GENETICS OF RESTRICTED ANTIBODIES TO STREPTOCOCCAL GROUP POLYSACCHARIDES IN MICE

1. Strain Differences of Isoelectric Focusing Spectra of Group A Hyperimmune Antisera

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The complexity of the immune responses of CBA mice to the DNP and 4-hydroxy-5-iodo-3-nitrophenacetyl (NIP)[1] ligand was most dramatically demonstrated by the use of isoelectric focusing (IEF) (1, 2). The molecular basis of this overwhelming antibody heterogeneity within this inbred strain remains a puzzle. In order to study the basis of such variability, however, it appears desirable to select systems of greater simplicity. This requirement appears to be related to both the antigen and the experimental animal (3). For example, streptococcal polysaccharide antigens may induce immune responses which are characterized by a high degree of restriction in selected rabbits and certain mouse strains (4–8).

IEF resolves specific antibodies at the level of single clone products (9). The method thus permits comparison of different antibody populations reflecting differences mainly in the variable region of heavy and light chains. Its advantage is the possibility of studying several different monoclonal antibodies of a single antiserum simultaneously.

This paper deals with differences observed in the serum levels and in the IEF patterns of streptococcal group A specific mouse antibodies obtained in inbred and hybrid mice. The latter approach is regarded as an alternative to idiotypic markers (10) for investigation related to antibody diversity.

Materials and Methods

Mice.—A/J, AKR/J, BALB/cJ, B10.D2 new/Sn, CBA/J, C3H/HeJ, C57BL/6J, DBA/2J, SJL/J, and some hybrid mice were obtained from the Jackson Laboratories, Bar Harbor, Maine. Outbred NMRI/HAN mice were obtained from Zentralinstitut für Versuchstierzucht, Hannover, Germany. The predominant proportion of the hybrid mice, C57BL/6 × BALB/c, BALB/c × C57BL/6, and C3H × BALB/c were bred from parent strain animals with known immune responses.

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[1] *Abbreviations used in this paper:* A-CHO, streptococcal group A polysaccharides; A-CHO-tyr-^{125}I, tyraminated and ^{125}I-labeled streptococcal group A polysaccharide; A-CHO-tyr-^{131}I, tyraminated and ^{131}I-labeled streptococcal group A polysaccharide; IEF, isoelectric focusing; NIP, 4-hydroxy-5-iodo-3-nitrophenacetyl; NS, preimmune serum.

Streptococcal Vaccines and Streptococcal Group A Polysaccharide.—Group A streptococci (J17A4) were used to prepare the vaccines for immunizations (5, 8). Cell suspensions used contained 250 μg of rhamnose/ml. The group A polysaccharide (A-CHO) was obtained by the method of hot formamide extraction of the cell walls (11).

Labeling of Group A Polysaccharide.—Isolated A-CHO was activated with cyanogen bromide (12) in a ratio of 5:1 (wt/wt) and then reacted with tyramine (Fluka, Buchs, Switzerland) under constant stirring at 4°C for 24 h. Unbound tyramine was removed by subsequent dialysis. Spectral analysis revealed covalent binding of tyramine to the A-CHO. Every 200 μg of tyraminated A-CHO was then labeled with 1 mCi of ^{131}I (Eidgenössisches Institut für Reaktorforschung, Würenlingen, Switzerland) after chloramine-T activation (13).

Immunization and Screening of Immune Sera.—3-mo old mice were immunized intraperitoneally and bled to obtain the immune sera as previously described (5, 8). These were screened for the presence of monodisperse Ig bands by microzone electrophoresis. All data presented in the subsequent discussion are from mice which had received a secondary immunization course (5, 8).

Quantitation of the Immune Response.—A modified Farr assay (14) was employed as described recently (6, 7) using tyraminated and ^{125}I-labeled A-CHO (A-CHO-tyr-^{125}I).

IEF and Staining of Plates.—Analytical IEF was performed with pH 5–10 gels as described previously (15). All gels contained 1–2 M urea freshly deionized before use (16). 10–20-μl serum samples were applied on strips of Whatman no. 1 filter paper after the addition of several crystals of urea (E. Merck AG, Darmstadt, W. Germany). Addition of urea is essential to increase the resolution of antibody patterns. Plates were focused at 450 V for 20 h and then for 3 h at 600 V to sharpen the Ig bands. pH gradients were determined by means of a flat membrane electrode (Dr. Ingold, Zürich). The gels were then overlayed in a moist chamber with a solution of A-CHO-tyr-^{131}I (10 μCi/plate) at 37°C for 40 min. This was followed by brief rinsing in distilled H_2O to remove unbound labeled A-CHO, fixation in 18% (wt/vol) Na_2SO_4 overnight, and treatment in "destain solution" (ethanol: distilled H_2O: glacial acetic acid = 3:6.5:0.5) for 20 min. Finally, plates were washed for 2 h in distilled H_2O to remove excess Na_2SO_4. Dehydration was achieved in 40% ethanol (20 min); the gels were then air-dried.

Agfa Gevaert Osray M3-TA-DW X-ray films were used for autoradiography. Exposure times varied between 12–72 h. Protein staining of the rehydrated plates (in destain solution) was performed with 0.2% bromphenol-blue.

