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Vaccination Against Acute Respiratory Virus Infections and Measles in Man

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Abstract

Several viruses may cause more or less severe acute respiratory infections in man, some of which are followed by systemic infection. Only for influenza and measles are licensed vaccines available at present.

The protection induced by influenza vaccines, which are based on inactivated whole virus or viral subunits, depends largely on the matching of vaccine strain and circulating virus. Measles vaccines, which are based on attenuated live virus, have been quite effective in controlling the disease in vaccinated populations in the industrialized world. In developing countries, severe measles infections occur in infants from six to nine months of age, which necessitates the vaccination of children of less than six months. At that time maternal antibodies, that may interfere with the induction of protection, may still be present. Therefore, instead of using the parenteral route, the possibility to use the mucosal route of primary immunization is also investigated for vaccination with attenuated live measles vaccines. The use of inactivated measles vaccines has resulted in a state of immunity which upon exposure to the virus may induce an atypical measles syndrome including a severe pneumonia. Measles virus proteins presented in an iscom matrix have recently been shown to induce functional B and T cell responses to both the surface glycoproteins of the virus. These responses could also be induced in the presence of virus neutralizing antibodies and they proved to be protective in several animal model systems.

Many of the problems that have been encountered in the development of measles vaccines, proved to be similar in the development of vaccines against other paramyxoviruses causing acute respiratory infections in man, including respiratory syncytial virus. Parenteral application of inactivated and attenuated live vaccines against these paramyxoviruses has generally had little success. Topical application of attenuated live vaccines has been more successful, and also the use of vaccinia recombinant viruses expressing foreign paramyxoviral glycoproteins has shown promising results in laboratory animals.

Live vaccines based on adenovirus types 4 and 7 in oral enteric-coated vaccines, which lead to virus replication in the intestines but not in the respiratory tract have been included in military vaccination programs. The possibility to replace e.g. the E3 region with foreign DNA makes adenoviruses also suitable as cloning vectors for proteins of other respiratory viruses. Although live attenuated vaccines against some of the serotypes of rhinoviruses have shown promising results, the generation of a multivalent vaccine against this epidemiologically most significant cause of acute respiratory infections will be almost impossible, due to the multiplicity of serotypes involved.

It may be expected that the new fields that are being explored in vaccine development at present, like the use of recombinant proteins of infectious agents, synthesized peptides

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Abbreviations: *HIV* = human immunodeficiency virus; *iscom* = immune stimulating complex; *MHC* = major histocompatibility complex; *SSPE* = subacute sclerosing panencephalitis; *MV* = measle virus

containing relevant B and T cell epitopes, anti-idiotypes and novel adjuvant and antigen presentation systems, will also lead to new generations of vaccines against many of the acute respiratory virus infections.

Introduction

Several virus infections may cause more or less severe acute respiratory infections in man, which are in some cases followed by a systemic infection. Of the viruses causing acute respiratory infections, rhinoviruses are by far the most prevalent in all age groups, as judged from the frequency of virus isolations (1). In industrialized countries, rhinoviruses account for about fifty percent of all acute respiratory illnesses in man. Infections with respiratory syncytial virus (RSV) and measles virus (MV) are predominantly found in children of younger age groups, whereas infections with influenzavirus, parainfluenzavirus and herpes simplex virus are more prevalent in adolescents and adults. Respiratory infections caused by adenoviruses and to some extent enteroviruses are predominantly seen in military recruits.

At present only for influenza and measles are licensed vaccines available, although in the military in the USA adenovirus 4 and 7 vaccines are also used. There is a need for vaccines against some of the other acute respiratory diseases in man and a number of these are at different stages of development. The present paper gives an overview of the current developments in this field.

New Generations of Virus Vaccines

The general requirements for safe and effective virus vaccines include their ability to induce a long-lasting immune response at the B and T cell level, which is immunodominant in the largest possible assessment of MHC haplotypes without causing adverse side effects. An extra requirement for vaccines that should provide protection against acute virus infection of the respiratory tract, is that they should preferably elicit a solid mucosal immunity at least in part based on the induction of secretory IgA antibodies. It has e.g. been shown for several viruses, like rhinoviruses and influenza A-virus, that virus replication and illness in the upper respiratory tract is largely limited by the presence of these antibodies. For infections with other viruses like MV and RSV, a solid systemic immunity is important to prevent systemic infections (for review see 2).

