Enzyme-linked immunosorbent assay for measuring serum IgE

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Summary. Serum IgE was measured by a sandwich method using polystyrene beads coated with anti-IgE γ-globulin and peroxidase labelled anti-IgE γ-globulin. The method was simple, and as sensitive as the radioimmunoassay.

Enzyme-linked immunosorbent assays (ELISA) are being applied widely in the medical field. We have developed an ELISA method for measuring the serum IgE level as described below.

Serum obtained from a patient with IgE myeloma (PS) was kindly supplied by Dr K. Ishizaka. IgE was purified from the serum using DEAE cellulose column chromatography followed by gel filtration. After elution of IgG with 0.005 M phosphate buffer, pH 8.0, from a DEAE cellulose column, E myeloma protein was eluted with 0.025 M phosphate buffer, pH 8.0. The solution was applied to a Sephadex G-200 column and eluted with borate buffered saline, pH 8.0. E myeloma protein was digested with papain to obtain an Fc fragment. The Fc fragment gave a single precipitin band with anti-IgE supplied by Ishizaka. A rabbit was immunized every 2 weeks with 1 mg of the IgE-Fc fragment in complete Freund's adjuvant and was bled 7 days after being immunized 5 times. This rabbit antiserum was absorbed on a Sepharose 4B column coupled with normal IgG, and showed a single precipitin line with the myeloma protein in immunoelectrophoresis. Rabbit γ-globulin fraction was obtained from this antiserum by the duplicate sodium sulfate precipitation method, first at 18% and then at 12%. Polystyrene beads used in an ELISA for insulin[2] in our laboratory were sensitized with 10 µg/ml of the previously obtained anti-IgE class-specific γ-globulin in 0.076 M, pH 6.4 phosphate buffered saline for 1 h at 37 °C by the method of Voller[3]. Horseradish peroxidase was coupled with the same anti-IgE γ-globulin as a second antibody by the method of Nakane[4]. The coupling ratio of globulin/enzyme in the conjugate used was about 1:2 in moles.

A volume of 0.025 ml of the serum sample being tested, and a standard IgE solution, were added to 0.5 ml of phosphate buffered saline containing 30% rabbit serum. An anti-IgE coated bead was added to each assay tube, and the tube was shaken thoroughly and incubated for 3 h at room temperature, followed by washing 3 times with 5 ml of saline containing Tween 20. Then 0.5 ml of peroxidase-labelled anti-IgE γ-globulin was added to each tube and incubated at 4.0 °C for 16 h. The experimental procedures thereafter were the same as in the ELISA for insulin[2].

The minimum and maximum concentrations of IgE measurable by ELISA were 6.25 and 1600 units/ml, respectively. The intraassay coefficient of variation was 6.2% at about 100 units/ml (n = 5) and 3.5% at about 400 units of IgE/ml (n = 5). The curve obtained from various serum dilutions ran parallel with a standard curve.

38 sera from normal subjects and atopic patients were tested with ELISA and with a commercially available radioimmunoassay (RIA) kit for IgE (Pharmacia). The correlation coefficient between the values measured by ELISA(Y) and RIA(X) was the regression equation $Y = 0.992 \times - 4.71$ ($\gamma = 0.990$). In summary, this ELISA is simple, and as sensitive an assay system as the radioimmunoassay.

ELISA methods for IgE have been reported by Hoffmann[5], Guesdon et al.[6], and Weltman et al.[7]. The first is a competitive immunoassay and requires a large amount of IgE for testing. A sandwich method similar to ours using a cellulose disc or single radial diffusion is employed in the latter two. They use glutaraldehyde for coupling. As compared with theirs, our procedure is simpler, and its sensitivity is higher.

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Cryoprotection of human bone marrow committed stem cells (CFU-c) by dextran, glycerol and dimethyl sulfoxide[1]

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Summary. Dextran, glycerol and dimethyl sulfoxide (DMSO), alone or in combination, were used for cryoprotection of human bone marrow cells. The viability of cryopreserved cells was assessed by culture of myelopoiesis-committed stem cells (CFU-c) in vitro. A significantly better protection against freezing injury was obtained by 9% dextran in combination with 3 or 5% DMSO, and also with 5 or 10% DMSO alone, than with either 15% glycerol or 9% dextran with 1% DMSO.

Modern antimitotic chemo- and radiotherapy of patients with malignant tumours frequently causes a life-threatening bone marrow aplasia. The haemopoietic depression can be successfully reversed by an infusion of cryopreserved autologous bone marrow cells[2,3]. According to experimental evidence, obtained on animals exposed to supralethal doses of irradiation, the repopulation of the aplastic marrow and functional recovery are positively correlated with