Immunology 1983 **49** 9

Fine specificity and idiotypic expression of monoclonal antibodies directed against poly(Tyr,Glu)-poly(DLAla)--poly(Lys) and its ordered analogue (Tyr-Tyr-Glu-Glu)-poly(DLAla)--poly(Lys)

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Accepted for publication 8 October 1982

Summary. In order to study the repertoire of poly (Tyr,Glu)-poly(DLAla)--poly(Lys) [(T,G)-A--L] specific antibodies, monoclonal antibodies were prepared by fusing myeloma cells with spleen cells from C3H.SW mice immunized with (T,G)-A--L and boosted with (Tyr-Tyr-Glu-Glu)-poly(DLAla)--poly (Lys)[(T-T-G-G)-A--L]. Eleven clones which secreted homogeneous antibodies were obtained. In general, two families of monoclonal antibodies were detected: those which bind exclusively (T-T-G-G)-A--L and those which bind both (T-T-G-G)-A--L and (T,G)-A--L. Analysis for idiotypic expression revealed that only two antibodies (clones no. 103 and 160), which were found to be similar in their fine specificity, cross-reacted with antibodies against the major idiotypes of (T,G)A--L specific antibodies. Guinea-pig antibodies against clone no. 160 reacted with the polyclonal (T,G)-A--L specific antibodies, whereas antibodies against 103 monoclonal antibodies did not react with C3H.SW anti-(T,G)-A--L antibodies, but did cross-react with four other monoclonal antibodies. It appears that the idiotypic determinants expressed on polyclonal (T,G)-A--L specific antibodies are heterogeneous, and consist of at least two serologically different idiotypes detected by clones no. 103 and 160.

Abbreviations: (T,G)-A--L, poly(LTyr,LGlu)-poly(DLAla)--poly(LLys); (T-T-G-G)-A--L, (LTyr-LTyr-LGlu-LGlu)-poly-(DLAla)--poly(LLys); (T-G-T-G)-A--L, (LTyr-LGlu-LTyr-LGlu)-poly(DLAla)--poly(LLys); (H,G)-A--L, poly(LHis, LGlu)-poly(DLAla)--poly(LLys); A--L, poly(DLAla)--poly-(LLys); Pro--L, poly(LPro)--poly(LLys), (G)-A--L, poly(L-Glu)-poly(DLAla)--poly(LLys); (T,G)-Pro--L, poly(LTyr,L-Glu)-poly(LPro)--poly(LLys); (T-T-G-G)-Pro--L, (LTyr-LTyr-LGlu-LGlu)-poly(LPro)--poly(LLys); (T-G-T-G)-Pro--L, (LTyr-LGlu-LTyr-LGlu)-poly(LPro)--poly-(LLys); Ig, immunoglobulins; Id-idiotypes or idiotypic determinants; GP, guinea-pig; IEF, isoelectric focusing; GaMFab, goat anti-mouse Fab anti-serum; GPaId, guinea-pig anti-idiotypic serum; GPaMIg, guinea-pig anti-mouse immunoglobulins; c.p.m., counts per minute.

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0019-2805/83/0500-0009$02.00

INTRODUCTION

A vast amount of information has been accumulated from various animal species and by different experimental approaches on the antigenic properties of the synthetic polypeptide antigen poly(Tyr,Glu)-poly (DLAla)--poly(Lys) designated (T,G)-A--L (McDevitt & Sela, 1965; Mozes & Shearer, 1972; Mozes, 1975). The ability to mount an antibody response to (T,G)-A--L is regulated by gene(s) that are linked to the major histocompatibility complex of the species (McDevitt & Tyan, 1968; McDevitt & Chinitz, 1969). Studies performed with ordered tetrapeptides of tyro-

sine and glutamic acid attached to multichain poly-DL-alanine--poly-L-lysine have indicated that the Tyr-Tyr-Glu-Glu sequence is the major determinant in the random (T,G)-A--L. Immunization with the ordered synthetic polypeptide (Tyr-Tyr-Glu-Glu)-poly(DLAla)--poly(Lys), [(T-T-G-G)-A--L] resulted in the same pattern of responses in the different mouse strains as observed after immunization with the random (T,G)-A--L (Mozes, Schwartz & Sela, 1974; Schwartz, Mozes & Sela, 1975).

The antibody responses of inbred strains of mice to the synthetic antigen (T,G)-A--L are characterized by the presence of major idiotypic determinants (Schwartz, Lifshitz, Givol, Mozes & Haimovich, 1978; Lifshitz, Schwartz and Mozes, 1978). These idiotypes were determined by the interaction between guinea-pig anti-idiotypic antiserum made against specifically purified C3H.SW (Igh-1^j, H-2^b) anti-(T,G)-A--L antibodies. The expression of the major idiotypes on (T,G)-A--L specific antibodies in the different inbred strains of mice was shown to be linked to heavy chain allotypes (Lifshitz, Schwartz & Mozes, 1980). In addition, results obtained with (T,G)-A--L specific sera of C58/J mice suggested that the V_L is also required for the expression of (T,G)-A--L site-related idiotypic determinants of C3H.SW mice (Lifshitz *et al.*, 1980).

