CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, May 1995, p. 272–276 Vol. 2, No. 3
1071-412X/95/$04.00+0

Effect of Age on Concentrations of Serum Antibodies to Viral, Bacterial, and Food Antigens in Elderly Swiss People

HARALD BRÜSSOW,[1]* JOSETTE SIDOTI,[1] ANNY BLONDEL-LUBRANO,[1] YVES BOREL,[1] JEAN-PIERRE MICHEL,[2] HENRI DIRREN,[1] AND BERNARD DECARLI[1]

Nestec Ltd., Nestlé Research Centre, Vers-chez-les-Blanc, CH-1000 Lausanne 26,[1] and Institutions Universitaires de Gériatrie, Geneva,[2] Switzerland

Received 10 August 1994/Returned for modification 13 October 1994/Accepted 20 February 1995

Serum antibody concentrations to two viral, five bacterial, and two food antigens were investigated in 307 elderly Swiss subjects, and the hypothesis of whether serum antibody titers decreased with age was tested. The cross-sectional part of the study consisted of 216 unselected consecutive patients hospitalized in one geriatric hospital. The patients were divided into two age groups (65 to 84 and 85 to 102 years old), and their antibody titers were compared. No age-related decreases in antibody titers were observed. The members of the two age groups were well matched for medical diagnosis and nutritional and inflammatory status. The prospective part of the study consisted of 91 healthy elderly subjects living in the community; they were 71 to 76 years old when they were enrolled in the study. Their serum antibody status was measured at the beginning of the study and 4 years later. We observed a significant decrease in diphtheria antitoxin levels and a significant increase in antibody titer to the capsular polysaccharide of *Streptococcus pneumoniae*. No change in antibody titer to rotavirus, respiratory syncytial virus, lipopolysaccharide of *Escherichia coli*, C polysaccharide of *S. pneumoniae*, or the polyribosyl-ribitol phosphate of *Haemophilus influenzae* was observed. Thus, no signs of B-cell immunosenescence were seen in these two groups of elderly Swiss people.

Elderly people have an increased incidence and severity of certain infections (1). Respiratory tract infections are the fourth leading cause of death in elderly people (1). Elderly people have a distinct predilection for developing nosocomial infection. *Streptococcus pneumoniae* may cause pneumonia in 10 to 20% of patients with nosocomial infection (1). In the community, respiratory syncytial virus is as important as influenza viruses as a cause of morbidity and mortality among elderly people (16). Elderly people also experience increased mortality from infectious diarrhea. Norwalk virus, rotavirus, and *Escherichia coli* have produced well-described outbreaks of diarrheal illnesses in elderly patients (19, 23). In addition, malignancies and autoimmune disorders are more common in elderly people. All of these trends may reflect deficiencies in the immune system. Age-related changes in immune responses have therefore been the focus of much recent work, and the topic has been reviewed extensively (11, 24). However, many studies used aged animals as a model, and among the studies in humans, few studies were longitudinal. Many published cross-sectional studies did not include appropriate controls for confounding factors. The underlying disease status of the elderly subjects (e.g., the presence of a carcinoma or inflammation) might influence the immune response independently of age. Many elderly people also suffer from nutritional deficiencies which could influence their immune status. An increased intake of drugs in elderly people could also influence their immune responses. In the present study we investigated the serum antibody concentrations to enteric (rotavirus, *E. coli*) and respiratory (respiratory syncytial virus, *S. pneumoniae*, *Haemophilus influenzae*) pathogens, childhood vaccine antigens (diphtheria toxoid), and food antigens (two cow's milk proteins) in 216 patients ranging in age from 65 to 102 years. Antibodies to diphtheria toxoid were measured to test for antibody levels as a result of vaccination received in earlier years, and β-lactoglobulin- and bovine serum albumin-specific antibodies were measured to see whether the immune tolerance to a food antigen was breached in old age.