Before the development of a vaccine against any of these virus infections is considered, it is important to define the final goals for its future application. This may be prevention of the disease, prevention of the infection or even local or «global» eradication of the infection. This last goal has e.g. successfully been achieved with smallpox and is presently being pursued for poliomyelitis and measles by collaborative efforts coordinated by the WHO.

The different strategies which are presently available for the development of virus vaccines and their advantages and disadvantages are summarized in Table 1. The classical vaccines used against virus infections are based on live attenuated virus or inactivated whole virus preparations.

The live attenuated viruses have the advantage of most closely mimicking infection with the wild type virus and hence, the immune response is the most similar to that elicited during natural infection. In most cases this will include B and T cell-mediated immune responses and, when safety requirements do not prohibit their local application, also a protective immune response at the level of the mucosal membranes. The major disadvantages of these types of viral vaccines are questions concerning their safety. Reversion to virulence may occur and their application in the immunocompromised host may cause specific problems. Especially at the present time when HIV has started its pandemic spread, this problem may need special attention. Since this type of vaccine depends on the replication of the attenuated vaccine virus in the vaccinee, other problems concerning the use of live attenuated virus vaccines are their susceptibility to interference by maternal antibodies present in young children, interference by intercurrent virus infections and the need for adequate «cold chain» facilities. However, it should be stressed that in spite of these disadvantages, this type of vaccine has been extremely successful, since it has enabled the first global eradication of virus infection of man: smallpox.

Inactivated whole virus vaccines have the advantage that, depending on the way of inactivation, all or most of the B and T cell epitopes are

Table 1. Strategies for the development of viral vaccines

Type of vaccine	Advantages	Disadvantages
Live attenuated	Mimics natural infection Good B and T cell responses	Safety problems Interference by maternal antibodies Interference by intercurrent infections «Cold chain» needed
Inactivated whole virus	«All» B and T cell epitopes presented	Not safe? No CTL response ($CD8^+$ class I MHC-restricted) Adjuvant systems needed Production expensive
Viral subunits (from virus/recombinant proteins/peptides)	Selected epitopes Safe	Adjuvant systems needed No CTL response ($CD8^+$ class I MHC-restricted) (exception: iscom presentation)
Live recombinants/ chimeras	(Carrier determined) B and T cell responses	Repeated boosters not possible? Limited immunogenicity
Anti-idiotypes	Elements of the (host) immune system	Limited immunogenicity/genetic restriction Research tool?

presented in a multimeric and natural presentation form to the immune system. However, since vaccination is not followed by virus replication, no *de novo* synthesis of viral proteins takes place. This is generally required for the induction of $CD8^+$ class I MHC-restricted cytotoxic T lymphocytes (CTL). This cell population may play an important role in the protection and recovery from virus infections. Novel types of adjuvant systems that circumvent this problem, like the iscom structure for antigenic presentation, may become available for human application in the near future. A practical problem concerning inactivated whole virus vaccines is also the relative high cost involved in their production, since the complete antigenic load should be prepared and presented in the vaccine. Several viral vaccines based on this principle like the Salk-type polio vaccine and inactivated rabies vaccines have been quite successful.

With the advent of the technological developments of the last decennia, the possibilities and expectations for the creation of new generations of vaccines has increased accordingly. It has become possible to define individual B and T cell epitopes with the help of monoclonal antibodies and cloned T cell populations. Recombinant DNA and peptide synthesis techniques have offered the opportunity to construct well-defined viral subunits produced from whole virus, recombinant proteins or synthetic peptides at a cost-effective way. They may be constructed in such a way that they only contain those components required for the induction of the desired immune response. This development has led to the increased need for suitable adjuvant systems that can be applied in man. The same technological developments have created possibilities to generate recombinant viruses and bacteria which can be used as live vectors for the expression of viral proteins. The advantages and disadvantages of the use of viral subunits and live recombinants coincide largely with those of the inactivated and live attenuated vaccines respectively.

