Effects of Ageing and Gender on Naturally Acquired Antibodies to Pneumococcal Capsular Polysaccharides and Virulence-Associated Proteins

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Received 18 March 2008/Returned for modification 18 April 2008/Accepted 26 June 2008

Elderly individuals are susceptible to pneumococcal infections. Although factors contributing to the increased susceptibility of the elderly to bacterial infections may be several, compromised immune function, a consequence of normal human ageing, is widely accepted to play a role. We evaluated the effect of ageing on the concentrations of naturally acquired antibodies to pneumococcal capsular polysaccharides (PPS) and protein antigens. The concentrations of immunoglobulin G (IgG) and IgM antibodies to the PPS of serotypes 3, 4, 6B, 9V, 14, and 23F and IgG antibodies to the pneumococcal virulence-associated proteins CbpA, LytC, PhdT and its C-terminal fragment (PhdT C), NanA, PspA fam1, and PspA fam2 were measured by enzyme immunoassay in the sera of younger (30 to 64 years of age) and elderly (65 to 97 years of age) adults. The concentrations of anti-PPS IgG against serotypes 3 and 6B, of anti-PPS IgM against serotypes 3, 4, 6B, 9V, and 23F, and of anti-protein IgG against all tested antigens were significantly lower in the elderly than in younger adults. A stronger decline in anti-PPS antibody concentrations was seen with age in women compared to men, while anti-protein antibody concentrations were mainly similar between the genders. Age, gender, and the nature of the antigen have substantial and varying effects on the antibody concentrations in the sera of adults.

Streptococcus pneumoniae causes a wide variety of infections, ranging from common upper respiratory tract infections to rare, severe, and potentially life-threatening conditions, including pneumonia, bacteremia, and meningitis. A major individual risk factor for pneumococcal infections is ageing (40), which can be seen by the increasing incidence of community-acquired pneumonia. Pneumococcal CAP (10), a common disorder among the aged.

Ageing of the immune system contributes to the increased susceptibility to infections in the elderly, although many coexisting chronic illnesses accumulated in elderly people likely act as important underlying cofactors (6). The mechanisms involved in the impaired immune defense are still poorly understood. Ageing is known to have widespread effects on the immune system, including decreases in B- and T-lymphocyte production, as well as perturbations in the function of mature B and T cells (24, 44). These age-associated changes lead up to an impairment of both humoral and cell-mediated immunity, causing a generalized decrease in immune responsiveness. As a consequence, the duration of humoral response is shorter and the quality of produced immunoglobulins is impaired in the aged compared to younger adults (21).

Exposure to S. pneumoniae induces natural antibodies against pneumococcus in the sera of children (29, 42) and adults (11). Existing data on the concentrations of antibodies against pneumococcal antigens acquired during periods of pneumococcal carriage and disease in an unvaccinated elderly population are limited. Concentrations of immunoglobulin G (IgG) antibodies to pneumococcal capsular polysaccharides (PPS) have been found to remain unchanged or decrease by age, depending on the serotype and the study (1, 33, 35). Age-specific development of antibody concentrations to pneumococcal proteins PsaA, PspA, and pneumolysin from young to old has been assessed in a Kenyan study with no decline in ageing adults (20). No previous data are available on the concentrations of IgM antibodies to PPS in the elderly, but a dramatic decline in the numbers of IgM memory B cells has been found with ageing (38).

We determined the concentrations of naturally acquired IgG and IgM antibodies in a large number of sera from younger (30 to 64 years of age) and elderly (≥65 years of age) adults to PPS of six serotypes commonly causing IPD in the elderly. In addition, the concentrations of IgG antibodies to seven essential pneumococcal virulence-associated proteins were analyzed. The antibody results of the elderly were compared to those of the younger adults to evaluate whether any age-associated changes could be demonstrated in the antibody concentrations. We found that age, gender, and the nature of the antigen have substantial and varying effects on the antibody concentrations in the sera of adults.

