III. Serum and sputum immunoglobulin E levels in respiratory diseases in adults

C. P. W. Warren,* M.B., F.R.C.P.[C] and K. S. Tse,† M.D., F.R.C.P.[C], *Winnipeg, Man.*

Summary: Using a solid-phase radioimmunoassay, serum IgE level was determined in 46 normal subjects, 53 patients with bronchial asthma, 44 patients with chronic bronchitis and/or emphysema, and 19 patients with restrictive lung disease. Sputum IgE was measured simultaneously in 51 of the subjects. The range of serum IgE concentration in the normal subjects was wide. It varied between 15 and 750 ng/ml with a mean of 135 ng. Asthmatic patients had significantly higher levels of serum IgE with a mean of 579 ng/ml, but only 30% fell outside the normal 95% confidence limits. Patients with chronic bronchitis, emphysema and restrictive lung diseases had normal IgE levels. There was a significant correlation between serum and sputum IgE levels.

Résumé: Par la réaction immunologique avec isotopes marqués, en phase solide, nous avons déterminé l'IgE sérique chez 46 sujets normaux, chez 53 malades souffrant d'asthme bronchique, chez 44 malades souffrant de bronchite chronique ou d'emphysème (ou des deux) et chez 19 malades présentant une pneumopathie restrictive. Chez 51 de ces malades, nous avons mesuré en même temps la concentration d'IgE dans le crachat. La gamme quantitative de la concentration d'IgE sérique chez les sujets normaux était très large: elle variait de 15 à 750 ng/ml, la moyenne étant de 135 ng. Chez les asthmatiques, la moyenne sérique d'IgE était nettement plus élevée, soit 579 ng/ml, mais on ne comptait que 30% des malades qui étaient en dehors des limites de confiance de 95%. Quant aux malades souffrant de bronchite chronique, d'emphysème et de pneumopathies restrictives, les concentrations d'IgE sérique étaient normales. On notait, par ailleurs, une corrélation assez constante entre les concentrations d'IgE du sérum et des expectorations.

From the MRC Group for Allergy Research, Department of Medicine and Immunology, Faculty of Medicine, University of Manitoba and the Clinical Investigation Unit, Health Sciences Centre, Winnipeg

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Reprint requests to: Dr. K. S. Tse, Director of Clinical Immunology, Health Sciences Centre — C3, 700 William Ave., Winnipeg, Man. R3E 0Z3

For many years reaginic antibody has been thought to play an important role in the pathogenesis of atopic diseases. Although much was known about the properties of the reaginic antibody, it was only in 1966 that Ishizaka and Ishizaka[1] demonstrated that it belonged to a new class of immunoglobulins now known as immunoglobulin E (IgE). IgE has since been shown to be the key molecule in the mediation of immediate-type hypersensitivity reactions. In 1967 Johansson and Bennich[2] reported the first case of an IgE-producing myeloma. The availability of large quantities of IgE from this and two other cases of IgE myeloma has enabled the properties of IgE to be studied and its quantities measured in various diseases. IgE is present in only minute quantities in serum and respiratory secretions, so that radioimmunoassay techniques are usually needed for its measurement. The purpose of this study is to describe the use of a solid-phase radioimmunoassay technique for the measurement of IgE, to report the serum and sputum levels of IgE in normal subjects and in patients with various respiratory diseases, and to examine the relationship between IgE levels in the serum and sputum in these individuals.

Materials and methods

Subjects

Serum samples were collected from each of 46 normal subjects, 53 patients with bronchial asthma,[3] 44 patients with chronic bronchitis[4] and/or emphysema,[5] and 19 patients with restrictive lung diseases (seven with extrinsic allergic alveolitis caused by exposure to avian antigens, five with sarcoidosis, two with carcinomatosis, five with intrinsic fibrosing alveolitis). These subjects all lived in Manitoba, were aged from 17 to 77 years and were of Caucasian, African or Asian origins. Sputum was collected from 46 patients and from five normal subjects; in the latter, sputum was induced by the inhalation of nebulized, heated hypertonic saline. The sputum specimens were homogenized with equal volumes of phosphate buffered saline (PBS) and the mucus was removed by centrifugation. Both the serum and sputum specimens were stored at −20°C until assayed. The atopic status of these subjects was determined by allergy skin tests with common inhalant allergens by the intracutaneous method.

