ANTI-IMMUNOGLOBULIN ANTIBODIES

III. Properties of Sequential Anti-Idiotypic Antibodies to Heterologous Anti-γ Globulins. Detection of Reactivity of Anti-Idiotype Antibodies with Epitopes of Fc Fragments (Homobodies) and with Epitopes and Idiotopes (Epibodies)*

By CONSTANTIN A. BONA, SCOTT FINLEY, STANLEY WATERS, and H. G. KUNKEL

From the Mount Sinai School of Medicine, New York 10029; and The Rockefeller University, New York 10021

The immune response against antigenic determinants of immunoglobulin (Ig) molecules (i.e., isotypes, allotypes, and idiotypes) has been extensively studied. In mice, it has been shown that the antibody response against allotypic determinants is under Ir gene control (1, 2).

The study of homologous and autologous anti-Ig responses has suggested that these responses may be uniquely similar among genetically distinct individuals. Thus, autologous anti-IgG antibodies in humans (rheumatoid factors) show a considerable degree of homogeneity, with many of those studied bearing a cross-reactive idiotype (IdX)[1] (i.e., Wa) and others bearing other cross-reactive idiotypes (Id) (3, 4). In mice and in rabbits, it has been shown that anti-allotype antibodies bear cross-reactive Id (2, 5, 6), indicating that the genes encoding the anti-allotype immune response are highly conserved. Similarly, syngeneic and homologous anti-Id antibodies share IdX in some cases. Because of these findings, the purpose of this investigation was to study the Id of the anti-Ig response in a heterologous system (murine/human), in which the immunogen was a human monoclonal IgM anti-γ-globulin bearing the IdX Wa (7).

Study of the idiotypy of murine anti-human V region of Gl antibodies produced by various strains of mice has shown that they share an IdX, despite the fact that the fine specificity of these antibodies is different from one strain to another. In addition, we found that A/J anti-Id antibodies recognize an epitope related specificity borne by the Fcγ fragment and shared by various human IgM monoclonal proteins. We have called these anti-Id antibodies epibodies. Finally, a fraction of anti-anti-Id antibodies bound to the antigen and therefore behaved as homobodies.

* Supported by research grants AG/A10271601A1 and AI10811 from the U. S. Public Health Service and grant PCM81105788 from the National Science Foundation.

[1] *Abbreviations used in this paper:* Ab_1, anti-epitope antibody; Ab_2, anti-Id antibody; Ab_3, anti-anti-Id antibody; Ab_4, anti-anti-anti-Id antibody; C region, constant region; epibody, Ab_2 antibody that is able to recognize similar "structures" or "shapes" borne by epitope and Ab_1; epitope, antigenic determinant; FCS, fetal calf serum; HA, hemagglutination; HI, hemagglutination inhibition; homobody, Ab_2 antibody that mimics the antigen and binds to Ab_1 and cell receptor for the antigen; IdI, individual idiotype; idiotope, individual antigen of the idiotype; IdX, cross-reactive idiotype; K, kappa chain; MHC, major histocompatibility complex; RIA, radioimmunoassay; SRBC, sheep erythrocytes; V region, variable region.

 J. Exp. Med. © The Rockefeller University Press · 0022-1007/82/10/0986/14 $1.00
Volume 156 October 1982 986-999

Materials and Methods

Mice. BALB/c, C.B20, C58/J, DBA/2, A/J, PL/J, P/J, CE/J, C57BL/6, and AKR/J mice, 6–8 wk old, were used in this study.

Human Proteins. $IgM_{K III}$ Gl and PG and $IgM_{K I}$ Lay with anti-IgG activity and $IgM_{K III}$ Dau, Ch, Ga, and Sal with no known specificity were used in this study. They are all monoclonal IgM proteins obtained from patients with macroglobulinemia or mixed cryoglobulinemia. IgM Gl was highly purified from the cryoglobulin of patient Gl by zone electrophoresis as the first step followed by G200 chromatography. The final preparation showed no detectable IgG by hemagglutination.

Preparation of Human Ig Fragments. Fab fragments of IgM Gl and PG, Fcγ fragments of IgG, and Fcμ fragments of IgM Gl were prepared according to previously described techniques (8).

Immunization of Mice. Various strains of mice were immunized intraperitoneally according to the method of Tung and Nisonoff (9) with 0.1 ml of an emulsified mixture of 10 mg/ml myeloma protein and complete Freund's adjuvant (1:9 ratio). Ascitic fluid was harvested after the fourth immunization.

Separation of Anti-V Region Gl Antibodies. An ammonium sulfate fraction of ascitic fluid was successively absorbed on Sepharose 4B coated with human IgG and $IgM_{K III}$ Ch columns. The effluent was precipitated with ammonium sulfate, dialyzed, and passed over an $IgM_{K III}$ Gl-Sepharose 4B column, and antibodies were eluted from this column with glycine-HCl buffer (pH 2.8) as previously described (2).

Preparation of Anti-Id Antibodies. Anti-V-region Gl antibodies separated from the ascitic fluid from single BALB/c mice were coupled with keyhole limpet hemocyanin (KLH), as previously described (2), and then used as immunogens to prepare syngeneic anti-Id (anti-V-region) antibodies, as previously described (4). Monoclonal anti-anti-V_H antibodies were prepared by fusion of Sp2/0 myeloma cells with spleen cells from CB6 F_1 mice immunized with syngeneic affinity-purified anti-V_H Gl antibody-KLH conjugate. Hybrids producing anti-anti-V_H Gl antibodies detected by the ELISA technique, as previously described (10), were cloned on thymocyte filler, and the clones were expanded in pristane-primed CB6 F_1 mice.

