Secretory Immunoglobulin A Antibody Response Is Conserved in Aged Mice following Oral Immunization with Influenza Virus Vaccine

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(Accepted 27 July 1989)

SUMMARY

Parenteral immunization of BALB/c mice at 3 months of age with inactivated influenza virus vaccine elicited a haemagglutinin (HA)-specific serum IgG antibody response. The magnitude of this response declined with advancing age at the time of vaccination. By contrast, HA-specific IgA and IgG antibody levels observed in lung lavage fluids of mice immunized at 1 and 2 years of age were comparable to those of 5 month old mice when inactivated influenza virus vaccine was administered intragastrically. The secretory immune response was not fully developed in the first 3 weeks of life. However, the HA-specific IgA and IgG responses to oral vaccination in sera were reduced in 1 or 2 year old mice when compared to 5 month old mice. These data demonstrated the preservation of the virus-specific secretory IgA response in the pulmonary fluids of aged mice after oral vaccination with inactivated influenza virus vaccine. An age-dependent difference of systemic and mucosal immunity was evident in orally immunized mice.

INTRODUCTION

There are many reports indicating that systemic antibody responsiveness declines with advancing age (Callard & Basten, 1978; Ershler et al., 1984; Goidl et al., 1976; Makinodan & Peterson, 1962; Price & Makinodan, 1972a,b). This deficiency is not simply due to the reduction or absence of responding lymphoid cells, since the numbers of reactive B and T cells remain at normal levels in aged mice (Price & Makinodan, 1972a). It may be caused by qualitative dysfunction of B cells, the loss of helper T cells, or the increased presence or activity of suppressor T cells (Goidl et al., 1976; Callard & Basten, 1978; Krogsrud & Perkins, 1977). On the other hand, the immunological function of gut-associated lymphoid tissues in aged mice remains competent (Szewcruk & Campbell, 1981; Szewcruk et al., 1981). The helper T cells from Peyer's patches of senescent mice retain their immune responsiveness to mitogens or alloantigens (Szewcruk & Campbell, 1981; Szewcruk et al., 1981; Ernst et al., 1987). These observations suggest a differential susceptibility to the deleterious effects of ageing when comparing systemic and mucosal immunity.

The elderly suffer significantly from influenza virus infection (Arden et al., 1986; Phair et al., 1978) and are not always fully protected by currently available parenteral vaccination. It has been suggested that secretory IgA in the respiratory tract may be more efficacious than serum antibody in providing resistance against influenza virus infection (Liew et al., 1984; Fazekas de St Groth & Donnelly, 1950). Previously we reported active synthesis of virus-specific secretory IgA in pulmonary secretions of young adult mice that had been immunized intragastrically with inactivated influenza virus vaccine (Chen et al., 1987; Chen & Quinnan, 1988). This study was designed to evaluate whether ageing affects the induction of virus-specific secretory antibodies in lungs of mice following intragastric immunization with inactivated influenza virus vaccine.
METHODS

Animals. Female BALB/c mice, ranging in age from 3 weeks to 2 years, were obtained from Charles River Laboratories and the National Cancer Institute.

Antigens and immunization procedures. Formalin-inactivated whole virion influenza vaccine containing 200 μg of influenza A/Philippines/2/82 (H3N2) haemagglutinin (HA)/ml was provided by Connaught Laboratories. The HA content of this virus, which is a reassortant between influenza A/Philippines/2/82 (H3N2) and influenza A/PR/8/34 (H1N1) virus (prepared by Dr E. D. Kilbourne, Mt Sinai School of Medicine), was measured by the single radial immunodiffusion assay reported by Williams et al. (1980). For subcutaneous immunization, vaccine containing 5 μg HA was administered twice at 3 week intervals to each mouse. Sera were collected 2 weeks after the second dose. For oral immunization, each mouse received an intramuscular injection of 1.2 mg Tagamet (Smith Kline & French Laboratories) to produce a neutral pH of the stomach fluid by reducing gastric secretion. One h later, 0.1 ml of aluminium hydroxide gel antacid (Wyeth Laboratories) was administered intragastrically with a blunt-ended animal feeding needle (Popper & Sons). Then, 0.2 ml of inactivated influenza virus (200 μg HA/ml) was administered by the same method. Four consecutive daily doses were administered initially and an identical regimen was given 3 weeks later as the booster dose (Chen & Quinnan, 1988). Blood collection and lung lavage procedures were performed 7 days after the booster immunization.

