Aging and the Immune Response to the *Haemophilus influenzae* Type b Capsular Polysaccharide: Retention of the Dominant Idiotype and Antibody Function in the Elderly

ALEXANDER H. LUCAS* AND DONALD C. REASON

Children's Hospital Oakland Research Institute, Oakland, California

Received 31 October 1997/Accepted 16 December 1997

Anti-*Haemophilus influenzae* b polysaccharide (Hib PS) antibodies elicited in elderly subjects following conjugate vaccination expressed a light-chain variable-region (V_L)-associated idiotype and had functional activities similar to those previously observed in children and younger adults. These findings indicate that advanced age is not accompanied by shifts in the major V_L component of the Hib PS-specific repertoire or by diminution of the protective function of antibodies.

Changes occurring in the immune system as a consequence of aging range from gross anatomical reorganization, such as involution of the thymus, to more subtle alterations at the cellular and molecular levels that include diminished germinal-center formation and hypermutation, shifts in variable (V)-region usage, decreased or altered patterns of cytokine secretion, and diminished lymphocyte responses (reviewed in reference 11). The extent to which these changes directly impact protective immunity in humans is largely unknown. The incidence of diseases caused by encapsulated bacteria such as *Streptococcus pneumoniae* and *Haemophilus influenzae* type b (Hib) is elevated significantly in elderly populations (3, 12, 13), although it is unclear whether this increased susceptibility results from intrinsic immune system defects or from factors such as diet, exercise, living conditions, and underlying illnesses. Compared to younger subjects, elderly individuals may produce decreased levels of serum antibodies and exhibit diminished memory responses following vaccination, and even when antibody levels do not appear to be diminished, antibody function may be compromised (11, 14).

Perhaps the most dramatic association between aging and altered antibody repertoire has been observed in the murine response to phosphorylcholine (PC), an antigenic determinant on the cell surface of *S. pneumoniae*. Anti-PC antibodies are elicited at near-normal levels in aged BALB/c mice following immunization with *S. pneumoniae* but have reduced affinity for PC and markedly reduced protective activity (14). Moreover, the anti-PC antibodies of aged mice utilize V gene segments not normally well represented in younger mice (13).

It is important to determine whether similar age-associated alterations of antibody repertoire occur in humans. The antibody response to the Hib polysaccharide (PS) serves as a good model for studying immunosenescence in humans, since it has been exceptionally well characterized and it parallels the murine antibody response to PC (reviewed in reference 6). Both of these antibody repertoires are oligoclonal, are associated with protective responses to encapsulated bacteria, and utilize a limited number of idiotypically cross-reactive V domains. In this study, we examined idiotype expression, avidity, and bactericidal activities of Hib PS antibodies elicited in elderly subjects following Hib PS-protein conjugate vaccination.

Serum samples were obtained from elderly subjects 30 days following vaccination with either PedvaxHIB (Merck Sharp & Dohme), a conjugate of Hib PS and an outer membrane protein complex of *Neisseria meningitidis* (Hib PS-OMP), or Hib-TITER (Lederle Praxis Biologicals), a conjugate of Hib PS oligomers and a nontoxic mutant diphtheria toxin, CRM197 (HbOC). The Hib PS-OMP group consisted of 15 subjects ranging in age from 69 to 82 years (mean age = 74.4 years). The HbOC group consisted of 15 subjects ranging in age from 69 to 80 years (mean age = 73.8 years). These subjects and their antibody levels before and after vaccination have been described in a previous report (5).