In the previous studies the antibody response to (T,G)-A--L was characterized using polyclonal antibodies. In order to get further insight into the fine specificity, repertoire and the idiotypes that compose the major idiotypic determinants of (T,G)-A--L specific antibodies, it was essential to dissect the heterogeneous immune response and to analyse monoclonal anti-(T,G)-A--L antibodies.

In the present study, hybridomas which secrete antibodies specific to (T,G)-A--L or its ordered analogue (T-T-G-G)-A--L have been established by fusing myeloma cells with spleen cells from C3H.SW mice primed with (T,G)-A--L and challenged with (T-T-G-G)-A--L. The monoclonal antibodies obtained could be divided into four groups according to their reactivity with different synthetic polypeptides related to (T,G)-A--L. Two of the hydrid clones secreted antibodies that express idiotypes which cross react with the major anti-(T,G)-A--L idiotypes.

MATERIALS AND METHODS

Animals and cell lines

C3H.SW and (C57BL/6 × BALB/c)F1 were used for immunization with antigen and production of ascites, respectively. Random bred DH albino guinea-pigs were used for production of anti-idiotypic antisera. The mice and guinea-pigs were obtained from the Experimental Animal Unit, The Weizmann Institute of Science. The P3-NS1/1-Ag4-1 (NS1) myeloma was obtained from Dr C. Milstein, MRC Cambridge, and the P3-X63.653 (X63) myeloma line from Dr G. Hammerling, DKFZ, Heidelberg.

Antigens

The antigens used in this study are poly(LTyr,LGlu)-poly(DLAla)--poly(LLys) designated (T,G)-A--L, (LTyr-LTyr-LGlu-LGlu)-poly(DLAla)--poly(LLys) [(T-T-G-G)-A--L], (LTyr-LGlu-LTyr-LGlu)-poly (DLAla--poly LLys) [(T-G-T-G)-A--L], poly(LHis,LGlu)-poly(DLAla)--poly(LLys) [(H,G)-A--L], poly(DLAla)--poly(LLys) [(A--L)], poly(LPro)--poly(LLys) [(Pro--L)], poly(LGlu)-poly(DLAla)--poly(LLys) [(G)-A--L], poly(LTyr,LGlu)-poly(LPro)--poly(LLys) [(T,G)-Pro--L], (LTyr-LTyr-LGlu-LGlu)-poly(LPro)--poly(LLys) [(T-T-G-G)-Pro--L] and (LTyr-LGlu-LTyr-LGlu)-poly(LPro)--poly(LLys) [(T-G-T-G)-Pro--L]. The synthesis and characteristics of these antigens have been previously described (Sela, Fuchs & Arnon, 1962; Fuchs & Sela, 1964; Jaton & Sela, 1968; Mozes *et al.*, 1974).

Cell hybridization and cloning

Splenic lymphocytes from immunized mice were fused with either NS1 or X63-plasmacytoma cells by using 41% polyethylene glycol (Serva, 1550) as described (Eshhar, Ofarim & Waks, 1980). Fifty million cells were fused with 10×10^6 myeloma cells. After fusion, cells were suspended in Dulbecco's modified Eagle's medium (DMEM) (high-glucose, Gibco, N.Y.) supplemented with glutamine (2 mM), pyruvate (1 mM), streptomycin (100 μg/ml), and penicillin (100 units/ml) and 15% horse serum. One millilitre aliquots containing 5×10^4 NS1 cells were dispensed into Costar tissue culture plates (Cluster^{24}, Costar no. 3524). After 24 hr, the culture supernatants were replaced by selective medium containing hypoxanthine aminopterin, and thymidine (HAT). Hybrid cells that secreted antibodies into the culture supernatants were cloned by either limiting dilutions in 96-well microtitre plates or on soft agar (Pluznik & Sachs, 1965). Hybrid clones were cultured at 37° in a humid atmosphere of 8% CO_2 in air.

Growth of hybridomas in mice

Cultured hybridoma cells ($5–10 \times 10^6$) were injected intraperitoneally (i.p.) into (C57BL/6 × BALB/c)F1 adult mice that had been treated 7–30 days previously with an i.p. injection of 2,6,10,14-tetramethylpentadecane (prystane). Ascitic fluid was harvested usually 10–20 days after the hybridoma cells were inoculated.

Purification of antibodies

Antibodies were purified by passing either supernatants or ascitic fluids through a column of (T-T-G-G)-A--L coupled to Sepharose 4B (Pharmacia, Uppsala). Elution of antibodies was performed with 0·1 M ammonia.

Production of guinea-pig anti-idiotypic serum to monoclonal antibodies

Guinea-pigs were immunized by multiple-site intradermal injections of 100 μg of the purified monoclonal antibodies (idiotypes) in Freund's complete adjuvant. Booster injections, with the same dose and route were given twice at 3-week intervals. The guinea-pigs were bled and the antisera were pooled and passed through an immunoabsorbent of C3H.SW normal mouse immunoglobulins in order to remove guinea-pig anti-MIG activity.

