Demonstration of a main immunogenic region on acetylcholine receptors from human muscle using monoclonal antibodies to human receptor

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Eleven cloned hybridomas which secrete antibodies to acetylcholine receptors from human muscle have been prepared. All of these monoclonal antibodies to have the same basic specificity as shown by competition for binding to the main immunogenic region on the receptor, but these antibodies differ in fine specificity as shown by reaction with denatured receptor subunits and interspecies cross-reaction.

Acetylcholine receptor *Human muscle* *Monoclonal antibodies* *Antibody specificity* *Antibody idiotype* *Myasthenia gravis*

1. INTRODUCTION

Monoclonal antibodies (mAbs) to acetylcholine receptors (AChRs) from human skeletal muscles should prove interesting because these AChRs are the object of an antibody-mediated autoimmune response which causes the muscular weakness and fatigability characteristic of myasthenia gravis (MG) (reviewed in [1]). In addition, they might prove valuable for comparing the structure of human AChR with the better characterized structure of AChR from the electric organ of *Torpedo*.

Studies of the antigenic structure of AChRs from the electric organs of *Torpedo californica* [2] and *Electrophorus electricus* [3] using mAbs have revealed that a majority of the antibodies made by rats to intact AChRs from these species are directed at the main immunogenic region (MIR). This is demonstrated by the ability of a single mAb bound to the MIR to prevent the binding of a large fraction of serum anti-AChR antibodies to AChR. AChRs have the subunit structure $\alpha_2\beta\gamma\delta$ (reviewed in [4]). The MIRs are distinct from the acetylcholine binding sites, but are also located on the extracellular surface of α subunits [5]. The MIR is relatively conformation dependent [2, 3] and relatively trypsin resistant [6]. mAbs to the MIR can crosslink AChRs and cause AChR loss from cells by antigenic modulation [7]. mAbs to the MIR can also passively transfer the complement-dependent acute form of experimental autoimmune myasthenia gravis [2]. mAbs to the MIR can inhibit the binding of a majority of the anti-AChR antibodies in MG patients, while mAbs specific for the β- and γ-subunits of AChRs can only inhibit the binding of a smaller fraction [8]. Antisera from rats immunized with human AChR in competition experiments also appear to be preferentially directed at the MIR [8].

Here, we report the preparation of 11 mAbs from rats immunized with human AChR, all of which are directed at the MIR.

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Abbreviations: AChR acetylcholine receptor; $^{125}I\alpha BGT$ ^{125}I-labeled α-bungarotoxin; mAb monoclonal antibody; MIR, main immunogenic region; SDS, sodium dodecyl sulfate

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2. EXPERIMENTAL

AChRs were purified by affinity chromatography on cobra toxin–agarose [9]. Hybridomas secreting mAbs to human AChRs were prepared in the same way as we prepared hybridomas to other AChRs in [2, 3]. Lewis rats were injected with 2 μg AChR emulsified in 200 μl complete Freund's adjuvant on day 0, 1 μg in adjuvant on day 32, and sacrificed on day 36. Spleen cells (1×10^8) were fused with non-secreting mouse myeloma cells (3×10^7 of S194/5.XXO.BD.1) using 50% polyethylene glycol 1500 by the basic technique in [10]. Culture supernatants were screened by radioimmunoassay using ^{125}IαBGT labeled AChR as antigen [9].. Immunoglobulin fractions of the media from cloned hybrids were isolated and concentratecd via ammonium sulfate precipitation.

Immunoglobulin subclasses were determined by radial immunodiffusion using typing antisera from Miles. Binding of mAbs to *S. aureus* (Pansorbin from Calbiochem-Behring) was tested by radioimmunoassay [2,3].

Binding to the MIR was determined by competition with mAb 35 (a rat anti-MIR mAb raised against AChRs from *Electrophorus electricus*) [3]. Binding to ^{125}I-labeled AChR subunits dissociated with SDS was measured by immune precipitation, electrophoresis, and autoradiography as in [2, 3]. Antibody titers against human, bovine and *Torpedo* AChRs were measured by immune precipitation using ^{125}IαGBT-labeled AChRs as antigen and titers were expressed in moles of ^{125}IαBGT binding sites bound per liter [9].

