IgE Antibody Measurements in Ragweed Hay Fever

RELATIONSHIP TO CLINICAL SEVERITY AND THE RESULTS OF IMMUNOTHERAPY

L. M. Lichtenstein, K. Ishizaka, P. S. Norman, A. K. Sobotka, and B. M. Hill

From the Johns Hopkins University School of Medicine at the The Good Samaritan Hospital, Baltimore, Maryland 21239

Abstract Specific IgE anti-ragweed antibodies (IgEAR) were measured over two years in two groups of highly sensitive patients treated (immunized) with either ragweed extract or placebo and in a third group of placebo-treated, relatively insensitive patients. The IgEAR on the patients' basophils were assessed by ragweed antigen E (AgE)-induced histamine release; blocking (IgG) antibodies were measured by their ability to inhibit AgE induced histamine release. These data were evaluated against the clinical severity of ragweed hay fever in each patient.

We found that: (*a*) In placebo treated patients IgEAR usually declined gradually prior to the ragweed season and were boosted by environmental exposure to ragweed pollen. (*b*) In immunized patients the IgEAR rose at the beginning of treatment, but fell as immunotherapy proceeded; by the end of the second year the levels had decreased in 18/19 patients. (*c*) The increase in blocking antibody during immunotherapy correlated significantly ($P < 0.05$) with the decrease in serum IgEAR. (*d*) Judged by their sensitivity to AgE induced histamine release, IgEAR on basophils correlated significantly with IgEAR in the serum of untreated patients ($P < 0.01$). (*e*) The highly sensitive placebo treated patients' symptom scores were significantly correlated with their IgEAR in serum ($P < 0.01$) and with the sensitivity of their basophils to AgE-induced histamine release ($P < 0.01$). Neither correlation was observed in the relatively insensitive patients. (*f*) In the treated group the IgEAR measurements predicted neither the degree of their illness nor their clinical improvement We conclude that IgE antibody measurements may be useful in the assessment of the severity of reaginic allergy in highly sensitive patients. Its use in modestly sensitive patients requires further study, as does the inverse association between IgE and IgG anti-ragweed antibodies.

Dr. Lichtenstein is the recipient of Research Career Development Award AI42373 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health.

Received for publication 2 August 1972 and in revised form 17 October 1972.

INTRODUCTION

The ability to measure immunologic parameters in allergic diseases has grown rapidly in the last five years as a result of the identification of the causative antibodies (reagins) as a unique class of immunoglobulins, IgE, and the discovery of first one and then several IgE myelomas (1–4). The availability of these large concentrations of IgE myeloma protein made possible the development of quantities of specific anti-IgE antisera sufficient to allow the measurement of total IgE levels in human sera by means of radioimmunoassay (5). Johansson, Bennich, Wide, their colleagues and others have carried out extensive surveys of serum IgE levels which have led to the following observations: the total serum IgE levels in normal, western man range from 60 to 1,800 ng/ml; elevated levels obtain, on the average, in atopic individuals whether their disease be hay fever, "allergic" asthma, or eczema; in spite of this average difference, the overlap between atopics and normals is significant and therefore IgE measurements in individual patients are unlikely to be clinically useful; finally, the highest serum IgE levels are seen in parasitized individuals (6–9).

A more recent technical advance was the development of the radioallergosorbent test (R.A.S.T.) by Wide, Bennich, and Johansson which allowed the measurement of specific IgE antibodies to various allergens (10). This promised to be more clinically useful in that it might allow the detection of specific allergic sensitivity

by a less laborious and more quantitative method than skin test or P-K (passive transfer) techniques. Indeed, there was a good, though certainly not perfect, correlation found between skin tests, provocative (intranasal or intrabronchial) tests, clinical history, and the IgE antibody levels as determined by the R.A.S.T. (11–14).

What is needed clinically, however, is not so much a simple positive or negative response to the question of allergic sensitivity, since history or simple skin tests provide that now. Rather, a quantitative statement is needed as to the *degree* of clinical sensitivity to a particular allergen in a particular patient. This can be achieved by an in vitro measurement of histamine release from peripheral human leukocytes challenged with a specific allergen, and we have recently shown that careful skin testing with standardized extracts has the same ability, albeit somewhat less precise (15, 16).

Atopic disease is not caused by circulating IgE antibodies but rather by those "fixed" to the target cells, tissue mast cells and basophils. The basic question which must be answered in assessing the usefulness of serum IgE antibody measurements, therefore, is the relationship, in a variety of situations, between these two parameters, i.e. circulating IgE antibody and cell-fixed IgE antibodies. These measurements must then be assessed against the degree of clinical illness. This paper reports a study of anti-ragweed IgE antibodies measured in the serum by a radioimmunoassay method and on the cells (basophils) by leukocyte histamine release in response to allergen challenge. The antibody levels were measured serially before and after the ragweed season and in treated (immunized) versus placebo-treated patients. These studies were carried out in matched groups of highly sensitive patients undergoing a clinical trial of the efficacy of immunotherapy. IgE antibody levels were also measured in a small group of the less sensitive patients (less sensitive by skin test, histamine release, and eventually by clinical criteria) who constitute more of a diagnostic problem. In each instance a correlation was sought between the antibody levels and the degree of clinical disease experienced by the patients during the ragweed season. Finally, the relationship of IgE antibodies to the IgG "blocking" antibodies induced by immunotherapy was assessed

METHODS

Patients. The study patients were selected by criteria previously described for a study of immunotherapy which is to be reported elsewhere (15, 16). Each was determined by clinical evaluation to be sensitive to ragweed pollen. The patients were all skin test positive to crude ragweed extract and ragweed antigen E, and the leukocytes of all patients released histamine when challenged with these materials. The patients ranged in age from 26 to 57; 70% were male. The leukocytes of 42 of the patients released 60–100% of their total histamine when exposed to antigen E or ragweed extract; these were matched by cell sensitivity (the concentration of antigen E required for 50% histamine release) and randomly placed into treatment groups (ragweed extract or placebo); other patients (15) released less than 50% of their total leukocyte histamine when challenged with the antigens: that is, their antigen dose response curves peaked at less than 50% histamine release and showed decreased release as the antigen dose was increased. These patients made up a special "low release" placebo treatment group. As the study progressed some patients dropped out (five in 1970 and three more in 1971); the reason for this attrition was usually the inconvenience of participating in the study, either due to new responsibilities or to moving. Five patients dropped out of the placebo group and three from the treated group. In some instances over the 18 month period of study a patient missed a particular bleeding period or there was a technical mishap. Because of this, the number of patients studied at each time period fluctuates somewhat but all data obtained at a given date were recorded.