Radioiodination

Proteins were radiolabelled with carrier-free Na[^{125}I] by the chloramine-T method (Hunter, 1970).

Solid phase radioimmunoassay of supernatants and ascitic fluids

The solid phase radioimmunoassay was performed as previously described (Eshhar, Strassmann, Waks & Mozes, 1979). Briefly, plastic microtitre plates were coated with solutions of the different antigens (50 μg/ml, phosphate-buffered saline, 100 μl per well). The antibodies were added for 2 hr followed by washing and addition of ^{125}I-labelled purified goat anti-mouse Fab antibodies (GaMFab) (1×10^5 c.p.m. of < 1 μCi/μg). After extensive washings, activity of each well was measured in a gamma spectrometer.

Binding of ^{125}I-(T-T-G-G)-A--L to monoclonal antibodies

Iodinated antigens (2–10 Ci/g) were reacted with purified monoclonal antibodies at different concentrations. All the experiments were performed in 5% normal mouse serum. Precipitates were obtained after addition of goat anti-mouse Fab serum.

Inhibition experiments

Inhibition of binding ^{125}I-(T-T-G-G)-A--L to antibodies by the different synthetic polypeptides or anti-idiotypic serum was performed with the amount of purified monoclonal antibodies which corresponded to the binding of 15–35% of the iodinated antigen. The monoclonal antibodies were incubated with different doses of inhibitors for 30 min at room temperature before addition of the radiolabelled antigen.

Isoelectric focusing (IEF) of monoclonal antibodies

IEF of anti-(T,G)-A--L or anti-(T-T-G-G)-A--L ascites was performed in 5% polyacrylamide gels as described by Awdeh, Williamson & Askonas (1968). Electrophoresis was done for 18–20 hr at 4° at 400 V. After measuring the pH gradient, gels were immersed in 18% sodium sulphate to precipitate the immunoglobulins and fixed in 0·2% glutaraldehyde. The focused gels were exposed to ^{125}I-(T-T-G-G)-A--L for 24–48 hr at 4°. After extensive wash with 5% foetal calf serum to remove the unbound ^{125}I-(T-T-G-G)-A--L, the gels were either developed on X-ray films or were cut out of the gel into slices (2 mm), and their radioactivity was measured.

Determination of heavy chain isotypes

The heavy chain class of the purified antibodies was determined in Ouchterlony plates using class-specific and subclass-specific antisera (Meloy Inc. Laboratories, Springfield, Virginia 22151).

RESULTS

Characterization of the monoclonal antibodies

Hybrid cell lines secreting antibodies were generated in two fusion experiments. In the first fusion, spleen cells from C3H.SW (H-2^b) mice primed to (T,G)-A--L and boosted with (T-T-G-G)-A--L were fused with the x63.653 non-Ig producing myeloma cells. In the second fusion, similarly primed spleen cells were fused with the Ig-producing non-secreting myeloma cells NS1. A variety of hybrid lines which secreted homogenous antibodies were isolated and cloned. The binding characteristic of these monoclonal antibodies and their heavy chain isotypes are shown in Table 1. As can be seen in this table, two families of monoclonal antibodies could be detected: those which bind exclusively (T-T-G-G)-A--L and those which bind both (T-T-G-G)-A--L and (T,G)-A--L. The monoc-

Table 1. Binding activity of monoclonal antibodies and their isotypes

Culture supernatant of hybrid clone*	Binding of ^{125}I-GaMFab (c.p.m.) to: †		Immunoglobulin class‡
	(T-T-G-G)-A--L	(T,G)-A--L	
X63–160	12,702	10,611	IgG3
X63–247	7379	7800	IgM
NS1–32	13,432	0	IgG_1,IgM§
50	14,553	13,732	IgG_2
100	16,356	0	IgG_1
101	11,637	0	$IgG_2{}^b$
103	21,370	19,398	IgG_2b
114	12,120	196	IgG_1
115	10,098	123	IgG_3
131	11,486	9617	IgG_3
139	9968	141	IgM

* Hybrid clones designated according to the myeloma parental cells and position of the original well in the culture plate.

† The binding activity of the various monoclonal antibodies was determined by solid phase radioimmunoassay using undiluted culture supernatant.

‡ Isotypes were determined by the double immunodiffusion technique.

§ Uncloned.

lonal antibodies express various heavy chain isotypes, such as IgM, IgG_1, IgG_2 and IgG_3.

The fine specificity of these monoclonal antibodies was further characterized by determining their direct binding to different synthetic antigens. As shown in Table 2, the specificity of the monoclonal antibodies can be divided into four groups. Hybrid clones no. 103 and 160 (group I) are producing antibodies which bind (T,G)-A--L, (T-T-G-G)-A--L and (T-G-T-G)-A--L and cross-react with (Phe,G)-A--L, (H,G)-A--L, (G)-A--L and A--L. The second group consists of antibodies which differ from the first group since they do not bind A--L. Antibodies in the third group bind (T-T-G-G)-A--L and clones no. 32, 100, 114 and 139

