Autoantibodies to Specific Enzymes: A Review*

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ABSTRACT

There are two categories of autoantibodies to specific enzymes: immunoglobulin-complexed enzymes and circulating autoantibodies directed to enzymes in tissue or tissues. Immunoglobulin-complexed enzymes may result in elevated serum enzyme activity. They are found more frequently in elderly patients and have limited clinical significance. Immunoglobulin association with the enzyme must be demonstrated to distinguish this macromolecule from other high molecular weight enzyme complexes. Autoantibodies to specific enzymes or regulators of enzyme activity do possess specific disease associations. The titers or presence of these autoantibodies may predict morbidity or response to therapy. These autoantibodies may be detected by Western blotting, enzyme-linked immunosorbent assays, tissue immunofluorescence, radioimmunoassay, immunoprecipitation flow cytometry or inhibition of enzyme activity. For example, anti-pyruvate dehydrogenase inhibits the activity of purified enzyme, but not relatively intact mitochondrial preparations. Most evidence suggests that the production of autoantibodies to specific enzymes represents an epiphenomenon secondary to tissue damage rather than a primary event in the pathogenetic pathway.
Introduction

Autoantibodies to specific enzymes may be classified into two general groups based on their location. The first group consists of immunoglobulin-complexed enzymes located in the circulation. The total activity of the immunoglobulin-complexed enzyme and unbound enzyme in sera may be elevated or within the reference range.1,2 The second group consists of circulating autoantibodies directed to autoantigens (enzymes) located on or expressed in only one tissue (thyroid peroxidase, gastric parietal cell H+, K+ ATPase) or in a variety of tissues (glutamic acid decarboxylase, pyruvate dehydrogenase).3,4 In vitro measurement of enzyme activity following addition of the appropriate autoantibody usually results in inhibition of enzyme activity. Therefore, if the total activity of this enzyme (autoantigen) is measured routinely in sera, circulating autoantibody-enzyme complexes would decrease, not increase total (bound plus unbound) activity as suggested by Hortin et al.5

The clinical significance of immunoglobulin-complexed enzymes is not as specific in relationship to disease association and/or disease severity as autoantibodies with regard to enzyme-autoantigens.1,2,3,4,5 The etiology of both types of autoantibodies with regard to specific enzymes may be secondary to altered B cell clonal proliferation or a disturbed immunologic network. The release of the specific enzyme from injured tissue followed by the proteolytic or other enzymatic modification of the autoantigen may initiate the immunologic response. Whether or not these autoantibodies are important in the pathogenesis of the disease with which they are associated is unresolved.

Although the autoantibodies to a specific enzyme-autoantigen inhibit enzyme activity in vitro, the large molecular weight immunoglobulin would need to traverse the plasma membrane as well as the outer membrane of any intracellular organelle to bind to the target. There is evidence to suggest that some autoantibodies can localize in intracellular organelles.6,7,8,9 For example, autoantibodies against adenosine diphosphate/adenosine triphosphate (ADP/ATP) carrier protein have been shown to penetrate into the mitochondria of myocardial cells, inhibit mitochondrial nucleotide exchange by blocking substrate binding to the inner mitochondrial membrane and disrupt in vivo energy metabolism of the cardiac muscle.10,11 However, most evidence suggests that the production of autoantibodies to specific enzymes represents an epiphenomenon secondary to tissue damage rather than a primary event in the pathogenetic pathway.

Immunoglobulin-Complexed Enzymes

The binding of an immunoglobulin to an enzyme circulating in blood results in a complex with a molecular weight larger than that expected for the enzyme or any of its isozymes. In table I are listed some enzymes which have been reported to form macroenzymes or immunoglobulin-complexed enzymes.1,2,5 Enzyme activity measured in sera may be elevated or within the reference range.1,2 This find-

<table>
<thead>
<tr>
<th>Immunoglobulin–complexed Enzymes</th>
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<tbody>
<tr>
<td>Creatine kinase</td>
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<tr>
<td>Lactate dehydrogenase</td>
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<tr>
<td>Alkaline phosphatase</td>
</tr>
<tr>
<td>Acid phosphatase</td>
</tr>
<tr>
<td>Amylase</td>
</tr>
<tr>
<td>Gamma-glutamyltransferase</td>
</tr>
<tr>
<td>Asparate aminotransferase</td>
</tr>
<tr>
<td>Alanine aminotransferase</td>
</tr>
<tr>
<td>Lipase</td>
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ing suggests that the autoantibody does not significantly alter the enzyme's active site. Clinically, the patients' symptoms are not characteristic of the elevated enzyme activity. These macroenzyme complexes are usually not associated with specific drug therapy, elevated antinuclear antibody titers or specific diseases. The incidence of immunoglobulin-complexed enzymes in the general population varies from 0.1 to 10 percent. Macro-creatine kinase (macro-CK1) has been associated with a variety of neoplasms.