Hemagglutination Assay (HA). HA titers of murine anti-V region Gl or BALB/c anti-(BALB/c anti-V region) antisera were measured in microtiter plates using sheep erythrocytes (SRBC) coated with human IgM myeloma protein Gl, PG, Ch, Sal, Ga, or BALB/c anti-V region Gl antibodies, respectively. The proteins were coated onto SRBC using Cr Cl_3 (2). In each case, the titer recorded as $1/\log_2$ of the highest dilution of antisera that caused agglutination.

Determination of Serum IdX Titer. A hemagglutination inhibition (HI) method described previously (2) was used to test sera for IdX. In these experiments, specifically purified BALB/c or P/J anti-V region Gl antibodies were coupled with SRBC. The capacity of various sera to inhibit hemagglutination of these cells by BALB/c anti(anti-V region) antibodies was determined. The titer reported as $1/\log_2$ of the highest dilution that caused inhibition of hemagglutination.

Radioimmunoassay (RIA). Purified BALB/c anti-anti-V region antibodies and goat anti-mouse Ig antibodies were labeled with ^{125}I, as described by Hunter (11).

The ability of anti-V region Gl antibodies to bind to various human IgM myeloma proteins was determined in microtiter plates that had been incubated for 18 h with 50 μg/ml myeloma proteins, followed by three washings in saline. The plates were incubated for 1 h with 50% fetal calf serum (FCS) and, after three washings, were incubated for 3 h with mouse anti-V region Gl antibodies. After three washings, the microplates were incubated for 3 h with 50 μl of ^{125}I-goat anti-mouse Ig (New England Nuclear, Boston, MA) (~50,000 cpm/50 μl). After incubations, the microplates were washed extensively, and the radioactivity on plates was measured in a γ-spectrophotometer. The titer of BALB/c anti-anti-V region Gl antibodies was measured with the use of microplates that had been incubated with 50 μg/ml purified anti-V region Gl antibodies.

ELISA Technique. Affinity-purified CB6 F_1 and anti-V_H Gl antibodies were labeled with alkaline phosphatase (grade I; Boehinger Mannheim, West Germany), as previously described (10). The specific activity of labeled antibodies was 930 international units of alkaline phosphatase per mg of enzyme-antibody conjugate. Labeled anti-V_H Gl antibodies were used in ELISA to test the reactivity of monoclonal anti-anti-V_H Gl antibodies as follows: microtiter

plates (Dynatech Laboratories, Inc., Dynatech Corp., Alexandria, VA) were coated with IgM Gl (10 μg/ml) for 18 h at 4°C. After three washings with phosphate-buffered saline containing 5% Tween, the microplates were incubated for 3 h at 4°C with labeled anti-V_H Gl antibodies (5 μl/ml). The labeled anti-V_H Gl antibodies were preincubated or not during 30 min at 23°C with various amounts of unlabeled monoclonal anti-anti-V_H Gl antibodies before being added to the microtiter plates. Finally, the microtiter plates were reincubated for 1 h at 37°C with *p*-nitrophenyl phosphate (1 tablet Sigma) in 5 ml of 10% (wt/vol) diethanolamine buffer (pH 9.8). The enzymatic reaction was stopped with 25 μl of 3 M NaOH, and the absorbance at 405 nm was carried out in an ELISA microreader (Dynatech Laboratories, Inc., Dynatech Corp.).

Results

Anti-V Region Gl Antibodies. Anti-V region antibodies were obtained by immunization of various strains of mice with IgM_{KIII} monoclonal protein Gl displaying IgG-binding activity and belonging to the human WaIdX subgroup. These were purified by elution from an IgM Gl–Sepharose 4B column after previous absorption and elution on an IgM Ch–Sepharose 4B column.

The agglutinating activity as well as the binding activity of these antibodies toward various human IgM monoclonal proteins was studied initially by HA and RIA assays, respectively. The HA assay results depicted in Table I show that antibodies produced in various strains of mice not only have a high agglutinating titer for IgM Gl and PG of the WaIdX subgroup, but that these antibodies, excepting those produced by C57BL/6, AKR/J, and C58/J, also agglutinated SRBC coated with IgM_{KIII} Wa^- Dau monoclonal protein. Thus, in further studies (Table II), we investigated with RIA the ability of anti-V_H Gl antibodies produced in various strains of mice to interact with several IgM_{KIII} monoclonal proteins. This study was performed after absorption of anti-V region Gl antibodies on human IgG and polyclonal IgM obtained from one individual.

The results depicted in Tables I and II indicate that anti-V region Gl antibodies produced by various strains of mice can be classified into several groups according to their specificity, as follows: (a) antibodies produced in certain strains (BALB/c, for example) were specific for IdI and IdX of human IgM myeloma protein Gl. The titer

TABLE I

HA Titer of Anti-Human V Region Antibodies Produced in Various Strains of Mice

Anti-V region Gl antibodies (100 μg/ml) from	HA titer (log 2 units)				
	SRBC coated with				
	IgMKIII Wa^+ Gl	IgMKIII Wa^+ PG	IgMKIII Wa^- Dau	IgMKI Po^+ Lay	IgMKIII Wa^- Ch
BALB/c	>12	6	2	1	0
DBA/2	>12	10	2	2	0
PL/J	7	7	5	0	0
C.B20	>12	2	4	1	0
C57BL/6	>12	2	0	0	0
AKR/J	7	2	0	0	0
C58/J	8	2	0	5	0
RIII	10	10	5	0	0
CE/J	7	6	7	1	0
P/J	11	10	7	0	0
BALB/c anti-Ch	0	0	6	5	>12

TABLE II

Reaction of Anti-V Region Antibodies Produced by Various Strains of Mice against Different Monoclonal IgM Proteins

