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What is This?
IMMUNOLOGIC MARKERS FOR DIFFERENTIATION OF AUTOIMMUNE RESPONSES

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Abstract—Several approaches have been directed at identifying accurate parameters for the measurement of disease activity in autoimmune disorders, both in humans and in experimental animal models. A great deal of previous effort has focused on determining what constitutes antigenic epitopes on various autologous proteins or tissue components, which then can generate an immune response in the host. Much of this work has been clouded by the fact that normal subjects (both animal and human) seem to mount an immune response to myriad autologous proteins, characterized by the formation of antibodies known as natural autoantibodies. During the course of certain autoimmune diseases such as systemic lupus erythematosus (SLE) or Wegener’s granulomatosis (WG), patients produce what appear to be autoantibodies reacting with autologous components such as DNA and Sm antigen (SLE) or proteinase-3 (WG). Low levels of these same autoantibodies are present in IgG derived from normal subjects. Recently, we have found that IgG anti-F(ab’)_2 from normal subjects, affinity-purified from immunoadsorbent columns of human F(ab’), Sepharose, exhibits not only anti-F(ab’)_2 but also anti-dsDNA and anti-Sm activity. These antibodies constitute an average of 0.02% of normal serum IgG. Similar findings have also been observed in SLE patients with active disease. Our findings suggest that perturbation of the idiotypic network may represent an important fundamental aspect of many autoimmune disorders.

Key words: Sjögren’s syndrome, autoantibodies, immune response.

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Many diseases considered to be initiated by various autoimmune mechanisms are also thought to be uniformly associated with the production of autoantibodies, which somehow distinguish them from normal physiologic processes exhibited during regular body homeostasis. The focus of this discussion is on immunological markers that may help both clinicians and researchers to identify dysfunctions or possible abnormalities in several specific disease processes, such as Sjögren’s syndrome, mixed connective tissue disease, systemic lupus erythematosus (SLE), and even rheumatoid arthritis (RA). Consideration of such autoimmune disorders almost simultaneously calls up the concept of salient or key pathogenic autoantibodies. Such a process, therefore, makes apparent pathogenic autoantibodies the object of attention and somehow empirically assigns such autoantibodies a central, primary causative role in the basic disease process. Actually, such reflex, automatic association may afford no real improvement in clinical or physiological understanding of autoimmune disease pathogenesis, since the presence of autoantibodies per se may not be a reliable method to differentiate normal physiologic mechanisms from processes occurring in autoimmune disease. Many naturally occurring autoantibodies are present in normal subjects and must serve some other type of physiologic function besides induction of any clear autoimmune disorder, distinct lesions, or frank disease process. Thus, Avrameas and other researchers have described multiple autoantibodies that can be identified using homogenates or extracts of normal mouse tissue and normal mouse serum (Avrameas, 1988, 1991; Berneman et al., 1992). Such naturally occurring antibodies not only show specificity for cell surface or membrane components, but also react with intracellular epitopes or relatively inaccessible molecules such as nucleolar proteins or components of highly conserved intracellular organelles. These natural antibodies have been distinguished from apparently pathogenic autoantibodies such as anti-nDNA, which is highly correlated with disease activity in florid SLE or SLE nephritis (Tan et al., 1966; Schur and Sandson, 1968; Koffler et al., 1971). How to distinguish apparently natural or normally produced autoantibodies from presumably nefarious or pathogenic autoantibodies or autoimmune responses is not an easy task, and many very difficult issues remain to be resolved. This brief review will focus on several presumed markers of autoimmune disease activity, including autoantibodies such as anti-neutrophil cytoplasmic antibody (anti-cANCA) and anti-F(ab’)_2, previously considered to be a general class of regulatory anti-idiotypic antibody.

ANTIBODIES TO NEUTROPHIL GRANULAR COMPONENTS

Anti-cANCAs were studied in patients with biopsy-confirmed Wegener’s granulomatosis (WG) or polyarteritis...
TABLE

REACTIVITY OF IgG ANTI-PR3 ANTIBODIES FROM PATIENTS WITH WEGENER’S GRANULOMATOSIS INTERACTING WITH SITES INVOLVED IN THE CATALYTIC TRIAD (ACTIVE SITE) OF PROTEINASE 3 AS A SERINE PROTEASE

<table>
<thead>
<tr>
<th>WG Serum IgG Tested</th>
<th>44H</th>
<th>91D</th>
<th>176S</th>
</tr>
</thead>
<tbody>
<tr>
<td>McG, active&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>34&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dif, active</td>
<td>1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Jon, active</td>
<td>1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fet, active</td>
<td>1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0</td>
<td>1&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Per, active</td>
<td>2&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0</td>
<td>3&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hug, active</td>
<td>3&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0</td>
</tr>
<tr>
<td>Car, active</td>
<td>±&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0</td>
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<tr>
<td>Har, active</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Gui, inactive&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lon, inactive</td>
<td>0</td>
<td>0</td>
<td>1&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> 44H, 91D, and 176S refer to the residues of histidine at position 44, aspartic acid at 91, and serine at position 176 involved in the catalytic triad or active site of PR3 serine protease activity.

<sup>b</sup> Degree of ELISA reactivity of serum IgG with this residue epitope, based on ELISA reactivity OD<sub>690</sub>.

<sup>c</sup> Patients designated as active had concurrent positive cANCA.

<sup>d</sup> Degree of ELISA reactivity of serum IgG with this residue epitope, based on ELISA reactivity OD<sub>690</sub>.

