# A RABBIT FAMILY OF RESTRICTED HIGH RESPONDERS TO THE STREPTOCOCCAL GROUP A-VARIANT POLYSACCHARIDE

## SELECTIVE BREEDING NARROWS THE ISOELECTRIC FOCUSING SPECTRA OF DOMINANT CLONES

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(Received for publication 23 May 1973)

Immunization of rabbits and inbred mouse strains with streptococcal vaccines may induce the production of high concentrations of group-specific antibodies that not infrequently show a considerable reduction in molecular heterogeneity (1, 2). Genetically, the degree of heterogeneity and the magnitude of the immune response to the streptococcal Group A and C polysaccharides appear to be independent variables (3). In addition, selective breeding for restricted antibodies is associated with limited idiotypic variability (4).[1]

Similar studies were now extended to the streptococcal Group A-variant polysaccharide as an antigen that chemically and serologically constitutes the backbone of the Groups A and C carbohydrates (5, 6). A random and a related group of rabbits were used for antibody production. The data suggest that immune responsiveness to the A-variant polysaccharide is also controlled by genetic factors that again influence the magnitude and the degree of restriction as independent variables. These systems appear to be different from the situation where the pneumococcal Types III and VIII antigens were used for immunization (7, 8), although recently suggestive evidence is at hand that in this latter situation genetic factors may be operative as well.[2]

### *Materials and Methods*

The major source of rabbits was the closed colony of the Statens Serum Institute, Copenhagen. A second group of rabbits consisted of progeny bred for high and restricted response to the Group C polysaccharide (3). They all originated from one breeding pair[3] (9).

Vaccines from Group A-variant, strain A486 var. M−, and Group C, strain C74, streptococci were prepared as previously described (9). The group polysaccharide concentration

[1] Braun, D. G., and A. S. Kelus. 1973. Idiotypic specificity of rabbit antibodies to streptococcal group-polysaccharides. Manuscript submitted for publication.

[2] Pincus, J. H., R. G. Mage, C. Alexander, and N. M. Chace. 1973. A familial incidence of structurally restricted antibodies to pneumococcal polysaccharides. Manuscript submitted for publication.

[3] We are very grateful to Dr. Richard M. Krause (The Rockefeller University, New York) who kindly supplied us with these rabbits.

was adjusted to 250 μg/ml as revealed by the determination of the rhamnose concentration (10). All vaccines were tested for sterility on blood agar plates.

Every rabbit, at the age of 6 mo, received a first (1°)[4] course of intravenous immunizations followed by a second (2°) course after a resting period of 6 mo. Bleedings were taken as previously described (3, 9).

The IgG concentration was determined as previously described (3, 9). Appropriate absorption studies with streptococcal Group A-variant cells and cell walls, as well as quantitative precipition analysis with isolated group carbohydrates, were used to determine the antibody concentration (9).

Analytical isoelectric focusing (IEF) was performed as described by Awdeh et al. (11). The gels were stained with 0.2% bromophenol blue or first developed with streptococcal Group A-variant polysaccharide tyraminated and labeled with $^{131}I$ [$^{131}I$](Av-CHO tyr) specifically and then stained.[5]

Classification of the immune sera into heterogeneous, restricted, and monoclonal followed the principles described previously (3). It was facilitated by the IEF patterns of the immune sera.

49 rabbits of the Copenhagen colony were immunized with Group A-variant vaccines. From these rabbits one monoclonal high-responder male rabbit, two restricted high-responder does, and one heterogeneous low-responder doe were selected for breeding. 12 rabbits (two litters) of Dr. R. M. Krause's colony were immunized with Group C vaccine and three restricted high responders (one male and two female rabbits) were selected for breeding. High responders contained more than 15 mg of antibody per ml of serum and low responders less than 12 mg (3).