Symptom evaluation. This was carried out by techniques previously reported (15,17). Briefly, a history form was filled out by each patient for each 12 h period of the ragweed season, recording the duration of sneezing, rhinitis, conjunctivitis, and cough. Antihistamine use was also recorded. The forms were collected weekly and the reported symptoms scored numerically and entered into a computer program. This yielded a symptom score for each patient (or each group of patients) for each day of the ragweed season. Daily symptom scores obtained in this way have been shown previously to be highly correlated with the daily ragweed pollen count (15, 18, 19). The scores were also averaged to yield a single number reflecting the clinical severity of the ragweed season for a single patient; these scores have previously been shown to correlate significantly with the patient's cell sensitivity measurements (15, 18). Each patient was evaluated bi-weekly by a physician who was not aware of the patient's therapeutic regimen. The physician's evaluation has been previously shown to correlate with the symptom scores reported by the patients (15, 18). We have found that the patients' symptom scores correlate better with both the pollen count and cell sensitivity measurements than do physician scores (15, 18). We have, therefore, presented only the former.

Immunotherapy. Placebo-treated patients received tris-buffered solutions containing histamine. Their "dose" was gradually increased so that they developed wheal and flare reactions to the histamine which mimicked the reactions reported by the other group. Specific therapy was with alum-precipitated crude ragweed extract prepared by Center Laboratories (20). Injections were started with 1 μg protein N (100 PNU) and doubled weekly unless this was prevented by severe local or generalized reactions. Injections were started April 22, 1970 and continued at weekly intervals until August 29, 1970. On the average a total of 15 injections were given. In this preseasonal course the patients received a total dose averaging 0.14 mg protein N of ragweed extract. The range was 0.02-0.38 mg. Immunization was begun again, after the ragweed season, on September 30, 1970, and continued until July 30, 1971. In this series of 20 injections the average total dose administered was 0.51 mg protein N. One patient could only tolerate 0.02 mg. The range for the rest was 0.21–1.15 mg.

Cell sensitivity determinations. The techniques involved have been previously published (21). We used May's modification of the technique described by Lichtenstein and

Osler (21, 22). Briefly, 50 cm^3 of blood was taken by venipuncture at least 7 days after any previous immunization. The white cells were separated by dextran sedimentation, washed free of platelets, red cells, and serum and suspended at a concentration of about 10^7 cells/ml in tris-buffered saline containing calcium, magnesium, and human serum albumin (HSA)[1] (0.03%). The buffer was serum free. Portions of this cell suspension were added to a series of tubes and challenged with appropriate concentrations of ragweed antigen E and crude ragweed pollen extract. The histamine released into the supernatant was measured by a modification of the fluorometric method of Shore, Burkhalter, and Cohn (21, 23). The total histamine in a portion of cells was determined after lysis of the cells with 8% $HClO_4$. The percentage of histamine released was calculated and an antigen dose response curve constructed, usually, from five separate points. From this, the concentration of antigen required for 50% histamine release was interpolated and defined as the cell sensitivity.

Blocking antibody determinations. The same histamine release system described above was employed using a technique described previously (24). All assays were carried out during a 3-wk period several months after the serum was drawn in September of 1971. The sera were stored at −20°C until that time. The antibody titers were measured using the leukocytes of three ragweed allergic, blood type O individuals. After determining the dose response curves of these individuals to ragweed antigen E a series of tubes was set up, each containing sufficient antigen to cause 60–80% histamine release. These were mixed with the allergic sera added in serial two- or threefold dilutions. A single AB normal serum was employed to keep the total serum concentration constant. Controls were run in AB serum alone. The allergic serum inhibits histamine release relative to the control; this allows the construction of a (five point) curve of percentage inhibition versus serum concentration. The concentration of serum required for 50% inhibition is interpolated from this curve and the reciprocal of this number is defined as the blocking antibody titer. We have previously shown that this blocking activity is essentially all due to IgG antibodies (25).

Antigens used for in vitro techniques. Antigens E and Fraction D were prepared as outlined in reference 26 and were the generous gift of Dr. T. P. King. Fraction D is obtained by precipitating whole ragweed extract in saturated ammonium sulfate and, after dialysis of the precipitate, fractionation of the active material on Sephadex G25 and DEAE Sephadex. Antigen E is derived from fraction D after chromatography on Sephadex G100. The allergenically active material in Fraction D is largely antigen E, which makes up 19% by weight of Fraction D; biological assays comparing antigen E and Fraction D have about this ratio (27).

IgE anti-ragweed fraction D measurements. IgE antibody against ragweed antigen was measured by the method of Wide (28). 400 mg of ultrafine Sephadex, (Pharmacia, Uppsala, Sweden) were activated by cyanogen bromide and reacted with 10 mg of Fraction D dissolved in 0.1 M $NaHCO_3$ for 24 h at room temperature with constant rotation. After washing thoroughly with 0.5 M $NaHCO_3$, acetate buffer (pH 4.0), and once with assay buffer,[2] the antigen-coupled immunosorbent was suspended in the same buffer. The final suspension contained 1 mg/ml of the immunosorbent.

Anti-IgE antiserum was prepared by immunization of rabbits with the Fc fragment of E myeloma protein (PS). The antiserum was absorbed with the $F(ab')_2$ fragment of the myeloma protein to render it monospecific for ϵ-chain. The antibody was then specifically purified using E myeloma protein-coupled immunosorbent prepared by the method of Porath, Axen, and Emback (29). 10 ml of Sepharose 2B (Pharmacia, Uppsala, Sweden) was coupled with 50 mg of E myeloma protein. The monospecific anti-IgE serum was passed through a column of IgE-immunosorbent and the antibody combined with the immunosorbent was eluted with 0.1 M glycine buffer, pH 3.0. Approximately 70% of the total protein in the purified antibody preparation was associated with precipitating antibody against the Fc portion of IgE. 60 μg of the purified antibody preparation in 0.01 ml was labeled using 2.5 mCi of ^{125}I (carrier free, high specific activity; Cambridge Nuclear Corp., Cambridge, Mass.) by the method of Klinman and Taylor using chloramine T (30). The ^{125}I-labeled antibody was diluted in 2 ml of 0.1% HSA and stored after dialysis. The final preparation gave 4×10^9 counts/min per ml.

In each test, serial twofold dilutions of a reference serum (AR) were used as a standard. The P-K titer of the reference serum was 1:4,000. $\frac{1}{10}$ ml of standard and test samples was incubated in duplicate with 0.5 ml of immunosorbent (1 mg/ml) at 4°C overnight with rotation. Control tubes contained immunosorbent alone. After overnight incubation at 4°C with constant rotation, the tubes were centrifuged, and the preparation washed three times with borate-buffered saline. A half-ml volume of a 1:10,000 dilution of [^{125}I] anti-IgE in assay buffer was then added to each tube and the suspension was rotated for 5 h at 4°C. The immunosorbent was washed three times with borate-buffered saline, and the radioactivity of the precipitates was measured by an automatic gamma counter (Nuclear Chicago Corp., Chicago, Ill.). The IgE antibody in the test samples was estimated from a standard curve which gave a straight line between the logarithm of the concentration of serum AR and the radioactivity (counts per minute) of the precipitate in the range of undiluted serum to a 1:1,024 dilution. Serum samples containing high concentrations of IgE antibody were diluted before titration. In view of the P-K titer of serum AR, the IgE antibody level in this serum was considered to be 4,000 U/ml and the IgE antibody levels in the test samples were expressed in terms of these units.