The use of idiotypic structures for the modulation of the immune response towards antiviral immunity, takes advantage of the observation that elements of the immune system may mimic or function as epitopes of external antigens. Although is has been shown in many model systems that this approach can be used for the induction of protective immunity against parasites, bacteria and viruses (for review see 3) there are no practical applications in human or veterinary medicine yet.

Influenza viruses

Licensed vaccines against influenza A and B virus infections are either based on whole formaldehyde inactivated viruses or on so-called split vaccines, which contain purified formaldehyde-treated virus disrupted with lipid solvents (for review see 4–7). They have only been licensed for parenteral use in humans. These vaccines are made from viruses produced in the allantoic cavity of fertilized chicken eggs, after inoculation with seed viruses. These seed viruses are naturally occurring virus strains or, in the

case of the influenza A virus components, may be reassortant viruses containing the genes coding for internal proteins of a high yielding laboratory strain (e.g. PR 8/34 [H_1N_1]) and the genes for the haemagglutinin (HA) and neuraminodase (NA) proteins of a wild-type virus. The influenza A and B virus strains used in the manufacturing of these vaccines are recommended annually by the WHO Expert Group on Influenza. Purification and concentration of the viral yield is accomplished by zonal ultracentrifugation or column chromatography and the virus yield is subsequently inactivated by formaldehyde treatment. The purification steps lead to a product that is standardized on the basis of its HA content, which should be 7–25 μg per component. The purification steps remove unwanted egg material, by which unwanted febrile reactions after inoculation can largely be avoided. The nature of this pyrogenic reaction has not been completely understood, but it has been speculated that viral proteins may also be involved. In general, split vaccines based on the solubilization of the HA and NA proteins with detergents, are believed to cause less pyrogenic reactions and it is recommended that in young seronegative children only split vaccines should be used. However, some of these split vaccines may be less immunogenic than whole virus vaccines. Epidemiological indications exist for an increased risk for the development of Guillain-Barré syndrome after influenza vaccination. Adaptation of human influenza A and B viruses to growth in chicken eggs has been shown to induce a selection of strains with an altered antigenic composition of the HA protein. Whether this selection has implications for their use as vaccine strains in humans is not clear at present.

The levels of HA and NA-specific antibodies induced after vaccination are related to the quantities of HA and NA present in the vaccine. Also the previous experience of the vaccinee is of major importance for the levels of HA and NA-specific antibodies that are elicited; adults tend to generate more broadly reactive antibodies than children. Another important factor is the route of immunization. The intradermal route has been compared with the normally used subcutaneous route. Although less febrile reactions were observed and less vaccine could be used, the consistency of the response proved to be unsatisfactory. The intranasal route by installation of drops or spray in the nose has not systematically been explored for inactivated influenza vaccines in comparison with other routes of application.

The protection induced by parenteral vaccination with inactivated influenza A and B vaccines is manifested by reduced levels of frequency and severity of illness after natural infection, although infections with influenza A and B viruses still occur after vaccination. The resistance induced is strongly correlated with the levels of haemagglutination inhibiting (HI) antibodies induced against the circulating virus. The discussions about the overall protective effect of influenza vaccination in general and annual revaccinations in particular have not been conclusive. The current inactivated vaccines are recommended primarily for those at high risk of death from influenza virus infection.

Table 2. Iscom approach for the development of viral subunit vaccines

Points in favor as concluded from several systems. For reviews see 30, 35, 36, 37.

I **B cell response**
- induction of broadly reactive and biologically active (e.g. VN) antibody responses
- induction of longlasting antibody responses
- induction of longlasting antibody responses in the presence of pre-existing antibodies

II **T cell response**
- induction of MHC class II restricted $CD4^+$ T cell responses (LST, DTH, cloning experiments)
- induction of MHC class II restricted $CD4^+$ T cell responses in the presence of pre-existing antibodies (DTH)
- induction of $CD8^+$ class I MHC-restricted cytotoxic T cell responses

III Good results with **local application**

IV **Protective immune response** in many systems (including those where other systems fail)

V **Absence of toxicity**
- accepted for animal vaccine production (licensed vaccines!)
- preliminary toxicological data favorable for human use

VI Adaptable to **large scale vaccine production**
- scaling up data favorable
- stability data favorable
- freeze drying possible

A new approach for the development of inactivated vaccines against influenza, is the development of a subunit vaccine, that contains the HA and the NA proteins. Experience in mice using the iscom matrix as a basis for antigenic presentation of influenza virus proteins proved to be quite successful (8). General considerations and points in favor of the use of the iscom matrix for virus vaccines are given in Table 2.

