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THE IMMUNE RESPONSE TO INFLUENZA VACCINES

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Summary. — Specific immunity to influenza is associated with a systemic immune response (serum haemagglutination inhibition antibody), local respiratory immune response (virus-specific local IgA and IgG antibodies in nasal wash), and with the cell-mediated immune response. Both inactivated and live influenza vaccines induce virus-specific serum antibody which can protect against infection with influenza virus possessing the same antigenic specificity. In the absence of serum antibodies, local antibodies in nasal wash are a major determinant of resistance to infection with influenza virus. In comparative studies in humans it was shown that nasal secretory IgA develops chiefly after immunization with live cold-adapted (CA) vaccine, but persistent nasal secretory IgG was detected in both CA live and inactivated vaccines. The origin of nasal wash haemagglutination inhibition (HI) antibodies is not completely known. Recently it was found that cytotoxic T-cells (CTL) play an important role in immunity against influenza and in clearance of influenza virus from the body. In primed humans, inactivated influenza vaccine stimulates a cross-reactive T-cell response, whereas the ability of inactivated vaccine to stimulate such immunity in unprimed humans has not been determined. Data on the T-cell response to live vaccine in humans are limited to the development of secondary T-cell responses in primed individuals vaccinated with a host-range (HR) attenuated vaccine. The data obtained have shown that immunity induced by inactivated influenza vaccines is presumably dependent on the stimulation of serum antibody. Live CA vaccines not only stimulate a durable serum antibody response, but also induce long-lasting local respiratory tract IgA antibody that plays an important role in host protection.

Key words: *influenza virus*; *live vaccine*; *inactivated vaccine; humoral immunity*; *cell-mediated immunity*

Introduction

Influenza has long been recognized as a problem of both developed and developing nations. For example, in 1957 in the U.S.A. the Asian strain of influenza virus caused as estimated 70 000 deaths; the Hong Kong strain

of influenza virus that appeared in 1968 caused about 30 000 deaths (Prevent. contr. infl., 1986). Even in years not associated with antigenic shift many people die as a result of influenza infection, so 10 000 or more excess deaths have been documented in the U.S.A. during the each of 18 different epidemics from 1957 to 1985 (Prevent. contr. infl., 1986). It was estimated also (New vaccine develop., 1985) that in the U.S.A. during an influenza outbreak, about 70 million people catch influenza at a cost of about 4.5 billion $.

For influenza prophylaxis two sorts of influenza vaccines are now available: inactivated concentrated and purified for parenteral administration and live attenuated CA recombinant vaccines destined for instillation or pulverization into the upper respiratory paths. Most information on the mechanism of immunity to influenza was obtained in animal models which suggests that resistance to the disease can be correlated with the presence of antibody to the surface proteins of the virus and that cell-mediated immunity plays an important role in the recovery from influenza infection (Ennis *et al.*, 1982; Ada and Jones, 1986). There is much less information available about the mechanism of protection of human against influenza viruses.

It is known that previous infection of humans with an identical strain of virus confers immunity upon later challenge. It should be noted that immunity to influenza can be both solid and long-lasting as shown by the resistance of the population older than 20—25 years to H1N1 influenza virus infection at the reappearance of this subtype in 1977 by 20 years after the last outbreak caused by this virus.

Humoral antibodies

It has been generally accepted that high titres of HI antibody are significantly associated with protection against a virus with a closely related haemagglutinin (HA) (Potter and Oxford, 1979). It was also shown that neuraminidase inhibiting antibody in serum contributed to the immunity to influenza (Murphy *et al.*, 1972; Couch *et al.*, 1981). The antibody response to influenza virus virus or vaccine depends on the recipient's prior antigenic experience. During primary infection of humans with influenza virus, IgM and IgG in the serum occur regularly and IgA less frequently; in nasal secretions IgA followed by IgM was the dominant response (Murphy *et al.*, 1982). Previously seropositive subjects produced antibodies of the IgG and IgA class more frequently than previously seronegative persons; in contrast, IgM antibodies occur more frequently in unprimed subjects than in primed ones (Beyer *et al.*, 1986).