Tissue specimens. Mice were bled from the orbital venous plexuses with glass capillary tubes and sera were stored at −20 °C. To obtain pulmonary secretions, mice were sacrificed by neck dislocation and lungs were lavaged with 0.7 ml phosphate-buffered saline with modifications from published methods (Scott & Sydiskis, 1976). An animal feeding needle was inserted intratracheally and fixed in place by tying with suture material. Lavage fluids were clarified by centrifugation at 15000 g for 2 min and stored at −20 °C.

ELISA. The HA-specific antibodies of each isotype in sera and lung lavage fluids were quantified by ELISA as described previously (Chen & Quinnan, 1988).

Statistics. Antibody titres for intragastrically immunized mice of different age groups, consisting of seven to 43 mice per group, were analysed by the Mann-Whitney U test for statistically significant differences. Correlations of antibody responses in sera following parenteral immunization were analysed by the least mean squares method of linear regression.

RESULTS

Systemic antibody response to parenteral immunization with inactivated vaccine in mice of various ages

Inactivated influenza virus vaccine containing 5 μg haemagglutinin (HA) per dose was administered subcutaneously twice at an interval of 3 weeks to BALB/c female mice of various ages. Antibodies in sera collected 2 weeks following the second immunization were titrated by an HA-specific, IgG-specific ELISA (Chen & Quinnan, 1988) as shown in Fig. 1. Young adult mice (12 weeks old) responded with the highest HA-specific IgG titres. The mean titres obtained in those mice were approximately 2.5-fold and 11-fold higher than were obtained in 52 and 104 week old mice, respectively. There was a significant correlation between declining antibody titres and advancing age, as evidenced by linear regression analysis (P = 0.0079; r = −0.6042; n = 18; y = 7868-602 − 307-5548x). This indicated that the intensity of systemic antibody response to the parenteral antigen declined with senescence.

Antibody responses to intragastric administration of inactivated vaccine

Mice received a daily oral dose of inactivated influenza virus vaccine containing 40 μg HA for 4 consecutive days in both primary and secondary immunizations. Titres of HA-specific antibodies in lung lavage fluids and sera are shown in Fig. 2 and 3. As shown in Fig. 2, the HA-specific IgA responses in lung lavage fluids were undetectable in 3 week old mice and were minimally detectable in mice immunized at 4 weeks of age. The intensity of responsiveness matured by 8 to 20 weeks of age. The responses at 52 and 104 weeks of age were comparable to those at 20 weeks. The earliest age at which HA-specific IgG responses were detectable was 4 weeks old. Responsiveness remained at similar levels up to 104 weeks of age.

The HA-specific IgG responses of mice inoculated intragastrically became detectable and matured in sera by 8 to 20 weeks of age (Fig. 3). The magnitude declined fourfold by 52 weeks of age and remained low at 104 weeks of age. Similarly, the HA-specific IgA response was fully developed at 20 weeks of age. By 52 weeks of age, the intensity fell fourfold (P < 0.05, Mann-Whitney U test) and remained at the depressed level with advancing age.
These results indicated that pulmonary antibody responses, especially those of the IgA class, to the ingested antigen was retained with senescence; this was contrary to the reduced systemic immune responsiveness.

**DISCUSSION**

Our studies showed that intragastric immunization with inactivated influenza virus vaccine induced both IgG and IgA antibodies specific for HA in lung lavage fluids and sera of adult mice. The predominant HA-specific isotype in pulmonary fluids was IgA. No detectable pulmonary immune responses occurred in 3 week old mice. Response capacity was matured fully by 8 weeks to 5 months of age and remained at equivalent levels in mice of 1 and 2 years of age. In contrast, the magnitude of HA-specific serum antibody responses, both IgA and IgG, declined with advancing age. The systemic immunity induced by parenteral immunization with inactivated influenza virus also diminished with ageing.

