GENETIC VARIATIONS IN SOMATIC CELLS

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ANTIGENIC VARIATIONS OF SOMATIC CELLS AND CYTOGENETIC ASPECTS OF CELL ANTIGENICITY - INTRODUCTION

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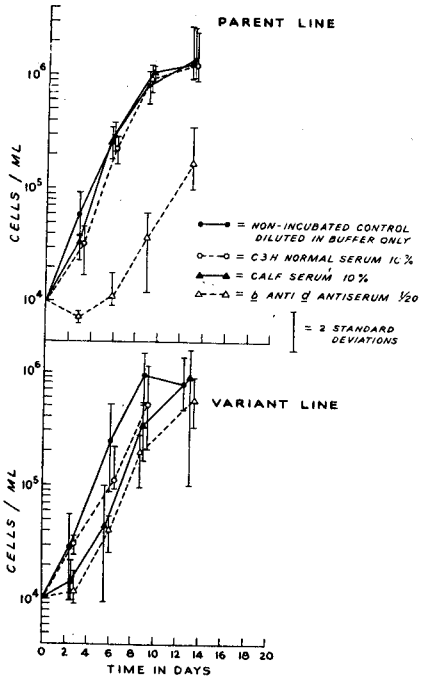

FIGURE 1

Growth of parent line cells (top) and resistant variant line cells (bottom) after treatment with C57BL anti-B10.D2 (H-2^b anti-H-2^d) antiserum diluted 1/20 as compared with controls.

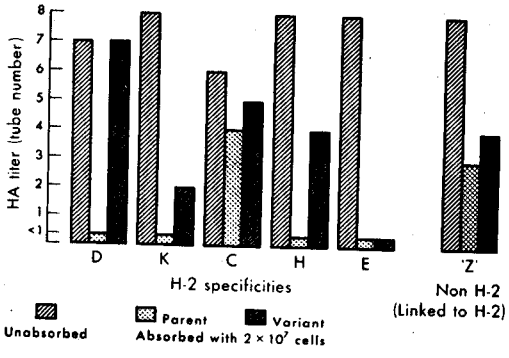

FIGURE 2

Absorption of hemagglutinins from unicomponent sera by the parent line and variant subline. The vertical bars indicate residual titers after absorption with 2 x 10^7 cells of the parent or variant lines compared to the unabsorbed control.

H-2 AND IMMUNOGLOBULIN ISOANTIGENS (ALLOTYPES) IN SOMATIC CELL GENETICS

(INTRODUCTION)

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Since the early experiments of Klein[1,2] and Mitchison[3], the H-2 locus has played a central role in studies of the genetics of mammalian somatic cells. Later on in this session we will hear of some of the latest work using this locus. I propose now to relate some of our attempts to select and analyze somatic cell variants for H-2 and then to describe the development of another potentially useful system for these sorts of investigations, the immunoglobulin allotypes (or isoantigens).

In 1963 Cann and Herzenberg described selection of H-2 variants of a cell culture line of a lymphoma originating in a DBA/2 ($H\text{-}2^d$) mouse.[4,5] These variants were selected by several exposures of the parental cell population to isoimmune ($H\text{-}2^b$ anti $H\text{-}2^d$) antiserum and guinea pig complement. The surviving cells and their progeny, after several years, were resistant to the cytotoxic action of anti $H\text{-}2^d$ antisera and complement. They were, however, still sensitive to heterologous antisera and complement. Hemagglutinin absorptions and indirect fluorescent staining[6] showed that these variant cells had substantially decreased amounts (or activity) of $H\text{-}2^d$ antigens on their surface membranes.

The permanence of the antigenic loss over at least seventy-five cell generations suggests that a genetic mechanism is responsible for the loss. But, the quantitative rather than qualitative change in a cell carrying at least two $H\text{-}2^d$ alleles has not been explained on any known genetic basis and I have no hypothesis to offer which is testable (useful).

Papermaster and I were then able to carry out similar investigations with a heterozygous lymphoma which I had succeeded in establishing as a continuous cultured line from a tumor kindly supplied by Dr. K. E. Hellström.[7] This cell type was originally $H\text{-}2^d/H\text{-}2^k$ and is readily killed by anti $H\text{-}2^d$ isoantisera and complement. Moderate cytotoxicity with anti $H\text{-}2^k$ and even with non-H-2 antisera is also obtained in the presence of complement. Complement alone is not cytotoxic.