Table 2. Specificity of monoclonal antibodies determined by direct binding

Antigen	I (103, 160)	II (50, 131)	III (32, 100, 101 114, 115, 139)	IV (247)	(T,G)-A--L specific antibodies
(T,G)-A--L	+	+	−	+	+
(T-T-G-G)-A--L	+	+	+	+	+
(T-G-T-G)-A--L	+	+	+ or ±	+	− or ±
(T, G)-Pro--L	−	−	−	+	+
(T-T-G-G)-Pro--L	−	−	−	+	+
(T-G-T-G)-Pro--L	−	−	−	−	−
(Phe,G)-A--L	+	+	−	−	+
(H,G)-A--L	+	+	−	−	+
(G)-A--L	+	+	−	−	+
Pro--L	−	−	−	−	−
A--L	+	−	−	−	+

Antibody activity was evaluated by direct binding of the different monoclonal antibodies (at $1:10^3$ and $1:10^4$ dilution of ascitic fluids) to microtire plates coated with the different antigens. (+) C.p.m. > 20,000; (±) c.p.m. 5000–15,000; (−) c.p.m. < 2000.

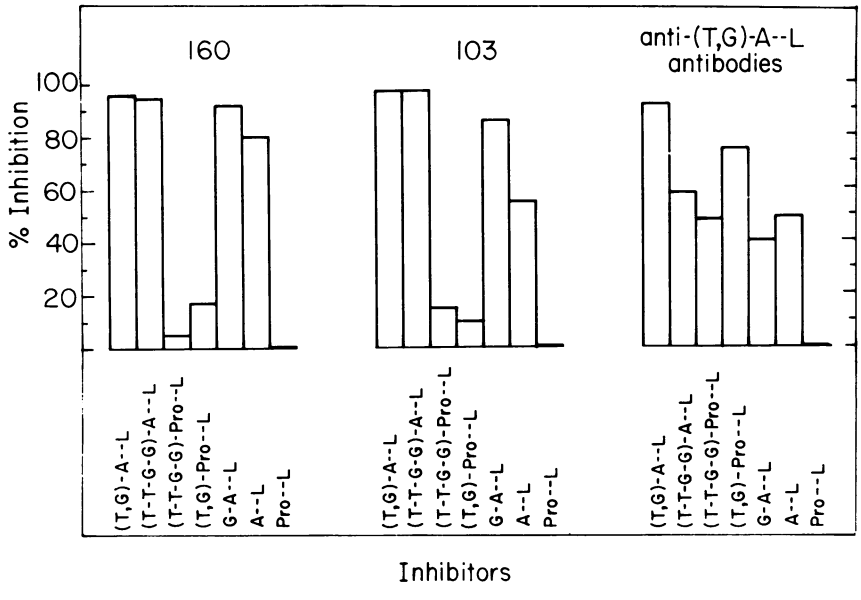

Figure 1. Inhibition of the binding of 160 and 103 monoclonal antibodies to ^{125}I-(T-T-G-G)-A--L by different synthetic antigens. One microgram of each inhibitor was used. The inhibitions of binding was performed with the amount of antibodies which corresponded to 15–35% binding of the iodinated antigens.

bind also (T-G-T-G)-A--L. The specificity of clone no. 247 (group IV) is different from that of antibodies in the other groups since it binds all the tyrosine containing polypeptides tested except (T-G-T-G)-Pro--L. The fine specificity of the monoclonal antibodies of group I was further confirmed by the inhibition of their binding to ^{125}I-(T-T-G-G)-A--L. Fig. 1 represents an experiment where the ability of the different polypeptides to inhibit the binding of the two monoclonal antibodies to ^{125}I-(T-T-G-G)-A--L was measured. Monoclonal antibodies 103 and 160 exhibit the same pattern in their specificity. Both can be well inhibited with (T,G)-A--L, (T-T-G-G)-A--L, G-A--L and A--L, but not with (T-T-G-G)-Pro--L or

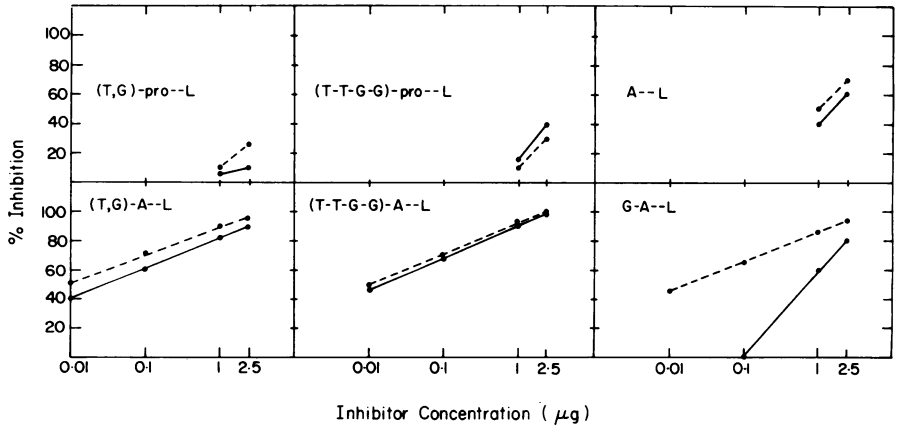

Figure 2. Inhibition of the binding of 160(– – –) and 103 (——) monoclonal antibodies to ^{125}I-(T-T-G-G)-A--L by different doses of the synthetic antigens. The inhibitions of binding were performed with the amount of antibodies which corresponded to 15–35% binding of the iodinated antigens.

