

Mass Spectrometric Characterization of Infectious Disease Epitopes Recognized by Human Monoclonal Antibodies

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As a response to an infection, the immune system produces antibodies. The determination of the antigenic structure recognized by the antibody through epitope mapping provides information about the interaction between antigen and antibody for the diagnosis of a disease on a molecular level, for characterizing the pathogenesis of the infectious material, and the development of interfering drugs or preventative vaccines. We have been using mass spectrometry to characterize the epitopes recognized by human monoclonal antibodies produced against infectious agents including the human immunodeficiency virus, HIV, anthrax bacillus, and the hepatitis C virus, HCV. To determine the antigenic region of the protein, we first bind the antigens to immobilized antibodies. Then, a combination of proteolytic enzymes with analysis of the products by direct analysis using MALDI/MS and MALDI/MS/MS is used to characterize the fine structure of the effective epitope. In these studies, we initially determined antigens on HIV proteins, e.g., p24 and gp120, recognized by monoclonal antibodies(1). The epitopes in our initial studies were continuous and linear in nature. Subsequent epitopes that we studied, however, appeared to be discontinuous or conformational in nature, as is the case with most B-cell epitopes. To adequately describe these epitopes, we incorporated differential surface modification reactions into our experimental repertoire. In differential surface modification, amino acid residues on the surface of bound antigens are chemically modified with reagents, such as acetic anhydride for lysines or hydroxyphenylglyoxal for arginines. The antigen in the absence of the antibody is chemically modified under identical conditions. The antigens are proteolyzed and the extent of modification of specific residues between the bound and unbound antigens are compared using tandem mass spectrometry. Residues within the epitope will be protected by the presence of the antibody. Based on our results, we observed that this approach, combined with crystallographic structural data or molecular model-based structures, enabled us to provide information about discontinuous epitopes as well as linear epitopes (2). Specific examples from human neutralizing anti-HIV antibodies will be presented.

1. Parker, C.E., D.I. Papac, S.K. Trojak, K.B. Tomer. 1996. *J. Immunol.* 157:198-206.
2. Parker, C.E., L.J. Deterding, C. Hager-Braun, J.M. Binley, N. Schulke, H. Katinger, J.P. Moore, K.B. Tomer. 2001. *J. Virol.* 75:10906-10911.