

CONGENIC MOUSE STRAINS: THE EXPRESSION OF A
HIDDEN IMMUNOGLOBULIN ALLOTYPE IN A
CONGENIC PARTNER STRAIN OF BALB/c MICE*

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It is generally believed that separate genes encode for the constant (C)¹ and variable (V) sequences of amino acids that characterize the polypeptide chains of immunoglobulins (1). Different C sequences define the particular class of immunoglobulin (Ig) chain and the fact that genetic markers for these regions are inherited in Mendelian fashion (2-7) supports the concept of one C gene (8) for each of the 10 or so known classes of Ig chains. Considerably more than 10 V genes are suspected to encode for V sequences since these regions of the Ig chain are responsible for the diversity of antibody specificity (9-12). Thus, a hypothesis for the translocation of antibody genes has become especially attractive with the discovery that different V region subgroups can be associated with the same C region and vice versa (13-16). Simply stated, translocation theories (9, 17) require that one of many tandemly linked V genes be transpositioned next to a C gene before transcription.

Of some consequence to the above viewpoint are the unexpected observations that we report here for a congenic partner strain of BALB/c mice. These mice were specifically bred so as not to differ from BALB/c mice in any known way except to carry Ig structural genes of the C57BL/Ka allotype. Mouse Ig allotypes serve as genetic markers for the closely linked C genes of Ig heavy chains (4, 5). And so the fact that the transfer of C57BL/Ka Ig genes to the BALB/c genome followed a Mendelian mode of inheritance was in accord with expectation. What was unexpected was that Ig of BALB/c allotype could be elicited in one of our congenic strains (ICR CB-17) that was defined to be homozygous for Ig of the C57BL/Ka allotype. The conditions necessary for the expression of this "hidden" allotype seemed to be specific. Further, the BALB/c allotype was detected only on the IgG (IgG2a) class and its appearance was usually very transient and unpredictable.

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¹ *Abbreviations used in this paper:* BC_n, number of introgressive backcrosses; C, constant; F_n, number of homozygous brother-sister generations; H chain, heavy chain; ICR, Institute of Cancer Research; ICR CB-n, an ICR congenic partner strain of BALB/c mice (C) where C57BL/Ka (B) is the source of Ig genes and (n) is the number of backcross generations; L chain, light chain; MOPC, plasmacytomas.

What follows is a presentation of the pedigree of our congenic BALB/cICR mouse strains, the occasions in which Ig of the "wrong allotype" appeared and a preliminary analysis of these mice.

Materials and Methods

Nomenclature.—To facilitate a clear distinction between Ig classes and allotypes we use the Potter-Lieberman nomenclature (in parentheses) (4) to designate the 7S classes of mouse IgG1 (IgF), IgG2a (IgG) and IgG2b (IgH); and as done elsewhere (18), we use the small case letters *a* and *b* to differentiate the Ig allotypes of BALB/c (Ig^a) and C57BL (Ig^b) mouse strains. Thus, for example, the allotype of the IgG class can be denoted as IgG^a or IgG^b.

Ig structural genes for the V and C segments of heavy (H) and light (L) chains are designated V_H, C_H, V_L, and C_L, respectively. Whenever convenient, Ig structural genes are further specified according to the class of H chain, as for example, Ig C_HA, Ig C_HG, etc.

History of BALB/c Mice Congenic for Ig Genes.—Congenic mouse strains result from experimental attempts to produce coisogenicity, a state in which two strains are genetically identical except for a difference at a single locus (19). Coisogenicity can be approximated when one or more genes of one strain are crossed introgressively onto the background of another strain (inbred partner strain). Here the intent of breeding is not necessarily the only consequence as contaminant genes may also be introduced into the congenic partner strain. However, as backcrossing of the specific foreign gene(s) continues, the probability of carrying contaminant genes decreases.

The use of congenic mice in this study applies to BALB/c mice that have different Ig structural genes, in which case the congenic partner strains carry Ig structural genes of the C57BL/Ka allotype. For these particular strains the foreign chromosomal segment is known to include Ig structural genes of the H chain linkage group (4).

The breeding of such mice was initiated in the laboratory of Dr. M. Potter (National Cancer Institute, Bethesda, Md.) as follows (4): F₁ hybrids of C57BL/Ka X BALB/c (♂) were backcrossed to BALB/c (♀); those progeny that showed Ig allotypes of both parental strains were again backcrossed to BALB/c (♀). Mating of this kind was continued through 14 and up to 20 backcross generations, at which times, the allotypically heterozygous mice were intercrossed. This yielded offspring of which approximately one-fourth were phenotypically homozygous for Ig of the C57BL/Ka allotype and from which the congenic line, CB-14 (BAB-14) and CB-20 were established by means of brother-sister matings.² Our own congenic partner strains of BALB/c mice (ICR CB-17, ICR CB-23, and ICR CB-26) were derived in the same manner from Potter's 13th backcross generation; the only difference being that inbred BALB/c mice of this institute (BALB/cICR) served as the inbred partner strain for all subsequent backcrosses and intercrosses.³

Strains of mice that are congenic for Ig genes can be designated (21) according to the parent strain (BALB/c), the strain source (C57BL/Ka) of new Ig genes (Ig^b), the number (n) of introgressive backcrosses (BC_n), and the number of homozygous brother-sister generations (F_n), e.g., BALB/cICR·C57BL/Ka Ig^b/Ig^b BC₁₇ F₄. In accord with the abbreviated notation of Potter (personal communication) we will use the symbols ICR CB-n to designate: (a) The institution (ICR) in which the congenic line is maintained; (b) the inbred partner strain, BALB/c (C); (c) the C57BL/Ka (B) source of Ig genes in the congenic partner strain;

² CB-14 is being maintained by Dr. L. Herzenberg (18) and also by Dr. M. Weigert (20); both of these investigators designate this congenic line as BAB-14.