Table 3. Expression of (T,G)-A--L specific idiotypes on monoclonal antibodies against (T,G)-A--L and (T-T-G-G)-A--L*

Source of antibodies	Concentration of antibodies μg/ml	Inhibition (%)	
		GPAId†	GPaMIg
103	0·35	92	4
160	0·5	73	1
50	6	−5	−13
131	5·6	1	ND‡
32	1·7	−4	ND
100	1·45	10	ND
101	1·45	−14	ND
114	3·1	−10	ND
115	2·8	−9	ND
139	0	−4	ND
247	10	−7	3
C3H.SW anti-(T,G)-A--L serum	1/250	31	ND
CWB anti-(T,G)-A--L serum	1/500	−1	ND

* Expression of (T,G)-A--L specific idiotypes on monoclonal antibodies was determined by the inhibition of the binding of these antibodies to ^{125}I-(T-T-G-G)-A--L by 5 μl of either guinea-pig anti-idiotypic serum against C3H.SW anti-(T,G)-A--L antibodies, or guinea-pig anti-mouse immunoglobulins.

† The inhibition experiments were performed with the amount of antibodies which corresponded to 15–35% binding of the iodinated antigen.

‡ N.D. not done.

§ Dilution of antiserum.

(T,G)-Pro--L. No inhibition was observed when Pro--L was used (Fig. 1). In order to check whether 103 and 160 monoclonal antibodies might differ in their affinity toward the various synthetic antigens, different doses of the inhibitors were used. As shown in Fig. 2, monoclonal antibodies 103 and 160 are similar in their pattern of inhibition. Clone no. 160 has higher apparent affinity with respect to its binding to (G)-A--L. No inhibition was observed with Pro--L even at very high doses of inhibitors. In a similar experiment (not shown), hybridomas no. 50 and 131 could only be inhibited with (T,G)-A--L, (T-T-G-G)-A--L and G-A--L. No inhibition was observed when (T-T-G-G)-Pro--L, (T,G)-Pro--L, A--L and Pro--L were used. The pattern of the specificity of these monoclonal antibodies is compared with that of polyclonal secondary anti-(T,G)-A--L antibodies of C3H.SW mice (Fig. 1).

Idiotypic expression of the monoclonal antibodies

Analysis of the expression of the major idiotypes of (T,G)-A--L specific antibodies on the monoclonal antibodies revealed that 103 and 160 monoclonal antibodies possess cross-reacting idiotypic determinants with C3H.SW anti-(T,G)-A--L antibodies. As shown in Table 3, only the binding of monoclonal antibodies 103 and 160 to ^{125}I-(T-T-G-G)-A--L could be inhibited by anti-idiotypic serum produced in guinea-pigs against C3H.SW anti-(T,G)-A--L antibodies. No inhibition was observed with guinea-pig anti-mouse immunoglobulins.

The two monoclonal antibodies, the idiotypes of which cross-reacted with those of anti-(T,G)-A--L antibodies, namely 103 and 160, were injected into guinea-pigs for the production of anti-idiotypic serum. The activity of the two anti-idiotypic sera is shown in Fig. 3. As can be seen anti-idiotypic serum against monoclonal antibodies 160 can completely inhibit the binding of clone no. 160 to ^{125}I-(T-T-G-G)-A--L and it can inhibit 37% of the binding of C3H.SW anti-(T,G)-A--L antibodies and 31% of the binding of C3H.SW anti-(T-T-G-G)-A--L antibodies to ^{125}I-(T-T-G-G)-A--L, but not of CWB anti-(T,G)-A--L antibodies and of 103 monoclonal antibodies. On the other hand, the anti-idiotypic serum against clone 103 can inhibit

