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Original Investigations

A Monoclonal Mouse Antiallotype Antibody Reacts with Certain Human and Other Vertebrate Immunoglobulins: Genetic and Phylogenetic Findings

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Abstract. A new mouse monoclonal antibody, 18.1, recognizes an allotypic determinant on mouse IgG_1 of the "a" allotype. We found that this alloantibody reacts with immunoglobulins of evolutionarily distant vertebrate species including man, and with only certain isotypes or allotypes in some of these species. Most mammalian sera are reactive, except those from lagomorphs, marsupials, and monotremes. Avian and reptilian sera are also positive, while the amphibian and fish sera tested were negative. In human sera, 18.1 detects an isotypic marker on IgG2 and an allotypic marker on IgG3. This reactivity parallels the distribution of the Gm non-g determinant. These findings are discussed in relation to the phylogeny of IgG and its subclasses.

Introduction

Domain homology suggests that gene duplication has played an important role in the evolution of the immunoglobulin (Ig) constant region. Both DNA (Honjo et al. 1979) and protein (Edelman et al. 1969) sequences provide primary evidence for this concept. On another level, the homologies between the Ig classes indicate more recent gene duplications. The first IgG-like immunoglobulin class, as opposed to the IgM class Igs, may have arisen in the original terrestrial vertebrates, the amphibia. Although the evolutionary relationships among avian, mammalian, and reptilian IgGs are not clear (Kubo et al. 1973), their IgG classes probably arose from one or more gene duplication events distinct from those yielding amphibian IgGs (Hadji-Azimi 1979). In any case, prior to the radiation of mammals, avian and mammalian prototype IgG differed considerably, as evidenced by their limited serological cross-reactivity. This constrasts with the widespread cross-reactivity amongst IgGs of mammals.

Further IgG-gene duplication and divergence were common, as shown by the appearance of several subclasses or isotypes of IgG within most mammalian species.

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In addition, allelic sequences within isotypes of a given species diverged, yielding the genetic polymorphisms known as allotypes. Allotypy has been studied most extensively in man (Van Loghem 1978, Grubb 1970), the mouse (Herzenberg and Herzenberg 1978), the rabbit (Oudin 1960), and the chicken (Foppoli et al. 1979). Usually, allotypes have been assigned by the use of alloantisera, although in the human system heteroantisera also have been used. It is clear that the allotypic forms of Igs differ from one another to a moderate degree, with allotypic determinants scattered throughout the molecule. In particular, the advent of hybridoma antibody technology (Kohler and Milstein 1975), which allows production of monoclonal, monospecific antibodies in large quantities, has led to rapid definition of a considerable number of allotypic determinants on mouse IgG_{2a} (Igh-1b) proteins (Oi and Herzenberg 1979; see Green 1979, for nomenclature). At least four separate allospecificities were demonstrated, mapping to the hinge, CH2, and CH3 portions of the molecule. We examined monoclonally derived antibodies directed against these and other allotypic determinants of the mouse for reactivitiy with human sera or immunoglobulins. This paper is about one monoclonal antibody, which recognizes an allotypic determinant on mouse IgG_1 (Igh-4a). This antibody also reacts with certain subclasses and allotypes of human immunoglobulins and with Igs of diverse phylogenetic origin, making it a useful reagent for evolutionary investigations of immunoglobulins.

Materials and Methods

Antibody 18.1. Hybridoma 18.1 was generated by Dr. Vernon Oi of our laboratory as previously described (Oi et al. 1978). The tumor parent was the myeloma cell line NS-1 and the spleen cell donor was an SJL (Igh^b) mouse immunized with pertussis-SJA (Igh^a) anti-pertussis antigen-antibody complexes. This immunization should yield antibodies which recognize the differences between a and b allotype immunoglobulin molecules. Hybrid 18 was derived from this fusion. Following incubation with antigen-coupled fluorescent beads, bright cells were cloned using the fluorescence-activated cell sorter as described by Parks and co-workers (1979). 18.1 is one of the resulting clones.