³ We are indebted to Dr. Michael Potter, who initiated this special breeding program, and from whom Dr. Weiler, formerly of this Institute, got the backcross mice of the 13th generation to start a congenic BALB/c mouse colony at ICR.

and (*d*) the number (*n*) of backcross generations that preceded the derivation of mice phenotypically homozygous for Ig^b .

Mouse Antisera.—Antiallotype sera was made in BALB/cICR and C57BL/6ICR mice by means of reciprocal immunizations with each other's Ig. Antisera to Ig of the C57BL/6 or C57BL/Ka allotype (Ig^b) was made in BALB/c mice following repeated injections of C57BL/6ICR hyperimmune sera (0.3 ml serum/injection), whereas C57BL/6ICR mice made antisera to Ig of the BALB/cICR allotype (Ig^a) only after many immunizations with rabbit red cell ghosts that were first agglutinated with BALB/cICR antibodies and emulsified in complete Freund's adjuvant. The monospecificity of both anti- Ig^a and anti- Ig^b for allotypic determinants unique to IgG was assured after absorption with IgF and IgH immunoglobulins derived from the following plasmacytomas (MOPC): IgF^a (MOPC 31), IgH^a (MOPC 195), IgF^b (MOPC 300), and IgH^b (MOPC 352).⁴ Fig. 1 demonstrates this kind of monospecificity, although indirectly for IgG^b since as yet there is no IgG^b MOPC tumor available.

Rabbit Antisera.—New Zealand rabbits were hyperimmunized with Fc fragments of either mouse IgF (MOPC 31c) or mouse IgG (MOPC 173). Appropriate combinations of IgM, IgA, IgH, and IgG or IgF from mouse plasmacytomas (MOPC 104E, MOPC 167, MOPC 195, and MOPC 173 or MOPC 31c) were insolubilized with glutaraldehyde (22) and used to absorb the rabbit antisera. In this way, we obtained class-specific antisera for IgF and IgG.

Papain Digestion and Immuno-electrophoresis.—Salt-precipitated Ig preparations that were chromatographically pure were subjected to papain digestion for 60–90 min according to the method of Porter (23). The resulting Fab and Fc fragments were separated by chromatography (24) over DEAE-cellulose using a linear salt gradient (0.005–0.3 M potassium phosphate, pH 8.0). Immuno-electrophoresis and papain digestion of mouse serum was done as prescribed by Minna et al. (25). Microimmuno-electrophoresis (LKB 6800A apparatus, LKB Produkter, Stockholm, Sweden) was carried out in 0.05 M barbital buffer, pH 8.6, at 7–9 volts/cm for 60–90 min.

RESULTS

Pedigree of Congenic BALB/cICR Mice.—The results of Figs. 2 and 3 show the inheritance of mouse IgG^b allotype throughout many backcrosses and intercrosses. Except for two of the twenty crosses, the observed fraction of heterozygotes (Fig. 2) and homozygotes (Fig. 3) fell within the 95% confidence interval that was expected of each sample of tested progeny, given a Mendelian mode of inheritance as the null hypothesis. These results, especially those of Fig. 3, confirm the unit-character inheritance of mouse Ig allotypes and served as the experimental basis for defining ICR CB-*n* congenic mice that were homozygous for Ig^b structural genes. Three such strains are being maintained presently: ICR CB-17, ICR CB-23, and ICR CB-26. The ancestors for the ICR CB-17 strain were derived from the first three intercrosses of Fig. 3.

What is not clear about the results in Fig. 2 is why the observed fractions of heterozygotes in the backcrosses approached the expected mean (0.5) only from the lower limit. Neither is it clear what significance, if any, should be attached to the fluctuation in the yield of heterozygous progeny from BC₂₂ to BC₂₆ (Fig. 2) or to the discrepancy in the yield of homozygotes from two different intercrosses of BC₂₃ (Fig. 3).

⁴ All mouse plasmacytomas were given to us by Dr. Michael Potter of the National Cancer Institute, Bethesda, Md.

