Immunoglobulins in the Hyperimmunoglobulin E and Recurrent Infection (Job's) Syndrome

Deficiency of Anti-*Staphylococcus aureus* Immunoglobulin A

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Abstract

Patients with the hyperimmunoglobulin E and recurrent infection syndrome (HIE) characteristically have frequent skin and respiratory infections caused by *Staphylococcus aureus.* We have developed a set of enzyme-linked immunosorbent assays that use whole *S. aureus* (Wood's strain) immobilized on 0.22-μm filters and highly specific, affinity-purified enzyme conjugates of goat anti-human IgE, anti-human IgD, anti-human IgG, anti-human IgA, and anti-human IgM. These reagents were used to determine *S. aureus*-specific immunoglobulin (Ig) levels. As previously published, 10 patients with HIE had markedly higher levels of anti-*S. aureus* IgE than did 5 patients with eczema and recurrent superficial *S. aureus* infections ($P < 0.001$). The HIE patients were also found to have a deficit of anti-*S. aureus* serum IgA as compared with 12 normal subjects, 12 patients with chronic granulomatous disease, 5 patients with chronic eczema and recurrent superficial *S. aureus* infections, and 3 patients with the Chediak-Higashi syndrome ($P < 0.01$ for each comparison). In addition, the HIE patients had an excess of anti-*S. aureus* IgM as compared with normal subjects ($P < 0.01$). An expected excess of anti-*S. aureus* IgG was absent. These abnormalities cannot be explained by variations of total serum Ig levels or by a general inability to produce antigen-specific IgA because levels of naturally occurring IgA antibody against *Escherichia coli* lipopolysaccharide and the antigens of the pneumococcal vaccine are normal. Parotid saliva from patients with HIE contained less salivary IgA per milligram of protein ($P < 0.01$) and less salivary anti-*S. aureus* IgA per milligram of protein ($P < 0.05$) than did normal controls. The incidence of infection at mucosal surfaces and adjacent lymph nodes correlated inversely with serum anti-*S. aureus* IgA ($r = -0.647$, $P = 0.034$), serum anti-*S. aureus* IgE ($r = -0.731$, $P = 0.016$), total serum IgE ($r = -0.714$, $P = 0.020$), and total serum IgD ($r = -0.597$, $P = 0.049$). These findings are evidence of a previously undescribed immunoregulatory defect in patients with HIE, which may contribute to the increased susceptibility to infection in this syndrome.

Introduction

The hyperimmunoglobulin E and recurrent infection (Job's) syndrome (HIE)[1] is a complex disorder characterized by onset early in life, markedly elevated serum IgE, and serious recurrent bacterial infections of the skin and sinopulmonary tract (1, 2).

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These skin infections are frequently "cold" subcutaneous abscesses caused by *Staphylococcus aureus,* the most common bacterial pathogen in this syndrome. The middle and external ear, mastoid processes, gingiva, bronchi, and lungs are the other frequent sites of bacterial infection. Additional characteristics of the syndrome include coarse facies, chronic eczematoid dermatitis, mild eosinophilia, and mucocutaneous candidiasis.

In addition to the hallmark of increased serum IgE, reported immunologic abnormalities include the presence of anti-*S. aureus* and anti-*Candida albicans* IgE (3–5), elevated total IgD (6), and a neutrophil chemotactic defect (7–9) possibly caused by suppressive factors released from mononuclear cells (10, 11). In addition, there are deficiencies of delayed hypersensitivity (2, 12), in vitro proliferative responses to *C. albicans* (1, 4, 12), proliferative responses in mixed lymphocyte cultures (1), anamnestic antibody responses to tetanus and diphtheria antigens (1), in vitro response to pokeweed mitogen (13) and concanavalin A (12), and suppressor T cell number and function (14).

The presence of anti-*S. aureus* IgE (3–5) led to the hypothesis that other abnormalities of the humoral immune response to *S. aureus* could be present and contribute to the marked predisposition to *S. aureus* infection in this syndrome (4). Therefore, we have measured the anti-*S. aureus* Ig levels in the serum and saliva from HIE patients and from control groups by use of a new, highly sensitive and specific enzyme-linked immunosorbent assay (ELISA) for anti-*S. aureus* IgG, IgA, IgM, IgE, and IgD. HIE patients have deficiencies of serum anti-*S. aureus* IgA, salivary IgA, and salivary anti-*S. aureus* IgA. In addition, there is an inverse correlation between the levels of serum anti-*S. aureus* IgA, serum anti-*S. aureus* IgE, total serum IgE, total serum IgD, and the number of infections at mucosal surfaces and in adjacent lymph nodes.

Methods

Subjects

Normal subjects. 39 healthy people (aged 20–39; mean±SEM 30.9±0.9) were studied. Serum samples from nine of them were obtained on a single morning, pooled in equal volumes, and divided into 1-ml aliquots. A freshly thawed sample of this reference pool was used to standardize each assay of anti-*S. aureus* IgG, IgA, and IgM. These subjects were not used to determine the normal range.

Patients with HIE. Total Ig levels were determined for 11 patients (aged 10–45; mean±SEM = 22.6±3.1) who were followed at the National Institutes of Health (NIH) and who manifested "classic" HIE

1. *Abbreviations used in this paper:* AU, arbitrary units; CGD, chronic granulomatous disease; CHS, Chediak-Higashi Syndrome; ELISA, enzyme-linked immunosorbent assay; HIE, hyperimmunoglobulin E and recurrent infection (Job's) syndrome; HRP, horseradish peroxidase; LPS, lipopolysaccharide.

(patients No. 1-5, 17, and 9-13 in reference 2). Anti-*S. aureus* Igs were studied in 10 of the patients. Five of the patients were studied 1-3 wk after the onset of an *S. aureus* infection (two, subcutaneous abscesses; one, bronchitis; one, lung abscess; and one, pneumonia). Six of the HIE group were in relatively good health but had experienced a *S. aureus* infection in the previous 2 yr (one, pneumonia; one, subcutaneous abscess; one, osteomyelitis; and three, bronchitis). On one occasion, sera from three of these patients were pooled, aliquoted, frozen, and used as an "among assay" reference standard for the quantification of serum anti-*S. aureus* IgE. Separate serum samples from these three patients were included in the anti-*S. aureus* IgE data. Nine of the patients had detectable anti-*S. aureus* IgE. Their medical records were reviewed to determine the incidence of infection from January, 1981 to the present. Infections included otitis (media and externa), mastoiditis, sialitis, lymphadenitis, acute bronchitis, pneumonia, urinary tract infection, pulmonary abscess, giardiasis, osteomyelitis, abscesses (subcutaneous, axillary, and perirectal), and mucocutaneous candidiasis. The presence of mucocutaneous candidiasis was considered to be a single infection episode even though it was a chronic problem. Infections occurring at sites where IgA may be an important host defense were considered separately. These sites included the oropharynx and associated lymph nodes, lungs, middle ear, gastrointestinal tract, and vagina.

