

PREG/MAN 5

## Serological Diagnosis with Recombinant Peptides/Proteins

Detection of antibodies directed against specific viral antigens has been the traditional manner of documenting prior infection. The viral antigens available for serological testing are usually in the form of a lysate prepared from whole virions. For those viruses that assemble and bud from host cell membranes, virion purification frequently results in copurification of host cellular proteins. These copurifying cellular proteins can be recognized by antibodies present in individuals who have been previously exposed to "foreign" antigens (e.g., via transfusion or transplantation, or prior pregnancies), resulting in false-positive reactions.

The dramatic revolution in serological testing within the past two decades is directly attributable to the explosive growth of the discipline of molecular biology. The ability to determine rapidly the entire genetic sequences of viruses and to clone and produce specific viral antigens in large quantities has permitted the development of homogeneous antigen preparations. In turn, these homogeneous preparations have permitted the development of highly reproducible serological assays. The absence of host cellular proteins in the recombinantly derived antigen preparations has also dramatically decreased the rate of false-positive reactions.

In addition to tests for Type 1 human immunodeficiency virus (HIV-1), recombinantly derived antigens have been used for the development of other serological assays for the detection of bloodborne infectious agents. Hepatitis C virus is the best example of the power of molecular biology techniques, both for identification of the causative agent and subsequent development of diagnostic reagents. The huge blood bank market has been the impetus for the commercial focus on developing serological assays for bloodborne infectious agents: all of the estimated 12 million units of donor blood collected per year need to be tested for the presence of transmissible agents. However, now that the needs of the blood banks have been adequately addressed, other "orphan" diseases (e.g., parasitic diseases), for which better diagnostic reagents are desperately needed and for which a global market exists, are finally being addressed.

HIV-1 "recombinant" antigens currently come in two forms: synthetic peptides and engineered recombinant proteins. Sequences for synthetic peptides are typically chosen by identifying areas of the viral genome most likely to induce an immune response. This identification is accomplished by entering the primary nucleic acid sequence of the entire viral genome into a computer program, which then determines the predicted amino acid sequence and generates a plot of hydrophobic and hydrophilic regions. Hydrophilic regions are more likely to be antigenic; thus, synthetic peptides corresponding to short segments of the hydrophilic regions (typically 10-20 amino acids) are synthesized and tested for their

ability to detect antibodies in sera obtained from known HIV-1-infected individuals. Recombinant proteins, in contrast, are known as "fusion proteins" and are constructed by splicing a specific viral-protein-coding sequence into an expressed bacteriophage gene. Translation is controlled by the bacteriophage gene, and the viral protein segment is translated as a part of a larger protein that includes the remainder of the bacterial gene into which the viral gene was spliced.

In this issue (Clin Chem 1991;37:1700-7), Pollet et al. report their favorable experience with a commercially available recombinant antigen-based assay (LIA HIV; Immunogenetics, Ghent, Belgium) as a "confirmatory" test for evidence of HIV-1 infection. This assay contains both recombinant antigens (produced in *Escherichia coli*) and synthetic peptides. Consistent with previously published accounts about similarly constructed HIV-1 confirmatory assays, they have also observed that the LIA HIV reliably detected HIV-1 antibodies and yielded fewer false-positive results than did conventional immunoblot. Pollet et al. are also to be commended for their rigorous documentation of the lot-to-lot variability of both qualitative and quantitative results observed with conventional immunoblot confirmatory assays.

A common misperception is that, if a serological assay is based on recombinant antigens, then results obtained by that assay must, by definition, be completely accurate. In fact, however, certain types of sera (e.g., those obtained from individuals with connective tissue disease or lipemic or hemolyzed sera) known to generate false-positive results in conventional serological assays can still give false-positive reactions in recombinant antigen-based assays. To the credit of the designers of the LIA HIV assay, no false-positive reactions were observed in a set of these known problematic sera. Secondly, if recombinant antigens are produced as fusion proteins and contain additional bacterial epitopes, samples from individuals who have antibacterial antibodies may react with the carrier portion of the fusion protein and not with the viral epitopes. In this study, "no reactivity patterns that might be attributable to interference of human anti-*E. coli* antibodies were observed." Thus, the two most likely sources of nonspecific reactivity were tested and did not interfere with the LIA HIV—with the reservation that the number of specimens tested in these categories was adequate for the study but was relatively small in comparison with the volume of such problematic sera seen daily in a clinical laboratory.

