

**17** Preclinical Assessment of a Novel Class of Immunoadjuvants

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We have developed a novel class of immunoadjuvants based on non-bacterial, phospholipid-polysaccharide conjugates. The best of these immunoadjuvants, namely the AMD, was tested using both particulate and soluble model antigens including formalin-inactivated *Klebsiella pneumoniae* (KP) and *Shigella flexneri* (SF) bacteria, UV-light inactivated Rubella virus (RV) particles and bovine serum albumin (BSA). BALB/c mice were subcutaneously immunized with 10 or 20 µg of protein according to a prime-boost regimen with either the antigen alone or in combination with AMD or one of the two control adjuvants, Rehydragel (RHG) and TiterMax Gold (TMG). The specific IgG antibody titers were monitored over a 3-month period by means of a non-competitive, solid-phase enzyme immunoassay. We have shown that both the primary and secondary antibody responses were overall superior when the antigen was formulated with AMD as opposed to TMG and RHG. This was particularly true with the SF antigen where the antibody titers were 6.7-32.0 times higher with AMD. With the KP antigen, the IgG titers remained elevated after the recall dose when TMG was used as the adjuvant, whereas these titers started to gradually decline 35 days after priming when both AMD and RHG were used. Although TMG exhibited the best safety profile, the moderate local inflammatory reactions induced by AMD were similar to the ones obtained with RHG, which is the only adjuvant approved by the FDA for human vaccines. Taken together, our results indicate that AMD is a potent and relatively safe novel immunoadjuvant. The ability of this latter immunoadjuvant to induce the synthesis of various IgG isotypes as well as other classes of immunoglobulins such as IgM and serum IgA is currently under investigation.

**18** Transcutaneous Immunization Using Cholera Toxin as Antigen and Adjuvant Induces Systemic and Mucosal Responses and Provides Immunoprotection

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Defining alternate routes of immunization is a current priority in vaccine research due to the need for multivalent delivery methods, multiple boosting strategies and the need to decrease the barriers to immunization associated with current injectable methods. Transcutaneous immunization is a new technique using an application of immunizing antigens to the surface of the skin. We recently reported that application of cholera toxin (CT) to the skin results in transcutaneous immunization and induces a systemic antibody response both to CT and co-administered antigens (Nature 1998, 391:861). We now show that anti-CT antibody responses rise rapidly, within two weeks of transcutaneous immunization and show classic boosting kinetics. Furthermore, anti-CT IgG and IgA antibodies were detected in sera, lung washes and stool samples from immunized mice, and a broad spectrum of IgG subclasses (IgG1, IgG2a, IgG2b and IgG3) was apparent in the sera. Mice challenged with a lethal dose of intrapulmonary cholera toxin exhibited significant levels of protection even after a single transcutaneous immunization suggesting that the immune responses induced by this method are clinically relevant. Similarly, antibodies to coadministered antigens such as diphtheria toxoid show classic immune kinetics and can be detected both systemically and at mucosal surfaces. Transcutaneous immunization appears to be a broadly applicable advance for vaccine delivery.

**19** CpG Motifs Establish Th1 Responses and Redirect Pre-Established Th2 Responses Against Hepatitis B Surface Antigen in Mice

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Unmethylated CpG dinucleotides in the context of certain DNA sequences may act as potent immune stimulators (CpG-S motifs). Oligonucleotides (ODN) containing such CpG-S motifs are a good candidate for vaccine adjuvants since they have been shown to induce strong immune responses in mice against the hepatitis B major surface protein (HBsAg). The CpG adjuvant, in contrast to alum, is strongly Th1. This is indicated *in vitro* by the type of cytokines secreted from stimulated splenocytes (IL-12, IFN-γ) and *in vivo* by predominantly IgG2a isotype of antibodies and strong CTL responses. This study further characterizes the ability of CpG-S motifs to induce Th1 type immune responses at different ages and investigates their ability to reverse a pre-established Th2 response. Adult or neonatal BALB/c mice were immunized with recombinant HBsAg using either alum and/or CpG ODN and some were boosted with the same or a different formulation. Antibodies against HBsAg (anti-HBs) in immunized adult mice were mostly IgG1 with alum and IgG2a with CpG ODN alone or mixed with alum. Furthermore, priming with alum and boosting with CpG ODN or vice versa both resulted in a Th1-type response. Thus the Th1 bias of CpG ODN dominates the Th2 effect of alum when they are given simultaneously and can also override a pre-established Th2 response.

**20** Characterization of Immune Responses Stimulated by Combinations of Oligomeric sGP140 and Cholera Toxin Derivatives

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Recent reports suggest that an effective vaccine strategy against HIV-1 may require an oligomeric envelope glycoprotein derived from a primary virus combined with a nontoxic mucosal adjuvant. Cholera toxin and its nontoxic derivatives are potent adjuvants that stimulate strong mucosal immune responses without destroying the antigenicity of the immunogen. To determine if non-toxic CT derivatives would be useful in an HIV-1 vaccine strategy, we evaluated the immunogenicity of cholera toxin holotoxin (CT), cholera toxin B subunit (CTB), or a CT non-toxic mutant (CTK63) combined with an oligomeric envelope glycoprotein derived from primary HIV-1 isolate 451 (sgp140). CT, CTB, and CTK63 stimulated vigorous sgp140-specific responses in the sera, IgG1 and IgG2b being the predominate isotypes. These responses lasted for at least 177 days post-inoculation. Three intranasal inoculations over a two-week period were required to generate sgp140-specific mucosal responses. Interestingly, both CT and CTB stimulated sgp140-specific mucosal IgG1 but no IgA. CTK63, however, stimulated no sgp140-specific mucosal responses. Adjuvant titration studies demonstrated that >5 µg/mouse of CTK63 were required to provide the adjuvanticity equivalent to 0.5 µg/mouse of CT or CTB. All CT, CTB, or CTK63/sgp140 combinations induced neutralizing antibodies against lab-adapted isolate IIIB. These studies suggest that the gp140 derived from HIV-1 451 can induce neutralizing antibodies but that careful evaluation of adjuvant/gp140 combinations are required since not all non-toxic derivatives of CT are equivalent.