Patients with chronic granulomatous disease (CGD). Total Ig levels were determined for 15 patients (aged 7-34; mean±SEM = 17.8±1.9) with CGD who had previously been evaluated at the NIH (15). Anti-*S. aureus* Igs were determined in 12 of the patients. Two patients were studied within 2 wk of a documented *S. aureus* infection (skin abscess, osteomyelitis). Three patients had had a documented *S. aureus* infection during the previous 2 yr (two, liver abscesses; one, pneumonia). These five patients are considered to have had recent *S. aureus* infection. Three patients had had recent serious infections (one, lymphadenitis, and three, pneumonia) with negative cultures. One patient had *Nocardia asteroides* pneumonia 1 yr before study. Four patients had been clinically well for >2 yr. Although data are shown as the geometric mean and relative SE (Figs. 4-6) for all CGD patients, statistical comparisons with other groups were performed with data from either the whole CGD group or the two subsets (presence or absence of recent documented *S. aureus* infection).

Patients with the Chediak-Higashi syndrome (CHS). Three CHS patients (aged 17-33; mean±SEM = 27.0±5.0) were studied (9). One had a history of recurrent superficial pustules caused by *S. aureus*, one had an acute perirectal abscess secondary to *Escherichia coli* at the time the serum was obtained, and one had been relatively free of significant infections for many years.

Patients with eczema and recurrent superficial S. aureus infections. The five patients (aged 6-39; mean±SEM = 22.2±6.3) in this category were referred to the NIH for consideration of the diagnosis of HIE because of markedly elevated IgE and a long history of recurrent but usually superficial *S. aureus* infections complicating chronic eczematoid dermatitis. One patient had an active *S. aureus* infection at the time of study, and four were clinically well except for diffuse eczema. All had experienced significant infections within the preceding year.

Sera

The serum samples were obtained before breakfast, allowed to clot at room temperature for 35-90 min, aliquoted, and stored at −70°C. Assays of anti-*S. aureus* Ig levels were performed with replicate aliquots of single serum samples. For assays of anti-*S. aureus* IgE, it was necessary to adsorb the serum samples with protein A (16) to remove an inhibitory effect that is seen at low dilutions and is presumably due to the displacement of IgE by IgG.

Saliva

Saliva samples from 8 HIE patients, 14 normal people, and 6 patients with CGD were obtained with a plastic cup held by gentle suction over Stenson's duct. With salivary secretion stimulated by oral instillation of lemon juice, 1-5 cc was collected on ice over 10-20 min, centrifuged immediately at 2,500 *g* at 4°C for 10 min, and stored at −70°C in 300-μl aliquots for up to 9 mo. Aliquots of a saliva sample from a normal person known to have a high titer of anti-*S. aureus* salivary IgA were used as a standard in each assay of salivary anti-*S. aureus* IgA. Data from this normal person are included in the data for normal subjects.

Microorganisms and bacterial antigens

S. aureus (Wood's strain) has been carried in our laboratory for many years (4). A clinical isolate of *S. aureus* was obtained from the NIH Clinical Laboratory and was shown to contain protein A. The lipopolysaccharide (LPS) antigen from *E. coli* strain J5 was prepared by List Biological Laboratories (Campbell, CA) and was a gift from Dr. Keith Joiner. The polyvalent pneumococcal polysaccharide vaccine was produced by Lederle Laboratories (Pearl River, NY).

Antibodies

Reference standards. An IgE reference standard was obtained from Pharmacia Fine Chemicals (Piscataway, NJ). A human IgD reference standard was obtained from the World Health Organization (Lausanne, Switzerland). Affinity-purified polyclonal human serum IgA was obtained commercially (Jackson Immunoresearch Laboratories, Inc., Avondale, PA).

Capture antibodies. The IgG fraction of goat anti-human IgA (Miles-Yeda, Rehovot, Israel), affinity-purified goat anti-human IgM (Cappel Laboratories, Cochranville, PA), affinity-purified goat anti-human IgG (Jackson Immunoresearch), and affinity-purified goat anti-human IgE (Tago Inc., Burlingame, CA) were used as capture antibodies (see below, Standard ELISA assays) without further purification. The IgG fraction of goat anti-human IgD (Cappel Laboratories) was adsorbed with solid-phase human IgG.

Enzyme conjugates. Affinity-purified horseradish peroxidase (HRP)-conjugated goat anti-human IgG (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD) was adsorbed against Sepharose-linked IgE and IgM. Affinity-purified HRP-goat anti-human IgA (Kirkegaard & Perry Laboratories, Inc.) was adsorbed against solid-phase human IgG and IgM. Affinity-purified HRP-goat anti-human IgM (Kirkegaard & Perry Laboratories, Inc.) was adsorbed against human serum IgG, serum IgA, and secretory IgA. Affinity-purified alkaline phosphatase-conjugated goat anti-human IgD (Sigma Chemical Co., St. Louis, MO) was used without further purification. HRP-anti-human IgE was prepared from goat antisera raised to IgE (myeloma PS). The goat antiserum was affinity-purified on an IgE (myeloma PS)-Sepharose column, with 3 M $MgCl_2$ used for desorption. The sample was then dialyzed, concentrated, labeled with HRP (Sigma Chemical Co.) (17), and further purified by crossadsorption with solid-phase IgG, serum IgA, and secretory IgA.

Material for solid-phase adsorbents and determination of HRP-anti-immunoglobulin specificity. The following purified antibody preparations were purchased: human serum IgG, serum IgA, and secretory IgA (Jackson Immunoresearch Laboratories, Inc.), and mixed myeloma human IgM (Cappel Laboratories). Human IgE (myeloma PS) was purified by DEAE ion-exchange and gel-permeation chromatography on Sephacryl S-200 (Pharmacia Fine Chemicals). Human IgD was purified from an HIE patient with elevated IgD by affinity chromatography (18) using an IgG fraction of goat anti-human IgD (Cappel Laboratories) linked to Sepharose 4B followed by adsorption with solid-phase anti-human IgG.

