philip S. Norman, MD, W. E. Waterbury, PhD,* and
N. Franklin Adkinson, Jr., MD Baltimore, Md.

Antihuman IgE is often used to study basophil- and mast cell-mediator release in vitro but is infrequently used in vivo. To evaluate in vivo skin reactivity to anti-IgE, an affinity-purified rabbit F(ab')₂ fragment of antihuman IgE was injected intradermally in 22 nonallergic and 27 allergic subjects. All 49 subjects (including a subject with <1 ng/ml of total serum IgE) had positive immediate cutaneous reactions to anti-IgE. Although total serum IgE level was weakly correlated ($r = -0.51$; $p < 0.005$) with in vivo skin reactivity to anti-IgE for the entire population, allergic subjects did not have significantly increased skin reactivity compared to nonallergic subjects ($p = 0.18$), despite having higher total serum IgE levels ($p < 0.002$). A late-phase cutaneous response (LPR) to anti-IgE occurred in 60% of the allergic and in 50% of the nonallergic subjects. Subjects with an LPR required approximately tenfold higher concentrations of anti-IgE to produce an immediate wheal of 10 mm compared to subjects who did not develop an LPR ($p = 0.02$), suggesting that the concentration of the stimulus injected is more important for the development of a LPR than the size of the immediate cutaneous response. Skin reactivity to codeine phosphate (a non-IgE-dependent secretagogue) was correlated with skin reactivity to anti-IgE ($r = 0.47$; $p < 0.05$), suggesting that in vivo skin mast cell degranulation is partially a function of mast cell releasability. No adverse reactions to intradermal injection of rabbit F(ab')₂ antihuman IgE occurred, and no increase in serum IgG antibodies to rabbit gamma globulin could be detected 3 weeks after skin testing. Antihuman IgE is therefore a skin test reagent that can be safely used to evaluate skin mast cell degranulation and LPR in vivo in nonatopic individuals and in those subjects whose allergic sensitivities are unknown. (J ALLERGY CLIN IMMUNOL 1989;83:1040-8.)

Since the early 1900s, human skin mast cell function has been studied by percutaneous injection of antigen to produce an IgE-dependent, wheal-and-flare response in allergic subjects.¹ In nonallergic subjects and those subjects whose allergic sensitivities are unknown, this approach has not been possible. Anti-IgE has proved quite useful in evaluating IgE-mediated

Abbreviations used

LPR: Late-phase response
F(ab')₂: Pepsin-digested immunoglobulin fragment bearing two antibody-binding sites
PIPES: Piperazine-*N,N'*-bis-2-ethanesulfonic acid
RGG: Rabbit gamma globulin

From The Johns Hopkins University School of Medicine, Division of Clinical Immunology at The Good Samaritan Hospital, Baltimore, Md.

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Reprint requests: N. F. Adkinson, Jr., MD, The Johns Hopkins University School of Medicine, Division of Clinical Immunology at The Good Samaritan Hospital, 5601 Loch Raven Blvd., Baltimore, MD 21239.

*Current address: Hollister-Stier, PO Box 3145, Spokane, WA 99220.

release mechanisms in human basophils and mast cells in vitro,² whereas its use in vivo has been more limited. Although anti-IgE is known to produce a wheal-and-flare reaction in human skin,³⁻⁸ it has not been clear whether this stimulus has uniform activity in all subjects or whether it discriminates between atopic or nonatopic individuals. If an anti-IgE stimulus in vivo can be demonstrated to be a "universal" skin test reagent (i.e., all subjects are reactive), it would be an alternative to the use of specific allergens for studies

of human skin mast cell activation. Alternatively, if atopic individuals were more sensitive to anti-IgE skin testing than nonatopic subjects, the reagent could have diagnostic potential or be useful in epidemiologic studies of atopy. We therefore chose to study the skin reactivity in vivo to a $F(ab')_2$ fragment of anti-IgE in well-defined patient sets.

Furthermore, certain immunoregulatory and cytotoxic drugs in clinical use are able to inhibit mast cell and basophil degranulation in vitro.^{9, 10} These, and other medications may be useful in the treatment of asthma,^{11, 12} as well as other allergic and inflammatory conditions. A universal skin test reagent would be useful to evaluate IgE-dependent mast cell function in vivo in patients receiving these therapies.

