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SERUM IMMUNOGLOBULIN E LEVELS IN PATIENTS WITH NEOPLASTIC DISEASE

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The serum immunoglobulin E (IgE) concentrations of groups of patients with neoplastic disease were determined with a double antibody radioimmunoassay technique. The IgE levels were normal in patients with most of the types of neoplastic disease studied. However, the serum levels of IgE were reduced in patients with neoplasms affecting the B cell (thymic independent) system of lymphocytes and plasma cells. Thus, the geometric mean serum IgE concentrations of patients with chronic lymphocyte leukemia (18 ng/ml), multiple myeloma with an IgG paraprotein (27 ng/ml), and multiple myeloma with an IgA paraprotein (36 ng/ml) were significantly reduced (p < 0.05) below the geometric mean of normal controls (96 ng/ml). The reduction in IgE levels is a feature of a more generalized pattern observed in these patients of reduced polyclonal immunoglobulin synthesis and reduced numbers of B lymphocytes bearing normal polyclonal surface immunoglobulins. This reduction in the number of normal B lymphocytes and in the concentration of polyclonal immunoglobulins may be due to the production, by the malignant plasma cells and lymphocytes, of humoral regulators of B cell proliferation and immunoglobulin synthesis.

In contrast to these findings in patients with malignancies of the B cell system there was a 9-fold elevation of the geometric mean serum IgE concentration to 945 ng/ml in patients with Hodgkin's disease, a disease associated with abnormalities of cellular immunity and disorders of T cell function. It is suggested that a reduction of thymic dependent, T cell, regulators of IgE synthesis may occur in such patients and that this reduction in T cell regulatory function may be the cause of the high IgE levels seen in patients with Hodgkin's disease.

Studies by Ishizaka and Ishizaka (1, 2) on the nature of antibodies associated with reaginic activity led to the definition of a new immunoglobulin, IgE. Antigenic analysis of this protein, as well as that of subsequently discovered myeloma proteins (3, 4) established IgE as an entirely distinct immunoglobulin class. The studies of IgE levels in disease states have provided insights into the role of IgE in the mediation of disease processes, into the factors controlling IgE biosynthesis, and into the nature of the immunologic defects in patients with immunodeficiency diseases. Markedly reduced IgE levels due to decreased IgE synthesis have been demonstrated in patients with congenital hypogammaglobulinemia (5, 6) or with primary acquired hypogammaglobulinemia (7, 8), that is in patients with a generalized defect in immunoglobulin synthesis. In addition, reduced IgE levels have been reported in patients with defects limited to IgE and IgA synthesis (9). The majority of patients with the immunodeficiency disease, ataxia telangiectasia, also have decreased IgA and IgE synthesis (9, 10). Finally, an isolated IgE deficiency has been demonstrated in some individuals (5, 9, 11). In contrast, markedly elevated serum levels of IgE have been demonstrated in patients with parasitic diseases (12–14), or with such disorders as extrinsic asthma (15), eczema (16), and the Wiskott-Aldrich syndrome (8, 17).

The purpose of the present study was to determine the serum IgE concentration in patients with neoplastic disease. In these investi-
gations we utilized a sensitive double antibody radioimmunoassay technique to determine the serum IgE concentration of groups of patients with different types of neoplastic disease. IgE deficiency was demonstrated in patients with chronic lymphocytic leukemia and multiple myeloma, that is in patients with malignancies of the B cell (thymic independent) system of lymphocytes and plasma cells. In contrast, elevated IgE levels were demonstrated in patients with Hodgkin’s disease, a disease associated with abnormalities of cellular immunity and disorders of T cell function.