## RESULTS

In the following section experimental data will be presented regarding the inheritance of the traits, magnitude, and restriction of the immune response to the streptococcal Group A-variant polysaccharide (Av-CHO) in rabbits originating from two unrelated colonies. The rabbit colony at the Statens Serum Institute in Copenhagen has been maintained as a closed colony for the last 24 yr, and it has been extensively used for the production of streptococcal group antisera (12, 13). The rabbit colony developed and maintained in Dr. R. M. Krause's laboratory was bred for restricted high responders to the streptococcal A-CHO and C-CHO (Groups A and C polysaccharide, respectively) (3). The degree of restriction of specific antibodies was routinely determined by analytical IEF combined with specific staining for antibody activity. The high degree of molecular uniformity of antibodies from six rabbits included in this study was also documented by partial amino acid sequence analysis for isolated heavy and/or light polypeptide chains (14).[6]

[4] *Abbreviations used in this paper:* 1°, primary; 2°, secondary; A-CHO, streptococcal Group A polysaccharide; Av, Group A-variant; Av-CHO, streptoccal Group A-variant polysaccharide; C-CHO, streptococcal Group C polysaccharide; IEF, isoelectric focusing; [$^{131}I$]Av-CHO tyr, streptococcal Group A-variant polysaccharide tyraminated and labeled with $^{131}I$.

[5] Cramer, M., and D. G. Braun. 1973. Cross-stimulation of monoclonal antibodies in anamnestic responses. Manuscript in preparation.

[6] Jaton, J.-C., D. G. Braun, A. D. Strosberg, E. Haber, and J. E. Morris. 1973. Homogeneous antibodies: amino acid sequences of rabbit H chains of allotype a1, a2 and a3 in the region of 80–94. Manuscript submitted for publication.

*Magnitude and Degree of Heterogeneity of the Response.*—A summary of the antibody levels as well as the degree of restriction observed in the random group of rabbits (Copenhagen) and in progeny from selective breeding combinations is given in Table I. 17 of the randomly chosen rabbits from the closed colony showed restricted immune responses and one rabbit a monoclonal response. Two breeding pairs consisted of maternal parents with high and restricted antibody populations (K4872 and K4878) and the monoclonally responding paternal parent. Immune responses in the offspring paralleled

TABLE I

*Antibody Levels to Streptococcal Av-CHO and C-CHO and the Frequency of Heterogeneous, Restricted, and Monoclonal Responders in Different Groups of Rabbits Analyzed*

| | Closed colony Copenhagen (Co.) | | | | Colony New York (N.Y.) | | Co. × N.Y. | |
|---|---|---|---|---|---|---|---|---|
| | | High response | | Low response | C-CHO high response | | Restricted high-response matings | |
| Origin of rabbits: | Random Av-CHO | F-1 Av-CHO | F-2 Av-CHO | F-1 Av-CHO | Selected C-CHO | F-1 Av-CHO | F-1′ Av-CHO | F-2 Av-CHO |
| No. tested: | 49 | 23 | 8 | 4 | 12 | 13 | 18 | 9* |
| Range of mg antibody (Ab)/ml | 3–38 | 5–30 | 16–50 | 8–14 | 11–48 | 21–58 | 15–56 | 18–28 |
| Average mg Ab/ml | 11 | 15.4 | 27.1 | 11 | 21 | 30 | 29.5 | 23 |
| No. of heterogeneous responders | 31 | 11 | 1 | 2 | 9 | — | 6 | 2 |
| No. of restricted responders | 17 | 11 | 4 | 2 | 3 | 11 | 11 | 3 |
| No. of monoclonal responders | 1 | 1 | 3 | — | — | 2 | 1 | 4 |

* These results come from 1° immunization only while all other data were obtained after 2° immunization.

the parental responses in both magnitude (on the average, a 5–10 mg increase was observed from one generation to the next two generations) and restriction of the antibody levels with increasing frequency from one generation to the next. Thus, after two generations of selective breeding by sibling and half-sibling matings, four out of eight rabbits showed restricted and three rabbits monoclonal responses (see also Fig. 1).

From four offspring of a low-responder mating with a high responder, two developed low and two intermediate antibody levels to the Av-CHO of the low number of surviving progeny tested. Two of these offspring had restricted responses similar to the one parent.

