Brief Definitive Report

MULTIPLE ORGAN-REACTIVE IgG ANTIBODY INDUCED BY AN ANTIIDIOTYPIC ANTIBODY TO A HUMAN MONOCLONAL IgM AUTOANTIBODY

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We have prepared a number of human monoclonal autoantibodies from patients with autoimmune disorders and from normal individuals (1, 2). Many of these monoclonal autoantibodies were found to react with antigens in multiple organs. One such multiple organ–reactive (MOR) antibody, designated MOR-h1, has been studied extensively. MOR-h1 is an IgM mAb that reacts with antigens in normal human anterior pituitary, thyroid, stomach, and pancreas (1). Studies on the nature of the antigens showed that MOR-h1 recognizes a 35 kD protein in the pituitary, thyroid, stomach and pancreas and growth hormone (hGH) in the pituitary (3). Recently, we prepared a mouse monoclonal anti–idiotypic antibody designated 4E6 and showed that it reacted with the paratope of MOR-h1 (4). Based on the network hypothesis (5), if the 4E6 paratope is complementary to the MOR-h1 paratope, then the 4E6 paratope might have a conformation similar to epitopes on hGH and the 35 kD protein. The current studies were initiated to see whether anti–idiotypic antibodies raised against 4E6 would have autoantigen-binding specificities similar to that of MOR-h1.

Materials and Methods

Immunoreagents and Preparation of Anti-Anti-Idiotypic Ig. Purified human growth hormone (hGH) was generously donated by Genentech, Inc., San Francisco, CA. The 35 kD protein from human stomach was purified as described earlier (3). All other reagents were commercially obtained (4). Preparation and affinity purification of human monoclonal autoantibody MOR-h1 and murine monoclonal anti–idiotypic antibodies (4E6, 3E5, and 3F6) that react specifically with MOR-h1 were described earlier (1, 4).

Anti-anti-idiotypic Ig (anti-4E6) was prepared by immunizing (5 ×) a rabbit with affinity-purified 4E6. Serum from the immunized rabbit was passed through a protein A–Sepharose 4B affinity column. The Ig bound to protein A was eluted and passed through a 4E6-affinity column. The eluate from this column was then passed sequentially through 356-1-affinity column (to remove Ig class–specific antibodies), and 183-4- and MOPC-21-affinity columns (to remove anti-mouse (IgG_1/κ) subclass–specific antibodies). The entire purification procedure was done twice. Ig from preimmune rabbit serum was affinity-purified on a protein A–Sepharose 4B column.

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TABLE I

Specificity of the Reaction of Anti-4E6 with 4E6

Wells coated with mouse mAbs	Ig class‡	Counts bound*		
		^{125}I-labeled anti-rabbit Ig§		^{125}I Anti-mouse Ig¶
		Anti-4E6	Preimmune Ig‖	
4E6	IgG_1 (k)	7,280 ± 415	91 ± 61	5,657 ± 316
3E5	IgG_1 (k)	274 ± 17	69 ± 73	5,726 ± 412
3F6	IgG_1 (k)	300 ± 70	151 ± 10	6,169 ± 756
183-4	IgG_1 (k)	218 ± 32	174 ± 5	5,517 ± 243
MOPC-21	IgG_1 (k)	187 ± 21	164 ± 12	5,558 ± 151
356-1	IgG_{2a} (k)	276 ± 221	187 ± 34	5,754 ± 229
204-4	IgG_{2a} (k)	292 ± 226	161 ± 5	5,714 ± 443

* cpm ± SD bound to wells.

‡ Determined by Ouchterloney technique.

§ Anti-4E6 1 mg/ml, diluted 1:25, was added to wells that had been coated with the different mouse mAbs. The amount of rabbit IgG that bound was detected by incubation with ^{125}I-labeled goat anti-rabbit IgG.

‖ Preimmune rabbit Ig.

¶ As a control, ^{125}I-goat anti-mouse IgG was added to ensure that all wells were coated with approximately equal quantities of mouse Ig.

Solid-Phase RIA. Microtiter plates were coated with antigen or antibody (1 μg/well) essentially as described previously (4). Samples were tested in triplicate and the binding of ^{125}I-labeled Ig was determined.

Immunofluorescence. The antibodies were tested for tissue reactivity by indirect immunofluorescence (1, 3).

Results

The specificity of affinity-purified anti-4E6 is shown in Table I. Anti-4E6 reacted with 4E6, but not with other mouse mAbs belonging to the same isotype and subclass.