Origin of anti-V region Gl antibodies (100 μg/ml)	IgM Gl*	IgM PG*	IgM Dau	IgM Sa	IgM Ga	IgM Ch
BALB/c	1,643 ± 40	1,174 ± 20	143 ± 23	166 ± 29	134 ± 46	42 ± 18
AKR/J	770 ± 27	249 ± 13	80 ± 23	104 ± 4	25 ± 23	0
DBA/2	2,641 ± 28	2,079 ± 421	230 ± 25	1,242 ± 28	1,420 ± 88	186 ± 36
C.B20	1,395 ± 115	543 ± 21	67 ± 6	350 ± 27	302 ± 9	152 ± 26
RIII	3,418 ± 36	2,164 ± 294	594 ± 32	521 ± 48	534 ± 44	324 ± 103
PL/J	2,834 ± 43	1,186 ± 32	1,716 ± 293	913 ± 51	510 ± 47	0
P/J	1,434 ± 199	893 ± 105	494 ± 42	562 ± 105	126 ± 62	105 ± 3
CE/J	1,935 ± 54	1,236 ± 59	1,106 ± 24	793 ± 76	1,043 ± 50	0
A/J	2,942 ± 528	2,734 ± 200	2,114 ± 62	2,494 ± 10	1,824 ± 52	209 ± 52

* Anti-γ-globulin.

‡ Counts per minute ± SD.

Microtiter plates were coated for 18 h at 4°C with 50 μg/ml of various myeloma proteins and washed three times and incubated 1 h with 50% FCS. After three washings, they were incubated for 3 h at 4°C with anti-V region Gl antibodies washed three times and then incubated for 3 h at 4°C with ^{125}I-goat anti-mouse antibodies (10,000 cpm). The nonspecific binding to microplates coated with 50% FCS was substracted. In the experiment illustrated in this table, the background was 385 ± 52. Each value represents the average of triplicates. SD was calculated on values after substraction of average of background.

and the specific binding of these antibodies were much higher for IgM Gl than for IgM PG. PG is an $IgM_{K III}$ myeloma protein with anti-γ-globulin activity that also belongs to the WaIdX subgroup. (b) Antibodies produced in other strains (DBA/2 for example) were mainly specific for WaIdX because the HA titers or the specific binding activity were roughly equal for Gl and PG $WaIdX^+$ monoclonal proteins. (c) Antibodies produced in PL/J mice, for example, recognized WaIdX and also framework determinants shared by certain IgM monoclonal proteins, which lack anti-γ globulin activity.

Id of Anti-V Region Gl Antibodies. BALB/c anti-Gl antibodies that are primarily specific for WaIdX and Gl IdI were used as immunogens to prepare syngeneic anti-Id (i.e., BALB/c anti-BALB/c anti-V region Gl antisera). These antisera were compared with BALB/c anti-anti-V region Ch antibodies, Ch being an IgM protein without anti-γ activity. They agglutinated BALB/c and DBA/2 anti-Gl antibodies at high titers and the control BALB/c anti-V region Ch antibodies at a very low titer. They did not interact with murine anti-Id (i.e., A48Id) or anti-allotype (i.e., IgG^b_{2a}) antibodies (Table III). From these results, we concluded that the syngeneic BALB/c anti-anti-V region Gl antibodies showed specificity for Id determinants of anti-V region Gl antibodies.

In further experiments, we used these syngeneic anti-anti-Id antisera to investigate the presence of IdX on anti-V region Gl antibodies produced by various strains of mice. The results presented in Table IV show that anti-V region Gl antibodies produced by various strains of mice share an IdX recognized by BALB/c anti-anti-V region Gl antibodies. The expression of this IdX is independent of the *MHC* or the *Igh* gene complex.

Therefore, our results indicate that murine anti-V region Gl antibodies share an

TABLE III

HA Titer of Syngeneic BALB/c Anti-Anti-V Region Gl Antibodies

SRBC coated with purified antibodies	HA titer (log_2 units)	
	Agglutinating antisera	
	BALB/c anti-anti-V region (Gl)	BALB/c anti-anti-V region (Ch)
BALB/c anti-V region Gl	8	1
BALB/c anti-V region Chr	0	5
DBA/2 anti-V region Gl	>8	0
RIII anti-V region Gl	3	0
BALB/c anti-A48Id*	0	0
BALB/c anti IgG^{b}_{2a} allotype‡	0	0

* ABPC48 is murine IgA_{κ} β2-6 fructosan-binding myeloma protein that expresses A48Id shared with UPC10 myeloma protein.

‡ These antibodies were obtained by immunization of BALB/c mice with CBPC 101 myeloma protein and purified on Sepharose 4B-C57BL/6 Ig columns.

TABLE IV

Expression of Cross-Reactive Id of BALB/c Anti-V Region Gl Antibodies in Various Strains of Mice

Anti-V region Gl antibodies from*	H2	IghC	HI titer (log_2 units)‡ of IdX antibodies		Number of mice expressing IdX	RIA
			BALB/c	P/J		^{125}I BALB/c anti(anti-V region Gl antibodies + microplates coated with anti-V region antibodies
BALB/c	d	a	9	9	8/11	5,467 ± 121
DBA/2	d	c	8	5	2/4	4,629 ± 70
PL/J	u	j	4	4	2/4	3,422 ± 283
C.B20	d	b	8	2	2/4	3,840 ± 417
C57BL/6	b	b	8	5	4/4	4,203 ± 60
AKR/J	k	d	9	6	3/4	3,784 ± 720
C58/J	k	a	3	5	5/5	2,896 ± 707
RIII	r	g	3	5	4/4	1,134 ± 351
P/J	p	h	6	8	2/4	4,417 ± 603
CE/J	k	q	2	2	2/6	1,704 ± 12
A/J	a	e	0	0	0/6	380 ± 88
Anti-A48Id(BALB/c)	d	a	0	0	—	197 ± 21

* In HI assay, the purified anti-V region Gl antibodies enumerated in column 1 were used as inhibitors, whereas in RIA they were used to coat the microtiter plates (at 50 μg/ml concentration).