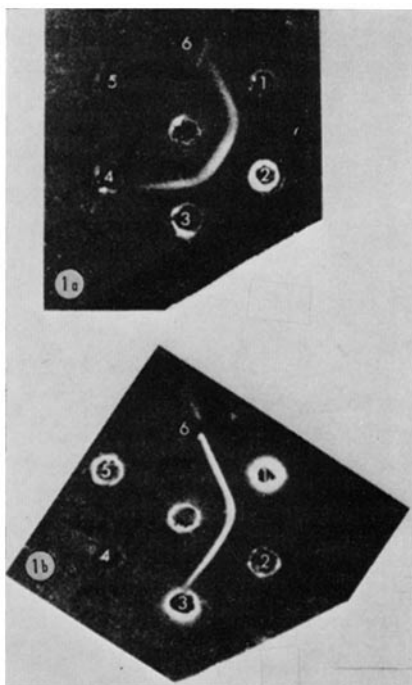


FIG. 1. The specificity of antiallotype sera to BALB/c immunoglobulin (Ig^a) and to C57BL immunoglobulin (Ig^b). (a) In the center well is C57BL/6 anti- Ig^a with the outer wells containing the following Ig antigens: IgG^a (MOPC 173) at 0.1 mg/ml (1); BALB/c normal serum (2); IgG^a (LPC-1) at 0.1 mg/ml (3); IgF^a (MOPC 31c) at 0.1 mg/ml (4); IgG^a Fab fragment (MOPC 173 Fab) at 0.2 mg/ml (5); and IgH^a (MOPC 195) at 0.1 mg/ml (6). (b) In the center well is BALB/c anti- Ig^b with the following Ig antigens contained in the outer wells: Fc (1) and Fab (3) preparations, respectively, of chromatographically pure 7S Ig fraction derived from C57BL/6 mouse serum (0.3 mg/ml); C57BL/6 normal serum (2); BALB/c normal serum (4); IgH^b (MOPC 352) at 0.2 mg/ml (5); and IgF^b (MOPC 300) at 0.2 mg/ml (6).

Linkage of Heavy Chain Genes.—The antiallotype sera used to define congenic BALB/cICR mice throughout all backcrosses and intercrosses (Figs. 2 and 3) were monospecific for allotypic determinants unique to IgG . Therefore, the detection of IgF^b , IgH^b , and the inferred presence of IgA^b in ICR CB-17 mice (Table I) demonstrates the close linkage of these Ig structural genes and confirms the work of others (4, 5).

Unexpected Observations in the ICR CB-17 Congenic Mouse Strain.—As a consequence of using ICR CB-17 mice as recipients of the IgM -synthesizing plasmacytoma, MOPC 104E, we first observed the appearance of the BALB/c allotype (ICR CB-17[MOPC-104E]mice). This is shown in Fig. 4. Although MOPC 104E is a BALB/c tumor, this finding was surprising because as yet no allotype marker for mouse IgM is known. Our immediate inclination was

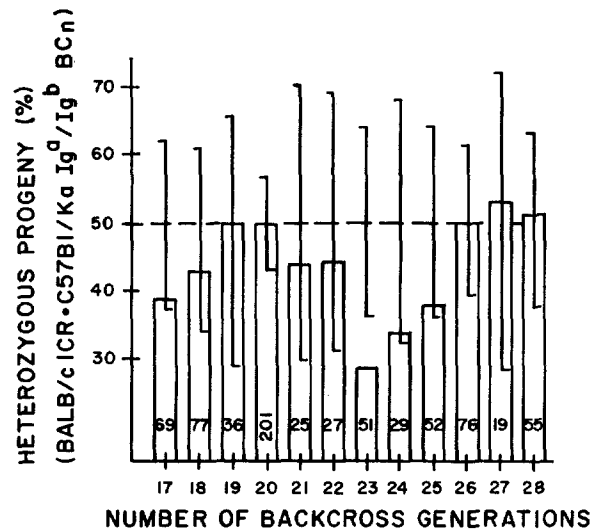


FIG. 2. Backcross matings of congenic BALB/cICR mouse strains: the percentage of allo-typically heterozygous progeny as a function of the number of introgressive backcrosses (BALB/cICR · C57BL/Ka Ig^a/Ig^b BC_n × BALB/cICR). The symbols designate the parent strain (BALB/cICR) and the strain source (C57BL/Ka) of the new immunoglobulin allotype (Ig^b) in mice of the respective BC_n. Progeny were tested in the range of 8–15 wk of age; the number within each histogram tells how many mice were tested. Given this number and the assumption that mouse immunoglobulin allotypes are inherited in Mendelian fashion, the 95% confidence interval for the expected fraction of heterozygotes at each backcross is shown. The total Chi-square between the fraction of heterozygotes observed and that expected is $\chi^2_{11} = 9.9$ ($0.5 > P > 0.4$).

to accept this as an example of an IgM clone switching to IgG^a analogous to the finding with the human myeloma patient, Til (16). At the same time, however, other ICR CB-17 mice that were carrying MOPC 195 (IgH^a) or MOPC 167 (IgA^a) showed the BALB/c allotype in their serum. Since our antisera were judged to be monospecific for allotypic determinants unique to IgG^a, it seemed as if all three conditions were reflecting a property of the host and not the plasmacytomas.