The subjects from the cross-sectional study were consecutively hospitalized patients from one geriatric hospital in Geneva, Switzerland. The study was conducted in the winter season of 1989 and 1990. The effect of age on antibody titers was analyzed and controlled for some confounding factors (sex, inflammation, nutritional status, and disease status). In addition, specific antibody concentrations were determined in serum samples obtained in 1989 and once again in 1993 from 91 healthy elderly subjects living in a Swiss city 80 km from Geneva. These subjects were part of a European longitudinal nutrition and health study (13). Also, in the present prospective study we investigated the effect of age on antibody titers. In both studies antibody titers to bacterial and viral pathogens did not decrease with increasing age.

MATERIALS AND METHODS

Serum samples (cross-sectional study). A total of 216 serum samples were obtained from the Geriatric Institute of the University of Geneva. The serum samples were collected between October 1989 and June 1990 from consecutively hospitalized patients. The medical diagnosis of the hospitalized patients was established, and the patients were grouped into four roughly defined subgroups (carcinoma, neurological and psychiatric diseases, falls and fractures, and internal and urological diseases). Many patients suffered from several diseases. These patients were classified according to the most serious clinical condition. For 33 patients no clear diagnosis was obtained or data were lacking. Sex and year of birth were documented for each patient.

Serum samples (prospective study). Serum samples were obtained from 91 noninstitutionalized elderly subjects living in Yverdon, a Swiss city 80 km from Geneva. The study population was part of the Euronut SENECA study conducted in 19 towns from 12 European countries (13). Yverdon was chosen because it showed a socioeconomic structure comparable to that of all of Switzerland. The protocol called for a random sample stratified by sex of elderly people born between 1913 and 1918. Nine of the research sites, including Yverdon, added a longitudinal component to the study. For subjects from Yverdon we received serum samples which were collected in 1989 and 1993.

Nutritional status. The weights of the study subjects were measured with standardized, good-quality weighing scales. Height was measured in the standing position with a stadiometer whenever possible. The body mass index (BMI) was

* Corresponding author.

TABLE 1. Characteristics of the study populations

Parameter	Elderly	Elderly	Very elderly
Study area	Yverdon	Geneva	Geneva
Time period	1989–1993	1989–1990	1989–1990
Study type	Prospective	Cross-section	Cross-section
Residence	Community	Hospital	Hospital
No. of subjects	91	119	97
Age (yr)			
Mean ± SD	73.2 ± 1.8[a]	79.2 ± 4.2	89.7 ± 3.7
Range	71–76[a]	65–84	85–102
Sex (% female)	45	61	77
Diagnosis (%)			
Cancer		14	20
Neurology		28	23
Internal medicine		32	36
Fractures		10	7
No data		16	14
BMI (mean + SD)	26.0 ± 3.8[a]	22.7 ± 3.7	22.2 ± 3.9
% of subjects with BMI of <20	4	21	30
% of subjects with BMI of >25	56	23	19
CRP concn (mg/liter [mean ± SD])	4.0 ± 6.8[b]	23.0 ± 38	25.6 ± 42
% of subjects with CRP concn of >10 g/liter	8	44	53
AGP concn (g/liter [mean ± SD])[c]	0.93 ± 0.25[b]	0.96 ± 0.38	0.95 ± 0.36
% of subjects with AGP of >1.4 g/liter	3	11	14
Albumin concn (g/liter [mean ± SD])	41.8 ± 2.7[a]	30.6 ± 6.0	29.4 ± 4.8
% of subjects with albumin concn of <30 g/liter	None	48	60
Prealbumin concn (g/liter [mean ± SD])	NT[d]	0.18 ± 0.06	0.16 ± 0.05
% of subjects with prealbumin concn of <0.15 g/liter	NT	32	40

[a] Status in 1989.
[b] Status in 1993.
[c] AGP, α-1-acid glycoprotein.
[d] NT, not tested.

calculated as weight/height^2. BMIs less than 20 kg/m^2 were considered underweight and BMIs greater than 25 kg/m^2 were considered overweight.

Serum protein determinations. C-reactive protein (CRP) was measured by latex-enhanced agglutination measured on a nephelometer. A commercial test kit was used (Behring). CRP concentrations greater than 10 mg/liter were considered indicative of a state of inflammation. Serum albumin, prealbumin, and α-1-acid glycoprotein concentrations were determined by nephelometry (Behring, Marburg, Germany) with a commercial kit providing adsorbed, specific antisera to albumin, prealbumin, and α-1-acid glycoprotein, respectively, and standardized reference sera (Behring). Albumin concentrations less than 30 g/liter and prealbumin concentrations less than 0.15 g/liter were classified as low. A serum α-1-acid glycoprotein concentration greater than 1.4 g/liter was considered an indicator of inflammation.