Fifty-seven percent of patients with acute bacterial infections had a specific IgG autoantibody which was bound to intestinal alkaline phosphatase through the Fab portion. IgA is bound to amylase more often than IgG. In most other examples, IgG is the most common autoantibody. The ratio of immunoglobulin to enzymes is 1:1 for alanine aminotransferase, creatine kinase and lactate dehydrogenase and 2:1 for alkaline phosphatase. The incidence of immunoglobulin-complexed enzymes increases with age for both sexes.

Immunoglobulin-complexed enzymes may be detected by a variety of methods (table II). Sucrose density gradient centrifugation is the most reliable but is labor intensive. Demonstration that an immunoglobulin species is bound to the enzyme is important to separate the macroenzyme from other larger molecular weight complexes like oligomers, or enzyme bound to substrate analogs.

| TABLE II
<table>
<thead>
<tr>
<th>Detection of Immunoglobulin–complexed Enzymes</th>
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<tbody>
<tr>
<td>Sucrose density gradient centrifugation</td>
</tr>
<tr>
<td>Column gel chromatography</td>
</tr>
<tr>
<td>Agarose or other type of electrophoresis</td>
</tr>
<tr>
<td>Gradient polyacrylamide gel electrophoresis</td>
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<tr>
<td>Separation of immunoglobulin bound to enzyme</td>
</tr>
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| TABLE III
<table>
<thead>
<tr>
<th>Alkaline Phosphatase Macroenzymes</th>
</tr>
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<tbody>
<tr>
<td>Immunoglobulin–complexed enzyme</td>
</tr>
<tr>
<td>Plasma membrane bound complex</td>
</tr>
<tr>
<td>Lipoprotein–X bound complex</td>
</tr>
<tr>
<td>Phenotypic variant</td>
</tr>
</tbody>
</table>

Macro-Alkaline Phosphatase

Macro-alkaline phosphatase may represent one of four species (table III). High molecular weight alkaline phosphatase is located at the top of the stacking gel (H1) or at the interface between the stacking gel and separating gels (H2) during polyacrylamide gel electrophoresis (figure 1). This finding is observed in sera from patients with cholestatic liver disease. This high molecular weight complex contains plasma membrane fragments with alkaline phosphatase attached to a glycosyl phosphatidylinositol anchor which is cleaved by the action of phosphatidylinositol-specific phospholipase C (Bacillus cereus phospholipase C) or circulating phospholipase D. In figure 1 is illustrated the effect of n-butanol (tube 2), Clostridium perfringens (tube 3), and B. cereus phospholipase C (tube 4) on a plasma membrane preparation from human endometrium compared to human kidney and human sera.

Endometrial plasma membranes were prepared by the technique for adipocytes after removal of blood from endometrial tissue with 0.9 percent saline and placing homogenized tissue in 0.25 M sucrose, 10 mM 3-(N-morpholino) propanesulfonic acid, pH 7.4, buffer containing the protease inhibitors, 1 mM phenylmethylsulfonyl fluoride and 0.1 mg/mL aprotinin. The plasma membrane fraction was separated on a discontinuous sucrose gradient and enriched by a factor of
FIGURE 1. Polyacrylamide gel electrophoresis of human endometrial plasma membranes (0.198 U total alkaline phosphatase activity/mg protein (tubes 1 to 4), human kidney homogenate (tube 5) and sera, unheated (tube 6) or heated at 56°C for 10 min (tube 7). Endometrial plasma were treated with n-butanol (tube 2), 50 U/mL *Clostridium perfringens* (Type IX) phospholipase C (tube 3) and *Bacillus cereus* phosphatidylinositol-specific phospholipase C (tube 4). In lane 7, high molecular weight alkaline phosphatase is designated H1, located at top of the stacking gel, or H2, located between the stacking gel and separating gel. Isozymes of human alkaline phosphatase are identified as I for intestinal form, B for bone form and C for liver form.

8.1 compared to the initial endometrial homogenate based on 5′-nucleotidase activity.