‡ Two systems were used to investigate the expression of IdX on anti-V region Gl antibodies as follows: BALB/c anti-anti-V region Gl antiserum and SRBC§ coated with BALB/c anti-V region Gl or P/J anti-V region Gl antibodies.

interstrain IdX. It should be mentioned that this IdX was not identified on two murine monoclonal antibodies specific for the human μ chain, kindly provided by J. Kearney, University of Alabama, for this study.

Polyclonal Syngeneic Anti-Anti-V Region Gl Antibodies Contain a Subset That Binds to Human Fc Fragment. In further studies, we investigated the ability of anti-anti-V region Gl

antibodies to bind to the human Fcγ fragment. The rationale of this investigation follows from several reports that suggest that anti-Id antibodies represent the internal image of antigen and from the fact that human IgM with anti-γ globulin activity was the initiator of the anti-anti-V region Gl antibody pathway. A comparative study of the binding of these anti-anti-V region Gl antibodies to plates coated with various human IgM myeloma proteins and Fcγ fragments demonstrated that a subset representing ~10% of this population of antibodies bound to Fc. This binding was inhibited by preabsorption of anti-anti-V region Gl antibodies with the Fcγ fragment but not with the Fab Gl fragment. No binding of heterogeneous ^{3}H-syngeneic anti-anti-A48Id antibodies to Fcγ fragments was observed in control experiments (Table V). These results suggest that a fraction of syngeneic (BALB/c) anti-anti-V region Gl antibodies displayed a binding activity similar to IgM Gl, the anti-γ monoclonal protein that was the initiator of this network pathway. In these terms, this subset of antibodies that bind to Fcγ fragments can be considered as homobodies (12).

Monoclonal Syngeneic Anti-Anti-V Region Gl Antibody That Bind to Human IgM Monoclonal Proteins. Several syngeneic CB6/F_1 monoclonal anti-anti-V region Gl antibodies were obtained by fusion of SP2/0 myeloma cells with spleen cells from CB6/F_1 mice immunized with anti-V region Gl antibodies. The majority of these monoclonal anti-anti-V region antibodies bound labeled anti-V region Gl antibodies as well as inhibited the binding of anti-V region Gl antibody to IgM Gl monoclonal protein.

Among these monoclonal anti-anti-V region antibodies, we found one, P1A2.3, that did not bind labeled anti-V region Gl antibodies but was able to inhibit the binding of anti-V region Gl antibodies to IgM Gl monoclonal protein (Table VI). Surprisingly, we found that this monoclonal antibody significantly bound to IgM Gl, PG, Sal, and Ga (IgM_{KIII}), but not to IgM Dau, Ch (IgM_{KIII}), Lay (IgM_{K1}), and human IgG (Table VII). The binding of P1A2.3 monoclonal antibody to human monoclonal proteins Gl and Ga was inhibited with Fab Gl and Fab PG, but not with Fcμ Gl (Fig.

TABLE V

Binding of BALB/C Anti (BALB/C Anti-V Region (Gl) Antibodies to Human Fc Fragment

Plates coated with 50 μg/m of	Absorption of anti-bodies	^{125}I-BALB/c anti-anti-V region	3H-BALB/C anti-anti-A48Id*
BALB/c anti-V region Gl	—	5,467 ± 121‡	332 ± 25‡
BALB/c anti-V region Ch	—	196 ± 10	356 ± 18
BALB/c anti-A48Id	—	197 ± 21	14,685 ± 2,135
IgM Wa^+ Gl	—	289 ± 112	342 ± 24
IgM Wa^+ PG	—	342 ± 65	333 ± 24
IgM Po^+ Lay	—	153 ± 42	342 ± 35
IgM Wa^- Da	—	220 ± 63	335 ± 26
Human IgG	—	484 ± 112	305 ± 38
Fcγ	—	651 ± 38	336 ± 28
Fcγ	Fab Gl§	648 ± 26	388 ± 122
Fcγ	Fcγ§	305 ± 18	312 ± 16
FCS (50%)	—	328 ± 33	289 ± 22

* This antibody used as a control is specific for the A48 Id of murine ABPC48 monoclonal protein (IgA kappa with B2-6 fructosan-binding activity).

‡ 50 μg/ml.

§ Counts per minute minus mean of triplicates ± SD.

TABLE VI

Binding and Inhibitory Activity of Two Monoclonal Anti-Anti-V Region Gl Antibodies (ELISA)

VIA Binding Activity

Microtiter plates coated with	Binding of labeled anti-V region antibodies (5 μg/ml)*
BSA	0
IgM (Gl)	0.52‡
IgM (PG)	0.15
PlA5.6	0.16
PlA2.3	0.02

Microtiter plates were coated for 18 h with proteins mentioned in column 1 and then incubated with alkaline phosphatase-labeled anti-V region Gl antibodies.

VIB Inhibitory Activity

Inhibitors	Binding of labeled anti-V region antibodies*
Nil	0.27‡
IgM (Gl)	0
IgM (PG)	0.13
PlA5.6	0.06
PlA2.3	0.08

Microtiter plates were coated with IgM Gl 50 μg/ml, and labeled anti-V region antibodies were preincubated for 1 h at 23°C with inhibitors before being incubated in microtiter plates.

* Alkaline phosphatase-labeled CB6 F_1 anti-V region Gl antibodies.

