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Immunopotentiating complexes

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Conventional vaccines consist of whole microorganisms or viruses. In many cases such vaccines have been effective and even excellent, e.g. smallpox was eradicated with such a vaccine. In other instances conventional vaccines were found not to be efficient, i.e. they did not induce protective immunity. For instance it has been difficult to formulate vaccines inducing protective immunity against viruses causing slowly developing diseases in contrast to viruses causing acute diseases. Also it has been difficult to develop effective vaccines against parasitic diseases.

After proteins inducing protective immunity had been defined, many attempts were made to base vaccines on these proteins, i.e. the protective antigens. Such vaccines are called subunit vaccines. There are at least three reasons to develop subunit vaccines. One reason is to avoid genetic material. Particularly the genes from herpes and retro-viruses are considered dangerous. A second reason is to avoid toxic components of the agent. In particular bacteria contain toxins which limit the use of whole bacteria as vaccines. The third reason to be mentioned is to reveal protective antigens or antigenic determinants which are hidden in the complete microorganism or virus (see measles below). There are other reasons too like autoimmunic and allergic reactions which might be avoided by use of vaccines with a restricted number of proteins.

The first attempts to make subunit vaccines were not successful. Most of these early experimental subunit vaccines were made with the envelope proteins of influenza viruses. Although a sufficient amount of the protective antigens, i.e. the hemagglutinin (H) and neuraminidase proteins, were included in the experimental vaccines they induced no or low immune response in contrast to the influenza vaccines based on whole virus particles (for references see Morein and Simons, 1985).

Monomers, micelles and virosomes as vaccines

In general protective antigens are surface proteins. As regards enveloped viruses the envelope proteins induce protective immunity. To find out how the

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envelope proteins should be presented in a vaccin to induce high antibody response and protective immunity three different and defined physical forms of Semlike Forest virus a (SFV) were prepared as experimental vaccines. The envelope protein was extracted from the virus with a non-ionic detergent Triton X-100 (TX-100). The three different forms were the monomer form – a complex between one protein-molecule and about 75 TX-100 molecules; the micelle form – an aggregation of eight envelope proteins; the virosome – where the envelope proteins are integrated into a lipid vesicle (liposome). In the micelles the proteins are aggregated by the hydrophobic region in the centre and the antigenic interesting part extended outwards. The micelles are virtually free of detergent and lipids. The virosomes are free of detergent but contain lipid. The monomers contain no lipid but detergent, the details of the work is described by Morein et al. (1978). Briefly, it was found that monomers are poorly immunogenic. Ten μg of protein in monomer form induced protection in mice against challenge with six LD_{50} of a virulent SFV strain causing a lethal encephalomyelitis in mice. The same amount of antigen in micelles or in virosomes induced protection against more than 10,000 LD_{50}.

In later experiments it was found that micelles containing the envelope proteins of parainfluenza 3 virus did not induce protection in lambs unless an oiladjuvant was included (Morein et al., 1983). From that point of view the micelles were equally good or bad as a whole virus vaccine (Wells et al., 1976). Although, there are effective adjuvants, they are unfortunately, only effective in doses when they also cause adverse side effects. Conventionally used adjuvants are mixed or emulgated with the antigens. That means to obtain a sufficient concentration of adjuvant at the same cells as the antigens to ewoke an enhanced immune response high doses are needed, because the adjuvant is resorbed independently from the antigen from the site of injection. The result is a local reaction and many times also general reactions. Ideally a vaccine should be limited to the desired antigens in a multimeric physically defined form – a submicroscopic particle, in which the adjuvant is built in.

I*mmuno* s*timulating* com*plex, i.e. iscom*

An iscom consists of a matrix – a glycoside named Quil A – on which the antigens are attached by hydrophobic interaction (Morein et al., 1984). Quil A is an extract of the bark of a South American tree Quilaja Saponaria Molina. Quil A – the matrix – is used as adjuvant in e.g. foot and mouth disease vaccine (Dalsgaard, 1978). The iscom has a cage like globular structure (Fig. 1) about 40 nm in diametre built up of circular subunits of about 12 nm in diametre. During the preparation of iscoms the membrane proteins can selectively be purified from other components.

Iscoms have been prepared with membrane proteins (i.e. envelope proteins) from about 20 different viruses, membrane proteins from cells, parasites and small peptides.