RESULTS

Changes in IgE anti-ragweed (IgEAR) levels. Figs. 1*A* and 1*B* show, for selected patients, the range of changes in IgEAR antibody levels from February, 1970 through the major part of the ragweed seasons (mid-August to the end of September) of 1970 and 1971. The antibody levels even in these highly sensitive individuals (chosen from the matched pairs treated either with ragweed antigens or placebo) range from about 10 U to several thousand units/ml. In the placebo-treated group (Fig. 1A) there is a tendency for the reagin titer to fall to its lowest value just before the annual environ-

[1] *Abbreviations used in this paper:* HSA, human serum albumin; IgEAR, IgE anti-ragweed antibodies.

[2] The assay buffer was composed of 500 ml 0.1 M phosphate buffer, pH 7.5, 500 ml 0.9% NaCl, 10 ml 5% sodium azide, 5 ml Tween 20 (City Chemical Corp., New York), and 2 g human serum albumin (ICN Nutritional Biochemicals, Cleveland, Ohio).

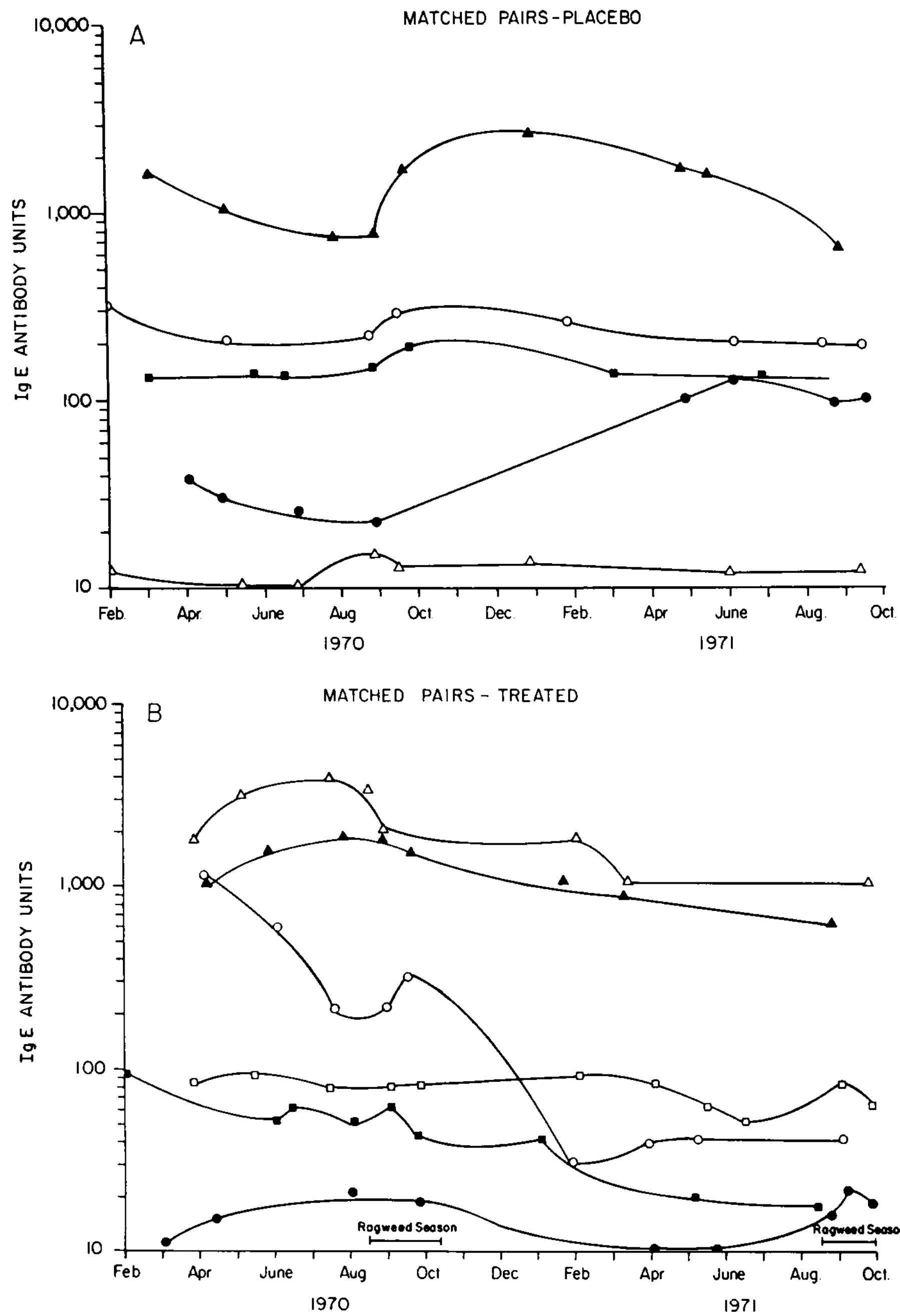

FIGURE 1 Serial IgE anti-ragweed antibody measurements in patients selected from the highly sensitive placebo-treated (*A*) or ragweed extract-treated (*B*) patients. The patients were selected to demonstrate diverse patterns (See Figs. 2 and 3 for complete data).

mental exposure to ragweed pollen and to increase just after the ragweed season. This tendency is reasonably regular. Fig. 2*A* compares the reagin titer measured in placebo treated patients in February 1970 with that found in July and August of 1970, just before the ragweed season. Of 19 patients studied only one showed a rise in IgEAR levels, in three the level remained the same, and the titers of the remainder of the group decreased. Fig. 2*B* shows the rise in IgEAR from the preseasonal specimen to mid-winter of 1970 in the same patients. This postseasonal rise in IgEAR is not seen in the immunized patients (Fig. 3*B*). Rather there is a tendency for the reagin levels to increase during the immunization procedure before the ragweed season (Fig. 3*A*). This ten-