Specificity. These antibody preparations were further immunoadsorbed and used to establish the cross-reactivity of the HRP-anti-immunoglobulin conjugates. For example, to determine the cross-reactivity of HRP-anti-IgG with IgA, serial dilutions of IgG and IgA were added to microtiter wells (on a single plate) coated with unconjugated anti-IgG and anti-IgA, respectively. After 2 h at room temperature, the plates were washed, the HRP-anti-IgG was added, and the reaction was completed (see below, Standard ELISA assays). The proportional difference between the amounts (weight/volume) of IgG and IgA necessary to give an absorbance at 490 nm of 0.100 determined the cross-reactivity of HRP-anti-IgG with IgA. By use of this assay system, the anti-IgE, anti-IgA, anti-IgM, and anti-IgD were found to have 10^4-fold specificity when tested against IgG, IgA, IgM, or IgE. Tested against IgD, the anti-IgE had a 10^4-fold specificity whereas the anti-IgM had a 10^3-fold specificity, and the anti-IgA had a 10^2-fold specificity. The anti-IgG had a 25-fold specificity when tested against IgD, a 10^2-fold specificity against IgM, a 10^3-fold specificity against IgA, and a 10^4-fold specificity against IgE. Thus, cross-reactivity of our conjugates with contaminating Ig's was negligible, and, in fact, the observed cross-reactivity may be secondary to the remaining impurities in the Ig preparations.

Assay of serum anti–S. aureus IgG and IgE (direct method)

S. aureus in log phase growth was harvested, washed twice in phosphate-buffered saline (PBS) with 10 μg/ml of gentamicin at pH 7.2 (buffer A), and resuspended (A_{650} = 0.400 for IgG, and A_{650} = 0.300 for IgE) in buffer A. Samples (100 μl) were collected by vacuum filtration with a manifold (Millipore Corp., Bedford, MA) onto prewetted 0.22-μm filters incorporated into a 96-well plastic plate (Millititer plate, type GV; Millipore Corp.). These amounts of *S. aureus* were demonstrated to give saturating values in the respective assays. The wells were washed with buffer B (0.01 M PBS, 10 μg/ml of gentamicin, and 1% bovine serum albumin, pH 7.4), and the underside was blotted dry. Duplicate 200-μl samples of serum (diluted in buffer B) were added to each well and left at room temperature for 4 h. The plates were then washed 10–12 times with four drying steps to remove pendant droplets. To each well 200 μl of conjugate (diluted 1:500 for HRP-anti-IgE, 1:1,000 for HRP-anti-IgG) diluted in buffer B was added. The plates were incubated overnight (14–18 h) at 4°C, washed again, and finally filled with 200 μl of substrate solution containing 0.4 mg/ml of *O*-phenylenediamine and 0.012% of H_2O_2 in phosphate citrate buffer (19) and left at room temperature. After 10 min for IgG and 15 min for IgE, the reaction was stopped by the addition of 50 μl of 8 N H_2SO_4. Supernatants were transferred via a drop guide (Millipore Corp.) into a clear microtiter plate (NUNC I; Gibco Laboratories, Grand Island, NY), and the absorbance (A_{490}) was read in a dual wavelength spectrophotometer (Dynatech Laboratories, Inc., Alexandria, VA). All samples were assayed in duplicate at two dilutions, and for each dilution, duplicate blanks (no *S. aureus*) were determined. Unit values were obtained by comparison of each unknown optical density to a standard curve generated by serial dilution of a freshly thawed sample of either the normal pool (IgG) or the HIE pool (IgE).

Examples of standard curves are shown in Fig. 1. As seen, the anti–*S. aureus* IgG standard curve (Fig. 1 *A*) is obtained with dilutions in the reference serum pool of from 1:10,000 to 1:160,000. In the anti–*S. aureus* IgE assay (Fig. 1 *B*), the HIE reference pool was used at dilutions ranging from 1:10 to 1:80. *S. aureus* Ig determinations are expressed as arbitrary units (AU) per milliliter, in which 1 AU/ml of anti–*S. aureus* IgG represents the amount present in a 1:10,000 dilution of the normal serum pool, and 1 AU/ml of anti–*S. aureus* IgE the amount present in a 1:10 dilution of the HIE pool.

Assay of serum anti–S. aureus IgA and IgM, and of salivary anti–S. aureus IgA (indirect method)

The samples were assayed as above with the following changes: 100 μl of *S. aureus* suspension (A_{650} = 0.100; ~2×10^8/ml), which yields saturating values in these assays, or of buffer A was placed into polypropylene tubes (12 × 75 mm), and 200 μl of serum or saliva diluted in buffer B was added. After incubation at room temperature with shaking for 4 h, 3 ml of buffer B was added, and the tubes were centrifuged at 7,000 *g* for 20 min at 4°C. The uppermost 2.9 ml were aspirated, and the pellets were transferred to millititer plates with three washes of 0.5 ml of buffer B. Samples were then handled as in the direct method except that the salivary anti–*S. aureus* IgA samples were read in the spectrophotometer with a 1.99 expansion factor. (Conjugates were diluted 1:2,000 for both HRP-anti-IgA and HRP-anti-IgM.) Examples of these standard curves are shown in Fig. 1. As can be

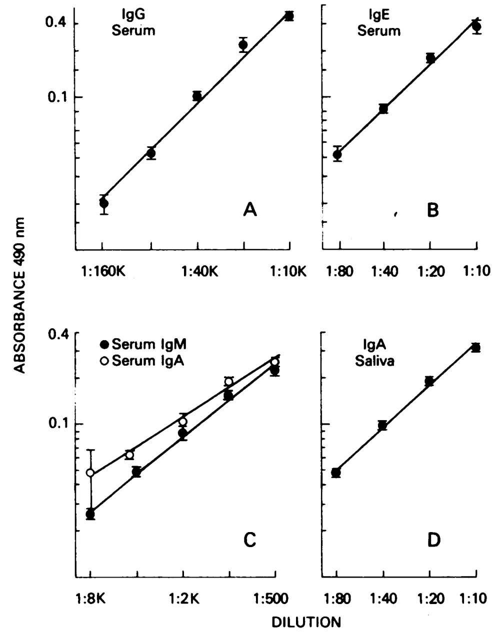

Figure 1. Assay of anti–*S. aureus* Ig's. Serum (*A–C*) or saliva (*D*) was diluted, as shown on the abscissa, and incubated with saturating amounts of *S. aureus*. An arbitrary value of 1 AU/ml was assigned to the normal serum pool at 1:10,000 for IgG (*A*) and 1:1,000 for IgA and IgM (*C*). 1:10 dilutions of the HIE pool and of the reference saliva were assigned a value of 1 AU/ml for anti–*S. aureus* IgE (*B*) and anti–*S. aureus* salivary IgA (*D*), respectively. All unknown samples were diluted as needed and assigned a value by comparison with a similar standard curve, which was run on each 96-well plate.