The monoclonally derived antibody was purified by ammonium sulfate precipitation and ion-exchange chomatography. The monoclonal origin of the protein was confirmed by two-dimensional electrophoresis (O'Farrell et al. 1977). Gel analysis also indicated the presence of the NS-1 light chain.

Iodinations. Iodinations were performed using the iodogen method (Fraker and Speck 1978) or, if the protein was bound to an immunosorbent (Herzenberg and Herzenberg 1978), using the chloramine T method. Usually, 0.5 mCi of ^{125}I (Amersham) per 50 µg protein was used. Free ^{125}I was separated from the labelled protein by chromatography over PD-10 columns (Pharmacia). CNBr-activated sepharose for immunosorbents was purchased from Pharmacia.

Solid phase radioimmunoassay. The general form and rationale of this assay has been well described (Tsu and Herzenberg 1978). For most assays reported here antibody at 30 µg/ml was coated onto the wells of a flexible microtiter plate (Cooke). Subsequent thorough washing with phosphate-buffered saline (PBS) plus 1% bovine serum albumin (BSA) saturated any free protein-binding sites on the plate. Ten µl of dilutions of various test sera or purified Igs were applied, along with radiolabelled antigen (30 000 dpm in 20 µl, approximately 5 mCi/mg). The labelled antigen in most cases was a known Igh-4a protein, MOPC 21. After 1 h incubation at room temperature, the wells were rinsed as above, removed, and counted. Inhibition of ^{125}I-MOPC 21 binding to the hybridoma indicates the presence of cross-reactive antigen in the test sample. To avoid nonspecific inhibition all test sera were microfuged.

Immunoglobulins and sera. The following investigators kindly provided sera or immunoglobulins: Dr. Donald Capra of Southwestern Medical School (mammalian sera, avian sera, fish sera, and subtyped human myeloma proteins); Dr. John Cebra and Dr. Jack Daiss of Johns Hopkins University (guinea pig sera and Igs); Dr. Ed Cooper of University of California at Los Angeles (reptilian and amphibian sera); Dr. James Goding of the Walter and Eliza Hall Institute, Melbourne, Australia (lower mammalian sera), Dr. John Kearny of the University of Alabama (affinity-purified anti-human kappa and anti-human gamma antisera); Dr. Jeffrey Ledbetter of Stanford University (rat hybridoma proteins, Ledbetter and Herzenberg 1979); and Dr. Mel Schanfield of the American Red Cross (Gm-typed human myeloma proteins).

Affinity purification of samples and electrophoretic analyses. SDS gel electrophoresis (Laemmli 1970) was used to examine the biochemical characteristics of molecules bearing the 18.1 antigen. ^{125}I-labelled protein preparations were incubated in wells which had previously been coated with antibody. After 1 hour, the wells were rinsed three times with PBS plus BSA, and three times with PBS alone. The antigens were then eluted from the plate with glycine-HCl, pH 3.2. This protocol allows removal of antigen, while the material which is directly, yet noncovalently, bound to the polyvinyl chloride surface (in this case, antibody and BSA) remains on the plate. Thus artifacts of gel overloading are avoided. The amount of radioactivity required per well varied according to the protein preparation. For example, in analyses of ^{125}I-labelled whole human serum, approximately 10^7 cpm per well were needed, while ^{125}I-radiolabelled papain digests of purified human IgG3 required only 10^6 cpm for analysis. Following elution, the pH was adjusted and the sample was placed in gel sample buffer with or without a reducing agent [25 mM dithioerythreitol (DTE)]. After electrophoresis, the gels were stained, dried and autoradiographed at $-70°$ using Cronex Lightning Plus enhancing screens and Kodak XR film. Molecular weight markers were purchased from Pharmacia.

