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...LTERATION IN H CHAIN V REGION AFFECTS COMPLEMENT ACTIVATION BY CHIMERIC ANTIBODIES[1]

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...imeric antibodies to the synthetic polypeptide ...Glu)-Ala—Lys ((T,G)-A—L) were used to ex...e C activation by human IgG1. Two IgG1 anti...s, which contained mouse L chains and H ...s with mouse V domains and human C do..., differed only in their V_H domain. Ag binding ...C activation by these antibodies were analyzed ...ELISA. When limiting amounts of Ag were used ...e assays, the antibodies required different ...ities of Ag for optimal binding, suggesting that ...ntibodies bind to different epitopes on the ...A—L molecule. However, when competitive ...ition assays were performed with an optimal ...ntration of Ag, there were no differences in ...ve binding affinities for (T,G)-A—L or dissocia... characteristics of the antibodies. C activation ...amined at optimal Ag concentration to en... equivalent binding of two IgG1 antibodies to ...fter combination with immobilized Ag, these ...ntibodies bearing different V regions exhibited ...ed differences in the binding of C components ...nd C3d. When present in equal amounts in the ..., antibody 10B activated C and bound more ...nd C3d than antibody B11. These results in...e that V region differences can affect C acti...n by IgG.

...nsist of V regions that are responsible for Ag ...g, and C regions that are responsible for effector ...ns such as C activation, antibody-dependent cell-...ytotoxicity, and FcR binding (1–4). It has been ...wn that after combination with Ag the antibody ...ges an allosteric change which allows greater ac...ity or higher affinity of C1q for the C1q-binding ...the Fc region of the antibody (5, 6). Physical data ...port this hypothesis are lacking. Others have ...e that Ag/antibody stoichiometry as well as anti...finity and avidity can affect Ig functions (7–14). ...ric antibodies consisting of human C regions and ...e V regions have been used to examine the effector ...ns of human Ig; this system affords the ability to examine antibodies of different IgG subclasses that have defined V regions (15). We have constructed antibodies to the synthetic polypeptide Ag ((T,G)-A—L),[3] using two different rearranged murine H chain V region genes joined to human IgG1 or IgG4 C region genes. Two IgG1 antibodies, which differ at the D and J regions of the H chain V domain (16), showed markedly different abilities to activate C. Differences in C1q binding were observed for antibodies with the same affinity for (T,G)-A—L when present at equal density in the assay. Our data indicate that V region changes can influence C activation by antibodies combined with Ag. We suggest that the pattern of IgG deposition on the Ag is important to the effector functions of the Ig.

MATERIALS AND METHODS

Reagents and cells. Synthetic polypeptides ((T,G)-A—L) and (GT) were purchased from ICN Biomedicals (Costa Mesa, CA). (T,G)-A—L (molecular mass approximately 200,000 Da) is a branched chain polymer consisting of exposed tyrosine and glutamic acid residues connected to a poly-lysine backbone by alanine residues. GT (molecular mass = 19,300 Da) is a linear polymer of randomly mixed glutamic acid and tyrosine residues at a 9:1 molar ratio. The same lot of each polypeptide was used throughout the study.

mAb T17 and T14 bind to the GT residues of (T,G)-A—L and to GT and were produced by Pincus et al. (17). T17 and T14 are encoded by the same V_H genes, but different D_H and J_H genes (16), (Fig. 1). We used H chain V genes from these mAb and human C region genes to form chimeric H chain constructs in the vector pSV-E2-neo (18). Human C region genes were gifts of Dr. Philip Leder (Harvard Medical School, Cambridge, MA) (IgG1) and Dr. Leroy Hood (California Institute of Technology, Pasadena, CA) (IgG4). The plasmid pSV-E2-neo was a gift from Dr. Thomas Simon (University of Koln, Cologne, Germany).