MATERIALS AND METHODS

The IgE concentration was determined on the serum of 331 patients with neoplastic disease including 32 patients with chronic lymphocytic leukemia, 16 patients with multiple myeloma and an IgG paraprotein, 17 patients with multiple myeloma and an IgA paraprotein, 56 patients with macroglobulinemia of Waldenström, and 19 patients with Hodgkin’s disease. The group of patients with Hodgkin’s disease included predominantly young individuals with advanced disease. Fifteen of the 19 patients were stage III or IV or had systemic symptoms. In addition, the IgE level was determined on the serum of 10 patients with malignant lymphoma of lymphocytic type, 10 patients with acute myelocytic leukemia, 10 patients with acute lymphocytic leukemia, 10 patients with chronic myelocytic leukemia, 11 patients with American Burkitt’s lymphoma, 25 patients with breast cancer, 41 patients with lung cancer, and 25 patients with carcinoma of the gastrointestinal tract including six with gastric cancer, and 19 with colonic cancer. In addition, IgE levels were determined on the serum of eight patients with thymoma and hypogammaglobulinemia and in 41 patients with thymoma without reduction in other immunoglobulin classes. The sera of these patients were obtained at the time of first admission to the hospital before surgical resection of the lesion and before radiotherapy or chemotherapy was initiated. The sera were stored at −20°C until analyzed.

Quantitation of serum immunoglobulins with the exception of IgE. The concentrations of immunoglobulin IgG, IgA, and IgM were determined by single radial diffusion in agar (18, 19).

Quantitation of serum IgE concentrations. The serum IgE determinations were performed by a double antibody radioimmunoassay procedure as previously described (9). A purified preparation of the IgE myeloma protein, ND, was kindly provided by Drs. S. Johannson, and H. Bennich. Purified IgE myeloma protein, PS, was obtained from the plasma of the patient by elution from a DEAE cellulose column at 0.025 M Tris-HCl, pH 8.0, and further purified by gel filtration on Sephadex G-200 columns.

Antiserum. An antiserum to IgE (PS) was produced in rabbits. Copolymers that contain bovine serum albumin, fetal bovine serum, IgG, IgA, IgM, PS light chains, and agammaglobulinemic plasma, prepared by ethyl chloroformate insolubilization (20) were used to render the antiserum specific for IgE. Specificity of the antiserum was confirmed by Ouchterlony double diffusion tests.

Radioiodination of IgE (ND). 125Iodine-labeled IgE (ND) was prepared by a chloramine-T method (21). Two millicuries of 125I in the presence of 25 μg of chloramine-T were added to 2 μg of purified IgE (ND). After 30 to 45 sec the reaction was terminated by the addition of 62 μg of sodium metabisulfite and the radioiodinated protein was separated from the inorganic 125I by gel filtration on sephadex G-75. The 125I IgE (ND) thus obtained was greater than 96% trichloroacetic acid precipitable and the specific activity was 50 to 75 μCi/μg.

Radioimmunoassay of serum IgE. Serum IgE was measured by the double antibody method with specific rabbit anti-IgE (PS) and 125I-labeled IgE (ND) according to the method of Gleich et al. (11). Standard inhibition curves were obtained by the addition of known amounts of purified unlabeled IgE (ND) or IgE (PS). A standard curve was constructed for each assay by plotting the logit of the percent specific counts bound vs log10 ng of IgE added to the reaction mixtures. These curves were linear from 0.4 to 28 ng of IgE in the reaction mixture. The IgE concentration of unknown serum samples was calculated from the coordinates of the least squares regression equation of the standard inhibition curve. The average standard deviation of duplicate determinations performed on different days was 2.6%.

Assay of serum IgE by single radial diffusion in agar. Serums with an IgE level greater than 1000 ng/ml as determined by the radioimmunoassay procedure were also quantitated by single radial diffusion in agar. The results with
this method agreed well with those of the double antibody radioimmunoassay procedure in contrast to the findings of Jacobs and co-workers (22) who found that many patients with untreated cancer had a low or undetectable serum IgE concentration as determined by radial immunodiffusion, but a very high level usually more than 100,000 units/ml as estimated with a solid phase radioimmunoassay procedure.