Papain digestions. Human IgG3 is very sensitive to proteolysis (Jefferis et al. 1968) due to its extended hinge region (Michaelson et al. 1977). Therefore, the papain digestion was performed under very mild conditions. Mercuripapain (Millipore) at 100 µg/ml in tris-HCl (50 mM, pH 8) plus 0.9% NaCl was activated by the addition of DTE and EDTA to final concentrations of 0.1 mM and 2 mM respectively. Activated enzyme was then added in a mg ratio of 1 : 100 enzyme to myeloma protein, in the same buffer, but containing no reducing agent. The final DTE concentration was 0.01 mM. Aliquots were removed at various time intervals and the reaction was terminated by oxidation with dehydroascorbic acid at 1 mM. SDS gel analysis under nonreducing conditions revealed an optimum digestion time of 30 min.

Results

We tested several mouse anti-mouse allotype antibodies (Oi et al. 1978) for reactivity with human sera in radioinhibition assay. These included eight anti-Igh-1a's, eight anti-Igh-1b's, two anti-Igh-4a's, and four anti-Igh-4b's. Antibody 18.1, which recognizes an allotypic determinant of mouse IgG_1 (Igh-4a), alone reacted. We therefore examined its binding to various vertebrate sera. A summary of these results is presented in Table 1. Most mammalian sera contained an antigen recognized by 18.1, with the exception of lagomorph and lower mammalian sera. The determinant was also detectable in avian and reptilian samples, but not in amphibian or fish sera.

Since a monoclonal antibody reacts with a single determinant, we felt it important to confirm that reactivity was confined to homologous molecules – IgGs. Pooled chicken gamma globulin was reactive, indicating that this Igh-4a-like determinant resides on an immunoglobulin molecule in chicken serum. However, either the affinity of the antibody is much lower for chicken IgG or only a fraction of the IgG molecules possess this determinant, since approximately ten times more chicken Ig than mouse IgG_1 is required for similar inhibition. Since reptilian sera

Table 1. 18.1 antigen in vertebrate sera

Species	18.1 antigen titer	Species	18.1 antigen titer
Mammals:		Birds:	
Rodents		chicken*	+
mouse "a" allotype	+ +	duck*	+
mouse "b" allotype	–	Reptiles:	
rat	+	*Chrysemys scripta* elegans* (turtle)	+/–
guinea pig	+ +	*Genthohotus multi-carinatus** (alligator lizard)	+/–
Carnivores		*Cneudephorius tigris** (whiptail lizard)	+/–
dog	+ +	Amphibia:	
siberian tiger*	+ +	*Rana pipiens**	–
Primates		*Xenopus**	–
man	+ +	Fish:	
chimpanzee	+ +	catfish*	–
capuchin monkey	+ + +		
squirrel monkey	+ + +		
Perissodactyls			
horse	+ +		
Artiodactyls			
cow	+ +		
sheep	+ + +		
goat	+ + +		
pig	+ + +		
Lagomorphs			
hare	–		
cottontail rabbit	–		
*Lepus timidus**	–		
Ochotona	–		
Marsupials			
oppossum*	–		
pandemelon wallaby*	–		
Monotremes			
echidna*	–		

* One individual serum from this species tested.
† Titers of 18.1 antigen were determined by solid-phase radioinhibition assay as described in *Materials and Methods*: – no detectable antigen, +/– 15–25 percent inhibition of MOPC 21 binding at lowest serum dilution, + 50 percent inhibition at serum dilution fourfold or more lower than "a" allotype mouse, + + 50 percent inhibition at serum dilution similar to "a" allotype mouse, + + + 50 percent inhibition at serum dilution fourfold or more higher than "a" allotype mouse.

showed marginal activity in the assay (about 20 percent inhibition at 1:5 serum dilution), it was necessary to determine if it could be attributed to Igs. Turtle serum was labelled with ^{125}I and any cross-reactive antigen was bound to antibody 18.1 coated onto the wells of a microtiter plate. Bound material was eluted and analyzed by SDS gel electrophoresis under reducing and nonreducing conditions. As seen in Figure 1, the bound material was a molecule slightly larger than human IgG and was composed of disulfide bonded subunits of about 71 K and 25 K molecular