seen, the anti–*S. aureus* IgA and IgM (Fig. 1 *C*) standard curves were obtained with dilutions of from 1:500 to 1:8,000. The anti–*S. aureus* salivary IgA standard curve (Fig. 1 *D*) used a 1:10 to 1:80 dilution of saliva. 1 AU/ml of anti–*S. aureus* IgM or of anti–*S. aureus* IgA was the amount present in a 1:1,000 dilution of the normal serum pool. 1 AU/ml of anti–*S. aureus* salivary IgA was the amount present in a 1:10 dilution of the reference saliva.

Standard ELISA assays

Immunoglobulins. Standard ELISA techniques were used to measure total serum IgE, total serum IgD, and salivary IgA (20, 21).

Assay of anti-E. coli J5 IgA. IgA directed against the *E. coli* strain J5 LPS was measured by a modification of the method of Ito (22). A solution of purified *E. coli* J5 LPS was sonicated and diluted (100 μg/ml) in PBS containing $MgCl_2$ (0.02 M), adsorbed to microtiter plates (100-μl aliquots) at 37°C for 1 h, and used immediately. The plates were handled as above except that PBS containing 20 mM $MgCl_2$ and 0.1% bovine serum albumin were used for the incubation and wash steps.

Assay of anti-pneumococcal polysaccharide IgA. Serum samples were tested for IgA antibodies directed against an array of pneumococcal polysaccharide antigens essentially as described by Kehrl and Fauci (23) except that the currently available pneumococcal polyvalent vaccine (Pnu-Imune 23; Lederle Laboratories) was used.

Serum IgA, IgM, and IgG

Total serum IgA, IgM, and IgG were measured in the NIH Clinical Chemistry Laboratory by automated nephelometry (24).

Protein

Salivary protein concentrations were determined by the method of Lowry (25) with bovine serum albumin (Sigma Chemical Co.) as a standard.

Statistical evaluation

All data were compared by use of log values and a two-tailed *t* test or by Spearman rank correlation coefficient.

Results

Total Igs

Values of serum IgE and IgD in HIE and eczema patients were determined. In agreement with previously published data (1, 2, 6), as compared with the normal subjects, the HIE patients had higher levels of serum IgE (geometric mean ×/÷ SEM = 4,496 ×/÷ 1.39 IU/ml, range 1,163 to 24,190 IU/ml vs. geometric mean ×/÷ SEM = 55.7 ×/÷ 1.21 IU/ml, range 25 to 133 IU/ml; $P < 0.01$) and higher levels of serum IgD (geometric mean ×/÷ SEM = 54.6 ×/÷ 1.49 IU/ml, range 12 to 384 IU/ml vs. geometric mean ×/÷ SEM = 9.1 ×/÷ 1.43 IU/ml, range 0.3 to 75 IU/ml; $P < 0.01$). Five patients with eczema and recurrent superficial *S. aureus* infections also had elevated IgE levels (geometric mean ×/÷ SEM = 2,704 ×/÷ 1.95 IU/ml, range 266 to 7,897 IU/ml, $P < 0.001$). Three patients had levels that were higher than the geometric mean for the HIE patients.

During the past 2 yr, 34 serum samples from 11 HIE patients, 7 samples from 5 eczema patients, and 23 samples from 15 CGD patients have been assayed for IgG, IgA, and IgM. Ig levels for single serum samples from 28 normal volunteers were also obtained. As compared with the normal population (Fig. 2), the HIE patients had higher levels of IgG (geometric mean, 1,593 vs. 1,280 mg/dl; $P < 0.01$), normal levels of IgA (geometric mean, 161 vs. 217 mg/dl; not statistically significant), and higher levels of IgM (geometric mean, 200 vs. 135 mg/dl, $P < 0.05$). Note that 3 of the 11 HIE patients had relatively low levels of total IgA.

The CGD patients as a group were found to have normal levels of total serum IgG and essentially normal levels of total serum IgA but unexpectedly low levels of serum IgM ($P < 0.05$) (Fig. 2). There was a wide difference in total IgM values for the HIE patients (geometric mean, 200 mg/dl) as compared with the CGD patients (geometric mean, 95 mg/dl; $P < 0.001$).

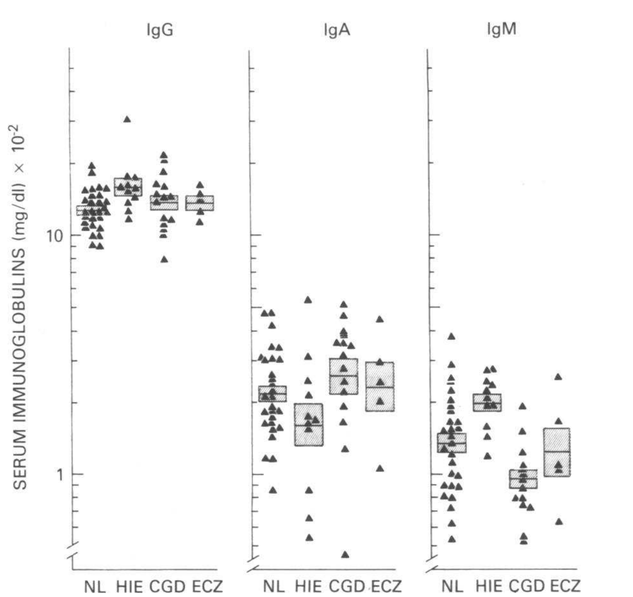

Figure 2. Total serum IgG, IgA, and IgM. The geometric mean and relative SE are shown for each group. For IgG: HIE vs. normal (NL) ($P < 0.01$). For IgM: HIE vs. NL ($P < 0.05$), CGD vs. NL ($P < 0.05$), and HIE vs. CGD ($P < 0.001$). Other comparisons were not significant. Eczema (ECZ).