The second, unrelated group of rabbits originated from rabbits that had

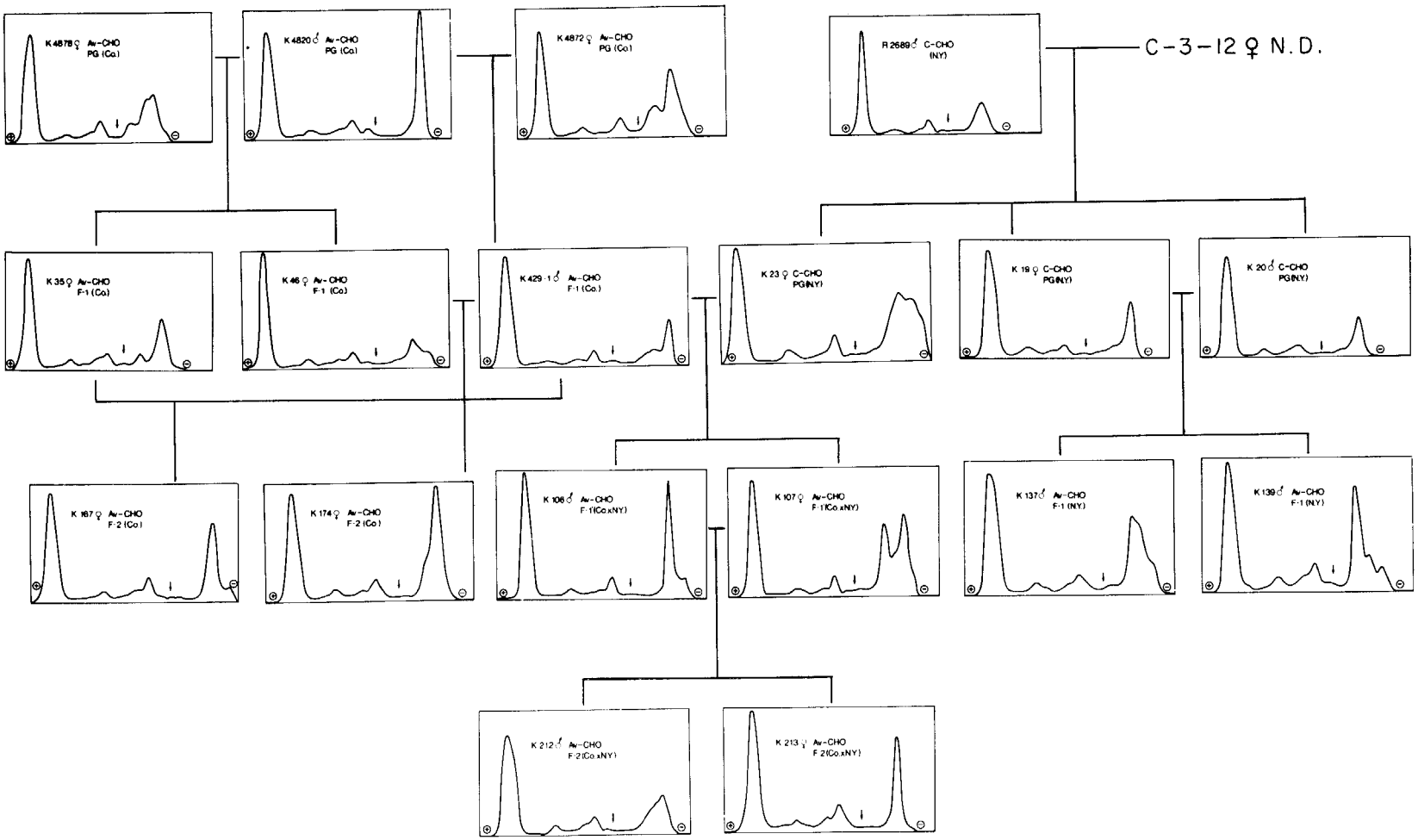


FIG. 1. Densitometric tracings of microzone electrophoresis patterns of representative immune sera within the pedigree. Peak areas to the right of the arrows (origin of migration) represent specific antibody. Selective breeding for monoclonal high responders was begun from members of two colonies: the left branch (K4878♀ and K4872♀, two restricted high responders, and K4820♂, the only monoclonal high responder to the Av-CHO of the random group as parent generation [PG]) originated from the closed colony of the Statens Serum Institute in Copenhagen (Co.) and the right branch bred from R2689♂ (3, 9), a restricted high responder of the colony in New York (N.Y.), and C-3-12♀, not immunized (N.D.). Three restricted high responders, of which K19♀ and K20♂ as one combination are shown here, were selected as PG to yield F-1 offspring K137♂ and K139♂. The heterogeneous high responder K23♀ (PG, N.Y.) was bred to the restricted high responder K429-1♂ (F-1, Co.) to yield the F-1′ (Co. × N.Y.) generation. From these matings, F-2 (Co. × N.Y.) progeny were obtained as shown for one breeding pair (K106♂ and K107♀) and two representative offspring.