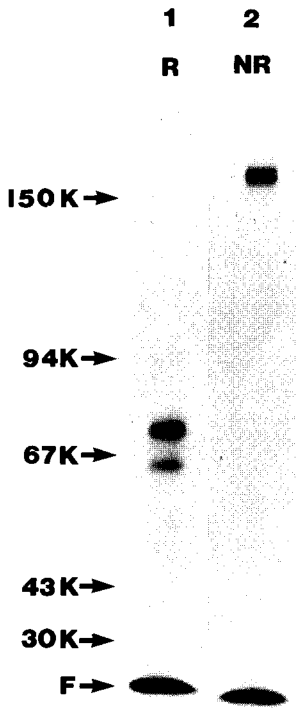

Fig. 1. SDS gel electrophoresis of 18.1 antigen-bearing molecules of turtle sera. Molecules possessing the 18.1 determinant were isolated from ^{125}I-labelled whole turtle serum as described in the text. Lane 1 contains the purified molecules electrophoresed under reducing conditions, lane 2 the same material under nonreducing conditions. At this acrylamide concentration (7.5 percent), the light chains run at the dye front, but the larger molecules are well resolved. The position of molecular weight markers is indicated to the left of the gel: human IgG, phosphorylase B, BSA, ovalbumin, carbonic anhydrase. The dye front is indicated by "F".

weight. This corresponds well to the molecular weight values of 7.5 S Ig seen in the turtle *Pseudemys scripta* (Leslie and Clem 1972). A fainter band was seen at 53 K Mr. The identity of this protein was not further pursued, but since it was more prominent in older preparations it could result from proteolytic cleavage. These molecular weight estimates, in particular the presence of 25 K Mr light chains, were confirmed by electrophoresis in 10 percent polyacrylamide gel.

The 18.1 determinant was also localized to IgG in three other species: guinea pig, rat, and man. Purified guinea pig IgG1 (strain 13) was strongly inhibitory, while IgG2 from the same strain showed no reaction. Serum from strain 2 reacted in a fashion similar to strain 13 serum. Individual nonimmune sera from 15 guinea pigs of the outbred Hartley strain differed considerably in reactivity, indicating quantitative or qualitative variation in the 18.1 determinant. In studies of rat Ig, the only purified hybridoma proteins to react were also of the IgG_1 class.

We were most interested to discover whether 18.1 detected isotypic or allotypic specifities on human Ig. Accordingly we tested purified myelomas of known subclass and allotype in this system. Figure 2 exemplifies the data from which our conclusions were drawn; Table 2 summarizes the results. Antibody 18.1 does not react with human IgG1 and IgG4. It does react with all IgG2s tested. Of the IgG3 proteins tested, 14 reacted, and one did not (IgG3 Cro). Since this result implies that 18.1 reacts with a determinant on IgG3 which is polymorphic, we felt it important to confirm that IgG3 Cro is indeed a bona fide IgG3. To do this we exploited characteristics unique to the IgG3 subclass: the lack of protein-A binding sites (Kronvall and Williams 1969) and a larger heavy chain. IgG3 Cro and other myeloma proteins were tested for their ability to inhibit the reaction of a known protein-A binding myeloma with *Staphylococcal* protein A coated onto plastic

