Immunoglobulin Levels in Saliva in Individuals with Selective IgA Deficiency: Compensatory IgM Secretion and Its Correlation with HLA and Susceptibility to Infections


Accepted: April 13, 1989

Total levels of IgA, IgM, and IgG were measured in unstimulated whole saliva and serum from 63 individuals with selective IgA deficiency. Values were compared with the incidence of upper respiratory tract infections, antibiotic treatments (necessitated by upper respiratory tract infections), and HLA antigens. A statistically significant increase in salivary IgM and IgG levels was noted in individuals with selective IgA deficiency compared to healthy normal individuals. Healthy individuals with selective IgA deficiency did not have increased concentrations of salivary IgM compared to infectious-prone patients. Nor was there any correlation found between proneness to infections and HLA antigens or between salivary IgM or IgG levels and HLA antigens in this patient material.

KEY WORDS: IgA deficiency; saliva antibodies; HLA; infections.

INTRODUCTION

The major immunoglobulin class in saliva of normal individuals is immunoglobulin A (IgA), which is transported through the secretory epithelial cells in the salivary glands into the oral cavity. Other immunoglobulin classes are also present in saliva, but in markedly lower amounts. Salivary IgA probably plays an important role as a first line of defense on mucosal surfaces against bacterial and viral antigens (1, 2).

IgA deficiency (IgAd) is the most common form of immunodeficiency in Sweden, with a reported frequency of about 1:500 (3, 4). The incidence of upper respiratory tract infections (sinusitis included) is increased in some of the IgAd individuals, with a reported frequency of 30–36% (5-7). Individuals with IgAd have low or no detectable levels of IgA in serum, with normal or increased concentrations of IgG and IgM (8). Elevated levels of IgG and IgM have also been found in secretions, such as saliva, in these individuals (9).

Certain HLA antigens are found at a higher frequency in IgAd individuals (10, 11) and a weak association with infection rate has been reported (12). The present study was undertaken in order to analyze further the possible influence of immunoglobulin levels and of HLA on susceptibility to infections in IgAd individuals.

MATERIALS AND METHODS

Patients. Serum was collected from 63 (35 females and 28 males) individuals with selective IgA deficiency (IgAd) with IgA levels ≤0.02 g/liter in serum. One of 63 investigated IgAd donors also had a concomitant IgG subclass deficiency. Fifty-eight IgAd individuals were tissue typed (HLA) for A/B, and forty-six for HLA-DR. Unstimulated whole saliva samples were also collected from the IgAd individuals and all lacked secretory IgA in saliva (<0.01 mg/liter). There was no apparent infection in any of the investigated patients at the time of sampling. A clinical questionnaire with special emphasis on the incidence of upper respiratory tract
infection days was completed by 46 IgAd individuals. The patients were divided into five groups (0, 1-7, 8-15, 16-30, and ≥31 days) according to the number of days suffering from upper respiratory tract infections, during the year of sampling. Forty-five IgA-deficient individuals completed the part of the questionnaire related to antibiotic therapy (used for treatment of upper respiratory infections) and they were divided into three groups (untreated and 1-3 and ≥4 antibiotic treatments). The control group for measurements of IgA, IgG, and IgM levels in saliva consisted of 20 healthy adult volunteers (10 females and 10 males).

Salivary Collection and Analysis. Unstimulated whole saliva was collected directly into tubes and all samples were frozen and kept at −70°C until analyzed. Samples were thawed and centrifuged for 15 min at 10,000 g at 4°C to remove cells and debris, and the supernatant was examined by enzyme-linked immunosorbent assay (ELISA) (13). Each sample of saliva was tested in duplicate at three fivefold dilution steps. All saliva samples were diluted in phosphate-buffered saline containing 0.05% Tween 20 (Polysorbatum 20, Apoteksbolaget, Sweden). Polystyrene microtiter plates (M129B, Dynatech, Denkendorf, West Germany) were coated with antiimmunoglobulins for 4 hr at 37°C and stored at 4°C until used. Each well was coated with 100 µl rabbit immunoglobulins directed toward human IgM (µ chains, Lot. 013F, DAKOPATTS, Copenhagen, Denmark), IgG (γ chains, Lot 100333C, Behringwerke AG, Marburg, West Germany) or secretory component (Lot 050A, DAKOPATTS) in 0.1 M carbonate-bicarbonate buffer (pH 9.6). The microtiter plates coated with antibodies against secretory component were incubated with 100 µl bovine serum albumin, 5 mg/ml (No. A-4378, albumin bovine, Lot 74F-9420, Sigma Chemical Company, Dorset, England), for 4 hr at room temperature before use, to reduce the background value. Diluted saliva samples were added in a volume of 100 µl to the microtiter wells and incubated overnight at room temperature. The peripheral wells were omitted to avoid disturbing "edge effects" (14).

Between the various incubation steps, the wells were rinsed five times with saline containing 0.05% Tween 20. Alkaline phosphatase (ALP)-conjugated immunoglobulin fractions of monospecific rabbit antisera to human IgG (γ chain), IgM (µ chain), and IgA (α chain) (DAKOPATTS) were added, 100 µl, diluted 1:1000 for 3 hr at room temperature. After the final wash, disodium p-nitrophenyl phosphate, 1 mg/ml (Sigma Chemical Company), in 10% diethanolamine buffer was added and the absorbance was measured at 405 nm at 10-min intervals using a Titertek Multiskan (Elflab, Helsinki, Finland).

Colostrum was collected from five individuals and pooled. The purification of IgA from colostrum was done by removing IgG and IgM by affinity chromatography, CNBr-activated Sepharose 4B (Pharmacia, Uppsala, Sweden). The purity was tested in double immunodiffusion against rabbit anti-human IgG, IgA, and IgM (DAKOPATTS). The purified colostrum IgA was also tested by ELISA by coating the microtiter plates with the colostrum diluted 1:2000. The plates were thereafter rinsed and ALP-conjugated immunoglobulins directed against IgG, IgA, and IgM (DAKOPATTS) were added. The contamination of IgM and IgG was below the background value and could not be determined in ELISA. The purified colostrum IgA was used as reference standard and for quantitation of sIgA. For measurements of IgG and IgM levels human serum (Lot 041015 F, standard human serum, Behringwerke AG) served as standard. All standards were diluted in phosphate-buffered saline containing 0.05% Tween 20 in six fivefold dilution steps and added in duplicate, 100 µl.

Serum Collection and Analysis. The collected sera were stored at −70°C until used. Serum IgA, IgG, and IgM levels were measured using commercially available immunodiffusion plates (Nordigen, Behringwerke AG).

Tissue Typing. Tissue typing (HLA-A/B/DR) was done according to routine methods (15, 16).

Statistical Comparison. The Mann–Whitney test and Spearman correlation was used for the statistical analyses.

RESULTS

Immunoglobulin Levels in IgAd Individuals Compared to Healthy Controls

Total immunoglobulin levels in saliva were measured in 63 IgA-deficient individuals and in 20 healthy volunteers. As expected, sIgA could not be detected in saliva from the IgA-deficient individuals, whereas IgM (P < 0.00001) and IgG (P < 0.0005) levels were increased (Table I).