MATERIAL AND METHODS

Subjects

Twenty-two nonallergic and 27 allergic subjects were skin tested with a rabbit $F(ab')_2$ antihuman IgE reagent. Nonallergic subjects had negative personal and family histories of seasonal rhinitis, allergic asthma, or atopic dermatitis and negative puncture skin tests to 17 common aeroallergens: *Cladosporium*, *Penicillium*, *Aspergillus*, *Alternaria*, *Mucor racemosus*, *Helminthosporium*, oak pollen, timothy-, orchard-, ryegrass-, and Bermuda-grass pollen, ragweed pollen, English plantain, *Dermatophagoides pteronyssinus*, *D. farinae*, and cat and dog (ALK Laboratories, Copenhagen, Denmark; Allergy Laboratories of Ohio, Columbus, Ohio; and 3M Diagnostics, Santa Clara, Calif.). Allergic subjects had positive personal and family histories of atopy and at least two positive puncture skin tests, defined as a wheal diameter more than one half the positive histamine control (10 ng/ml). In the allergic group, all 27 subjects had seasonal rhinitis, 11 also had allergic asthma, and one subject had atopic dermatitis. Puncture skin tests were done with a bifurcated Wyeth needle (Wyeth Laboratories Inc., Philadelphia, Pa.) with a technique previously described.¹³ No subject had received immunotherapy within the previous 3 years.

Intracutaneous tests

Intradermal injections were performed with a technique previously described.¹³ Briefly, approximately 0.02 ml was injected, in duplicate, on the volar aspect of the lower arm, raising a 3 to 4 mm bleb. After 15 minutes, the site was inspected, and the longest and midpoint orthogonal wheal-and-flare diameters were measured and recorded. The sum of the longest and midpoint orthogonal diameters was divided by two, and the means of the duplicates were recorded. The first 24 subjects (12 allergic and 12 nonallergic subjects) were tested with rabbit $F(ab')_2$ antihuman IgE antibody starting at 0.001 $\mu\text{g/ml}$ diluted with sterile albumin saline with phenol (Hollister-Stier Laboratories, Spokane, Wash.). Increasing tenfold concentrations (0.01, 0.1, 1, 10, and 100 $\mu\text{g/ml}$) were used until a 10 mm wheal diameter

was obtained or the maximal concentration (100 $\mu\text{g/ml}$) was reached. The next 25 subjects were skin tested starting with a concentration of 0.1 $\mu\text{g/ml}$ of rabbit $F(ab')_2$ antihuman IgE antibody. The concentration was then serially increased or decreased tenfold so that the wheals obtained spanned the 10 mm midpoint diameter. In 18 subjects, the skin site where anti-IgE caused an immediate wheal >10 mm was evaluated for a cutaneous LPR, defined as erythema and induration 4 to 8 hours after intradermal injection. Similar concentrations of $F(ab')_2$ fragments of normal rabbit IgG were also evaluated for skin test reactivity.

The initial 20 subjects (10 allergic and 10 nonallergic subjects) were skin tested with codeine phosphate starting at 0.0003 mg/ml. Increasing tenfold concentrations (0.003, 0.03, 0.3, and 3 mg/ml) were used until a 10 mm wheal diameter was obtained or the maximal concentration (3 mg/ml) was reached. The subsequent 29 subjects were evaluated with 0.3 mg/ml of codeine phosphate. All subjects were also skin tested with histamine phosphate (0.275 mg/ml) (Center Laboratories, Port Washington, N.Y.) and sterile albumin saline diluent. All subjects denied the use of antihistamines for at least 96 hours before skin testing.

Test material

Affinity-purified rabbit $F(ab')_2$ antihuman IgE and control IgG fragments were prepared as follows: male New Zealand white rabbits (Don Morton, Rathdrum, Idaho) were immunized subcutaneously with 100 μg of purified IgE (PS myeloma) in complete Freund's adjuvant, followed by five biweekly injections of 50 μg of IgE (PS myeloma) in incomplete Freund's adjuvant. Ten days after the last injection the animals were bled by cardiac puncture. Serum was collected and frozen until further processing. Control sera was obtained from nonimmunized rabbits.

Serum was thawed, sterile filtered, and precipitated with ammonium sulfate (33%). The precipitate was centrifuged and dialyzed with a 12,000 dalton exclusion membrane. The material was applied to a diethylaminoethyl Affi-gel blue column (Bio-Rad Laboratories, Richmond, Calif.) with the effluent passed over a human gamma globulin affinity column to remove any cross-reactive antihuman IgG, IgA, or IgM antibodies. Next, the effluent was adjusted to pH 4.5 with glacial acetic acid and digested with pepsin (1:50 pepsin per protein in 0.01 N HCl) for 18 hours at 37°C. The pH was then adjusted to 7 with 50% NaOH. The digested gamma globulin was next passed over a *Staphylococcus* protein A affinity column with the effluent applied to a human IgE (PS myeloma) affinity column. After washing with 20 ml of 0.5M NaCl in phosphate-buffered saline, followed by 20 ml of phosphate-buffered saline, the column was eluted with 0.1 mol/L of glycine and 1 mol/L of HCl, pH 2.5. Fractions containing protein (A_{280} absorbing) were pooled and applied to a high-performance liquid chromatography molecular sieve, and the $F(ab')_2$ fractions (molecular weight 95,000) were pooled. Control serum from nonimmunized rabbits was prepared similarly, except passage over a human IgE affinity column was substituted with a