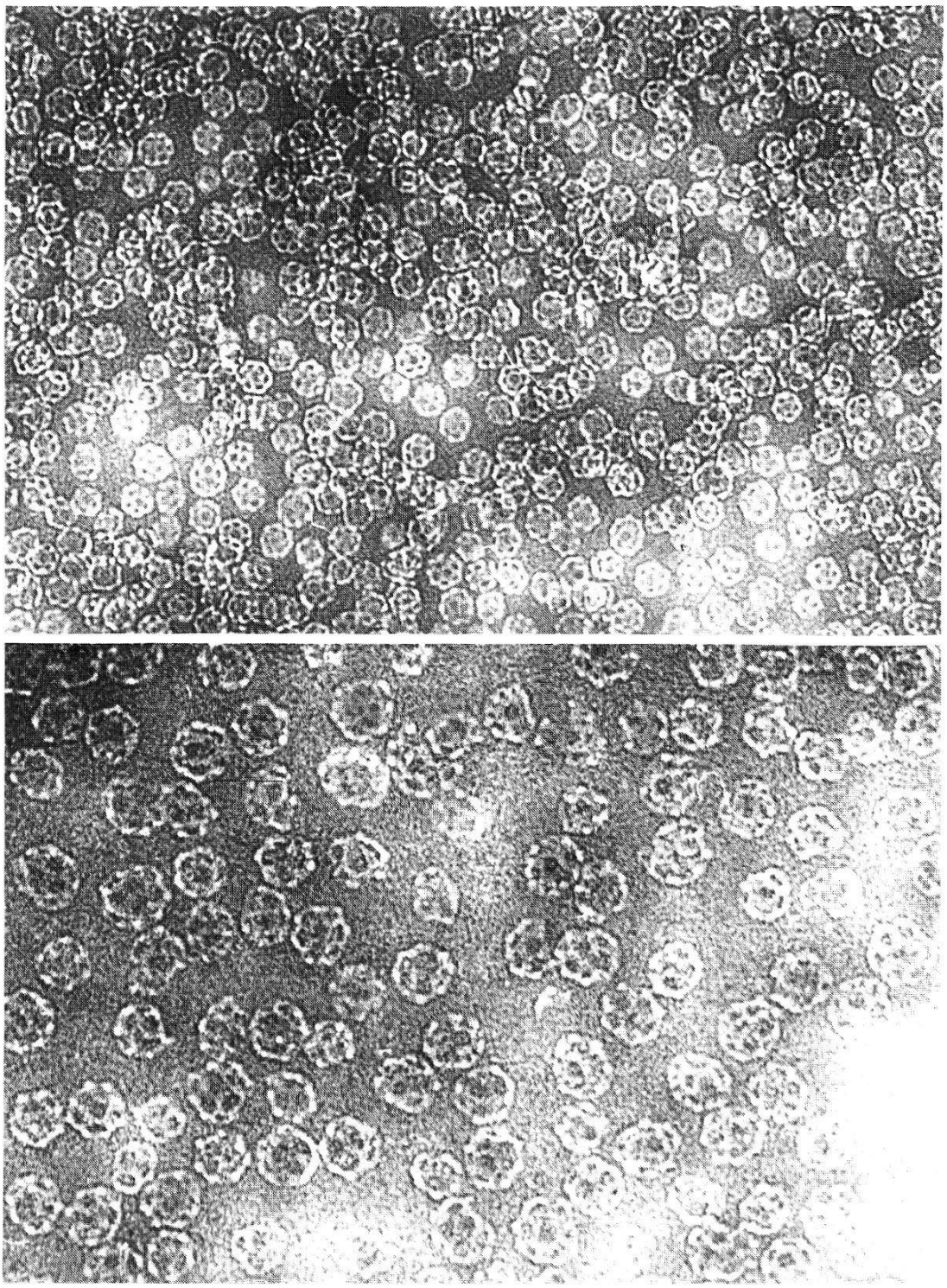

Fig. 1. Electron micrographs of iscoms containing the envelope glycoproteins in influenzavirus. A. Magnification ×1000,000; B. magnification ×200,000.

Most immunization experiments have been performed with virus antigens and the general results can be summarized as follows:

1. A given dose of antigen in iscoms induce about ten times higher antibody response than it does in micelles or in situ in the virus particle.

2. In the iscom the antigen dose can be reduced to one tenth of that in a micelle or in a virus particle and still ewoke a similar or higher magnitude of immune response.
3. The iscom gives a longer lasting immune response than the micelle or the whole virus particle.

Such results have e.g. been shown with the envelope proteins from influenza, parainfluenza 3 and measles viruses. With rabies virus it was shown that the iscoms induce cell mediated immunity as measured by the lymphocyte stimulation test or delayed hypersensitivity (Osterhaus, personal communication).