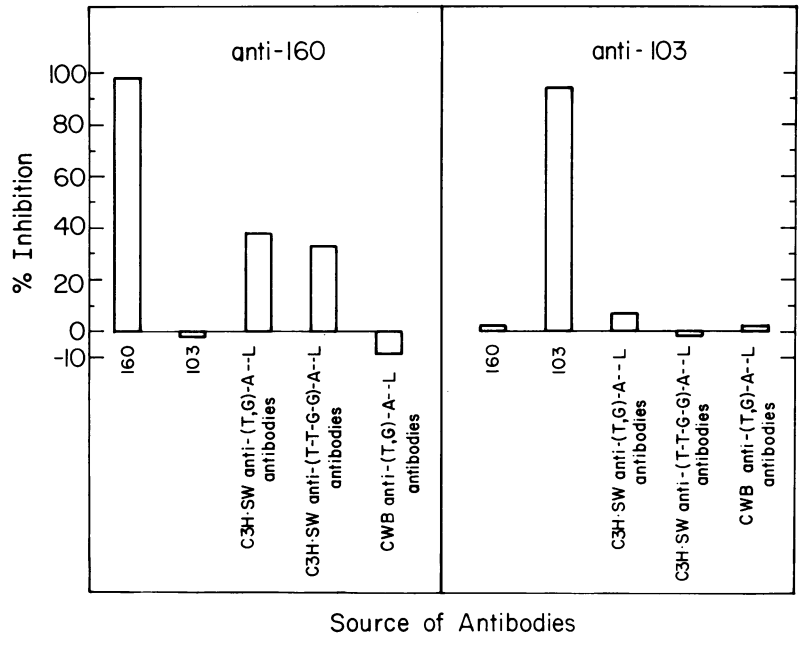

Figure 3. Characterization of anti-idiotypic serum against 160 and 103 monoclonal antibodies. The presence of anti-idiotypic activity in the guinea-pig sera was detected by their ability to inhibit the binding of 103, 160 monoclonal antibodies and (T,G)-A--L specific antibodies to ^{125}I-(T-T-G-G)-A--L. The inhibitions were performed with the amount of antibodies which corresponded to 15–35% binding of the iodinated antigen.

Table 4. Cross-reactive idiotypic determinants on (T,G)-A--L and (T-T-G-G)-A--L binding monoclonal antibodies*

Source of antibodies	Inhibition of antigen binding (%) in the presence of†	
	GP anti-160	GP anti-103
C3H.SW anti-(T,G)-A--L serum	45	3
CWB anti-(T,G)-A--L serum	−1	8
Monoclonal antibody 103	2	91
Monoclonal antibody 160	92	−7
Monoclonal antibody 50	1	−10
Monoclonal antibody 131	18	14
Monoclonal antibody 32	5	90
Monoclonal antibody 100	7	93
Monoclonal antibody 101	−14	90
Monoclonal antibody 114	−4	90
Monoclonal antibody 115	2	3
Monoclonal antibody 139	33	−5
Monoclonal antibody 247	3	8

* Cross-reactive idiotypic determinants were determined by the inhibition of binding of monoclonal antibodies to ^{125}I-(T-T-G-G)-A--L by guinea-pig anti-160 and guinea-pig anti-103.

† The inhibitions were peformed with the amount of antibodies which corresponded to 15–35% binding of the iodinated antigen.

completely the binding of 103 to ^{125}I-(T-T-G-G)-A--L, but not the binding of C3H.SW anti-(T,G)-A--L, anti-(T-T-G-G)-A--L, CWB anti-(T,G)-A--L antibodies and 160 monoclonal antibodies. We have further screened the other monoclonal antibodies for the presence of cross-reactive idiotypic determinant with guinea-pig anti-idiotypic serum against the monoclonal antibodies 103 and 160. As can be seen in Table 4, anti-idiotypic serum against 103 completely inhibits the binding of monoclonal antibodies from clones 32, 100, 101, 114, but not significantly the binding of monoclonal antibodies 160, 50, 131, 115, 139 and 247 to ^{125}I-(T-T-G-G-)-A--L. On the other hand, anti-idiotypic serum against 160 monoclonal antibodies inhibits the antigen binding to the homologous antibodies, to 139 monoclonal antibody and to the C3H.SW anti-(T,G)-A--L antibodies.

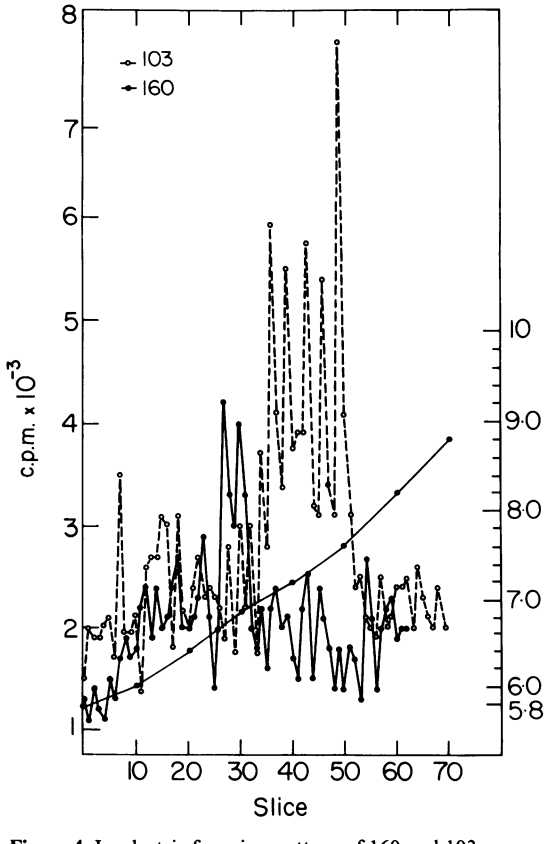

Figure 4. Isoelectric focusing pattern of 160 and 103 monoclonal antibodies. Fifteen microlitres of ascites were focused on 5% polyacrylamide gel.