N-butanol treatment (tube 2) resulted in removal of alkaline phosphatase activity from the stacking gel and migration into the upper portion of the separating gel. This migration to approximately one-third of the serum liver isozyme (tube 6, 7) is similar to that observed in the sera from some patients who had either immunoglobulin-complexed alkaline phosphatase or membrane fragments containing alkaline phosphatase.19

*B. cereus* phospholipase C caused some of the alkaline phosphatase activity to migrate from the gel origin to a position above the serum bone isoform. Incubation of *Cl. perfringens* (Type IX) phospholipase C with endometrial plasma membranes resulted in four new bands: two bands significantly below the serum liver isoform, one band immediately below the serum liver isoform, and one band above the serum bone isoform.

Electrophoresis of untreated human kidney homogenate resulted in activity located at the top of the stacking gel as
well as two diffuse bands: one with about 50% of the mobility of the liver isoenzyme and one just above the bone isoenzyme, similar to B. cereus phospholipase C-treated endometrial plasma membranes.

Human endometrial alkaline phosphatase has been reported to consist of the liver/bone/kidney and placental isoenzymes\(^{20,21,22,23}\) and migrate “more anodally” than human intestinal alkaline phosphatase on polyacrylamide gel electrophoresis.\(^{24}\) The observed mobility in figure 1 suggests that the alkaline phosphatase in human endometrium may consist of a renal-type isoform or a unique isoform. Sequencing data of the endometrial alkaline phosphatase gene will resolve this issue. However, these data illustrate that not all macromolecules represent immunoglobulin-complexed enzymes.

**Autoantibodies to Specific Enzymes**

Autoantibodies have been reported against specific enzymes\(^{3,4,25-44}\) as well as regulators of enzyme activity\(^{40,45}\) resulting in a variety of specific disease association (table IV). Methods of detection are listed in table V. The search for autoantibodies associated with disease may begin with separation of proteins from a specific tissue by molecular weight using polyacrylamide gel electrophoresis. These proteins are immunoblotted using the Western technique with sera from controls and from patients with disease.\(^{46,47,48}\) Antigen-autoantibody complexes are identified. The identity of the protein antigen bound by autoantibodies found only in sera from patients with a specific disease, and not in sera from controls, may be determined by partial protein sequencing and then searching for the mRNA or cDNA in tissue libraries.\(^{45,49,50}\) Once identified, the protein’s function may be defined as enzyme, regulator of enzyme activity, cytoskeletal protein, etc.

Complement-fixing autoantibodies to thyroid peroxidase, associated with autoimmune thyroid disease, are found in approximately 95 percent of patients with Hashimoto thyroiditis and 80 percent with Graves’ disease.\(^{4}\) Since this enzyme is expressed only in the thyroid, autoantibody reactivity has not been observed in other tissues. Molecular cloning and expression of a rat cDNA encoding for one of the major mitochondrial autoantigens\(^{51}\) and subsequent identification of this autoantigen as the E2 component of the multimeric pyruvate dehydrogenase (PDH) enzyme complex\(^{52}\) located within the inner mitochondrial membrane, have identified that PDH is a specific autoantigen in primary biliary cirrhosis.

The PDH complex is a macromolecule composed of multiple copies of the E1 component (pyruvate decarboxylase, consisting of alpha and beta subunits), the E2 component (dihydrolipoamide acetyl transferase), the E3 component (dihydrolipoamide dehydrogenase), and protein X (involved in high affinity binding of E3 to E2).\(^{53}\) It plays a pivotal role in the energy yielding pathway from the decarboxylation of pyruvate to acetyl coenzyme A and CO\(_2\). Anti-pyruvate dehydrogenase has been detected by Western blotting, immunofluorescence, enzyme-linked immunosorbent assay, enzyme inhibition and a flow cytometric method using antigen-coated polystyrene microbeads.\(^{27,28,29}\) The flow cytometric method has a sensitivity of 94 percent and specificity of 100 percent.\(^{29}\)

Anticytoplasmic islet cell antibodies react to heterogeneous islet antigens, including the enzyme, glutamic acid decarboxylase (GAD). The GAD has two isoforms of molecular weight, 65,000 (GAD65) and 67,000 (GAD67), each encoded on a different gene. Anti-GAD65 to conformation epitopes occur in 70 to 80 percent of insulin-dependent diabetes mellitus patients and linear
### TABLE IV
Autoantibodies to Specific Enzymes or Regulators of Enzyme Activity