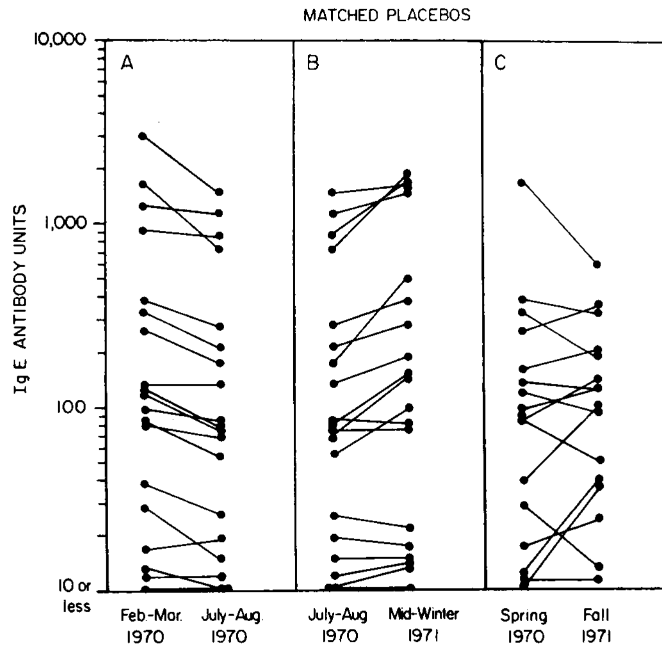

FIGURE 2 Changes in IgE anti-ragweed antibodies for the time periods stated in all of the highly sensitive placebo-treated patients. There is a tendency for the levels to fall prior to the ragweed season (*A*) and to rise thereafter (*B*).

dency is not as striking as reported during "rush" desensitization by Berg and Johansson, inasmuch as only about half of the patients demonstrate a rise in IgEAR (31). This increase, however, must be contrasted with the consistent pattern of falling IgEAR levels in the placebo-treated patients over the same period. After this

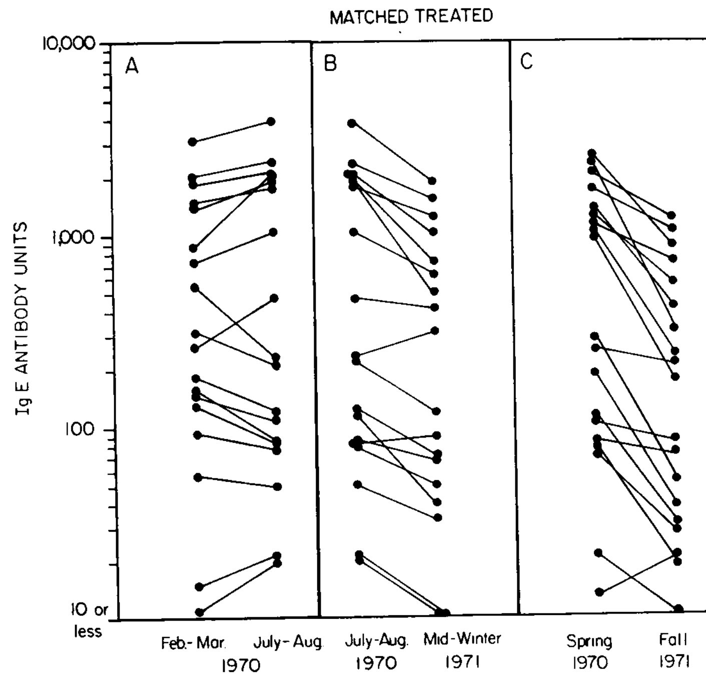

FIGURE 3 Changes in the IgE anti-ragweed antibody levels in all of the highly sensitive treated patients for the periods stated. There is a tendency in the treated group for the IgEAR levels to increase at the beginning of immunization (*A*) and then to fall (*B* and *C*).

original rise in IgEAR, the levels in the immunized patients start to fall. No increase is seen during the 1970 ragweed season and by mid-winter of 1971 the levels were decreased in 16 patients (Fig. 3*B*). At the time of the ragweed season of 1971 their IgEAR titers had fallen appreciably. Fig. 3*C* presents this data for the entire group of treated patients: 18 of 19 show a decrease in IgEAR. In the placebo-treated group over the same period there is no consistent change (Fig. 2*C*).

Relationship of IgEAR level to cell sensitivity. Cell sensitivity measurements, although indirect, are currently the only accurate technique available for assessing the IgEAR antibody on target cells. It has been shown by Levy and Osler, in passive sensitization experiments, that the sensitivity of a population of leukocytes varies inversely with the concentration of IgEAR with which the cells are sensitized (32). That is, the concentration of antigen required to elicit a given response decreases as the concentration of IgE antibody is increased. It is likely, however, that the concentration of antigen needed to effect 50% histamine release depends not only on the number of IgEAR molecules on the cell but also upon the "effectiveness" of the histamine releasing mechanism on these cells (33). That is, even with two evenly sensitized populations of basophils there are likely to be variations in the complex secretory processes which lead to histamine release. Nonetheless, these two parameters are likely to vary independently and population studies should, therefore, show a relationship between circulating IgEAR and cell-fixed IgEAR if one exists. Fig. 4 presents a plot of each patient's cell sensitivity against the IgEAR antibody level in the same patient. These values were obtained at the start of this study before any of the patients had received immunotherapy and the graph includes the entire group of patients studied. There is clearly a relationship between the two measurements in question: the Spearman Rank correlation coefficient is 0.74 ($P < 0.001$) for the matched group of highly sensitive patients (closed circles). There is, nevertheless, a great variability in that patients with the same IgEAR level may differ in cell sensitivity by 100 to 1,000-fold. In the less sensitive group for whom, by definition, no cell sensitivity measurement is possible, 13 of the 15 patients had very low IgEAR antibody levels (open circles).

This relationship between cell sensitivity and IgEAR continued at a highly significant level in the placebo-treated individuals throughout the course of this study. This was not the case, however, in the patients who were immunized: by fall of 1970 this group no longer showed a significant association between cell sensitivity and IgEAR (data not shown).

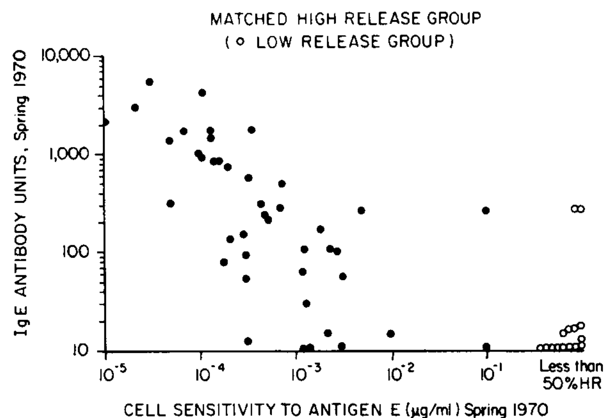

FIGURE 4 The relationship between a patient's serum level of IgEAR and the IgEAR on his basophils as judged by antigen E-induced histamine release (HR). The Spearman Rank correlation coefficient for the highly sensitive patients (closed circles) is 0.74 $p < 0.01$. All but two of the relatively insensitive patients (open circles) had very low IgEAR levels.