Several approaches for the development of live influenza vaccines based on the principle of reassortment are being explored. The strategy involves in all cases the possibility to transfer HA and NA genes from an epidemic virus to an attenuated virus. By doing so, the reassortant virus is attenuated and expresses the HA and NA genes which are of major importance for the induction of the strain-specific immunity. Four attenuated donor viruses have been studied for this purpose: * viruses attenuated by multiple passages; * temperature-sensitive virus mutants; * cold-adapted virus strains; * avian influenza viruses; (for review see 7). Clear advantages of these live influenza vaccines would be their capacity to induce a better local immunity in the respiratory tract and an overall more «complete» protection.

Measles

The first attenuated measles vaccine (Edmonston B strain) was prepared only a few years after MV had been isolated and adapted to *in vitro* cell culture systems (9). The side reactions induced by these vaccines could be

diminished by simultaneous administration of anti-measles immunoglobulin. Recently developed attenuated measles vaccine strains are less reactogenic.

The use of the live measles vaccine has been successful in many cases. If properly used, the frequency of seroconversion after vaccination exceeds 95 % (10) and after seroconversion the protective efficacy of the vaccine is very high. Cases of infection of vaccinated individuals are most likely due to inadequate vaccination, since analysis of serum antibody titres after infection often showed a MV-specific IgM response, which is indicative for a primary measles virus infection. Attenuated MV strains are not transmitted from one individual to another (9, 11).

Side reactions upon vaccination were shown to be very mild compared to natural MV infection. A slight fever beginning at about the fifth day after vaccination was observed in 5 to 15 % of the vaccinated individuals and rash was observed in approximately 5 % of the cases. The frequency of convulsions after vaccination is about 10-fold less than after natural infection. Since the introduction of live vaccines the incidence of neurological complications, like MV encephalitis and SSPE, has also decreased in populations with a high percentage of vaccinated individuals (12, 13).

Although live measles vaccines have proven to be highly effective, there are still considerable disadvantages associated with their use. Vaccination is recommended after the age of 14 months, since maternal antibodies may interfere with virus replication. However, in developing countries measles is responsible for a large number of deaths of children younger than one year of age. The use of an inactivated measles vaccine, that would be effective in the presence of maternal antibodies, may be more successful in these countries than the use of live vaccines. Furthermore, immunocompromised individuals have been shown to develop severe clinical signs upon vaccination with attenuated virus. Other problems associated with the use of live vaccines are the interference by other infections and the loss of infectivity upon improper storage of the vaccine. Although the newly developed vaccines are more stable, maintenance of a cold chain during transport is still required. Finally, the virtual disappearance of measles in communities will reduce the acceptability of side-effects and complications induced by the use of live vaccines.

Almost simultaneously with the introduction of live attenuated vaccines, Tween-ether or formaldehyde inactivated virus preparations were introduced as measles vaccines. Although the majority of these preparations were shown to induce virus neutralizing (VN) and HI antibodies, these antibodies have been shown to persist only for a relatively short period compared to antibodies induced by natural MV infection. However, more important was the observation that individuals vaccinated with these vaccines were not protected against measles, and in some cases the symptoms in these individuals after infection were more severe than in individuals, who had never been in contact with inactivated MV preparations. Cases of atypical measles were more frequently observed after natural MV infection

in individuals vaccinated with inactivated measles virus, than after revaccination with live measles vaccine (14).

In 1975 NORRBY et al. (15) reported that after natural infection or vaccination with attenuated virus strains non-HI-haemolysis inhibiting (HLI) antibodies were induced, whereas after vaccination with inactivated MV preparations these antibodies were not induced. It was suggested by these authors that the non-HI-HLI antibodies played an important role in the protection against infection. The importance of the F protein in the induction of a protective immune response was also supported by the findings of MERZ et al. (16) that antibodies against the F protein of simian virus 5 (SV5), another member of the family Paramyxoviridae, are important in the prevention of virus spread by cell-cell fusion *in vitro*. Recently it was shown that the purified F protein of CDV induces protection against canine distemper (17). On basis of these results, it has been suggested that for the construction of an effective inactivated measles vaccine, the F protein has to be presented in a proper antigenic form.