<table>
<thead>
<tr>
<th>Type</th>
<th>Intracellular Location</th>
<th>Disease Association</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Autoantigen: Enzymes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thyroid peroxidase</td>
<td>Microsomes</td>
<td>Autoimmune thyroid disease</td>
</tr>
<tr>
<td>Steroid 21–hydroxylase</td>
<td>Microsomes</td>
<td>Autoimmune Addison disease</td>
</tr>
<tr>
<td>Pyruvate dehydrogenase</td>
<td>Mitochondria</td>
<td>Primary biliary cirrhosis</td>
</tr>
<tr>
<td>H+, K+–ATPase</td>
<td>Plasma membrane</td>
<td>Autoimmune gastritis</td>
</tr>
<tr>
<td>Acetylcholinesterase</td>
<td>Plasma membrane</td>
<td>Myasthenia gravis</td>
</tr>
<tr>
<td>Glutamate decarboxylase</td>
<td>Cytosol</td>
<td>Insulin–dependent diabetes mellitus</td>
</tr>
<tr>
<td>GAD65</td>
<td></td>
<td>Stiff–man syndrome; polyendocrine syndrome II with insulin–dependent diabetes mellitus</td>
</tr>
<tr>
<td>GAD67</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbonic anhydrase II</td>
<td>Cytosol</td>
<td>Endometriosis; autoimmune cholangitis</td>
</tr>
<tr>
<td>Triosephosphate isomerase</td>
<td>Cytosol</td>
<td>Hemolysis in Epstein–Barr virus or hepatitis A virus infection</td>
</tr>
<tr>
<td>Mn++ superoxide dismutase</td>
<td>Cytosol</td>
<td>Epstein–Barr virus infection</td>
</tr>
<tr>
<td>Prostate specific antigen</td>
<td>Cytosol</td>
<td>Benign prostatic hyperplasia</td>
</tr>
<tr>
<td>Tissue–type plasminogen activator</td>
<td>Cytosol &amp; sera</td>
<td>Systemic lupus erythematosus</td>
</tr>
<tr>
<td>DNA helicase II (Ku)</td>
<td>Nucleus</td>
<td>Scleroderma, systemic lupus erythematosus</td>
</tr>
<tr>
<td>Topoisomerase I (Scl 70)</td>
<td>Nucleus</td>
<td>Scleroderma</td>
</tr>
<tr>
<td>Histidyl–tRNA synthetase (Jo–1)</td>
<td>Nucleus</td>
<td>Polymyositis–dermatomyositis</td>
</tr>
<tr>
<td>Alanyl–tRNA synthetase (PL–12)</td>
<td>Nucleus</td>
<td>Polymyositis</td>
</tr>
<tr>
<td>Threonyl–tRNA synthetase (PL–7)</td>
<td>Nucleus</td>
<td>Polymyositis</td>
</tr>
<tr>
<td>RNA polymerase I/III</td>
<td>Nucleus</td>
<td>Scleroderma</td>
</tr>
<tr>
<td><strong>Autoantigen: Inhibitors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasminogen activator inhibitor 1</td>
<td>Sera</td>
<td>Systemic lupus erythematosus</td>
</tr>
<tr>
<td>Calpastatin</td>
<td>Sera</td>
<td>Rheumatoid arthritis</td>
</tr>
</tbody>
</table>

epitopes in 60 percent of patients with stiff-man syndrome (SMS).\textsuperscript{54} Anti-GAD67 is observed in patients with SMS, palatal myoclonus and epilepsy,\textsuperscript{55} as well as polyendocrine syndrome type II with insulin-dependent diabetes mellitus.\textsuperscript{56} Radiobinding assays for anti-GAD65 are more sensitive than ELISA or enzymatic immunoprecipitation assays.\textsuperscript{57} Anti-carbonic anhydrase II has been reported in 70 percent of patients with endometriosis,\textsuperscript{34} 83 percent with autoimmune cholangitis,\textsuperscript{33} 30 percent with systemic lupus erythematosus, polymyositis and systemic sclerosis\textsuperscript{36} and 21 percent with Sjögren’s syndrome.\textsuperscript{50} There are at least seven isozymes of carbonic anhydrase distributed in a variety of tissues.\textsuperscript{58}
Autoantibody-reactivity to carbonic anhydrase III to VII has not been investigated to date. The enzyme catalyzes the reversible hydration of carbon dioxide to bicarbonate and hydrogen ions. The finding of carbonic anhydrase II autoantibodies was a consequence of immunoblotting the molecular weight markers with patients' sera during Western blotting. Carbonic anhydrase II is commonly included in commercially available mixtures of protein used for molecular weight determination during polyacrylamide gel electrophoresis.