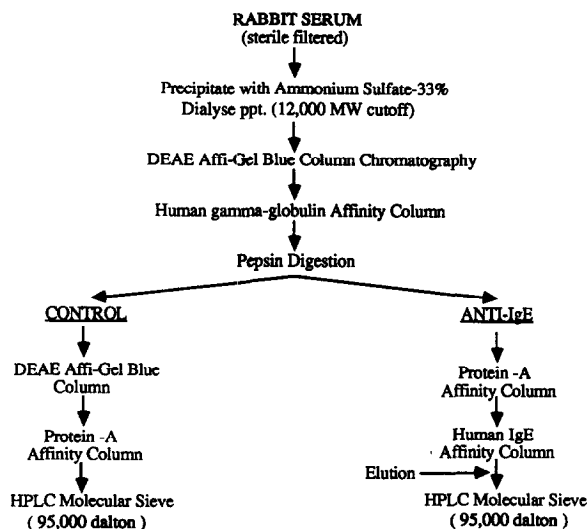


FIG. 1. Flow diagram for the preparation of both rabbit F(ab')₂ antihuman IgE and normal rabbit IgG (control) F(ab')₂ fragments. Rabbits were immunized with purified IgE (PS myeloma), whereas control serum was obtained from nonimmunized rabbits.

second pass through a diethylaminoethyl Affi-gel blue column (Fig. 1). The sterile, filtered material was freeze-dried in vials containing 50 µg of F(ab')₂. The vials were reconstituted immediately before use in 0.5 ml of sterile albumin saline diluent providing a top concentration of 100 µg/ml of F(ab')₂. Before human use, the F(ab')₂ anti-IgE and control fragments were evaluated *in vitro* for their ability to release histamine from human basophils. The rabbit anti-IgE F(ab')₂ fragments released histamine in a IgE-dependent manner with maximal histamine release equivalent to that observed with goat antihuman IgE (normally used in *in vitro* studies). The rabbit anti-IgE fragment had a dose-response curve that was parallel to, but that required about 10 times the concentration of, the goat antihuman IgE antibody to cause a similar amount of histamine release. No basophil histamine release occurred with up to 100 µg/ml of control fragments. Approval was granted for investigational use in humans by the Institutional Review Board and the Office of Biologics Research and Review, Center for Drugs and Biologics, Food and Drug Administration (BB-IND 2280).

Total serum IgE measurements

Total serum IgE levels were measured with a direct (non-competitive) radioimmunosorbent test previously described in detail.¹⁴ The assay is sensitive to ≤1 ng/ml IgE and has a midrange coefficient of variation of 5%.¹⁵

Buffers

PAG contains 25 mmol/L of PIPES, 110 mmol/L of NaCl, 5 mmol/L KCl, 0.003% of human serum albumin, and 0.1% of D-glucose adjusted to pH 7.40 with 10 mol/L of NaOH. PC₂G contains the above materials with 2

mmol/L of CaCl₂ instead of the human serum albumin. Carbonate buffer (pH 9.6) contains 0.015 mol/L of Na₂CO₃ and 0.035 mol/L of NaHCO₃. ELISA buffer (pH 7.4) contains 0.05 mol/L of Na₂HPO₄, 0.5 mol/L of NaCl, 0.05% Tween 20, and 0.2% of bovine albumin.

In vitro basophil histamine release in study subjects

After informed consent, basophils were obtained from peripheral blood, as previously described.¹⁴ Briefly, 30 ml of blood was mixed with 6.25 ml of 6% clinical dextran and 2.5 ml of 0.1 mol/L of ethylenediaminetetraacetic acid and allowed to sediment by gravity for 90 minutes. The leukocyte-containing plasma layer was then removed, and cells were sedimented by centrifugation (150 g, for 10 minutes at 21° C), washed twice in PAG (150 g for 8 minutes at 4° C), and resuspended in PC₂G; 5 × 10⁴ basophils were challenged with 0.1 µg/ml of goat antihuman IgE (generously provided by Dr. Kimishige Ishizaka, The Johns Hopkins University) in a final volume of 90 µl. After incubating for 45 minutes at 37° C, the reactions were stopped by the addition of 0.75 ml of cold PAG. The tubes were then centrifuged (1000 g for 2½ minutes at 21° C) and aliquots of the cell-free supernatant were assessed for histamine content by the automated fluorometric assay of Siraganian.¹⁶ The results were based on the mean of duplicate determinations and were expressed as percent of histamine release, by dividing by total cellular histamine after subtracting for spontaneous release of unstimulated basophils. Total cellular histamine was obtained by lysing cells in 2% perchloric acid.

