New Against the Flu

An adjuvanted influenza vaccine to protect the young and elderly may be just around the corner in the U.S.

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EVERYONE KNOWS that getting the flu can be a miserable, temporarily debilitating annoyance. But for young children, the elderly and people with certain chronic diseases, contracting seasonal influenza can sometimes lead to hospitalization with bacterial pneumonia or other serious complications and death. The reason is starkly simple: Natural protective immunity in young children is still underdeveloped, while in the elderly it is in a long decline.

Ironically, for these two particularly vulnerable ends of the age spectrum, immunization with seasonal influenza vaccine is less effective in preventing the flu than it is for older children and non-elderly adults, who mount a stronger protective immune response to the vaccine antigens and, subsequently, the circulating influenza virus itself. While conventional flu vaccines generally provide protection to 70 percent to 90 percent of healthy young adults, the protection rate is far lower in young children and people in their mid-60s and older.

This obvious need for a more immunogenic flu vaccine for the young and elderly who most need it has driven intensive research efforts for decades. Finally, a vaccine that promises to fill this void may be nearing approval. And in an echo of groundbreaking work by British physician Edward Jenner, who in 1796 reported the first successful vaccination against smallpox by use of cowpox from skin lesions of milkmaids, the origins of this new kind of "adjuvanted" influenza vaccine can be traced to keen observation and experimentation.

Adjuvants Provide a Boost

The journey begins in 1925, when a French veterinarian named Gaston Ramon noticed that horses that developed abscesses at the site of injection of diphtheria toxin vaccine produced higher antitoxin titers than horses without abscesses.[1] Soon thereafter, he discovered that sterile abscesses generated by the injection of various substances — lecithin, tapioca, even bread crumbs — along with the diphtheria toxoid also increased the animals' immune response to the toxoid.[2] Ramon coined the term "adjuvant," from the Latin word *adjuvare,* which means to aid or help, to describe these vaccine potentiators.

Shortly following Ramon's discovery, U.S. scientists reported that diphtheria toxoid precipitated with aluminum salts (alum) induced better antibody responses

than toxoid alone, particularly in young children.[3,4] But while alum salts or gels have an excellent safety record and are used in a number of licensed human vaccines, they are relatively weak adjuvants and rarely induce a cell-mediated immune response to combat certain bacteria, parasites and viruses — including the influenza virus.

Then in 1937, a Hungarian-born immunologist named Jules Freund described what immunologists still refer to as the "gold standard" adjuvant: a mineral oil-in-water emulsion containing killed mycobacteria called Freund's Complete Adjuvant (FCA).[5] While far more potent than alum-based adjuvants, FCA was found to be relatively toxic, frequently inducing keloid formation and abscesses at the site of inoculation. A Freund's Incomplete Adjuvant (FIA), formulated without the killed mycobacteria, proved far less toxic. Clinical trials of mineral oil-based influenza vaccines, first conducted in 1953, demonstrated high and sustained antibody responses and protective immunity compared with standard nonadjuvanted trivalent inactivated influenza vaccine (TIV).[6] Adjuvants similar to FIA were incorporated in some human influenza vaccines, but small numbers of delayed side effects including cystic swelling and persistent muscle induration prompted manufacturers to discontinue their use by the mid-1960s.

This didn't stop experimentation with other oil-based emulsions to find a safe and effective vaccine adjuvant. Vegetable oil, sesame oil and peanut oil, among others, were tried, all with disappointing results.[5] Not knowing the underlying mode of action complicated the search for a good adjuvant to potentiate the immunogenic effect of vaccines for which a boost was needed. Finally, scientists at what today is Novartis Vaccines and Diagnostics focused on squalene, a natural lipid produced in plants and animals, including humans. Squalene is found in abundance in human skin, where it acts as a natural moisturizer, and in tissues throughout the body.

After years of clinical testing, in 1997 Novartis introduced Fluad, a seasonal influenza vaccine containing a squalene-in-water microemulsion dubbed "MF59," in Europe for immunization of persons ages 65 years and older. Earlier this year, Canadian health authorities approved Fluad to target this same age group, which accounts for some 70 percent of influenza-related hospitalizations and 90 percent of deaths.

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Fluad: Safety and Immunogenicity in the Elderly

With advancing age, the likelihood of a protective antibody response to conventional TIV steadily diminishes. By age 85, there is a 16-fold higher risk of dying from any flu-related cause, and a 30-fold higher likelihood of dying directly from influenza infection or secondary pneumonia than those between age 65 and 69.[7]

In five pivotal trials involving 1,168 subjects ages 65 and older, those immunized with Fluad experienced consistently higher hemagglutinin-inhibition (HI) antibody titers than subjects who received conventional TIV. Greater percentages also achieved seroconversion or a significant increase in HI titers for homologous virus strains.[8]

The safety profile for Fluad approved in Canada is based on 39 studies in which a total of 12,889 subjects were exposed to at least one dose, 492 of whom received a second consecutive dose one year later, and 150 a third dose the following year. Pooled safety data showed that the most frequently reported local adverse events within four days of vaccination were injection site pain (26 percent in the Fluad group vs. 14 percent in the comparator group) and a "warm" or "hot" temperature at the injection site (18 percent vs. 11 percent). Generally of mild or moderate intensity, these reactions usually resolved within two or three days. Systemic reactions, most notably headache, fatigue, malaise and myalgia, were reported by similar percentages of subjects after the first, second and third vaccinations in both the Fluad and comparator vaccine groups.