produced high and restricted antibody levels to the C-CHO (Table I). This family is related to the New York colony through three parental high responders. All F-1 progeny from two matings (one brother-sister mating, see K19♀ and K20♂ in Fig. 1, and one mating between K20♂ and K31♀) were identified as high responders to the Av-CHO with average antibody levels of 30 mg/ml of antiserum. Two of these 13 offspring were monoclonal while the remaining 11 were restricted responders. These findings therefore suggest that high responsiveness to the C-CHO is also paralleled by a similar trait in response to the Av-CHO.

In the breeding studies reported here, most success so far was obtained by an intercolony combination (Fig. 1) of a restricted high responder to the Av-CHO (K429-1♂, an F-1 progeny of the Copenhagen colony) and a heterogeneous high responder to the C-CHO (K23, a littermate to rabbits K19 and K20). None of the 18 offspring immunized with Group A-variant (Av)-streptococci failed to develop high antibody levels. Among these F-1′ progeny, one rabbit responded with a single dominating clone and 11 of the remaining 17 rabbits with two to three very prominent clones. Two representative examples are shown in Fig. 1.

Excessive high responsiveness is usually seen after a 2° immunization course (3, 9).[6] The as yet limited number of F-2 generation offspring from brother-sister matings within the F-1 generation of the intercolony cross, however, developed an average antibody level of 23 mg of antibody/ml of antiserum already after 1° immunization (Table I). Four out of nine rabbits had limited their response to a single dominating clone and three rabbits were restricted high responders. Two examples are depicted in Fig. 1 as densitometric tracings of the microzone electrophoretic patterns.

*Selection of Dominating Clones.*—The degree of antibody restriction in immune sera is rapidly assayed by IEF. If combined with specific staining employing $^{131}$I-labeled antigen, the specificity of monoclonal antibodies is easily determined. For the present paper, [$^{131}$I]Av-CHO tyr was used as antigen. As depicted in Fig. 2 for a representative example, all dominant monoclonal antibodies in the sera analyzed bind Av-CHO specifically.

In the first set of experiments (Fig. 3 *A*), patterns of antisera from unrelated rabbits with high levels of monoclonal antibody populations were compared. Within the pH range depicted clearly visible bands represent specific antibody. Under such conditions, individual streptococcal Group A-variant antisera with electrophoretically restricted antibody populations generally express a series of nonoverlapping monoclonal antibodies. The situation was different if Av-CHO-specific antisera from closely related rabbits were focused (Fig. 3 *B* and *C*). Clearly, siblings of the F-1′ generations (Fig. 1) obtained by the intercolony mating (K429-1♂ × K23♀) expressed a series of repetitive monoclonal antibody patterns. Upon comparison with the pattern identified first in K4820 antiserum (Fig. 3 *A* 5*, Fig. 3 *C* PR A5), similar isoelectric

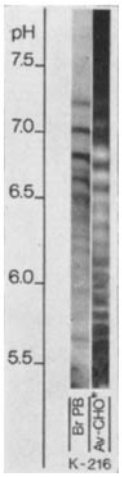


FIG. 2. Isoelectric focusing (IEF) patterns of a restricted group Av-CHO-specific rabbit antiserum. The left pattern was obtained by staining with 0.2% bromophenol blue (*Br PB*), the companion pattern to the right (*Av-CHO**) by specific staining with [$^{131}$I]Av-CHO tyr. All major bands of the left pattern bind labeled homologous antigen specifically.

focusing characteristics are apparent, suggesting a greater degree of similarities among expressed monoclonal antibodies. Brother-sister inbreeding (K106♂ was bred to K107♀ , K108♀ , and K109♀ ) yielded F-2 generation offspring that in a high proportion expressed dominant Av-CHO-specific antibody clones at average pI's between pH 7.1–7.2 and pH 7.5–7.6 (Fig. 3 *C*). Slight charge differences are obvious among these monoclonal antibodies; however, their appearance is very similar to K4820 antibody (PR A5). Besides others, monoclonal antibody focusing at pH 7.1–7.2 was clearly expressed as a dominant clone in the antiserum of parent F-1′a (K107♀ ) and not in the other, antiserum F-1′c (K106♂).