Szewcruk et al. (1981) have reported that the responses of splenic antibody-producing cells (APC) from old mice to a parenterally administered T-dependent antigen, trinitrophenylated bovine gammaglobulin, are greatly diminished in comparison to young mice. In contrast, antigen-specific APC from mucosa-associated lymphoid tissues, after intragastric or intraperitoneal immunization, are not impaired in aged mice. The responsiveness of T lymphocytes from Peyer's patches to mitogens and alloantigens is also not affected by increasing age, suggesting that helper T cells in mucosa-associated lymphoid tissues retain their function during ageing (Szewcruk & Campbell, 1981; Ernst et al., 1987). Previously we hypothesized that plasma cells synthesizing antibodies in lung tissue originate from Peyer’s patches after oral immunization with inactivated influenza virus vaccine (Chen et al., 1987). Thus, the conserved reactivity of antibody synthesis in pulmonary tissues of senescent mice might be explained by the presence of functional helper T cell reactivity and B cells at both Peyer’s patches and bronchial-associated lymphoid tissues. The present study showed that the immunocompetence of secretory immunity at a distant mucosal site, such as the lung, was preserved in 2 year old mice. These results extended previous notions that secretory and serum humoral immunity constitute
Fig. 2. Pulmonary antibody responses in mice of various ages that were vaccinated intragastrically with inactivated influenza virus vaccine. HA-specific antibody titres of IgA and IgG isotypes at 7 days after the last dose of booster immunization were monitored. The curves represent the geometric mean antibody titres (x ± s.e. of several independent experiments with time. The geometric mean titres (n = 4 to 12/age/experiment) of HA-specific IgA in individual experiments are indicated by solid symbols and HA-specific IgG by open symbols.

Separate compartments (Ernst et al., 1987). Earlier, we also examined the distribution of dye following its intragastric administration and found that it was present in the gastrointestinal tract only and not in the lung (Chen et al., 1987). It is possible that this delivery route for the vaccine might inadvertently contaminate the lung tissue and cause pulmonary immune responses.

Waldman et al. (1987) have recently reported significantly lower lung and serum antibody titres in four 2 year old mice following oral immunization with live influenza virus. Our study confirmed the reduction in serum IgG and IgA responsiveness to oral immunization with advancing age, but the secretory IgA and IgG responses in pulmonary fluids were conserved. The reasons for this discrepancy is unclear. However, our finding is consistent with their data from human subjects in that the magnitude of the antibody response in nasal secretions of a geriatric population vaccinated orally with inactivated influenza virus vaccine is comparable to that induced in young adults (Waldman et al., 1987).

The immune responsiveness at other mucosal sites, such as the eye and saliva, have been shown to be either conserved (Sullivan & Allansmith, 1988) or decreased in salivary secretion (Smith et al., 1983) of aged rodents in comparison with the young adults. In these reports,
Fig. 3. Serological responses in mice of various ages that were vaccinated intragastrically with inactivated influenza virus vaccine. HA-specific antibody titres for IgA and IgG isotypes at 7 days after the last dose in the booster immunization were monitored. The curves represent the geometric mean antibody titres (×) ± S.E. of several independent experiments with time. The geometric mean titres (n = 4 to 12/age/experiment) of HA-specific IgA in individual experiments are indicated by solid symbols and HA-specific IgG by open symbols.

However, the IgA serum concentration was similarly elevated with the ageing process. This is contrary to the findings of Szewcruk et al. (1981) and to our current report of the inverse relationship between mucosal and systemic immune responses during ageing. There is no explanation for the discrepancy.

We have demonstrated the unique retention of pulmonary secretory immune responsiveness with ageing in contrast to the diminished systemic humoral immune reactivity. Since the elderly are one of the primary human target populations afflicted with influenza virus infection and the current parenteral vaccine is not optimally protective, oral vaccination might induce secretory immunity in their pulmonary tissues and be more efficacious.

We thank E. Staton and B. Rangi for their excellent technical assistance, the cell biology staff of the Division of Virology, and the animal care laboratory.
REFERENCES


(Received 6 June 1989)