ELISA. The relative serum antibody concentrations were determined by enzyme-linked immunosorbent assays (ELISAs) in serum samples diluted 1:100 in phosphate-buffered saline solution containing 0.05% Tween 20. Dilutions of 1:100 were chosen because at this dilution the optical density (OD) readings were linearly proportional to the relative antibody concentrations, measured by endpoint titration. OD readings obtained with the sera on plates covered with coating buffer only were subtracted from the test values. Reference sera were included on all test plates to correct for plate-to-plate variations. All ELISA procedures were described previously and were validated in seroprevalence studies (2–8). Bound antibody was revealed with affinity-purified antibody to human immunoglobulin G (IgG) or IgM coupled to alkaline phosphatase by using Sigma 104 substrate. Conjugate dilutions were chosen so that 10 ng of IgG or IgM per ml produced an absorbancy of 0.1 OD unit.

RESULTS

Clinical characteristics of the study populations. The cross-sectional study group consisted of 216 consecutive patients hospitalized in one geriatric hospital. The patients ranged in age from 65 to 102 years. They were subdivided into two age groups, those up to 84 years old (elderly patients) and those 85 years old and older (very elderly patients). Table 1 provides a clinical comparison of the elderly and the very elderly patients. No difference in the disease type leading to hospitalization was found. No difference in mean BMI was detected between the two groups, although underweight patients were more prevalent among the very elderly patients. In both groups about half of the patients showed elevated concentrations of CRP indicating a state of inflammation. The CRP concentration was positively correlated with α-1-acid glycoprotein concentration ($r = 0.54$) and was negatively correlated with prealbumin and albumin concentrations ($r = -0.44$ and -0.32, respectively). Albumin and prealbumin concentrations were correlated with each other ($r = 0.66$), but not with BMI. Therefore, low levels of albumin and prealbumin in serum are not indicators of a

poor nutritional status but of negative acute-phase proteins. Twenty-three patients showed CRP concentrations of >50 mg/liter, 10 suffered from bronchopneumonia, 3 suffered from cancer, 3 showed myocardial infarcts, and an additional 4 patients showed various conditions associated with inflammation. As expected, the prevalence of female patients was higher among the very elderly patients than among the elderly patients.

Because of the inclusion criteria, the subjects enrolled into the prospective study were younger than the hospitalized patients and the sexes were equally represented. The study participants answered questionnaires with respect to self-perceived health status, presence of chronic diseases, and use of medications. Among the subjects from Yverdon, 84% claimed a good health status, and only 3% reported a poor health status. Twenty-three percent reported that they had a chronic disease, the major causes being arthrosis, heart disease, and hypertension, and 30% took medications. Very few subjects showed a BMI indicative of being underweight, and none had serum albumin levels of less than 30 g/liter. A total of 8 and 3% of the subjects showed elevated CRP protein and α-1-acid glycoprotein concentrations, respectively; both measures were correlated ($r = 0.59$). Subjects who were overweight were significantly more prevalent among the subjects of the prospective study than among the hospitalized patients of the cross-sectional study.

Effect of age on antibody concentration. We measured titers of antibodies to a number of viral, bacterial, and food antigens in the sera collected for both studies to test whether specific antibody concentrations decreased with age. In the cross-sectional study the serum antibody concentrations to viral (rotavirus and respiratory syncytial virus), bacterial (*S. pneumoniae*, *H. influenzae*, *E. coli*, and *Corynebacterium diphtheriae*), and food (β-lactoglobulin and bovine serum albumin) antigens determined by ELISA did not differ significantly between the elderly and the very elderly patients (Table 2). The only exception was the IgG antibody titer to rotavirus, which was significantly, but not substantially, higher in the very elderly patients than in the elderly patients. We calculated the correlation coefficient between age and the concentration of each antibody specificity. The highest was that between age and rotavirus antibody titer ($r = 0.18$).