Again, by growing out the survivors of treatment of the parental line with anti $H\text{-}2^d$ serum and complement, a variant line specifically resistant to the cytotoxic action of anti $H\text{-}2^d$ sera was obtained (Figure 1). Several of the component specificities of the $H\text{-}2^d$ and $H\text{-}2^k$ alleles were estimated in the parental and this variant line by the method of hemagglutinin absorption. Figure 2 summarizes the important results of these studies. It is seen that the variant has no detectable D antigen, a product of the $H\text{-}2^d$ allele, a reduced amount of H, which is controlled by both the $H\text{-}2^d$ and $H\text{-}2^k$ alleles and an insignificant decrease in K, a product of the $H\text{-}2^k$ allele. The C ($H\text{-}2^d$ and $H\text{-}2^k$) and E ($H\text{-}2^k$) components are not different in parent and variant.

TABLE 1

Distribution of Ig-1 Alleles In Inbred Mouse Strains [12]

Ig-1[a]			Ig-1[b]		Ig-1[c]	Ig-1[d]	Ig-1[e]	Ig-1[f]	Ig-1[g]	Ig-1[h]
C3H/HeJ*			C57BL/10J*		DBA/2J*	AKR/J*	A/J*	CE/J*	R111/J*	SEA/Gn*
AU/Ss	C58/J	PBR/Ao	B10.D2(new)/Hz	SJL/J	DBA/1J	AL/Ks	A2G/	DE/J	DA/Hu	BDP/J
BALB/CGa	F/Ao	PL/J	B10.D2(old)/Hz	SM/J	DBA/10Hu	AL/N	NZB/Bl	N/Ao	FZ/Di	BSL/Di
BUB/Bn	H-2G/Go	POLY1/Ao	C57BL/H	STA/Je	HD/Hu		NZO/Bl	NH/N	STB/Je	P/J
CBA/J	JK/Bi	POLY2/Ao	C57BL/Ka	WB/Re	I/Ao		NZW/Bl			SEAC/J
CH1/Ao	MA/J	PRUNT/Ao	C57BL/6J	WC/Re	JB/Di					SEC/Gn
C3H/Hz	MA/MyJ	ST/J	HALB/Hu	WH/Re	RF/J					
C3H.SW/Hz	MYA/Hij	STR/N	Hg/Hu	WK/Re	SWR/J					
C57BR/cdJ	NZC/Bl	T6/H	H-2H/Go	58N/Sn						
C57L/J	NZY/Bl	129/RrGa	H-21/Go	101/Ri						
			LP/J							

* Type strain for each allele is underlined.

The H-2 changes in this variant of a heterozygous lymphoma can be interpreted on the basis of somatic crossing-over and segregation as suggested by Klein and his colleagues for similar H-2 changes in tumors.[8,9,10] The crossover would be to the left of D in Figure 3, assuming the centromere is to the left again as suggested by the work of Klein's laboratory. However, a real decrease in H in the variant was found. This requires an ad hoc additional interpretation such as partial deletion or loss of the entire chromosome carrying the H-2^d allele. Although such a clearcut genetic mechanism is a likely explanation for the qualitative changes seen in immunoselected variants, the concomitant quantitative changes in these heterozygous cells and the quantitative changes in homozygous cells seen in our earlier studies demand extreme caution in accepting such explanations.

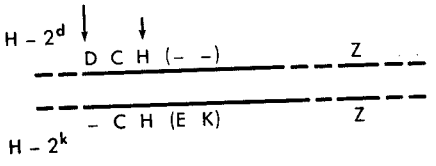

FIGURE 3

Genotypic map of the antigenic components arranged in the linear order proposed by Stimpfling and Snell.[35] The components indicated are those detected on the parent and variant sublines as in Figure 2. The arrows point to the components absent or reduced in the variant.

Immunoglobulin Isoantigens or Allotypes

Another system of genetically controlled antigenic differences has been developed recently in the mouse. The immunoglobulin allotypes or isoantigens with which we and others[11-15] have been working promise a great utility in studies of somatic cell genetics. Further, they offer an added opportunity to learn something of the genetic control of antibody structure and even synthesis.