T17.2 cells secrete only the L chain of T17 antibodies and were derived as follows. Hybridoma T17 expressing T17 mAb (17) was treated with three successive rounds of anti-mouse IgM and C. Surviving cells were cloned, and one clone, T17.2, secreted κ-chain only. Southern analysis of T17.2 DNA revealed that these cells had lost the H chain genes. All cell lines were maintained in DMEM supplemented with 10% FCS (Hyclone, Logan, UT) plus 3 mg/ml glutamine, 62.5 μg/ml gentamicin, and 200 IU/ml penicillin. Cell lines transfected with chimeric H chain genes were selected and maintained in medium containing 1 mg/ml geneticin (G-418, GIBCO, Grand Island, NY).

All enzymes (restriction endonucleases, DNA ligase, polynucleotide kinase, and Klenow fraction of DNA polymerase) were purchased from New England Biolabs, Beverly, MA. Monoclonal anti-mouse kappa (19) was conjugated to alkaline phosphatase by using 0.2% glutaraldehyde, and anti-C3d (Genzyme, Boston, MA) was biotinylated by using biotin-*N*-hydroxysuccinimide (The Binding Site, San Diego, CA).

Construction and expression of Ig genes. H chain IgG genes were constructed in the plasmid pSV-E2-neo (18). First, human C region

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[3] Abbreviations used in this paper: (T,G)-A—L, synthetic branched chain polypeptide (Tyr, Glu)-Ala—Lys; GT, linear polymer consisting of Glu and Tyr at a 9:1 molar ratio; DD_{50}, 50% dissociating dose; PCR, polymerase chain reaction, CH_2, second constant domain of IgG; K_a, association constant; PEG, polyethylene glycol; CDR, complementarity-determining region.

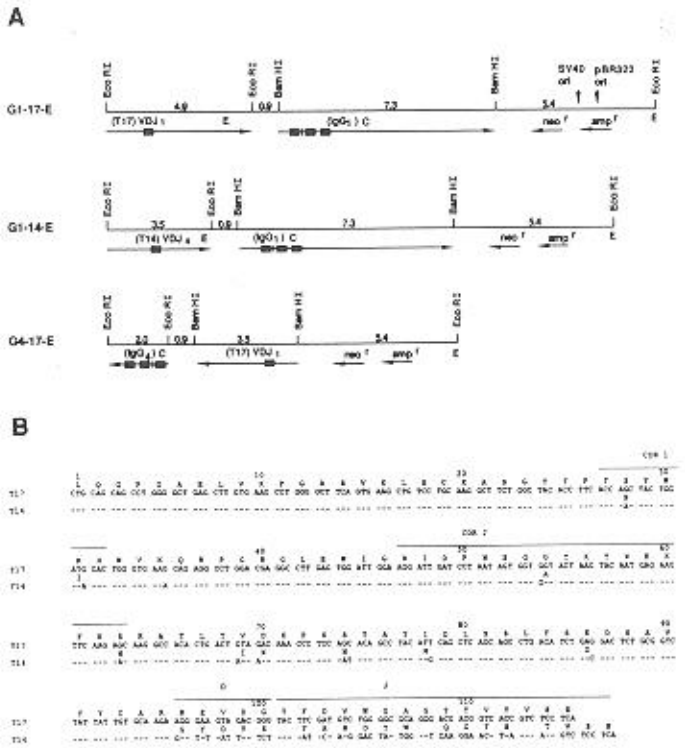

Figure 1. *A*, H chain constructs containing murine V region genes and human C region genes. Rearranged mouse H chain V region genes derived from mAb T17 and T14 were joined with human genomic C region genes in the expression vector pSV-E2-neo as shown. The exons corresponding to VDJ, and H chain C regions CH_1, hinge, CH_2, and CH_3 are depicted as blocks. *E* denotes the location of the murine Ig H chain enhancer. The size in kilobases of each portion of the constructs is noted. The presence of additional enhancer sequences in G1-17-E and G1-14-E did not result in increased expression of these constructs (data not shown). *B*, sequence comparison of T17 and T14 V region genes. The sequences of the two V_H genes used for the constructs were previously determined (16). Identity is indicated by (-).