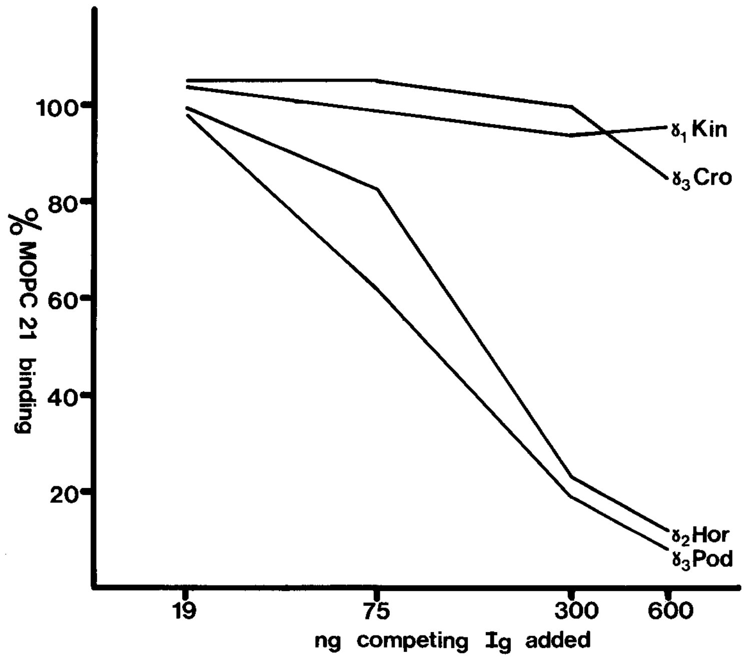

Fig. 2. Isotype and allotype specificity of monoclonal antibody 18.1: radioinhibition assay with human myeloma proteins. The assay was performed as described in *Materials and Methods* with antibody 18.1 as plate coat. The amount of the test myeloma added per well is indicated on the *abscissa*. 18.1 antigen on the sample is detected by inhibition of the binding of a radiolabelled Igh-4a protein, MOPC 21, as indicated on the *ordinate*. MOPC 21 binding in the absence of any competitor is defined as 100 percent.

Table 2. 18.1 antigen on human IgG. Allotype and isotype specificity

Subclass	Allotype		18.1 reactivity
IgG1	f	(9)	–
15 kappa	za	(6)	–
10 lambda			
3 n. d.	zax	(4)	–
IgG2	n	(6)	+
10 kappa			
4 lambda	n. d.	(8)	+
IgG3	b	(11)	+
11 kappa			
4 lambda	g	(1)	–
IgG4	n. d.	(3)	–
3 kappa			

wells. Like other IgG3's, IgG3 Cro did not inhibit binding to protein A, while proteins of other IgG subclasses clearly did (Fig. 3). Figure 4 shows that IgG3 Cro possesses the 60 K Mr heavy chain appropriate to the IgG3 subclass.

These findings suggest that the Igh-4a-like determinant is polymorphic on human IgG3. Allotypes are known on IgG3, with the most common forms possessing either G3m b (Gm 5) or G3m g (Gm 21) specificities (for review see Van Loghem 1978, Grubb 1970). IgG3 Cro is typed as a G3m g protein, while the remaining IgG myelomas are typed as G3m b. We decided to confirm this correlation with Gm type by studying the reaction of 18.1 with normal serum IgG. IgG from individuals of known Gm type were purified by binding to anti-IgG covalently coupled to sepharose, and labelled with ^{125}I while still bound to the immunosorbent. Following elution, the labelled material was partially absorbed with protein A to enrich for IgG3 and then bound to either affinity purified anti-human IgG or to antibody 18.1 coated onto the wells of a plate. After rinsing, the bound material was again eluted and examined by SDS polyacrylamide gel electrophoresis under reducing conditions. As can be seen in Figure 5, samples of both type g (lane 3) and type b (lane 4) contain IgG heavy chains of 50 K Mr which bind to 18.1. This we expect to be IgG2. However, in the 60 K Mr region, only material from the sample type b is bound to 18.1. Examining the eluate from anti-human IgG, we see roughly equivalent amounts of IgG3 (60 K Mr heavy chain) in