Resistance to influenza infection in humans has been correlated with HI antibody nasal washings (Couch *et al.*, 1974; Clements *et al.*, 1983; 1984) and resistance to wild type influenza A infection has been demonstrated in adults with nasal wash neutralizing antibody, but without detectable serum antibody (Murphy *et al.*, 1973). On the other hand a contribution of serum antibody alone has been inferred from studies of the correlation of resistance

to illness caused by influenza A virus with the level of maternally transferred antibody in the neonates (Puck *et al.*, 1980). Perhaps in humans antibody present in either the local or systemic compartment can contribute to resistance to illness caused by influenza virus, but it is possible that to be protective, HA antibody must be present at the mucosal surface, having been produced either locally or derived from serum.

Cell mediated immunity

As to recovery from influenza infection it is possible to assume that antibody is not essential because patients with agammaglobulinaemia recover from influenza infection. Data available at present show that cell-mediated immunity is a host factor responsible for the recovery process from influenza infection (Ennis *et al.*, 1982; Ada and Jones, 1986). In several laboratories in studies on mice it was found that Tc lymphocyte response to influenza infection is required for recovery from influenza pneumonia (Ada and Jones, 1986).

Studies on human volunteers support the contention that Tc cells are important in recovery from influenza virus infection. Mitchell *et al.* (1985) have shown that Tc-cell memory in peripheral blood lymphocytes correlated with the rapid clearing of administered virus in individuals, some of whom lacked specific antibody to HA or NA.

Since local and serum antibodies and also cytotoxic T-cells (Tc-cells) appear to be mediators of immunity to influenza infection, it is important that influenza vaccines induce all these components of immunity.

Inactivated vaccines

Inactivated influenza vaccines in primed individuals induced a protective level of serum HA antibody in over 85% of recipients (Potter, 1982). The induction of a secretory antibody response to inactivated influenza vaccines is dependent both on the route of administration and on the recipient's prior antigenic experience. In unprimed recipients local antibody responses are of low magnitude and occur infrequently after both parenteral and intranasal administration of the vaccine. Parenteral administration of inactivated vaccine produced a local IgG response in 94% of primed recipients, whereas local IgA responses developed in only 38% (Clements *et al.*, 1985). In contrast, after intranasal administration of inactivated vaccines local IgA response developed in the majority of primed recipients (Wright *et al.*, 1983).

A substantial difference between the response to natural infection and inactivated vaccines is seen when the dynamics of the antibody response are compared (Potter, 1982). Serum and anti-HA titres gradually decrease over the first six months after infection and may then persist for several years, possibly due to subsequent infections by related virus strains. The duration of serum anti-HA after vaccination with inactivated vaccine varies — primed subjects retain protective levels of antibody for at least one year, whereas antibody levels decline rapidly in unprimed subjects.

There are very few studies on Tc response after immunization of humans with influenza vaccines.

McMichael *et al.* (1981) have demonstrated that inactivated vaccines in primed humans stimulated a cross-reactive Tc cell response. However this response is not universal and appears to be related to the pre-immunization level of Tc memory.

Live cold adapted vaccines

Murphy *et al.* (1982) found in studies with CA live influenza vaccines that young children vaccinated with these vaccines (H3N2 or H1N1) had serum IgG, IgM, and IgA antibody response. In nasal washes of most of the vaccinees were found IgA and IgM antibody and in 50% of vacciness, IgG antibody. Most of the IgA and IgM antibody was actively secreted locally, whereas only some of the IgG HA antibody could be shown to be actively secreted into the respiratory tract. These data indicate that intranasal vaccination of susceptible children with CA live influenza A vaccines efficiently stimulates both systemic and local antibody responses. It should be no'ed that CA live influenza vaccine (H3N2) can induce local IgA antibody not only in the majority of seronegative but also seropositive vaccines (Clements *et al.*, 1985).

Feldman *et al.* (1985) found that after vaccination of seronegative children with CA live H1N1 influenza vaccine, only 57% of vaccinees showed serological responses. But a natural H1N1 challenge which occurred shortly after completion of the vaccination showed that most vaccinees were protected against infection and symptomatic illness despite its failure to stimulate high levels of serum HI antibody.

