predominantly as a delivery system, to promote the uptake of coadministered vaccine antigens into APC (32,33). However, there does not appear to be a need for the antigen to be directly associated with the emulsion droplets. Rather, MF59 recruits and activates APC to the injection site, which take up and process coadministered antigens. It has been found that MF59 activates human monocytes and granulocytes in vitro. On monocytes, MF59 leads to increased endocytosis, enhanced surface expression of MHC (major histocompatibility complex) class II and CD86, and downregulation of the monocyte lineage marker CD14. These are phenotypic changes consistent with differentiation toward DC lineages. MF59 also induces monocytes and granulocytes to produce chemokines, including CCL2 (MCP-1), CCL4 (MIP-1β), and CXCL8 (IL-8), which are all involved in recruitment of immune cells from the blood into peripheral tissue. In experimental conditions where monocytes differentiate into DC by addition of GM-CSF and IL-4, the presence of MF59 enhances the acquisition of a mature DC phenotype, as monitored by the expression of MHC II, CD86, and CD71. On maturing DC, MF59 leads to an earlier and overall higher expression of the maturation marker CD83 and the chemokine receptor CCR7, which is crucial for homing of DC from peripheral tissue into lymph nodes. Hence, following parenteral vaccination, MF59 increases recruitment of immune cells into the injection site, accelerates and enhances maturation of monocytes, augments Ag uptake, and facilitates migration of differentiating monocytes into the draining lymph nodes. Consequently, MF59 creates a local immune stimulatory environment within the muscle, following immunization, which greatly enhances immune responses to coadministered antigens.

The Composition of MF59

MF59 is a low-oil-content o/w emulsion. The oil used for MF59 is squalene, which is a naturally occurring substance found in plants, in the livers of a range of species, including humans, and is a component of the secretions of the sebaceous glands in humans. Moreover, squalene is an intermediate in the human steroid hormone biosynthetic pathway and is a direct synthetic precursor to cholesterol. Therefore, squalene is biodegradable and biocompatible. Eighty percent of shark liver oil is squalene, and shark livers provide the natural source of the squalene, which is used to prepare MF59. MF59 also contains two nonionic surfactants, Tween 80 and Span 85, which have been used in other biomedical products and here, are designed to optimally stabilize the small emulsion droplets. Although single vial formulations can be developed with vaccine antigens dispersed directly in MF59, MF59 can also be added to antigens immediately prior to their administration. Even though a less favorable option, combination prior to administration may be necessary to ensure optimal stability for some more labile antigens.

Manufacturing of MF59

Details of the manufacturing process for MF59 have previously been described (34). The process involves dispersing Span 85 in the squalene phase and Tween 80 in the aqueous phase, before high-speed mixing to form a coarse emulsion. The coarse emulsion is then passed repeatedly through a microfluidizer to produce an emulsion of uniform small droplet size (~165 nm), which can then be sterile filtered and filled into vials. Methods have also been published describing the preparation of MF59 at small scale, for use in research studies (35).

Preclinical Experience with MF59

Preclinical experience with MF59 is extensive and has been reviewed on several occasions previously (34,36,37). MF59 has been shown to be a potent adjuvant in a diverse range of species, in combination with a broad range of vaccine antigens, to include recombinant proteins, isolated viral membrane antigens, bacterial toxoids, protein polysaccharide conjugates, peptides, and VLPs. MF59 is particularly effective for inducing high levels of antibodies, including functional titers (neutralizing, bactericidal, and opsonophagocytic titers), and is generally more potent than alum. A preclinical study that directly compared MF59 and alum for several different vaccines confirmed that MF59 was more potent, although alum performed well for bacterial toxoids (38). MF59 has also shown enhanced potency over alum when directly compared by protein polysaccharide conjugate vaccines (39) and by a recombinant viral antigen in nonhuman primates (35). In preclinical studies, MF59 is a more potent adjuvant for influenza vaccines, in comparison with various alternative adjuvants.

In addition to immunogenicity studies, extensive preclinical toxicology studies have been undertaken with MF59, in combination with a range of different antigens in a number of species. MF59 has shown no evidence of either mutagenic or teratogenic effects, and does not induce sensitization in an established guinea pig model to assess contact hypersensitivity. The favorable toxicological profile for MF59 allowed extensive clinical testing with a number of different vaccine candidates.

Clinical Experience with MF59 Adjuvant

The largest clinical experience with MF59 has been obtained with the adjuvanted influenza vaccine (Fluad®), which was initially licensed in Italy in 1997, and is now licensed in more than 20 countries. More than 45 million doses of this product have been used in humans. The adjuvanted influenza vaccine was initially targeted for vaccination of the elderly, since conventional vaccines do not provide optimal protection in this age group (40). For this reason, most of the clinical trials with MF59 adjuvanted influenza vaccines have been performed in elderly subjects, in which a significant adjuvant effect has been consistently observed (41). The increased immunogenicity of MF59 adjuvanted influenza vaccine was shown to be particularly important in subsets of the elderly population, which have a higher risk of developing influenza and its most severe complications, including subjects with a low preimmunization titer and subjects affected by chronic diseases (41,42). Additionally, immunogenicity against heterovariant flu viruses was enhanced by MF59, a feature that is particularly beneficial when the vaccine antigens do not match completely those of the circulating viruses (41,43). Importantly, the addition of MF59 to influenza vaccine did not affect the safety profile of the vaccine, which was very well tolerated (41). MF59 was also evaluated as a potential adjuvant for pandemic influenza vaccines and was shown to induce a highly significant enhancement of antibody titers (44,45). Importantly, MF59 also allowed a significant reduction in the antigen dose, an observation that might be very important to increase the vaccine production capacity when a real pandemic occurs (Fig. 3). As already shown for the interpandemic vaccine (41,43,46), broader cross-neutralization against heterovariant pandemic strains was also an additional benefit of an MF59 adjuvanted vaccine (47). This is an important observation, which might favor the use of MF59 adjuvanted pandemic vaccines for stockpiling purposes.