Allotype Determination.—Allotypes were determined by double diffusion in agar with specific immune sera[2] (17).

Histocompatibility Typing.—Histocompatibility types were determined with antisera kindly obtained by Dr. D. Shreffler. They included the following specificities: anti-*H-2.3*, anti-*H-2.4*, and anti-*H-2.31* to determine the *H-2*^{d} type of BALB/c mice, and anti-H-*2.5* and anti-*H-2.33* for the *H-2*^{b} type of C57BL/6 mice (18, 19). The cytotoxicity test performed with lymph node cells was used with both the dye exclusion (eosin) and the fluorochrome uptake (fluorescein) test[2] (20, 21).

RESULTS

Strain Differences of the Immune Responses.—Previous studies on inbred mouse strains had suggested differences in the magnitude and the restriction of the response to the A-CHO (5–8). The mouse strains immunized in this work indicate that magnitude and degree of heterogeneity of the responses are properties unique for every strain tested. Upon intraperitoneal immunization, large

[2] The authors are indebted to Dr. Ethel B. Jacobson, The Basel Institute for Immunology, for the generous supply of antimouse allotype antisera; and to Dr. D. Bernoco, The Basel Institute for Immunology, for evaluation of the dye exclusion and fluorochrome uptake test.

amounts of antibody (>10 mg/ml serum) were made by BALB/c mice. AKR and B10.D2 mice produced somewhat intermediate levels of antibody, while A/J, SJL, CBA, DBA/2, C57BL/6, C3H, and NMRI mice were low responders (<5 mg/ml) (Fig. 1). Among the mice tested only one A/J and one NMRI mouse did not fit this classification. Using a different immunization schedule (2 × 6 intravenous injections), Eichmann found that A/J mice are high responders (6, 10). These findings show a strong dependence of the response on the route of immunization, as was already pointed out by Krause for the rabbit model (3).

IEF was used to evaluate the degree of heterogeneity on the level of single clone products in different mouse strains (Fig. 2). In principle, a monoclonal antibody (e.g., in BALB/c antisera) may give as many as three–four bands which are characteristically spaced throughout a certain pH range (9). Diffuse patterns (e.g., C57BL/6 mice) without distinct banding (Fig. 2 *a*) were interpreted to reflect a high degree of heterogeneity of specific antibodies. Preimmune sera did not bind the A-CHO. Restricted antibody populations were seen in 97/105 BALB/c, 6/9 B10.D2, 5/9 A/J, 3/8 AKR, 5/15 SJL, 3/13 C3H, 3/15 DBA, 2/14 C57BL/6, 1/8 NMRI, and 0/6 CBA mice.

IEF Patterns of BALB/c, C57BL/6, and C3H Immune Sera.—Since BALB/c

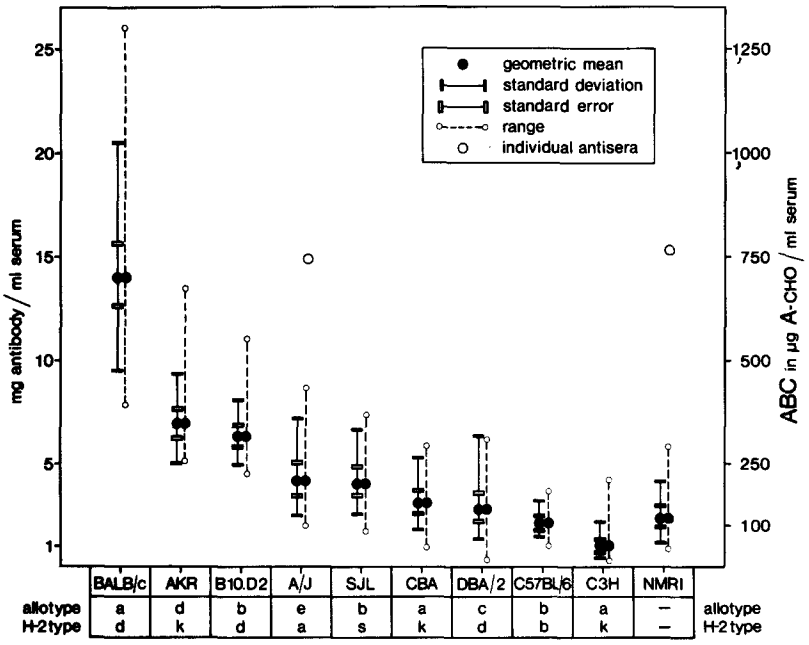

FIG. 1. Immune response of 10 mouse strains to the streptococcal group A polysaccharide (A-CHO) quantitated for individual mouse sera by a modified Farr assay. The mean antibody levels are taken from tests of 9–14 single antisera. ABC symbolizes antigen-binding capacity of A-CHO antibodies. Selection of strains considered IgG allotypes (17) and *H-2* types (18).