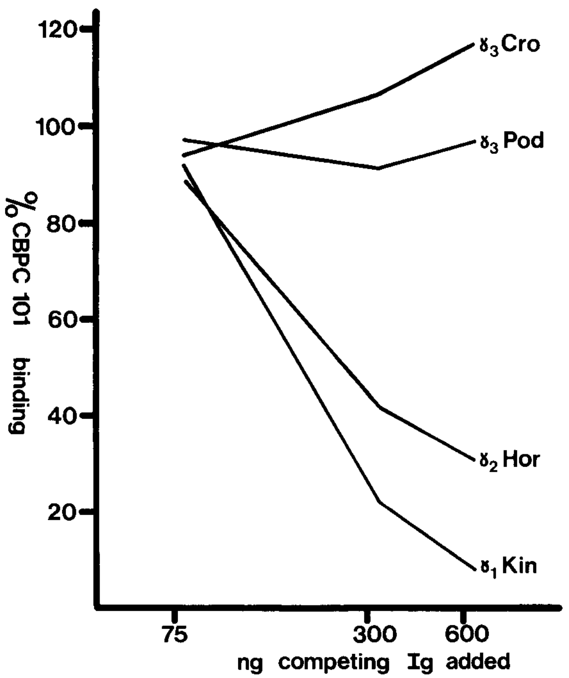

Fig. 3. Protein A reactivity of human myeloma proteins. Protein A was coated onto the wells of a plate at 50 μg/ml. The amount of test myeloma added is indicated on the *abscissa*. Residual binding of a radiolabelled, protein-A binding mouse myeloma protein, CBPC 101, in the presence of the competing human Ig is indicated on the *ordinate*.

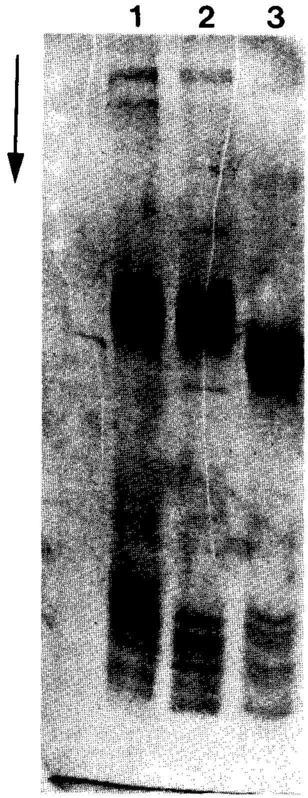

Fig. 4. SDS gel electrophoresis of human myeloma proteins. Proteins (0.5 mg/ml) were reduced with 25 mM DTE and 10 µl was loaded into the wells of a 4.75% stacking gel. The separating gel was 10% polyacrylamide. After electrophoresis the gels were stained for protein with coomassie blue and crocein scarlet. Lane 1 contains IgG3 Cro, lane 2 IgG3 Pod, and lane 3 IgG1 Kin.

both sample tracks (lane 1 and 2). We conclude that antibody 18.1 recognizes IgG3 from the type b individual, but not that from the type g individual.

Since we know that 18.1 reacts with the Fc portion of murine IgG_1 (V. T. Oi and L. A. Herzenberg, unpublished results), we thought it interesting to localize the determinant on the human IgG molecule. The fragments obtained from a limited papain digest of a human IgG3 kappa myeloma were radioiodinated. These fragments were bound to, and then eluted from, in parallel, three antibody preparations coated onto a plate: 18.1, polyvalent anti-human IgG, and polyvalent anti-human kappa. SDS polyacrylamide gel electrophoresis under nonreducing conditions revealed that 18.1 bound exactly the same fragments (Michaelsen and Natvig 1973) as did anti-IgG, and did not bind the Fab fragment eluted from the anti-kappa coat (data not shown).

No evidence was found for reaction of 18.1 with proteins in human serum other than IgG. In this experiment, ^{125}I-labelled whole human serum was incubated with 18.1 previously coated onto the plate. After thorough rinsing, the bound material