‡ OD, 405 nm

TABLE VII

Binding of P1A2.3 Monoclonal Anti-Anti-V Region Gl Antibody to Various Human IgM Monoclonal Proteins

Microtiter plates coated with	Binding of			
	BALB/c anti-human Ig	CB6 F_1 anti-V region Gl	P1A2.3	P4D2.7
IgM Gl	18,392 ± 1,184*	12,575 ± 2,600	6,059 ± 799	1,544 ± 1,146
IgM PG	20,236 ± 2,819	11,792 ± 3,191	11,068 ± 18	252 ± 257
IgM Dau	23,586 ± 989	2,898 ± 1,782	1,678 ± 373	2,363 ± 408
IgM Sal	16,639 ± 1,629	403 ± 385	6,610 ± 193	0
IgM Ga	16,957 ± 57	681 ± 635	2,744 ± 470	0
IgM Lay	12,926 ± 870	1,088 ± 202	0	0
IgM Ch	18,645 ± 1,058	85 ± 43	905 ± 147	115 ± 16
IgG	18,046 ± 521	354 ± 300	959 ± 958	0

Microtiter plates were coated with 50 μg/ml of human IgM monoclonal proteins mentioned in column 1 and then with 50 μg/ml of purified heterogeneous BALB/c anti-human Ig or CB6 F_1 anti-V region Gl antibodies or with P1A2.3 or P4D2.7 monoclonal CB6 F_1 anti-anti-V region Gl antibody. Their binding was estimated in RIA by incubating the plates with 50,000 cpm ^{125}I-goat anti-mouse Ig. The binding is expressed as mean ± SD of cpm after the subtraction of the background (i.e., the binding ^{125}I-goat anti-mouse Ig to human IgM proteins).

* Results expressed as cpm minus mean of triplicates ± SD.

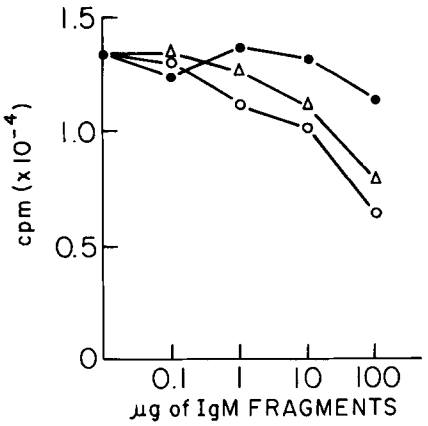

FIG. 1. Inhibition of binding of PIA2.3 monoclonal antibody to human IgM Gl by IgM fragments. Microtiter plates were coated with human IgM Gl (50 μg/ml) overnight, washed three times, and then incubated for 1 h with 50% FCS. PIA2.3 was preincubated for 1 h with human IgM fragments and then added for 3 h to IgM Gl-coated plates. After three washings, the microplate was incubated for 3 h with ^{125}I-goat anti-mouse antibody, washed extensively, and radioactivity was counted in a γ-spectrometer. ○, Fab GL; △, Fab PG; ●, Fcμ GL.

1). It should be mentioned that 50% inhibition was reached with high amounts of Fab fragments (100 μg/ml). This particular monoclonal anti-anti-V region Gl antibody, which interacts with anti-V region antibodies as well as with antigenic determinants of V region Gl, which we have called epibodies.

Special Properties of A/J Anti-V Region Gl Antibodies. Special studies were carried out on A/J anti-V region Gl antibodies. These antibodies, obtained by the same regimen of immunization used for other strains, exhibited two peculiar properties: (*a*) they bound in an equal manner to most IgM myeloma proteins independent of anti-IgG activity (Table II); and (*b*) they did not express the IdX recognized by BALB/c anti-anti-V region Gl antibodies.

Because of these particular properties of A/J anti-V region Gl antibodies, we studied their binding to human IgG. The data presented in Table VIII showed that anti-V region Gl antibodies produced in A/J mice, but not in other strains, exhibited the ability to bind to human IgG. In further experiments, we studied the effects of absorption of A/J anti-V region Gl antibodies with various IgM proteins, Fab Gl, Fcγ, and IgG on their ability to agglutinate IgM Gl-SRBC or to bind to microtiter plates coated with Fcγ fragment. The results presented in Table IX showed that the ability of A/J anti-V region antibodies to agglutinate SRBC was partially inhibited after absorption with IgM Gl, Sal, Dau, Fab Gl fragments, IgG, and Fcγ fragments. Similarly, the binding of these antibodies to Fcγ fragments was inhibited by the same proteins. We explain these results by a possible related epitope between the Fcγ fragment (for which the IgM Gl is specific) and framework determinants of various other IgM proteins. Therefore, these anti-Id antibodies that bind to the V region of human IgM proteins and to the Fcγ fragment can also be considered epibodies.

Discussion

The results presented in this communication indicate that various strains of mice, in response to immunization with a monoclonal human IgM protein with anti-γ

TABLE VIII

Binding of anti-V Region Gl Antibodies Produced in Various Strains of Mice to Human IgG

Antibodies	cpm
Nil	3,536 ± 349
BALB/c anti-human Ig	22,959 ± 521
BALB/c anti-V region Ch	3,673 ± 519
BALB/c anti-V region Gl	3,826 ± 471
DBA/2 anti-V region Gl	5,050 ± 318
RIII anti-V region Gl	6,353 ± 545
P/5 anti-V region Gl	4,570 ± 533
A/J anti-V region Gl	17,340 ± 1,463
C.B20 anti-V region Gl	3,412 ± 240
PL/J anti-V region Gl	8,732 ± 316
C58/J anti-V region Gl	2,721 ± 528
AKR/J anti-V region Gl	1,665 ± 203
CE/J anti-V region Gl	5,446 ± 164

Microtiter plates were coated for 18 h at 4°C with 50 μg/ml human IgM, washed three times, and incubated for 1 h with 50% FCS, washed three times and then coated with 50 μg/ml antibodies mentioned in column 1. After three washings, the plates were incubated for 3 h at 4°C with 50,000 cpm of ^{125}I-goat anti-mouse Ig. Results are expressed in cpm mean ± S.D.