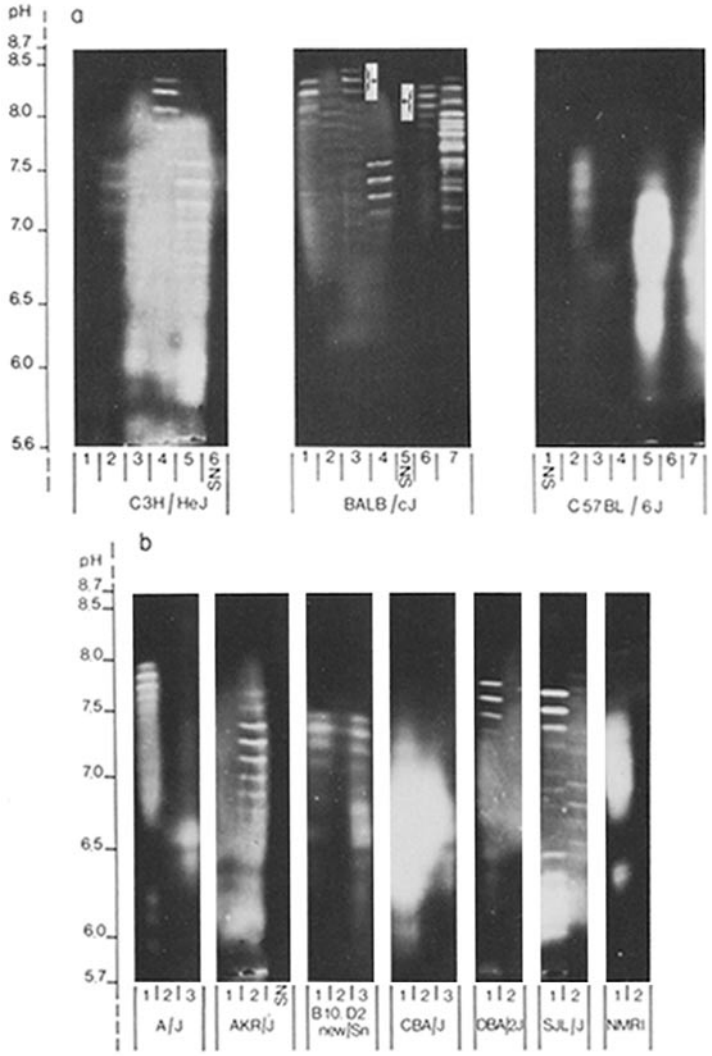

FIG. 2. (*a*) Analytical IEF patterns of 15 μl of individual antisera were developed with A-CHO-tyr-^{131}I. Exposure time to the X-ray film: BALB/c, 16 h; C3H and C57BL/6, 70 h. Two monoclonal antibodies produced are marked by *. NS show no antigen binding. (*b*) IEF patterns of 15 μl of individual antisera developed with A-CHO-tyr-^{131}I. Exposure time to X-ray films, 30 h. Patterns DBA/2-1 and SJL-1 represent rare cases in these strains, where prominent clones dominate the diffuse background, while all the other sera are representative for the patterns generally observed in the different strains.

immune sera showed the highest degree of restriction associated with high amounts of antibody, we chose to study the immune response of this strain in greater detail. For comparison, we selected C57BL/6 and C3H mice because BALB/c and C57BL/6 mice differ by both the Ig allotypes of their heavy

chains and the *H-2* types (17, 18). Furthermore, their immune response to the A-CHO was significantly lower and of greater heterogeneity than that of the BALB/c strain. C3H and BALB/c mice on the other hand share the Ig allotype *a* of the heavy chain (17), but differ by their *H-2* type (18).

Earlier studies had suggested: (*a*) that the allotype *a* marker, shared by various strains, could be associated with both low and high responsiveness (5); (*b*) that high responsiveness in this system seemed to be unlinked to the *H-2* type (5, 6); and (*c*) that magnitude and restriction of the response to the A-CHO once driven from IgM to IgG production were exclusively determined at the level of the bone marrow-derived cell (5).

Although 87% BALB/c mice were classified as restricted high responders considerable variations were seen in the number of clones identified by IEF (8). In general, every BALB/c immune serum has its individual antibody pattern (Fig. 2 *a*). Some of these sera are rather complex with as many as five–six clones; others are fairly restricted with as little as one dominant clone. Occasional sera contained some diffuse background staining on top of which prominent monoclonal patterns were superimposed. Side by side analysis of 40 BALB/c sera by this technique revealed that the BALB/c strain can express at least 30–40 different predominant antibody clones specific for the A-CHO. Among these 40 mice three antibody clones were shared by eight individual mice. These mice fell into three different groups consisting of two groups with three and one group of two mice, respectively. This is a considerably lower number than was reported for shared idiotypes in A/J mice with the A5A clone (10). IEF patterns of these specific immune sera therefore suggest that the BALB/c strain may express a very large number of A-CHO specific antibodies, and that the antibody pool size is larger than 30.

Altogether different IEF patterns were obtained with immune sera from C57BL/6 and C3H mice. The lack of distinct banding in the majority of the sera tested was taken as evidence for a high degree of heterogeneity of A-CHO specific antibodies (Fig. 2 *a*). Very typically, even in the presence of urea, antibodies in these immune sera smeared over a wide range. C3H mice no. 4, 5, and 7 (Fig. 2 *a*) were exceptional in expressing distinct clones to predominance among the total of 13 C3H immune sera tested. It was concluded from these data that low responsiveness in these two strains was generally associated with the specific stimulation of a large pool of A-CHO antibody-forming cells.