genes for IgG1 and IgG4 were inserted at the *Bam*HI and *Eco*RI sites, respectively. Then, V region genes from the two anti-(T,G)-A—L mAb, T17 and T14 (16), were inserted at the unoccupied *Eco*RI or *Bam*HI site (Fig. 1). The H chain constructs were transfected into T17.2 hybridoma cells by electroporation (20, 21) with the use of a Gene Pulser (Biorad, Richmond, CA). Transfectants were selected for resistance to the antibiotic geneticin (G-418). Culture supernatants were assayed for anti-(T,G)-A—L antibody by ELISA (see below) on days 14 to 28 after transfection. Cultures positive for antibody were cloned by limiting dilution, clones were grown in DMEM supplemented with 1 mg/ml G-418. Chimeric antibodies were purified from the supernatants on protein-G Sepharose (Zymed Laboratories, San Francisco, CA). The isotypes of the chimeras were confirmed by ELISA with the use of alkaline phosphatase-conjugated anti-human IgG1 and IgG4 (Sigma Chemical Co., St. Louis, MO) as the second antibody.

Characterization of Ag binding. Chimeras bearing the two different V regions were examined for binding to (T,G)-A—L or GT by ELISA. Microtiter plates (Immunlon II, Dynatech Laboratories, Chantilly, VA) were coated with 10 μg/ml (T,G)-A—L (1 μg/well) or 50 μg/ml GT (5 μg/well) in PBS (0.13 M NaCl, 0.023 M sodium phosphate (pH 7.4)) unless otherwise specified. At these concentrations, about 0.5 μg (T,G)-A—L and about 0.2 μg GT were bound/well. Culture supernatant or purified antibody was allowed to bind to the plate for 1 h at 37°C, then washed, and bound antibody was detected with either alkaline phosphatase-conjugated anti-mouse kappa, anti-human IgG1, or anti-human IgG4. The relative affinities for (T,G)-A—L and GT were determined by competitive inhibition ELISA (22, 23). First, antibodies were titered to determine the amount of antibody that gave the same OD_{405} reading (approximately 0.3 to 0.5). At this input, the binding of antibody was inhibited by increasing amounts of free (T,G)-A—L or GT. The amount of inhibitor that caused a 50% decrease in binding was reported as ID_{50}, for 50% inhibitory dose. To determine the ID_{50}, antibody and inhibitor were added to the plates simultaneously, incubated for 1 h at 37°C, and washed, and bound antibody was detected with either anti-mouse kappa or anti-human IgG1 or IgG4. In other experiments, antibody was on the plate for 1 h before addition of the inhibitor, then a further hour before the second (detecting) antibody was this case, the amount of inhibitor that caused the diss 50% of control-bound antibody (in the absence of inhi reported as DD_{50} for dissociating dose. The association for GT was measured in solution by using 7.5% PEG to immune complexes formed with the chimeric antibodies creasing amounts of ^{125}I-labeled GT (24) and calculated Arend and Mannik (25). We were unable to measure the K A—L by using the PEG assay, because (T,G)-A—L pre the presence of 7.5% PEG.

C activation assays. C activation was examined by the binding of C1q and C3d to antibodies bound to immobil A—L after incubation with normal human C or purified Experiments were performed with the same batch of fre human serum obtained from a single donor. For the C antibodies were incubated on plates coated with (T,G)-A— at 37°C, then washed and reacted with 100 μl of norma serum (1/50 dilution in PBS supplemented with 1% BSA, $CaCl_2$ and 0.5 mM $MgCl_2$) for 30 min at 37°C. After washi bound C1q was detected with alkaline phosphatase-conju human C1q (The Binding Site). Alternately, purified C1q (San Diego, CA) diluted to 10 μg/ml in the same buffer was u for whole C. C3d binding was measured similarly, exce incubation with whole C, bound C3d was detected with anti-C3d and alkaline phosphatase-conjugated streptavi Binding Site). When either BSA or nonspecific human fraction II, Sigma) was substituted for the anti-(T,G)-A—L ics, no measurable C1q or C3d binding was detected.