ELISA to detect anti-RGG

An ELISA was used to evaluate whether skin testing with the reagent induced an IgG antibody response against RGG (Chemicon International Inc., El Segundo, Calif.). Polystyrene microtiter plates (PGC Scientific Laboratories, Gaithersburg, Md.) were coated with 100 µl of 10 µg/ml of RGG in carbonate buffer, and incubated overnight at 4° C. After washing three times with ELISA buffer, 100 µl of a 1:10 dilution of sera obtained immediately before and 3 weeks after skin testing were added in triplicate to antigen-coated wells. Inhibition studies were performed with 100 µg/ml of soluble RGG, which was demonstrated to inhibit >95% the binding of mouse antirabbit IgG antibody to microtiter wells coated with RGG. Sera were preincubated with RGG or buffer (in triplicate) 30 minutes before addition to the microtiter well. After overnight incubation of sera at 4° C, the plates were washed three times with ELISA buffer, and 100 µl of mouse monoclonal antihuman IgG antibody (lot No. 3598R, clone HP60640) (Unipath Inc., Birmingham, U.K.), diluted 1:10,000 in ELISA buffer, was added to each well. After a 2-hour incubation at 37° C, the plates were washed three times with ELISA buffer, and 100 µl of affinity-purified alkaline phosphatase-conjugated goat antimouse IgG (lot No. 50-15-02) (Tago Inc., Burlingame, Calif.), diluted to 1:2,000, was added for 2 hours at 37° C.

After three washes with ELISA buffer, 100 μ l of 4-methylumbelliferyl-phosphate was allowed to react at room temperature for 30 minutes and read on an automated microtiter fluorometer (3M Diagnostics Inc., Mountain View, Calif.) with 405 nm for excitation.

Expression and analysis of data

The concentration of rabbit F(ab')₂ antihuman IgE antibody causing a 10 mm wheal was obtained by linear regression of concentrations causing wheals above and below the 10 mm midpoint. Mann-Whitney U test was used to compare the response of allergic and nonallergic subjects to antihuman IgE antibody, codeine phosphate, and histamine phosphate and the slopes of the in vivo dose-response curves to anti-IgE. Nonpaired Student's *t* test was used to compare log total serum IgE levels. Pearson's correlation coefficient was used to evaluate associations. Human IgG anti-RGG antibody in the before and after skin test sera were compared by paired Student's *t* tests on the ELISA response that was inhibitable by soluble RGG (optical density of serum plus buffer, minus optical density of serum plus 100 μ g/ml of RGG).

RESULTS

F(ab')₂ anti-IgE skin testing

Seven male and 15 female nonallergic subjects, aged 18 to 64 years (mean 31.4), and 13 male and 14 female allergic subjects, aged 22 to 47 years (mean 32.6), were evaluated. In Fig. 2, the full dose-response curves to the rabbit F(ab')₂ antihuman IgE reagent are illustrated in the first 12 consecutive allergic and nonallergic subjects who were all studied with a full dose range. All the 49 subjects evaluated demonstrated in vivo skin reactivity to the rabbit F(ab')₂ antihuman IgE antibody, including one subject with <1 ng/ml of total serum IgE. No subjects had a wheal-and-flare reaction to intracutaneous injections of F(ab')₂ fragments of normal rabbit antibodies at the same concentrations.

Skin sensitivity for all 49 subjects is illustrated in Fig. 3. Although there was a trend toward increased skin reactivity to rabbit F(ab')₂ antihuman IgE antibody in allergic subjects compared to nonallergic subjects, this did not reach statistical significance ($p = 0.18$). The mean coefficient of variation for duplicate skin tests causing wheals around the 10 mm midpoint was 6.1%. There was no correlation between in vitro basophil histamine release and in vivo skin reactivity to antihuman IgE ($r = -0.22$; $p > 0.25$) (Fig. 4). Nor did in vitro basophil histamine release to 0.1 μ g/ml anti-IgE correlate with wheal size ($r = 0.12$; $p > 0.25$) or flare size ($r = 0.13$; $p > 0.25$) to 0.1 μ g/ml antihuman IgE (data not presented). As expected, the allergic subjects had higher