Quantity and Quality of A-CHO Antibodies in F_1 Hybrid Mice.—46 C57BL/6 × BALB/c and 34 C3H × BALB/c F_1 hybrid mice were immunized. 87% and 81% respectively of immune sera of both hybrid strains contained A-CHO antibodies with restricted electrophoretic properties (Fig. 3). Quantitation of these responses revealed that C57BL/6 × BALB/c F_1 hybrids made levels very close to the high responder parent strain while C3H × BALB/c F_1 hybrids produced levels of antibody intermediate between both parental strains (Fig. 4). These results indicate that responsiveness of C57BL/6 × BALB/c F_1 hybrid mice to the A-CHO is inherited as a dominant genetic trait from high re-

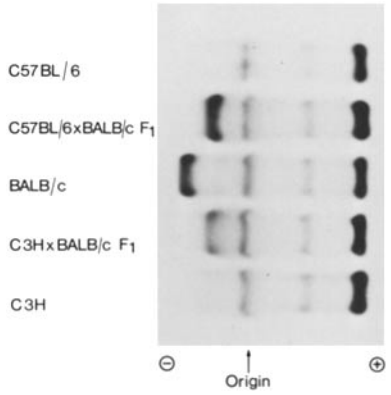

FIG. 3. Microzone electrophoretic analysis of five A-CHO immune sera of the three parental and two F_1 hybrid strains.

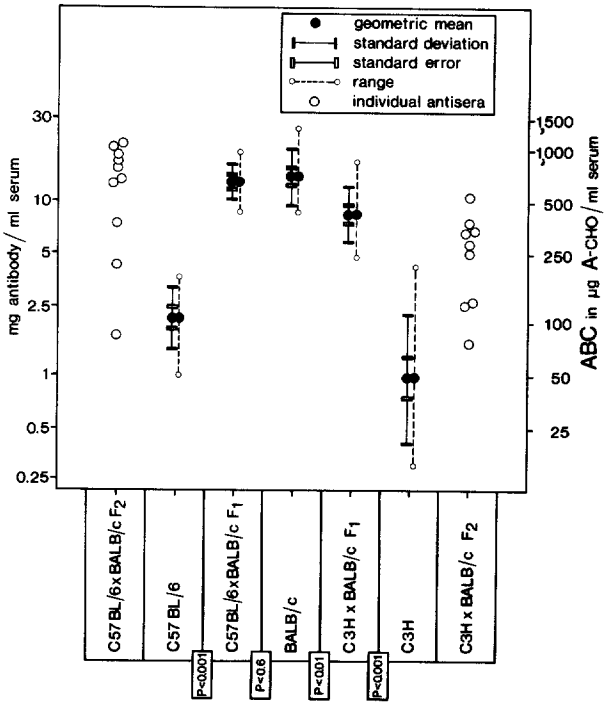

FIG. 4. Immune responses of the high responder strain BALB/c and low responder strains C57BL/6 and C3H are shown together with the corresponding F_1 hybrids. Mean antibody levels are taken from tests of 10–14 individual antisera. *P* values (*t* test) are included showing that immune responses of BALB/c and C57BL/6 × BALB/c F_1 are not significantly different, while all the others are. The immune responses of individual F_2 generation mice are added for comparison.

sponder BALB/c mice. In the second F_1 hybrid situation, responsiveness behaves like a codominant trait.

By IEF C57BL/6 × BALB/c F_1 hybrid immune sera disclosed a rather characteristic pattern of A-CHO antibodies (Fig. 5 *a*). In the pH 7–8 range a slow clone (B) and a more acidic clone (A) were overlapping among 81% of the immune sera. Distinct banding of A-CHO antibodies was also noted in the pH 6–7 range. However, within this range overlapping patterns were not observed in different immune sera (Fig. 5 *a*). Altogether C57BL/6 × BALB/c F_1 immune sera contained three–six distinct A-CHO antibody clones and a rather intense background staining very typical for C57BL/6 immune sera.

The high rate of expression of clones A and B conferred unique IEF characteristics to C57BL/6 × BALB/c F_1 hybrid immune sera. On the basis of these criteria F_1 immune sera were well distinguishable from patterns obtained with both BALB/c and C57BL/6 hyperimmune sera.

IEF patterns obtained with hyperimmune sera of C3H × BALB/c F_1 mice were far less uniform as was typical for the above combination. Only 13 out of 34 hybrids tested shared a clone A′ similar to but distinct from clone A. A rather basic clone C was present in eight individual antisera of these mice (Fig. 5 *b*).