In the prospective study, the concentrations of six of nine antibody specificities did not change significantly among the elderly subjects within the 4-year survey period when they were tested by paired *t* tests (Table 3) (as an example, see rotavirus-specific IgG antibody titers in Fig. 1A). The serum antibody concentrations to diphtheria toxoid, however, showed a significant decrease over this 4-year period when they were analyzed by paired *t* test (Table 3) and by scatter plot (Fig. 1B). In contrast, antibody to capsular polysaccharides of *S. pneumoniae* showed a significant increase in titer within the survey period (Table 3; Fig. 1C). Age-related increases in titers were of borderline significance for antibody to lipopolysaccharides of *E. coli* (Table 3; Fig. 1D).

Effect of sex on antibody concentration. Because the sex ratio among the very elderly patients differed from that among the elderly patients, the hospitalized patients were stratified by sex and investigated for the effect of age on antibody concentrations. No differences were found. In the prospective study only one antibody specificity showed a statistically significant difference with sex: male patients showed higher IgG antibody concentrations to the C polysaccharide of *S. pneumoniae* (means ± standard deviations for male subjects in 1989 and 1993, 0.43 ± 0.08 and 0.42 ± 0.08, respectively, versus 0.35 ± 0.0.09 and 0.34 ± 0.08, respectively, for the female subjects; $P < 0.0001$ in both cases).

TABLE 2. Serum antibody concentrations in ELISA to viral, bacterial, and food antigens in elderly and very elderly hospitalized patients from the cross-sectional study in Geneva

Antibody to indicated antigen[a]	Mean OD ± SD		P^b
	Elderly patients	Very elderly patients	
T-cell-dependent antigens (IgG class)			
Rotavirus	0.37 ± 0.11	0.41 ± 0.12	0.009
Respiratory syncytial virus	0.20 ± 0.05	0.20 ± 0.05	NS[c]
Diphtheria toxoid	0.05 ± 0.03	0.05 ± 0.04	NS
β-Lactoglobulin	0.08 ± 0.06	0.07 ± 0.06	NS
Bovine serum albumin	0.06 ± 0.06	0.05 ± 0.03	0.03
T-cell independent antigens			
IgM class			
E. coli LPS	0.12 ± 0.09	0.11 ± 0.07	NS
Hib PRP	0.20 ± 0.10	0.20 ± 0.09	NS
S. pneumoniae CPS	0.18 ± 0.11	0.17 ± 0.09	NS
IgG class			
E. coli LPS	0.11 ± 0.07	0.10 ± 0.07	NS
Hib PRP	0.12 ± 0.05	0.12 ± 0.06	NS
S. pneumoniae CPS	0.32 ± 0.14	0.34 ± 0.17	NS
S. pneumoniae C polysaccharide	0.48 ± 0.14	0.45 ± 0.14	NS

[a] LPS, lipopolysaccharides from *E. coli*; PRP, polyribosyl-ribitol phosphate antigen from *H. influenzae* type b (Hib); CPS, capsular polysaccharides from *S. pneumoniae.*
[b] Probability for difference between both groups; *t* test.
[c] NS, difference not significant.

Effect of nutritional status, inflammation, and disease type on antibody concentration. In the cross-sectional study underweight patients (defined by either BMI < 20 or, more strictly, by BMI < 18.5) and overweight patients (BMI > 25) did not differ from patients with BMIs of between 20 to 25 with respect to any antibody concentration. None of the antibody concentrations differed among patients with elevated CRP concentrations (>10 mg/liter) and patients with lower CRP concentrations. No significant differences in antibody concentrations were seen among patients with different diagnoses.

In the prospective study, the concentration of any antibody in overweight subjects did not differ from that in subjects with normal BMIs. Too few subjects showed poor nutritional status, inflammation, or disease to allow for a meaningful analysis of a correlation with antibody concentrations.

DISCUSSION

In contrast to numerous studies of age-associated alterations of T-cell function, those involving B-lymphocyte function or humoral immunity are relatively few (24). A general decrease in antibody response to T-cell-dependent and -independent antigens with increasing age has been reported in aging mice (9, 17, 20). Several studies in humans have revealed that both primary and secondary antibody responses to immunization with hepatitis B virus (14), influenza virus (21), pneumococcal (25), and tetanus (18) vaccines are impaired in elderly subjects. However, essentially normal antibody responses by elderly people to pneumococcal (22) and tetanus (26) vaccines have been reported by others.