Relationship between the severity of ragweed pollenosis, IgEAR levels, and cell sensitivity measurements in highly sensitive patients. In the placebo-treated group there was a significant relationship between cell sensitivity and the symptom scores recorded during the 1970 and 1971 ragweed pollen seasons (Figs. 5*A* and 5*B*). This correlation is similar to that observed regularly in our laboratory since 1964 (15, 18, 19); in these several years the correlation coefficient between cell sensitivity and symptom scores in groups of 20–30 placebo-treated patients has varied only between 0.70 and 0.75. In 1970, with a group of 21 patients, the Spearman Rank correlation coefficient was 0.70, $P < 0.01$; in 1971 with data on only 15 patients, the correlation coefficient was 0.65, $P = < 0.01$. The same relationship between symptom scores and IgEAR is examined in Figs. 6*A* and 6*B*. In 1970 (Fig. 6*A*) there is a clear relationship between the two; the Spearman Rank correlation coefficient is 0.69 $P < 0.01$. In 1971, however, when the group fell to 15 patients, the association was not so obvious and, in fact, was no longer statistically significant. This is surprising since, as mentioned above, there remained in 1971, in this placebo-treated group, a significant association between the IgEAR antibody level and cell sensitivity and between cell sensitivity and symptom scores. Figure 6*A* presents data which for the first time demonstrates an association between the IgE antibody levels to a particular allergen and the *severity* of the allergic disorder caused by exposure to that allergen. We have examined the failure of this pattern to repeat in 1971 in detail: there are shifts in both symptom scores and IgEAR but no obvious trend explains the change. Presumably, it was merely due to a decrease in the number of patients available for study.

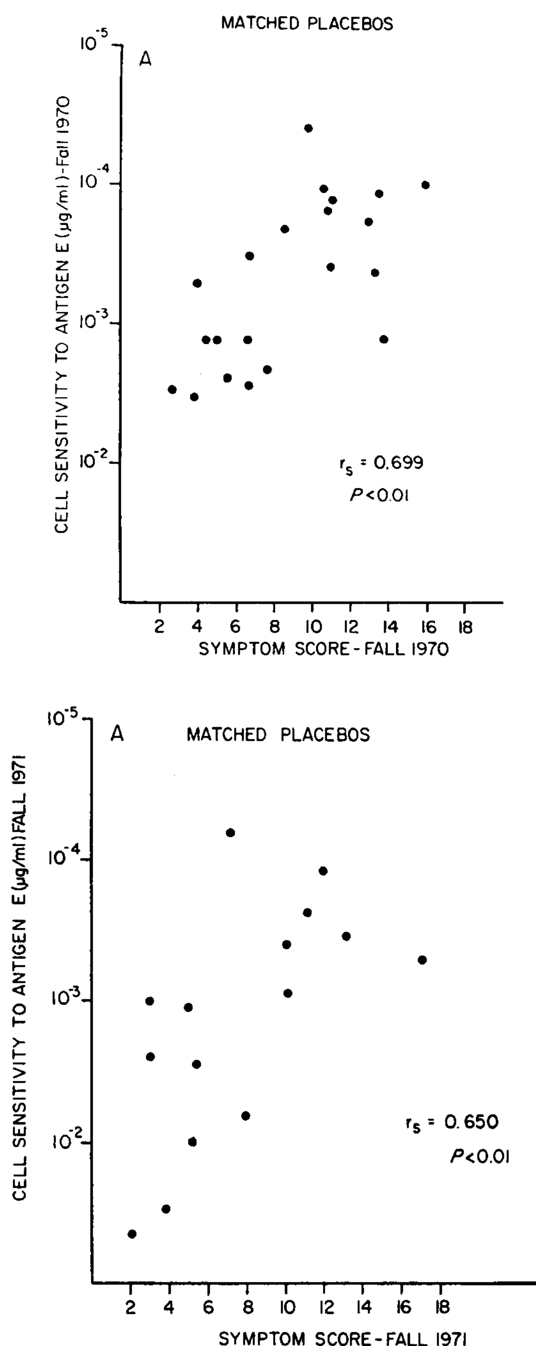

FIGURE 5 The relationship between the sensitivity of a patient's basophils to antigen E (the concentration of antigen E required for 50% histamine release) and the severity of his ragweed hay fever in the highly sensitive placebo-treated group in 1970 (*A*) and 1971 (*B*).

Cell sensitivity tends to decrease with immunotherapy, and while a significant number of patients who have improved demonstrate this change we have never noted a quantitative association between the cell sensitivity and symptom scores in treated patients (15, 18, 19); neither did we in this study (data not shown). Similarly, there is no association between the IgEAR and symptom scores in the treated patients. Moreover, we were not able to find an association between the change (decrease) in IgEAR and the degree of clinical relief noted by the immunized donors (data not shown). This association was sought by examining the correlation coefficient between the decrease in IgEAR in treated patients from spring 1970 to the ragweed season of 1971 (*cf.* Fig. 8) and either the absolute symptom scores or the decrease in symptom scores from those expected on the basis of cell sensitivity measurements, using the cell sensitivity symptom score relationship noted in the placebo treated patients as a point of reference.

IgEAR levels in less sensitive patients. These patients had leukocytes which released less than 50% of their histamine (by definition precluding cell sensitivity measurements). Skin testing to a 50% end-point with antigen E and ragweed extract showed them as a group to be less sensitive and, on the average, their symptom scores were significantly lower than the matched placebo-treated group of more highly sensitive donors. There was, however, a wide range in symptom scores in this group and skin testing and histamine release studies were not helpful in deciding how an individual patient would respond clinically. These data will be presented elsewhere.[8] IgEAR antibody measurements were not more useful than the other techniques in predicting the clinical course of this group. Fig. 7 presents the 1970 IgEAR levels for these patients plotted against their symptom scores. There is no correlation between the two parameters.

The relationship between IgEAR and IgG blocking antibodies. IgG blocking antibodies regularly increase with immunization in a reproducible fashion, such that the antibody response is proportional to the dose of antigen administered (15). While there is no direct relationship between the magnitude of the rise in blocking antibody and clinical success in immunotherapy, there is a statistical association such that treated patients who develop high levels of blocking antibodies have somewhat fewer symptoms than immunized patients who develop lower levels (19). When the absolute level of IgEAR in the treated group was plotted against the blocking antibody titer no association was noted. However, as shown in Fig. 8, if the magnitude of the drop in IgEAR from the onset of treatment through the end of the ragweed season of 1971 was plotted against the blocking antibody level determined on sera drawn during the 1971 ragweed season there was a significant correlation (0.49, $P < 0.05$).

[8] Norman, P. S., K. Ishizaka, and L. M. Lichtenstein. Manuscript in preparation.