Statistical methods. Serum immunoglobulin concentrations are not distributed in a normal manner. However, the logarithm to the base 10 of the immunoglobulin concentration is distributed more normally. For this reason the geometric mean rather than arithmetic mean was used to estimate the median for IgE for the different populations. The log~10 of the immunoglobulin concentration was used in all statistical tests. Statistical analyses were performed by Student’s t-test.

RESULTS

The range of serum IgE concentrations of 74 normal adults was 6 to 5000 ng/ml with a geometric mean of 96 ng/ml and a 90% interval of 15 to 592 ng/ml. Results of estimates of the serum IgE concentrations in patients with different forms of neoplastic disease are shown in Figures 1 and 2. In general, the serum levels of IgE were reduced in patients with neoplasms affecting the immunoglobulin-bearing B lymphocytes, or plasma cells (Fig. 1, Table I). Thus, the IgE levels of 32 patients with chronic lymphocytic leukemia were extremely reduced with a geometric mean serum IgE concentration of 18 ng/ml, a value that differed significantly from that of the normal population (p < 0.001). In 63% of the patients with chronic lymphocytic leukemia, IgE was either undetectable in the serum (i.e. < 4 ng/ml) or was below 15 ng/ml, that is below the 10th percentile for normal adults. In general, the reduced serum IgE levels in patients with chronic lymphocytic leukemia paralleled reductions in the other immunoglobulin classes in the patients with a normal IgE level. It should be noted, however, that although the mean levels of all classes of immunoglobulin in the serum were reduced in patients with chronic lymphocytic leukemia the level of IgE, when expressed as a percentage of the normal mean (20% of normal), was more depressed than that of any of the other immunoglobulin classes (Table I).

The mean IgE concentration was also significantly lower than normal (p < 0.05) in multiple myeloma associated with an IgG or IgA paraprotein. The 16 patients with multiple myeloma and an IgG paraprotein had a geometric mean serum IgE concentration of 27 ng/ml and the 17 patients with multiple myeloma and an IgA paraprotein had a geometric mean IgE level of 36 ng/ml. The 56 patients with macroglobulinemia of Waldenström studied had a geometric mean IgE level of 67 ng/ml. Although this value is lower than the normal mean the difference was not significant (p > 0.1).

The IgE levels were depressed in another subgroup of patients with neoplasia, that is in patients with thymoma and hypogammaglobulinemia. No IgE was detectable in the serum of five of the six patients studied who had a thymoma, reduction of all classes of immunoglobulin molecules, and absence of eosinophils. In the remaining patient of this group the IgE level was 8 ng/ml. Similarly no IgE was detectable in the serum of the two patients studied with thymoma and markedly reduced serum concentrations of IgG and IgA but elevated serum concentrations of IgM.

In contrast to the groups of patients with neoplasia and the disorders discussed above, patients with advanced Hodgkin’s disease had elevated IgE levels. The geometric mean serum IgE concentration of 19 patients with Hodgkin’s disease studied was 945 ng/ml, nine times the control value (p < 0.001).

The geometric mean serum IgE concentration was slightly, but significantly, elevated above normal in the patients with lung cancer (p < 0.05) but did not differ significantly from normal in groups of patients with other forms of neoplasia including malignant lymphoma of lymphocytic type, American Burkitt’s lymphoma, thymoma without hypogammaglobulinemia, acute and chronic myelocytic and lymphocytic leukemia, as well as cancer of the breast or gastrointestinal tract (Fig. 2).
Figure 1. The serum IgE concentration of patients with chronic lymphocytic leukemia, thymoma and hypogammaglobulinemia, multiple myeloma, macroglobulinemia of Waldenström, and Hodgkin’s disease. The geometric mean and the 90% interval for the serum IgE concentration of 73 normal adults are indicated by the central line and cross-hatched area, respectively.

