antigen delivery (116), as too have polymers which self-assemble into particulates (poloxamers) (117), or soluble polymers (polyphosphazenes) (118). However, the potency, safety, and tolerability of many of these approaches require further evaluation. Although advantages are often claimed for these approaches over the more established PLG, the advantages are often not clear and are rarely demonstrated in comparative studies.

IMMUNE-STIMULATING COMPLEXES

ISCOM adjuvants are a particulate complex containing saponin, cholesterol, and phospholipids. The saponins used for ISCOMs have varied over the years, depending on the manufacturer, but is usually a multicomponent fraction extracted from the bark of the *Quillaia saponaria* tree. *Quillaia* saponins have been known for many years to exhibit potent immunomodulatory activity, and have been used in animal vaccines, but the crude preparations are not suitable for human vaccines because of toxicity (119). Therefore, better-defined fractions of *Quillaia* were developed for human use, including QS21 (120) and ISCOPREP™ (121). ISCOPREP™ saponin is a well-defined, multicomponent fraction, which has been included in ISCOM formulations that have been extensively evaluated in humans and appear to be reasonably well tolerated (122).

During the manufacture of ISCOM adjuvants, the cholesterol interacts with saponin to form an extremely strong bond, which is the basis of the unique particulate structure of ISCOMs, and likely contributes to the stability of the adjuvant (123). The interaction with cholesterol also substantially reduces the hemolytic activity of the saponins and is important for safety. Phosphatidylcholine (PC) was traditionally used in the early ISCOM vaccines, usually egg derived. More recently, dipalmytoylphosphatidylcholine (DPPC) has been identified as the optimal phospholipid to be used for ISCOMs manufacturing and stability.

A molecular structure for ISCOMs was originally proposed by Kersten et al. (124), who suggested the adjuvant had a "soccer ball" arrangement with the multiple micelles held together by hydrophobic interactions. In this model, the saponin molecules create pores in cholesterol/DPPC vesicles with only the triterpenoid core of the saponin interacting with the lipid bilayer. An important physical property of ISCOMs is the negative surface charge of approximately −20 mV, which enables the adjuvant to form a stable colloidal dispersion. The 40 nm size and particulate structure of the adjuvant is thought to be important for potency, promoting the delivery of antigens to APC. ISCOM adjuvants have been shown to induce potent humoral and cellular immune responses in all species in which they have been evaluated, including nonhuman primates. The immune responses generated in response to immunization with ISCOM adjuvants have recently been reviewed (119).

In the 1990s, clinical studies were performed using ISCOMs as adjuvants for influenza vaccines. These studies involved almost 900 participants and showed the induction of both antibody and cellular immune responses (125,126). However, the ISCOM adjuvant was considered too reactogenic to allow further development for human vaccines. Nevertheless, the adjuvant subsequently underwent extensive optimization, to improve tolerability, and the modified adjuvant has now been used in a number of additional clinical studies. NY-ESO-1, an antigen found on a number of cancer types including melanoma and breast, has been formulated with ISCOMs and evaluated in a clinical trial. The vaccine was safe, well tolerated, and induced both humoral and cellular immune responses (127). Human papilloma virus (HPV) type 16 E6 and E7 proteins were also formulated with ISCOMs and evaluated in two studies. In the second study, the vaccine was found to be immunogenic (humoral and cellular responses), safe, and well tolerated. An HCV core ISCOM vaccine has also been evaluated, and shown to be safe and well tolerated, while inducing both humoral and cell-mediated immune responses. Hence, it appears that a new generation of ISCOM adjuvants are now available, which are capable of inducing potent immune responses in humans, while being better tolerated than the first generation of ISCOMs.

INTERCELL'S ADJUVANT COMPOSITION

Intercell has described a vaccine adjuvant formulation that comprises particulate structures made by combining a cationic peptide with an oligonucleotide that does not contain a CpG motif (128). The adjuvant formulation can be added to antigens of interest and is capable of inducing potent immune responses in preclinical models (129). Recently, this adjuvant formulation was progressed into clinical evaluation as an adjuvant for a new generation TB vaccine and appeared to be well tolerated (K. Lingnau, personal communication). The suitability of this new adjuvant formulation for a wide range of vaccines remains to be established, as does its clinical acceptability for further human use.

VIRUS-LIKE PARTICLES

The rationale for the use of VLPs as an antigen delivery system came from observations that a glycoprotein antigen was highly immunogenic when presented to the immune system as an ordered array on the surface of virions but not when presented in a soluble or membrane bound form (72). It appears that only highly repetitive antigens are able to efficiently cross-link surface antigen receptors on B cells and to induce potent immune responses (130). VLPs are nonreplicating virus capsids made from recombinant DNA technology that mimics the structure of native viruses. They are generally formed from a single viral structural protein, which self-assembles to form a defined particulate structure, following expression in mammalian cells, yeast, *Escherichia coli*, or in baculovirus systems. VLPs are noninfectious, as they lack the viral genome and can sometimes be comprised of more than one viral structural protein. However, the most well-known and commonly used VLPs are expressed as a single protein (HPV, HBV core, or calicivirus). VLPs mimic the native structure of the protein comprising their structure and they are potently immunogenic. VLPs are being explored in many different ways as subunit virus vaccine candidates, they can also be used as carriers for heterologous antigens, co-expressed with the VLP protein, or conjugated after preparation. VLPs are a particularly attractive approach if the native virus cannot be easily grown in culture (e.g., HPV). Potentially, VLPs can stimulate both arms of the immune response, including antibodies and T cells, and have significant safety advantages over live-attenuated viral vectors. Antigens can be displayed on VLPs either as genetic fusions (131), by streptavidin-biotin conjugation (132) or by chemical cross-linking (133), with the density of antigen on the surface of the particle apparently playing a critical role in the induction of strong antibody responses (133,134). VLP-based vaccines have been tested in clinical trials to induce immune