A summary of the data obtained for a representative branch of the colony is given in a histiogram plot for three different generations (Fig. 4). For this plot, the average charge of a typical three-band pattern characterizing a single monoclonal antibody was recorded. While monoclonal Av-CHO-specific antibodies identified in the random group of rabbits were scattered between pH 5.5 and 8.8, a somewhat greater restriction was seen in the F-1 generation, originating from the only monoclonal responder rabbit (paternal parent) and three does that all had shown patterns of much greater complexity. Judged by their net charge, some very similar monoclonal antibody patterns were apparent (e.g., at pH 6.4–6.5, 6.6–6.7, and 7.2–7.3).

Mechanisms placing constraints on the overall charge of the antibody clones stimulated in response to the Av-CHO appeared more obvious in the F-1′ generation. Here 63 definitive monoclonal antibodies were apparent in 17 antisera, scattering around 6 maxima (e.g., pI 6.0, 6.3, 6.6, 7.2, 7.3, and

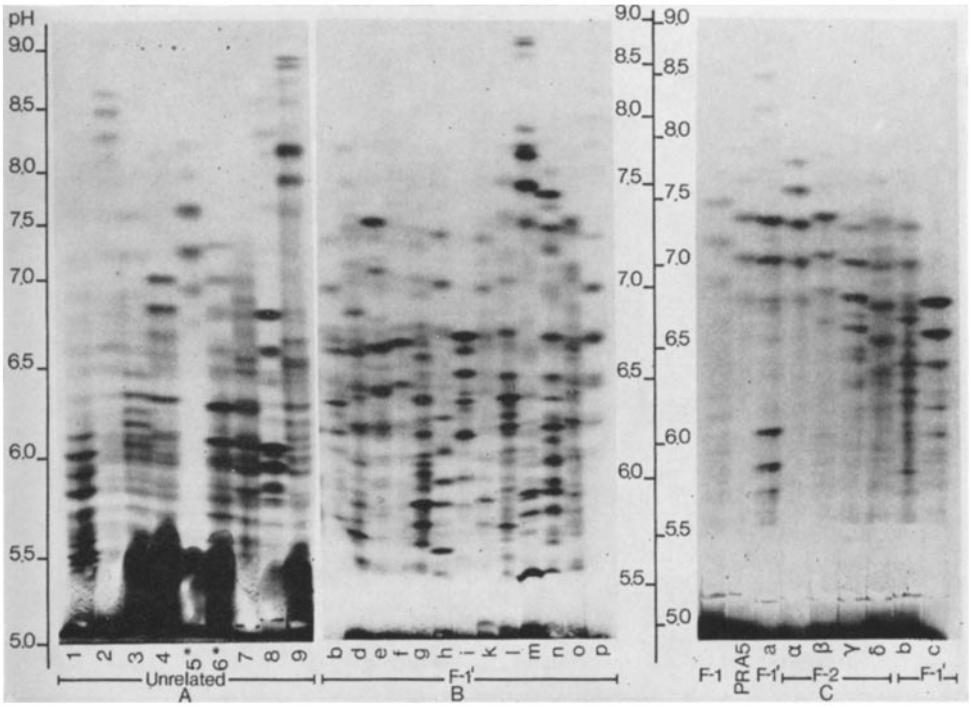


FIG. 3. IEF patterns of rabbit immune sera to the Av-CHO of three different groups of rabbits developed by staining with bromophenol blue. (*A*) Except for patterns nos. 5* and 6* (son [5]-mother [6] relationship), all patterns of restricted or monoclonal immune sera to Av-CHO were obtained from unrelated rabbits. Monoclonal antibodies (3–4 band patterns) focus differently in individual antisera. Pattern no. 5 was obtained with K4820♂ antiserum, pattern no. 7 with K4872♀ antiserum (see Fig. 1). (*B*) These are representative patterns obtained with immune sera from F-1′ progeny (*b-p*) of the intercolony mating (K429-1♂ × K23♀). Repetitive banding patterns at various pI's are apparent (e.g., pH 6.6–7.3; *e, h, k, o, p*). (*C*) These are representative patterns of immune sera from F-2 progeny (F-2 α-δ) of the intercolony matings (N.Y. × Co.). For direct comparison, the patterns of the parent from the random group K4820♂ (*PR A5*) and of his son K429-1 (*F-1*) as well as of the F-1′ parental generation (F-1′a = K107♀, F-1′b = K108♀, F-1′c = K106♂) were included. Great similarities of the monoclonal patterns in the average range of pH 7.1–7.2 are apparent.

7.9). Thus, although the number of clearly expressed clones was much higher in this group of rabbits compared with the random rabbits and the F-1 offspring, their distribution was suggesting nonrandomness.

In an attempt to further narrow the IEF patterns by inbreeding, four rabbits were selected from the F-1′ generation for brother-sister matings (one male and three female rabbits, see previous paragraphs) whose monoclonal antibodies were fairly representative for the scatter observed in this