To see whether anti-4E6 reacted with the same epitope on 4E6 with which MOR-h1 reacted, various concentrations of MOR-h1 were mixed with a constant amount of anti-4E6 and added to 4E6-coated wells. As seen in Fig. 1 *A*, MOR-h1, and not pooled human IgM, inhibited the binding of anti-4E6 to 4E6. The data suggest that anti-4E6 and MOR-h1 interact with the same epitope on 4E6.

To determine if the antigen-binding specificity of anti-4E6 was similar to that of MOR-h1, the binding of these antibodies to wells coated with hGH, 35 kD protein, or gelatin was tested. Table II shows that both anti-4E6 and MOR-h1 reacted with hGH and 35 kD protein, but not with gelatin.

To test whether MOR-h1 and anti-4E6 were reacting with the same or different epitopes on the hGH molecule, various concentrations of MOR-h1 were added, along with a constant amount of anti-4E6, to hGH-coated wells. Fig. 1 *B* shows that MOR-h1 competed with anti-4E6 for binding to hGH. This argues that MOR-h1 and anti-4E6 are recognizing the same epitope or epitopes in close proximity to each other on hGH.

To see whether the binding of anti-4E6 to hGH could be inhibited by 4E6, various concentrations of 4E6 were mixed with a constant amount of anti-4E6 and added to hGH-coated wells. Fig. 1 *C* shows that the binding of anti-4E6 to hGH was inhibited by 4E6 and not by MOPC-21. Taken together, these experiments strongly suggest that both 4E6 and hGH are reacting with the paratope of anti-4E6.