Hemolysis in acute hepatitis A infection or Epstein-Barr virus infection is caused by IgM antibodies to the Embden-Meyerhoff pathway enzyme, triosephosphate isomerase. Anti-prostate specific antigen was reported in sera of 59 percent of patients with benign prostatic hypertrophy. Prostate specific antigen is a 34 kD serine protease with sequence homology to the kallikrein family. Sera from 57 percent of patients with rheumatoid arthritis had autoantibodies to the endogenous protein inhibitor, calpastatin. Calpastatin inhibits the calcium-dependent cysteine protease, calpain. Calpain in synovial fluid may damage the cartilage in patients with joint disease.

A variety of antinuclear antigens have been identified as enzymes located in the nucleus. For example, the Ku antigen has been identified as the human DNA helicase II. This ATP-dependent DNA unwinding enzyme binds to partially unwound duplex DNA and unwinds in the 3' to 5' direction.

### Autoantibody Inhibition of Enzyme Activity

Inhibition of enzyme activity by specific autoantibodies has been used as a method for detection and/or quantitation of autoantibodies in sera (table V). In table VI are listed the effects of some autoantibodies on enzyme activity. For example, several investigators have demonstrated the *in vitro* inhibition of purified PDH activity by antimitochondrial antibodies present in the sera of patients with primary biliary cirrhosis (PBC). The majority of anti-PDH autoantibodies react with the lipoic acid binding domain, a functionally critical site within the enzyme complex. Inhibition of purified PDH activity by sera from patients with PBC have raised questions about the relevance of the *in vitro* phenomenon in the pathogenesis of this disease.

To investigate the ability of sera from patients with primary biliary cirrhosis to inhibit mitochondrial PDH activity, their effect on the activity of a purified enzyme preparation was compared to that of minimally disrupted mitochondria purified from rat adipocytes. It is demonstrated in figure 2 that the addition of individual sera of 11 PBC patients that reacted with PDH by immunoblotting caused profound inhibition (92.5 ± 4.3 percent) of purified PDH activity. The same PBC sera had no significant inhibitory effect on native PDH within minimally disrupted mitochondria (figure 2). However, when the mitochondria were maximally disrupted either by sonication...
cent inhibition at a concentration of 17.6 μg/mL). Addition of purified IgG from normal individuals or addition of normal sera depleted of IgG resulted in no inhibition of PDH activity (figure 4).

**TABLE VI**

<table>
<thead>
<tr>
<th>Type</th>
<th>Effect</th>
</tr>
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<tbody>
<tr>
<td>Glutamate decarboxylase&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Inhibited</td>
</tr>
<tr>
<td>Steroid 21-hydroxylase&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Inhibited</td>
</tr>
<tr>
<td>Alanyl-tRNA synthetase&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Inhibited</td>
</tr>
<tr>
<td>Threonyl-tRNA synthetase&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Inhibited</td>
</tr>
<tr>
<td>Triosephosphate isomerase&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Inhibited</td>
</tr>
<tr>
<td>Manganese superoxide dismutase&lt;sup&gt;f&lt;/sup&gt;</td>
<td>Inhibited</td>
</tr>
<tr>
<td>Pyruvate dehydrogenase&lt;sup&gt;g&lt;/sup&gt;</td>
<td>Inhibited: purified</td>
</tr>
<tr>
<td></td>
<td>No effect: mitochondrial</td>
</tr>
<tr>
<td></td>
<td>preparation</td>
</tr>
<tr>
<td>Carbonic anhydrase II&lt;sup&gt;h&lt;/sup&gt;</td>
<td>Inhibited</td>
</tr>
<tr>
<td>Tissue-type plasminogen activator&lt;sup&gt;i&lt;/sup&gt;</td>
<td>No effect</td>
</tr>
</tbody>
</table>

<sup>a</sup> Schmidli, RS, Colman, PH, Bonifacio E, Bottazzo GF, Harrison LS. *Diabetes* 1994;43:1005-9.
In figure 4, it is also demonstrated that IgG was twice as inhibitory (90.0 ± 6.8 percent) as compared to the fraction containing IgG₁, and IgG₂, and IgG₄ (47.6 ± 10.1 percent). Both fractions were tested at a similar immunoglobulin concentration of 16 μg/mL.