Figure 2. The serum IgE concentrations of patients with different malignancies.
TABLE I

Immunoglobulin levels of patients with various forms of malignancy

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>IgE</th>
<th>IgG</th>
<th>IgA</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (74)</td>
<td>96 (24–386)</td>
<td>11.8 (9.5–14.6)</td>
<td>2.0 (1.16–3.4)</td>
<td>1.3 (0.87–2.0)</td>
</tr>
<tr>
<td>Chronic lymphocytic leukemia (32)</td>
<td>18.4 (3.5–96)</td>
<td>7.2 (4.7–11.2)</td>
<td>0.97 (0.35–2.7)</td>
<td>0.44 (0.10–2.0)</td>
</tr>
<tr>
<td>Thymoma and hypogammaglobulinemia (8)</td>
<td>4.4 (3.4–6.0)</td>
<td>3.4 (1.7–7.1)</td>
<td>0.37 (0.08–1.7)</td>
<td>0.16 (0.03–0.8)</td>
</tr>
<tr>
<td>Multiple myeloma with IgG paraprotein (16)</td>
<td>27.4 (11–71)</td>
<td>62 (36.6–105)</td>
<td>1.0 (0.017–0.59)</td>
<td>0.14 (0.07–0.29)</td>
</tr>
<tr>
<td>Multiple myeloma with IgA paraprotein (17)</td>
<td>35.9 (7.5–172)</td>
<td>4.0 (2.5–6.7)</td>
<td>36.9 (18–75)</td>
<td>0.09 (0.013–0.57)</td>
</tr>
<tr>
<td>Macroglobulinemia of Waldenström (56)</td>
<td>67.3 (15–305)</td>
<td>5.0 (2.8–8.9)</td>
<td>0.22 (0.03–1.5)</td>
<td>26.3 (8.7–79)</td>
</tr>
<tr>
<td>Hodgkin’s disease (19)</td>
<td>945 (162–5512)</td>
<td>12.5 (9.4–16.6)</td>
<td>2.7 (1.16–4.35)</td>
<td>1.4 (0.63–3.0)</td>
</tr>
</tbody>
</table>

a Number of patients in group in parentheses.
b Data presented as geometric mean with 67% interval in parentheses.
c Significantly different from normal p < 0.05.
d IgM for only the six patients with reduction in all classes of immunoglobulins are included.

DISCUSSION

In the present study patients with thymoma and hypogammaglobulinemia, chronic lymphocytic leukemia, or multiple myeloma had significantly reduced serum IgE concentrations. Chronic lymphocytic leukemia and multiple myeloma represent neoplasms of the system of cells involved in antibody production, the humoral component of the immune response. Multiple myeloma represents a neoplasm of the cells involved in immunoglobulin synthesis whereas chronic lymphocytic leukemia is a neoplasm of the class of lymphocytes (B cells) that is bone marrow derived. Chronic lymphocytic leukemia cells from the majority of patients studied have been shown to be B cells in that they have easily demonstrated membrane-bound monoclonal immunoglobulins as well as receptors for the third component of complement and for IgG aggregates on their surface (23–26). The finding of reduced IgE levels in patients with chronic lymphocytic leukemia or multiple myeloma is consistent with previous observations that the serum levels of the normal polyclonal immunoglobulins are significantly reduced in patients with these neoplasms affecting the B cell system of lymphocytes (27–31) and plasma cells (28, 30–32). In addition, patients who have chronic lymphocytic leukemia or multiple myeloma (33) have been shown to have a significant reduction in the percentage of their circulating lymphocytes that have normal surface immunoglobulins. However, an individually specific or idiotypic monoclonal immunoglobulin marker was shown to be present on a relatively high percentage of the peripheral lymphocytes from the patients with multiple myeloma (33) and a monoclonal immunoglobulin was shown to be present on the surface of the lymphocytes of patients with chronic lymphocytic leukemia (23, 24). Such patients with multiple myeloma or chronic lymphocytic leukemia have been shown to have a reduced ability to synthesize antibody in response to antigenic challenge and to have an increased incidence of infection with highly pathogenic encapsulated bacteria (28, 30, 34). In addition, utilizing an in vitro culture system, we have shown that the peripheral blood lymphocytes of patients with chronic lymphocytic leukemia or multiple myeloma have a significantly reduced rate of synthesis of polyclonal immunoglobulin after stimulation with pokeweed mitogen when compared to the rate of immunoglobulin synthesis by lymphocytes from normal individuals.