An experimental horse influenza iscom vaccine was found superior to commercial vaccines. After the three initial vaccinations the iscom vaccines induced immune response lasting above the protective levels for more than a year compared with a period of three to six months for commercial vaccines (Mamford et al., submitted).

Bovine virus diarrhea virus (BVDV), a Toga virus, causes among other symptoms abortions in cattle and sheep. Iscoms containing the envelope proteins from BVDV protected sheep completely against challenge. No abortions occurred in the group of 12 vaccinated animals while 10 out and 12 non-vaccinated eves aborted (Sundquist, personal communications). The iscom vaccine was prepared with envelope proteins from a bovine strain while the challenge virus was an ovine strain.

About 20 years ago a measles virus vaccine was prepared containing formaline killed virus. This vaccine induced high levels of neutralizing and hemagglutination inhibition serum antibody titres. However, when some of the vaccines became exposed to a natural infection, they developed an atypical measles with bad rash and giant cell pneumonia. Later it was considered that this killed vaccine induced an incomplete immune response (Norrby et al., 1975; Merz et al., 1980). A complete immune response from protective point of view should include immune response to both envelope proteins, i.e. the hemagglutinin, which is responsible for the attachment of the virus to the cells as well as the fusion protein which facilitates fusion between the virus and the cell so that the nucleic acid can be introduced to the cell. The killed virus did induce antibody to the hemagglutinin but not to the fusion proteins. Measles iscoms like the now used live attenuated measles virus vaccines induce high levels of antibody response to both envelope proteins, i.e. the hemagglutinin and fusion proteins (Morein et al., 1984).

Iscoms have been prepared consisting of the major S gene products of the hepatitis B virus genome (HBs AG) (Howard et al., in preparation). The HBs AG particles were expressed in yeast. Immunization of BALB/c mice with a single dose of hepatitis B iscoms induced high antibody titres to HBs Ag. In contrast, the original HBs AG preparation required and adjuvant to produce equivalent amounts of antibody. Analysis of the sera from mice immunized with hepatitis

B iscoms revealed antibodies directed against the major determinants of HBs AG. High secondary antibody response was observed in animals previously immunized with a subimmunogenic dose of HBs AG. These results indicate that hepatitis B iscoms may represent a suitable immunogen for use in individuals in whom a course of immunization with currently licenced hepatitis B vaccines failed to produce a significant anti-HBs response.

An experimental iscom vaccine with the envelope protein gp 70 from feline leukemia virus (Fe LV) induced neutralizing antibodies and protected cats against challenge with FeLV as measured by virus isolation. Non-vaccinated control cats became viremic after challenge. These experiments are repeated in a larger scale (Osterhaus et al., 1985).

Iscoms have been prepared containing membrane proteins from *Toxoplasma gondii.* Four major proteins and three major antigens were detected. These toxoplasma iscoms were highly immunogenic in mice and sheep. Preliminary studies indicate that toxoplasma iscoms may induce protection against challenge in mice (prolonged survival) and in sheep by protecting them from abortion upon challenge with a virulent toxoplasma strain. The vaccine was prepared with envelope proteins from a mouse laboratory strain of *Toxoplasma gondii* which probably is not the best choice of strain.

Toxoplasma iscoms have been used as coating antigens in ELISA for detection of antibody to *T. gondii.* This ELISA was as sensitive and more specific than the conventional toxoplasma ELISA. The coating antigen in the iscom ELISA is restricted to membrane proteins while the coating antigen in the conventional ELISA consists of soluble, cytoplasmatic, *T. gondii* tachyzoite antigen. Calves experimentally infected with *Sarcocystis cruzi* developed antibodies which were detected with the conventional toxoplasma ELISA but not in the iscom ELISA. The iscom technique can be used for selective preparation and isolation of membrane proteins (Lövgren et al., in press: J. vet. Med. 1987).

The iscoms have also been used to present small molecules and peptides. The iscom system proved to be more effective than carrier systems employing albumine or KLH supplemented with Freund's complete adjuvant (FCA). Besides that with the iscom system no adverse side effects were noticed following immunization of the mice in contrast to systems, where FCA has to be included (Lövgren et al., 1986).

In conclusion the iscom system can be used to enhance the immune response of antigens prepared from microorganisms, viruses, cells or from antigens produced by genetically engeneered cells. The system can also be used to enhance the immunogenicity of peptides and small molecules.

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