Protein determinations

Total protein concentration in the sputum was determined by the Lowry method[6] with human serum albumin as the standard.

IgE measurements

Measurement of IgE was performed by a slight modification of the solid-phase radioimmunoassay technique described by Smith, Ozkaragoz and Gokcen.[7] Disposable flexible polyvinyl microtitration plates were used.* Each plate contains 96 wells, each of 0.2 ml volume.

Anti-IgE was prepared by immunization of a sheep with a chromatographically purified fraction of myeloma IgE.** The antiserum was further purified by absorption with human cord plasma and proved to be monospecific to myeloma IgE on immunoelectrophoresis and immunodiffusion. In each well was placed 0.1 ml of anti-IgE at a concentration of 1 mg/ml in 0.15 molar borate buffer pH 9.4. The wells were sealed with a plastic cover to prevent evaporation and left for 72 hours at 4°C. The anti-IgE became physically adsorbed to the plastic. The plates were then washed three times with PBS and the wells subsequently filled with 0.2 ml of 1% human serum albumin (HSA). After incubation for six hours during which any bare plastic became coated with HSA, the plate was again washed three times with PBS. The plate was then ready to be used immediately or could be stored at 4°C and used at the end of several weeks.

The serum or sputum samples were diluted 1 in 10 in PBS or 1 in 100 in 1% HSA. Serial dilutions of known standard concentrations of IgE† were also prepared. Duplicate aliquots of 0.1 ml of the unknown sample or the known standards were pipetted into each well and incubated at room temperature for six hours. In at least three wells, only PBS was introduced and in these the level of IgE was zero.

*Cooke Laboratory Products, Alexandria, Virginia 22314.

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†Pharmacia Ltd., Montreal, Que.

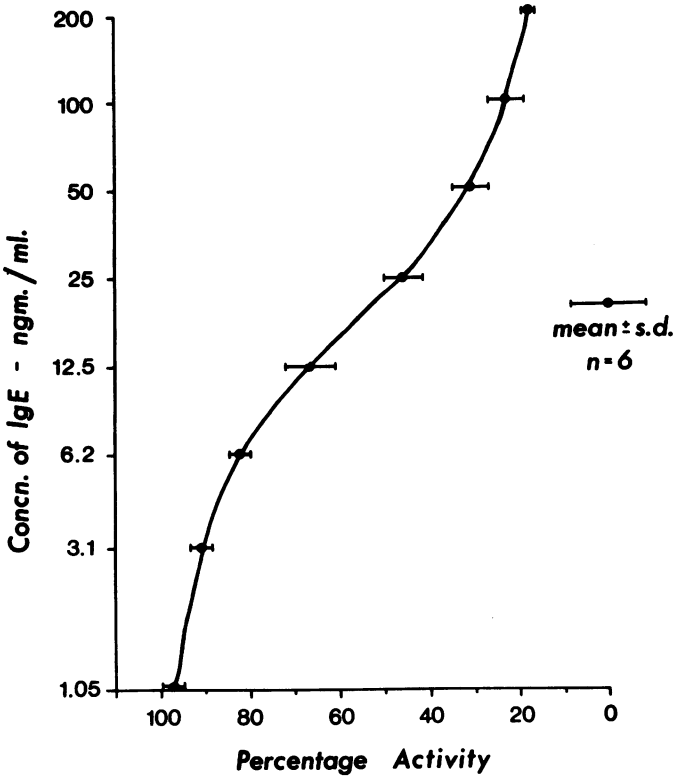

FIG. 1—Standard curve for radioimmunoassay of IgE, which gives the concentration of IgE in ng/ml on a $\log_{10}$ scale on the vertical axis and the percentage activity of bound ^{125}I-IgE on the horizontal axis. The mean and standard deviation of the percentage activity for each of six measurements of known concentration of IgE are also shown.