This caused us to check the entire colony of ICR CB-17 breeder mice for possible IgG^a beginning in March of 1972. The results are shown in Fig. 5 a. The first group (B) of mice tested included all our foundation breeders (150 mice) of which 48 mice exhibited IgG^a in their serum. In the second group (C) of 35 production breeders, only one mouse was positive for IgG^a. We were surprised to find that there was no obvious inheritance of the IgG^a phenotype among the foundation weanlings of Group B as can be seen in Group D. Moreover, when Group B was retested in May of 1972, of the original 48 mice that had detectable IgG^a in their serum, only one remained positive for IgG^a. However, even this mouse (no. 17 ICR CB-17) lost its IgG^a phenotype and

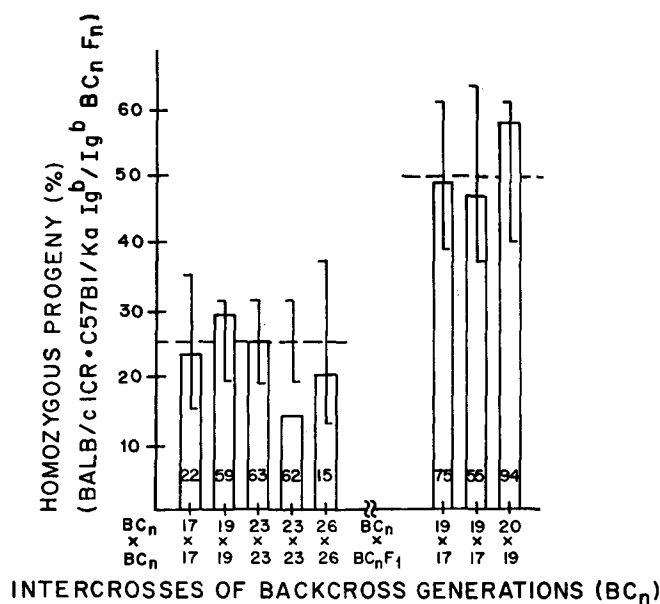


FIG. 3. Intercross matings of congenic BALB/cICR mouse strains: the percentage of allotypically homozygous progeny as a function of the number of successive intercrosses (BALB/cICR·C57BL/Ka Ig^a/Ig^b BC_n × BALB/cICR·C57BL/Ka Ig^a/Ig^b BC_n). The symbols designate the parent strain (BALB/cICR) and the strain source (C57BL/Ka) of the new Ig^b in mice of the respective BC_n and F_n. Progeny were tested in the range of 8–15 wk of age; the number within each histogram tells how many mice were tested. Given this number and the assumption that mouse immunoglobulin allotypes are inherited in Mendelian fashion, the 95% confidence interval for the expected fraction of homozygotes at each intercross is shown. The total Chi-square between the fraction of homozygotes observed and that expected is $\chi^2 = 4.8$ ($0.4 > P > 0.3$).

TABLE I
Allotypes of Different Ig Classes in BALB/c, C57BL and ICR CB-17 Mouse Strains

Ig class and allotype	Mouse strain		
	BALB/c	C57BL	ICR CB-17
IgF ^a	+	—	—
IgF ^b	—	+	+
IgG ^a (G ^{1,6,7,8})	+	—	—
IgG ^b (² G)	—	+	+
IgH ^b (H ¹⁶)	—	+	+
IgA ^a (A ^{12,13,14})	+	—	—

The numbers in parentheses are the assigned allotypic determinants of Lieberman and Potter (4) for the respective Ig classes of BALB/c (Ig^a) and C57BL (Ig^b) mouse strains. Determinant ²G is still unassigned but is presumably on IgG^b molecules. We thank Rose Lieberman for typing the IgH and IgA classes, as well as for confirming the presence of IgG^b in ICR CB-17 mice.

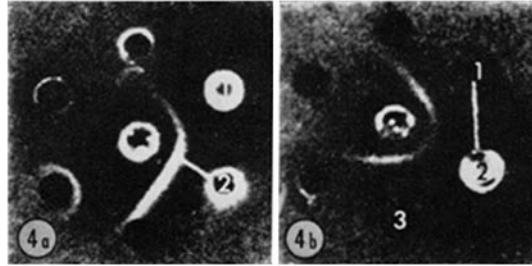


FIG. 4. The detection of IgG^a in ICR CB-17 mice bearing MOPC 104E plasmacytoma (ICR CB-17 [MOPC 104E]); (a) in outer wells are serum samples of ICR CB-17 (MOPC 104E) mice (well 1) and of BALB/c normal mice (well 2) vs. anti- IgG^a in the center well; (b) in outer wells are serum samples of ICR CB-17 (MOPC 104E) mice (well 2) and ICR CB-17 mice bearing IgG^a plasmacytomas (LPC-1 in well 1 and MOPC 173 in well 3).