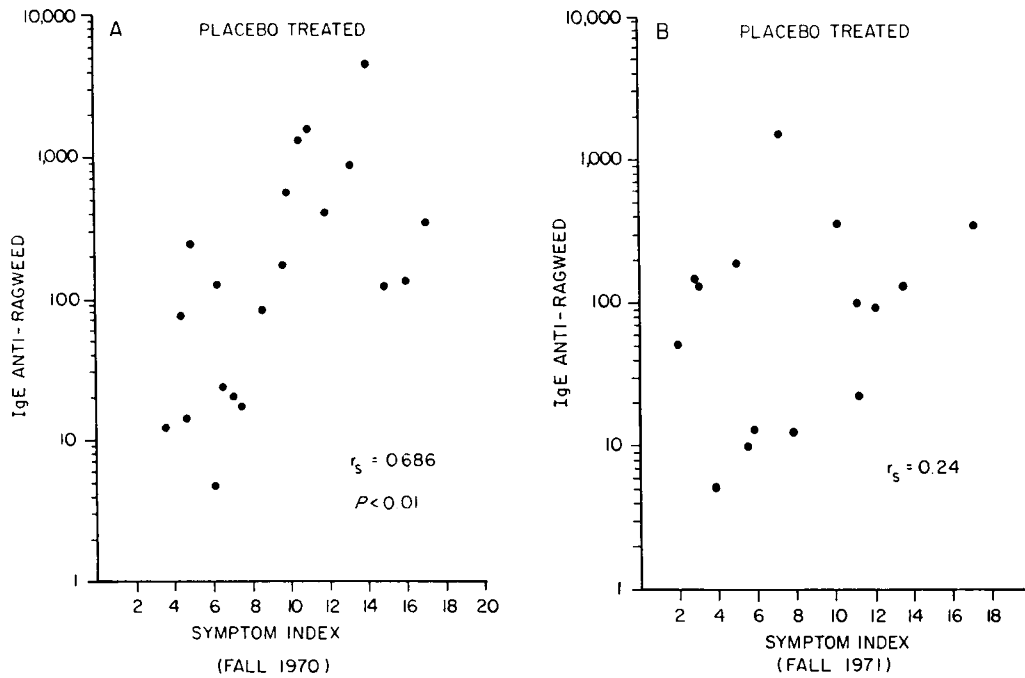

FIGURE 6 The relationship between a patient's IgEAR level and the severity of his ragweed hay fever in the highly sensitive placebo-treated group in 1970 (*A*) and 1971 (*B*).

DISCUSSION

This study has confirmed a number of observations related to the changes in reaginic antibodies which were made earlier, using biologic assays to detect IgE antibodies. The annual rise in reagins after the pollen season followed by a gradual fall until the next environmental exposure has been reported by Levy and Osler (34). Similarly, a rise in reagin levels immediately after

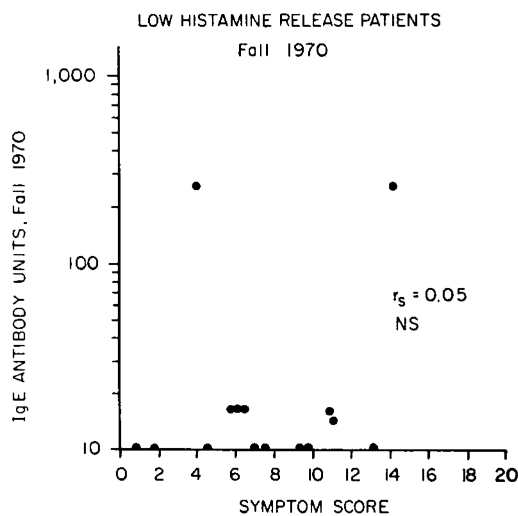

FIGURE 7 The relationship between a patient's IgEAR level and the severity of his ragweed hay fever in the relatively insensitive placebo-treated patients.

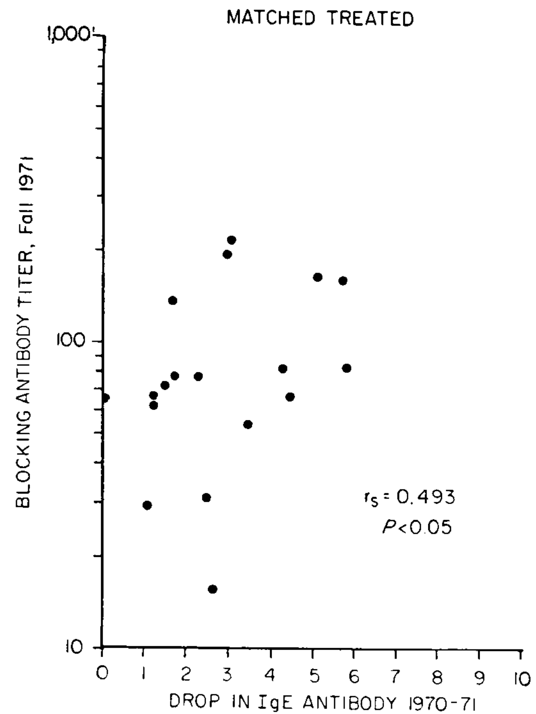

FIGURE 8 The relationship between a patient's blocking (IgG) antibody titer and the drop in his IgEAR level from the beginning of therapy to the ragweed season of 1971. The numbers refer to the ratio of initial IgEAR titer: final IgEAR titer, i.e. an initial titer of 2,000 falling to 500 is considered to be a fourfold drop and is recorded as "4" on the abscissa.

the onset of immunotherapy followed by a gradual decrease as the therapy is continued was suggested by Sherman, Stull, and Cooke (35). The greater precision possible with the present radioimmunoassay, however, has let us examine individual patterns of response more closely and it is clear that the above observations are only generalizations; each individual seems to have a fairly unique way of responding.

The major aim of this study was to investigate critically three points: (*a*) the relationship between circulating IgE antibodies and those which are fixed to the target cells as judged by cell sensitivity; (*b*) the relationship between the IgE antibodies to a specific allergen and the clinical severity of the atopic disease related to this allergen in untreated patients, as well as the usefulness of this assay in predicting the clinical results of immunotherapy with the allergen and, finally, (*c*) the relationship between IgE antibodies and the IgG blocking antibodies which result from therapeutic immunization.

The first question is the most difficult to investigate and we have not fully answered it. We were, at the outset, less than sanguine about the validity of this relationship for two reasons. First, was the observation that reagin titers fall in childhood and adolescence and are considerably lower in adults than in children (36). This decrease is *not* reflected in skin testing and cell sensitivity measurements (or probably in the clinical severity of atopic disease). Thus, it is readily demonstrable that for a given level of skin or cell sensitivity an adult has a lower reagin titer than does a child. Secondly, we and others have observed, in children and adults treated by immunotherapy, a marked fall in cell sensitivity even to the point of no response to very high antigen concentrations (19, 37, 38). The serum of these patients, however, continued to show significant levels of reaginic antibody, which were sufficient to passively sensitize other leukocytes, obtained from normal donors (38).

