Researchers are also using microarray technology to identify genes differently expressed in response to alteration in environmental parameters and to evaluate mutations or key factors in regulatory and metabolic pathways (66). This can aid in the functional characterization of protein antigens with as yet unidentified roles.

Gene expression can be analyzed in either pathogen or host, thus allowing investigation of both sides of the host-pathogen interaction. Understanding the mechanism of protection of a vaccine is important for developing a new generation of vaccines. Some more recent studies have been directed toward obtaining immunological profiles of gene expression responses in individuals/in vitro models through DNA microarray following vaccination to assess the correlation of these parameters with protection. Global gene expression profiling is an ideal platform to compare induction of immune-response parameters following vaccination and challenge. For instance, human immunological responses to the *F. tularensis* live vaccine were monitored with transcriptome analysis (67) to gain a better understanding of the mechanism of protection afforded by the vaccine and perhaps the responses necessary for long-lasting immunity.

Many similar studies have been involved in the emerging field of ''vaccinomics,'' which encompasses immunogenetics and immunogenomics as applied to understanding the mechanisms of heterogeneity in immune responses to vaccines (68). This growing area of inquiry and importance seeks to understand the influence of immune-response gene polymorphisms on the heterogeneity of humoral, cell-mediated, and even innate immune responses to vaccines at both the individual and population levels.

Proteomics

Recent years have seen the accelerated development of technologies that study proteins in high throughput. Referred to as functional proteomics, these methods support the global study of protein interactions, enzymatic activities, and immune responses. The complete complement of proteins of an organism following separation by two-dimensional protein separation methods can be analyzed/identified by mass spectrometric analyses. These analyses can be from both a qualitative and a quantitative point of view. Global protein expression profiles from two different conditions can be generated and compared using proteomics to identify up- or downregulated proteins.

Proteomics can define proteins that are differentially located or secreted to outside of the cell (i.e., to the media or host cell). Rodríguez-Ortega et al. (69) described a new procedure using proteolytic enzymes to ''shave'' the group A streptococcal (GAS) surface, and the peptides generated were then separated and identified. This approach provided an extensive map of the surface antigens, namely, the ''surfome'' of the GAS strain, and enabled the identification of a new possible vaccine target. Use of this technique can provide a detailed picture of surface protein organization in any pathogenic bacterium.

The combination of proteomics with serological analysis has led to the development of a new approach termed serological proteome analysis. After a two-dimensional separation of a pathogen's protein sample, sera from individuals known to have been infected is used to identify immunoreactive proteins against which the patient has mounted a response. This method is invaluable for identification of in vivo immunogens suitable as vaccine candidates (70–72).

Protein Array Technology

Many groups are now involved in using the available genome sequence of an organism to construct a comprehensive gene collection for expression of the entire proteome of the organism. The proteins can then be spotted onto arrays, and these tools allow comprehensive analyses of immune responses and system-wide functional studies. LaBaer and colleagues have developed full proteomic arrays for the pathogens *F. tularensis* (73) and *V. cholerae* (74). Proteins expressed by pathogenic organisms can be screened with serum from convalescent patients to identify immunodominant antigens, leading to good vaccine candidates. A second application of protein microarrays is in examining protein function in high throughput by assessing their interactions and biochemical activities (75). JPT Peptide Technologies in Berlin, Germany, is examining similar applications, with microarrays displaying overlapping peptides comprising the full *M. tuberculosis* proteome. The analysis of reactivity profiles provides a wealth of novel information about the immune response against microbial organisms that would pass unnoticed in analysis of reactivity to antigens individually. Extension of this approach to a genome-wide fraction of the proteome may expedite the identification of correlates of protection and vaccine development against microbial diseases.

In summary, coupled with new technologies such as protein microarrays, proteomics might allow the functional characterization of and the documentation of immune responses to each protein of a pathogen. These results in turn may lead to better understanding of the pathogen biology and new vaccine and therapeutic strategies.

STRUCTURAL PREDICTION AND STRUCTURAL GENOMICS

After the storm of the ''omics'' era, there has been an increased understanding that it is also necessary to return to characterization of the individual proteins, putting a ''face'' on the molecules themselves. An increased effort to elucidate the structures of surface molecules is under way, and ongoing technological advances in protein biochemistry have allowed high-throughput platforms for structural resolution (76). Current structural genomics projects are being driven by two main goals: (*i*) to produce a representative set of protein folds that could be used as templates for comparative modeling purposes and (*ii*) to provide insight into the function of currently unannotated protein sequences. In target selection, a strong emphasis has been put to disease- and drug-related proteins. The number of high-resolution structures available in public databases today is approaching 50,000, including almost 30,000 protein structures, which will definitely aid in structure-based vaccine and drug design. The ultimate goal is to have at least one structure, and possibly multiple structures, from members of each protein family described to allow more accurate modeling.

We believe that a systematic approach to the structural properties of immunodominant and immunosilent epitopes can provide the scientific rationale that in future may allow us to engineer immunodominant epitopes. A rational approach to the three-dimensional structure of antigens (structural vaccinology) is one of the basic aspects of vaccine research that should be a priority (77).