With polyclonal IgG antibody in sera from patients with active anti-cANCA-positive uncontrolled disease. In particular, two major linear antigenic regions represented by the sequences ATQQLPQ and RVGAHDP, at positions (108-114) and (132-138), respectively, constituted major PR3 antigenic sites. This was demonstrated by absorption of whole WG sera with PR3 peptide, which resulted in marked reduction of anti-whole PR3 by ELISA assay or abolition of neutrophil cytoplasmic staining (cANCA) in the immunofluorescence cANCA assay (Williams <i>et al.</i>, 1994). During the course of this work, it became clear that low levels of IgG anti-AVTQQLPQ peptide or anti-RVGAHDP peptide antibody could also be demonstrated in sera of many normal human subjects tested as parallel controls. These findings, therefore, suggested that the occurrence of high levels of anti-neutrophil cytoplasmic antibody in patients with active WG or PAN might be merely an accentuation of a normal self-directed immune response against the serine protease PR3. Great interest were our findings during this study that a high proportion of anti-cANCA IgG antibodies from WG patients actually reacted with discrete regions on PR3 involved in the active site or catalytic triad of the PR3 serine protease enzyme. Representative data illustrating this point are shown in the Table. It can be seen that virtually all WG patients with active disease showed serum IgG antibodies reacting with at least one part of the catalytic triad. These findings are reminiscent of a similar pattern of autoantibody specificity, previously reported by Tan, in which, in numerous instances, many other autoantibodies with antinuclear specificity appeared to react with physiologic functional sites of key molecules involved in basic cell functions (<i>e.g.</i>, RNA polymerase, RNA splicing, and others) (Tan, 1982, 1992).

IgG ANTI-F(ab')<sub>2</sub> ANTIBODY

Anti-F(ab')<sub>2</sub>, was studied in SLE and normal sera by means of a 1:1,000 dilution of serum and an ELISA method previously described in detail (Silvestris <i>et al.</i>, 1984, 1985). IgG anti-F(ab')<sub>2</sub> antibodies were isolated from whole serum IgG by means of affinity columns of F(ab')<sub>2</sub> prepared from Cohn FrII (Silvestris <i>et al.</i>, 1984).

Anti-F(ab')<sub>2</sub>, in both normal and pathologic sera, may represent a collection of anti-idiotypic antibodies. Previous work from our laboratory has demonstrated that serum IgG reacting with determinants on the Fab or F(ab')<sub>2</sub> portions of IgG may indeed represent anti-idiotypic antibody activity somehow involved in modulation of the humoral response of the host (Silvestris <i>et al.</i>, 1984, 1985, 1986). When SLE patients become ill, their IgG serum anti-F(ab')<sub>2</sub> levels frequently fall far below the normal mean. As the patients improve, their levels of anti-F(ab')<sub>2</sub> rise to normal, or sometimes slightly higher than normal, values (Silvestris <i>et al.</i>, 1984). Recently, our group has been re-examining this phenomenon in studies of 108 SLE patients followed at the University of Florida Clinics and inpatient service during the last 4-5 years. Very low levels of serum IgG anti-F(ab')<sub>2</sub>...
are frequently associated with general flares of SLE disease activity; often, the lowest serum anti-F(ab')$_2$ levels appear to correlate with very active lupus nephritis, as documented by concurrent renal biopsy and clinical profiles of these patients. We have also found that eluates of IgG from these SLE renal biopsies contain markedly higher relative concentrations of both IgG anti-DNA and IgG anti-F(ab')$_2$ than are concurrently present in patients' serum or plasma. It has become clear in studying these eluates that, in many instances, a population of IgG antibodies can be eluted from sonicated SLE renal biopsy material, which shows an unusual dual anti-DNA/anti-F(ab')$_2$ specificity. This has been demonstrated by adsorption of these renal biopsy eluates to DNA-Sepharose immunoabsorbents and then elution of the IgG adsorbed to the DNA cellulose by means of low-pH (2.5) glycine-saline buffers. When such eluted IgG is immediately brought back to neutral pH, it can also be demonstrated through ELISA assay to have anti-F(ab')$_2$ reactivity. Thus, our recent studies appear to indicate that IgG anti-F(ab')$_2$ antibodies can show dual specificity, reacting with nDNA as well as with sites on F(ab')$_2$. It is still not clear whether this doubly-reacting population of IgG anti-F(ab')$_2$/anti-DNA antibodies has any preferential affinity for particular anatomic sites within the body, such as the glomeruli, which may be related to specific disease lesions or organ involvement.

Finally, we have also recently found that IgG anti-F(ab')$_2$ in normal subjects' serum also appears to show the same sort of dual specificity, since such affinity-isolated anti-F(ab')$_2$ shows both anti-F(ab')$_2$ and anti-DNA specificities (Williams et al., 1994). Furthermore, this unusual cross-specificity appears to be present in anti-F(ab')$_2$ of SLE patients in remission. Surprisingly, when direct affinity measurements of affinity-purified anti-dsDNA and anti-F(ab')$_2$ are conducted both in normal subjects and in patients with SLE, the relative affinity of IgG anti-F(ab')$_2$ antibodies is much higher for DNA than for F(ab')$_2$. This difference in affinity is present in normal subjects as well as in patients with SLE. It therefore appears that a relatively restricted idiotypic network involving anti-DNA, anti-F(ab')$_2$, and perhaps a limited number of other autoantigens may be at work both in patients with active SLE and in normal subjects. The manner in which these findings may relate to the presence and ultimate progression of renal disease or other tissue-damaging lesions in SLE remains to be elucidated by ongoing work.

ACKNOWLEDGMENTS

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