Cell sensitivity measurements are not the ideal way of assessing the load of specific IgE antibodies on a target cell membrane. There is presently, however, no alternative since fluorescent or autoradiographic techniques do not yield quantitative data (39). Moreover, this test is a reasonable standard inasmuch as it correlates regularly with the severity of clinical disease (15). In spite of these potential difficulties Fig. 4 shows a significant correlation between the level of circulating IgE antibodies to ragweed antigen and those fixed to the target cells as judged by ragweed antigen-induced histamine release. Whether the variability in the association between IgE antibody and cell sensitivity shown in Fig. 4 represents differences in the efficacy of the histamine release mechanism in different cells or truly represents a discrepancy between the levels of circulating and cell-fixed IgEAR remains to be demonstrated. Most likely it is a combination of both factors. It would appear, however, that at least in an adult population there is a definite correlation between these two parameters although it is not precise. A similar correlation was found between semiquantitative skin testing and the IgE antibody levels in data to be presented elsewhere.[3]

With respect to the second question, in the highly sensitive, homogeneous group of patients who were treated with placebo injections there was, in 1970, a significant relationship between IgEAR levels and the symptom scores which reflect the severity of clinical illness. This pattern was similar but not significant in 1971 in a somewhat smaller group and it is evident that the question must be further studied. We can, therefore, comment only briefly on the usefulness of these IgE antibody measurements in the diagnosis of allergic diseases. As mentioned above, the problem is not one of determining whether there is any sensitivity to an allergen, but whether the sensitivity is of clinical importance. This can only be answered by accurately assessing the clinical course of the disease in its natural state while at the same time carrying out the antibody measurements. Our data show that the IgEAR assay can select those patients who have severe disease about as well as histamine release studies or skin tests.

It is not more helpful than the other assays in the gray area of moderate to insignificant sensitivity, which presents the real clinical problem. At the moment, the IgE antibody assay seems to offer no theoretical advantage over adequate skin testing. There may be practical advantages, however, because skin testing as it is presently carried out in a clinical situation is, for many reasons, rarely performed rigorously. The R.A.S.T., however, is readily susceptible to standardization and automation. To this can be added the desirability of not subjecting the patient to the time or inconvenience of skin testing, which may be a major advantage. Certainly, however, the measurement of IgE antibodies is not a panacea for the diagnosis of allergic diseases, and its ultimate usefulness will have to be assessed by careful clinical and laboratory studies.

There seems little reason, from our data, to conclude that the measurements of IgE antibody levels in patients therapeutically immunized will provide prognostic information. Immunotherapy produces a modest, but easily demonstrable, clinical ameliorative effect (15, 18, 19). At the same time it prevents the seasonal rise in IgE antibody levels after environmental exposure to allergen and, in fact, leads to a gradual fall in the reagin titer. Unfortunately, these events do not seem to be quantitatively associated with one another, so that IgE antibody levels can not be used to ascertain the clinical efficiency of immunotherapy in a particular individual.

Finally, and perhaps most interestingly, we have reported here for the first time a significant correlation between a rise in the IgG blocking antibody level and a fall in specific IgE antibody measured over a 2 yr course of immunotherapy. It is known that the production of antibodies associated with different immunoglobulin classes is different depending on the dose and route of administration of antigen, the period after immunization, and the number of booster injections administered. In rodents, the reaginic antibody response to a high dose of antigen is transient in nature and a booster injection does not give a secondary antibody response, but rather terminates reaginic antibody formation (40). The gradual decrease of IgE antibody associated with the rise in the IgG blocking antibody level during immunotherapy suggests the possibility that a similar mechanism may be involved. Tada and Okumura showed that the administration of IgG antibody to the immunized rat resulted in a decrease of pre-existing reaginic antibody formation within 2 days and suggested the possibility that the effect of immunotherapy might be due to feedback inhibition of IgE antibody synthesis by IgG antibody (41). However, our recent studies showed that the administration of large amounts of IgG antibody to an inbred strain of mice sensitized with a low dose of antigen did not terminate pre-existing reaginic antibody formation nor prevent a secondary response (42). Since the reaginic antibody response in the mouse, as in hay fever patients, is persistent, whereas antibody formation in the rat is transient in nature, the gradual decrease of IgE antibody during immunotherapy may well not be due to feedback inhibition by IgG antibodies. Quantitative measurements of both IgG and IgE antibodies during the course of immunotherapy are underway to elucidate the mechanism involved.

ACKNOWLEDGMENTS

This is publication No. 54 of the O'Neill Research Laboratories.

This work was supported by Grants AI7290, AI8270, and AI4866 from the National Institutes of Health, Grant GB27862 from the National Science Foundation, and a Research Grant from the John A. Hartford Foundation.

