influenza virus and two palmitic acid groups attached to the peptides through a lysine residue, failed to signal through TLR2, mature DC, or trigger IL-12 secretion at levels where Pam2Cys- and Pam3Cys-containing lipopeptides were highly active (89). Perhaps increased stability and persistence of the MHC-peptide complex contributes to enhancing lipopeptide immunogenicity as found with a palmitoylated version of a vaccine candidate for human papillomavirus in HLA-A2 transgenic mice (122).

The innate and adaptive immune systems provide multiple points at which lipids can have an effect, and the interplay between the two systems often enables downstream amplification effects; Pam3Cys- and Pam2Cys-based lipid moieties, for example, cause macrophages and monocytes to release proinflammatory cytokines as well as chemokines that attract neutrophils and other white cells (123–125). Expression of TLRs on tissues other than DCs provides opportunities for input of additional stimuli into the immune system; B cells present in the nasal-associated lymphoid tissues constitutively express TLR2 and engagement of MALP-2 causes the upregulation of class II molecules and the expression of costimulatory molecules. Such stimulation could improve these cells' antigen-presenting properties as well as making them more accessible to the help provided by their interaction with $CD4^+$ T cells. Many of these properties are retained by lipid-conjugated vaccine epitopes and antigens including lipopeptides containing a single palmitoyl lysine residue (126).

It is clear that the ability of some lipopeptide-based vaccines to specifically target DCs and to be efficiently transported across the cell membrane through interaction with surface receptors ensures efficient antigen uptake by DCs. The ensuing events of cellular maturation including expression of class II and costimulatory molecules that can also occur clearly improve the efficacy of lipopeptide-based vaccines. The fact that the underlying mechanisms of action are understood also admits them into the repertoire of rationally designed vaccines.

CODELIVERY OF LIPOPEPTIDES WITH ANTIGEN

The immunostimulatory properties of lipopeptides have also been exploited by admixing with antigens in much the same way that adjuvants are admixed with antigen prior to administration. Such lipopeptides generally have little or no intrinsic immunogenicity. The water-soluble lipohexapeptide Pam3Cys-Ser-(Lys)4 was shown to improve the antibody response to a variety of antigens (127–129) and has also been shown to promote type 1 cytokine responses (130).

In a direct comparison of different adjuvants, Pam3Cys-Ser-(Lys)4 either administered with malaria CTL epitope or when covalently attached to it was found to be superior to other formulations (131). As a consequence of the finding that simple lipopeptide-based adjuvants can be administered with antigen to enhance ensuing immune responses, a great deal of effort has been applied to optimizing these structures (78,132–135) for maximum biological function.

Recent work in this area has focused on the use of a synthetic version of MALP-2 [which contains Pam2Cys as the lipid component) as a co-delivered adjuvant for both mucosal and systemic delivery (136) and has been reported to enhance the antibody response to the Tat (137) and matrix (138)] proteins of HIV. In addition, intranasal co-inoculation of MALP-2 with live-attenuated measles vaccine virus lead to the induction of higher titers of neutralizing antibodies leading to protective immunity in cotton rats (139). Some studies have shown that covalent attachment of the lipid to the peptide immunogen is more efficient for a given dose of peptide (56,132), but the codelivery approach does have the advantage of utility with vaccine candidates that are not readily synthesized.

The strong immunostimulatory effects of lipopolysaccharide have also attracted immunologists for many years but its toxic effects have prevented its adoption into the field of vaccinology. Attempts to identify an adjuvanting, nontoxic component of LPS resulted in the development of monophosphoryl lipid (MPL) A. MPL is a mixture of six glycolipids and is obtained through sequential acid-base hydrolyses of bacteria-derived LPS (140). MPL A has been demonstrated to possess many of the adjuvant properties of LPS with fewer side effects (141). By 2005, over 273,000 MPL doses had been administered in clinical trials, with a high degree of safety and superior adjuvant activity compared with alum (141). These results have led to GlaxoSmithKline's MPL-adjuvanted hepatitis B vaccine, Fendrix, receiving marketing approval in Europe. Synthetic analogues of LPS have also been synthesized (142,143), which tend to be single chemical entities as opposed to mixtures of compounds.

The majority of MPL studies have investigated its administration by parenteral routes in admixture with various antigens. MPL has also been demonstrated to have a potent mucosal adjuvant activity, which is most probably associated with expression of TLR4 on mucosal tissues (144). Preclinical mucosal immunization studies have been performed by the nasal routes for hepatitis B, influenza and tetanus (145), HIV-1 (25), *Streptococcus mutans* (146), and by the oral route for *Mycobacterium tuberculosis* (147). In general, the results of administration by these mucosal routes was reported to result in the induction of antigen-specific mucosal IgA and systemic IgG antibodies as well as increased levels of cell-mediated immunity.

Because of the unique biological effects, and strong adjuvant activity of MPL, investigations have sought to produce self-adjuvanting vaccines by conjugating various antigens to MPL (or MPL analogues). In one study a trinitrophenyl (TNP) group was attached via a 6-aminocaproic acid linker to *Escherichia coli* J5 MPL. Intraperitoneal immunization of mice with this construct yielded high titers of anti-TNP IgM and IgG antibodies (148), suggesting that MPL can act as a carrier as well as an adjuvant when conjugated to haptenic antigens.

CONCLUSION

The feasibility of lipopeptide-based vaccines has been demonstrated by numerous groups, and we are now seeing some evidence of the proof of principle of the approach where totally synthetic epitope-based vaccines that carry a lipid moiety have entered clinical trials. Among the first human studies was the demonstration that hepatitis B virus-specific CTL responses could be induced by lipopeptide vaccination and that these were of comparable magnitude to those elicited by acute viral infection (149–151). In addition, the French National Agency for AIDS Research (ANRS) have been developing T-cell-inducing lipopeptide vaccines against HIV since 1994 (reviewed in Ref. 152). In a series of trials, over 200 healthy volunteers and 48 infected patients have been vaccinated with lipopeptides alone, in combination with adjuvants or in a prime-boost regimen with canary pox vectored antigen. A common theme from these studies is that of safety and immunogenicity of lipopeptide vaccines. Whether they will impact on the viral load in chronic disease is the next hurdle, and potency will have to be