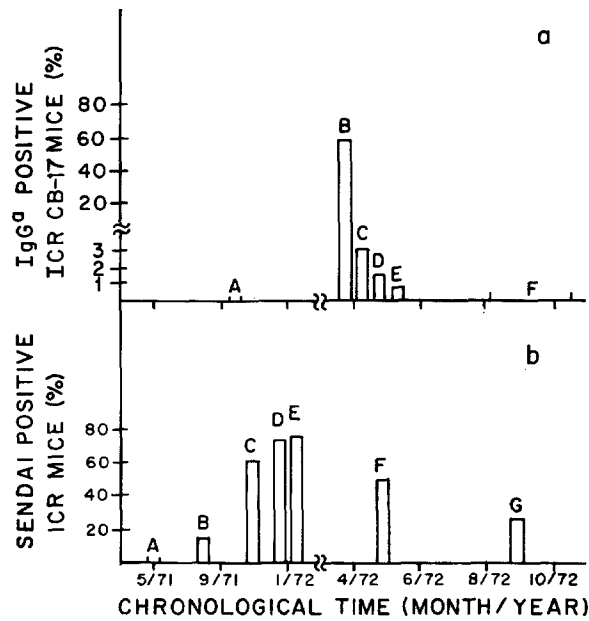


FIG. 5. (a) The appearance of IgG^a in the ICR CB-17 mouse colony; the percentage of mice positive for IgG^a as a function of chronological time. The age range and the number of mice positive for IgG^a per number tested is as follows: Group A, 8-12-wk old production stock, 0/115; Group B, 1-13-mo old foundation breeders, 48/150; Group C, 12-25-wk old production breeders, 1/35; Group D, 5-9-wk old foundation weanlings, 3/211; Group E, retesting of Group B, 1/150; and Group F, 9-20-wk old production stock, 0/236. (b) Random sampling of ICR mouse colony for the presence of Sendai virus; the percentage of mice serologically positive for Sendai virus as a function of chronological time. The number of mice tested at each interval is as follows: Group A, 30; Group B, 30; Group C, 27; Group D, 20; Group E, 25; Group F, 24; and Group G, 100.

died shortly thereafter (see Fig. 6). To round out this exhaustive screen of ICR CB-17 mice, it was fortuitous that earlier we had checked some 200 ICR CB-17 production mice for IgG^a and IgG^b in October of 1972 (Group A); subsequent testing of ICR CB-17 production stock has consistently failed to reveal IgG^a (Group E).

The transient appearance of the IgG^a phenotype in the ICR CB-17 mouse colony suggested to us that the expression of IgG^a might have been a consequence of some infectious agent. Further support for this idea came from the results of serological tests for Sendai virus in mice representative of all ICR

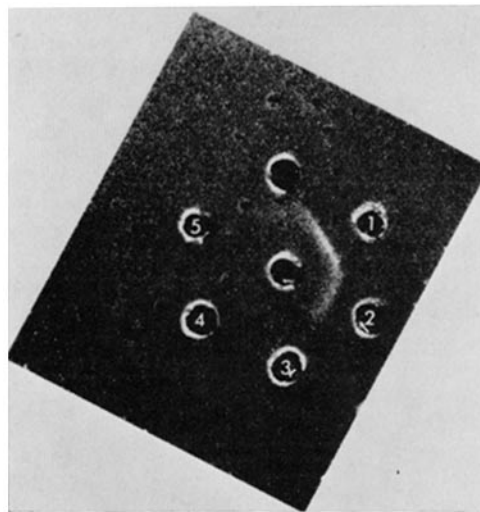


FIG. 6. The transient appearance of IgG^a in mouse 17 ICR CB-17 of Fig. 5 a, Group E Serum samples that were obtained at different intervals of time (5/23, 5/25, 5/30, 6/7, and 6/13) are in the outer wells numbered 1-5, respectively. Anti-IgG^a is in the center well.

strains (Fig. 5 *b*). These and other tests are performed routinely to monitor the general health in the ICR animal colony. What is clear from Fig. 5 *b* is that the entire ICR mouse colony experienced a Sendai epizootic in the fall of 1971 which reached its peak in February of 1972. As can be seen in Fig. 5, the time-course of the Sendai infection preceded that of the IgG^a detection in ICR CB-17 mice. This was consistent with the idea that some infectious agent (not necessarily Sendai virus) could be partly responsible for the transient expression of IgG^a in ICR CB-17 mice. Possibly the putative agent was acting as an antigen to stimulate an unusual IgG^a immune response.

The Appearance of an IgG^a-Synthesizing Plasmacytoma in an ICR CB-17 Mouse.—To further test the idea that an infectious agent was in some way responsible for IgG^a in ICR CB-17 mice, preparations of bacterial cultures that were obtained from the blood samples of sick-looking ICR CB-17 mice

(organisms that were later identified in these cultures included *Pasturella pneumotropica* and *Streptococcus faecalis* plus Sendai and Reo virus) were injected into six healthy ICR CB-17 recipients. About 1 mo after three such i.p. injections (0.3 ml/injection/mouse), some of the recipients suddenly died. The three surviving ICR CB-17 mice (numbered 2, 3, 4) were bled; no. 3 ICR CB-17 and no. 4 ICR CB-17 showed IgG^a in their serum and 1 wk later so did mouse no. 2 ICR CB-17 (mouse 4 ICR CB-17 died 1 day after being bled). The results for no. 3 ICR CB-17 and no. 2 ICR CB-17 are shown in Fig. 7. The lymph node pathology of mouse 3 ICR CB-17 indicated it had a