Precipitating and indirect agglutinating isoantibodies to immunoglobulins have been obtained in the mouse by immunization with purified gamma globulins in adjuvants, antibody specific precipitates and agglutinates, and with antibodies to the tissues of the recipient animal (presumably due to complexing in vivo). This latter technique has been most useful and has been previously described.[12] In order to work sensitively with the relatively small quantities of antisera and antigens provided by mice, we have mostly used precipitation of isotopically labelled antigens as described[12] but double diffusion in agar and indirect (Coombs) agglutination have also been used.[13]

Most of the antisera we have produced react with the γG(7S)-globulin antigens and I will discuss these antigens first. The γG-globulins of each of the nearly 100 inbred strains now

to the question of allelism depends on identification of the polypeptide on which the Ig-1 antigens are found. Fahey et al.[18] have shown that the anti-Ig-1^a antisera precipitate myeloma proteins of the γG_{2a} subclass of γG-globulins. Further, Mishell and Fahey have localized these antigens, in some strains, at least, to the F_c fragments and therefore to the γ^{2a} (H) polypeptide chains.[19]

As new antisera were made, we found some that react with γA immunoglobulins from normal serum and with γA myeloma proteins.[11] The isoantigens (allotypes) defined by these antisera, were shown also to be on the F_c fragments after papain digestion of the γA proteins and therefore on the α(see ref.20)$_{(H^{\gamma A})}$ polypeptide chains. These antigens were designated Ig-2 antigens and at least three alleles were described.[11]

The α and γ^{2a} chains are different polypeptides both when studied immunochemically with heterologous antisera and by comparison of their tryptic peptide fingerprints. Thus, it seems correct to conclude that the Ig-1 and Ig-2 allotypes, which are on these two polypeptides respectively, are controlled by two distinct genetic loci. However, these two loci are sufficiently closely linked that no recombinants between them have yet been found in some 150 animals tested.[11]

Quantitative Allotype Estimation

A sensitive method of assaying for individual allotypes was developed based upon the inhibition of precipitation of isotopically labelled antigen. The slow γG_2-globulins or a γG_{2a} myeloma protein are purified by ammonium sulfate precipitation, zone electrophoresis and Sephadex G-200 gel filtration and then labelled by the chloramine-T method[21] with I^{125} to have a specific activity of 10^5 -10^6 counts per minute per μg. Fifty μl portions of a solution of one of these antigens in 3% BSA in 0.05M Tris buffer, pH 7.6, are pipetted into 6 x 50mm test tubes. To each tube is then added 25 μl of S-dil (3% BSA and 10% normal rabbit serum in 0.05M Tris buffer, pH 7.6) containing a varied amount of a standard or unknown (inhibitor) serum of the same allotype as the labelled antigen. Finally each tube receives 25 μl of S-dil containing a constant amount of anti-allotype serum which has previously been found to be sufficient to specifically precipitate about 80% of the labelled antigen when no inhibitor is present. With most antisera this requires 0.02 - 0.2 μl of antiserum per tube. Thus each tube receives a total volume of 100 μl. The tubes are incubated at 37°C for three hours and then briefly chilled and centrifuged at 10,000 X g for 15 minutes of 4°C. Fifty μl samples of the supernatant are removed and counted in a well type scintillation crystal counter. An example of the results obtained when the reciprocal of the percent of counts precipitated is plotted against increasing amount of inhibitor is shown in Figure 4. It is seen that a straight line over a useful range is obtained. This standard curve can then be used to read off the content of an unknown relative to the standard. To make it more convenient to analyze hundreds of individual dilutions we have used a simple program on a LINC computer[22] to "read the curve". The hundreds of additions and

tested cross react with those of many other strains when tested with different isoantisera. By grouping strains which behave identically in their cross reactions we have defined 8 allele groups named Ig-1^a through Ig-1^h. These groups and their antigenic component specificities are shown in Tables 1 and 2. The specificities are derived from the observed cross reactions and have the same significance as the specificities in the H-2 system. A detailed derivation of this table has been presented with the exception of several additional strains[16] and specificities 9 and 10 whose definition will be published in detail.[17]