3. RESULTS AND DISCUSSION

Eleven stable hybridoma lines producing mAbs to human skeletal muscle AChR were made by fusing spleen cells of rats immunized with purified AChR with mouse myeloma cells.

The properties of the constant regions of these mAbs were studied (table 1). Both IgM and several subclasses of IgG antibodies were obtained. As expected of rat immunoglobulins, many did not bind well to fixed *S. aureus* cells.

More importantly, the properties of the variable regions of these mAbs were also studied (table 1). By competitive binding studies with mAb 35, all of the mAbs to human AChR were directed at the MIR. All of the mAbs were also mutually competitive for binding to human AChR. One of the

Table 1

Properties of rat mAbs to human muscle AChR

mAb[a]	Constant region properties		Variable region properties		
	Immunoglobulin class	Binding to *Staph. aureus*	Binding site specificity	Species specificity % crossreaction[b]	
				Bovine AChR	Torpedo AChR
189	?	–	MIR	100	0
190	IgG2b	–	MIR	37	0
192	IgG2b	–	MIR	68	0
195	IgG1	+	MIR, α	98	0
196	IgM	+	MIR	0	0
197	IgM	–	MIR	0	0
198	IgG2a	–	MIR, α	100	100
202	IgG1	+	MIR, α	93	0
203	IgG2a	+	MIR, α	100	100
204	?	+	MIR, α	100	0
207	IgG2a	–	MIR, α	100	0

[a]mAb 189 reacted with none of the isotyping sera used, while mAb 204 reacted with all of the isotyping sera used

[b]Percent crossreaction is calculated by dividing the titer against the antigen (in moles of ^{125}I-αBGT binding sites of AChR bound/liter of antibody solution) by the titer against human AChR

mAbs (198) was tested on sucrose gradients with ^{125}IαGBT-labeled bovine AChR. As in the case of mAbs to the MIR on AChR from *Torpedo* and *Electrophorus* [7], mAb 198 could crosslink AChR monomers and bind 2 mAbs/AChR in mAb excess (not shown). Six of these mAbs crossreacted with ^{125}I-labeled, SDS denatured α subunits of AChR from bovine muscle. Two of these mAbs (198 and 203) also crossreacted with ^{125}I-labeled, SDS-denatured α-subunits of AChR from *Torpedo* electric organ. These results are consistent with those obtained with other anti-MIR mAbs in showing that the MIR is a conformation-dependent antigenic determination, but that some crossreaction at lower affinity is retained with denatured α-subunits.

Even though it was expected that immunization with nondenatured AChR should produce a disproportionate response to the MIR, it is striking that all of the mAbs are of this specificity. One contributing factor is probably that the amputated human leg muscle is subject to autolysis and the AChR purified from human leg muscle lacked intact β, γ and δ subunits. Because the MIR is relatively resistent to proteolysis [6], by contrast with most other antigenic determinants on the AChR, the effect of this proteolysis is probably to destroy many other potentially immunogenic sites.

Most of these mAbs (9/11) crossreact very well with bovine AChR, while only two crossreact well with *Torpedo* AChR (table 1). These results are consistent with the interspecies crossreactivity of anti-AChR sera and reflect the high crossreactivity of other anti-MIR mAbs, apparently due to the conserved structure of this antigenic determinant [2, 3, 8]. The observation that all of these mAbs are mutually competitive for binding to the same structure on AChR, yet that some are highly species specific (e.g., 196 and 197) while others are not (e.g., 198 and 203), probably reflects the range of different anti-body idiotypes capable of binding to the MIR.

These mAbs should prove valuable in further studies of MG and of the AChR molecule. For example, mAb 203 and other anti-MIR mAbs raised against AChRs from *Torpedo, Electrophorus*, and cattle are capable of specifically labeling neurons in the lateral spiriform nucleus of chicken brain [11].

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