Anti–S. aureus Igs

Anti–S. aureus IgE and IgD. Anti–*S. aureus* IgE values (Fig. 3) were elevated in 9 of 10 patients with HIE (3.0 AU/ml), confirming earlier reports (3, 4). The one patient with undetectable anti–*S. aureus* IgE was unusual in that his primary infectious process was cryptococcal esophagitis (26), although he had had occasional *S. aureus* skin abscesses. As expected, HIE patients with more IgE have more anti–*S. aureus* IgE, with a significant correlation between total IgE and anti–*S. aureus* IgE ($r = 0.84$, $P = 0.002$). In contrast with published reports (5) and in agreement with a recent observation (27), our assay indicated that patients with eczema and recurrent superficial *S. aureus* infections did not have detectable anti–*S. aureus* IgE (lower limits of detectability, 0.1 AU/ml). Normal serum plus myeloma IgE (1,500 ng/ml) did not have detectable anti–*S. aureus* IgE. Anti–*S. aureus* IgD was found in low titer in five HIE patients and in four normal control subjects without evidence of marked differences (data not shown).

Anti–S. aureus IgG. Anti–*S. aureus* IgG levels (Fig. 4) were significantly higher in CGD patients with recent *S. aureus* infections (solid and open circles) (geometric mean = 32.2 × 10^3 AU/ml; $P < 0.01$) and in patients with eczema and recurrent superficial *S. aureus* infections (geometric mean = 24.0 × 10^3 AU/ml; $P < 0.05$) as compared with normal

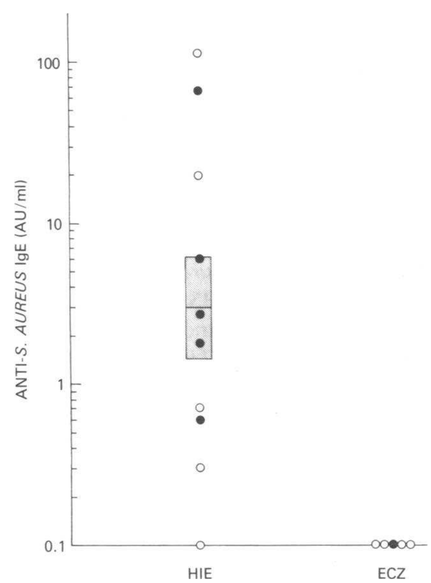

Figure 3. Anti–*S. aureus* IgE. Sera were adsorbed with protein A-Sepharose and assayed at dilutions ranging from 1:10 to 1:250. Samples that were negative for anti–*S. aureus* IgE at a 1:10 dilution were reassayed without absorption of IgG by protein A-Sepharose. At dilutions of 1:5 and 1:2, and even undiluted, anti–*S. aureus* IgE was still undetectable in their samples. ●, Patients with acute *S. aureus* infections; ○, other patients with a history of recent (within 2 yr) *S. aureus* infections. The geometric mean and relative SE are shown for each group. HIE patients vs. eczema (ECZ) patients ($P < 0.001$).

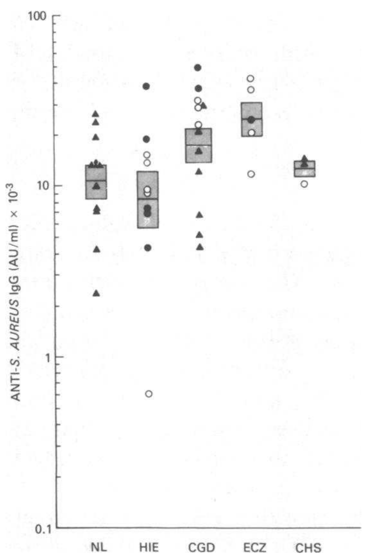

Figure 4. Anti–*S. aureus* IgG. Sera were assayed at dilutions ranging from 1:250 to 1:160,000. ●, Patients with acute *S. aureus* infections; ○, other patients with history of recent (within 2 yr) infections; ▲, normal control subjects (NL) and patients with no history of recent *S. aureus* infections. The geometric mean and relative SE are shown for each group. To simplify the figure, only one SE bar is shown for the CGD patients. However, for statistical comparison, these patients can be considered as a whole (CGD) or as two groups, those with recent *S. aureus* infections (CGD*) and those without recent documented *S. aureus* infections (CGD‡). CGD* vs. NL ($P < 0.01$), CGD‡ ($P < 0.01$), and CHS ($P < 0.001$). Eczema (ECZ) vs. NL ($P < 0.05$). HIE vs. CGD* ($P < 0.02$). Other comparisons were not significant.

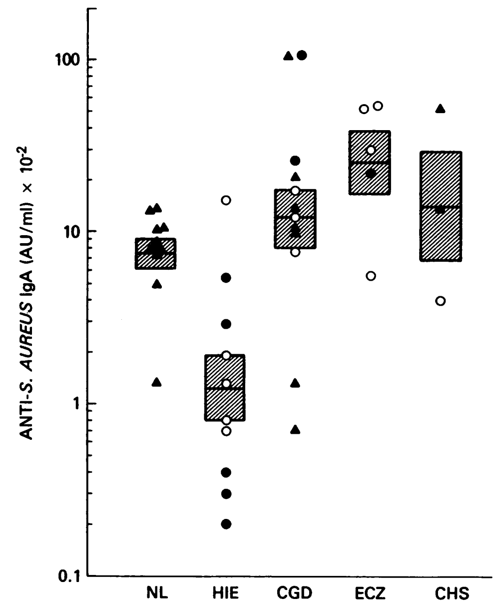

Figure 5. Anti–*S. aureus* IgA. Sera were assayed at dilutions ranging from 1:60 to 1:32,000. ●, Patients with acute *S. aureus* infections; ○, other patients with history of recent (within 2 yr) infections; ▲, normal control subjects (NL) and patients with no history of recent *S. aureus* infections. The geometric mean and relative SE are shown for each group. To simplify the figure, only one SE is shown for the CGD patients. However, for statistical comparison, these patients can be considered as a whole (CGD) or as two groups, those with recent *S. aureus* infections (CGD)* and those without recently documented *S. aureus* infections (CGD).‡ HIE vs. NL ($P < 0.001$), CGD ($P < 0.01$), CGD‡ ($P < 0.05$), CGD* ($P < 0.01$), eczema (ECZ) ($P < 0.001$), and CHS ($P < 0.02$) patients. CGD* vs. NL ($P < 0.02$) patients. ECZ vs. NL ($P < 0.01$) patients. Other statistical comparisons were not significant.

people (geometric mean = 10.3×10^3 AU/ml). It is important that the HIE patients (geometric mean = 8.3×10^3 AU/ml) lacked an expected high level of anti–*S. aureus* IgG as compared with normal people (geometric mean = 10.3×10^3 AU/ml; not statistically significant) or to recently infected CGD (geometric mean = 32.2×10^3 AU/ml; $P < 0.02$) subjects (circles). The GCD patients with recent *S. aureus* infection had higher anti-*S. aureus* IgG than did CGD patients without a history of recent *S. aureus* infections (triangles) (geometric mean = 10.7×10^3 AU/ml, $P < 0.01$).