PCR amplification and sequencing. Genomic DNA transfectomas was prepared (26) and used as a template PCR. Oligonucleotide primers were synthesized on a Biosea ONE DNA synthesizer (San Rafael, CA), and the Gene (Perkin Elmer Cetus, Norwalk, CT) was used to amplify fragments corresponding to the hinge and the second C regi The primers used to amplify the hinge regions exactly nucleotides 557–576 and 1001–1020 (negative strand) lished nucleotide sequence for IgG1 H chain C region (primers had 5′ extensions that provided *Eco*RI recogni Primers used to amplify CH_2 regions exactly matched 1001–1020 and 1431–1450 (negative strand), also with gnition sequences. The reaction conditions were as follo at 95°C, 30 s at 50°C, and 30 s at 72°C for 30 cycles. The products were gel purified and cloned into the *Eco*RI plasmid pT7T3 (Pharmacia, Piscataway, NJ). dsDNA seque performed in both directions by using the Sequenase Cleveland, OH) and the PCR primers.

RESULTS

Expression of chimeric antibodies. Chimeric ies were expressed from murine V region genes man IgG1 or IgG4 C region genes by the murine oma cell line T17.2. The antibodies were purifie culture supernatants by protein G-Sepharose chr raphy and analyzed by electrophoresis on 12.5% acrylamide gels. Figure 2 shows the SDS-PAGE of the chimeras under reducing and nonreducing tions. H and L chain bands of expected m.w. appropriate ratio can be observed. The antibodie not aggregated as determined by their migratio single ca. 150-kDa band on a nondenaturing gradi (data not shown). The isotypes of the chimeric ant were determined by ELISA (Table I) to confirm th hybridomas expressed the introduced genes.

Ag-binding activity of chimeric antibodies. A ies bearing the H chain V domains of T17 and T1 in the D and J regions and in 9 amino acid p encoded by the V genes of the V region (16) (Fig. elucidate the effect of their different V domains binding, we examined the binding of the chimer bodies to (T,G)-A—L and GT. Four IgG1 antibodi with T17 V_H (10B and 9A) and two with T14 V_H (C6), showed similar Ag-binding characteristics.

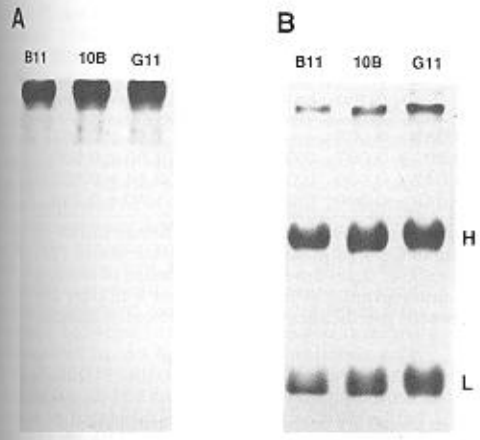

Figure 2. Polyacrylamide gel analysis of protein G-purified chimeric antibodies. Purified antibodies were run on 12.5% gels containing 0.1% SDS and stained with Coomassie brilliant blue. (*A*), samples were loaded without reduction of samples. (*B*), samples were heated at 100°C for 5 min in the presence of 0.7 M 2-ME before loading. The size of the H and L chains correspond to the migration pattern of (H) and (L) chains of standard human IgG (Cohn fraction II, Sigma).

...of a competitive inhibition ELISA, we measured the amount of free Ag required to inhibit the binding of antibody to Ag (ID_{50}) or dissociate antibody from Ag (DD_{50}) (Table II). The ID_{50} and DD_{50} of antibodies with either V region were not significantly different from each other (p ...). This was true when GT, as well as (T,G)-A—L, was used as inhibitor. It was not surprising that GT was better inhibitor of binding to (T,G)-A—L than (T,G)-A—L itself because the antibodies recognize glu and tyr residues of (T,G)-A—L (17) and more epitopes are likely to be present per microgram on GT in solution than on (T,G)-A—L. K_a values for GT binding were measured in solution by PEG precipitation of ^{125}I-labeled GT/antibody complexes (Table II). The K_a of binding to GT for antibodies with the different V regions were not significantly different ($p > 0.2$). We were not able to make similar measurements for (T,G)-A—L binding because (T,G)-A—L precipitated in 7.5% PEG in the absence of antibody.