The serum Hib PS-specific antibody repertoire of infants and adults is oligoclonal and dominated by antibodies encoded by the kII-A2 V-region gene. The dominance of A2 antibodies has been demonstrated by analysis of the expression of HibId-1, an idiotypic marker for antibodies having A2 V regions (4, 6, 9). To examine whether advanced age was associated with altered A2 expression, we evaluated HibId-1 levels in the elderly subjects described above. The percentage of the total serum anti-Hib PS expressing HibId-1 was determined by measuring the extent to which anti-HibId-1 inhibited 125I-Hib PS binding as previously described (9). HibId-1 antibodies were present in 9 of 15 (60%) Hib PS-OMP-vaccinated subjects and 12 of 15 (80%) of HbOC vaccinated subjects (Fig. 1). These values agree well with previous studies of children and younger adults immunized with plain and protein-conjugated Hib PS vaccines that show frequencies of HibId-1 positivity ranging from 55 to 80% (4, 7, 9). The average percentages of the total serum Hib PS antibody expressing HibId-1 were 68 and 55% for the HbOC and Hib PS-OMP groups, respectively (Fig. 1), and these means resemble those observed with younger subjects (Fig. 1). These data indicate that advanced age is not generally associated with alterations in either the frequency of expression or the levels of anti-Hib PS antibodies encoded by the A2 V gene.

To determine whether antibody quality might be diminished in the elderly, we examined avidity and bactericidal activity of immunoglobulin G (IgG) anti-Hib PS antibodies isolated from serum pools of the respective vaccine groups. IgG antibodies were used to minimize the variables of multivalence and complement fixation associated with antibodies of the IgM and IgA classes, which could confound interpretation of the avidity and bactericidal-activity determinations. Hib PS-OMP and HbOC serum pools were made by combining individual sera within
each vaccine group. To obtain a representative sample, a volume of each serum corresponding to approximately 40 μg of IgG anti-Hib PS was used to make the pools. The pools were heat inactivated at 56°C for 30 minutes, and the IgG anti-Hib PS populations were isolated by affinity chromatography as previously described (8). The resulting preparations were analyzed by enzyme-linked immunosorbent assay (4, 8) and contained IgG anti-Hib PS antibodies with <0.1% contamination with IgM or IgA.

Table 1 shows the mean avidity indices and bactericidal activities of IgG antibodies from the serum pools. Again, these values are comparable to those observed in younger subjects. Avidities of infant and adult anti-Hib PS antibodies have been shown to range from 1 to 7 nmol⁻¹ (1, 4, 8, 15), and bactericidal indices generally range from 0.05 to 0.7 μg/ml (1, 4, 8, 10, 15). The values for antibodies from the elderly subjects fell within these ranges. There was a trend for HbOC antibodies from elderly subjects to have higher avidity and bactericidal activity than the Hib PS-OMP antibodies from the same group, but the differences were not statistically significant. A similar pattern is seen in the infant response to these conjugate vaccines. Following HbOC vaccination, infants produce anti-Hib PS antibodies that have significantly higher mean avidity and bactericidal activity than antibodies induced by Hib PS-OMP vaccination (4, 8, 15).

Our findings demonstrate that antibodies to Hib PS, elicited in the elderly by conjugate vaccination, express HibId-1 and possess functional activities similar to those of antibodies of younger subjects. Thus, we find no evidence that the repertoire is impaired as a consequence of advanced age. It is conceivable that the expression of V regions other than A2 may have been influenced by increasing age, as has been shown to occur in the first 2 years of life (7). However, if these putative changes in V-region repertoire have occurred, they have neither changed representation of the A2 V region nor diminished the functional quality of IgG antibody.

These results with Hib PS antibodies are in striking contrast to studies of the murine antibody repertoire to PC that show dramatic alterations in V-region expression and diminished functional activity as consequences of advanced age (13, 14). These differences in immunosenescence between the human Hib PS and the murine PC repertoires are particularly noteworthy given the similarities between them, such as oligoclonality, idiotypic and V region dominance, and specificity for a bacterial capsule-associated epitope. Thus, it may be difficult to draw general conclusions concerning the effect of age on immunocompetence from surveys with a limited number of model antigens. Further studies of other clinically relevant human antibody repertoires are required before humoral immunosenescence can be considered a general property of elderly immune systems.

This work was supported by Public Health Service grant AI-25008 from the National Institute of Allergy and Infectious Diseases.

**REFERENCES**


8. Lucas, A. H., and D. M. Granoff. 1995. Functional differences in idiotypically defined IgG1 anti-polysaccharide antibodies elicited by vaccination with...


Editor: J. R. McGhee