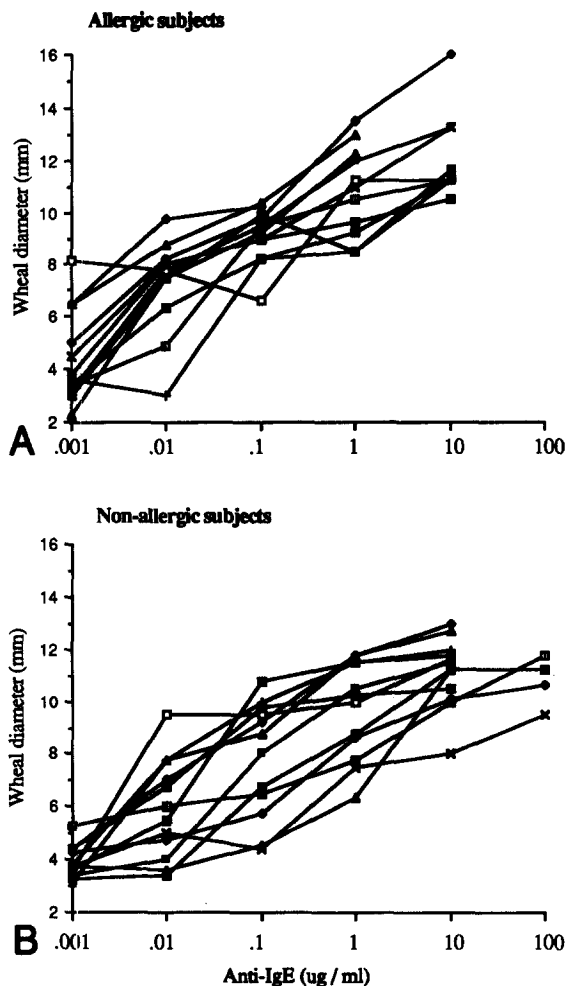


FIG. 2. Dose-response curves for skin responses to anti-human IgE in 12 allergic (A) and 12 nonallergic subjects (B). Intradermal injections initially raised 3 to 4 mm blebs. Wheal size was read in 15 minutes and recorded as the average of the longest and the midpoint orthogonal diameter (mean of duplicates). Each subject's data are illustrated with a different symbol. The slopes of least-squares regression lines of the dose-response curves of allergic and nonallergic subjects were not different ($p = 0.3$) by Mann-Whitney U Test.

total serum IgE levels than the nonallergic subjects with geometric means of 267 and 38, respectively ($p < 0.002$) (data not presented). There was a significant correlation between the total serum IgE level and in vivo skin reactivity to antihuman IgE ($r = -0.51$; $p < 0.005$) (Fig. 5), which was not obtained for basophil histamine release to anti-IgE ($r = 0.11$; $p > 0.25$) (data not presented).

Adverse responses to F(ab')₂ anti-IgE

All subjects tolerated skin testing without respiratory, cardiovascular, or objective generalized cuta-

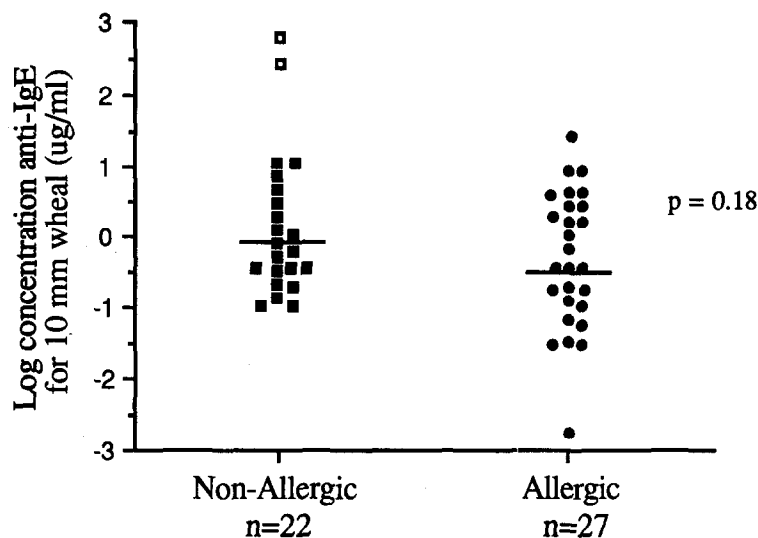


FIG. 3. Comparison of skin reactivity to rabbit F(ab')₂ antihuman IgE reagent between nonallergic and allergic subjects. Concentration of reagent causing 10 mm wheal was obtained by linear interpolation of points above and below 10 mm. Horizontal bars represent medians of each group. Data were analyzed with Mann-Whitney U test. Median for nonallergic subjects is 0.13, and for allergic subjects, -0.5 ($p = 0.18$). Open squares represent subjects with <1 and 7 ng/ml of total serum IgE antibody.