When antibody binding was measured at various Ag concentrations, differences were observed for antibodies 10B and B11 (Fig. 3). Antibody 10B bound similarly to (T,G)-A—L at Ag concentrations from 1 to 30 μg/ml, whereas B11 showed less binding to (T,G)-A—L at Ag concentrations less than 3 μg/ml. The differences between 10B and B11 were even more evident in their binding to GT. 10B bound to GT optimally at GT concentrations of 10 μg/ml or higher; B11 required at least 50 μg/ml for optimal binding. Thus, approximately threefold more (T,G)-A—L and at least fivefold more GT were required for optimal binding of B11, compared with 10B. We also examined binding of antibody G11, an IgG4 bearing T17 V_H domains, to (T,G)-A—L and GT. Like 10B, G11 bound (T,G)-A—L equally at Ag concentrations of 1 to 30 μg/ml, and bound GT equally at Ag concentrations of 10 to 50 μg/ml (data not shown). These data concur with others (15) that recognition of the epitope is dependent on the V region regardless of isotype. Our data suggest that antibodies with different V regions bind to different epitopes that are present at different densities on (T,G)-A—L and GT. At higher Ag concentrations, the different epitopes are present at sufficient density to allow for equivalent binding of antibodies 10B and B11 (see Fig. 4*A*).

Higher GT concentrations were required for optimal binding than (T,G)-A—L because GT bound less well to the plate than (T,G)-A—L. Plates coated with 5 μg GT/well bound 0.2 μg, whereas (T,G)-A—L plates coated with 1 μg/well bound 0.5 μg. The apparent discrepancy between the need for more GT for binding in this experiment and the ability of GT to inhibit binding to (T,G)-A—L at very low concentrations as shown in Table II may also be caused by a different arrangement of epitopes when GT is immobilized on a plate as compared to being free in solution.

C activation by chimeras. The abilities of the two IgG1 chimeras, 10B and B11, to activate C when combined with (T,G)-A—L were compared. Antibodies were incubated on plates coated with 10 μg/ml (T,G)-A—L, and the resulting immune complex was reacted with C (normal human serum diluted 1/50). C1q and C3d binding to the immobilized Ag/antibody complex was detected by subsequent binding of anti-C1q and anti-C3d as described in *Materials and Methods*. To determine whether equivalent amounts of antibody bound to the plates in these experiments, anti-human IgG was used to detect the quantities of 10B or B11 antibody bound to the immobilized (T,G)-A—L (Fig. 4*A*). With equal amounts of antibody bound, 10B activated C better and bound more C1q and C3b than did B11 (Fig. 4*B* and *C*). As expected, the IgG4 chimera G11 did not bind C1q or C3d. When purified C1q (10 μg/ml) was used in place of whole C, the results were similar to those with whole C (Fig. 4*B*).

Sequence of PCR amplified C region domains. To determine if mutations had occurred in the C region genes to account for the differences in C activation by antibodies 10B and B11, we used PCR to amplify hinge and CH_2 exons from cellular DNA extracted from the transfectomas. Alterations in either region might affect C fixation

TABLE I

Isotype analysis of chimeric antibodies[a]

Antibody	Construct	Second Antibody (Alkaline Phosphatase Conjugated)					
		Anti-κ^{mu}	Anti-IgG1^{hu}	Anti-IgG4^{hu}	Anti-IgM^{hu}	Anti-IgG^{mu}	Anti-IgM^{mu}
T17	Parent	+[b]	−[c]	−	−	−	+
T14	Parent	+	−	−	−	+	−
B11	G1-14-E	+	+	−	−	−	−
10B	G1-17-E	+	+	−	−	−	−
G11	G4-17-E	+	−	+	−	−	−

[a] Culture supernatants from cloned, antibody positive cultures were tested for specific isotype by ELISA. Supernatants were allowed to bind to (T,G)-A--L-coated microtiter plates, and bound antibody was detected with different alkaline-conjugated second antibodies. Only the isotype of the introduced genes was detected for each clone. (mu, murine; hu, human.)