Our cross-sectional study with hospitalized geriatric patients did not provide indications of decreased serum antibody levels

TABLE 3. Serum antibody concentrations by ELISA to viral, bacterial, and food antigens in noninstitutionalized elderly people from the longitudinal study in Yverdon

Antibody to indicated antigen[a]	Mean OD ± SD		r^b	P^c
	1989	1993		
T-cell-dependent antigens				
IgG class				
Rotavirus	0.25 ± 0.06	0.25 ± 0.06	0.87	NS[d]
Respiratory syncytial virus	0.19 ± 0.06	0.19 ± 0.06	0.78	NS
Diphtheria toxoid	0.12 ± 0.07	0.10 ± 0.06	0.91	<0.00001
β-Lactoglobulin	0.10 ± 0.06	0.11 ± 0.07	0.63	NS
IgM class, rotavirus	0.13 + 0.05	0.13 + 0.04	0.85	NS
T-cell-independent antigen (IgG class)				
E. coli LPS	0.15 ± 0.08	0.16 ± 0.09	0.88	0.03
Hib PRP	0.16 ± 0.07	0.15 ± 0.07	0.81	NS
S. pneumoniae CPS	0.35 ± 0.13	0.39 ± 0.13	0.82	<0.0001
S. pneumoniae C polysaccharide	0.39 ± 0.09	0.38 ± 0.09	0.78	NS

[a] LPS, lipopolysaccharides from *E. coli*; PRP, polyribosyl-ribitol phosphate antigen from *H. influenzae* type b (Hib); CPS, capsular polysaccharides from *S. pneumoniae.*
[b] Correlation coefficient in the Pearson test.
[c] Probability in paired *t* test that the difference is distinct from 0; two-tailed test.
[d] NS, difference not significant.

in very elderly patients compared with those in elderly patients. We considered the possibility that the antibody levels in the elderly patients were already decreased in comparison with those in members of younger age groups such that no further decreases could be observed when elderly and very elderly patients were compared. To this purpose the antibody levels of the elderly patients were compared with the titers found in a collection of 40 blood donors; no decreased titers were observed (data not shown).

A major problem with studies on immunological changes with age in humans is the separation of these effects from the physiological changes that are secondary to disease that fre-

