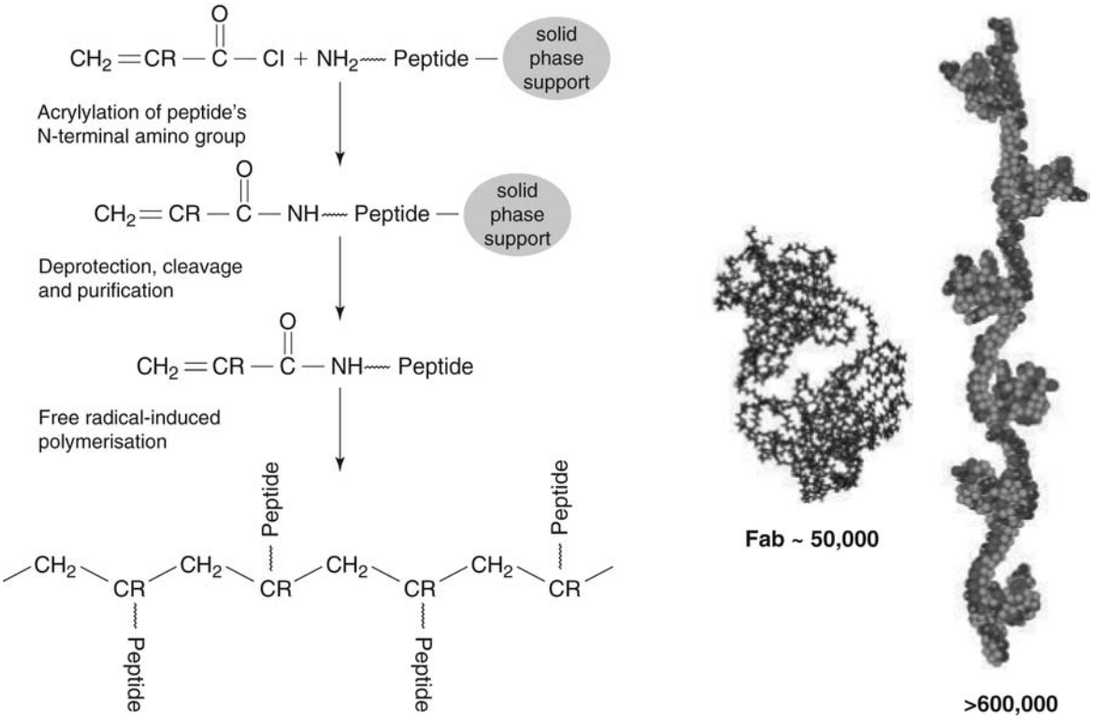

Figure 1 Scheme for the preparation of synthetic peptide–based polymers. Peptides are assembled on solid phase supports and then acylated at the N-terminus with acryloyl chloride. Following removal of the peptide from the support and concomitant removal of the side chain protecting groups, the peptide epitopes are purified and polymerized by exposure to free radical. The molecular models at the right represent, to the same scale, an IgG Fab fragment (~50,000 Da) and a portion of a polymer formed by the free radical-induced polymerization of multiple peptide epitopes. R=H or R=CN.

stimulated with a series of peptides from the target protein. Such assays are very sensitive and, by judicious use of 18-residue and approximately 9-residue peptides, allow the identification of $CD4^+$ and $CD8^+$ T-cell epitopes.

Ab and T-cell epitopes identified by each of these methods now become candidates for incorporation into a single synthetic structure for inducing broad-based immunity. This strategy has advantages over using a recombinant protein because epitopes from multiple and relevant proteins can be included and deleterious sequences can be excluded. Caution, however, must be taken in the selection of T-cell epitopes especially when the disease pathology is caused by inappropriate T-cell activity. The topic of epitope identification has been the subject of many reports in the scientific literature and is the subject of the laboratory handbook *Epitope Mapping Protocols* (76).

STRUCTURES OF LIPOPEPTIDE-BASED VACCINES

Representative structures of bacterial-derived or synthetic lipid moieties that potentially have the capacity to provide an adjuvanting effect for peptide epitopes, including points of modification, are shown in Figure 3. Sites of modification that have been investigated include the (*i*) the N-linked fatty acids (R^1), (*ii*) the O-linked fatty acids (R^2 and R^3), (*iii*) the chirality of the glycerol backbone, (*iv*) cysteine residue chirality, (*v*) sulphur atom substitutions within the cysteine residue (R^4), and (*vi*) variations in the carboxyl-terminal peptide sequence (R^5).

Changes to the O-linked fatty acids incorporated within the lipid moiety appear to have the greatest effect on adjuvant activity. For both Pam_2- and Pam_3-Cys analogues, the presence of O-linked palmitoyl groups (C16) provides the best adjuvant activity, with shorter (<C16) fatty acids resulting in reduced activity (78,84–88).

The design of peptide-based structures that incorporate the adjuvanting lipid range from simple, linear, and branched structures to the large and complex. Among the simplest are linear and branched structures that posses either a single (target) epitope, usually a CTL epitope, or those that possess a helper T-cell epitope in addition to the target epitope. Examples of such structures that we (27,53,55,56,89) have used are shown in Figure 4. In those cases where multiple epitopes were needed, specific ligation or polymerization techniques to produce polyvalent lipopeptide vaccines have been used (19,63,90).

The lipid core peptide (LCP) system (91) incorporates a lipid adjuvant that is produced using synthetic lipidic amino acids (92) and glycine spacers superimposed on a polylysine MAP system (46). Alternatives to the approach make use of a carbohydrate (93) scaffold instead of