[b] + Denotes $OD_{405} > 0.20$ in the ELISA.

[c] − Denotes $OD_{405} < 0.01$.

TABLE II

Ag-binding characteristics of chimeric antibodies[a]

Antibody	[n]	Construct	(T,G)-A—L/(T,G)-A—L[b]		(T,G)A—L/GT[c]		GT/GT[d]	GT[e]
			ID_{50}	DD_{50}	ID_{50}	DD_{50}	ID_{50}	$K_a(M^{-1} \times 10^{-7})$
B11	(6)	G1-14-E	0.54 ± 0.22	1.20 ± 0.01	0.006 ± 0.00	0.035 ± 0.005	ND	1.41 ± 0.361
C6	(5)	G1-14-E	0.54 ± 0.29	1.55 ± 1.0	0.005 ± 0.007	0.30 ± 0.007	0.003 ± 0.001	2.36 ± 0.209
10B	(5)	G1-17-E	0.72 ± 0.20	1.50 ± 0.14	0.003 ± 0.001	0.042 ± 0.000	0.006 ± 0.003	2.44 ± 0.525
9A	(3)	G1-17-E	0.32 ± 0.03	1.75 ± 0.07	0.003 ± 0.001	0.048 ± 0.006	0.005 ± 0.001	1.70 ± 0.218

[a] The characteristics of antibody binding to (T,G)-A—L and GT were measured by competitive inhibition ELISA to determine relative affinities and strengths of interaction. The K_a for binding to GT was measured by solution phase PEG precipitation assay as described. ID_{50} denotes the amount (micrograms) of inhibitor that caused a 50% decrease in binding when added at the same time as the antibody. DD_{50} denotes the amount (micrograms) of inhibitor added 1 h after the antibody, which caused a 50% dissociation of bound antibody. Values represent the mean ± SD of the indicated number of replicate samples (*n*).

[b] Binding to immobilized (T,G)-A—L was inhibited by free (T,G)-A—L.

[c] Binding immobilized (T,G)-A—L was inhibited by free GT.

[d] Binding to immobilized GT was inhibited by free GT.

[e] K_a was measured for binding to GT only, since (T,G)-A—L precipitated in the assay in the absence of antibody.

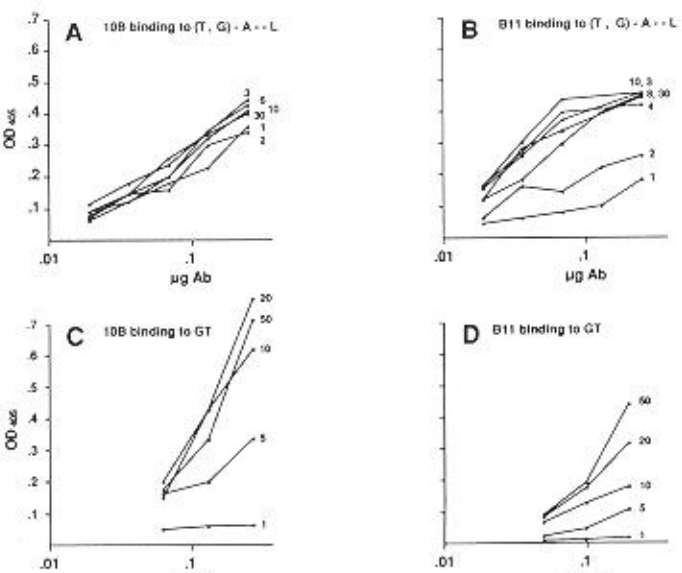

Figure 3. Binding of chimeric antibodies to (T,G)-A—L and GT at various Ag concentrations. ELISA plates were coated with various concentrations of (T,G)-A—L or GT. The binding of 10B (*A* and *C*) and B11 (*B* and *D*) was measured at increasing antibody input. (*A* and *B*) show binding to (T,G)-A—L and (*C* and *D*) show binding to GT. Ag concentrations (micrograms per milliliter) are indicated adjacent to each curve. Optimum binding of antibody to (T,G)-A—L was obtained at 1 μg/ml and 3 μg/ml for 10B and B11, respectively. Optimum binding of antibody to GT required 10 μg/ml for 10B and at least 50 μg/ml for B11. The points on the graphs represent the average of duplicate samples. There was always ≤10% difference between the duplicates.

because the hinge may be involved in the activation of C (5, 28, 29), and the C1q-binding site is present in CH_2 (30). We found no differences between the expected sequences (27) and those of the amplified DNA exons from either 10B or B11 cellular DNA.