TABLE IX

Absorption of Human Fc-Binding Activity of A/J Anti-V Region Gl Antibodies

Absorption of A/J anti-V region Gl antibodies with*	HA($\log_2$ units) titer*	RIA (cpm)‡
None	7	8,897 ± 23
Fab Gl	2	6,667 ± 342
Fc	4	5,669 ± 156
IgG	3	4,467 ± 156
Polyclonal IgM	6	7,128 ± 525
IgM Wa^+ Gl	3	6,380 ± 394
IgM Wa^- Sal	5	5,836 ± 144
IgM Wa^- Ga	7	8,587 ± 488
IgM Wa^- Dau	5	6,117 ± 1
IgM Wa^- Ch	7	8,767 ± 460
IgM Po^+ Lay	7	8,839 ± 485

* SRBC coated with IgM Gl.

‡ Microplates coated for 18 h with Fcγ (30 μg/ml) washed three times and then incubated further with 50% FCS. After three washings, the microplates were incubated for 3 h with A/J anti-V antibodies washed three times and the incubated for 3 h with ^{125}I-goat anti-mouse antibodies. Results are expressed in cpm, mean of triplicates ± SD. Adsorption was performed by mixing equal volumes (i.e., 100 μg/ml) of anti-A/J anti-V region (Gl) antibodies and various proteins for 30 min at room temperature before incubation in RIA or use in HA assays.

globulin activity and bearing the WaIdX, produced multiple antibodies with specificities for IdI, WaIdX, and against framework-associated antigenic determinants of the monoclonal protein used as the immunogen.

The anti-Id antibodies produced by various strains of mice also share an interstrain

IdX. The expression of this IdX is independent of the *MHC* or *Igh* gene complexes. This suggests that the response to idiotypic determinants of antibodies produced across heterologous as well as homologous (2, 5, 6) and syngeneic (3, 13) barriers is highly conserved. The existence of an interstrain IdX that is expressed in the majority of the individuals of several strains strongly suggests that this response is encoded by a germ line gene.

The study of the binding activity of anti-V region and anti-anti-V region Gl antibodies, obtained by hetero or syngeneic immunization, respectively, showed that some of these antibodies display special binding activity. Thus, we found that a fraction of anti-anti-V region Gl antibodies exhibited antigenic-binding activity similar to the IgM Gl monoclonal protein that was the initiator of our network pathway. These antibodies, which represent ~10% of total anti-anti-V region antibodies, bound to Fcγ and can be considered homobodies, as defined previously (12).

In our system, Ab_1, i.e., human IgM monoclonal protein with anti-γ globulin activity, recognizes epitopes of the Fcγ fragment. Immunization of mice with Ab_1 induces the synthesis of anti-V region antibodies, which can be considered as Ab_2 or anti-Id antibody. Some idiotopes of the Ab_2 antibodies are probably similar to epitope(s) of the Fcγ fragment and represent their internal image. Therefore, immunization of a second mouse with these anti-V region antibodies elicited the production of a family of anti-anti-V region antibodies; among these, a small subset recognizes some epitopes of the Fcγ fragment as well as the idiotopes of Ab_2 antibodies. The existence of these homobodies implies, first, a certain degree of similarity of structure between the antigen and anti-V region antibodies and, second, that some anti-Id antibodies represent the "internal image" of the antigen within the immune system. A prediction of the internal image concept is that homobodies might bind to antigen, displacing Ab_1, because the same interaction takes place between antibody and antigen as between homobodies and antigen.

There are several examples that suggest that homobodies represent topochemical copies of the antigen. Thus, Ab_4 (i.e., anti-anti-anti-Id antibodies), obtained in rabbits and in mice in the *M. lysodeikticus* or bacterial levan systems, display binding activity similar to Ab_2 (i.e., anti-Id antibodies) to idiotypic determinants of Ab_1 (14, 15). If the idiotopes of Ab_1 are considered as epitopes in these systems, then Ab_4 represents the internal image of the Ab_2-combining site, because Ab_1 and Ab_3 share similar idiotopes.

The internal image concept predicts that homobodies can display biological functions of the antigen. Sege and Peterson (16) have shown that anti-Id antibodies against anti-insulin antibodies can mimic some functions of insulin. Schreiber et al. (17) found that anti-Id antibodies against rabbit anti-alprenelol antibodies compete with the binding of dehydroalprenelol to β-adrenergic receptors and can stimulate basal adenylate cyclase activity in the cells bearing these receptors.

A second category of antibodies observed in our system was quite different and exhibited the ability to bind to both the epitopes of the antigen and idiotopes of the antibodies. We found two types of antibodies that exhibited this peculiar property as follows: (*a*) a monoclonal anti-anti-V region antibody (Ab_3) that binds to IgM Gl as well as several IgM proteins. This binding was inhibited by Fab Gl and PG fragments but not by Fcμ Gl fragments; and (*b*) a polyclonal A/J anti-V region Gl antibody (Ab_2) that was able to recognize some epitopes of Fcγ fragments as well as framework determinants of several IgM monoclonal proteins (Ab_1).