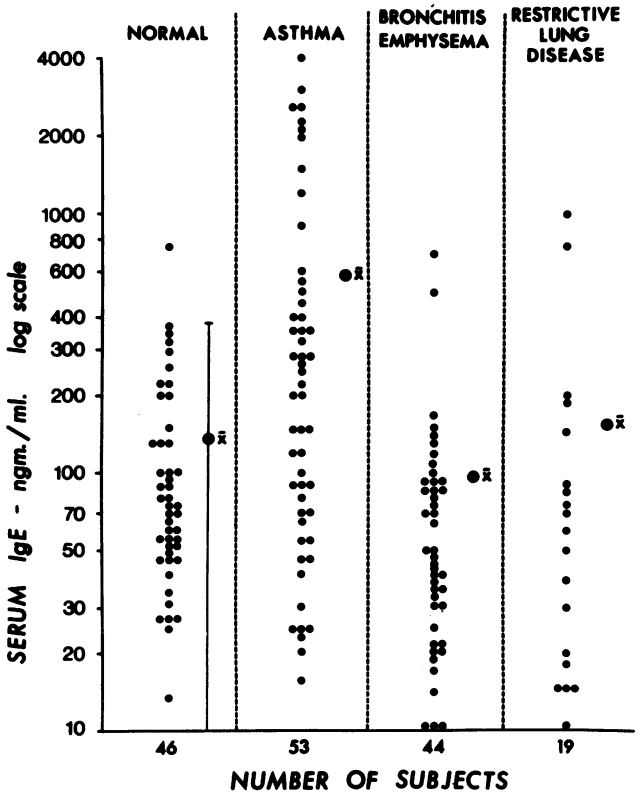

FIG. 2—Serum IgE levels in 162 subjects divided into diagnostic groups. The IgE concentrations in ng/ml on a $\log_{10}$ scale is given on the vertical axis. The small dots indicate the values in individual subjects; the large dot designated $\bar{x}$ indicates the mean for the group. The line and bars show the 95% confidence limits for the normal group.

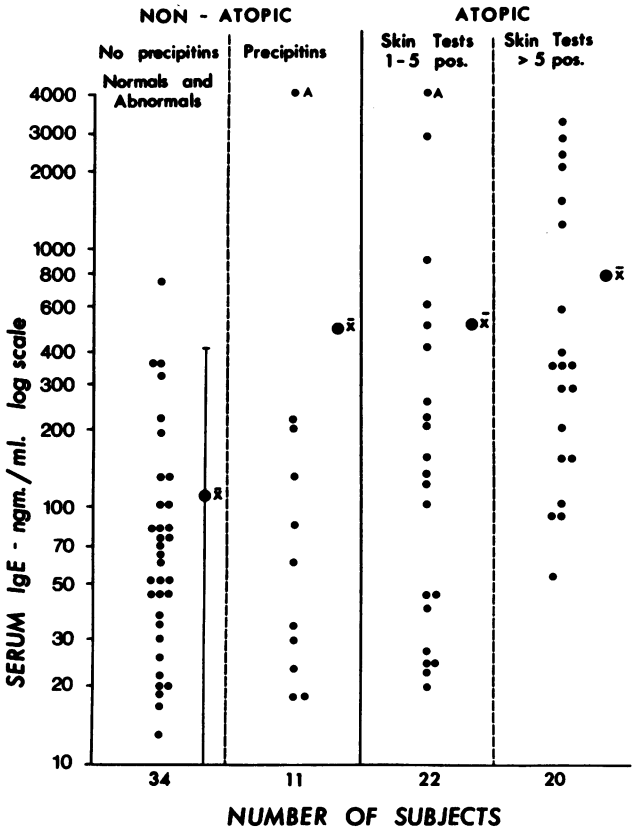

FIG. 3—Serum IgE levels in 87 subjects grouped according to their immunological status as determined by skin testing with common allergens. The same key and scale are used as in Fig. 2. Individual A has bronchopulmonary aspergillosis.