DISCUSSION

Mutations in the genes encoding antibody molecules have helped to elucidate the structure and function relationship of Ig (28, 31–36). In most cases, alterations in the V region affect Ag-binding characteristics (31–33, 36) whereas alterations in the C region affect C activation (28, 37–39). However, in some instances, mutations in the C region resulted in altered Ag binding (34, 35). In other studies, altered effector functions of IgG antibodies directed against the same Ag stem from differences in affinity or avidity (7–10) or in antibody/Ag stoichiometry (11–14). We present evidence that differences in Ag binding that are not related to avidity or antibody/Ag stoichiometry can affect the C activation of closely related antibodies.

We constructed and expressed chimeric Ig genes using two different cloned H chain V region genes mAb to the synthetic peptide (T,G)-A—L. The chi Ig express human H chain C regions, murine H ch regions, and murine L chains. The L chains of th meric antibodies are identical. Other studies (15) that murine L chains can be paired with chime chains to produce antibodies with the expected ef functions. The V_H region genes were previousl quenced and shown to differ at the D and J region at 9 amino acid positions encoded by the V genes Although this region comprises part of the third h variable region or CDR3, which is involved in Ag nition (40), antibodies expressing these two genes similar (T,G)-A—L binding characteristics when as at high Ag density. However, they appear to bi different epitopes on the Ag, as shown by the amo Ag necessary for optimal binding (Fig. 3). Even th the antibodies may recognize different epitopes on A—L, the relative affinities (estimated by ID_{50} (22 strength of binding (estimated by DD_{50}) to either A—L or GT were similar for antibodies with eit region (Table II).

Although the binding to (T,G)-A—L at optimal Ag centration was the same for both 10B and B11 activation of C by these two antibodies differed sub tially. 10B activated C and bound significantly mor and C3d than B11 (Fig. 4). Differences in C activati IgG antibodies can be attributed to a variety of fa We have ruled out differing densities of the two ant ies on the plate by showing that equal amounts o and B11 bound to the (T,G)-A—L plates (Fig. 4*A*). both antibodies exhibit the same relative affinity f and the same dissociation characteristics, so the dis ancy in C activation cannot be explained by a diffe in the strength of the interaction between Ag and body.

Although the antibodies were constructed with same C region genes, mutations incurred during expression scheme could affect the C activation o resulting chimeric antibodies. Previous studies hav scribed C region mutations that caused gross chang the constant region domains (34, 35, 41–44). We out such mutations in our antibodies, because F analysis of the purified chimeras did not reveal an