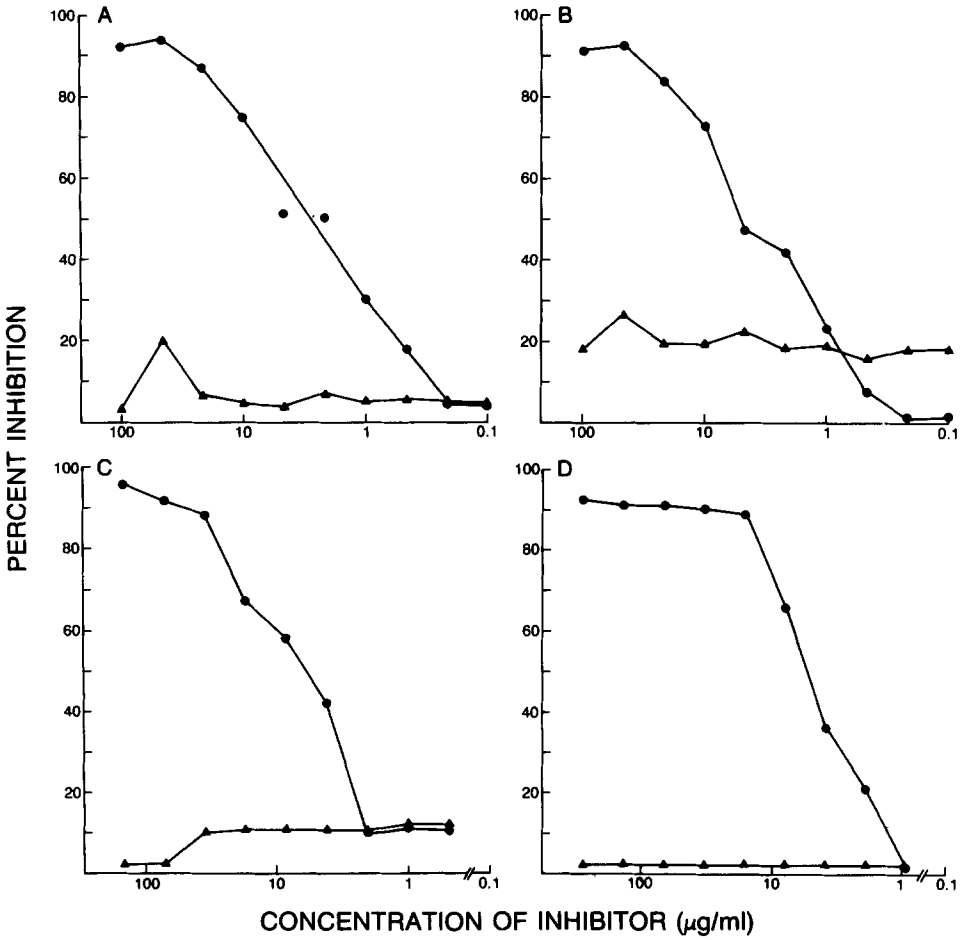

FIGURE 1. Competitive inhibition experiments (*A*) Inhibition of binding of anti-4E6 to 4E6 by MOR-h1. Serial twofold dilutions of either MOR-h1 (●) or pooled human IgM (▲) were mixed with an equal volume of anti-4E6 (10 μg/ml) and added to 4E6-coated wells. Bound anti-4E6 was quantitated using ^{125}I-labeled anti-rabbit IgG. Maximum binding was ~3400 cpm. (*B*) Inhibition of binding of anti-4E6 to hGH by MOR-h1. Serial twofold dilutions of either MOR-h1 (●) or pooled human IgM (▲) were mixed with an equal volume of anti-4E6 (10 μg/ml) and added to hGH-coated wells. Bound anti-4E6 was quantitated as described above. Maximum binding was ~4300 cpm. (*C*) Inhibition of binding of anti-4E6 to hGH by 4E6. Serial twofold dilution of either 4E6 (●) or MOPC-21 (▲) were mixed with an equal volume of anti-4E6 (10 μg/ml) and added to hGH-coated wells. Bound anti-4E6 was quantitated as described above. Maximum binding was ~3800 cpm. (*D*) Inhibition of binding of anti-4E6 to 4E6 by hGH. Serial twofold dilution of either hGH (●) or gelatin (▲) were mixed with an equal volume of anti-4E6 (10 μg/ml) and added to 4E6-coated wells. Bound anti-4E6 was quantitated as described above. Maximum binding was ~4800 cpm.

To determine what proportion of the Ig molecules in our anti-4E6 preparation were capable of binding to hGH, different concentrations of hGH were incubated with a constant amount of anti-4E6 and then added to 4E6-coated wells. Fig. 1 *D* shows that the binding of anti-4E6 to 4E6 was inhibited by hGH but not by gelatin. Moreover, when anti-4E6 was absorbed with hGH, >90% of its reactivity with the tissues was eliminated (not shown). Taken together, these studies

TABLE II

Reactivity of Anti-4E6 with hGH and 35kD Protein

First antibody	Second antibody‡	Wells coated with:*		
		hGH	35 kD protein	Gelatin
Anti-4E6	Anti-rabbit IgG	2,538 ± 371§	3,812 ± 350	96 ± 9
MOR-h1	Anti-human IgM	2,065 ± 139	2,842 ± 100	117 ± 42
NR serum‖	Anti-rabbit IgG	168 ± 25	154 ± 21	179 ± 16
Anti-hGH	Anti-rabbit IgG	6,417 ± 931	84 ± 3	77 ± 11
None	Anti-rabbit IgG	96 ± 14	97 ± 4	106 ± 11
None	Anti-human IgM	99 ± 8	98 ± 8	92 ± 13

* Wells were coated with 0.5 μg/well of either hGH, 35kD protein, or gelatin and then incubated with the first antibody.

‡ The binding of anti-4E6, normal rabbit serum and anti-hGH antibody was quantitated with ^{125}I-labeled anti-rabbit IgG, and the binding of MOR-h1 was quantitated with ^{125}I-labeled anti-human IgM.

§ cpm ± SD bound to wells.

‖ Preimmune normal rabbit serum.

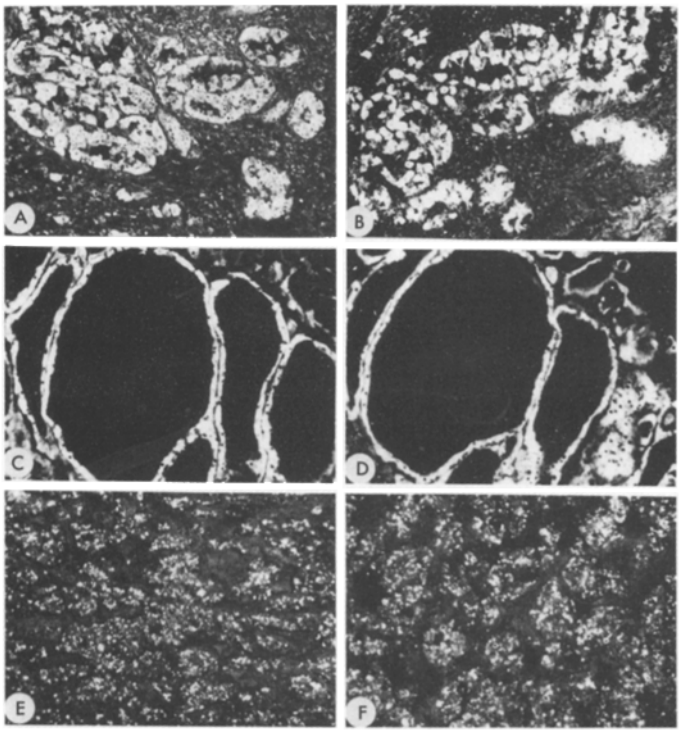

FIGURE 2. Indirect Immunofluorescence. Reactivity of MOR-h1 (*A*, *C*, and *E*) and anti-4E6 (*B*, *D*, and *F*) with stomach (*A* and *B*), thyroid (*C* and *D*), and anterior pituitary (*E* and *F*). Original magnification × 100.

strongly argue that the binding specificity for both 4E6 and hGH reside in the same Ig molecules of the anti-4E6 preparation and that the majority of them are of the internal image type.