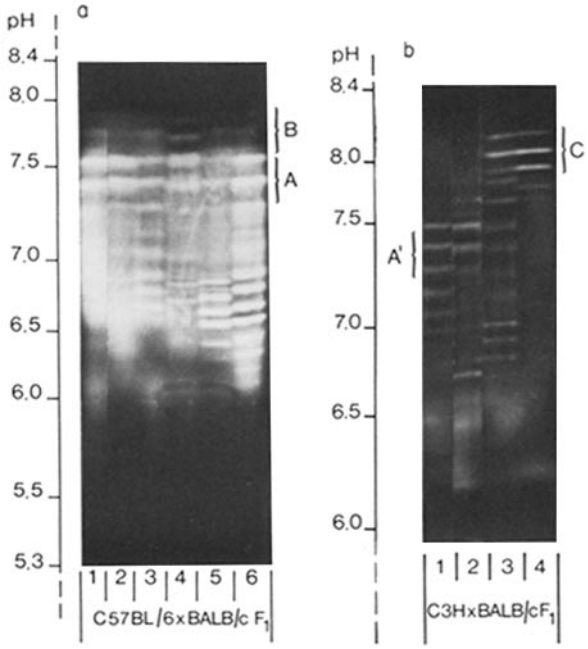

FIG. 5. IEF patterns of individual antisera (10 μl) of C57BL/6 × BALB/c F_1 (*a*) and C3H × BALB/c F_1 hybrid mice (*b*) developed with A-CHO-tyr-^{131}I. Repetitive clones are marked by A, A′, B, and C.

In order to explore further the allotypes of the various antibody components constituting the typical IEF pattern of C57BL/6 × BALB/c F_1 hybrids each 30 μl of one F_1 mouse serum (Fig. 5 *a*, pattern 6) was passed over an anti-$Ig^{a/a}$ Sepharose column and an anti-$Ig^{b/b}$ Sepharose column. IEF analysis of the eluates revealed that the clones A and B were absorbed to the anti-$Ig^{b/b}$ column only (Fig. 6). Attempts to recover this material by washing with a solution containing 1 N acetic acid and 1 M NaCl were unsuccessful. Conversely, the restricted clones focusing at pH 6.2–6.9 (Fig. 6) were selectively absorbed to the anti-$Ig^{a/a}$ Sepharose column.

In another series of experiments 50 μl of C57BL/6 × BALB/c F_1 of antisera 1 and 6 (Fig. 5 *a*) were focused in polyacrylamide plates, then precipitated with 18% Na_2SO_4 and sliced out. The recovered material was labeled with ^{125}I (13) and analyzed in coprecipitating double diffusion analysis with anti-$Ig^{a/a}$ and anti-$Ig^{b/b}$ antisera, respectively. The data for one serum are given in Fig. 6.

The combined evidence of these two experiments suggests that significant

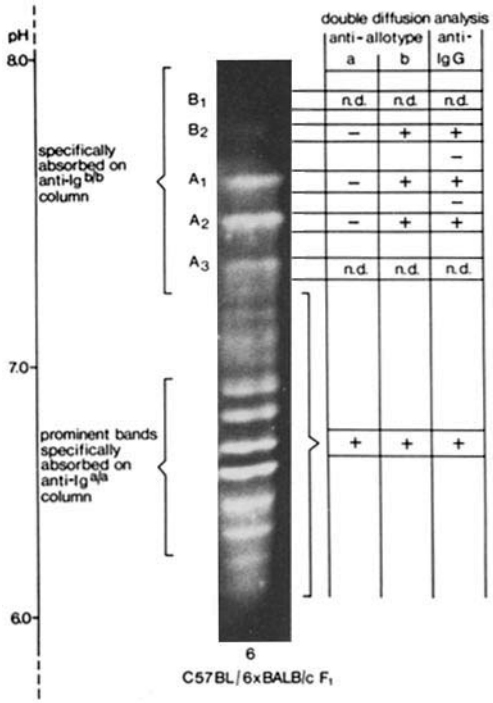

FIG. 6. Allotype analysis of clones A and B in C57BL/6 × BALB/c F_1 antiserum 6. For double diffusion analysis, coprecipitation was done with carrier Ig at equivalence. A clearly visible precipitin band on the X-ray film at different dilutions was taken as positive (+), the absence as negative (−). A control analysis run in parallel employed sheep antimouse IgG (The authors wish to thank Dr. A. S. Kelus, The Basel Institute for Immunology, for kindly donating this antiserum.). n.d., not done.

levels of restricted anti-A-CHO antibodies induced in C57BL/6 × BALB/c F_1 hybrids can be of C57BL/6 origin. This interpretation is supported by the fact that clones A and B were not found in BALB/c antisera, and by results obtained with F_2 generation mice discussed below.

Anti-Streptococcal A-CHO Response in F_2 Generation Progeny.—All F_2 progeny analysed originated from F_1 parents with known immune responses to the A-CHO. Analysis of the magnitude of the responses by microzone electrophoresis and absorption with antigen revealed that 79% (50/63) of the F_2 mice from C57BL/6 × BALB/c F_1 matings were high responders similar to BALB/c and F_1 hybrid mice. 21% (13/63) of these mice showed low levels of A-CHO antibody similar to C57BL/6 mice. These results were in good agreement with data obtained with 10 of these C57BL/6 × BALB/c F_2 sera in the Farr assay (Fig. 4). A similar distribution was found for F_2 progeny from C3H × BALB/c F_1 matings. Six out of nine mice responded with high to intermediate levels and three out of nine mice with low levels of A-CHO-specific antibody (Fig. 4).

