

5 Reaping the Fruits of Biotechnology: Development of Better Tuberculosis Vaccines

Douglas R Young, Imperial College School of Medicine, London, UK

Tuberculosis currently causes around 3 million deaths every year and there is widespread recognition of the need for improved tools for disease control. Elucidation of the sequence of the 3,924 open reading frames that make up the total genome of *Mycobacterium tuberculosis* provides the starting point for development of a wide range of new tuberculosis vaccine candidates based on protein and DNA subunits or on live attenuated mutants. Formidable challenges remain, however, in the selection of the most promising candidates from this vast repertoire, and in the design of feasible protocols for human trials. The immune response to mycobacterial infection involves a complex array of cellular and molecular interactions and the mechanisms involved in protective immunity are only partially understood. Selection of new vaccine candidates requires a combination of rational evaluation based on current understanding of immune mechanisms, together with a more empirical assessment of efficacy in experimental models. Initial infection with *M. tuberculosis* is generally contained in a quiescent form with the potential for reactivation to cause clinical disease. Potential vaccine targets include augmentation of this initial response to produce sterilizing immunity, or subsequent boosting of responses to prevent reactivation.

6 Molecular Mechanisms of MHC Class II-Restricted Antigen Processing

P. Cresswell, Section of Immunobiology, Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, Connecticut

MHC class II molecules assemble in the endoplasmic reticulum (ER) as a nonameric complex of three class II $\alpha\beta$ dimers bound to a core trimer of invariant (I) chain molecules. The $\alpha\beta$ I complex leaves the ER upon completion of assembly and at the Trans Golgi Network (TGN) it is diverted into the endocytic pathway. Here the I chain is degraded, generating class II $\alpha\beta$ dimers associated with a residual fragment of the I chain (CLIP) in the peptide binding groove. The interaction of $\alpha\beta$ CLIP with another class II-like molecule, called HLA-DM in humans, in late endocytic compartments causes CLIP release which is followed by binding of peptides generated by proteolysis of endocytosed proteins. These newly formed $\alpha\beta$ -peptide complexes are subsequently expressed on the plasma membrane and screened by CD4 positive T-cells. In B-cells, peptide loading is modulated by another class II-like heterodimer, HLA-DO, which inhibits peptide loading by DM. A novel interferon- γ -inducible thiol reductase, which functions in the endocytic pathway, will also be described. This enzyme may play a role in unfolding endocytically acquired proteins prior to their proteolysis, and thus facilitate the generation of MHC class II-restricted epitopes.

7 Modulation of Proteasome Structure and Function for Antigen Processing

John J. Monaco. Howard Hughes Medical Institute, Department of Molecular Genetics, Biochemistry and Microbiology, University of Cincinnati College of Medicine, Ohio

CD8⁺ (cytotoxic) T cell immunity to intracellular infection requires that native antigen be processed and presented at the cell surface in association with MHC class I molecules. Although several different mechanisms have been reported, the major pathway for presentation of endogenous antigens in most cell types involves proteolysis by the proteasome, energy-dependent transport of the resulting peptides into the lumen of the endoplasmic reticulum by the TAP transporter, and binding to MHC class I heterodimers. As each of these three steps of antigen processing has inherent specificity for peptide length and sequence, non-responsiveness to particular epitopes of foreign antigen may result from failure of that epitope to be recognized at any one of these steps. While the specificity of MHC binding for many MHC class I alleles has been characterized, and there has been some characterization of the specificity of the TAP transporter, relatively little is known about proteasome specificity. Moreover, recent work indicates that proteasome structure and specificity are modulated during the immune response via the induction of three catalytic subunits by the cytokine γ -interferon. The basics of proteasome structure will be discussed, as well as its catalytic mechanism and methodologies for study of the cleavage specificities of its catalytic sites.

8 IMMUNE SURVEILLANCE OF VIRAL INFECTIONS

Kenneth L. Rock¹, Luis Sigal¹, Shane Crotty² and Raul Andino². ¹Department of Pathology, University of Massachusetts Medical School; Department of Microbiology and Immunology, UCSF.

MHC class I molecules display oligopeptides derived from a cell's expressed genes on the surface of all cells in the body. This allows cytotoxic T lymphocytes (CTL) of the immune system to detect and eliminate cells expressing "foreign" sequences (e.g. from a viral infection or mutation). In many cases, two antigen presentation pathways are involved in this process. To initiate responses, antigens must be displayed on class I molecules of professional antigen presenting cells (e.g. dendritic cells). All other cells, when infected, are unable to directly stimulate CD8 T cell responses. In such situations, e.g. tissue tropic viral infections, the obligatory pathway for stimulating CTL immunity requires that the professional antigen presenting cells acquire the viral antigens from the extracellular fluids, e.g. from dying parenchymal cells. The mechanisms that underlie this process and their potential to be exploited for vaccine delivery will be discussed. Once cytotoxic T lymphocytes are stimulated they then seek out all cells that are synthesizing the "foreign antigen" and displaying its fragments on class I molecules. In this pathway of antigen presentation, the viral proteins synthesized in infected cells are degraded in the cytoplasm to oligopeptides which are then supplied to class I molecules.