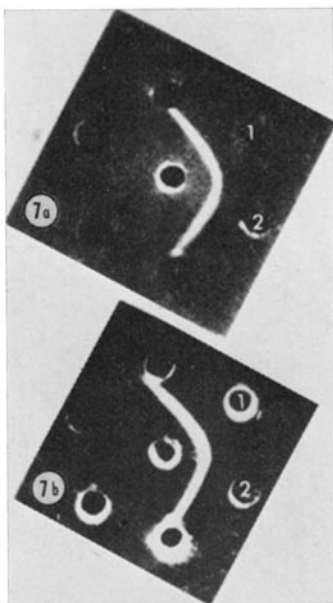


FIG. 7. The detection of IgG^a in sick ICR CB-17 mice. (a) The outer wells contain serum samples of mouse 2 ICR CB-17 (well 1) and mouse 3 ICR CB-17 (2) vs. anti-IgG^a in the center well; (b) The outer wells contain serum samples of mouse 3 ICR CB-17 (1) and an ICR CB-17 mouse bearing MOPC-173 (2) vs. anti-IgG^a in the center.

plasma cell leukemia which we were able to transplant successfully into other ICR CB-17 mice. This tumor (ICR PC 3) has now been passaged for 11 generations, during which time its pathology has progressed to that of a plasmacytoma.⁵ The ICR PC 3 plasmacytoma continues to synthesize large quantities of IgG^a. Although the lymphoid tissue of mouse 2 ICR CB-17 was judged to be that of an incipient leukemia, the transfer of this tissue to X-irradiated ICR CB-17 mice did not result in a tumor or an IgG^a response in these recipients.

We have since observed IgG^a in other ICR CB-17 mice that were either made

⁵ The tissue pathology of these mice was diagnosed by Dr. Philip Custer of this Institute.

sick with X-irradiation or were generally debilitated with a transplanted plasmacytoma (MOPC 104E). Whether utilizing the lymphoid tissue of these mice for cell transfer to other ICR CB-17 recipients or using identical procedures to those mentioned above, we have not been able to reliably elicit IgG^a in ICR CB-17 mice. When we succeeded in doing so, the IgG^a phenotype usually disappeared in a few weeks or the mice died. Also, the IgG^a phenotype in ICR CB-17 serum was not removed by absorption with the previously used bacterial preparations. Other attempts to induce IgG^a production in ICR CB-17 mice by injection with live Sendai virus, or repeated injections with different immunogens (dextran, R-36A pneumococcal vaccine, strep-DNP, lipopolysaccharides, various red cell antigens, and protein antigens) have failed.⁶

Analysis of ICR CB-17 Mice Showing IgG^a.—Using the serum of several ICR CB-17 mice that had detectable IgG^a, we determined what strain source of IgF was present (Fig. 8). Following papain digestion and immunoelectrophoresis of these serum samples, we used rabbit antiserum monospecific for mouse IgF to differentiate the IgF of BALB/c (IgF^a) from that of C57BL (IgF^b). This is possible because the Fc fragment of IgF^a migrates farther to the anode than the Fc fragment of IgF^b (4, 25). Accordingly, in Fig. 7 it can be seen with two examples (sera of mouse 2 ICR CB-17 and of ICR CB-17 [MOPC 104E] mice) that only IgF^b was found as opposed to the presence of both IgF^a and IgF^b in the serum of F₁ BALB/cICR X ICR CB-17 hybrid mice. We presume that the lack of detectable IgF^b in the serum of mouse 3 ICR CB-17 was because the large quantity of tumor protein (IgG^a) caused a depression in the normal synthesis of IgF^b.

As with IgF, the Fc fragments of BALB/c IgG migrate farther to the anode than those of C57BL. Therefore, with the same techniques as above, we used rabbit antiserum directed against mouse IgG to assure ourselves that our anti-allotype sera was not precipitating the IgG^a in ICR CB-17 mice on the basis of some unusual determinants (idiotypes) that might be associated with the combining site specificity of these IgG^a molecules.

A summary of what allotypes were found among Ig classes of the heavy chain linkage group is shown in Table II for ICR CB-17 normal sera, sera of mouse 2 ICR CB-17 and of ICR CB-17 (MOPC 104E) mice. It should be emphasized here that whenever IgG^a was detected in ICR CB-17 mice, it was always in addition to the normal presence of IgG^b. Also, it was not possible to determine if IgH^a was present in these ICR CB-17 experimental mice, because antisera monospecific for allotypic determinants unique to IgH^a was not available.

⁶ The injection of ICR CB-17 mice with Sendai virus was carried out at Oak Ridge National Laboratory, Oak Ridge, Tenn., by Dr. Conrad Richter. ICR CB-17 mice were immunized to dextran by Dr. Martin Weigert of the Salk Institute, San Diego, Calif. R-36A pneumococcal vaccine was given to us by Dr. Alex Tomasz of The Rockefeller University, New York.