TABLE 2

Antigenic Specificities Present in Ig-1 Alleles

Allele	Type Strain	Antigenic Specificities									
		1	2	3	4	5	6	7	8	9	10
Ig-1^a	C3H/HeJ	1*	2	-**	-	-	6	-	8	9	10
Ig-1^b	C57BL/10J	-	-	-	4	-	-	7	-	9	-
Ig-1^c	DBA/2J	-	2	3	-	-	-	7	-	9	-
Ig-1^d	AKR/J	1	2	-	-	5	-	7	ND†	-	-
Ig-1^e	A/J	1	2	-	-	5	6	7	8	-	-
Ig-1^f	CE/J	1	2	-	-	-	-	-	8	9	ND
Ig-1^g	RIII/J	-	2	3	-	-	-	-	-	9	-
Ig-1^h	SEA/Gn	1	2	-	-	-	6	7	-	9	10

* Number means specificity is present.
**Dash means specificity is absent.
† Not done.

That these groups of strains differ with respect to the Ig-1 alleles they possess was shown by straightforward genetic testing as Mendel would have done it 100 years ago if he could have scored for this character. Of course, in addition to the four laws he enunciated he would also have discovered codominance if he had the means to detect isoantigens. As is generally so for antigens, the Ig-1 antigens are codominant. However, our present sophistication leads us to ask if progeny testing,in a higher organism such as the mouse where only in the order of hundreds of offspring are practicably obtained in a given cross, is sufficient to rule out close linkage of two or more genes in contrast with true allelism.

We have argued elsewhere[11] that observing no genetic recombinants in some several hundred animals tested merely indicates that the two characters have a recombination frequency of somewhat less than about 1%. This means there may be room for many genes between the genes tested. On the other hand, the genes may be truly allelic. To distinguish between these possibilities we must use some molecular considerations. The definition of allele I will use is, "alleles are alternate forms of a length of DNA coding for a single polypeptide". Then the answer

allotypes are readily distinguished. In fact by using the method of inhibition of precipitation of I^{125} labelled antigen, as little as 0.05% of one allotype in the presence of one of the others can be reliably estimated. We have applied this method in studies of lymphoid chimeras[26,27] and maternal versus paternal (or progeny) type γ-globulin in young mice.[28] In the former, an interesting finding was a dissociation of donor-skin-graft tolerance and donor-type γ-globulin production in radiation chimeras. By taking small blood samples from such chimeras and analyzing them for donor and host type allotype it was possible to follow changes in the activity of the two genetically different components of the chimeras without sacrificing the animals. The latter application is discussed elsewhere in this session so I will not discuss it here.

Somatic Antigenic Variation of Immunoglobulins

The allotypic variation we have discussed is within the sphere of a classical genetic polymorphism. Its relevance to a session on somatic cell variation lies in considering immunoglobulin heterogeneity. The allotypes are expressed on one or another of the classes of immunoglobulins. In homozygotes they appear to be on all the molecules of this class but in heterozygotes only on about half of these molecules. Pernis and his collaborators have shown that this is due to an ever-present somatic cell variation. That is, some cells produce one allotype and other produce the other.[29] It has long been known that each plasma cell tumor (myeloma) produces a very restricted number of distinct immunoglobulin molecules.[30] Each antibody producing cell in an immunized animal produces one or only a small number of antibody types. These facts, well known to you, document the tremendous somatic cell variation responsible for immunoglobulin and antibody heterogeneity. I will not here attempt to review the extensive literature and theories of this fascinating field but will conclude with a few of the questions to which we are addressing ourselves about somatic variation and immunoglobulins.

In Table 2 we listed several isoantigenic (allotypic) specificities controlled by each allele. Do all molecules carrying one of these specificities carry them all? We do not yet have a complete answer. In several cases, including one published,[11] the answer is yes. However, one case has been found[17] of a myeloma protein with specificity 5 but lacking specificities 1, 2, 6 and others usually found in association with it under the control of allele $Ig\text{-}1^e$. It may be that these observations will be explainable by a somatic crossing-over mechanism as has been referred to earlier for H-2 variation.

