sufficient information built into the native sequence that the correct conformation is promptly attained and antibodies readily elicited (27,54–56). In the case of T-cell epitopes, which are not recognized in their native structure as in the intact protein but only when enzymatically processed and loaded onto MHC molecules, conformation is not an issue and T-cell epitope-based vaccines can be readily synthesized.

3. The T- and B-cell epitopes defined in a single host of a particular MHC type may be inadequate for eliciting immunity in outbred populations with polymorphic MHC molecules. In addition, many diseases are caused by organisms where the target antigens show a high degree of genetic variation, creating multiple serologically distinct variants. An important consideration therefore is to provide sufficient numbers of different epitopes. Protein antigens contain multiple B-cell epitopes and often also possess epitopes that will be recognized by helper T cells, providing the help that is necessary for antibody production. Likewise a pathogen may have a range of different $CD8^+$ cytotoxic T-cell epitopes that can be used as vaccine targets despite the fact that only one or two may dominate in the response to the whole organism. In other words, whole proteins or whole pathogens usually contain all of the information that is needed to produce a poly-specific immune response. Thus, for epitope-based vaccines to elicit such responses, all this information should ideally be incorporated into the vaccine. A number of methods for doing this are available and include the multiple antigenic peptide (MAP) approach of Tam and colleagues (46,57) involving assembly of multiple peptides onto a branched oligolysine support, and the synthesis of peptides on cross-linked acrylamide supports where the cross-links are cleaved on exposure to trifluoroacetic acid, resulting in a long single chain polyamide to which multiple copies of the peptide are attached (58).

There are, however, limitations to the degree of purity of the materials that are achieved with these approaches. As the valency of the vaccine candidate increases, the heterogeneity of the product also increases, making quality assurance difficult. Although the elegant chemical ligation approaches described by Rose (59) and Tam (60,61) addressed these issues providing greater flexibility by permitting the conjugation of different purified peptides onto a template support, the number of different peptide epitopes that can be incorporated into these structures is still limited by the number of orthogonal chemistries available.

A number of approaches permit peptides to be synthesized, purified, and then assembled into polymers. Using the technique of free radical–induced polymerization of peptides (16,17,62), very large (>600,000 Da) molecular species can be assembled using virtually any number of the same or different epitopes. The method allows purification of the individual determinants prior to polymerization and thereby avoids errors inherent in long sequential syntheses. The approach has been successfully used to design and assemble GAS vaccines containing multiple epitopic variants present in different isolates (19) and also hepatitis C virus vaccines based on multiple B-cell epitopes in the form of a library covering possible variants of the hypervariable region of the surface antigen of the virus (63) (Fig. 1).

Polyvalent, self-adjuvanting vaccines have also been produced using the multiple-antigen lipophilic adjuvant carrier (MALAC) system (Fig. 2) (64–67), which utilizes site-specific conjugation of purified peptide epitopes into a lipoaminoacid-based scaffold. Using this approach, GAS lipopeptide vaccines containing different peptide epitopes have been synthesized in good yield and purity and been demonstrated to elicit high-titer antigen-specific antibodies (67).

EPITOPE IDENTIFICATION

Before a synthetic vaccine can be designed, appropriate epitopes must be identified. Epitope identification is and has been a continuing endeavor of immunology, and as a consequence a large number of different methods have been applied to the identification of these, the smallest, elements that are recognized by antibodies and T-cell receptors. The suite of methodologies available range from simple ELISA and even Ouchterlony-related methods to the use of panels of synthetic peptides representing complete sequences of antigens and the sophisticated techniques of electron microscopy, NMR, X-ray crystallography, and the use of mass spectrometry to characterize protease or chemically derived fragments. Many of these disparate techniques can be mixed and matched to identify epitopes in almost any antigen. The plethora of protocols available reflects the number of solutions available and invites an eclectic approach to the solution of epitope mapping.

Epitope mapping has often been done using monoclonal antibodies (MAbs), and although there is no doubt that MAbs provide exquisite specificity, the case should be made that the antibody response is polyclonal and more attention should perhaps be paid to polyclonal antibodies. Furthermore, antisera exhibit properties not always exhibited by MAbs. These include the ability to refold antigens through concerted and multiple antibody-binding events; some isolated subunits of antigens with quaternary structure are not bound by MAbs but are recognized by antisera. Immunoglobulin (Ig) purified from such antisera can be useful where MAbs are not. Furthermore, it is antisera from patients recovering from disease that may provide very useful information concerning identification of biologically important epitopes. If patients have their serum antibodies capable of neutralizing a virus, then information about those epitopes is present in the binding sites of those antibodies. Ig isolated from individuals with past or current infection can therefore be used to ''mine'' a panel of peptide-based epitopes that represents the complete amino acid sequence of any protein to discover epitopes of significance. Peptides that are bound by Ab can then be isolated and identified by a process of ''epitope extraction.'' This general approach to epitope identification was pioneered by Suckau et al. (68) and more recently utilized by others (69,70). The method has lately been applied to the identification of novel, and potentially neutralizing, epitopes of hepatitis C virus (71).

In the case of T cells, the epitopes that they recognize can now be quickly and easily identified using peripheral blood mononuclear cells (72,73) or with techniques using whole blood–based assays (74,75). Longitudinal studies of immune responses in various disease states can provide insights into the relevant $CD8^+$ or $CD4^+$ T-cell responses that correlate with recovery, which can be further validated in models of the target disease using mice that are transgenic for the MHC alleles expressed by humans. In either case, lymphocytes are