Anti–S. aureus IgA (Fig. 5). The HIE patients' geometric mean levels of serum anti–*S. aureus* IgA (1.2×10^2 AU/ml) were significantly lower than those of the normal control subjects (7.5×10^2 AU/ml, $P < 0.001$), all patients with CGD (12.4×10^2 AU/ml, $P < 0.01$), CGD patients with recent *S. aureus* infections (21.6×10^2 AU/ml, $P < 0.001$), CGD patients without documented recent *S. aureus* infection (8.3×10^2 AU/ml, $P < 0.05$), patients with CHS (14.1×10^2 AU/ml, $P < 0.02$), and patients with eczema and recurrent superficial *S. aureus* infections (25.4×10^2 AU/ml, $P < 0.001$). There is also a positive correlation between total serum IgA and serum anti–*S. aureus* IgA ($r = 0.81$, $P = 0.004$). However, a significant correlation could not be demonstrated between total IgA and total IgE, or between anti–*S. aureus* IgA and anti–*S. aureus* IgE, or between salivary IgA (total or anti–*S. aureus*) and serum IgE (total or anti–*S. aureus*).

The CGD patients with recently documented *S. aureus* infections and the patients with eczema and recurrent superficial *S. aureus* infections had significantly higher anti–*S. aureus* IgA than did the normal population, suggesting that the lack of anti–*S. aureus* IgA in HIE is peculiar to this syndrome and is not an effect of frequent *S. aureus* infection. The low levels of anti–*S. aureus* IgA in HIE (1.2×10^2 AU/ml) are even more marked when compared with the larger amounts in the recently infected control groups (eczema patients, 25.4×10^2 AU/ml; recently infected CGD patients, 21.6×10^2 AU/ml).

Anti–S. aureus IgM. In patients with HIF, the levels of anti–*S. aureus* IgM (geometric mean = 29.6×10^2 AU/ml), (Fig. 6) were significantly higher than those in normal control subjects (10.6×10^2 AU/ml, $P < 0.001$), all CGD patients (9.4×10^2 AU/ml, $P < 0.01$), and patients with CHS (12.0×10^2 AU/ml, $P < 0.01$), Even those HIE patients without acute infections (open circles) tended to have higher than normal values of anti–*S. aureus* IgM.

Further investigation of IgA in HIE

Serum IgA directed against E. coli J5 LPS and the polyvalent pneumococcal vaccine polysaccharides. To evaluate the specificity of anti–*S. aureus* IgA deficiency in HIE, we chose to measure the levels of serum IgA directed against both the *E. coli* J5 LPS (22) and the antigens of the polyvalent pneumococcal vaccine in eight HIE patients and seven normal people, none of whom had been vaccinated with the pneumococcal vaccine (Fig. 7). Most of the HIE patients had normal or above normal serum anti–*E. coli* J5 LPS IgA. Similar data were obtained for anti-pneumococcal IgA. It is interesting that one patient had an undetectably low level of anti–*E. coli* J5 LPS IgA. She also had the lowest value of anti-pneumococcal polysaccharide IgA and among the lowest values for anti–*S. aureus* IgA in both serum and saliva (see below) and the lowest value of total IgA (54 mg/dl; normal range, 65–415 ng/dl). Thus, she may have a more generalized inability to mount

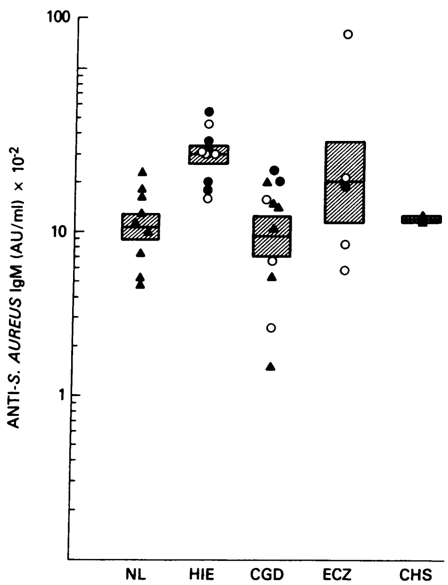

Figure 6. Anti-*S. aureus* IgM. Sera were assayed at dilutions ranging from 1:500 to 1:32,000. ●, Patients with acute *S. aureus* infections; ○, other patients with history of recent (within 2 yr) infections; ▲, normal control subjects (NL) and patients with no history of recent *S. aureus* infections. The geometric mean and relative SE are shown for each group. To simplify the figure, only one SE is shown for the CGD patients. However, for statistical comparisons, these patients can be considered as a whole (CGD) or as two groups, those with recent *S. aureus* infections (CGD)* and those without recently documented *S. aureus* infections (CGD).‡ HIE vs. NL ($P < 0.001$), CGD ($P < 0.01$), CGD‡ ($P < 0.01$), CGD* ($P < 0.01$), and CHS ($P < 0.01$). Other comparisons were not significant. Eczema (ECZ).

an antigen-specific IgA response. Nonetheless, in HIE patients as a group, the serum IgA against these common bacterial antigens was normal. This was in marked contrast to the deficiency of anti-*S. aureus* IgA shown in Fig. 5 and, in conjunction with the evidence of normal total serum IgA (Fig.

