Anti-idiotypic antibodies for the diagnosis of infectious diseases

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Immunoassays are well-established as practical, rapid tests for detecting many infectious agents in the clinical laboratory. Immunoassays for infectious pathogens include the traditional radioimmunoassay and the more recent enzyme-linked immunosorbent assay (ELISA), which allows numerous different configurations of antigen and antibody. Most immunoassays use primary antibodies (either polyclonal or monoclonal) directed against specific epitopes of the infectious agent; the amount of primary antibody bound via a second antibody subsequently is quantitated by tagging with an easily identifiable marker. Despite the general success of this approach, effective immunoassays for certain infectious agents have been very difficult to develop, prompting investigators to search for alternatives in the design of rapid immunological tests.

A recent strategy taken by the authors’ laboratory has exploited the idiotypic network of the immune system to develop novel reagents for improved detection of microbial antigens. The highly specific antibodies generated during an immune response contain combining sites with structural complementarity to the epitope of the target antigen. The hypervariable region of the immunoglobulin molecule, which forms the antibody-combining site and is responsible for defining the specificity of the antibody, also carries a unique set of antigenic determinants, which collectively are termed the idiotypic of that antibody. These idiotypic determinants are antigenic, and as such may stimulate the formation of anti-idiotypic antibodies (Figure 1). Studies from many laboratories have confirmed that the combining site of some of the anti-idiotypic antibodies functionally will mimic the conformation of the original epitope, representing its ‘internal image’ (1-5). These particular anti-idiotypic antibodies, thus, can substitute for the antigen in primary antibody binding.

Much of the evidence for the ability of anti-idiotypic antibodies to act as surrogate antigens has been provided by research into the development of anti-idiotypic vaccines. In recent years, anti-idiotypic antibodies have been used to immunize experimental animals against a variety of viruses, bacteria and parasites (6,7). The anti-anti-idiotypic response induced in the hosts against the nominal pathogen has included development of protective immunity (eg, for hepatitis B virus, Streptococcus pneumoniae and Trypanosoma rhodesiense), production of viral neutralizing antibodies (eg, for poliovirus type II and rabies virus) and stimulation of cell-mediated immunity (eg, for Sendai virus and reovirus type 3).

Observations such as these have not only demonstrated the vaccine potential of anti-idiotypic antibodies, but also suggested that they might be useful tools for improved immunodetection of infectious agents. As for other immunoassays, the particular details of any protocol may be varied considerably. But the essence of an anti-idiotypic immunoassay lies in the structural similarity between the anti-idiotypic and the target epitope for the primary antibody, such that one may substitute for the other in binding to that antibody. An example of an anti-idiotypic assay is shown in Figure 2.

In an inhibition assay, when antigen (present in the test sample) is allowed to bind to a specific antibody, subsequent interaction between the antibody and complementary anti-idiotypic antibody will be blocked (compared with a control in which no antigen is present). The inhibition of binding between idiotypic and anti-idiotypic can be quantitated, and will reflect the concentration of antigen present in the test sample. Alternatively, competitive immunoassays could be established, with labelled anti-idiotypic antibodies competing with antigen for binding to a specific antibody.

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Figure 1) Idiotypes and anti-idiotypes. Antigen induces the formation of primary specific antibody, which bears an idiotypic determinant with complementarity to the microbial epitope. Since the idiotypic determinants are antigenic, the anti-idiotypic antibodies formed against this primary antibody may mimic the structure of the original epitope.

Given the variety of existing immunoassays for infectious agents, what advantages might be expected from anti-idiotypic assays? First, there are many complex microbial antigens which are difficult to obtain in purified form; by generating anti-idiotypic antibodies, purified protein substitutes for these antigens can be used. Second, certain antigens may have few exposed epitopes to any particular antibody; thus, sandwich immunoassays – which require a minimum of two accessible binding sites – are not successful. Third, in certain configurations such as competitive assays, it may be possible to achieve greater sensitivity using anti-idiotypic antibodies than otherwise is possible.

As a model system to test the feasibility of this concept, Brodeur et al recently developed an anti-idiotypic ELISA for human cytomegalovirus (HCMV). HCMV is a slow growing virus, and current immunoassays for the virus in the clinical laboratory are not ideal. The authors used a highly neutralizing anti-HCMV antibody (CMVBl) and generated monoclonal anti-idiotypic antibodies against it. The anti-idiotypic antibodies exhibited properties that indicated they were ideal candidates for assay development; in particular, they completely inhibited the reactivity of CMVBl towards its viral antigen. The anti-idiotypic inhibition ELISA was developed with a laboratory strain of HCMV, following the strategy outlined in Figure 2. The assay was able to detect HCMV and measured viral antigen in a dose-dependent manner over a clinically relevant range (20 to 0.6x10^3 plaque forming units per millilitre of urine). These encouraging results have prompted the authors’ further evaluation of the assay with clinical specimens.

There is widespread interest in idiotypes and anti-idiotypes for their role in regulating the immune response, as probes for various cellular receptors and as possible vaccines against tumours and infectious agents. Anti-idiotypic antibodies as potential reagents for in vitro immunoassays can now be added to this list. To the current workers’ knowledge, no other reports in the scientific literature have investigated the applicability of this idea. Nonetheless, it seems likely that anti-idiotypic antibodies are poised to take their place among other immunological reagents as a valuable option in the design of future immunodiagnoses for infectious agents.

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REFERENCES