Chapter 10

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Design and Evaluation of a Safe and Potent Adjuvant for Human Vaccines

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1. RATIONALE FOR AND DESIGN OF MICROFLUIDIZED OIL/WATER EMULSIONS

Advances in recombinant DNA technology have made possible the advent of a new generation of safer, better-defined subunit vaccines. Because vaccines based on these weakly immunogenic antigens require an adjuvant for efficacy, we undertook the development of a safe and efficacious adjuvant suitable for widespread human administration. Vaccines formulated with aluminum salts (alum), the only adjuvant thus far utilized with vaccines approved in the United States for human administration, were necessarily adopted as a benchmark for the minimum acceptable activity of a new adjuvant. Our goal was to develop an adjuvant that significantly exceeded aluminum hydroxide in potency, while retaining equally low toxicity. By the early 1990s a wide variety of approaches to adjuvant development had been described (Allison and Byars, 1990; Edelman, 1980; Gregoriadis and Panagiotidi, 1989; Warren et al., 1986). Two major mechanisms of adjuvant activity have been repeatedly cited in this literature: the depot effect, whereby long-term release of antigen results in increased immune response; and coadministration of immunostimulators, which specifically activate portions of the immune system in, as yet, incompletely defined fashions. The prototypic strong adjuvant, complete Freund's adjuvant (CFA),
combined these functions by releasing a mixture of immunostimulatory mycobacterial cell
wall components along with antigen from a water/mineral oil/Arlacel A emulsion depot
over an extended period of time (Freund, 1956). CFA remains the reference standard for
potent adjuvant activity; however, it is now considered too toxic in many cases for use
even in laboratory animals. In addition to the aluminum salts, several adjuvants based on
the depot effect alone have been studied. These include incomplete Freund's adjuvant
(IFA), which lacks the potent, but toxic, cell wall components, and Adjuvant 65 (water/pea-
nut oil/mannide monooleate), a yet further detoxified water/oil formulation (Hilleman et
al., 1972a,b). Despite extensive study, neither formulation was approved for human
administration. We chose to avoid water/oil emulsions. A more recent version of the depot
approach, controlled release of antigen from synthetic polymer microspheres, remains a
promising area of study (Cohen et al., 1991; O'Hagan et al., 1991) but appears to have an
unacceptably long development time for our purposes.

A major part of the long ongoing effort to develop immunostimulators as adjuvants
has been devoted to characterization and synthesis of mycobacterial cell wall compo-
nents and their analogues (White et al., 1964). The most studied component of the cell
wall, the muramyl peptide N-acetylmuramyl-L-alanyl-D-isoglutamine (MDP), was
synthesized by 1975 (Kotani et al., 1975; Merser et al., 1975) and the activity of
a number of analogues has since been described (Ott et al., 1992). We initially set
out to develop an adjuvant that used the amphiphilic muramyl tripeptide, MTP-PE
[sodium N-acetyl-muramyl-L-alanyl-D-isoglutaminyl-L-alanyl-2-(1',2'-dipalmitoyl-sn-glycero-3'-phospho)ethylamide] (Gisler et al., 1986). MTP-PE was se-
lected because of an established clinical record of high potency and low toxicity
(Fidler, 1988; Fidler et al., 1981) as well as the availability of sufficient quantities of
injectable-grade material.

Because muramyl peptides alone have been reported to be no more effective an
adjuvant than alum (Audibert et al., 1980) they have been formulated with a variety of
vehicles. The use of liposomes containing muramyl peptides has been described for several
systems (Brynestad et al., 1990; Gregoriadis and Manesis, 1980; Ullrich and Fidler, 1992).
It has been our experience that more robust antibody responses are obtained with oil/water
emulsions (Sanchez-Pescador et al., 1988). Complex squalene/water emulsions containing
a muramyl peptide component, trehalose dimycolates, and monophosphoryl lipid A have
been shown to be effective adjuvants (Masihi et al., 1986; Ribi et al., 1976). A body of
work has been devoted to generation of less complex and better defined variations of these
formulations. Similar emulsions have been used to formulate a variety of hydrophobic
spreading agents (Woodward, 1989). A family of potent synthetic hydrophobic agents, the
pluronic block polymers, have been used with both squalene emulsions (Hunter and
Bennett, 1984; Hunter et al., 1981) and squalane emulsions in combination with the
synthetic muramyl peptide, threonyl MDP (Byars and Allison, 1987; Byars, et al., 1990).
Finally, the hydrophobic muramyl peptide B30-MDP has also been formulated with a
Ribi-like emulsion (Tsujimoto et al., 1986).

Our initial formulations were designed with the intention of binding MTP-PE to the
surface of a squalene/water emulsion. The formulation MTP-LO, a prototype formulation
generated by multiple intersyringe passages, had excellent adjuvant activity in guinea pigs
(Sanchez-Pescador et al., 1988). Poor physical stability and the modest adjuvant activity