Monoclonal Antibodies Binding Renal Renin

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SUMMARY Somatic-cell fusion of normal antibody-producing spleen cells with cells from a plasmacytoma culture results in a culture of hybrid cells from which a monoclonal line may be selected. These lines are immortal and may be amplified as tumors in syngeneic animals to produce large quantities of antibodies characterized by molecular homogeneity. We report the application of this technique to the production of antibodies binding canine renin. Balb/c mice were immunized with pure canine renal renin and their spleen cells fused with the NS-1 myeloma line. In two separate fusions, nine clones of cells were isolated that bound canine renal renin but did not cross-react with a number of protein antigens tested. One of these antibodies cross-reacted with renins of several different species, including human renin. Binding inhibition studies carried out with one of these monoclonal antibodies demonstrated a dissociation constant for renin of \(10^{-9}\) M. These monoclonal antibodies have great potential in answering significant questions concerning the structure, biosynthesis, tissue localization, and physiologic actions of renin. (Hypertension 3 (suppl II): II-4-II-8, 1981)

**Key Words** • renin antibody • monoclonal antibody

ANTIBODIES specific for renin have recently become available with the purification of the enzyme from tissues of several different species. These antibodies have been used in the direct measurement of renin by immunoassay as inhibitors of renin in vivo, in the identification of extra-renal renin-like enzymes, and in the examination of renin biosynthesis. Although these studies have yielded information of interest, antibodies produced by the process of conventional immunization are necessarily limited with respect to quantity, reproducibility, and homogeneity. The recent development of methods for the production of monoclonal antibodies has overcome these problems; antibodies can now be obtained as homogeneous proteins in unlimited quantity. We have applied these methods to the selection and propagation of hybrid clones from mice immunized with canine renin and report here the preliminary characterization of nine monoclonal antibodies binding this antigen.

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### Methods

#### Immunization

Balb/c mice were injected intraperitoneally with 50 \(\mu g\) of purified canine renal renin in complete Freund's adjuvant 4 weeks prior to fusion. Three days before fusion the animals were intravenously boosted with 5 \(\mu g\) of the same antigen.

#### Cell Fusion

The procedure for fusion is a modification of one reported by Marshak-Rothstein et al. We fused 10⁶ spleen cells from an immunized mouse with 10⁷ mouse myeloma cells from the NS-1 line( kind gift of Dr. Malcolm Gefter) in the presence of 30% polyethylene glycol for 5 minutes. After 48 hours of growth in Delbecco's modified Eagle medium (DMEM) supplemented with 20% fetal calf serum, 50 \(\mu g\)/ml gentamicin, and 580 \(\mu g\)/ml glutamine, the cells were resuspended in hypoxanthine aminopterin thymidine (HAT) medium (1 \(\times\) 10⁻⁴ M hypoxanthine, 4 \(\times\) 10⁻⁷ M aminopterin, and 1.6 \(\times\) 10⁻⁴ M thymidine) and distributed into four Costar 96-well microtiter plates. After 14 days of growth in the HAT medium, supernatants from wells exhibiting cell growth were assayed utilizing an immunoradiometric assay for renin-specific antibody.
Immunoradiometric Assay

The general approach described by Klinman et al. was utilized. Flexible polyvinyl chloride microtiter plates (Cooke Laboratories) were first coated with a solution of canine renin or one of the following control antigens: pepsin, trypsin, hemoglobin (or bovine serum albumin). The plates were then washed with 20% horse serum followed by incubation with varying dilutions of culture supernatants, ascites fluid, or fractionated monoclonal antibody. After a water wash, 1IgI goat antimouse Fab (the antibody is directed at Fab so that all hybridoma protein isotypes may be detected by the reagent) was added to each well; and, after a final wash, the wells were separated and counted in a gamma scintillation counter. To determine the capacity of soluble renin to inhibit the binding of monoclonal antibodies to immobilized renin, increasing concentrations of renin were added to the antibody prior to application to the microtiter plate.

Subcloning and Expansion of Hybridomas

Utilizing mouse 3T3 cells or non-immune spleen cells as a feeder layer, cells from wells exhibiting renin-specific antibody were subcloned by limiting dilution. Positive clones were injected into pristane-primed Balb/c mice (0.5 ml pristane i.p. 1–2 weeks prior to cell injection) at a dose of 10⁶ cells per mouse. After visible evidence of ascites production, the abdomens of the mice were tapped every other day, and fluid from several animals pooled.