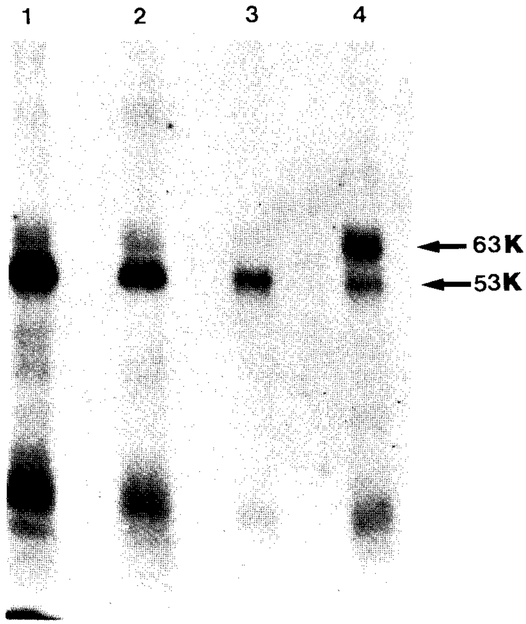

Fig. 5. SDS gel analysis of 18.1-antigen-bearing molecules in human sera of different allotype. Isolation of radiolabelled antigen is described in the text. The material was reduced with 25 mM DTE and electrophoresed in 10% polyacrylamide gel. Sample 1: gamma-determinant-bearing molecules from G3m g serum. Sample 2: gamma-determinant-bearing molecules from G3m b serum. Sample 3: 18.1-determinant-bearing molecules from G3m g serum. Sample 4: 18.1-determinant-bearing molecules from G3m b serum.

was eluted and examined by SDS polyacrylamide gel electrophoresis under reducing conditions. Only bands corresponding in molecular weight to IgG heavy and light chains were seen. This technique, however, would not detect proteins in very low concentrations or which did not label well with ^{125}I under the conditions employed. In addition, a series of irrelevant proteins was tested for reactivity with this antibody: yeast hexokinase, bovine pancreas ribonuclease, bovine liver catalase, glyceraldehyde dehydrogenase, egg albumin, porcine heart malic dehydrogenase, and BSA. All were negative.

Discussion

Hybridoma 18.1, generated by fusion of alloimmunized mouse spleen cells and the tumor cell line NS-1, synthesizes antibody detecting a polymorphic determinant of mouse IgG_1. It is striking that this antibody reacts with IgGs of evolutionarily distant species and reacts with only certain isotypes or allotypes in some of these species. Using monoclonal antibodies detecting monomorphic determinants on human HLA-A, B, C antigens, Parham and Brodsky (1979) found polymorphisms in other primate species. In their work, the immunogens were proteins of a type in which extensive variation may be an evolutionary advantage.

We have explored the pattern of 18.1 reactivity as a function of the phylogenetic relationships of Igs. Since certain physical properties of mammalian and avian IgGs differ (Kubo et al. 1973) and since serological cross-reactivities between the two have been reported rarely at most (Oriol et al. 1972), many workers have felt that mammalian IgG and avian IgG arose through separate gene duplications. Our results, however, might indicate that an Igh-4a like determinant was present on a primordial IgG molecule before the divergence of aves and mammals. The last common ancestor of mammals and birds can be found in the *Reptilia*. The modern day turtle represents the reptilian Anapsid group, thought to be the stem group from which other reptiles evolved, including the Therapsid ancestors of mammals and the Archosaurian ancestors of birds (Rosser 1976). Thus, reactivity of 18.1 with an IgG-like molecule in turtle sera and with sera from members of another reptilian group, the lizards, could imply that the gene duplication event yielding the prototype IgG occurred before the diversification of reptiles. Further support for this view might be gathered from the fact that a similar (probably identical) molecular species in turtle sera possesses protein-A binding sites (our unpublished results), as do most mammalian IgGs (see Goding 1978 for review). However, to date no reaction of avian IgG with protein A has been found.

Amphibia, the first group on the phylogenetic tree to possess a distinct IgG-like immunoglobulin class, apparently do not possess the 18.1 determinant. This is compatible with the view that 7S amphibian Ig is not directly related evolutionarily to mammalian IgG. Data supporting this conclusion include amino-acid analyses (Hadji-Azimi 1979). Our sole representative of teleost fish, a catfish, also lacked the 18.1 antigen. Fish do not have an IgG class.