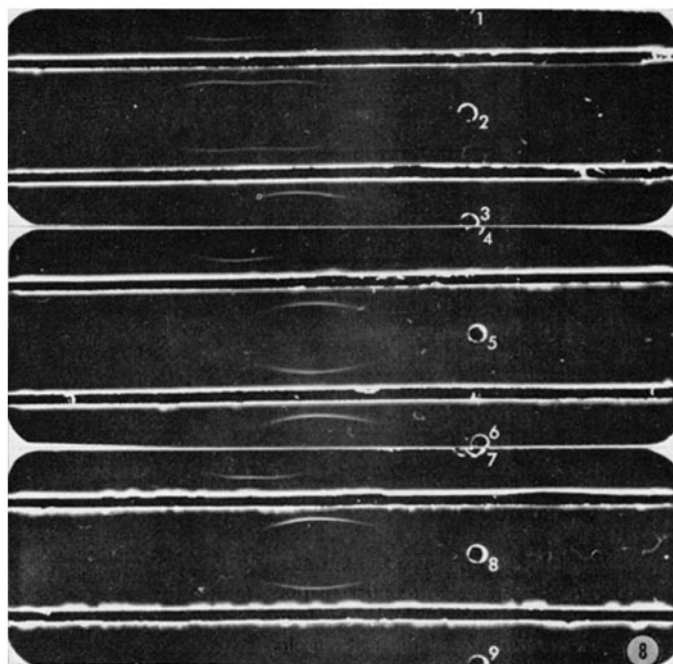


FIG. 8. Immunoelectrophoretic migrations of Fc fragments of IgF derived from papain-digested BALB/cICR and ICR CB-17 normal mouse sera and from papain-digested ICR CB-17 experimental mouse sera. All troughs contained rabbit antiserum that was mono-specific for mouse IgF, i.e., for determinants on the Fc portion. The wells were loaded with the following digested sera: BALB/cICR (wells 1, 4, and 7); ICR CB-17 (3 and 6); F₁ hybrid BALB/cICR × ICR CB-17 (2); ICR CB-17 (MOPC 104E) (5); mouse 2 ICR CB-17 (8); and mouse 3 ICR CB-17 (9).

TABLE II

Allotypes of Different Ig Classes in No. 2 ICR CB-17 and ICR CB-17(MOPC 104E) Experimental Mice and in ICR CB-17 Normal Mice

Ig class and allotype	ICR CB-17 mice		
	No. 2 ICR CB-17	ICR CB-17 (MOPC 104E)	ICR CB-17
IgF ^a	—	—	—
IgF ^b	+	+	+
IgG ^a (G ^{1,6,7,8})	+	+	—
IgG ^b (² G)	+	+	+
IgH ^b (H ¹⁶)	+	+	+
IgA ^a (A ^{12,13,14})	—	—	—

The numbers in parentheses are the assigned allotypic determinants of Lieberman and Potter (4) for the respective Ig classes of BALB/c (Ig^a) and C57BL (Ig^b) mouse strains. Determinant ²G is still unassigned but is presumably on IgG^b molecules. We thank Rose Lieberman for typing the IgH and IgA classes, as well as for confirming the presence of IgG^a and IgG^b in the ICR CB-17 experimental mice.

DISCUSSION

The preceding results showed that the transient appearance of IgG^a in ICR CB-17 mice was correlated with general sickness or overall debilitation resulting from the growth of transplanted plasmacytomas. This suggested to us that an infectious agent normally latent in BALB/c mice might have caused ICR CB-17 mice to produce IgG^a.

In our attempts to account for hidden IgG^a structural genes in ICR CB-17 mice, we will consider the following possible sources: (a) that BALB/c recombinant genes reside in some ICR CB-17 mice, the detection of which requires specific antigenic stimulation and possibly the action of specific regulator genes; (b) that IgG^a-like genes are carried on the C57BL/Ka chromosome in a repressed state (these same genes might be derepressed under appropriate conditions when introduced to the BALB/c genome); and (c) that viruses normally latent and indigenous to BALB/c mice carry some IgG^a information. We would presume that some forms of stress place ICR CB-17 cells at great risk to viral transformation, an event that results in transient IgG^a synthesis and possible lymphoid neoplasia.

The first consideration accounts for the origin of IgG^a by postulating that some ICR CB-17 mice carry a recombinant Ig C_HG^a gene(s) that was derived from the BALB/c mouse strain during the introgressive backcrossing of ICR CB-17 ancestors. As the first of two explanations for the normal silence of this recombinant gene, we postulate the following: (a) that the recombinational event included one Ig C_HG^a gene and a few associated V_H genes; and (b) that specific amplification of C_H gene expression (clonal cell proliferation) is dependent on V_H gene stimulation. The latter postulate is consistent with evidence for the close linkage of V and C genes (26, 27); with the selective stimulation of antibody allotypes by different haptens in F₁ hybrid C57BL/6 × C3H/HeN mice (28); and with that reported by Tosi et al. (29), namely, that the quantitative expression of allotype markers in the C_H region of rabbit Ig molecules is influenced by those in the V_H regions. Further evidence can be cited in support of the regulation of C gene expression by V gene stimulation. For example, the ratio of λ/κ-chains varies greatly in different animal species (13, 30, 31) as does the ratio of different rabbit κ-chains (those with five half-cystine residues vs. those containing seven half-cystine residues) with different κ-chain allotypes (32).