Recently we have shown that $CD8^+$ class I MHC-restricted CTL's may play a major role in the recovery from measles (18). As the inactivated vaccines used, probably did not elicit this population of T lymphocytes, it may be speculated that this phenomenon may have largely contributed to the failure of inactivated vaccines to protect against natural infection.

An iscom that contains intact antigen of MV proved to be effective in protecting mice from experimental MV infection (19), and MV-F iscoms were shown to induce non-HI-HLI antibodies and T cell immunity (Table 2, 37). This together with the observation that iscoms containing intact viral proteins are capable of inducing $CD8^+$ CTL's (20) indicates that this may be a successful candidate vaccine against measles. The recent observation that the immunogenicity of MV iscoms in the presence of MV-specific antibodies, also indicates the potential of iscoms for the development of a future subunit iscom measles vaccine.

The genomes of the two membrane proteins have been cloned and expressed by vaccinia recombinant viruses (21). It has been shown that the F protein recombinant virus protected mice against fatal encephalopathy, whereas the haemagglutinin (H) protein recombinant virus induced only partial protection. Although the protective properties of several vaccinia recombinant viruses have been shown to be promising candidate vaccines, the pathogenicity of the vector itself is still a disadvantage for using this type of live vaccines. Therefore, at present it seems unlikely that MV vaccinia recombinant viruses will be acceptable as measles vaccines.

However, the ability of new expression systems to produce high yields of the viral glycoproteins, which may subsequently be used with the appropriate adjuvant system e.g. as a subunit iscom vaccine, should be explored in the near future. Finally the potential of live attenuated vaccines applied locally in the upper respiratory tract, to induce protective immunity in the presence of maternal antibodies, should also further be investigated.

Respiratory syncytial virus and parainfluenza viruses

Many of the problems that have been encountered in the development of measles vaccines proved to be similar in the development of vaccines against other paramyxoviruses including respiratory syncytial virus (RSV). For protection against RSV infection both antibodies and cell-mediated immunity seem to play an important but as yet not fully understood role. Serum antibodies mainly protect against infection of the lower respiratory tract, whereas secretory IgA antibodies are more important in protecting the upper respiratory tract. Cellular immune response to RSV seems predominantly involved in recovery from the infection (22, 23).

Attempts to develop a safe and effective RSV vaccine for children have all been unsuccessful. A formaldehyde inactivated vaccine failed to induce protection and even had a disease-enhancing effect upon subsequent infection with the virus (24–26). This observation is quite similar to that made with inactivated measles vaccines. A model for this enhancement of pulmonary histopathology has been developed in the cotton rat, which develop a pulmonary Arthus-type reaction, when immunized with the formaldehyde inactivated RSV prior to live RSV challenge. A disbalance in antibody and in cell-mediated immune responses has been implicated in this phenomenon in man.

Several attempts to produce live attenuated RSV vaccines have also met with failure (for review see 23). These candidate vaccines were either insufficiently attenuated or overattenuated and parenteral immunization failed to induce protective immunity. However, positive results have been observed when recombinant vaccinia viruses were constructed which expressed the F or the glyco (G) protein of RSV during infection in cell culture. RSV neutralizing antibodies could effectively be induced in rodents and monkeys by infection with either of these recombinant viruses. Cotton rats and BALB/c mice immunized with recombinant vaccinia-F showed a considerable immunity towards infection of the respiratory tract with RSV. Similar results were also observed in owl monkeys. This immunity mainly involved the lower respiratory tract, still allowing the upper respiratory tract to become infected. In this way a solid immunity could be established after initial protection against the development of serious disease.

As we also suggested for measles, the generation of a subunit vaccine based on the iscom matrix (Table 2) may be another interesting approach that has so far not been explored.

