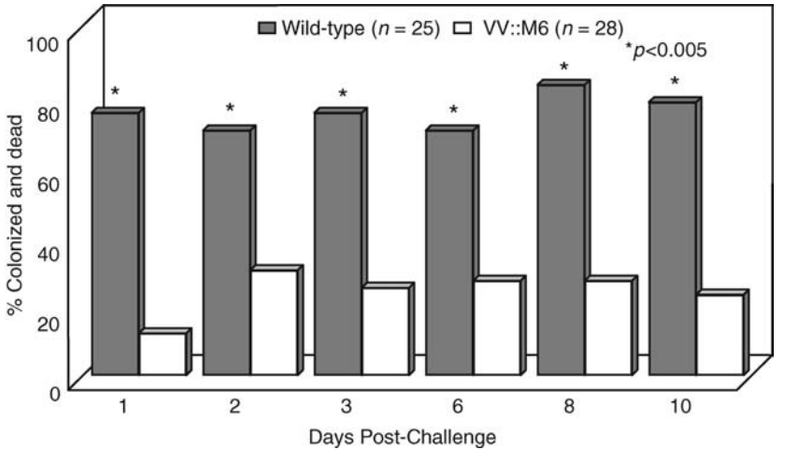

Figure 7 The extent of colonization of mice challenged with group A streptococci after oral immunization with recombinant vaccinia virus containing the gene for the whole conserved region of the M6 protein or vaccinia virus alone. Orally immunized mice were swabbed each day after challenge with M14 streptococci and plated on blood plates to determine the extent of colonization compared with mice vaccinated with wild-type vaccinia only. Plates showing group A streptococci were scored as positive.

virus were found to produce the conserved region of the M6 molecule. Animals immunized intranasally with only a single dose of recombinant virus were significantly protected from heterologous streptococcal challenge compared with animals immunized with wild-type virus (Fig. 7). When the extent of colonization was examined in those animals immunized with wild-type or the VV::M6 recombinant, the VV::M6-immunized animals that exhibited positive swabs showed a marked reduction in overall colonization compared with controls, indicating that mucosal immunization reduced the bacterial load on the mucosa in these animals. Animals immunized intradermally with the VV::M6 virus and challenged intranasally showed no protection.

The approaches described above proved that induction of a local immune response was critical for protection against streptococcal colonization and that the protection was not dependent on an opsonic response. However, in the event that the *Streptococcus* was successful in penetrating the mucosa and establishing an infection, only then would type-specific antibodies be necessary to eradicate the organism. This idea may perhaps explain why adults sporadically develop a streptococcal pharyngitis, that is, a mucosal response may be breached when a large number of streptococci are encountered on the mucosal surface. The success of these strategies not only forms the basis of a broadly protective vaccine for the prevention of streptococcal pharyngitis but may offer insights for the development of other vaccines. For instance, a vaccine candidate that does not evoke protective immunity by the parenteral route may prove to be successful by simply changing the site of immunization. Furthermore, these results emphasize the fact that in some instances antigens need to be presented to the immune system in a specific fashion to ultimately induce a protective response.

STREPTOCOCCUS GORDONII AS A VECTOR

The importance of the C-terminal region in the attachment of surface proteins in gram-positive bacteria was previously demonstrated using the protein A from *Staphylococcus aureus* as a model system (96,97). Surface proteins in gram-positive bacteria (which could number more than 20 in a single organism) are synthesized and exported at the septum, where new cell wall is also being produced and translocated to the surface (55,98). Thus, the C-terminal hydrophobic domain and charged tail in these proteins function to control the export and anchoring process by acting as a temporary stop to position the LPXTG motif [the anchor motif common to >100 surface proteins on gram-positive bacteria (Fig. 8) (99)] precisely at the outer surface of the cytoplasmic membrane. This sequence motif, which is an enzyme recognition sequence, is cleaved resulting in the attachment of the surface-exposed segment of the protein to a cellular substrate (96). This idea is supported by studies indicating that the C-terminal hydrophobic domain and charged tail are missing from the streptococcal surface M protein extracted from the cell wall (57,96). Since the anchor region is highly conserved among a wide variety of surface molecules within several different gram-positive species, could it be fused to a foreign antigen and used to deliver the resulting fusion protein to the surface of a gram-positive bacterium, ultimately anchoring it to the cell? To answer this, the streptococcal M protein was employed in a model system.

Pozzi et al. (100,101) were the first to deliver a fusion protein to the surface of the gram-positive human oral commensal *Streptococcus gordonii*. The approach utilized knowledge

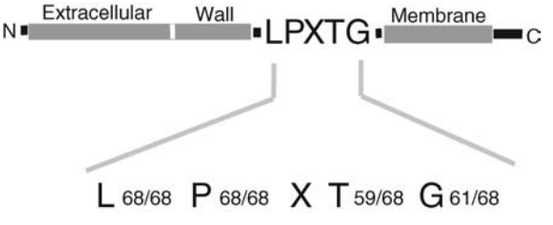

Figure 8 Conservation of the LPXTG motif at the C-terminal end of surface proteins on gram-positive bacteria. Sixty-eight surface proteins from gram-positive organisms were compared as to the number of times L, P, T, and G were found. As seen, L and P are found 100% of the time in this position and T and G more than 86% of the time.