In mice CA live influenza vaccine can induce a primary Tc cell response and can sensitize the lungs for a secondary Tc cell response (Mak *et al.*, 1982; 1984). It was found that the dose of a live CA vaccine strain required to induce the same level of Tc response was 100 to 1000 times greater than that of the parenteral wild strain (Mak *et al.*, 1982). The difference in dosage required for priming could be overcome by giving two small doses of CA virus three weeks apart (Tannock *et al.*, 1984). Using this approach CA live vaccine may induce in mice cross-protection against different subtype viruses (Tannock *et al.*, 1987).

Inactivated versus live vaccines

In comparative studies of Johnson *et al.* (1985) of antibody responses of young children vaccinated with intranasal CA live vaccines (H3N2 or H1N1) or intramuscular inactivated influenza vaccine (H3N2), it was found that six weeks after vaccination the titres of HAI antibody were more or less the same. In other studies it has been reported that parenteral vaccination with inactivated influenza vaccine stimulated systemic antibodies in humans more efficiently than does intranasal vaccination with live attenuated vaccine (Mann *et al.*, 1986; Zahradnic *et al.*, 1983; Clark *et al.*, 1983*a*, *b*; Clements *et al.*, 1984).

Clements and Murphy (1986) also found that inactivated influenza vaccine induced serum IgA and IgG in most vaccinees in comparison with CA live vaccine and induced higher titres of serum antibodies than did the live vaccine But in contrast only 38% of inactivated virus vaccines had local IgA responses compared with 83% of vaccinees immunized with CA live vaccine. The same results were obtained by Zahradnic *et al.* (1983) who showed that parenteral inactivated vaccine was relatively ineffective in stimulating neutralizing secretory antibodies when compared with intranasal CA live vaccine.

Johnson *et al.* (1985) found in comparative studies of inactivated (intramuscular) and CA live (intranasal) vaccines on young children that nasal secretory IgA developed almost exclusively in vaccines with live vaccines and persisted for up to one year (vaccine H3N2) and after vaccination with the CA live vaccine H1N1, nasal IgA was demonstrable as long as 30 months. Persistent nasal secretory IgG was detected in vaccinees vaccinated with both inactivated and live vaccines but the titre of this antibody was higher in vaccinees with the CA live vaccine.

In subsequent studies by Johnson *et al.* (1986) 59 young children were divided into four groups based on prior exposure to influenza A(H3N2) virus, natural infection, live CA vaccine given intranasally, inactivated vaccine given intramuscularly, and no previous exposure. Virus challenge with homologous live CA vaccine occurred 12 months after vaccination or natural infection. It was found that prechallenge local IgA detected almost exclusively in subjects naturally infected or vaccinated with CA live vaccine was associated with protection against shedding. Any detectable nasal IgA ($\geqq 1 \log_2$) suppressed viral shedding. Effect of nasal IgG was not as sharply defined in this study; however, at higher levels ($>4 \log_2$) the shedding was reduced. It should be noted that although inactivated vaccine failed to produce signification local IgA during the primary response, it primed infants to a better response to both nasal IgA and IgG after challenge with CA live virus.

Clements *et al.* (1986*a*) investigated the role of serum and nasal wash antibodies in resistance of humans vaccinated with CA live or inactivated influenza A vaccines to experimental challenge with influenza A wild-type virus. Protection of vaccines receiving inactivated vaccine for infection or illness correlated with the level of HI antibody and neuraminidase-inhibiting antibody in serum and local anti-HA IgG (but not IgA) antibody. Protection of vaccinees receiving CA live vaccines against infection correlated with local anti-HA IgA antibody and neuraminidase-inhibiting antibody in serum, but not with HI antibody in serum. The authors suggest that live vaccine-induced immunity may involve different compartments of the immune system but sufficient antibody in either serum or nasal secretion is capable of conferring resistance.

The origin of nasal-wash HA antibodies induced by influenza vaccines is not completely known. There are at least three possibilities: 1) Local production of a J chain containing IgA dimers (or IgM pentamers) which are actively transported externally by nasal epithelium possessing secretory

component to which polymeric IgA and IgM bind (Bienenstock and Befus, 1980); 2) Local synthesis of non-chain containing immunoglobulin by plasma cells in the nasal mucosa followed by its passive transport toward this lumen; 3) Passive transsudation of serum-derived antibodies. In the case of intranasal vaccination with live influenza vaccine there is evidence that secretory IgA and to some extent IgM and IgG are synthesized by nasal epithelium cells and actively secreted locally (Butler *et al.*, 1970; Bienenstock and Befus, 1980; Murphy *et al.*, 1982). It should be noted that peripheral blood lymphocytes obtained from children immunized with CA live vaccine produced *in vitro* IgG but not IgA antibody (Edwards *et al.*, 1986). The absence of IgA-producing B-cells in the peripheral blood of children vaccinated with live vaccine may reflect that these cells belong to local mucosal sites.