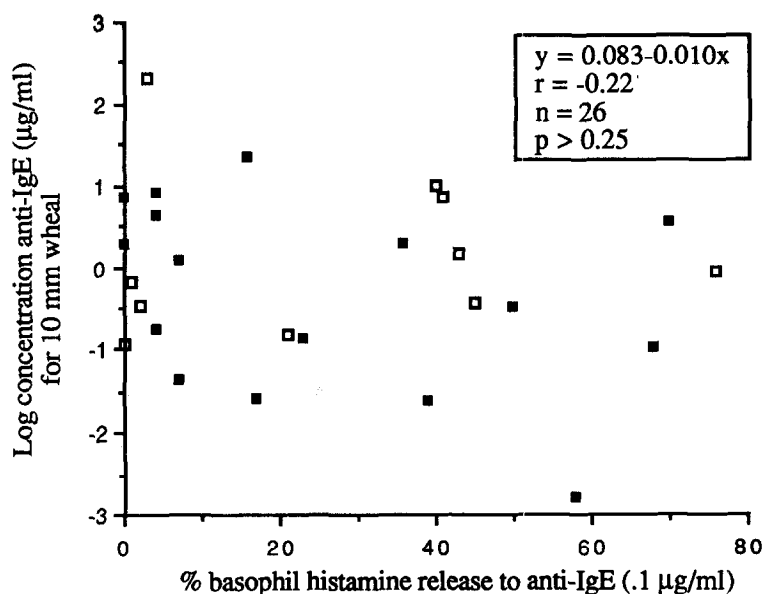


FIG. 4. Correlation between in vitro basophil histamine release to 0.1 $\mu\text{g/ml}$ of goat anti-IgE (abscissa) and in vivo skin reactivity to rabbit F(ab')₂ anti-IgE as measured by the logarithmic concentration causing a 10 mm wheal (ordinate) in 16 allergic (■) and 10 nonallergic (□) subjects. No correlation was found for the group as a whole ($r = -0.22$; $p > 0.25$) or for allergic ($r = -0.38$; $p > 0.05$) or nonallergic ($r = 0.04$; $p > 0.25$) subgroups.

neous reactions. One subject complained of mild axillary pruritus that she had experienced frequently in the past when she was skin tested with relevant allergens, and one subject complained of mild scalp pruritus.

Antibody against RGG

Forty-one of the 49 subjects evaluated had small quantities of IgG antibodies recognizing RGG in pre-skin test sera, as determined by RGG inhibition (data not presented). Anti-RGG antibody levels were not

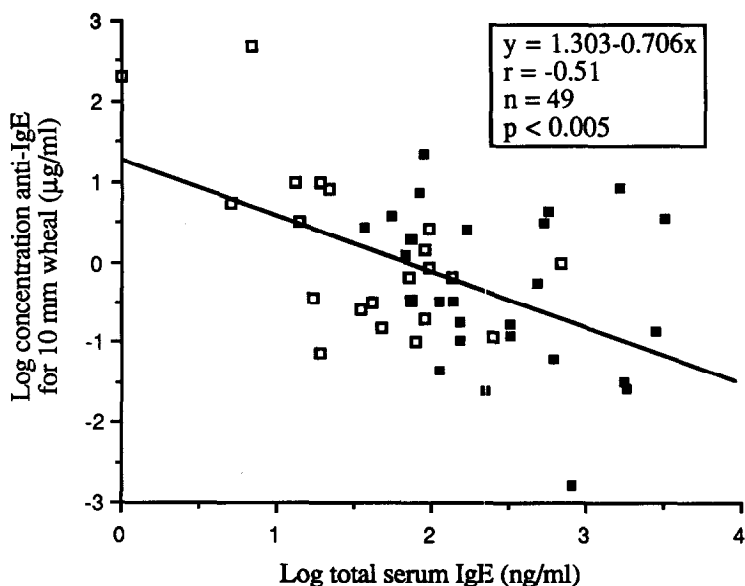


FIG. 5. Correlation between total serum IgE concentration (abscissa) and in vivo skin reactivity to the anti-IgE reagent (ordinate) in 27 allergic (■) and 22 nonallergic (□) subjects. For the group as a whole, $r = -0.51$; $p < 0.005$. The correlation coefficients for allergic ($r = -0.31$; $p > 0.10$) and for nonallergic ($r = -0.65$; $p < 0.005$) subjects were not statistically different with the z transformation of correlation coefficients ($p > 0.1$).

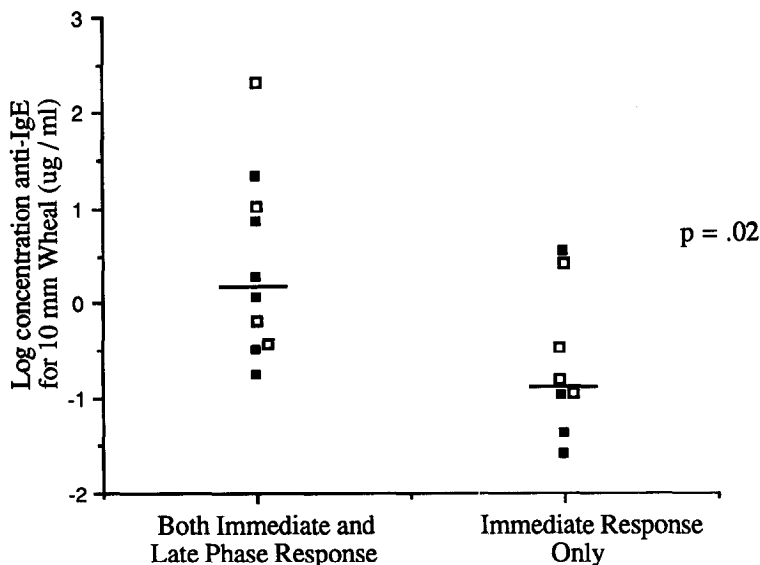


FIG. 6. Eighteen subjects in whom LPRs were evaluated with skin sites where immediate skin wheal was >10 mm. Open squares represent nonallergic subjects and closed squares represent allergic subjects. Horizontal bars designate group medians. Data were analyzed with Mann-Whitney U test. Median for subjects with LPRs is 0.16, and for those with immediate response only, -0.88 ($p = 0.02$).

significantly increased in any of the 49 subjects 3 weeks after skin testing with rabbit F(ab')₂ fragments.