IEF patterns of their immune sera yielded additional information (Fig. 7). 24 of the 52 C57BL/6 × BALB/c F_2 mice disclosed patterns like F_1 hybrid mice, 20 like BALB/c mice, and six like C57BL/6 mice. Two F_2 antisera could not be

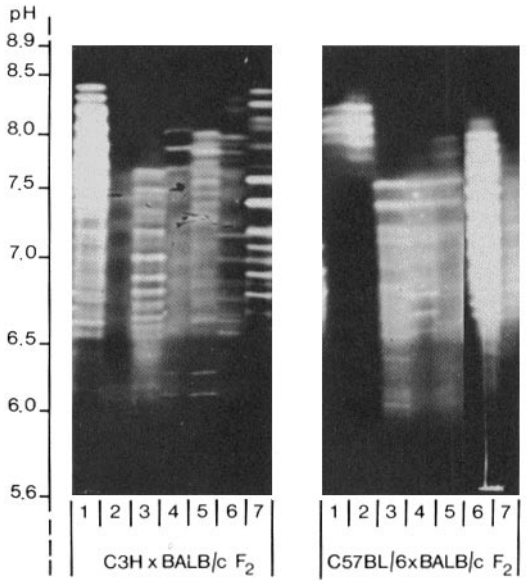

FIG. 7. IEF patterns of seven individual antisera of C57BL/6 × BALB/c F_2 and C3H × BALB/c F_2 mice developed with A-CHO-tyr-^{131}I. C3H × BALB/c F_2 patterns 1, 6 and 7, are typical for BALB/c antisera, patterns 3–5 are representative for C3H × BALB/c F_1 hybrid mice pattern 2 stands for C3H mouse antiserum. C57BL/6 × BALB/c F_2 antisera 1, 2 and 6 represent BALB/c-like patterns, antisera 3, 4 and 5 stand for F_1 patterns and antiserum 7 is typical for patterns of C57BL/6 mice.

classified by their IEF characteristics (Table I). Among the 20 mice with BALB/c patterns two showed completely overlapping monoclonal antibodies (Fig. 7). These mice were littermates.

Similar results were obtained with C3H × BALB/c F_2 antisera (Fig. 7), although only a total of nine mice were tested. Under these conditions, three mice showed F_1 hybrid, five BALB/c, and one mouse C3H patterns. Two different clones were shared by four mice suggesting the presence of molecules with overlapping pI's.

Such results indicate that responsiveness as a dominant trait segregates in F_2 generation progeny in the C57BL/6 × BALB/c strain combination to yield approximately 25% low and 75% high responders. The quality of the immune response to the A-CHO documented by the clonal patterns also follows simple genetic traits achieving a segregation within two generations. Segregation of antibody levels and clonal pattern is independent of hair color and sex (Table I).

Distribution of Allotypes in Hyperimmune C57BL/6 × BALB/c F_2 Progeny.— The heavy-chain allotypes *a* (BALB/c) and *b* (C57BL/6) of F_2 progeny were determined in whole antisera using antiallotype *a* antisera obtained in C57BL/6

TABLE I

Immune Response of C57BL/6 × BALB/c F_2 Mice. Correlation to Hair Color, Sex and Ig Allotype

		Serum antibody levels		IEF characteristics			
	Total	High*	Low†	BALB/c§	F_1 hybrid‖	C57BL/6¶	?**
No. of mice tested:	52	42	10	20	24	6	2
Hair color							
White	12	11 (9.7)‡‡	1 (2.3)	6 (4.6)	4 (5.6)	1 (1.4)	1 (0.4)
Black-agouti	20	14 (16.2)	6 (3.8)	7 (7.7)	10 (9.2)	2 (2.3)	1 (0.8)
Brown-agouti	7	5 (5.6)	2 (1.4)	1 (2.7)	4 (3.2)	2 (0.8)	— (0.3)
Black	10	10 (8.1)	— (1.9)	4 (3.8)	6 (4.6)	— (1.2)	— (0.4)
Brown	3	2 (2.4)	1 (0.6)	2 (1.2)	— (1.4)	1 (0.3)	— (0.1)
Sex							
♂	24	18 (19.4)	6 (4.6)	8 (9.2)	12 (11.1)	3 (2.8)	1 (0.9)
♀	28	24 (22.6)	4 (5.4)	12 (10.8)	12 (12.9)	3 (3.2)	1 (1.1)
Ig allotypes							
aa	9	7 (7.3)	2 (1.7)	8 (3.5)	— (4.2)	1 (1.0)	— (0.3)
ab	31	23 (25.0)	8 (6.0)	10 (11.9)	15 (14.3)	5 (3.6)	1 (1.2)
bb	12	12 (9.7)	— (2.3)	2 (4.6)	9 (5.5)	— (1.4)	1 (0.5)

* Antibody levels like BALB/c and F_1 hybrid mice.
‡ Antibody levels like C57BL/6 mice.
§ Highly restricted to monoclonal IEF patterns.
‖ Typical F_1 hybrid clones A and B present.
¶ No restriction in IEF patterns.
** Antisera could not be classified by their IEF characteristics.
‡‡ Numbers in parenthesis express the expected figures assuming a random distribution and thus no genetic linkage between the parameters listed.

and antiallotype *b* antisera prepared in BALB/c mice. Since BALB/c and C3H Ig heavy chains share the allotype *a* marker F_2 generation immune sera of this combination could not be included. The data for C57BL/6 × BALB/c F_2 progeny were summarized in Table I. As is apparent, high responsiveness to the A-CHO is not linked to the Ig allotype.