Looking at animals more closely related to the mouse, sera from both orders of lower mammals were negative for this determinant. Amongst higher mammals, each family shows similar patterns of reactivity, which may reflect subclass differentiation. Thus, some subclasses have lost the original determinant (mouse IgG_{2a}, IgG_{2b}, IgG_3, guinea pig IgG2, and human IgG1 and IgG4), while others (mouse IgG_1, guinea pig IgG1, human IgG2 and IgG3) have retained it. It is interesting to note here the work of Melamed (1976) who observed less evolutionary distance between guinea pig IgG1, mouse IgG1, and all human subclasses, than between guinea pig IgG2 and the same. He postulates early divergence of the progenitor genes for guinea pig IgG1 and IgG2, with the IgG1 precursor giving rise to modern day mouse IgG1 and all of the subclasses of human IgG. Similar work (Adetugbo 1978) has shown that murine IgG_1 and IgG_2 genes probably diverged prior to speciation, whereas primate and ungulate subclasses (Conde et al. 1975), for example, are of much more recent evolutionary origin, and are of IgG_1-like nature.

Such hypotheses lead us to an explanation as to why certain sera, i. e., monkey or cow sera, show stronger reactivity with 18.1 than do sera of "a" allotype mice. Since the IgG subclasses in the former species only recently diverged, it is quite possible that all of their IgGs react with 18.1, or at least that the major subclass does. Because IgG_1 is not the major serum subclass in the mouse, our findings could be explained without invoking heteroclitic antibody.

Most inbred strains of mice possess the "*a*" allele at the *Igh-4* locus. Considering the fact that many of these strains have common ancestors, this is not surprising. In wild mice, the "*a*" allele is also most common (Lieberman and Potter 1969). Our data indicate that the "*a*" allele is the prototype of mouse IgG_1, at least at the specific site we examined. The "*b*" allele would represent a later variant. It is worth mentioning here that this phylogenetic study has discriminated between two hitherto indisinguishable Igh-4a determinants, those recognized by antibodies 18.1 and 10.9 (Oi et al. 1978). The antibodies both react with the Fc portion of IgG_1 and cross block one another's binding to antigen. However, 10.9 reacts with mouse IgG_1 only.

While the data fit a pleasing phylogenetic pattern, we must caution that if selection is very strong perhaps only a few amino-acid substitutions might be tolerated in this region of the molecule. Thus, we could be looking at a case of convergent evolution, where progenitor sequences differed but selection later minimized differences. We cannot be certain that these serologically cross-reacting determinants are homologous, but they have been localized to the Fc portion of chicken IgG (R. Kennedy and A. Benedict, personal communication), human IgG3, and murine IgG_1. We do feel that antibody 18.1 has given us important clues to the evolutionary relationships of IgGs, but we must emphasize the necessity of obtaining more serological and structural data before drawing firm conclusions.

One of our most interesting findings concerns the reactivity of 18.1 with human IgGs, where it shows both isotype and allotype specificity. A series of allotypic specificities is known on human IgG3, with G3m b (G3m 5) and G3m g (G3m 21) being most commonly utilized in genetic studies. The b and g markers are not located at the same position on the molecule, as revealed by proteolytic fragmentation (Natvig and Turner 1970, Natvig and Turner 1971). At the position complementary to marker b in type g proteins is the non-b marker, which is also found on all IgG1 and IgG2 proteins. A similar situation occurs at the position of the g marker, with non-g being allelic on IgG3, but monomorphic on IgG2. Such isoallotypes (or "nonmarkers") have been described as unique to human immunoglobulins, but it would not be surprising to find them in other species where the IgG subclasses only recently diverged. Tempered by the fact that IgG3 is a minor subclass of human serum (comprising approximately 5 percent of total serum Ig, Morell et al. 1972), and that its serum levels are influenced by allotype (Yount et al. 1967, Morell et al. 1972), the lack of an 18.1-reactive IgG3 in the G3m g serum suggests that 18.1 recognizes an allotypic determinant on this subclass. The myeloma data indicate that the 18.1 monoclonal antibody defines an isoallotype of human IgG3 paralleling the non-g marker in human immunoglobulin subclass and allotype distribution. This is the first human allotypic marker found which is shared with a nonprimate species. Family and population studies of the 18.1 marker and further correlations with traditional Gm markers will be the subject of another paper.

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