LPR to anti-IgE

Four of eight nonallergic and six of 10 allergic subjects tested developed a cutaneous LPR to the con-

centration of rabbit antihuman IgE causing an immediate wheal of >10 mm. The concentration of anti-IgE causing an initial 10 mm wheal was significantly greater (on average about 10 times) in subjects with an LPR, compared to subjects without an LPR ($p = 0.02$) (Fig. 6).

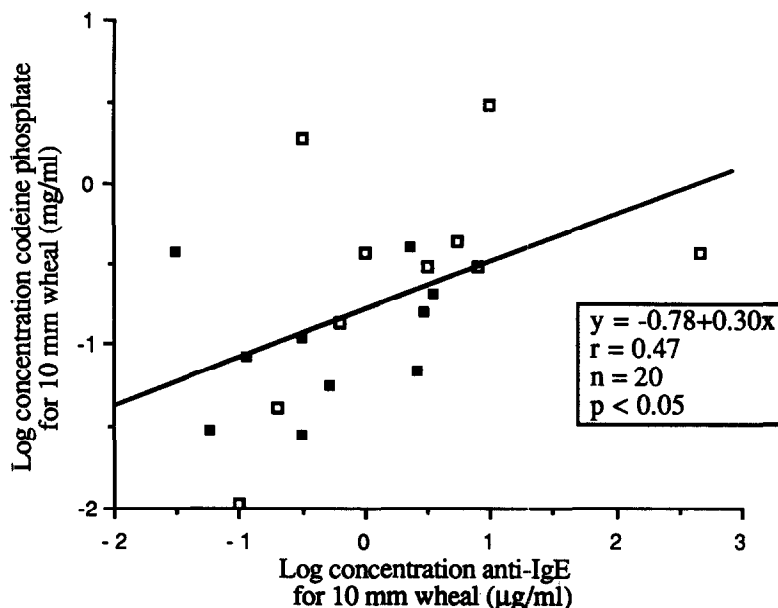


FIG. 7. Correlation between skin reactivity to anti-IgE (abscissa) and skin reactivity to codeine phosphate (ordinate) in 10 allergic (■) and 10 nonallergic (□) subjects who were studied with full-dose titration. For the group as a whole, $r = 0.47$ ($p < 0.05$). For allergic subjects, $r = 0.23$ ($p > 0.05$), and for nonallergic subjects, $r = 0.47$ ($p > 0.05$).

Histamine and codeine skin tests

No difference in wheal or flare size to histamine phosphate (0.275 mg/ml) was found between allergic and nonallergic subjects ($p > 0.2$ and $p > 0.1$, respectively) (data not presented). There also was no significant difference in immediate skin reactivity to codeine phosphate in allergic subjects compared to nonallergic subjects ($p = 0.1$) (data not presented). However, there was a positive correlation ($r = 0.47$; $p < 0.05$) between skin reactivity to anti-IgE and codeine phosphate, a non-IgE-dependent secretagogue (Fig. 7).

DISCUSSION

All subjects evaluated had an immediate cutaneous wheal-and-flare reaction to affinity-purified rabbit F(ab')₂ antihuman IgE antibody, including a subject with a total serum IgE level <1 ng/ml. Conroy et al.¹⁷ demonstrated that the amount of IgE molecules bound to human basophils is highly correlated ($r = 0.89$) with the total serum IgE level. The ability of F(ab')₂ anti-IgE to cause an immediate wheal-and-flare reaction in a subject with <1 ng/ml of total serum IgE and, hence, a small amount of cell-bound IgE, is consistent with the study of MacGlashan and Lichtenstein¹⁸ that demonstrated that basophil histamine release could be initiated by cross-linking fewer than 100 molecules of cell-surface IgE. Presumably, these observations may apply to human mast cells as

well. Taken together, these findings suggest that antihuman IgE is an excellent "universal" skin test reagent and that even in individuals with very low concentrations of cell-bound IgE an immediate cutaneous response is elicited. A 100% response to the anti-IgE stimulus in vivo contrasts with studies in vitro in which about 80% to 85% of basophil donors respond to anti-IgE.^{19, 20}

Despite the fact that total serum IgE level is directly correlated with in vivo skin reactivity to the anti-IgE reagent (Fig. 5) and that, as a group, allergic subjects have higher total serum IgE levels than nonallergic subjects (data not presented), the skin reactivity of the two groups overlap enough that allergic subjects cannot be distinguished from nonallergic subjects with the reagent (Figs. 2 and 3). This is in agreement with a previous study by Newcomb and Ishizaka.⁴