It was regarded as critical to investigate a possible linkage between the Ig allotypes and the IEF patterns. The data obtained are summarized in Table I. The appearance of clones A and B which are typical for a C57BL/6 × BALB/c F_1-IEF pattern is exclusively associated with the presence of allotype *b*, indicating that these clones are of C57BL/6 origin. Apart from this correlation the figures found are close to an expected random distribution if non-linkage existed between the Ig allotypes and IEF patterns ($\chi^2 = 5.62$).

H-2 types of F_2 Progeny from C57BL/6 × BALB/c Combinations.—Immune responsiveness to various synthetic polypeptide antigens is linked to the *H-2* type of mice (22). Unlike these systems responsiveness to the A-CHO was reported to be not linked to the *H-2* type of BALB/c and DBA/2 strains (5, 6). Since BALB/c and C57BL/6 mice differ in both responsiveness and *H-2* type, the question of a possible linkage was investigated with F_2 progeny mice. Lymph node cells from 14 of the 52 F_2 mice were *H-2*-typed by both the dye exclusion (eosin) and dye-uptake (fluorescein) assay (20, 21) in every experiment. The results are summarized in Table II. It appears that high responsiveness and restricted antibody heterogeneity associated with the expression of a BALB/c-like IEF pattern, is independent of the *H-2*-type of the mouse, e.g., mice 2/7 and 3/17, Table II. Conversely, mouse 2/16 that was homozygous for $H\text{-}2^d$ responded with low antibody levels and an IEF pattern typical for F_1 hybrid mice. Statistical analysis (χ^2-test) of the data presented in Table II also supports this interpretation.

DISCUSSION

The data in this paper suggest that BALB/c mice may display a repertoire of at least 30–40 different A-CHO antibodies that can be stimulated to clonal predominance.[3] Within our study the antibody pool size of the heterogeneous responder strains remains unestimated.

Despite a considerable pool size of A-CHO antibodies in A/J mice as revealed by IEF, it was possible to stimulate the A5A clone to predominant expression (6, 10, 23). This clone was also identified by its idiotype,[4] and IEF pattern in A/J mice immunized within this work. There are some other cases of high preponderance of

[3] If a first course of intraperitoneal injections is followed by a subsequent intravenous course, additional monoclonal antibodies were found (Cramer and Braun, unpublished observations), suggesting that every mouse used only a fraction of the available antibody clones upon a specific stimulus.

[4] This determination was kindly provided by Dr. K. Eichmann, Cologne.

TABLE II

Immune Response and H-2 Types of C57BL/6 × BALB/c F_2 Mice

No.	Sex	Hair color	Ig allotype	Response*	IEF‡	*H-2*
2/1	♂	Black-agouti	a b	Low	F_1	d b
3	♂	Black-agouti	a b	High	F_1	d b
4	♂	Black	a b	High	F_1	d b
7	♀	Brown	a b	High	BALB/c	*b b*
9	♂	Black-agouti	a b	High	F_1	d b
16	♀	Black-agouti	a b	Low	F_1	*d d*
17	♀	Brown-agouti	*b b*	High	F_1	d b
3/7	♀	Black	*a a*	High	BALB/c	d b
11	♂	White	a b	High	BALB/c	*d d*
14	♂	Black-agouti	a b	Low	BALB/c§ C57BL/6	d b
17	♀	White	a b	High	BALB/c	*b b*
19	♂	Brown-agouti	a b	Low	C57BL/6	d b
22	♂	White	*a a*	High	BALB/c‖	d b
23	♂	Black	*a a*	High	BALB/c‖	*d d*

* High, antibody levels like BALB/c and F_1 hybrid mice; Low, antibody levels like C57BL/6 mice.

‡ BALB/c, monoclonal or restricted pattern; C57BL/6, heteroclonal pattern; F_1, typical clones A and B present.

§ This antiserum could not be classified by its IEF characteristics.

‖ Mice 3/22 and 3/23 share an identical clone on IEF (Fig. 7).

idiotypic intrastrain cross-reactions of specific antibodies stimulated in A/J and BALB/c mice (24, 25). Recently, Pawlak and Nisonoff (26) extended this observasion for anti-*p*-azo-phenylarsonate antibodies of A/J mice to closely related mouse strains, while antibodies raised in mice from unrelated strains failed to cross-react idiotypically (10, 26).

From the work presented here, which permitted identification of single antibody forming clones by their IEF characteristics, it appears that the clonal pattern of A-CHO antibodies of most inbred mouse strains is more complex than was suggested by studies using the idiotype and the IEF pattern of predominant clones of the A/J strain as markers (6, 10). It is believed that IEF and idiotypy score for different characteristics of the variable region (27). Since A-CHO antibodies of BALB/c mice were reported to belong predominantly to the IgG_{2a} subclass (5), differences in the isoelectric points of monoclonal antibodies within the investigated pH range should not be due to substitutions of charged amino acids in the constant region of the heavy chain. It may therefore be concluded that the great heterogeneity encountered with homogeneous mouse A-CHO antibodies reflects the general picture while predominant expression of a similar or identical antibody is the exception, dependent on the strain and the antigen used.