It should also be apparent, however, that false-positive reactions can occur with recombinant antigen-based assays for reasons unique to recombinant technology. For example, immunoreactive epitopes may rely on either primary amino acid sequence or conformational shape for antigenicity. Therefore, nonspecific reactivity may be

observed if similar epitopes exist on different viruses. In fact, Pollet and colleagues cite two articles reporting epitopes common to HIV-1 p24 and the picornaviral VP2 coat protein or the core protein of human T-cell leukemia virus-V (HTLV-V). Although only a single isolate of HTLV-V has been described thus far, picornaviruses are widespread and are usually responsible for yearly epidemics of gastrointestinal and respiratory "flu." It is easy to speculate that the widespread exposure to picornaviruses would result in a small subset of infected individuals who would make antibodies against the epitope shared with HIV-1 p24. This could explain the observation that HIV-1 p24 is the HIV-1 protein most prone to "false-positive" reactions (i.e., reacts with antibodies in the sera of individuals not infected with HIV-1). We would have to infer, however, that this subset of picornavirus-infected individuals who make antibodies reactive with HIV-1 p24 must be small, because the sensitivity and specificity of all HIV-1 assays in use (recombinant- or non-recombinant-based) approach 99.9%.

Another consideration unique to recombinant technology is that viral proteins produced as fusion proteins do not typically represent the entire viral protein as encoded, translated, and processed according to the virus-specified lifecycle. The viral component of fusion proteins is typically the segment of the viral genome that coincidentally is located between two sites recognized by the restriction enzymes in use. In this manner, new epitopes may be created by engineered viral protein segments that do not contain junctions between two proteins that ordinarily would be cleaved during the virus maturation process or by truncation of the complete viral protein, generating new epitopes at either the N- or C-terminus. Some recombinant-based assays have avoided this potential problem by engineering "authentic" HIV-1 viral proteins through manipulation of the expression vector to include the virally encoded protease and ensure faithful protein processing after translation within the host bacterium.

A third consideration is unique to synthetic peptide antigens. Synthetic peptides are only short segments of amino acids, corresponding to highly immunogenic regions of viral proteins. The shortness of these peptides may preclude recognition of epitopes, which may be dependent on conformational shape and involve linking of distant sites via disulfide bonding or hydrophilic/hydrophobic interactions. Alternatively, a short peptide may in fact represent an epitope found commonly in nature and may not show a reaction specific for the virus under consideration.

Another potential problem exists for all serological assays, regardless of source of antigen. The first antibodies produced in response to antigenic challenge are typically broadly cross-reactive and are produced by CD5+ B cells. Continued antigen exposure results in recruitment of a different subset of B cells (CD5-), which are destined to differentiate into mature plasma cells that secrete antibodies with high affinity and avidity for the inciting antigen. For those medical conditions associated with an increased number of cir-

culating CD5+ B cells (e.g., rheumatological disorders), the nonspecific immunoglobulins produced might also react with antigens, but in a nonpredictable, nonuniform fashion. For recombinant antigen-based assays, this could result in a pattern of reactivity sufficient for an indeterminate or even positive interpretation.

The idiotype network theory of antibody regulation predicts a potential problem in individuals who have been treated with recombinant CD4 peptides/proteins. CD4 binding of HIV-1 gp120 is the primary mechanism by which HIV-1 binds and infects CD4+ T cells. Continued exposure to recombinant CD4 would permit the host immune system to generate anti-CD4 antibodies, some of which will be directed against the CD4 site directly involved with HIV-1 gp120 binding. Anti-idiotypic antibodies could then be generated against these anti-CD4 antibodies. Some of these anti-idiotypic antibodies would undoubtedly react in any HIV-1 serological assay in a manner indistinguishable from an authentic anti-gp120 antibody.

The issue of which criteria to use for LIA interpretation so as to avoid false-positive results is well addressed by Pollet and colleagues. Although the opinion is frequently voiced that reactivity against a single HIV-1 envelope protein (gp41) should be sufficient for confirmation of HIV-1 infection, most laboratorians would take the more conservative approach and demand to see evidence of reactivity against proteins derived from at least two different HIV-1 genes. This conservatism arises from the fact that false-positive reactions have been observed with every single HIV-1 protein, recombinant or authentic—an important consideration, given the ramifications of a diagnosis of HIV-1 infection.

Lastly, one must consider the cost of performing these recombinant antigen-based assays. Currently they are quite expensive, with reagent costs for HIV-1 assays priced to be comparable with conventional immunoblot (~\$20-30 per strip), and assays of hepatitis C virus are priced at \$7-8 per single microtiter well. (In comparison, reagent costs for biochemical enzyme assays are typically <\$1 per assay.) Although recombinant antigens should be much cheaper to produce (because the host bacteria are grown in great quantities in fermentation vats) than whole viral lysates (which require tissue-culture facilities), they will probably not become cheaper until the associated research and development costs are recovered. Although the time necessary to recoup costs cannot be predicted, perhaps in the near future recombinant-based serological assays could be as competitively priced as standard clinical laboratory biochemical tests, whereupon they would be embraced with great enthusiasm by the clinical laboratories. (Recom = EXPENSIVE \$\$\$  
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