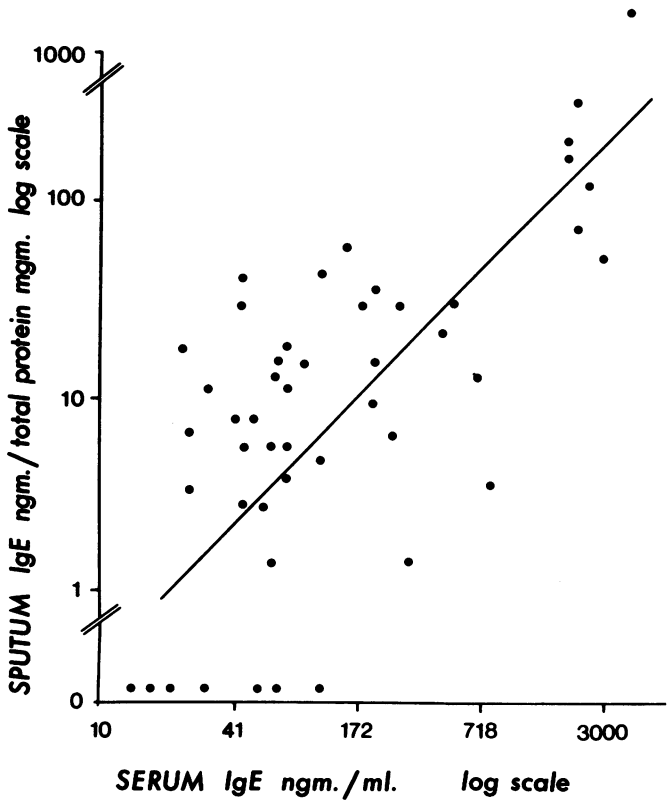

FIG. 4—The correlation of serum IgE and sputum IgE/protein ratio. Both scales to $\log_e$. γ=0.686, P=0.001, N=51.

Iodine-125-labelled IgE† was diluted to obtain a 20 ng/ml solution; 0.05 ml (1 ng) was added to each well and incubated for 24 hours. The cold and radiolabelled IgE compete for binding with the anti-IgE so that the quantity of the latter bound is dependent on the concentration of the former. After the incubation period the plates were washed three times with PBS to remove the excess IgE. The wells were cut out individually and placed in test tubes. The radioactivity of each well was then determined by using a gamma counter.

The wells which had not been filled with any cold IgE (zero wells) were considered to have counts equal to 100% activity. The counts obtained from the wells containing known concentrations of IgE, in range of 1 to 200 ng/ml, were divided by the count of the zero wells so as to give the percent activity. A standard curve obtained from this method is shown in Fig. 1. The concentrations of IgE in ng/ml is shown by a log scale. The line connects the means of six estimates of the percent activity for each concentration of IgE and the standard deviation is shown by the bars. The most discerning range is from 6 to 100 ng/ml. The standard curve was reproducible within batches of plates, but not between batches. Therefore, a standard curve should be prepared for each new batch of assays.

Statistical analysis

Because of the skewed distribution, the serum IgE levels were transformed to natural logarithms and the mean of each group was compared with that of the normal using the Wilcoxon signed-rank test. The serum and sputum IgE levels were transformed to natural logarithms before estimation of the regression line and correlation coefficient. Sputum in which IgE levels were undetectable was assigned a concentration of 0.1 ng/mg protein for statistical analysis.

Results

Serum IgE levels in the four groups of subjects are shown in Fig. 2. In the normal group the range was from 15 to 750 ng/ml with a mean of 135 ng and an upper 95% confidence limit of 382 ng/ml. The asthmatic group had a range of 18 to 4200 ng/ml with a mean of 579 ng, which was significantly different from the normal ($P<0.01$). Thirty percent of the asthmatic subjects' IgE levels were above the 95% confidence limits of the normal group. The means of the other two diagnostic groups did not differ from those of the normal group, and their ranges were similar.

Allergy skin tests were done on 87 subjects and these were divided into two groups. Those with positive skin tests were considered atopic and the others non-atopic. The non-atopic group was divided into those with precipitating-antibody-mediated lung disease (seven with hypersensitivity pneumonitis, four with asthma) and those without. The latter subgroup included normal subjects and patients with lung disease. The atopic group was divided into those with one to five positive skin tests and those with six or more. The serum IgE levels in these four subgroups are shown in Fig. 3. The two atopic subgroups did not have significantly different mean IgE levels. Both, however, differed significantly in their means from the normal non-atopic group ($P<0.01$). The subject designated A had allergic bronchopulmonary aspergillosis and so was included both in the subgroup with precipitin-mediated lung disease and in the atopic group. If the value from this patient is ignored, the mean IgE level of the precipitin-mediated subgroup is not significantly different from that of the other non-atopic subgroup.