The use of affinity-purified F(ab')₂ fragments of rabbit antihuman IgE would be expected to reduce or eliminate the immunogenicity of rabbit immunoglobulins, since this preparation eliminates the Fc portion of the molecule what contains most if not all of the antigenic sites for human rheumatoid factors.^{21, 22} This lack of immunogenicity of the reagent was confirmed with an ELISA. Before skin testing, 41 of the 49 subjects had some detectable IgG antibodies that recognized RGG, but skin testing did not boost the RGG IgG in any patient. The nearly ubiquitous preexisting IgG anti-RGG may reflect prior dietary exposure or

low levels of cross-reacting antibodies. No serious adverse reactions developed after use of the reagent. That skin reactivity was due to the antihuman IgE specificity of the antibody was confirmed by the fact that no subject developed an immediate cutaneous reaction to similar concentrations of rabbit F(ab')₂ fragments of normal rabbit IgG.

We were surprised by the lack of correlation between in vivo skin reactivity and in vitro basophil histamine release to the anti-IgE stimulus (Fig. 4) since prior studies with specific allergens have demonstrated a correlation between in vivo and in vitro challenge.^{23, 24} The lack of correlation observed with anti-IgE could be due to the fact that the basophil histamine release studies were done with only one concentration of anti-IgE, rather than a full dose-response curve that was used in prior studies with specific allergens.^{23, 24} The alternative explanation is that anti-IgE acts somewhat differently on human mast cells and basophils. Previous in vitro studies have demonstrated human basophils to be 10 to 30 times more sensitive to antihuman IgE than mast cells.²⁵

Although Dolovich et al.⁶ previously reported that all 10 subjects skin tested with anti-IgE developed a LPR, we found an LPR reaction rate of about 50%. Although small numbers of subjects were evaluated, the rates of LPRs in allergic and nonallergic subjects were similar; 6 of 10 (60%) and 4 of 8 (50%), respectively. In our study, the concentration of F(ab')₂ anti-IgE causing an immediate wheal >10 mm in diameter was used to evaluate LPRs. As can be observed in Fig. 6, for the development of the same immediate 10 mm wheal size, subjects who developed an LPR required about tenfold higher concentrations of anti-IgE compared to subjects who did not develop an LPR. Umemoto et al.²⁶ demonstrated that for a given individual, increasing concentrations of anti-IgE (or allergen) injected resulted in a larger immediate cutaneous response and that both the concentration of anti-IgE (or allergen) injected and the immediate wheal size were correlated with the size of the LPR. Our study suggests that the development of an LPR in the skin is dependent more on the concentration of the IgE-dependent stimulus injected than the size of the immediate cutaneous response. The study of Lai and Holgate²⁷ supports the concept that the concentration the IgE-dependent stimulus, and not the size of the immediate response, may be responsible for the development of an LPR. Using allergen bronchoprovocation, they converted two of 10 single early responders with asthma into dual (early and late) responders with asthma by pretreatment with large doses of terfenadine that blunted the immediate fall in pulmonary function

after allergen inhalation. This allowed inhalation of 30- to 60-fold more allergen without increasing the immediate fall in FEV₁. Higher concentrations of anti-IgE (and allergen) could cause LPRs by producing qualitatively different mast cell degranulation or by uniquely interacting with cell-surface IgE bound to low-affinity IgE receptors that have been described on eosinophils, macrophages, platelets, and lymphocytes.²⁸ Persistence of anti-IgE (or allergen) at the skin site is not likely to be the cause of the LPR based on prior studies by Warner et al.²⁹

Just as for anti-IgE, skin reactivity to codeine phosphate, a non-IgE-dependent secretagogue, was not significantly different in allergic and nonallergic subjects. This result agrees with Cohen and Rosenstreich.³⁰ The positive correlation between the in vivo responsiveness to codeine and anti-IgE (Fig. 7) suggests that in vivo reactivity to both stimuli may in part be a function of skin mast cell releasability. We believe that anti-IgE is more useful than codeine for studying mast cell degranulation in vivo, since it is believed that anti-IgE works through the same IgE-dependent mechanism as does allergen.

The ability of the antihuman IgE stimulus to function as a universal skin test reagent in vivo will permit a variety of investigations of IgE-dependent human mast cell function to be undertaken in both nonallergic subjects and allergic subjects in whom the relevant allergens are unknown. Subjects undergoing therapeutic interventions with cytotoxic or immunoregulatory drugs or biologic agents that may effect mast cell function can be evaluated in vivo with such an anti-IgE reagent. Another possible use of anti-IgE skin testing would be in evaluating the biologic effects of therapeutic interventions that may effect mast cell-IgE antibody interactions, which are involved in diseases, such as allergic asthma, rhinitis, and certain drug allergies.

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