Whether the superior immunogenicity of Fluad to TIV translates into reduced influenza-related complications and mortality remains to be answered by future clinical studies.

Fluad Appears Protective in Young Children

Fluad's safety and immunogenicity record in the elderly population has raised hopes that this adjuvanted seasonal flu vaccine can be shown safe and protective in the next-largest at-risk group: children under 6 years of age. Findings from a newly published study involving 4,707 previously unvaccinated German and Finnish children ages 6 to 72 months appear to have justified these hopes.[9]

Over two influenza seasons, children were stratified first by age — 6 months

to less than 36 months and 36 months to less than 72 months — and then randomly assigned in a ratio of 2:2:1 to receive two doses, 28 days apart, of 1) MF59 adjuvant-containing TIV (ATIV; Fluad), 2) conventional TIV with hemagglutinin antigens from the same three viral subtypes, or 3) a non-influenza "control" vaccine.* Key efficacy results are summarized in Table 1.

Table 1. Efficacy of MF59-Adjuvant Trivalent Influenza Vaccine (Fluad), TIV and Control (Noninfluenza) Vaccine Against Confirmed Influenza Over Two Seasons in Children Ages 6 to <72 Months[9]

	Relative Efficacy			
	6 to <72 months	6 to <24 months	6 to <36 months	6 to <72 months
Fluad vs. Control	86 (74 to 93)	77 (37 to 92)	79 (55 to 90)	92 (77 to 97)
Fluad vs. TIV	75 (55 to 87)	73 (29 to 90)	64 (23 to 83)	86 (59 to 95)
TIV vs. Control	43 (15 to 61)	11 (-89 to 58)	40 (-6 to 66)	45 (6 to 68)

Over both influenza seasons, the absolute efficacy of Fluad against all influenza strains was 86 percent (95 percent confidence interval [CI], 74 to 93) and 89 percent against vaccine-matched strains (95 percent CI, 78 to 95). Just 13 confirmed cases of influenza occurred among 1,937 children immunized with Fluad — an attack rate of less than 0.7 percent. By contrast, 47 of 993 control group children (4.7 percent) contracted influenza. Relative to TIV, Fluad was 75 percent effective (95 percent CI, 57 to 87) against all flu strains.

Fluad was effective relative to both control vaccine (77 percent) and TIV (93 percent), albeit with wide confidence intervals due to the low (2.3 percent) overall influenza attack rate.

A subanalysis showed that Fluad was efficacious in both younger and older age groups. Fluad efficacy against all flu strains was 79 percent in children 6 months to less than 36 months and 92 percent in those 36 months to less than 72 months of age. TIV efficacy versus controls was just 40 percent (with 95 percent CI overlapping zero) and 45 percent in the younger and older age cohorts, respectively.

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Even more striking was the superior efficacy of Fluad in infants from 6 years to less than 24 months of age — the least immunocompetent and thus the least responsive to conventional flu vaccine. While TIV didn't show significant efficacy in relation to control vaccination (11 precent, 95 percent CI, -89 to 58),

As with previous studies of Novartis' MF59-adjuvanted seasonal and pandemic flu vaccines,[10,11] Fluad induced a significantly greater antibody response than TIV, both against homologous (vaccine) and other flu strains. Remarkably, the response to the first of two Fluad injections in these young children met the standard seroprotection threshold (HI antibody titer ≥40) for both A-subtype viruses.

Vaccine-related adverse events were generally mild to moderate in both age cohorts. Systemic reactions, including mild fever, were slightly more frequent in older children after Fluad, but they were mostly mild and of short duration. Rates of serious adverse events were similar in the TIV and Fluad group, and confirm previous experience with MF59 adjuvant in trials of other vaccines involving some 33,000 children.

Approval Prospects Look Good — with a Caveat

With these excellent supportive data, together with experience from more than 50 million Fluad doses supplied to the elderly population since 1997 and twice that number of doses of MF59-adjuvanted pandemic influenza vaccine administered to all age groups, the prospects appear good that Fluad will eventually become available in the U.S.

A new U.S. Phase III clinical trial is now under way to evaluate Fluad in persons ages 65 years and older. Novartis expects to file for regulatory approval of the product for use in this age group in 2012. Meanwhile, the company filed in

*Meningococcal C conjugate vaccine given in 0.25 mL doses in children 6 to <12 months of age, and tickborne encephalitis vaccine given in 0.5 mL doses to children 12 to <72 months of age.

2010 for approval of Fluad in European Union countries for pediatric use.

But still lingering in some minds are safety questions raised by studies in small animal models describing induction of arthritis-like inflammation and lupus autoantibodies following administration of small quantities of squalene, as well as other endogenous lipids.[12,13] A core concern is whether injection, year after year, of even the minute quantity of squalene (about 10 mg in a 0.5 mL dose) in Fluad could trigger immune cross-reactivity with endogenous squalene found in the joints, nervous system or other parts of the body. This hypothetical concern that injection — rather than ingestion — of an important lipid tissue component could trigger autoimmune disease likely figures into the conservative, "go slow" approach of the U.S. Food and Drug Administration (FDA) with respect to vaccines generally that include oil-in-water emulsions.

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