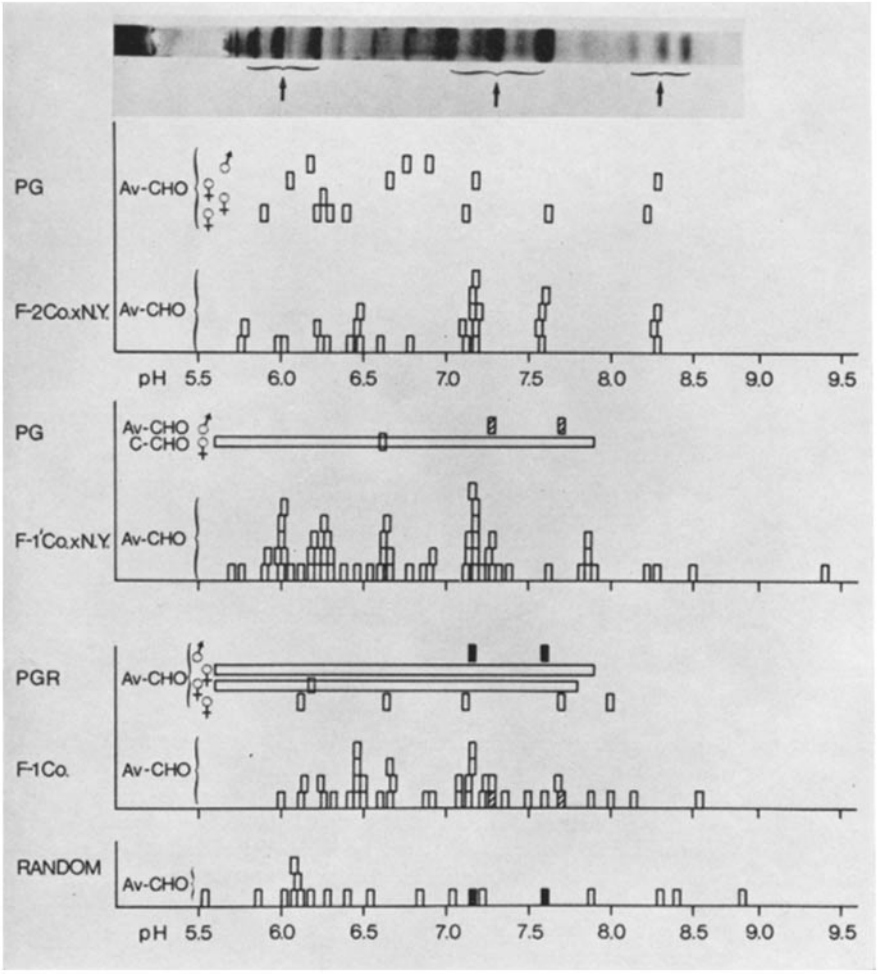


FIG. 4. Histiogram plots of the average pI's of the clearly expressed monoclonal antibodies in rabbit immune sera of the various generations analyzed. Every square is representative for one monoclonal antibody. The serological specificity of these antibodies is indicated. IEF pattern of antiserum K107 chosen for clarification of selection of monoclonal antibodies on top of the histiogram; their average pI's are indicated by ↑. *Random:* 20 distinct monoclonal antibodies were identified in 6 out of 49 random rabbit antisera. The filled-in squares (▮) indicate the pI's of K4820 monoclonal antibodies. *F-1 Co.:* 38 distinct monoclonal antibodies were identified in F-1 generation antisera. Hatched squares indicate the pI's of K429-1 monoclonal antibodies. *PGR:* Schematic representation of antibody patterns of parent antisera to yield the F-1 Co. generation. Open bars indicate the presence of multiple not clearly identified antibodies in the respective range of pH. *F-1' Co. × N.Y.:* 63 Av-CHO-specific monoclonal antibodies were identified in 17 offspring originating from the mating between K429-1♂ and K23♀ (PG). *F-2 Co. × N.Y.:* 28 Av-CHO-specific monoclonal antibodies were found in nine offspring of brother-sister matings (K106♂ was mated to K107♀, K108♀, and K109♀) between F-1' Co. × N.Y. progeny (PG).

group (Fig. 4). Nine offspring rabbits of the resulting F-2 generation survived a 1° course of immunization. A total of 28 monoclonal antibodies was expressed by them, distributed over four very restricted pH ranges with pI's at pH 6.5, 7.2, 7.6, and 8.3. Among these dominant monoclonal Av-CHO-specific antibodies, those with charge properties similar to K4820 antibody (pI 7.2 and 7.6, Fig. 5) were expressed in high frequency. Table II summarizes the number of progeny in which considerable expression of similar populations to these two monoclonal antibodies was suggestive by the IEF patterns of whole antisera. Comparison is made as percent of the total number of