The (*a*) type of antibody was a clear-cut reaction of a monoclonal antibody from a mouse that had not received human Ig, but where the anti-anti-V region antibody reacted with the human IgM. The (*b*) type antibody might conceivably have arisen because the original IgM Gl used to give rise to anti-V region antibodies, despite its known purity, was contaminated with small amounts of IgG that produced anti-IgG antibodies. A number of observations argue against this possibility: (*a*) the reaction of the antiserum with IgM Gl was markedly reduced by absorption with IgG that could only have been the case if there was a large contamination of IgM Gl with IgG. This is known not to be the case (*b*) The reaction of A/J anti-V region Gl antibodies with IgM Gl was virtually lost by absorption with the Fab fragment of IgM Gl. (*c*) None of the other mouse strains produced such antibodies when injected with IgM Gl.

The best explanation of the peculiar binding activities of these antibodies is that they recognize some idiotopes or framework determinants of anti-Id antibodies that mimic the shape of the epitopes of the immunizing antigen, which was the initiator of the idiotypic network pathway. We have called these epibodies. Fig. 2 shows the various antibodies studied and the specific reactions of the two types of epibodies.

One may speculate about the structural homology and cellular mechanisms that lead to the occurrence of epibodies. There are few data on the 3-dimensional structures of V domains of an antibody molecule that allow it to recognize epitopes and to be recognized by an antibody that generally is defined as an anti-Id antibody (18, 19). There is little knowledge of structural correlates of internal image.

However, from our study, it clearly appears that the mice immunized with a human IgM respond to several epitopes, such as individual antigenic specificities borne by IgM Gl (IdI), WaIdX, and framework determinants of IgM V regions. Therefore, among the B cells of these mice, it is possible that there are clones that bear an "idiotype" that represents the internal image of one of the epitopes and that, subsequent to the binding to IgM Gl, can be stimulated to produce antibody that will recognize the epitope. This explanation is supported by the data regarding the properties of monoclonal anti-anti-V region Gl antibodies (Ab_3), which can inhibit the binding-V region antibodies (Ab_2) to IgM Gl (Ab_1), but that has a very low binding activity to Ab_2, as assessed in ELISA. Therefore, the epibodies appear to be combining site-induced antibodies, rather than classical anti-Id antibodies induced by Id. The epitopes recognized by epibodies probably do not include the dominant epitope that induces the synthesis of Ab_1, and may represent bystander epitopes that resemble idiotopes or framework antigenic determinants of a subset(s) of Ab_1 antibodies (i.e., of human IgM Gl monoclonal protein).

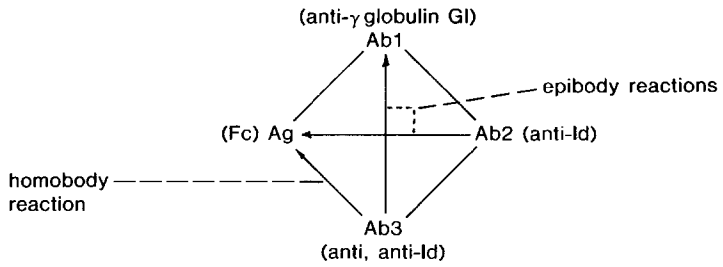

FIG. 2. Diagram showing the series of antibodies studied and their specific interactions. The arrows indicate the epibody and homobody reactions.

Wordfang and Rubin (20) reported some results that can be interpreted as epibodies. They prepared in tolerized rabbits a highly specific anti-Id antiserum against B6 anti-CBA (Ia^K) antibodies called 5936 Id. The anti-5936 Id antiserum binds not only to B6 anti-Ia^K cytotoxic antibodies, but also to antibody devoid of cytotoxic activity. Among various explanations provided by the authors, one that they take into account is that 5936 Id-bearing antibodies that lack anti-Ia^K cytotoxic activity may represent anti-(anti-CBA) antibodies that share 5936 Id with B6 and CBA antibodies.

These data, when considered collectively, indicate that anti-Id antibodies represent a heterogeneous population composed of at least four major categories: (*a*) conventional anti-Id antibodies, directed against idiotopes associated with the combining site (hapten inhibitable); (*b*) anti-Id antibodies directed against idiotopes associated with the framework of the V region (not inhibited by hapten); (*c*) epibodies that interact with epitope or idiotope-related structures of antibodies (Ab_1) or anti-Id antibodies (Ab_2), respectively; and (*d*) homobodies that may mimic the antigen (Ab_2 homobodies).

The existence of epibodies and homobodies strongly supports the concept of an internal image of the antigen within the immune system (21). This concept, wherein the average of the three-dimensional shapes of all V domains could be cross-reactive to the average shapes of epitopes, is significant for the understanding of the genetic, regulatory, and recognition mechanisms of the immune system. The implications of this concept include: (*a*) each antibody molecule can be regarded as an anti-Id antibody for another Ig; (*b*) the immune system does not consist of two separate entities (anti-epitope and anti-idiotope antibodies), as has been proposed (21), but a unitary system of multiple antibodies; and (*c*) that the antigens are not completely foreign because the immune system already contains structures (i.e., Id) that are similar to the antigens (epitopes).

Research in progress is designed to test this concept by studying the binding activity of several monoclonal anti-murine Id antibodies to human myeloma proteins as well as of murine anti-V region Gl antibodies to murine myeloma proteins.

Summary

Murine anti-V region antibodies against a human monoclonal protein Gl with anti-γ-globulin activity and bearing the Wa cross-reactive idiotype were prepared in several strains of mice. Antibodies were obtained that were specific for the Gl idiotype, the Wa cross-reactive idiotype, and for various framework antigenic determinants that were distinguished by a variety of procedures. Synthesis of such antibodies were found to be independent of *MHC* and *Igh* gene complexes. These anti-V region antibodies, produced by a majority of mouse strains investigated, also share a cross-reactive idiotype recognized by BALB/c anti-anti-V region Gl antibodies. A fraction of BALB/c anti-anti-V region Gl antibodies displayed human Fcγ binding activity and, therefore, can be considered homobodies, as described in other systems.