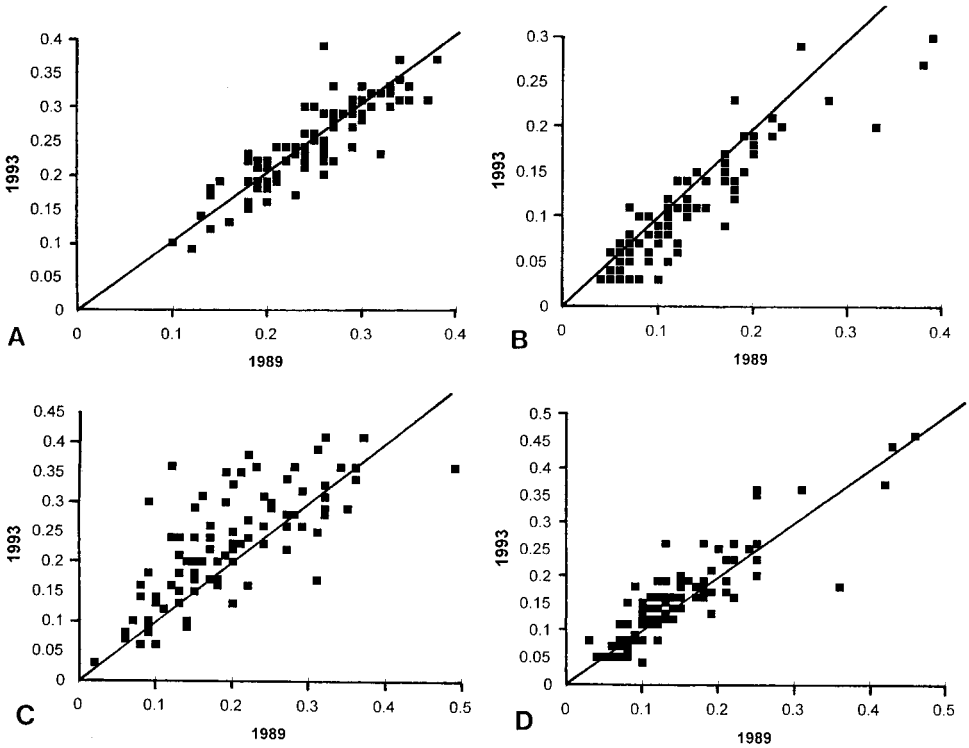

FIG. 1. Scatter plot of specific antibody titers (expressed as OD reading in ELISA) in serum samples obtained in 1989 and 1993 from the same 91 elderly Swiss subjects in the prospective study. (A) Data for rotavirus IgG antibody ($r = 0.87$; $P = 0.53$ in paired *t* test [two-tailed] for a significant deviation from difference of 0). (B) Data for IgG antibody to diphtheria toxoid ($r = 0.91$; $P < 0.00001$). (C) Data for IgG antibody to capsular polysaccharides of *S. pneumoniae* ($r = 0.76$; $P < 0.0001$). (D) Data for IgG antibody to lipopolysaccharides of *E. coli* ($r = 0.88$; $P = 0.03$).

quently accompany aging. Our cross-sectional study suffers from the fact that a complicated group of geriatric patients with a multiplicity of underlying diseases was studied. Comparison of the elderly and the very elderly patients, however, did not reveal major differences in diagnosis or nutritional or inflammatory status between these groups. Stratification for these factors did not reveal an association with antibody levels.

We addressed the question of decreased antibody response with increasing age with an additional study group living in a community not far away from the geriatric hospital. Serum sampling at the two sites started at the same time. The prospective character of this second survey ensured that we could measure the difference in antibody titer over a 4-year time period in healthy elderly patients who did not demonstrate significant changes in inflammatory or nutritional status (data not shown). Significant age-related changes were not demonstrated for most antibody concentrations. The exceptions were antitoxin antibody titers, which decreased with age, and anti-*S. pneumoniae* antibody titers, which increased with age. The results might not be contradictory because two different types of immune response were measured. Antitoxin antibodies were induced by childhood vaccination and were rarely if ever refreshed by revaccination. This component of the serum antibody response might thus demonstrate some kind of immunosenescence. On the other hand, most of the elderly people might have encountered many of the pathogens to which antibody responses were evaluated in the present study. It is well-known that elderly people are more susceptible to respiratory pathogens, especially *S. pneumoniae*; therefore, the increase in antibody titers to capsular polysaccharides of *S. pneumoniae* might reflect recent reinfection. The observation of increased antirotavirus antibody titers in the older geriatric patients could reflect their exposure to rotavirus in nursing homes and hospitals. However, one cross-sectional study (15) reported a decrease in rotavirus antibody titers when elderly and very elderly hospitalized patients in Britain were compared, but the statistical power of that study was limited because only 12 patients older than 80 years were investigated.

Certain old individuals are particularly at risk of malnutrition: those living alone, those with chronic systemic diseases, those with mental impairment, the very old, and the very poor. Anthropometric evaluation provides an objective method for the diagnosis of protein-energy malnutrition (10). The simultaneous assessment of nutritional status and immune responses and subsequent correlation analysis have suggested that impaired immunity in elderly people may be due in part to associated nutritional deficiencies (10). Prealbumin levels have been reported to correlate with impaired immune responses (10). These observations have led to proposals to improve immunocompetence by nutritional intervention in elderly people. In fact, one study reported an improved antibody response to influenza vaccine in elderly people by providing nutritional support (12). In the present study we did not find a correlation between nutritional status and serum antibody concentrations. The power to detect such an effect, however, was low in the present study. In the prospective survey the nutritional status of most participants was excellent, and in the geriatric patients serum albumin and prealbumin levels were not informative because they were low because of inflammation.

ACKNOWLEDGMENTS

We thank Andreas Rytz for help with the statistical evaluations and Claude Cavadini for helpful discussion.

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