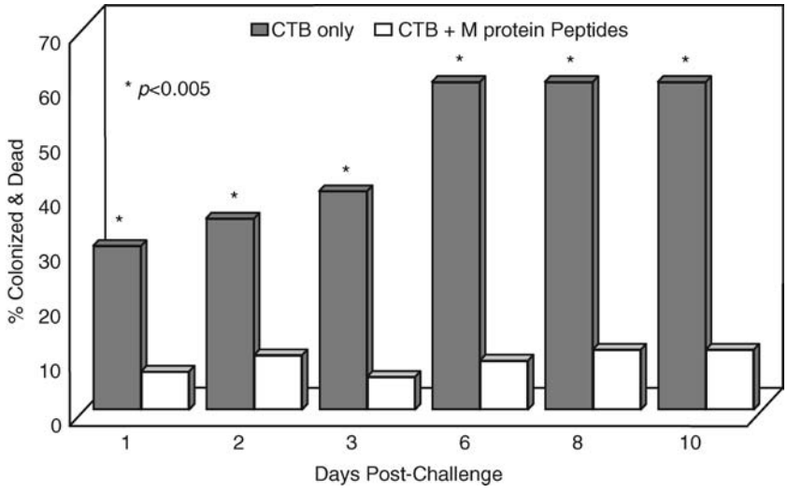

Figure 6 The extent of colonization of mice challenged with group A streptococci after oral immunization with M protein–conserved region M6 peptides linked to CTB or CTB alone. Orally immunized mice were swabbed each day after challenge with M14 streptococci and plated on blood plates to determine the extent of colonization compared with mice vaccinated with CTB only. Plates showing group A streptococci were scored as positive. *Abbreviation*: CTB, cholera toxin B.

to cause death by first colonizing and then invading the mucosal barrier resulting in dissemination of the organism to systemic sites. Using this model, it was first examined if sIgA delivered directly to the mucosa plays a role in protecting against streptococcal infection. Live streptococci were mixed with affinity-purified M protein–specific sIgA or IgG antibodies and administered intranasally to the animals (87). The results clearly showed that the anti–M protein sIgA protected the mice against streptococcal infection and death, whereas the opsonic serum IgG administered by the same route was without effect. This indicated that sIgA can protect at the level of the mucosa and may preclude the need for opsonic IgG in preventing streptococcal infection. These studies were also one of the first to compare purified, antigen-specific sIgA and serum IgG for passive protection at a mucosal site.

Passive protection against streptococcal pharyngeal colonization was also shown by the oral administration of purified lipoteichoic acid (LTA) but not deacylated LTA prior to oral challenge in mice (88). The addition of anti-LTA by the same route also protected mice from oral streptococcal challenge. While several in vitro studies showed the importance of M protein (89–91) and LTA (92) in streptococcal adherence, these in vivo studies, together with those presented above, suggest that both M protein and LTA may play a key role in the colonization of the mouse pharyngeal mucosa. However, it is uncertain whether this is also true in humans.

ACTIVE IMMUNIZATION AT THE MUCOSAL LEVEL WITH CONSERVED REGION PEPTIDES

To determine whether a local mucosal response directed to the conserved exposed epitopes of M protein can influence the course of mucosal colonization by GAS, peptides corresponding to these regions were used as immunogens in a mouse model (83,84). Overlapping synthetic peptides of the conserved region of the M6 protein were covalently linked to the mucosal adjuvant cholera toxin B (CTB) subunit and administered intranasally to mice in three weekly doses and boosted 30 days after the last dose with the peptide mixture. Ten days later, animals were challenged intranasally with live streptococci (either homologous M6 or heterologous M14), and pharyngeal colonization by the challenge organism was monitored for 10 to 15 days. Mice immunized with the peptide-CTB complex showed a significant reduction in colonization with either the M6 or M14 streptococci compared with mice receiving CTB alone (83,84) (Fig. 6). Thus, despite the fact that conserved region peptides were unable to evoke an opsonic antibody response (61), these peptides have the capacity to induce a local immune response capable of influencing the colonization of GAS at the nasopharyngeal mucosa in this model system. These findings were the first to demonstrate protection against a heterologous serotype of GAS with a vaccine consisting of the widely shared C-repeat region of the M6 protein.

Confirmation of these findings was later published independently using different streptococcal serotypes as the immunizing and challenge strains (93). In a separate study, Pruksakorn et al., found (66), using a different criteria for streptococcal opsonization than previously published (47), reported that when a peptide derived from the conserved region of the M protein was used to immunize mice, it induced antibodies capable of opsonizing type 5 streptococci and streptococci isolated from Aboriginal and Thai rheumatic fever patients. These findings are in sharp contrast to the studies of Jones et al. (61), who showed that antibodies to the conserved region of M protein are not opsonic. However, since the peptide reported by Pruksakorn et al. (66) is similar to one of the peptides used by Bessen and Fischetti (83,84) in mucosal protection studies (see above), the induction of serum IgG during mucosal immunization may offer added protection against streptococcal infection.

VECTORING THE M PROTEIN WITH VACCINIA VIRUS

To further verify the validity of using the M protein–conserved region as a streptococcal vaccine, experiments were repeated in a vaccinia virus vector system. In these studies, the gene encoding the complete conserved region of the M6 molecule (from the pepsin site to the C-terminus, see Fig. 3) was cloned and expressed in vaccinia virus producing the recombinant VV::M6 virus (94,95). Tissue culture cells infected with this