In keeping with the tandem arrangement of V_H and C_H genes proposed by others (9, 17), in which a single cluster of many V_H genes is followed by single copies of different C_H genes, most reciprocal recombinations involving C_H genes would include associated V_H genes.⁷ However infrequent such recombinations, we expect that random translocations of the involved V_H genes to adjacent C_H genes would lead to the serological detection of recombinant C_H

⁷ This is not to exclude the possibility that repeating clusters of V_H and C_H genes follow in series or that clusters of repeating V_H genes alternate with different C_H genes (existing in one or more copies).

genes. Therefore, in scoring for ICR CB-17 homozygotes (see Fig. 3), one would select against most recombinant mice as these would be indistinguishable from the heterozygotes. If, on the other hand, a gene recombination included only one C_H gene and very few associated V_H genes, it might not be detected for lack of antigenic stimulation. In other words, any antibody precursor cell of an ICR CB-17 recombinant mouse that synthesizes IgG^a would go undetected unless the appropriate antigen were normally present to induce enough clonal proliferation of these cells to make IgG^a detectable in the serum. The specific candidate that caused proliferation of cells expressing IgG^a recombinant genes would presumably be some infectious agent that acted as an antigen. Thus, our inability to reliably cause ICR CB-17 mice to synthesize IgG^a would result from the ill-defined conditions of stress that activate the putative agent and also from not knowing which ICR CB-17 mice were the recombinant mice.

Relevant to our observations are those of Blomberg et al. (20) that pertain to a relative of the ICR CB-17 congenic line. These investigators found that the BAB-14 congenic line (see footnote 2) gave an unexpected antibody response to dextran; i.e., BAB-14 mice responded as well to dextran as did BALB/c mice, instead of responding poorly as do C57BL/6 mice. A poor antibody response was expected since the use of recombinant inbred strains indicated that responsiveness to dextran was linked to C_H genes. Therefore, the BAB-14 antibody response was best explained by postulating a recombinational event between the C_H gene locus of C57BL and the BALB/c locus containing V_H genes for dextran.

What is inconsistent with the first explanation for the silence of a recombinant $Ig C_H G^a$ gene was our inability to remove the IgG^a in ICR CB-17 serum after absorption with the previously used bacterial preparations. And so perhaps the lack of IgG^a detection in normal ICR CB-17 mice relates to a more basic deficiency than that of not having the appropriate antigen. Therefore, a second explanation to account for the quiescence of a recombinant $Ig C_H G^a$ gene is as follows: (a) that the differentiation of stem cells into Ig -bearing precursor cells requires the derepression of specific regulator genes to initiate the joining of any one V_H gene with a C_H gene; (b) that the specificity of a given "joining enzyme" is restricted to one of two allelic sets of V_H and C_H genes; and (c) that during the backcrossing of ICR CB-17 ancestors, one selected against the acquisition of BALB/c regulator genes on the ICR CB-17 chromosome but not necessarily against the accumulation of recombinant V_H and C_H genes. According to this line of reasoning, the $Ig C_H G^a$ recombinant gene in ICR CB-17 mice would not be transcribed for lack of a BALB/c regulator gene(s). How this regulator gene deficiency might have been circumvented or altered in ICR CB-17 mice that exhibited IgG^a remains unclear.

The above explanation could also be applied to a second consideration for the genetic origin of IgG^a in ICR CB-17 mice, namely, that the structural genes for the IgG^a molecule reside in C57BL/Ka mice in a repressed state. In this

context, it becomes especially important to know whether the unassigned ²G allotypic determinant in C57BL mice (33) is indeed a true genetic marker for IgG.

As a last possible consideration, we feel it must be conceded that the information for the IgG^a molecule in ICR CB-17 mice could be carried as an episome (virus). Normally latent and indigenous to BALB/c mice, such a virus would not have been selected against in the ICR CB-17 pedigree. However, under some forms of stress to the lymphoid system, the virus may become infectious and possibly cause incipient neoplasia accompanied by IgG^a production. The final outcome may lead to a tumor. This explanation succeeds fairly well in accounting for our observations, especially the no. 3 ICR CB-17 leukemia mouse. Clearly, more experiments with ICR CB-17 mice are required before it can be decided which of the three considerations, if any, is likely to be correct.

SUMMARY

This paper derives from the unexpected observations of the "wrong immunoglobulin allotype" in a congenic partner strain of BALB/c mice from the Institute of Cancer Research (ICR CB-17). These mice were specially bred so as not to differ from BALB/c mice in any known way except to carry immunoglobulin structural genes of the C57BL/Ka allotype. In this respect, ICR CB-17 mice were defined as allotypically homozygous according to the Mendelian inheritance of mouse allotype markers. The homozygosity of these mice was challenged, however, when in certain instances immunoglobulins of the BALB/c allotype appeared in the serum of some ICR CB-17 mice. The appearance of this hidden allotype was usually transient and only associated with immunoglobulins of the IgG (IgG2a) class. The implications of these findings for the inheritance and expression of immunoglobulin structural genes are discussed.

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