The studies of persistence of the serum and nasal-wash IgA, IgG, and IgM antibody have shown that 12—24 months after vaccination with CA live vaccine had significantly less decay of serum HAI and IgG antibody in contrast to the antibody induced by inactivated influenza vaccine (Clark *et al.*, 1983; Johnson *et al.*, 1985; Wright *et al.*, 1986) which declines significantly in the first six months after vaccination. Serum IgM antibody persisted for one year after vaccination with inactivated vaccine (Wright *et al.*, 1986). As to secretory IgA antibody it was found that in adults such antibody induced by live influenza vaccines is relatively short-lived (Butler *et al.*, 1970; Clements and Murphy, 1986; Wright *et al.*, 1986). However, data from studies in seronegative children have indicated that nasal IgA antibody could be detected for one or more years in about 50% of naturally infected or vaccinated with live influenza vaccine children but in only 5% of inactivated vaccine recipients (Johnson *et al.*, 1985).

The results presented above show that inactivated and live influenza vaccines stimulate both systemic and secretory antibodies in children and adults, and that inactivated vaccine is more potent in inducing serum antibodies; but live vaccines are superior in stimulation of secretory antibodies that may play an important role in host protection. Following the cell mediated response Ennis *et al.* (1981, 1982) found that in primed volunteers immunized with live or inactivated influenza vaccines (H1N1) both types of vaccines induced HLA-restricted T-lymphocyte responses specific for influenza A virus. But by six months after vaccination, both the memory of Tc cell activity and the directly detected Tc cell activity had returned to pre-immunization levels. It should be noted that in these studies there was no absolute correlation between antibody responses and an increase in Tc cell activity: there were several volunteers who had antibody responses without increase in specific Tc cell activity and vice versa.

The ability of influenza vaccines to stimulate the Tc cell responses in unprimed humans has not been determined. It was also found that stimulation of Tc cell response in mice by live virus infection was superior to inactivated virus (Webster and Askonas, 1980).

Several authors found that human and murine Tc cells can recognize not only glycoproteins of outer membranes of influenza virions as HA and

neuraminidase but also other viral-specific proteins with common antigenic specificity such as NP, Mt, PA, PB1, PB2, and NS1. Although Tc cells cannot per se prevent infection, infection in primed persons may be modified early in its course. As Tc cells recognize the common internal viral proteins this protective effect can be broader than that of antibody response. In fact it was found recently that local injection of mice with purified NP antigen of influenza virus primes for influenza A virus cross-reactive Tc memory cells and leads to protection of hosts against intranasal infection with a lethal dose of influenza virus (Wraith *et al.*, 1987). But at the moment there are no data that Tc cells which can recognize internal proteins of influenza virus really take part in the immunity of humans.

In spite of convincing data obtained on mice showing that influenza vaccine stimulates the Tc cell response the ability of inactivated and live influenza vaccines to induce cell-mediated immunity in unprimed and primed humans should be determined. The protection afforded by influenza vaccines was evaluated in volunteers and in field trials. One of the first comparative studies of protective effects of live and inactivated influenza vaccine (H1N1) was done by Clark *et al.* (1983*a*, *b*). In a study of short term immunity young adults were vaccinated with CA live vaccine (H1N1) or inactivated vaccine. One month after vaccination, protection against challenge with homologous CA live vaccine was equivalent in the two groups as assessed by rises in titres of HAI antibody. The same data was obtained in a study of long term immunity when groups of young adults were challenged eight months after vaccination. But it should be noted that this study did not include investigation of local antibody and viral shedding data.