REFERENCES

1. Ishizaka, K., and T. Ishizaka. 1967. Identification of γE-antibodies as a carrier of reaginic activity. *J. Immunol.* **99**: 1187.
2. Ishizaka, K., T. Ishizaka, and W. D. Terry. 1967. Antigenic structure of γE-globulin and reaginic antibody. *J. Immunol.* **99**: 849.
3. Johansson, S. G. O., and H. Bennich. 1967. Immunological studies of an atypical (myeloma) immunoglobulin. *Immunology.* **13**: 381.
4. Ogawa, M., S. Kochwa, C. Smith, K. Ishizaka, and O. R. McIntyre. 1969. Clinical aspects of IgE myeloma. *N. Engl. J. Med.* **281**: 1217.
5. Johansson, S. G. O., H. Bennich, and L. Wide. 1968. A new class of immunoglobulin in human serum. *Immunology.* **14**: 265.
6. Johansson, S. G. O. 1968. Serum IgND levels in healthy children and adults. *Int. Arch. Allergy.* **34**: 1.
7. Johansson, S. G. O., H. Bennich, T. Berg, and C. Högman. 1970. Some factors influencing the serum IgE levels in atopic diseases. *Clin. Exp. Immunol.* **6**: 43.
8. Johansson, S. G. O., T. Mellbin, and B. Vahlquist. 1968. Immunoglobulin levels in Ethiopian preschool children with special reference to high concentrations of immunoglobulin E (IgND). *Lancet.* **1**: 1118.
9. Gleich, G. J., A. K. Averbeck, and H. A. Swedlund. 1971. Measurement of IgE in normal and allergic sera by radioimmunoassay. *J. Lab. Clin. Med.* **77**: 690.
10. Wide, L., H. Bennich, and S. G. O. Johansson. 1967. Diagnosis of allergy by an in-vitro test for allergen antibodies. *Lancet.* **2**: 1105.
11. Berg, T., H. Bennich, and S. G. O. Johansson. 1971. In vitro diagnosis of atopic allergy. I. A comparison between provocation tests and the radioallergosorbent test. *Int. Arch. Allergy.* **40**: 770.
12. Johansson, S. G. O., H. Bennich, and T. Berg. 1971. In vitro diagnosis of atopic allergy. III. Quantitative estimation of circulating IgE antibodies by the radioallergosorbent test. *Int. Arch. Allergy.* **41**: 443.
13. Strenius, B., L. Wide, W. M. Seymour, V. Holford-Strevens, and J. Pepys. 1971. Clinical significance of specific IgE to common allergens. *Clin. Allergy.* **1**: 37.
14. Yuninger, J. W., and G. J. Gleich. 1972. Seasonal changes in IgE antibodies to ragweed antigen E. *J. Allergy.* **49**: 118. (Abstr.)
15. Lichtenstein, L. M., P. S. Norman, W. L. Winkenwerder, and A. G. Osler. 1966. In vitro studies of human ragweed allergy: changes in cellular and humoral activity associated with specific desensitization. *J. Clin. Invest.* **45**: 1126.
16. Norman, P. S., and W. L. Winkenwerder. 1965. Suppression of hay fever symptoms with intranasal dexamethasone aerosol. *J. Allergy.* **36**: 284.
17. Norman, P. S., B. V. Rhyne, and E. D. Mellits. 1966. The evaluation of agents for the treatment of seasonal respiratory allergies. *In* International Encyclopedia of Pharmacology and Therapeutics. L. Lasagna, editor. Pergamon Press Ltd., Oxford. **2**: 639.
18. Lichtenstein, L. M., P. S. Norman, and W. L. Winkenwerder. 1968. Clinical and in vitro studies on the role of immunotherapy in ragweed hay fever. *Am. J. Med.* **44**: 514.
19. Lichtenstein, L. M., P. S. Norman, and W. L. Winkenwerder. 1971. A single year of immunotherapy for ragweed hay fever. Immunologic and clinical studies. *Ann. Int. Med.* **75**: 663.
20. Norman, P. S., W. L. Winkenwerder, and L. M. Lichtenstein. 1972. Trials of alum-precipitated pollen extracts in the treatment of hay fever. *J. Allergy Clin. Immunol.* **50**: 31.
21. Lichtenstein, L. M., and A. G. Osler. 1964. Studies on the mechanisms of hypersensitivity phenomena. IX. Histamine release from human leukocytes by ragweed pollen antigen. *J. Exp. Med.* **120**: 507.
22. May, C. D., M. Lyman, R. Alberto, and J. Cheng. 1970. Procedures for immunochemical study of histamine release from leukocytes with small volume of blood. *J. Allergy.* **46**: 12.

23. Shore, P. A., A. Burkhalter, and V. H. Cohn, Jr. 1959. A method for the fluorometric assay of histamine in tissues. *J. Pharmacol. Exp. Ther.* **127**: 182.
24. Lichtenstein, L. M., and A. G. Osler. 1966. Studies on the mechanisms of hypersensitivity phenomena. XII. An in vitro study of the reaction between ragweed pollen antigen, allergic human serum and ragweed-sensitive human leukocytes. *J. Immunol.* **96**: 169.
25. Lichtenstein, L. M., N. A. Holtzman, and L. S. Burnett. 1968. A quantitative in vitro study of the chromatographic distribution and immunoglobulin characteristics of human blocking antibody. *J. Immunol.* **101**: 317.
26. King, T. P., and P. S. Norman. 1962. Isolation studies of allergens from ragweed pollen. *Biochemistry.* **1**: 709.
27. Lichtenstein, L. M., T. P. King, and A. G. Osler. 1966. In vitro assay of allergenic properties of ragweed pollen antigens. *J. Allergy.* **38**: 174.
28. Wide, L. 1969. Radioimmunoassays employing immunosorbents. *Acta Endocrinol. Suppl.* **142**: 207.
29. Porath, J., R. Axen, and S. Ernback. 1967. Chemical coupling of proteins to agarose. *Nature (Lond.).* **215**: 1491.
30. Klinman, N. R., and R. B. Taylor. 1969. General method for the study of cells and serum during the immune response, the response to dinitrophenyl in mice. *Clin. Exp. Immunol.* **4**: 473.
31. Berg, T., and S. G. O. Johansson. 1971. In vitro diagnosis of atopic allergy. II. IgE and reaginic antibodies during and after rush desensitization. *Int. Arch. Allergy.* **41**: 434.
32. Levy, D. A., and A. G. Osler. 1966. Studies on the mechanisms of hypersensitivity phenomena. XIV. Passive sensitization in vitro of human leukocytes to ragweed pollen antigen. *J. Immunol.* **97**: 203.
33. Lichtenstein, L. M., and H. R. Bourne. 1971. Inhibition of allergic histamine release by histamine and other agents which stimulate adenyl cyclase. *In* Second International Symposium on the Biochemistry of Acute Allergic Reactions. E. L. Becker and K. Frank Austen, editors. Blackwell Scientific Productions, London. 161.
34. Levy, D. A., A. G. Osler. 1967. Studies on the mechanisms of hypersensitivity phenomena. XVI. In vitro assays of reaginic activity in human sera: effect of therapeutic immunization on seasonal titer changes. *J. Immunol.* **99**: 1068.
35. Sherman, W. B., A. Stull, and R. A. Cooke. 1940. Serologic changes in hay fever cases treated over a period of years. *J. Allergy.* **11**: 225.
36. Levy, D. A., L. M. Lichtenstein, E. O. Goldstein, and K. Ishizaka. 1971. Immunologic and cellular changes accompanying the therapy of pollen allergy. *J. Clin. Invest.* **50**: 360.
37. Pruzansky, J. J., and R. Patterson. 1967. Histamine release from leukocytes of hypersensitive individuals. II. Reduced sensitivity of leukocytes after injection therapy. *J. Allergy.* **39**: 44.
38. Sadan, N., M. B. Rhyne, D. Mellits, E. O. Goldstein, D. A. Levy, and L. M. Lichtenstein. 1969. Immunotherapy of pollinosis in children: an investigation of the immunological basis of clinical improvement. *N. Engl. J. Med.* **280**: 623.
39. Ishizaka, T., R. DeBernardo, H. Tomioka, L. M. Lichtenstein, and K. Ishizaka. 1972. Identification of basophil granulocytes as a site of allergic histamine release. *J. Immunol.* **108**: 1000.
40. Vaz, N. M., and A. Prouvost-Danon. 1969. Behavior of mouse mast cells during anaphylaxis in vitro. *Prog. Allergy.* **13**: 111.
41. Tada, T., and K. Okumura. 1971. Regulation of homocytotropic antibody formation in the rat. I. Feed-back regulation by passively administered antibody. *J. Immunol.* **106**: 1002.
42. Ishizaka, K., and H. Okudaira. 1972. Reaginic antibody formation in the mouse. I. Antibody-mediated suppression of reaginic antibody formation. *J. Immunol.* **109**: 84.