The PBC sera containing anti-PDH autoantibodies had no inhibitory effect on PDH activity of minimally disrupted mitochondria. No inhibition of mitochondrial PDH activity was observed, even when PBC sera were used at very high concentrations (1:10). However, when the mitochondrial membrane was maximally disrupted, PBC sera were capable of exerting substantial inhibition of mitochondrial PDH similar to that observed with purified PDH. These results indicate that anti-PDH antibodies cannot traverse the mitochondrial membrane and cause inhibition of PDH activity within intact mitochondria. These results, however, do not completely rule out a possible pathogenic role for anti-PDH autoantibodies through the interference with cytosolic PDH, present as a primary translation product. Entry of anti-PDH antibodies could be accomplished by receptor mediated endocytosis possibly through a PDH cross-reactive epitope on the surface of PBC bile ducts.

Both IgG purified from PBC sera as well as PBC sera depleted of IgG, but containing IgM, caused inhibition of purified PDH activity. Sundin has reported that inhibition of PDH activity by PBC sera was exclusively due to IgG and not IgM. The fractionation procedure described by Sundin would yield pentameric (19S) IgM which was used in the assay of purified PDH. However, PBC sera contain the pentameric IgM as well as a monomeric form (7S) of IgM. Our IgG depleted sera may have both forms of IgM present. In light of Sundin’s findings, it has been proposed by us that inhibition and recognition of PDH by IgM may be mediated by monomeric or oligomeric forms of IgM and not by the pentameric form. It has also been shown that inhibition of PDH activity by IgG, present in PBC sera, is mediated primarily by IgG₃. This finding supports the earlier observation of the predominance of IgG₃ subclass in the recognition of PDH.

Although the inhibition of PDH activity by antimitochondrial antibodies may not be the pathophysiologic effect leading to PBC, anti-PDH autoantibodies, specifically the IgG₃ subclass, may play a role in progressive bile duct injury in PBC. The binding of such autoantibodies to their target antigen within dam-
Figure 4. Inhibition of purified pyruvate dehydrogenase (PDH) activity by different Ig isotypes present in primary biliary cirrhosis (PBC) sera. Activity of PDH was assayed by the release of $^{14}$CO$_2$ from (1-14C) pyruvate. Original PBC sera consisted of a pool of eleven PBC sera which individually inhibited purified PDH activity. These sera were used at a 1:400 dilution. Purified IgG, free of any other immunoglobulins, was obtained from pooled PBC sera by protein G affinity column and used at a concentration of 43 μg/mL. Pooled PBC serum depleted of any IgG was used at a concentration of 17.6 μg/mL IgM. The concentrations of the purified IgG and IgM represents the concentration of both immunoglobulins in the unfractinated pooled PBC serum specimen, diluted 1:400. PBC IgG$_3$ was obtained by protein A absorption of the other IgG subclasses from purified PBC IgG. Protein A eluted fraction contained IgG$_1$, IgG$_2$, and IgG$_4$, but no IgG$_3$. Both fractions were tested in the PDH assay at a concentration of 16 μg/mL each. Purified normal IgG and normal sera depleted of IgG were obtained from a pool of 10 normal individuals and prepared in a similar manner to that described for PBC sera. All experiments were done in triplicate and the results reflect the mean ± SD. Control value for purified PDH was 56.7 ± 3.2 ng/mg protein/min.

Aged biliary epithelium and disrupted mitochondria may lead to activation of the complement cascade and potentiation of antibody dependent cellular cytotoxicity. Such an inflammatory reaction may even extend to affect undamaged neighboring structures within the portal triad. Suggestive evidence for this mechanism is seen in liver biopsies of PBC patients, especially those with stage II disease, where in addition to the mononuclear infiltrate, characteristic of PBC, acute inflammatory cells are commonly found.  

Summary

Immunoglobulin-complexed enzymes are found more frequently in elderly patients and have limited clinical significance. However, autoantibodies to specific enzymes or regulators of enzyme activity do possess significant disease association. The immunology laboratory of the future will be requested to quantify their presence. Future investigation is required to determine the etiology of autoantibody production specifically the role of B cells and/or T cell responses.
Finally, the titers or presence of these autoantibodies may predict patient morbidity or response to therapy.

Acknowledgment

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