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Research paper

A new sensitive and specific enzyme-linked immunosorbent assay for IgD

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Abstract

We have developed a new highly specific ELISA for IgD, and then used it to measure levels of circulating IgD in the serum of 480 un-selected patients from the East Anglia region of UK. The assay is both extremely sensitive and specific, with a minimum detected IgD concentration of 30 pg/ml and more than 10,000-fold specificity for IgD over all other human immunoglobulins. The assay shows linear dilution characteristics with both purified IgD and human serum, and spiking of purified IgD into either purified immunoglobulins or human serum shows c. 100% recovery. Furthermore, intra-assay and inter-assay coefficients of variation for repeated measurements of the same samples are below 10% and 15% respectively. Measurement of IgD levels on the un-selected patient population showed levels to range from <300 pg/ml to over 100 μg/ml, with a geometric mean of 8 μg/ml. The distribution is approximately normal after log transformation. Levels of circulating IgD were higher in men than in women. There was a significant negative correlation between levels of IgD and age in women, but not in men. Moreover, after adjustment for age and sex, there were statistically significantly higher levels of circulating IgD in male (but not female) smokers, compared to their non-smoking counterparts. These results highlight the care that needs to be taken to control for age, sex and cigarette smoking when examining levels of circulating IgD in future studies.

1. Introduction

Although IgD was first discovered in 1965 (Rowe and Fahey, 1965), relatively little is known about its function in vivo, compared with the other immunoglobulin isotypes. It is produced in two variants, a membrane-bound form, which is a major component of the B lymphocyte B-cell receptor (Preud'homme et al., 2000) and a secretory form, whose function is largely unknown. Deletion of the gene encoding IgD in the mouse results in a phenotype that is not materially different from wild type animals (Nitschke et al., 1993; Roes and Rajewsky, 1993), suggesting that under some conditions IgD is not essential. However, IgD may, in some circumstances, be able to perform some of the functions of IgM, as deletion of the gene encoding IgM in a mouse model leads to IgD replacing membrane-bound and secretory IgM during B-cell development (Lutz et al., 1998).

Abbreviations: ELISA, Enzyme-linked immunosorbent assay; Ig, Immunoglobulin; RT, Room temperature.

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Levels of secretory IgD have been measured in the circulation previously (see Preud'homme et al., 2000; Vladutiu, 2000 for reviews). Levels of circulating IgD differ throughout life, with most studies reporting low levels of IgD at birth (c. 0.2 μg/ml), rising during childhood until early adulthood, and then declining with age thereafter (Leslie et al., 1975; Zegers et al., 1975; De Greef et al., 1992). In adults, circulating IgD has been shown to be either higher in men than in women (Stoica et al., 1980), in women than in men (Leslie et al., 1975) or the same in both sexes (Dunnette et al., 1978; Levan-Petit et al., 2000). All studies agree that there is considerable variation in the levels of human IgD in normal populations (typically from 0.1–300 μg/ml), although the shape of the distribution has been variously described as unimodal (Levan-Petit et al., 2000), bimodal (Kholmogorova and Stefani, 1982) or trimodal (Dunnette et al., 1978). The reported geometric mean concentration of IgD in healthy adults also varies, from around 8 to 40 μg/ml (Peng et al., 1991; Levan-Petit et al., 2000; Preud'homme et al., 2000). Differences in circulating IgD have been found between volunteers of different ethnic backgrounds and Gm haplotypes (Litwin et al., 1985). A major environmental influence, smoking, has also been shown to be associated with increased levels of circulating IgD in one small study (Bahna et al., 1983).

IgD antibodies have been shown to be specific for antigens from various infectious agents (Sewell et al., 1978; Mortensen et al., 1989), allergens (Zhang et al., 1994) and auto-antigens (Luster et al., 1976). Recently, we have shown that IgD antibodies specifically bind various carbohydrates, especially those related to the α-gal moiety (Galα1-3Galβ1-4GlcNAc) that is believed to be the major antigen acting as an obstacle for xenotransplantation (Mosedale et al., 2006).

Various methods have been used to measure IgD in the studies described above, and this variation may contribute to the differences in results from one study to another. The majority of measurements have been made using radioimmuno assays, which do not readily lend themselves to be used to measure hundreds of samples. More recently, an ELISA has been described which has a sensitivity greater than most radioimmunodiffusion assays. However, the report describing this assay only showed it to be specific for IgD over other immunoglobulins by a factor of 1000. This is insufficient to guarantee specificity of the assay versus other immunoglobulins, as the normal range of human IgG concentrations in serum is up to about 20 mg/ml.

We have generated and optimised a novel ELISA for IgD that is highly specific and sensitive for IgD. We validated the assay by measuring the levels of total IgD in over 400 healthy subjects from the East Anglia region of the UK, one of the largest cohorts of healthy volunteers assayed for IgD. As expected from previous studies, levels of IgD differed widely within the normal population, from < 30 pg/ml to >100 μg/ml. We found that the distribution of IgD was approximately log-normal. On average, levels of IgD were higher in men than in women. We also found an inverse association between IgD levels and age in women but not in men. IgD levels were also associated with smoking status in men but not in women. However, we found no statistically significant evidence to indicate that sex modifies the association between smoking and IgD levels.

2. Materials and methods

2.1. Purification of monoclonal anti-IgD from JA11 cell culture supernatant

JA11 hybridoma cells were maintained in RPMI 1640 media (Sigma, Poole, UK) containing 10% foetal calf serum, 3 mM glutamine, 60 μg/ml penicillin, 100 μg/ml streptomycin and 25 μg/ml gentamycin at 37 °C in an atmosphere containing 5% CO_2. Cells were subcultured when confluent, approximately every 3–4 days and reduced to 2×10^5 cells/ml. When collecting tissue culture supernatant for purification of the JA11 antibody, cells were maintained for extended periods of time in the same media — up to 14 days with regular supplementation with 1/25 volume of 625 mM HEPES, pH 7.2 containing 25% (w/v) glucose.

A protein L column (Perbio Science, Helsingborg, Sweden) was equilibrated with binding buffer (100 mM phosphate containing 150 mM NaCl, pH 7.2). The tissue culture supernatant medium was diluted 1/1 (v/v) with binding buffer and applied to the column. Following extensive washing with binding buffer the bound immunoglobulin was eluted with 10 ml of 0.1 M glycine, pH 3 and immediately returned to pH 7.2 with 1.1 ml of 1 M Tris, pH 7.2. The column was regenerated by washing with 10 ml of 0.1 M glycine, pH 2.5, followed by at least 30 ml of binding buffer. The purified immunoglobulin was buffer-exchanged into phosphate-buffered saline (PBS: 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 137 mM NaCl, 2.7 mM KCl, pH 7.2) and concentrated to 1 mg/ml using Ultrafree centrifugal concentrators (Millipore, Bedford, MA) for storage.

2.2. IgD ELISA

ELISA plates (Nunc Maxisorp, Roskilde, Denmark) were coated overnight at room temperature (RT) in the dark with 0.5 μg of mouse anti-IgD (JA11; prepared as above) in 200 μl of 50 mM Na_2CO_3, pH 9.6. Following 3