MATERIALS AND METHODS

Study population and clinical samples. Serum samples for the purposes of the present study came from the Health 2000 Study, a nationally representative health survey of 9,922 adults aged 18 years or older, carried out in Finland in 2000 to 2001 (http://www.ktl.fi/health2000). A serum sample of each participant aged 30 years or older has been reserved for infectious disease serology. The study protocol was approved by the project group of the Health 2000 study and evaluated by the ethics committee of the National Public Health Institute. Altogether, 600 randomly picked serum samples were received: 300 samples from younger adults (aged 30 to 64 years; 150 men and 150 women), with a mean age of 48 years, 300 samples from elderly adults (aged 65 to 97 years; 150 men and 150 women), with a mean age of 77 years. The 300 younger adults were further stratified into three age groups with mean ages of 37 (30 to 44 years; n = 100), 48 (45 to 54 years; n = 100), and 59 (55 to 64 years; n = 100) years. The 300 elderly adults were further stratified into three age groups with mean ages of 69 (65 to 74 years; n = 120), 79 (75 to 84 years; n = 100), and 90 (85 to 100 years; n = 80).
FIG. 1. Effect of ageing and gender on naturally acquired serum IgG antibodies against PPS in adults. (A) Anti-PPS IgG antibodies in the sera of younger (*n = 300) and elderly (*n = 300) adults. *, *P < 0.05 (Student t test). (B) Anti-PPS IgG antibodies in the sera of younger men (*n = 150) and women (*n = 150) and in the sera of elderly men (*n = 150) and women (*n = 150). *, *P < 0.05 (Student t test).

RESULTS
Effect of ageing and gender on naturally acquired anti-PPS IgG. Concentrations of IgG antibodies to PPS, especially to serotype 4, were low in both younger and elderly adults (Fig. 1A). The GMCs of antibodies to serotype 3 and 6B were
significantly lower in elderly than in younger adults ($P < 0.001$ and $P = 0.017$, respectively). Stratification of data to six age groups showed a clear decrease in antibody concentration to serotype 3 with ageing and a slight decrease in antibody concentration to serotype 6B with ageing (Fig. 2). For other serotypes tested, the GMCs of antibodies did not differ significantly between younger and elderly individuals. Further stratification of data did not provide additional information; there was variation in antibody concentrations with age (data not shown).

At younger ages, women tended to have higher antibody concentrations than men to all tested serotypes, except serotypes 4 and 9V (Fig. 1B). Younger women had significantly higher concentrations of anti-6B ($P = 0.030$) and anti-23F ($P = 0.007$) antibodies than younger men, while men had higher concentrations of anti-4 antibody than women ($P = 0.037$) (Fig. 1B). In elderly men and women the concentrations of anti-PPS antibodies to all serotypes were similar (Fig. 1B). Elderly women had significantly lower anti-PPS antibody concentrations than younger women to serotypes 3 ($P < 0.001$), 6B ($P = 0.009$), and 23F ($P = 0.006$), while elderly men had lower antibody concentrations than younger men to serotype 3 only ($P < 0.001$).

**Effect of ageing and gender on naturally acquired anti-PPS IgM.** The GMCs of IgM antibodies to PPS were significantly lower in elderly than in younger adults for all tested serotypes, except for type 14 (Fig. 3). Stratification of data showed an age-dependent decrease in IgM antibody concentrations to all serotypes; types 3, 9V, and 14 represent various effects of ageing on IgM concentration (Fig. 4).

Younger women had significantly higher anti-PPS IgM concentrations than younger men for all serotypes, while in elderly men and women the anti-PPS IgM concentrations were similar, except for serotype 14 (Fig. 3B). In women, the GMCs of anti-PPS IgM antibodies decreased significantly with age for serotypes 3, 4, 6B, 9V, and 23F. In men, the decreases were significant for serotypes 4 and 9V only.

**Effect of ageing and gender on naturally acquired anti-protein IgG.** The GMCs of antibodies to pneumococcal virulence-associated proteins were significantly lower in elderly than in younger adults for all antigens tested (Fig. 5A). Stratification of data to six age groups showed a clear decrease in anti-protein antibody concentrations with ageing (Fig. 6).