Clements *et al.* (1984) in comparative studies on seronegative adult volunteers who were vaccinated with CA live vaccine (H3N2) or inactivated vaccine showed that after challenge with the homologous wild-type virus five to eight weeks after vaccination, recipients of live vaccine were completely protected against illness compared with a 72% efficacy in the inactivated vaccine recipients. Wild-type virus was recovered from only 13% of live vaccine vaccinees compared with 63% of inactivated vaccine vaccinees. The few infected vaccinees immunized with CA live vaccine shed 1000 times less wild-type virus than did infected inactivated vaccine vaccinees or unvaccinated control. This strinking reduction in virus shedding suggests that influenza transmission may be more efficiently interrupted with the live than with the inactivated vaccine.

In subsequent studies by Clements *et al.* (1986*b*) of resistance of vaccinees to challenge with influenza wild-type virus seven months after vaccination it was found that vaccine efficacy, measured by reduction in febrile or systemic illness in vaccinees, compared with that in controls was 100% for the CA live H3N2 vaccine, 87% for the inactivated H3N2 vaccine, 79% for the CA live H1N1 vaccine, and 67% for the inactivated H1N1 vaccine. The authors concluded that CA live influenza vaccine induced significantly greater resistance to wild-type influenza virus one to two months after vaccination but at seven months post-vaccination, the resistance induced by live vaccines is only slightly greater than that induced by inactivated vac-

cine. But it should be noted that in the studies of Johnson *et al.* (1986) and Wright *et al.* (1986) vaccinees immunized with CA live (H3N2) or inactivated vaccine and challenged with homologous live CA vaccine or natural challenge with wild-type influenza virus 12 months after vaccination, live vaccine significantly reduced virus shedding after challenge compared with inactivated vaccine.

As to the efficacy of inactivated or live influenza vaccines it should be noted that at the moment there are no publications on comparative studies on the efficacy of the two types of vaccines during the same field trials. But as many studies have shown, adults who have experienced one or more infections by influenza virus and have been immunized with inactivated vaccines, may have protection against antigenically homologous influenza virus for at least one to two years. In unprimed individuals, usually young children, this immunization is less protective, probably because of the poor ability of parenterally administered inactivated virus to prime for a local humoral or Tc cell response (Ada and Jones, 1986).

The efficacy of live influenza vaccines in primed adults is more or less similar to the efficacy of inactivated vaccines. Efficacy studies of CA live vaccines on unprimed children has shown (Wright *et al.*, 1982; Belshe and Van Voris, 1984; Belshe *et al.*, 1984) that children receiving a H3N2 CA live vaccine appeared to be protected against subsequent natural infection with related strains of influenza virus. CA live influenza A vaccine was studied in a controlled field trial among more than 16 000 children between three and 15 years of age vaccinated with bivalent vaccine (H1N1 + H3N2) (Alexandrova *et al.*, 1986). Protective efficacy of both components of the vaccine was developed in two time decrease in morbidity of vaccinees during outbreaks of influenza A/H1N1 and A/H3N2.

Another important problem related to the efficacy of influenza vaccines is the protective effect of vaccine against drift variants of influenza virus. (1979) showed that the protective effect of inactivated influenza vaccine was limited to non-immune schoolchildren who were vaccinated for the first time with the inactivated vaccine made from the prevailing strain of influenza virus. Revaccination with inactivated vaccine produced from the later prevailing strain did not provide protection against a new drift variant. On the other hand, natural infection with live influenza virus afforded almost complete protection during successive outbreaks involving drift variants for more than four years. As the specificity of the antibody respones after vaccination with inactivated vaccine and after infection with live virus is similar, differences in the extent of cross-reactive Tc cell responses to vaccination and infection may account for the lesser degree of heterotypic protection seen after vaccination with inactivated vaccine. Taking this into account it is possible to suggest that live influenza vaccine may have the advantage over inactivated vaccines in protection against new influenza virus drift variants.

It was found recently that adaptation of influenza viruses to growth in embryonated eggs resulted in selection of variants which were antigenically and biologically distinguishable from viruses isolated from the same source

in mammalian cell line MDCK (Schild *et al.*, 1983; Oxford *et al.*, 1987). It was also found that HI or virus neutralizing antibodies in human sera can be detected more frequently, and to a higher titre, in tests employing virus grown exclusively in MDCK cells than in tests with virus adapted tc growth in embryonated eggs (Oxford *et al.*, 1987). Because the substrate for production of influenza vaccines — inactivated or live — is embryonated chicken eggs, these findings have raised concern regarding the suitability of eggs for cultivation of influenza viruses used in vaccine production.

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