Two of the anti-idiotype antibodies obtained in this system exhibited a peculiar property: they interacted not only with their own antigen (IgM Gl), but also with the Fc fragment with which IgM Gl reacts. Thus, A/J anti-V region Gl antibodies bind to the human Fcγ fragment in addition to IgM Gl. Similarly, a monoclonal CB6 F_1 anti-anti-V region Gl antibody interacted with the V region of IgM Gl as well as with

the syngeneic anti-V region antibodies. We called these anti-idiotypic antibodies epibodies because they interact with epitopes of the primary antigen. These homobodies and epibodies, obtained by immunization across heterologous barriers, represent new examples of recognition of the internal image of the antigen within the immune system.

Received for publication 22 June 1982.

References

1. Lieberman, R., and W. Humphrey. 1972. Association of H-2 types with genetic control of immune responsiveness to IgA allotype in mouse. *Proc. Natl. Acad. Sci. U. S. A.* **68:**2510.
2. Bona, C., P. K. A. Mongini, K. E. Stein, and W. E. Paul. 1980. Anti-immunoglobulin antibodies. I. Expression of cross-reactive idiotypes and Ir gene control of the response to IgG_{2a} of the b allotype. *J. Exp. Med.* **151:**1334.
3. Kunkel, H. G., V. Agnello, F. G. Joslin, R. J. Winchester, and J. D. Capra. 1973. Cross-idiotypic specificity among monoclonal IgM protein with anti-γ-globulin activity. *J. Exp. Med.* **137:**331.
4. Agnello, V., A. Arbetter, I., DeKasap, R. Powell, E. Tan, and J. Joslin. 1980. Evidence for a subset of rheumatoid factors that cross-react with DNA-histone and have a distinct cross-idiotype. *J. Exp. Med.* **151:**1514.
5. Roland, J., and P. A. Cazenave. 1979. Mise en evidence d'idiotypes anti-b4 apparentes chez les lapins de phenotype $a3^+$ immunisés contre l'allotype b4. *C. R. Acad. Sci. Paris.* **288:**571.
6. Gilman-Sachs, A., S. Dray, and W. J. Horng. 1980. A common idiotypic specificity of rabbit antibodies to the a2 allotype of the immunoglobulin heavy chain variable region. *J. Immunol.* **125:**96.
7. Kunkel, H. G., R. J. Winchester, F. G. Joslin, and J. D. Capra. 1974. Similarities in the light chains of anti-γ-globulins showing cross-reactive specificities. *J. Exp. Med.* **149:**128.
8. Nisonoff, A., F. C. Wissler, and L. N. Lipman. 1960. Properties of the major component of a peptic digest of rabbit antibody. *Science (Wash. D. C.).* **132:**1770.
9. Tung, A. S., S. T. Ju, S. Sato, and A. Nisonoff. 1976. Production of large amounts of antibodies in individual mice. *J. Immunol.* **116:**676.
10. Hiernaux, J., and C. Bona. 1982. Shared idiotypes among monoclonal antibodies specific for different immunodominant sugars of lipopolysaccharide of different gram negative bacteria. *Proc. Natl. Acad. Sci. U. S. A.* **79:**1616.
11. Hunter, R. 1970. Standardization of the chloramine-T method of protein iodination. *Proc. Soc. Exp. Biol. Med.* **133:**986.
12. Lindemann, J. 1979. Homobodies: do they exist. *Ann. d'Immunol. (Inst. Pasteur).* **130C:**313.
13. Bona, C., and J. Kearney. 1981. Anti-immunoglobulin antibodies II. Expression of individual and cross-reactive idiotypes on syngeneic and homologous anti-idiotype antibodies. *J. Immunol.* **127:**491.
14. Wikler, M., J. D. Franssen, C. Collignon, O. Leo, B. Mariame, P. Van de Walle, D. De Groote, and J. Urbain. 1979. Idiotypic regulation of the immune system. Common idiotypic specificities between idiotypes and antibodies raised anti-idiotypic antibodies in rabbits. *J. Exp. Med.* **150:**184.
15. Bona, C., E. Heber-Katz, and W. E. Paul. 1981. Idiotypic-antiidiotype regulation. I. Immunization with a levan binding myeloma protein leads to the appearance of auto-anti-(anti idiotypic) antibodies and to the activation of silent clones. *J. Exp. Med.* **153:**951.
16. Sege, K., and P. A. Peterson. 1978. Use of anti-idiotypic antibodies as cell-surface receptor probes. *Proc. Natl. Acad. Sci. U. S. A.* **75:**2443.

17. Schreiber, B. A., P. O. Courand, C. André, B. Wray, and A. D. Strosberg. Anti-alprenelol anti-idiotypic antibodies bind to β-adrenergic receptors and modulate catecholamine-sensitive adenylate cyclase. *Proc. Natl. Acad. Sci. U. S. A.* **77:**7.
18. Clevinger, B., J. Schilling, L. Hood, and J. Davie. 1980. Structural correlates of cross-reactive and individual idiotypic determinants on murine antibodies to α 1 $\rightarrow$ 3 dextran. *J. Exp. Med.* **151:**1009.
19. Pawlita, M., E. Mushinski, R. J. Feldman, and M. Potter. 1981. A monoclonal antibody that defines an idiotope with two subsites in galactan-binding myeloma proteins. *J. Exp. Med.* **154:**1946.
20. Nordfang, D., and B. Rubin. 1981. Idiotypic regulation of mouse anti-H-2 antibody response I Induction of 5936-idiotype bearing Ig molecules upon immunization with H-2^k alloantigen. *Immunogenetics.* **12:**197.
21. Jerne, N. K. 1976. The immune system a web of V-domains. Harvey Lectures Academic Press, Inc. New York. **70:**93.