Isoelectric focusing pattern of clones 103 and 160

The two monoclonal antibodies 103 and 160 were found to be similar in their fine specificity, but they induce non-cross-reactive antibodies in guinea-pigs. It was of interest to compare their isoelectric focusing pattern. Results shown in Fig. 4 demonstrate differences in the pattern of binding of the two monoclonal antibodies to ^{125}I-(T-T-G-G)-A--L. Monoclonal antibody 160 displayed two bands that focused in a range between pH 6·6–7·0 and 103 antibody displayed five bands in a range between pH 7·2–7·6. It is noteworthy that the range of focusing of the two monoclonal antibodies overlaps with the pH range where the polyclonal (T,G)-A--L specific antibodies were found to be focused (Mozes, Sela & Chedid, 1980; Lifshitz, Gitler & Mozes, 1981).

DISCUSSION

The aims of the present study were: first, to obtain monoclonal anti-(T,G)-A--L antibodies in order to characterize the clonal heterogeneity and fine antigenic specificity following immunization with (T,G)-A--L; second, to analyse their idiotypic expression in relation to the major idiotypes found on C3H.SW anti-(T,G)-A--L antibodies; and third, if possible, to correlate the specificity pattern with the presence of the anti-(T,G)-A--L specific idiotypes.

Using somatic cell hybrids between myeloma cells and splenocytes primed to (T,G)-A--L and challenged with (T-T-G-G)-A--L, eleven monoclonal antibodies were obtained. This protocol of immunization was designed in order to increase the frequency of clones specific to (T-T-G-G)-A--L, the main antigenic determinant of (T,G)-A--L (Mozes *et al.*, 1974), which elicits antibodies that express the major idiotypes of (T,G)-A--L specific antibodies (Schwartz *et al.*, 1978). Each of the antibodies secreted had been specifically purified by affinity chromatography and exhibited a very limited electrophoretic heterogeneity as determined by isoelectric focusing. The data show that there is no preferential subclass distribution among the monoclonal antibodies since they exhibited IgG_1, IgG_2, IgG_3 and IgM heavy chain determinants in equal proportions (Table 1). Similar results were reported for hybridomas obtained after secondary immunizations and secret anti-p-azophenylarsonate (Lamoyi, Estess, Capra & Nisonoff, 1980) or anti-(4-hydroxy-3-

nitrophenyl) acetyl antibodies (Reth, Hammerling & Rajewsky, 1978) which express all immunoglobulin subclasses. However, using the poly (Glu^{60} Ala^{30} Tyr^{10}) system (GAT; Pierres, Ju, Waltenbaugh, Dorf, Benacerraf & Germain, 1979), sperm whale myoglobin (Kohno, Berkower, Buckenmeyer, Minna & Berzofsky, 1981), poly (Glu^{60} Ala^{40}) (GA; Ju, Pierres, Germain, Benacerraf & Dorf, 1980) or hen egg white lysozyme (Metzger, Furman, Miller & Sercarz, 1981), there was preferential Ig distribution, namely, most of the monoclonal antibodies elicited against these antigens were of the IgG1 subclass.

Analysis of the specificities of the monoclonal antibodies indicated that they can be divided into two major groups: those which bind only (T-T-G-G)-A--L and those which bind both (T-T-G-G)-A--L and (T,G)-A--L (Table 1). The fine specificity was determined by two different techniques: (i) direct binding of monoclonal antibodies to different synthetic antigens; and (ii) the inhibition of binding of these antibodies to ^{125}I-(T-T-G-G)-A--L. The results of both techniques are comparable and show that the monoclonal antibodies can be further subdivided into four groups. The specificity of the monoclonal antibodies altogether reflects the spectrum of specificites observed in the polyclonal anti-(T,G)-A--L antibodies. Some of the antibodies (e.g. clones no. 103, 160, 50, 131, 247) exhibit a range of cross-reactivity which is very similar to that of the polyclonal antibodies whereas monoclonal antibodies of group III (Table 2) display a very restricted specificity expressed by the cross-reactivity with only the ordered peptides coupled to the A--L region. It is noteworthy that on the level of polyclonal antibodies no cross-reaction was observed between antibodies elicited to (T-T-G-G)-A--L and (T-G-T-G)-A--L and *vice versa* (Mozes *et al.*, 1974). Although the eleven monoclonal antibodies described herein recognize the antigenic determinants towards which the polyclonal (T,G)-A--L specific antibodies react, they by no means cover the whole spectrum of clones than can be triggered in high-responder mice by the random branched synthetic polypeptide (T,G)-A--L.

Screening of all the monoclonal antibodies for the presence of the major idiotypes of (T,G)-A--L specific antibodies demonstrated that two out of the eleven monoclonal antibodies express the idiotypic determinants of the anti-(T,G)-A--L antibodies. This was evident by the inhibition of binding of 103 and 160 monoclonal antibodies to ^{125}I-(T-T-G-G)-A--L by the anti-idiotypic serum raised in guinea-pigs against C3H.SW anti-(T,G)-A--L antibodies. The specificity of the inhibition was confirmed by the inability of guinea-pig anti-mouse immunoglobulin to inhibit this binding. Indeed, clones 103 and 160 are similar in their specificity and apparent affinity towards the (T,G)-A--L related polypeptides (Table 2; Figs. 1, 2).