Several approaches have been evaluated for the development of vaccines against parainfluenza virus types 1, 2 and 3 (PIV1–3) of man (24, 25). Standard procedures involving the production, purification and inactivation of these viruses, resulted in candidate vaccines which did induce VN and HI serum antibodies in children. However, upon parenteral administration, no protection was achieved against the respective infections, probably due to the failure to induce secretory IgA antibody and the relevant T cell responses. Other attempts to generate candidate parainfluenza vaccines

have been carried out in animal model systems. These included experiments with vaccinia virus recombinants expressing the F and HN protein of PIV3 in cotton rats and monkeys, in which protection was induced (27). Cotton rats could also be protected by immunization with the HN expressed in baculovirus (28). Immunization of cotton rats with a bovine PIV3 strain also protected them from infection with human PIV3 (29). Experiments with iscom and micelle subunit vaccines against parainfluenzavirus) have shown that mice and sheep may be protected with these types of vaccines (for review see 30).

These data indicate indeed that protection may be induced in animal model systems, when the relevant proteins are presented in the proper immunogenic form.

Rhinoviruses

Although rhinovirus infections are by far the most common cause of acute respiratory infections in man, the vast number serotypes that play a role in its epidemiology have made the development of an effective vaccine virtually impossible. Attempts to produce inactivated vaccines representing multiple strains have been made and these have been evaluated in experimental immunization and challenge experiments in man. They proved to provide a certain degree of protection against these strains, by reducing virus shedding and severity of the disease (31, 32). Some initial attempts to produce live attenuated rhinovirus vaccines have not been followed up.

Adenoviruses

Live vaccines based on adenovirus types 4 and 7, have been used in military vaccination programs, but have not been licensed for general administration (33, 34). They are given orally in enteric-coated capsules, which do not allow contact of the viruses with the epithelium of the respiratory tract. Replication takes place in the intestine, where an asymptomatic infection elicits a neutralizing antibody response. Discussions concerning the safety of this type of vaccine have concentrated in the past on the presence of SV_{40} as a contaminant virus on possible oncogenicity of the adenoviruses used and the possible spread to non-vaccinated household contacts. The contaminant SV_{40} is no longer present in the vaccines used and no oncogenicity has ever been shown for any of the human adenoviruses in man. The issue of virus spreading is still considered a problem in young children, from which the viruses has been shown to spread to household contacts who may develop clinical illness. The spread does not seem to be a major problem in adults.

Finally it should be realized that adenoviruses may be used as vectors for other viral proteins, by e.g. replacing the non-essential E3 region with DNA coding for other viral proteins.

Conclusions

To date licensed vaccines against acute respiratory infection are only available for influenza and measles and these vaccines are still based on the classical approaches of vaccine production. Although these vaccines have their limitations, they have contributed to the control of the disease against which they should protect. Vaccines against human adenoviruses have been widely used in the USA military in enteric coated capsules and proved to be safe and efficacious.

For many of the other viruses that may cause acute respiratory illness in man, vaccines are being developed at present. With the help of new developments in immunology and molecular biology, new approaches are being explored for many of these. It is expected that these will indeed lead to new generations of vaccines. However, it should be realized that for the development of these vaccines the availability of suitable animal models and adjuvant systems acceptable for general use in humans, will be of crucial importance.