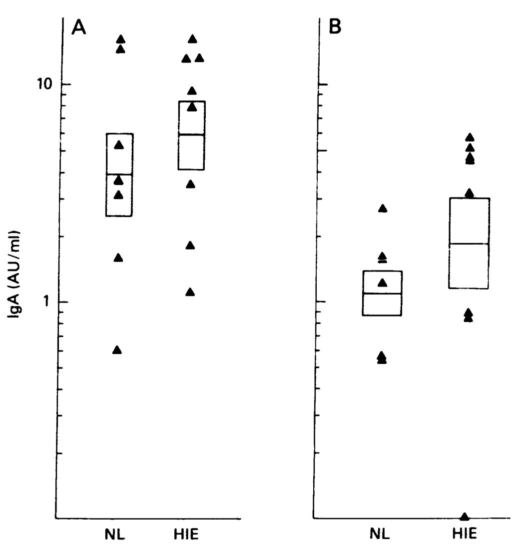

Figure 7. Serum IgA directed against the LPS from *E. coli* J5 (*A*) and against the polyvalent pneumococcal polysaccharide vaccine. (*B*). Sera were diluted 1:10–1:250 in *A* and 1:50–1:500 in *B*. The geometric mean and relative SE are shown for each group. No significant difference can be demonstrated between groups in either assay.

2), suggested that this abnormality of circulating anti-*S. aureus* IgA was probably restricted to idiotypes recognizing a specific antigen or set of antigens.

Salivary anti-S. aureus IgA

The finding that patients with HIE have a deficit of anti-*S. aureus* serum IgA led to the evaluation of salivary IgA and salivary anti-*S. aureus* IgA in these patients. As Fig. 8 shows, the patients with HIE have significantly decreased levels of salivary IgA per milligram of protein (Fig. 8 *A*) and decreased salivary anti-*S. aureus* IgA per milligram of protein (Fig. 8 *B*) as compared with the normal controls ($P < 0.01$ and $P < 0.05$, respectively).

One of the six CGD patients had undetectable levels of anti-*S. aureus* salivary IgA and thus is unusual. As compared with the five CGD patients with detectable salivary anti-*S. aureus* IgA, the HIE patients have a marked deficit of salivary anti-*S. aureus* IgA per milligram of protein ($P < 0.01$). If the data are re-expressed as anti-*S. aureus* salivary IgA (AU) per microgram of total salivary IgA, the HIE patients have a deficit (geometric mean = 0.478) as compared with the five CGD patients with detectable anti-*S. aureus* salivary IgA (geometric mean = 1.858; $P < 0.05$).

Correlation with infection

9 of the 10 HIE patients studied had detectable anti-*S. aureus* IgE. In these patients, infections occurring at sites (see Methods) where IgA may be an important host defense accounted for 75% (42 of 56) of all infections. 52% of these infections were due to *S. aureus*, 17% to *Haemophilus influenzae*, and 17% to *C. albicans*. There was an inverse correlation between serum anti-*S. aureus* IgA and the number of infections (Fig. 9 *A*; $r = -0.647$, $P = 0.034$). A similar negative correlation was seen between serum anti-*S. aureus* IgE and the number of infections (Fig. 9 *B*; $r = -0.731$, $P = 0.016$). Negative correlations with the number of infections were also noted for total serum IgE (Fig. 9 *C*; $r = -0.714$; $P = 0.020$) and for total serum IgD (Fig. 9 *D*; $r = -0.597$, $P = 0.049$). Other Ig's including serum anti-*S. aureus* IgG, serum anti-*S. aureus* IgM, salivary IgA, and anti-*S. aureus* IgA were not significantly correlated with the number of infections. If all infections (regardless of organism or site) were included, the negative correlation with anti-*S. aureus* IgE retained significance ($r = -0.593$, $P = 0.050$) as did the correlation with total serum IgD ($r = -0.729$, $P = 0.016$), but the other negative correlations lost their significance. When the 14 nonmucosal infections (80% of which were *S. aureus* abscesses) were considered separately, no significant correlations were found.

Discussion

The immunologic defect in HIE is not understood, but recent evidence suggests that there is an abnormality in the production of Ig after stimulation in vitro with pokeweed mitogen (13) and exposure of patients to exogenous antigens in vivo (1, 28). Information about humoral immunity to *S. aureus* in HIE is scant. Serum anti-*S. aureus* agglutinin titers, when reported (29, 30), have been normal in three patients and absent in one. To further our understanding of this syndrome, we have developed a new method to measure the anti-*S. aureus* specificity of Ig isotopes in HIE and have compared this patient population with appropriate control groups that have

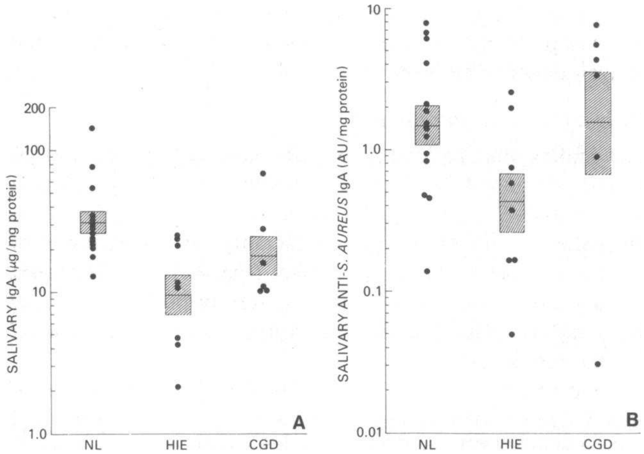

Figure 8. Samples of parotid gland saliva were assayed for total IgA (*A*) and for anti-*S. aureus* IgA (*B*). Data are expressed per milligram of protein. Saliva samples were diluted from 1:1,000 to 1:16,000 in *A* and 1:5 to 1:80 in *B*. The geometric mean and relative SE are shown for each group. (*A*) HIE vs. NL ($P < 0.01$), HIE vs. CGD (NS). (*B*) HIE vs. NL ($P < 0.05$), HIE vs. CGD (NS).

frequent *S. aureus* infections. Our findings indicate that, in HIE, elevated serum levels of total IgE and IgD are accompanied by increased total IgG, normal total IgA, and elevated total IgM (Fig. 2). In addition, total salivary IgA is decreased (Fig. 8 *B*). HIE is characterized both by the presence of markedly elevated serum anti-*S. aureus* IgE (Fig. 3) and by a deficiency of anti-*S. aureus* IgA in both serum (Fig. 5) and saliva (Fig. 8 *B*). Furthermore, in HIE the incidence of infection at mucosal surfaces and adjacent lymph nodes (Fig. 9) is inversely proportional to the low levels of serum anti-*S. aureus* IgA and the elevated levels of serum anti-*S. aureus* IgE, total serum IgE, and total serum IgD.