The pathophysiology of the reduced concentration of these normal polyclonal immunoglobulins in patients with multiple myeloma or chronic lymphocytic leukemia has been studied with radioiodinated immunoglobulins in metabolic turnover studies. In patients with multiple myeloma the reduced concentrations of polyclonal IgM, IgA, and IgG were shown to be
primarily due to decreased synthesis of these proteins (35–38). An expanded plasma volume, and an increased fractional catabolic rate for IgG were contributing factors in the reduction of polyclonal IgG in patients with IgG para-proteins (35–37). The reduced IgG concentration in patients with chronic lymphocytic leukemia was shown to be due to a reduction in the rate of IgG synthesis in the majority of patients (35, 39, 40). In metabolic turnover studies with radioiodinated IgE the patients with chronic lymphocytic leukemia or multiple myeloma in the present study were shown to have an extreme reduction in the rate of IgE synthesis. This reduced synthetic rate was the primary metabolic disorder resulting in the low serum IgE concentration observed in these patients.

A number of mechanisms may be considered as potential factors leading to the reduced number of peripheral blood lymphocytes bearing polyclonal surface immunoglobulins, the reduced serum concentration of polyclonal immunoglobulin molecules, and the reduced serum concentration of IgE noted in patients with multiple myeloma or chronic lymphocytic leukemia. Early views that a reduction in cells producing immunoglobulin molecules may be due to replacement of normal immunocompetent cells by malignant cells or be due to deviation of metabolites from normal lymphocytes or plasma cells would not appear to be valid since the reduction in immunoglobulin synthesis is restricted to patients with malignancy of the B cell system and is not seen in patients with other forms of cancer, including patients with chronic and acute myelocytic and acute lymphocytic leukemia. Furthermore, a reduction in immunoglobulin levels and antibody production is also present in rodents with localized plasmacytoma (41, 42). An alternative explanation for the reduction in immunoglobulin levels and synthesis in patients with myeloma is suggested by the work of Heller, Yakulis and co-workers (43–45). These authors showed that there was an alteration of surface immunoglobulin determinants of circulating lymphocytes of BALB/c mice in the presence of a plasmacytoma producing a monoclonal immunoglobulin. After the implantation of such a plasmacytoma they found that the proportion of lymphocytes bearing normal polyclonal immunoglobulin determinants on their cell surface decreased and lymphocytes with surface determinants characteristic of the plasmacytoma immunoglobulin appeared and increased with progression of the disease (43). In further studies these changes in the character of surface immunoglobulins of normal lymphocytes were reproduced in vitro by incubating normal lymphocytes with an RNA preparation obtained by hot phenol extraction from the plasmacytomas (44, 45). The authors suggest the possibility that RNA from the plasmacytomas may cause a functional impairment of the immune system by reprogramming the protein synthesis of normal lymphocytes so that they produce the monoclonal immunoglobulin determinant characteristic of the plasmacytoma and incorporate it into their cell surface membrane.

Another possible explanation for the reduction of IgE and other polyclonal immunoglobulins by patients with multiple myeloma, or chronic lymphocytic leukemia is the production of a tissue-specific humoral mitotic inhibitor or chalone which acts as a feedback inhibitor regulating the growth of clones of cells destined to produce normal immunoglobulins. Salmon (46) has proposed that myeloma cells may produce such a B cell chalone which would inhibit the production of normal immunoglobulin-bearing and producing cells by the marrow. Experimental evidence has been presented for such a lymphocyte chalone by a number of laboratories (47–49). In addition, the work of Zolla (42) provides support for such a chalone concept. She found that in the mouse subcutaneous implantation of a plasmacytoma causes a depressive effect on normal splenic immune cell proliferation, apparently by a humoral mechanism. It is clear that further work is necessary in order to define the relative contributions of RNA from malignant cells redirecting immunoglobulin biosynthesis and of B cell chalones regulating B cell proliferation to the decreased immunoglobulin synthesis observed with patients who have B cell malignancies. However, the identification and characterization of such humoral factors may be of major importance in the understanding of control mechanisms that regulate B cell proliferation and immunoglobulin synthesis.