References

1. Couch, R. B. 1990. Rhinoviruses: In: Virology, 2nd ed., Bernard N. Fields, David M. Knipe et al. (eds.). Raven Press Ltd., New York, p. 607.
2. Murphy, B. R., and R. M. Chanock. 1990. Immunization against viruses. In: Virology, 2nd ed., Bernard N. Fields, David M. Knipe et al. (eds.). Raven Press Ltd., New York, p. 469.
3. Osterhaus, A. D. M. E., and F. G. C. M. Uytdehaag. 1990. Idiotype Networks in Biology and Medicine. Excerpta Medica International Congress Series 862. Elsevier Science Publ., Amsterdam, 310 pp.
4. Kilbourne, E. D. 1988. Inactivated influenza vaccines. In: Vaccines, S. A. Plothin and E. A. Mortimer (eds.). Saunders, Philadelphia, p. 420.
5. Meyer Jr., H. M., H. E. Hopps, P. D. Parkman, and F. A. Ennis. 1978. Review of existing vaccines for influenza. Am. J. Clin. Path. **70:** 146.
6. Potter, C. W. 1982. Inactivated influenza virus vaccine. In: Basic and applied influenza research, A. S. Beare (ed.). CRC Press, Boca Raton, p. 119.
7. Murphy, B. R., and R. G. Webster. 1990. Orthomyxoviruses. In: Virology, 2nd ed., Bernard N. Fields, David M., Knipe et al. (eds.). Raven Press Ltd., New York, p. 1091.
8. Lövgren, K., H. Kaberg, and B. Morein. 1990. An experimental influenza subunit vaccine (iscom) induction of protective immunity to challenge infection in mice after intranasal or subcutaneous administration. Clin. exp. Immunol. **82:** 435.
9. Katz, S. L. 1965. Immunization with live attenuated measles virus vaccines: Five years experience. Arch. ges. Virusforsch. **16:** 222.
10. Brunell, P. A., K. Weigle, D. Murphy, Z. Shehab, and E. Cobb. 1983. Antibody response following measles-mumps-rubella vaccine under conditions of customary use. J. Am. Med. Ass. **250:** 1409.
11. Brandling-Bennett, A. D., P. J. Landrigan, and E. L. Baker. 1973. Failure of vaccinated children to transmit measles. J. Am. Med. Ass. **224:** 616.
12. Landrigan, P. J., and J. J. Witte. 1973. Neurologic disorders following live measles-virus vaccination. J. Am. Med. Ass. **223:** 1459.
13. Modlin, J. F., J. T. Jabbour, J. J. Witte, and N. A. Halsey. 1977. Epidemiologic studies of measles vaccine and subacute sclerosing panencephalitis. Pediatrics **59:** 506.

14. Fulginiti, V. A., and R. E. Helfer. 1980. Atypical measles in adolescent siblings 16 years after killed measles virus vaccine. J. Am. Med. Ass. **244:** 804.
15. Norrby, E., G. Enders-Ruckle, and V. Ter Meulen. 1975. Differences in the appearance of antibodies to structural components of measles virus after immunization with inactivated and live virus. J. Infect. Dis. **132:** 262.
16. Merz, D. C., A. Scheid, and P. W. Choppin. 1980. Importance of antibodies to the fusion glycoprotein of paramyxoviruses in the prevention of spread of infection. J. Exp. Med. **151:** 275.
17. Norrby, E., G. Utter, C. Örvell, and M. Appel. 1986. Protection against canine distemper virus in dogs after immunization with isolated fusion protein. J. Virol. **58:** 536.
18. Van Binnendijk, R. S., M. C. M. Poelen, P. de Vries, F. G. C. M. Uytdehaag, and A. D. M. E. Osterhaus. 1991. A role for $CD8^+$ class I MHC-restricted CTLs in recovery from measles: Implications for the development of inactivated measles vaccines. In: Vaccines 91: Proceedings of the meeting «Modern Approaches to New Vaccines Including the Prevention of AIDS», 12–16 September 1990. Cold Spring Harbor Laboratory Press, p. 299.
19. De Vries, P., R. S. van Binnendijk, P. van der Marel, A. L. van Wezel, H. O. Voorma, B. Sundquist, F. G. C. M. Uytdehaag, and A. D. M. E. Osterhaus. 1988. Measles virus fusion protein presented in an immune-stimulating complex (iscom) induces haemolysis-inhibiting and fusion-inhibiting antibodies, virus-specific T cells and protection in mice. J. gen. Virol. **69:** 549.
20. Takahashi, H., T. Takeshita, B. Morein, S. Putney, R. N. Germain, and J. A. Berzofsky. 1990. Induction of $CD8^+$ cytotoxic T cells by immunization with purified HIV-1 envelope protein in iscoms. Nature **344:** 873.
21. Drillien, R., D. Spehner, A. Kirn, P. Giraudon, R. Buckland, F. Wild, and J. P. Lecocq. 1988. Protection of mice from fatal measles encephalitis by vaccination with vaccinia virus recombinants encoding either the hemagglutinin or the fusion protein. Proc. Natl. Acad. Sci. USA **85:** 1252.
22. Fishaut, M., D. Tubergen, and K. McIntosh. 1980. Cellular response to respiratory viruses with particular reference to children with disorders of cell-mediated immunity. J. Pediat. **96:** 179.
23. McIntosh, K., and R. M. Chanock. 1990. Respiratory syncytial virus. In: Virology, 2nd ed., Bernard N. Fields, David M. Knipe et al. (eds.). Raven Press Ltd., New York, p. 1045.
24. Chin, J., R. L. Magoffin, L. A. Shearer, J. H. Schieble, and E. H. Lennette. 1969. Field evaluation of a respiratory syncytial virus vaccine and a trivalent parainfluenza virus vaccine in a pediatric population. Am. J. Epidem. **89:** 449.
25. Fulginiti, V. A., J. J. Eller, O. F. Sieber, J. W. Joyner, M. Minamitani, and G. Meiklejohn. 1969. Respiratory virus immunization. I. A field trial of two inactivated respiratory virus vaccines: an aqueous trivalent parainfluenza virus vaccine and an alum-precipitated respiratory syncytial virus vaccine. Am. J. Epidem. **89:** 435.
26. Kapikian, A. Z., R. H. Mitchell, R. M. Chanock, R. A. Shvedoff, and C. E. Stewart. 1969. An epidemiological study of altered clinical reactivity to respiratory syncytial (RS) virus infection in children previously vaccinated with an inactivated RS virus vaccine. Am. J. Epidem. **89:** 405.
27. Spriggs, M. K., B. R. Murphy, G. A. Prince, R. A. Olmsted, and P. L. Collins. 1987. Expression of the F and HN glycoproteins of human parainfluenza virus type 3 recombinant vaccinia viruses: contributions of the individual proteins to host immunity. J. Virol. **61:** 3416.
28. Coelingh, K. L. V. W., B. R. Murphy, P. L. Collins, A.-M. Lebacq-Verheyden, and J. F. Battery. 1987. Expression of biologically active and antigenically authentic parainfluenza type 3 virus hemagglutininneuraminidase glycoprotein by a recombinant baculovirus. Virology **160:** 465.
29. Coelingh, K. L. V. W., C. C. Winter, E. L. Tierney, W. T. London, and B. R. Murphy. 1988. Attention of bovine parainfluenza virus type 3 in nonhuman primates and