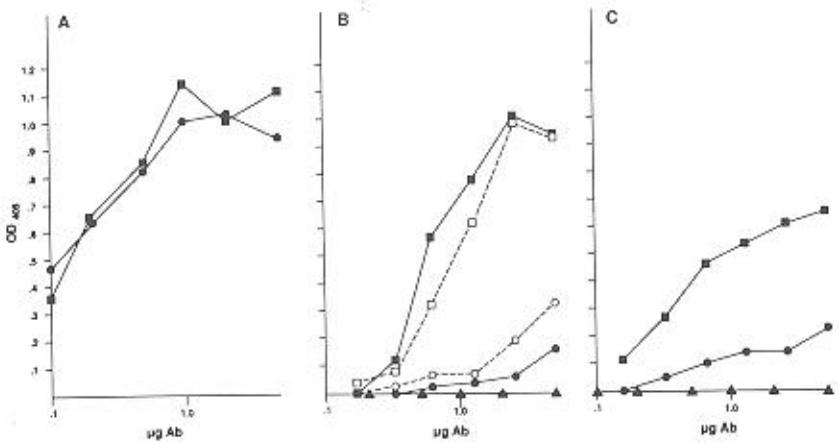

Figure 4. C activation by chimeric antibodies. *A*, comparison of 10B and B11 binding to (T,G)-A—L at high antibody input. The binding of antibodies 10B (■) and B11 (●) to plates coated with 10 µg/ml (T,G)-A—L was measured under the same conditions as used for C-binding assays. Equal amounts of both antibodies bound to the plates per microgram of antibody added. In this experiment, anti-human IgG1 was used as the detecting antibody; similar results were observed when anti-mouse kappa was used (data not shown). *B*, binding of C1q by chimeric antibodies bound to immobilized (T,G)-A—L. C1q binding was measured by ELISA as described. Antibodies 10B (■, □) and B11 (●, ○) were bound to plates coated with 10 µg/ml (T,G)-A—L, then incubated with whole C (*solid lines*) or purified C1q (*dotted lines*). C1q bound to the antibodies was detected with anti-human C1q. An IgG4 chimeric antibody with the T17 V_H gene, G11 (▲), demonstrated no binding of C1q in this assay. *C*, binding of C3d by chimeric antibodies bound to immobilized (T,G)-A—L. C3d binding by 10B (■), B11 (●), and G11 (▲) was measured after the antibodies were bound to (T,G)-A—L plates and incubated with whole C as described in *Materials and Methods*.

differences in the size of the H chains of 10B and B11 (Fig. 1). Furthermore, the equal size of the H chains from the two antibodies suggest these are similarly glycosylated. Because the second constant domain (CH_2) contains the binding site for C1q and mutations in this region can affect C activation (30, 37), we amplified and sequenced the CH_2 domains of 10B and B11 DNA. There were no differences in the sequences of the amplified CH_2 domains of 10B and B11. The hinge region may also affect activation (5, 28, 29), but we found no sequence differences in the amplified hinge regions of 10B and B11. Thus, the difference in C activation by 10B and B11 was not caused by C region mutations.

Our failure to find C region mutations to account for differences in C activation by 10B and B11 suggests that major changes in the CDR3 of V_H (which is encoded by D and J genes, and is involved in Ag recognition) and/or minor changes (9 amino acid changes) elsewhere in the V region can influence the effector functions of these antibodies. There are at least two possible explanations for this. First, it is possible that the antibodies, although present in equal quantities, were arranged differently on the immobilized Ag such that the Fc portions had an inconsistent spatial distribution. This could cause them to activate C differently. In fact, we have demonstrated that antibodies 10B and B11 recognize (T,G)-A—L somewhat differently (Fig. 3), and therefore could bind to the Ag in a different pattern. Alternately, the difference in the V_H domain may influence the ability of the IgG to undergo a conformational shift upon combination with Ag that allows for C activation such as has been described for IgM (5). This type of "second signal" has been proposed for the activation of IgG effector functions (4, 6, 45, 46), although direct evidence for such a phenomenon is not available. Our data are consistent with the premise that variations in the CDR3 portion of the Ag-binding site cause differences in recognition of Ag, which leads to a different pattern of IgG deposition on the immobilized Ag. This arrangement affects C activation by the IgG.

Studies with chimeric mouse/human antibodies have been instrumental in the evaluation of the effector functions of human IgG (15, 47, 48). From experiments on antibodies consisting of identical V regions and C regions of different IgG subclasses, one can determine relative efficiencies of C activation, Fc receptor interaction, antibody-dependent cellular cytotoxicity, or other functions. This information is useful for the design of rIg for immunotherapy. However, we have just shown that two IgG1 antibodies with only slightly different Ag-binding specificities have quite different C activation capabilities. Similar differences within subclasses may exist in antibodies directed against other Ag. One should be cautious in choosing antibodies for immunotherapy if the effector functions of the administered antibodies are important to the therapy. The characteristics of one set of antibodies may differ from those of a second set of antibodies directed against a different epitope on the same Ag.

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