In spite of the observed cross-reactivity between the guinea-pig anti-idiotypic serum and clones no. 103 and 160, these two monoclonal antibodies probably define different non cross-reactive idiotype markers that exist on the anti-(T,G)-A--L antibodies. This was suggested by the fact that anti-idiotypic antibodies raised in guinea-pigs against either of the monoclonal antibodies inhibited only the binding of the homologous antibodies to the ^{125}I-(T-T-G-G)-A--L (Fig. 3).

Among the anti-idiotypic sera produced in guinea-pigs against these two monoclonal antibodies, it appears that the antibodies against clone no. 160 resemble more the antibodies against polyclonal (T,G)-A--L specific antibodies. Thus, anti-idiotypic sera against 160 monoclonal antibodies inhibited 37% of the binding of polyclonal C3H.SW anti-(T,G)-A--L antibodies to ^{125}I-(T-T-G-G)-A--L and 31% of the binding of C3H.SW anti-(T-T-G-G)-A--L antibodies. These results are in agreement with those obtained in previous studies in which the anti-idiotypic serum against anti-(T,G)-A--L antibodies bound specifically 30% of the iodinated anti-(T,G)-A--L antibodies and cross-reacted with the idiotypes of anti-(T-T-G-G)-A--L antibodies (Schwartz *et al.*, 1978). Lack of inhibition of the binding of CWB (Ig-1^b) anti-(T,G)-A--L antibodies to ^{125}I-(T-T-G-G)-A--L, with the guinea-pig sera against clone no. 160 (Fig. 3) may suggest that expression of cross-reactive idiotypic determinants on the 160 monoclonal antibody is linked to the heavy chain allotypes, as shown for the idiotypes on polyclonal anti-(T,G)-A--L antibodies (Lifshitz *et al.*, 1980). Antibodies against clone 160 reacted partially with clone no. 139 and not at all with any of the other monoclonal antibodies (Table 4). Guinea-pig serum against 103 monoclonal antibodies did not inhibit either the binding of anti-(T,G)-A--L or of anti-(T-T-G-G)-A--L antibodies to the iodinated antigen whereas it inhibited completely the binding of most of the monoclonal antibodies of group III to ^{125}I-(T-T-G-G)-A--L (Table 4).

In further studies we have raised antibodies against clone 103 in mice. Some of the individual antisera reacted with the $F(ab')_2$ of (T,G)-A--L specific polyclonal antibodies (unpublished data). It therefore

appears that cross-reactive idiotypic determinants on 103 monoclonal antibody are not sufficiently immunogenic in the guinea-pigs to elicit detectable levels of the corresponding anti-idiotypic antibodies. Instead, determinants other than the (T,G)-A--L cross-reactive idiotypes (private determinants) act in an immunodominant fashion and are responsible for the observed cross-reaction with the monoclonal antibodies of group III. The existence of both private and public idiotypic determinants on monoclonal antibodies has been also reported for other antigenic systems such as GAT and GA (Ju *et al.*, 1980; Ju, Pierres, Waltenbaugh, Germain, Benacerraf & Dorf, 1979).

The isoelectric focusing (IEF) gel of the two monoclonal antibodies 103 and 160 shows different pattern of binding to ^{125}I-(T-T-G-G)-A--L (Fig. 4) despite the fact that their combining sites are similar with respect to binding to different (T,G)-A--L related synthetic polypeptides. The observed differences in the IEF pattern may reflect the differences in the expression of non-cross-reactive idiotypic determinants on the two monoclonal antibodies, however the possibility that they may be due to differences in their Ig subclasses (Table 1) cannot be excluded.

Our data suggest that idiotypic determinants of the two monoclonal antibodies against which the guinea-pig sera are directed are associated with the antigen-combining site (Table 3; Fig. 3) and correlation exists between expression of certain specificity and the presence of the major idiotypes. A similar observation was made by analysing idiotypes of polyclonal anti-(T,G)-A--L antibodies (Schwartz *et al.*, 1978) and also with monoclonal antibodies against other antigenic systems (Ju *et al.*, 1979, 1980). However, in some instances it has been shown that idiotypes can be dissociated from specificity. Typical examples are the idiotypes of group A streptococcal carbohydrate (*A5A*) (Eichmann, Coutinho and Melchers, 1977) that can occur on immunoglobulin molecules of A/J mice that do not bind streptococcal A carbohydrates, and the hen egg white lysozyme system in which monoclonal antibodies that react with different regions of this protein antigen share common idiotypes (Eichmann *et al.*, 1977; Metzger, Miller & Sercarz, 1980).

Monoclonal anti-(T,G)-A--L antibodies allow the dissection of the specificities recognized by the anti-idiotypic sera. The anti-idiotypic sera against monoclonal antibodies provide tools to analyse further the expression of idiotypic determinants on (T,G)-A--L specific T cells and their products.

ACKNOWLEDGMENTS

We are grateful to Ms N. Zinberg and Mr M. Rotman for their excellent technical assistance and to Drs C. Milstein and G. Hammerling for supplying the myeloma cells. Z.E. is an Incumbent of the Recanati Development Chair in Cancer Research.

This paper was supported in part by the Stiftung Volksvagenwerk.

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