Results

Two separate fusions, F5 and F9, yielded renin antibody-secreting clones (table 1). Antibodies of the isotypes IgG₁ and IgM were identified. Canine renin appears to be an effective antigen in the mouse, and the yield of positive clones is abundant, exceeding the laboratory's capacity for isolation. The antibodies bound renin at a considerable dilution. In this assay the half-maximal point of antibody binding to the immobilized antigen is both a measure of concentration and avidity. Representative dilution plots are shown in figure 1 for antibodies from fusions 5 and 9. The antibodies representative of each of the two fusions may be diluted considerably with continued antigen-bind-

<table>
<thead>
<tr>
<th>Fusion</th>
<th>Wells assayed (no.)</th>
<th>Cell growth (%)</th>
<th>Renin antibody production</th>
<th>Clones isolated</th>
<th>Isotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>F5</td>
<td>400</td>
<td>95</td>
<td>9</td>
<td>1G11-2E7</td>
<td>IgM</td>
</tr>
<tr>
<td>F9</td>
<td>400</td>
<td>80</td>
<td>78</td>
<td>2B3-2H10</td>
<td>IgG₁</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2B3-1H4</td>
<td>IgG₁</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2B3-IA6</td>
<td>IgG₁</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2B3-IA5</td>
<td>IgG₁</td>
</tr>
</tbody>
</table>

**Table 1. Yields of Antibody-Producing Clones in Fusions F5 and F9**

**Figure 1.** Left: Solid-phase immunoradiometric assay of antibody 2E7. Renin is bound to the plastic microtiter plate and then incubated with the monoclonal antibody solution. The ordinate represents binding of 1IgI goat antimouse Fab to IgM monoclonal antibody 1G11-2E7. The abscissa represents dilution of the antibody solution that had an initial concentration of 1.2 × 10⁻⁶ M. Right: Immunoradiometric assay of antibody 2B3-2H10, using same method. Abscissa is molar concentration of antibody.
ing activity. Figure 1 right, which examines the IgG₁ antibody, 2B3-2H10, from fusion 9, is particularly instructive. The antibody concentration was determined in this experiment, and it is remarkable that half-maximal binding to insolubilized antigen occurs at $8 \times 10^{-14}$ M antibody concentration, indicating considerable avidity for immobilized renin.

The antibodies appear to be specific for purified renin. Table 2 indicates that no significant binding occurred to pepsin, trypsin, hemoglobin, digoxin conjugated to hemocyanin, or to the mixture of proteins contained in either 10% horse serum or 20% fetal calf serum. Cross reactivity can be demonstrated with renins of other species, as can be seen in table 3. It is of considerable interest that antibody 2E7, while being fully specific for canine renin, recognized the renins of several other species equally well, including human renin. Apparently this is an antibody to an epitope common among mammalian renins. This epitope is not prominently recognized in conventional renin antisera since there is usually little antigenic cross activity between human and animal renins. As expected, another mouse monoclonal antibody specific for digoxin (our laboratory code: 26-10) did not bind renin in this assay.

Measurement of the binding of antibody to immobilized renin affords only a measurement of the ill-defined quantity, avidity. Since there is opportunity for both monovalent and bivalent binding to antigen, the half-maximal concentration for antibody binding is dependent in part on the density of antigen substitution on the solid support. Affinity is best determined utilizing soluble antigen. Since monoclonal antibodies recognize but a single determinant on the surface of a non-repeating antigen such as renin, affinity may be measured utilizing the same approach applied to antibody-hapete binding. In figure 2 the binding of IgG₁ antibody to immobilized renin is examined in the presence of varying concentrations of soluble renin. Half-maximal binding occurs at a concentration of $10^{-7}$ M renin, which corresponds to the dissociation constant of the antibody for the single epitope on renin that is bound.

### Table 2. Binding of Monoclonal Antibodies to a Variety of Antigens

<table>
<thead>
<tr>
<th>Monoclonal antibody</th>
<th>Horse serum (10%)</th>
<th>Fetal calf serum (20%)</th>
<th>Digoxin-hemocyanin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A5</td>
<td>257</td>
<td>1740</td>
<td>281</td>
</tr>
<tr>
<td>2H10</td>
<td>136</td>
<td>182</td>
<td>199</td>
</tr>
<tr>
<td>1A6</td>
<td>238</td>
<td>1402</td>
<td>264</td>
</tr>
<tr>
<td>1H4</td>
<td>225</td>
<td>265</td>
<td>250</td>
</tr>
<tr>
<td>Medium</td>
<td>121</td>
<td>1145</td>
<td>100</td>
</tr>
</tbody>
</table>