In contrast to the group of patients with neoplasms of the B cell system the patients with Hodgkin’s disease in the present study had elevated IgE levels. The geometric mean serum IgE concentration of the patients with advanced
Hodgkin's disease studied was 9 times that of the normal control group. None of the patients with Hodgkin's disease had such known causes of elevated IgE levels as detectable parasitism, eczema, or the Wiskott-Aldrich syndrome. However, six of the patients did have asthma, hay fever, or hives. The IgE levels of these six patients were very high with a geometric mean of 2900 ng/ml. The geometric mean IgE level of the remaining 14 patients who did not have a previously known cause for a high IgE level was also significantly elevated to 755 ng/ml, over 7 times normal (p < 0.001). In preliminary turnover studies with radioiodinated IgE these elevated IgE levels were shown to be due to elevated rates of IgE synthesis. The patients with Hodgkin's disease in the present and previous studies (31) did not have major alterations in the levels of other classes of immunoglobulins. However, they have a progressive deficit of cell-mediated immunity with reduced ability to reject skin grafts and reduced ability to develop delayed allergic reactions after appropriate stimulation (34, 50–53). A number of pathophysiologic mechanisms are possible for the high serum IgE levels in patients with Hodgkin’s disease. One attractive hypothesis that associates the high IgE level with the disorder of cell-mediated immunity and T cell function is suggested by the work of Okumura and Tada and co-workers (54–56). These authors conclude that T cells are important regulators of IgE antibody production. They found that there is an augmentation and prolongation of the reaginic (IgE type) antibody response to immunization in rats after x-irradiation, thymectomy, splenectomy, or treatment with anti-lymphocyte serum. The authors suggest that these procedures result in the depletion of thymic-dependent regulatory cells that function to terminate ongoing IgE antibody responses and thus limit the synthesis of IgE type antibody. Furthermore, they have demonstrated that the passive transfer of thymocytes from appropriately immunized donors terminates the ongoing production of specific reaginic antibody by the recipients, thus providing direct evidence for the importance of regulatory T cells in controlling the production of antibodies of the IgE class (54). Although the inhibitory effect demonstrated in these studies was specific and was due to carrier-specific T cells it is quite possible that a general reduction in the number of regulatory T cells of all specificities would have the broader effect of reducing the ability to terminate IgE antibody response to most antigens. Thus a reduction in T cell regulatory function would be expected to lead to a persistent production of IgE antibodies to a variety of antigens that would in turn cause an elevation of the serum concentration of IgE. It should be noted that in addition to the carrier-specific T cell regulatory effect observed by Okumura and Tada, that evidence has been presented from a number of laboratories that indicates that a population of activated T cells can exert a nonspecific inhibitory effect on antibody production (57, 58) and polyclonal immunoglobulin synthesis (T. Waldmann, Broder, S., Suer, M., Blackman, M., unpublished observations). In light of these studies it is possible that the high IgE levels of patients with Hodgkin’s disease are related to the anergy and abnormalities of T cell function present in these patients. These disorders in T cell function may be associated with a reduction in the number or function of regulatory T cells that are normally involved in terminating IgE responses. This concept of elevated IgE levels secondary to a reduction in T cell regulatory function is supported by the observation of elevated serum IgE levels in patients with the Wiskott-Aldrich syndrome (8, 17), some patients with the DiGeorge syndrome (6, 59), and certain patients with recurrent infections and impaired cellular immunity (60). In each of these disorders the elevated IgE levels are associated with defects in T cell function in patients who have the capacity to synthesize immunoglobulin molecules including IgE.

REFERENCES