its ability to confer immunity to human parainfluenza virus type 3 challenge. J. Infect. Dis. **157:** 655.
30. Höglund, S., K. Dalsgaard, K. Lövgren, B. Sundquist, A. Osterhaus, and B. Morein. 1989. Iscoms and immunostimulation with viral antigens. In: Subcellular Biochemistry, Vol. 15, H. R. Harris (ed.). Plenum Press, New York, p. 39.
31. Douglas jr., R. G., and R. B. Couch. 1972. Parenteral inactivated rhinovirus vaccine: Minimal protective effect. Proc. Soc. Exp. Biol. Med. **139:** 899.
32. Perkins, J. C., D. N. Tucker, H. L. S. Knopf et al. 1969. Evidence for protective effect of an inactivated rhinovirus vaccine administered by the nasal route. Am. J. Epidem. **90:** 319.
33. Top jr., F. H. 1975. Control of adenovirus acute respiratory disease in US army trainees. Yale J. Biol. Med. **48:** 185.
34. Top jr., F. H., E. L. Buescher, W. H. Brancroft, and P. K. Russell. 1971. Immunization with live type 7 and 4 adenovirus vaccines. II. Antibody response and protective effect against acute respiratory disease due to adenovirus type 7. J. Infect. Dis. **124:** 155.
35. Morein, B., K. Lövgren, and S. Höglund. 1989. Immunostimulating complex (iscom). In: Immunological Adjuvants and Vaccines, G. Gregoriadis and A. C. Allison (eds.). Plenum Press Publishing Corporation, p. 153.
36. Morein, B. 1990. The iscom: an antigen presenting system. Immunol. Lett. **25:** 281.
37. Osterhaus, A. D. M. E., P. de Vries, F. G. C. M. Uytdehaag, I. K. G. Visser, and B. Morein. 1990. Induction of protective immunity with morbillivirus iscom preparations. In: Vaccines 90: Proceedings of the meeting «Modern Approaches to New Vaccines Including the Prevention of AIDS», 20–24 september 1990. Cold Spring Harbor Laboratory Press, p. 145.

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