dipalmitoyl phosphatidylethanolamine (MTP-PE) (57,145). Because MDP in water provides only a modest adjuvant effect in mice (155,156) and humans (152), threonyl-MDP and MTP-PE have been administered in oil emulsion vehicles in attempts to improve potency. The Syntex Adjuvant Formulation (SAF) preparation (Syntex Research, acquired by Roche in 1995) is an O/W emulsion vehicle. The vehicle contains 5% squalane, 2.5% *Pluronic*™ L121, and 0.2% polysorbate 80 (*Tween*™ 80) in phosphate-buffered saline, pH 7.4 (149,157). Squalane, used in several modern adjuvant emulsions, is metabolizable oil used in many over-the-counter drugs and cosmetics. Pluronic 121 is a nonionic block copolymer discussed below. SAF elicits both cell-mediated (lymphocyte blastogenic) and humoral responses, but it is highly reactogenic so it is no longer studied as a vaccine adjuvant. GSK Biologicals is also developing a proprietary O/W emulsion-based AS for use in a prepandemic H5N1 influenza vaccine to provide heterologous immunity (115). Finally, MF-59 has been studied by Chiron in various experimental vaccines for HIV, herpes simplex, and HPV (57,90,94,158).

Monophosphoryl Lipid A

The adjuvant effect of LPS was described in 1956 (159). Most of the adjuvanticity and toxicity of LPS are associated with the lipid A region of the molecule (160). The LPS of *Salmonella minnesota* R595 has been detoxified without destroying its adjuvant activity by exposing the LPS to mild hydrolytic treatment (161). The resultant monophosphoryl derivative of lipid A, called MPL, is a highly adaptable molecule that can be used effectively in many adjuvant formulations (162). The immunopotentiating nature of MPL may be associated with its capacity to induce cytokines such as IL-12 (163), IFN-γ, IL-1, and IL-2 in mouse and human macrophages (164–166). MPL promotes antigen-specific DTH and a predominant murine IgG2a immunoglobulin response characteristic of TH1 help (167). Numerous animal and human studies testify to the utility of MPL as an adjuvant, used alone or combined effectively with other adjuvants and vehicles for capsular polysaccharide, protein, and peptide antigens (74,123,162). In the past decade, many clinical studies have utilized MPL or DETOX™ (MPL plus cell wall skeleton of *Mycobacterium phlei* in a squalane-in-water emulsion vehicle) as vaccine adjuvants in volunteers (75,76,79–82,95,119,123,130,131,143,168–170) (Table 2). More recently, synthetic lipid A mimetics (aminoalkyl glucosaminide 4-phosphates) that share most of the properties of MPL, have been developed by Corixa, and are now being developed by GSK (83,171,172).

Several AS under development by GSK Biologicals contain MPL combined with O/W emulsions. AS02 (formerly known as SBAS2) is a proprietary O/W emulsion containing MPL and QS-21 that causes strong antibody responses as well as Th1 and CTL cellular responses. Phase 1/2 studies have been conducted in hepatitis (82), HIV (95), and with multiple HIV vaccine formulations (96). AS02 has been broadly studied in malaria, most recently with RTS,S, a circumsporozoite (CS) subunit antigen fused to the hepatitis S antigen (119,130,132–134), or with FMP1, a 42-kDa fragment of the merozoite surface protein-1 (131,143). RTS,S with AS02 demonstrated efficacy in Phase 2b field trials in The Gambia (168) and Mozambique (135–137). AS04 (described above) is comprised of aluminum salts and MPL for use in licensed vaccines. This AS has also been studied in HSV (173).

Exotoxins

Recombinant LT, which is one of the most potent mucosal adjuvants (174), has been shown to be safe and immunogenic by transcutaneous immunization (TCI) in humans (175). Antigens can be formulated with LT and delivered using a topical patch for delivery to the dense population of dendritic cells that are resident in the epidermis. LT-specific IgG and IgA antibodies were present in both stool and urine, implying the induction of a strong mucosal immune response. The potent activation of epidermal Langerhans cells allows LT to adjuvant the response to a coadministered enterotoxigenic *Escherichia coli* (ETEC) antigen as well (176). Serological and antibody-secreting cell (ASC) responses to the LT and the *E. coli* surface antigen CS6 were comparable to those seen following a protective oral challenge, suggesting that TCI can potentially elicit effective immunity similar to natural infection with ETEC, although local rashes may limit its broad applicability (176). LT is being studied as an immunostimulant to be used with TCI in conjunction with an injected influenza vaccine (104,177). Other groups are using detoxified mutants of LT to explore the potential for oral or intranasal vaccination (178–180), however, there is at least a theoretical concern that the LT could traffic to the brain and could cause inflammation there (72).

Saponins

Saponins are triterpene glycosides that can be isolated from the bark of the *Quillaja saponaria* Molina tree, a species native to South America (181). A partially purified saponin, Quil A, has been used widely as an adjuvant in veterinary vaccines (182). Quil A is a heterogeneous mixture of glycosides. Analysis by high performance liquid chromatography (HPLC) reveals at least 24 peaks that vary in their adjuvanticity and toxicity in mice (183). Quil A has also been tested extensively as part of immune-stimulating complexes known as *ISCOMs*™, which are colloidal cage-like 40 nm particles consisting of antigen, cholesterol, phospholipids, and Quil A (184). Despite their potent adjuvanticity, ISCOM vaccines have only recently been administered to humans because of the local and systemic toxicity of Quil A in mice (184,185). An influenza-ISCOM vaccine for humans containing a less toxic saponin fraction is under development, which shows a strong cellular immune response (103,186).

QS-21 (*Stimulon*™) is one of at least 24 structurally distinct triterpene glycosides isolated from Quil A, and is being developed by the Antigenics, Inc. (Framingham, Massachusetts, U.S.). It demonstrated the proper balance of low mouse toxicity and maximum adjuvanticity, and it eliminated the problem of lot-to-lot variation characteristic of Quil A (183). QS-21 is novel in that it can improve the immunogenicity of protein and polysaccharide antigens (187) in a variety of small animals, dogs, or primates. It also uniquely stimulates both humoral and cell mediated immunity, including potent class I-restricted cytotoxic T lymphocyte responses to subunit antigens (188). The addition of QS-21 to malarial peptide vaccines promoted CD4 and CD8 T cell responses (189). As noted above QS-21 is synergistic with MPL in stimulating the response to several vaccines in AS02.

Nonionic Block Copolymers

The copolymer adjuvants are simple linear chains or blocks of polymers of hydrophobic polyoxypropylene, flanked by two