Secreted immunoglobulin D (IgD) is present in small amounts in human serum, and membrane-bound IgD is a major component of the B-cell antigen receptor (1, 2). The role of secreted IgD is largely unknown (3). Results from a recent study of IgM-deficient mice suggest that IgD is able to substitute for IgM in most of its functions (4). However, the phenotype of IgD-deficient mice is virtually indistinguishable from IgM-deficient mice (5). The serum IgD half-life is known to be short, which is probably attributable to its high sensitivity to proteolysis (13). IgD Abs specific for viruses, bacteria, allergens, and autoantigens have been detected (14). Serum polyclonal IgD concentrations are increased in various diseases, including the hyper IgD syndrome and allergic disorders, which raises the question of a possible role of IgD in such disorders (15, 16). Conversely, some recent studies aimed at measuring IgD in patients with immunodeficiency or Henoch–Schönberg purpura reported undetectable serum IgD (1). The detection threshold of the assay used in the latter studies was relatively high (17, 18).

We considered it useful to develop a sensitive assay able to measure IgD in all normal sera, whatever the age of the subjects. Such an assay could allow laboratories to provide and compare values below the detection limits of previous assays. We hence developed an ELISA that permits the measurement of low concentrations of human IgD in biological fluids such as neonatal sera. We also studied by ELISA and Western blotting the behavior of IgD in stored sera and the effects of storage on the reliability of IgD measurements.

Adult sera were collected from 124 randomly chosen blood donors (62 women and 62 men; ages, 20–55 years). Sera from neonates (ages, 0–1 month; n = 105) and infants (ages, 1–12 months; n = 22) were collected during their routine management at entrance into the pediatric unit of Poitiers University Hospital, as approved by the local Ethics Committee. Criteria for subject inclusion were the absence of inflammatory syndrome (serum C-reactive protein <5 mg/L and body temperature <37.5 °C) and of treatment with corticosteroids or cyclosporin A. Neonates were classified as premature (<38 weeks amenorrhea; n = 75) or term-born (n = 30). Sera were stored at −20 °C before assays. For the study of serum IgD stability, three samples were kept for 30 days at 4 °C or for 1 day at 20 or 37 °C in the absence or presence of protease inhibitors (5 mmol/L e-amino caproic acid and 1 mmol/L phenylmethylsulfonyl fluoride; Sigma Chemical).

For the IgD ELISA, preliminary experiments led us to select the combination of capture and detection commercial Abs and working concentrations on the basis of the highest signal-to-noise absorbance ratio and sensitivity. Flat-bottomed microtiter plates (Maxisorp; Nunc) were coated for 18 h at 4 °C with polyclonal rabbit anti-human δ-chain Ab (0.5 mg/L in 0.1 mol/L carbonate buffer, pH 9.6; Dako). The plates were washed three times with 0.5 mL/L Tween 20 (Sigma Chemical) in phosphate-buffered saline, pH 7.3, and saturated for 1 h at 37 °C with 200 μL of phosphate-buffered saline containing 100 mL/L fetal calf serum. All samples were analyzed in duplicate. Calibrators were obtained from Behring (OTRD; Behring) and diluted to the appropriate concentrations in phosphate-buffered saline containing fetal calf serum; 100 μL was added to each well. Usually, sera were diluted from 1:100 to 1:40 000. After a 2-h incubation at 37 °C, the wells were washed three times and incubated with a biotinylated polyclonal goat anti-human δ-chain antiserum (0.1 mg/mL; Sigma) for 2 h at 37 °C. After three additional washes, the plates were incubated for 30 min with streptavidin-polyperoxidase (Tebu). Peroxidase activity was assayed using 100 μL of 0.05 mol/L citrate buffer, pH 5, containing 0.5 g/L o-phenylenediamine and 0.4 mL/L hydrogen peroxide per well. The reaction was stopped with 50 μL of 1.15 mol/L sulfuric acid per well, and absorbances were measured at 490 nm. Absorbances values obtained with increasing concentrations of IgD calibrator were used to construct the calibration curve, allowing IgD measurement in the studied samples. The specificities of the anti-human δ-chain antisera were controlled by ELISA with monoclonal immunoglobulins of various isotypes. Data were expressed as means ± SD. Differences between groups of subjects were tested by the Mann–Whitney test.