**Table 3. Cross Reactivity of Monoclonal Antibody 2E7 with Renins of Several Species**

<table>
<thead>
<tr>
<th>Species</th>
<th>Bound $^{125}$I goat antimouse Fab (CPM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog</td>
<td>2700</td>
</tr>
<tr>
<td>Human</td>
<td>2500</td>
</tr>
<tr>
<td>Hog</td>
<td>2600</td>
</tr>
<tr>
<td>Bovine</td>
<td>2000</td>
</tr>
<tr>
<td>Mouse</td>
<td>2300</td>
</tr>
<tr>
<td>Control</td>
<td>80</td>
</tr>
</tbody>
</table>

**Figure 2. Competitive radioimmunometric assay of antibody 2B3-1A6.** The assay is carried out as indicated in figure 1 except that varying concentrations of soluble renin (absissa) are added to the antibody solution prior to its application to immobilized renin.
Discussion

There are a number of significant research problems that could be attacked effectively if monoclonal antibodies specific for renin were available. Most obvious is the value of renin antibody or Fab fragments as specific physiological reagents to delineate the role of renin in the control of the normal or diseased circulation. While these studies can be carried out with conventionally derived antibodies, the difficulty in isolating renin limits the amount of antigen available, and thus, supplies of antibody are also severely limited. Since each antiserum is comprised of a different mixture of antibodies varying in both specificity and affinity, comparative experiments among different laboratories is difficult. We have noted an additional problem. The effectiveness of a mixed immunoglobulin population in inhibiting the enzymatic activity of renin is markedly reduced when papain cleavage to produce Fab is carried out. This may be explained by demonstrating that the IgG fraction of a renin-specific antiserum is comprised of antibodies that recognize not only the catalytic site of renin but also other epitopes that are remote from this site. In fact antibodies, being bivalent, precipitate renin and thus take it out of solution, regardless of whether the catalytic site or another part of the molecule is bound. On the other hand, Fab, which is monovalent, will only inactivate the enzyme if the catalytic site is bound. Since a minority of antibodies are catalytically site specific, the effectiveness of Fab from a mixture of antibodies is far less as has been observed.

Monoclonal antibodies solve all these problems. A small amount of antigen can yield an infinite amount of antibody because of the immortality of the cell culture that produces the antibody. The availability of large quantities of antibody will allow for comparative studies among different laboratories, but more importantly, the selection of catalytic site-specific monoclonal antibodies will solve the difficulty of producing ample quantities of Fab, a useful physiological reagent.

A major question in renin research relates to the identity of inactive renins found in various physiological fluids. Are these prorenins in a biosynthetic sense, or do they represent a post-synthetic modification? The mapping of epitopes on the surface of renin and prorenin with a variety of monoclonal antibodies should resolve this problem.

There has been considerable interest in the identification of renin-like proteins in tissues other than the kidney. Conflicting results have been reported with different antisera. Are these related to cross-reacting antibodies recognizing epitopes common to several proteins, or is renin really present in a number of tissues? Mapping the profile of renal renin with a set of monoclonal antibodies should allow identification of the cross-reacting species of other tissues. In this preliminary communication, we have described a set of monoclonal antibodies that appear to be specific for renin. They bind immobilized renin with seemingly different avidities, but do not cross react with the other antibodies tested. A monoclonal antibody specific for digoxin does not bind renin in the assay used. One of the monoclonal antibodies recognizes an unusual epitope that is common to the renins of a number of species, including man. None of the antibodies inhibits the enzymatic activity of renin. This may be a product of the unique specificity characteristic of monoclonal antibodies in that they are directed against a single epitope on the surface of a molecule. The monoclonal antibodies examined thus far may not recognize renin's catalytic site or a region sufficiently near it to hinder access of substrate. An alternative possibility is that the antibodies are not specific for native renin but recognize a denatured form of the enzyme. An epitope on denatured renin may not be shared by the native enzyme. A remote explanation, though unlikely in view of the number of monoclonal antibodies examined, is that the antibody is directed to a contaminating protein present in a quantity too small to be detected by conventional analytical procedures and not to renin itself. The interest and excitement of this line of investigation will be found in future studies that further characterize these as well as other renin-specific hybridoma proteins.

References

Monoclonal antibodies binding renal renin.
V Dzau, M Mudgett-Hunter, G Kapler and E Haber

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