The sputum IgE level was estimated in 51 subjects. In seven the IgE level was not detectable. Since the protein concentration of the sputum may vary considerably between different specimens, the IgE concentration in the sputum is expressed as ng of IgE per mg of total protein in the sputum extract. In Fig. 4 the relationship between the serum IgE and sputum IgE levels is shown. There was a significant correlation between the two.

Discussion

Several different radioimmunoassay procedures are available for the quantitation of IgE. The Phadebas kit† uses coupling of the anti-IgE to Sephadex particles which have been activated by cyanogen bromide.[8] Catt and Tregear[9] first utilized the adsorption of antibodies to polystyrene tubes as a method of solid-phase radioimmunoassay. Although rigid polystyrene was the usual plastic used, we have found that flexible polyvinyl plastics were equally effective. This method required only 1 ng of ^{125}I-labelled IgE for each estimate. Since the latter is expensive it meant the test becomes relatively more economical. The anti-IgE was adsorbed to the wells in the plastic plate. This simplified washing since there was no need for centrifugation between washings, which is necessary when the antibody is bound to particles as in fluid-phase assay. Although adsorption of antibodies to the plastic should occur within a few hours, we have obtained better reproducibility in our results by increasing the period for adsorption to

72 hours and the pH of the buffer to 9.4. The solid-phase radioimmunoassay did not prove to be as accurate as in the hands of Smith, Ozkaragoz and Gokcen,[7] judged by the range of counts on their standard inhibition curve. It is not as sensitive as the double-antibody radioimmunoassay method of Yunginger and Gleich[10] in which values as low as 0.1 ng/ml could be detected. Nevertheless, the assay was easy to perform and was reasonable and economical in terms of cost and time.

The wide range of serum IgE in normal individuals has been observed previously by many authors using several different techniques.[7,11-13] Although the mean serum IgE level for the asthmatic group was significantly higher than the normal, only 30% of the asthmatics fell outside the 95% confidence limits. That the wide range of IgE concentrations found in normal subjects is not related to a latent atopic status is indicated by a similarly wide range found in subjects who were skin-test negative. The normal serum IgE levels in patients with precipitin-mediated lung disease has been reported by Patterson *et al.*[14] At present it would seem that the estimation of serum IgE in adult asthmatic subjects is less informative than a history and skin testing, since the latter provide information on the allergen specificity of the IgE-mediated hypersensitivity. The recent report of high serum IgE levels in allergic bronchopulmonary aspergillosis[14] was confirmed in the one subject with this disease included in our study.

The serum IgE levels were normal in patients with chronic bronchitis and/or emphysema, indicating that immediate hypersensitivity probably plays no significant role in their pathogenesis. Serum IgG, IgA and IgM levels in these diseases are usually normal or elevated,[15,16] while immunoglobulin levels in the sputum are no different from those of control subjects.[17] Serum IgE level in patients with cystic fibrosis was found to be increased.[12] Whether this is related to infection or hypersensitivity to *Aspergillus fumigatus* is unknown.[18] The mean serum IgE level was also normal for the group of patients with restrictive lung diseases, including seven patients with precipitin-mediated hypersensitivity pneumonitis. This is not unexpected since immediate hypersensitivity is thought to be of no, or limited, importance in their pathogenesis.

The correlation between serum and sputum IgE levels in our study was similar to that observed by Yunginger and Gleich[10] with respect to the IgE levels in the serum and nasal washings in normal subjects and patients with hay fever.

Other investigators have also demonstrated the presence of IgE[19] and IgE antibodies[20,21] in respiratory secretions. It is not clear whether or not there is active secretion of IgE into the respiratory secretions, as in the case of secretory IgA. IgE-secreting plasma cells have been shown to be present in the submucosa of the respiratory tract.[22] By comparing the IgE/IgG ratio in the sputum and serum Ishizaka and Newcomb[19] indicated that there might be an active secretion of IgE into the respiratory secretions. However, since IgE appears to function only through its fixation to target cells such as mast cells, it would seem likely that the IgE present in the serum and respiratory secretions merely represents the excess of this immunoglobulin that is not attached to the target cells.

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