Plasma cell tumors have very recently been induced in Ig-1 heterozygous mice.[31] The myeloma protein produced by each of these which produces a γG_2 myeloma protein has been found to have the allotypic specificities of only one of the Ig alleles represented in the mouse in which the tumor arose.[17] Are there myeloma proteins which have specificities of both allelic types of the mouse of origin of the tumor producing the protein?

Is there any correlation of antibody specificity with allotype? Others have asked this question

withdrawals of 25 and 50 µl quantities has been greatly facilitated by the use of a semi-automatic micropipetting system designed and constructed locally.[23]

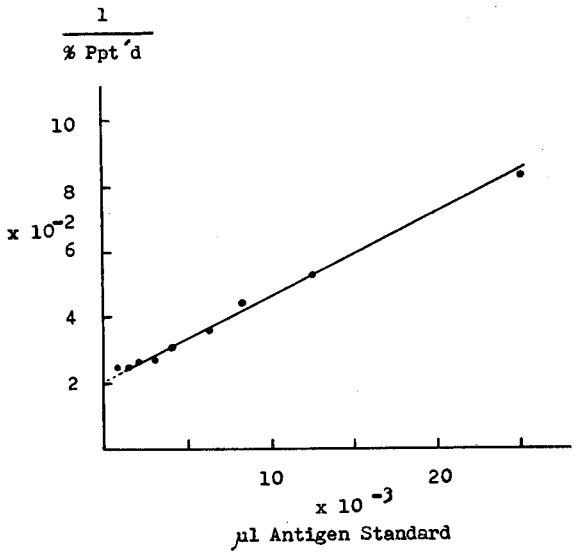

FIGURE 4

Inhibition of precipitation of I^{125}-labelled antigen. Each tube contained approximately 0.05µg I^{125}-labelled C57BL/6 (Ig-1^b) γG_2-globulin, 0.05µl BALB/C (Ig-1^a) anti-C57BL/6 (Ig-1^b) anti-allotype serum and indicated amounts of a standard pool of C57BL/6 "inhibitor" serum. The final volume of 100µl in each 6 x 50mm tube contained 3% w/v Bovine Serum Albumin and 5% v/v normal rabbit serum in 0.05M Tris -HCl buffer, pH 7.6. The mixtures were incubated for 3 hours at 37°C, centrifuged at 4°C at 10,000 X g for 15 minutes and then 50µl samples of the supernantants were removed and counted. The percent precipitated was calculated relative to controls without the antiserum.

A recent modification of this method involves conjugating the antibodies by diazotization to polyaminopolystyrene (PAPS).[24] We estimate the allotype content of an unknown by its ability to inhibit binding to the resin-antibody conjugate using a plot of the same type as Figure 4.

The method has also been applied in reverse to estimate anti-allotype antibody content of an antiserum. Antibody was purified by absorption to PAPS-antigen conjugate and elution at acid pH. It was then I^{125} labelled. Inhibition of binding of this labelled antibody to PAPS-antigen when plotted as a reciprocal of the percent of counts versus inhibitor amount also gives a straight line.[25] In this application, the influence of dissociation constant on the slope of the line remains to be extensively explored.

The potential use of the Ig-1 antigens as cellular genetic markers is indicated best by describing some applications already utilized. As was shown in Table 1, each of the inbred strains C57BL/6, C3H and DBA/2 carries a different Ig-1 allele and these three γG-globulin

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and found very suggestive differences between the allotype ratios of specific antibodies from an animal and of the whole serum from the same animal.[32,33] Slight modifications of the I^{125} methods described earlier make it possible to use the mouse, which can be obtained in large numbers with identical genotypes, for answering the question.

Are there a number of structural (antigenic) components of the H chains of immunoglobulins shared by fractions of the immunoglobulin population? We have now produced rabbit antisera against a set of myeloma proteins from one mouse strain.[34] These antisera are being absorbed with the other members of the set in all combinations and then tested for their ability to precipitate each member of the set. From these results we can tentatively answer this question affirmatively.

I have ranged over much territory in this short introduction to a session on "Antigenic Variation of Somatic Cells" and only hope that I have indicated some of the possibilities immunoglobulin genetics offers to investigate the same kinds of questions to which the remainder of this session will be addressed.

ACKNOWLEDGEMENTS

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