In contrast to anti-PPS antibodies, the anti-protein IgG concentrations in younger men and women were similar (Fig. 5B). Elderly women had significantly lower concentrations of antibodies to CbpA ($P < 0.001$) and PspA1 ($P = 0.001$) than elderly men (Fig. 5B). Nevertheless, the GMCs in the oldest age group ($\geq 85$ years of age) were similar in men and women (data not shown). Elderly women had significantly lower IgG antibody concentrations than younger women to all protein antigens but PspA2. In men, the decreases were significant for CbpA, PhtD, PhtD C, LytC, and PspA1.

**DISCUSSION**

The objective of the present study was to determine the effect of ageing on serum concentrations of naturally acquired antipneumococcal antibodies. The IgG concentrations especially to pneumococcal virulence-associated proteins, as well as to some PPS, and the IgM concentrations to PPS decrease with increasing age. By subdividing the groups, age-dependent and relatively steady decreases in antibody concentrations were seen. A stronger decline in anti-PPS antibody concentrations was seen with age in women compared to men, while the effects of ageing on anti-protein IgG concentrations were similar between the genders.

Information on the concentration of antibodies to pneumococcus acquired during the periods of pneumococcal carriage and disease in an unvaccinated elderly population is sparse. The concentrations of naturally acquired anti-PPS IgG antibodies have been shown to either decrease or remain unchanged with ageing. In a recent seroepidemiological study (1), the serotype-specific IgG concentrations were shown to remain high in older adults. However, the vaccination status of the subjects was not reported, and the authors suggest that the sustained antibody concentrations in adults aged $\geq 65$ years may partly result from 29% uptake of PPS vaccine in this group. In an analysis of naturally acquired anti-PPS antibodies
in Finnish adults reported in 1996 (35), the elderly subjects had significantly lower GMCs of antibodies than the younger persons to five (serotypes 4, 6B, 9V, 19F, and 23F) of six studied serotypes, while in the present study the GMCs of antibodies to two of six serotypes were significantly lower in elderly compared to younger adults. The difference between these two studies may be partly associated with the lower specificity of the EIA method used in 1996 compared to the currently used EIA optimized for PPS specificity by an additional adsorption step with the PPS of serotype 22F (14). In both studies, the lowest GMCs for anti-PPS were detected with serotype 4, which may be explained by poor immunogenicity of this PPS and/or by the low carriage rate of serotype 4 in the adult population (11, 30). The varying effect of ageing on IgG antibody concentrations to different PPS is in agreement with previous studies (33, 35). It may reflect the differences in the antigenic properties of pneumococcal capsular antigens combined with different immune properties of young and elderly adults and/or differences in the prevalence of pneumococcal carriage by various serotypes.

The effect of ageing on serum IgG antibody concentrations was found to be related to the nature of the pneumococcal antigen: anti-protein IgG concentrations against all analyzed pneumococcal proteins decreased significantly with age, while the decrease in anti-PPS IgG was less pronounced and serotype dependent. This finding was interesting, but not surprising. In general, B-cell responses to foreign protein antigens are T cell dependent, performed by B2 cells and modulated by cytokines, while carbohydrate antigens are T cell independent and stimulate B1 cells. Ageing has been suggested to compromise antibody response of B2 but not B1 lymphocytes (15). Accordingly, antibody responses to T-cell-dependent antigens have been shown to be significantly more affected by ageing than those to T-cell-independent antigens (23, 41). Changes in B-cell function during ageing have been largely attributed to
underlying deficiencies in T-cell function (24), as well as to changes in the B-cell repertoire (44).