The detection threshold was 0.1 μg/L, calculated as the IgD concentration corresponding to the mean ± 4 SD of 10 absorbance determinations of the blank absorbance values. Within-run imprecision (CV) was 6.0–9.3% at 0.9, 1.0, 4.7, and 5.5 μg/L (n = 20 each). The interassay CV was 10%, as determined from 20 consecutive measurements of a serum diluted 1:20 000. The apparent recovery of IgD in a mixture of 2.5 μg/L IgD from sera and 1.25 μg/L from supernatant was 103%, and was 101% for 1.25 μg/L IgD from sera and 2.5 μg/L from supernatant. To analyze the linearity of the assay, a diluted serum containing 10 μg/L IgD was further serially diluted to 5, 2.5, 1.25, and 0.675 μg/L, and each dilution was tested in quadruplicate. In comparison with theoretic values, the recoveries were 97%, 100%, 101%, 101%, and 113%, respectively. Mono-
clonal IgG, IgM, IgA, or IgE (purified in the laboratory) at 100 mg/L yielded negative results. The concentration of IgD in dilutions of a purified IgD myeloma protein (15 g/L; Bio-Rad protein assay) was in very close agreement with the total measurement of proteins, demonstrating the validity of the calibrators used in the ELISA. This assay is now used for measurements of serum IgD in our hospital laboratory. It could help to discriminate between hyper IgD syndrome and other hereditary fevers. It uses commercial Abs and calibrators. The intra- and interassay repeatability, dilution, and recovery experiments showed that it is accurate, reproducible, and specific. It appears to be more sensitive than previously reported ELISAs (thresholds, 1 and 4 μg/L) or radioimmunoassays (0.5 μg/L) for IgD (9, 15, 16).

Because IgD has been described to be highly sensitive to

Fig. 1. Distribution of IgD in 124 normal adult sera (A) and in 105 neonate sera (B). The distribution of very low concentrations in adult sera (up to 1 mg/L) is shown in the inset.
proteolysis (13), we measured IgD concentrations in three normal sera maintained for 0, 1, 3, 5, 7, and 30 days at 4 °C, in the presence or absence of protease inhibitors. IgD concentrations remained stable with no difference according to the presence or absence of protease inhibitors, as confirmed by Western blot analysis of these sera (data not shown). No evidence of proteolysis was found under these conditions. In agreement with these data, Dunnette et al. (9) previously observed that the treatment of a normal serum for 5 h at 37 °C or 10 cycles of freezing and thawing did not significantly alter the IgD concentration. Recently, Drenth et al. (16) reported that they were able to measure similar IgD concentrations in normal sera maintained up to 6 months at 4 °C.

As shown in Fig. 1A, the observed distribution of IgD in 124 normal adult sera was nongaussian and extremely heterogeneous (mean, 36 mg/L; range, 0.1–213 mg/L), thus confirming previous studies (7, 8). The geometric mean was 14.5 mg/L, a value located between lower (8.4 mg/L) and higher (53.5 mg/L) previously reported geometric means [reviewed in Ref. (15)]. In agreement with Dunnette et al. (19), we observed no difference according to gender, whereas Leslie et al. (20) reported that women tended to have higher concentrations than men. Previous studies reported on low or undetectable IgD concentrations in neonates (8, 11, 20, 21). At variance, we detected IgD in the sera of all neonates studied, and IgD concentrations were not significantly different between premature and term neonates, in agreement with a previous report (8). In neonate sera, the distribution of IgD concentrations was unimodal and homogeneous (0.26 ± 0.18 mg/L; range, 0.04–1.2 mg/L; Fig. 1B). IgD was significantly higher in infants 1–12 months of age than in the neonate group, and the distribution appeared to be multimodal (0.95 ± 1.06 mg/L; P <0.0001). A bimodal distribution was described previously in the latter age group, but IgD was undetectable in most subjects in this study (11). We hypothesized that major changes in the regulation of IgD production might occur early in life. IgD synthesis is likely to be regulated by genetic and environmental factors. We recently showed that in vitro IgD production by normal peripheral blood human B cells is regulated positively by Th2 cytokines and negatively by Th1 cytokines (22), and hypothesized that cytokines might play a role in the heterogeneity of the distribution of serum IgD. In any case, the present ELISA provides an inexpensive, sensitive, and easy tool to study IgD in biological fluids, even at low concentrations, and in cell culture supernatants. It will be a challenge to further characterize mechanisms responsible for the dispersion of IgD in sera.

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References