origin. However, the inclusion of immune potentiators often raised concerns about the safety of the formulation and indeed, has generally restricted the further development of many of these novel approaches.

Nevertheless, based on the long history of the use of emulsions as adjuvants, including the widely used Freund's incomplete adjuvant (FIA), several groups investigated the development of emulsion formulations as adjuvants for use in humans. Scientists at Syntex developed an oil-in-water (o/w) emulsion adjuvant using the biodegradable oil, squalane, and used this as a delivery system for a synthetic immune potentiator called *N*-acetylmuramyl-L-threonyl-D-isoglutamine (threonyl-MDP) (20). This o/w emulsion adjuvant was called the Syntex adjuvant formulation (SAF). A closely related immune potentiator to threonyl-MDP, *N*-acetyl-L-alanyl-D-isoglutamine (MDP), had been originally identified as the minimal structure isolated from the peptidoglycan of mycobacterial cell walls, which had adjuvant activity (21). However, the parent MDP compound was pyrogenic and induced uveitis in rabbits (22), making it unacceptable as an adjuvant for human vaccines. Therefore, various derivatives of MDP were synthesized in an effort to identify a molecule with an acceptable toxicology profile, which retained adjuvant activity, including threonyl-MDP. MDP and its related compounds were later shown to activate immune cells through interaction with NOD, which acts as an intracellular recognition system for bacteria, and is a PRR (14). In addition to threonyl-MDP, SAF also contained a pluronic polymer surfactant (L121), which was included to help bind antigens to the surface of the emulsion droplets. However, clinical evaluations of SAF as an adjuvant for an HIV vaccine showed it to have an unacceptable profile of reactogenicity (23).

As an alternative adjuvant formulation to SAF, Chiron scientists initially developed an o/w emulsion based on the biodegradable oil, squalene, as a delivery system for an alternative synthetic MDP derivative, muramyl tripeptide phosphatidylethanolamine (MTP-PE). MTP-PE had a phospholipid tail attached to it, to allow it to be more easily incorporated into lipid-based formulations, particularly liposomes, and to reduce toxicity (24). Unfortunately, clinical testing showed that emulsions of MTP-PE also showed an unacceptable degree of reactogenicity, making them unsuitable for routine clinical use (25,26). Nevertheless, these studies highlighted that the squalene o/w emulsion alone, without the added MTP-PE immune potentiator, was well tolerated and induced comparable immune responses to the emulsion containing the immune potentiator (26,27). Hence, these observations resulted in the development of the squalene-based o/w emulsion alone as an adjuvant, which was called MF59. The composition of MF59 is shown in Figure 2. The small droplet size of MF59 emulsion, generated through the use of a high-pressure homogenizer, called a microfluidizer, in the preparation process, was crucial to potency, but also enhanced stability and allowed the formulation to be sterile filtered. MF59 emulsion adjuvant, without additional immune potentiators, proved sufficiently potent and safe to allow the successful development of a new generation influenza vaccine containing this adjuvant (28). Hence the experience with MF59 showed that o/w emulsions alone, without additional immune potentiators could be highly effective adjuvants with an acceptable safety profile. Moreover, the early clinical experience with MF59 also served to highlight the need for careful selection of immune potentiators, should it prove necessary to include them in adjuvant formulations.

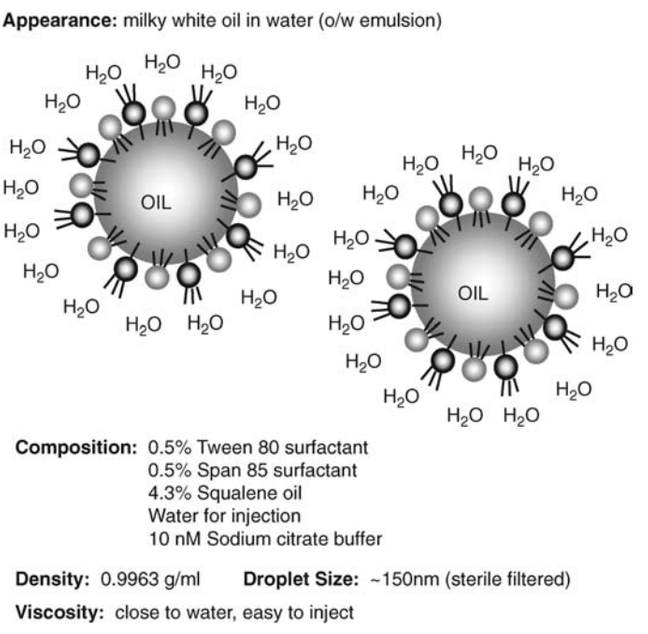

Figure 2 The composition of MF59 adjuvant.

The Mechanism of Action of MF59 Adjuvant

Early studies designed to determine the mechanism of action of MF59 focused on the possibility of establishing a depot effect for coadministered antigens, since there had been suggestions that emulsions may retain antigen at the injection site. However, it was shown that an antigen depot was not established at the injection site with MF59, and that the emulsion was cleared rapidly (29). The lack of an antigen depot was confirmed in later studies (30), which also established that MF59 and antigen were cleared rapidly. Subsequently, it was thought that perhaps the emulsion acted as a ''direct delivery system'' and was responsible for promoting the uptake of antigen into APC. This was linked to earlier observations with SAF emulsion, which contained a pluronic surfactant and that was thought to be capable of binding antigen to the emulsion droplets (20). However, studies with recombinant antigens showed that MF59 was an effective adjuvant, despite no evidence of association of the antigens to the oil droplets. Moreover, an adjuvant effect was still observed if MF59 was injected up to 24 hours before the antigen, or up to 1 hour after, confirming that direct association with the emulsion was not required (29). Nevertheless, administration of MF59, 24 hours after the antigen, resulted in a much reduced adjuvant effect, suggesting that the emulsion was activating immune cells, which were able to better process and present the coadministered antigen. A direct effect on cytokine levels in vivo following administration of MF59 has also been observed, supporting the theory of immune activation (31). Moreover, more recent studies have confirmed the ability of MF59 to have a direct effect on immune cells, triggering the release of chemokines and other factors responsible for recruitment and maturation of immune cells.

Hence, although the exact mechanism of action of MF59 adjuvant remains to be better defined, it appears to function