To see whether anti-4E6 also reacts with multiple organs, normal tissues were stained with MOR-h1 and anti-4E6. As seen in Fig. 2, both MOR-h1 and anti-4E6 reacted with stomach (Fig. 2, *A* and *B*), thyroid (*C* and *D*), and anterior

pituitary (Fig. 2, *E* and *F*). By double immunofluorescence (3), the same cells were stained by both antibodies (not shown). Thus, anti-4E6 is an IgG multiple organ–reactive antibody with a specificity similar to that of MOR-h1.

Discussion

Previously, we reported the production of human IgM monoclonal autoantibodies (Ab1) in vitro that reacted with multiple organs (1, 2). The present experiments show that animals immunized with a monoclonal anti–idiotypic antibody (Ab2) to an Ab1 can make IgG antibodies that have MOR activity. Thus, MOR antibodies are not an artifact of in vitro procedures, nor are they limited to the IgM class. Moreover, these experiments suggest that it is possible to raise autoantibodies without immunizing the host with autoantigens.

Studies from several laboratories have shown that Ab2 made to paratopes of Ab1 that react with certain ligands (e.g., insulin and Bis Q) can bind to corresponding receptors for these ligands and, in some cases, can trigger an experimental autoimmune disease (6, 7). It is thought that the paratope of these Ab2s are complementary to the paratope of Ab1. Several groups have also made anti-anti-idiotypic antibodies (Ab3). These latter antibodies have shown several different types of reactivity (5, 8). Some are directed against framework determinants on Ab2 and do not show Ab1 like activity; others react with the paratope of Ab2 but are not internal images and fail to show Ab1-like antigen binding activity; and still others react with the paratope of Ab2 and show Ab1-like antigen-binding activity. Our Ab3 belongs to this latter category. The 4E6 paratope (Ab2) appears to have a conformational resemblance to an epitope on hGH and the 35 kD protein and thus can elicit an antibody (Ab3) that reacts with these ligands.

Since some Ab2s have conformational similarities to the ligands with which Ab1 react, they have been used with success to elicit antibodies against viruses, trypanosomes, and histocompatibility antigens (9). The present work and the recent report by Zanetti et al. (10) show that anti–idiotypic antibodies can also induce autoantibodies. This raises the possibility that stimulation of autoreactive B lymphocytes present in the normal B cell repertoire (11) by Ab2 may be one of the mechanisms for triggering autoimmunity. In fact, some anti–idiotypic antibodies are known to have public specificity (4, 8), and several Ig families bearing specific idiotypes have been implicated in certain autoimmune diseases (12). Along these lines, it is interesting to speculate whether stimulation of a family of B lymphocytes by broadly reactive anti–idiotypic antibodies could result in the production of a spectrum of autoantibodies with different organ specificities as in systemic lupus erythematosus or polyendocrine disease. If this turns out to be the case, then it is conceivable that clonal proliferation of a single anti–idiotypic antibody could trigger an autoimmune cascade (2).

Summary

MOR-h1 is a human multiple organ–reactive (MOR) monoclonal autoantibody (Ab1) that reacts with human growth hormone (hGH) and a 35 kD protein found in the anterior pituitary, thyroid, stomach, and pancreas. 4E6 is a mouse mono-

clonal anti-idiotypic antibody (Ab2) that reacts with the paratope of MOR-h and is ligand inhibitable. In the present study, we immunized a rabbit with 4E and purified an IgG fraction (anti-4E6) from the sera. Competitive inhibitio experiments showed that anti-4E6 (Ab3) binds to the same epitope on 4E6 an to the same antigens (i.e., hGH and 35 kD protein) as does MOR-h1. B immunofluorescence, anti-4E6, an IgG antibody, shows the same multiple orga reactivity with tissues as does MOR-h1, an IgM antibody. From these and othe studies, we conclude that the 4E6 paratope (Ab2) has a conformational resem blance to an epitope on hGH and the 35 kD protein. This raises the possibilit that antibodies made in response to certain anti-idiotypic antibodies may be on of the mechanisms for triggering an autoimmune response.

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