There may be differences between men and women in the immune response to different types of antigens and in the extent that ageing affects these responses. In earlier studies, the IgG antibody concentrations against PPS antigens have been lower in elderly women than in elderly men (32, 35). In the present study, the mean antibody concentrations in elderly

![Graph 1](image1.png)

**FIG. 5.** Effect of ageing and gender on naturally acquired serum anti-protein IgG antibodies in adults. (A) Anti-protein IgG antibodies in the sera of younger (n = 300) and elderly (n = 300) adults. *, P < 0.05 (Student t test). (B) Anti-protein IgG antibodies in the sera of younger men (n = 150) and women (n = 150) and in the sera of elderly men (n = 150) and women (n = 150). *, P < 0.05 (Student t test).

![Graph 2](image2.png)

**FIG. 6.** Effect of ageing on naturally acquired serum antibodies against pneumococcal proteins in further stratified age groups with mean ages of 37, 48, 59, 69, 79, and 88 years (n = 100, 100, 100, 120, 120, and 60, respectively).
women did not differ from those in elderly men, but a more pronounced decrease in the anti-PPS IgG and IgM concentrations was seen in women with ageing. At a younger age, the concentrations of anti-PPS antibodies, especially IgM, were found to be higher in women than in men. This difference may partly reflect the higher exposure by younger women to the pathogen when the household includes young children. However, the difference was not seen in anti-protein antibody concentrations, which tended to be slightly lower in women than in men.

Men generally have a greater risk for pneumococcal diseases at all ages: both IPD (2, 3, 34) and CAP (17) are more common among men than women. The factors contributing to the greater susceptibility of men to IPD or CAP are not fully understood. A recent study in the United States has, however, found a slight but significant increase in the risk of IPD with the pediatric serotypes among elderly women compared to elderly men (9). Whether this reflects differences in exposure to children between elderly men and women remains unclear. Our antibody data are difficult to link with the disease susceptibility: elderly men and women had similar antipneumococcal antibody concentrations, but the decreases in antibody concentrations with ageing were more pronounced in women. An explanation for the differences may partially lie in the functionality of antibodies or in other immunological factors. Also, physiological and lifestyle risk factors for pneumococcal infections, such as coronary artery disease, alcoholism, history of smoking, and smoking-related lung diseases (3, 18, 22, 43), which are more common in men than women, may play an important role. Smoking at all ages is associated with more numerous pneumococcal infections (26) and a higher carrier rate for pneumococci (13), which may in turn result in increased concentrations of antipneumococcal antibodies among smokers, as suggested by our preliminary data (data not shown).

Age-related changes in serum antipneumococcal antibody concentrations were mainly subtle, and their possible effect on the age and/or gender distribution of pneumococcal diseases remains uncertain. Interestingly, while IgG and IgM antibody concentrations specific to pneumococcal antigens tended to decrease with ageing, the serum levels of total IgG and IgM antibodies have been shown to increase and remain unchanged, respectively, with age (12). These data, combined with the previous data showing a pronounced decrease in the anti-PPS IgG and IgM concentrations of naturally acquired antipneumococcal antibodies among smokers, as suggested by our preliminary data (data not shown).

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ACEWALOOGMENTS

The present study was supported by the Academy of Finland, the Päiviikki and Sakari Sohlberg Foundation, and the Program of Healthy Aging of the National Public Health Institute.

We thank Jan Poolman and coworkers from GlaxoSmithKline Biologicals (Rixensart, Belgium) and D. Briles and S. Hollingshead from the University of Alabama (Birmingham) for providing the pneumococcal protein antigens for EIA. We thank Sinikka Grönholm, Teija Jaakkola, Leena Saarinen, and Pekka Väisänen for technical assistance with the antibody measurements. We thank Esa Ruokokoski and Arja Vuorella for skilled data management and Sirkka Rinne, Pirko Alha, and Harri Rissanen for assistance with the Health 2000 Study samples. H.K. has provided consultancies on advisory boards for Sanofi Pasteur and GSK Bio; has had travels paid for by Sanofi Pasteur, GSK Bio, and Wyeth Vaccines as an invited speaker or expert at symposia; and has received honoraria from Sanofi Pasteur, GSK Bio, and Wyeth Vaccines.

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