Because of this complexity within most mouse strains, we chose to concentrate our efforts mainly on hybrids which arose from C57BL/6 × BALB/c and BALB/c × C57BL/6 matings and their F_2 progeny. The two parent strains differ by both their Ig allotypes and their *H-2* types. BALB/c mice are restricted high responders and C57BL/6 mice are heterogeneous low responders to the A-CHO. As expected, high responsiveness was found to be dominant over low responsiveness in this strain combination, similar to analogous traits reported for a variety of antigens (22). High responsiveness proved to be independent of the genotype of the mothers. It is therefore concluded that this trait is autosomal, in contrast to an X-linked gene, which controls responsiveness of mice to the pneumococcal SIII polysaccharide (28). In the second combination, C3H × BALB/c F_1 hybrid mice, responsiveness was codominantly inherited.

Analytical IEF revealed uniform expression of two distinct clones of A-CHO antibodies in 80–90% of C57BL/6 × BALB/c F_1 hybrid mice. Since the bands of these clones A and B appear to be products of the low responder H-chain genotype, their variable regions are likely to be of C57BL/6 origin as variable and constant region genes of the H chains are closely linked (10, 25, 29). The absence of clones A and B in $Ig^{a/a}$ F_2 progeny but their occurrence in $Ig^{b/b}$ F_2 mice (Table I) points in the same direction. The possibility was not excluded that antibodies of clones A and B represent hybrid molecules in which the light chains originate from the BALB/c strain.

Isoelectric spectra of A-CHO antibodies produced by C57BL/6 × BALB/c F_2 mice segregated into about 40% BALB/c, 48% F_1 and 12% C57BL/6 patterns. Such segregation, although not following simple unigenic rules, indicates strain-specific, heritable mechanism(s) that control clonal expression. Selection of this kind, distinct from that by affinity (30), must be considered as regulatory, and it occurs independent of the *H-2* type. Concomitantly, responsiveness is independent of the *H-2* type similar to the response to multichain polyproline antigens (31) and the expression of the BALB/c idiotype of anti-α-1,3-dextran antibodies (29). It therefore can not be regulated by genes coding in the *Ir-1* region of the *H-2* complex (22). Since responsiveness and clonal patterns were to be found unlinked to the agouti locus (calculated from data given in Table I), it is, furthermore, excluded that genes of the *Ir-2* locus, which controls the production of antibodies to the *Ea-1*-erythrocytic antigens of *Mus*-musculus in certain strain combinations (32), are involved in the control of immune responsiveness to A-CHO.

The control mechanism(s) that regulate clonal propagation to yield isoelectric spectra of A-CHO antibodies of restricted heterogeneity in BALB/c mice occurs independent of the heavy-chain allotype (Table I) and is thus of a different nature than the heritable factors that determine the idiotype of mouse antibodies and the respective allotype of the strain (10, 23, 25, 29, 33). If present, this mechanism also exerts its control on antibody clones of different genetic origin such that $Ig^{b/b}$ C57BL/6 × BALB/c F_2 mice may respond with clonal

isoelectric patterns typical for BALB/c mice; while, if absent, $Ig^{a/a}$ F_2 progeny may show patterns as do C57BL/6 mice. The predominant phenotypic expression of clones A and B in C57BL/6 × BALB/c F_1 hybrids suggests a similar interpretation.

Further studies are concerned with these heritable regulatory factors acting on the selection and expression of monoclonal antibodies. Preliminary data obtained with backcross mice point to a limited number of genes involved in the expression of strain-specific IEF patterns.

SUMMARY

The immune response of nine inbred and one outbred strain of mice to the streptococcal group A polysaccharide was investigated with respect to magnitude and restriction. Analytical isoelectric focusing served as a tool to estimate the degree of restriction of Group A polysaccharide-specific antibodies.

It proved feasible to distinguish low and intermediate from high responder strains, and to delineate strain-specificity of isoelectric focusing spectra of the immune sera. For example, immune sera of BALB/c mice, restricted high responders, and of C57BL/6 mice, heterogeneous low responders, had distinct focusing properties.

Responsiveness was a dominant autosomal genetic trait in C57BL/6 × BALB/c F_1 hybrid mice, irrespective of the maternal and the paternal genotype; the immune sera of these mice had their own, rather uniform isoelectric focusing spectra whereby structural genes of the low responder strain were expressed to predominant levels in 81% of the hybrids.

Responsiveness in C57BL/6 × BALB/c F_2 progeny segregated into 79% high and 21% low responders, and showed no genetic linkage to the following characteristics: hair color, sex, *H-2* type, and Ig allotype of the heavy chain. The isoelectric focusing properties of these immune sera indicated segregation into patterns like BALB/c mice (40%), F_1 hybrids (48%), and C57BL/6 mice (12%). Since this segregation is independent of any of the above criteria in these F_2 mice a regulatory gene(s) is postulated that controls the clonal pattern of the immune response.

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