The method for assay of anti-*S. aureus* Ig's that we developed is similar to the assays used by Schopfer et al. (3), Berger et al. (4), and Walsh et al. (5) to measure anti-*S. aureus* IgE in that all antigenic moieties of the Wood's strain of *S. aureus*, both soluble and insoluble, are present in the assay. The advantages of our ELISA are the absence of radioactivity and direct measurement of the Ig bound to *S. aureus* as compared with the measurement of the removal of iodinated antibody from solution. In addition, we took great care to evaluate the specificity of the enzyme-linked anti-Ig antibodies with the same ELISA technology used in the anti-*S. aureus* Ig assay.

Although anti-*S. aureus* IgE is correlated with total IgE in HIE, the presence of anti-*S. aureus* IgE cannot be explained solely on the basis of elevated total IgE, and exposure to *S. aureus*. Specifically, we have shown that the control patients with eczema, elevated IgE, and recurrent superficial *S. aureus* infection have evidence of an immunologic response to *S. aureus* (elevated anti-*S. aureus* IgG, IgA, and IgM) (Figs. 4–6) but do not have detectable anti-*S. aureus* IgE (Fig. 3 and reference 27).

The deficit of anti-*S. aureus* IgA is not explained solely on the basis of lower total IgA, as the total serum IgA is normal. In addition, seven of eight HIE patients had normal anti-*E. coli* J5 LPS IgA, and eight of eight had normal, naturally occurring IgA antibodies against the antigens present in the pneumococcal polysaccharide vaccine. These findings are compatible with apparently normal immunity (in HIE) to gram-negative organisms and *Streptococcus pneumoniae*. Although it is impossible to repeat this work with every strain of *S. aureus*, we have checked for the presence of anti-*S. aureus* IgA by the use of a clinical isolate of *S. aureus* containing protein A and we have found (in the presence of saturating amounts of rabbit Fc fragments to block nonspecific binding of Ig to protein A) that the observed deficit in anti-*S. aureus* IgA is still detectable (data not shown). Patients with HIE are known to have IgE directed against *C. albicans* as well as against *S. aureus* (4), and, therefore, a deficiency in anti-*C. albicans* IgA might be expected. In a preliminary study (data

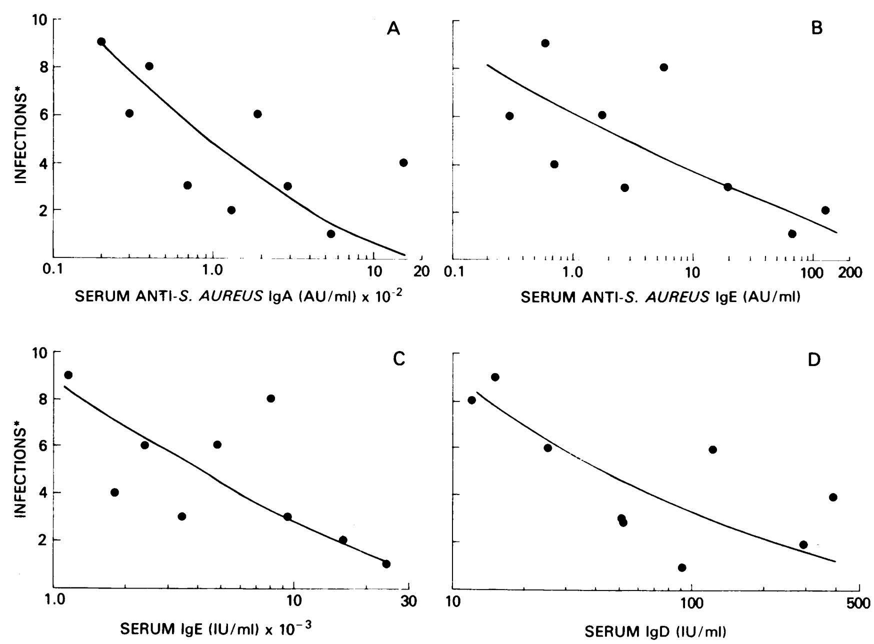

Figure 9. Correlation of serum Ig levels in HIE with number of documented infections from January, 1981 to the present. *Infections at mucosal surfaces and in adjacent lymph nodes (see Methods). (*A*) Anti-*S. aureus* IgA ($r = -0.647$, $P = 0.034$); (*B*) anti-*S. aureus* IgE ($r = -0.731$, $P = 0.016$); (*C*) total IgE ($r = -0.714$, $P = 0.020$); and (*D*) total IgD ($r = -0.597$, $P = 0.049$). Lines are drawn for the purpose of interpretation.

not shown) of anti–*C. albicans* serum IgA in HIE, we found a marked deficit in two of six patients tested and a moderate deficit in one other. Thus, there is a deficit of serum IgA against *C. albicans,* but it does not appear to be as prominent as the deficit of anti–*S. aureus* serum IgA.

The role of serum IgA is unknown, although there is evidence that it is important in the body's defense against *Neisseria meningitis* (31). Secretory IgA on the other hand has a well-defined role in interfering with bacterial adherence (32, 33) and may be important in the phagocytosis of bacteria by oral neutrophils (34). The possibility that deficiencies of organism-specific serum and secretory IgA contribute to the recurrent mucosal infections in HIE is intriguing and merits additional investigation.

The negative correlation between serum levels of total IgE, anti–*S. aureus* IgE, and total IgD with the number of infections (Fig. 9, *A–D*) suggests that these substances are not responsible for recurrent infections in HIE and may be protective. If IgE were detrimental, one would expect a positive correlation between the incidence of infection and the IgE levels. Alternatively, IgE and anti–*S. aureus* IgE may play a dual role such that the presence of large amounts of IgE is detrimental (35) but that even larger amounts of IgE are protective. Evidence for a protective role for IgE has been published (36). Rat IgE can mediate rat eosinophil-dependent cytotoxicity against *Shistosoma mansoni* shistosomula (37) and has been shown to interact with monocytes from atopic patients and to mediate monocyte cytotoxic function against IgE-coated target cells (38).

The demonstration of a deficiency of anti–*S. aureus* IgA (in serum and saliva) in conjunction with elevated serum anti–*S. aureus* IgE, elevated serum anti–*S. aureus* IgM, and the lack of an expected excess of serum anti–*S. aureus* IgG describes a unique abnormality of the humoral immune system in HIE. These findings offer new insight into the basis of increased susceptibility to infection in the HIE syndrome and open new avenues for the investigation of isotype-specific responsiveness to common pathogens in man.

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