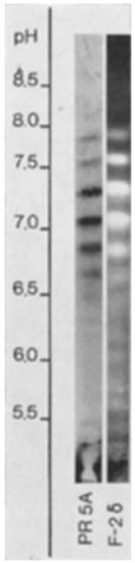


FIG. 5. IEF patterns of K4820 antibodies (*PR5A*) stained with bromophenol blue and of the antiserum of an F-2 Co. × N.Y. offspring (*F-2δ*) developed specifically with [$^{131}$I]Av-CHO tyr. The net charges of its dominant two monoclonal antibodies binding to antigen is very similar to that of K4820 antibodies.

TABLE II

*Expression of Distinct Monoclonal Antibodies (m Ab) of Similar Isoelectric Points (pI's) in Progeny as Parent K4820 Antibody*

| pI | Random group (6 rabbits) | | F-1 Co. (22 rabbits) | | F-1′ Co. × N.Y. (17 rabbits) | | F-2 Co. × N.Y. (9 rabbits) | |
|---|---|---|---|---|---|---|---|---|
| | No. of rabbits | % of total m Ab | No. of rabbits | % of total m Ab | No. of rabbits | % of total m Ab | No. of rabbits | % of total m Ab |
| 7.1–7.2 | 1 | 5 | 2 | 5.3 | 9* | 12.7 | 6* | 25 |
| 7.5–7.6 | 1 | 5 | 1 | 2.6 | 1 | 1.6 | 4 | 14.3 |
| Total no. of m Ab | 20 | | 38 | | 63 | | 28 | |

* Two distinct m Ab within 0.1 of a pH unit expressed in some rabbits.

distinct monoclonal antibodies expressed in every generation of rabbits. As a result of selective inbreeding, six out of nine F-2 generation progeny expressed dominant monoclonal antibody with pI's close to or at pH 7.2 and four out of nine rabbits, antibody with pI's of about 7.6. One-quarter of the dominant monoclonal antibody focused around pH 7.2, which was about double as many as in the F-1 progeny.

Thus it appears that genetic information for these two monoclonal antibody populations expressed to dominance in the random paternal parent was transmitted to yield a high penetration rate of very similar antibodies in F-2 generation progeny. These data would therefore suggest that selective breeding for restricted high-response rabbits to the Av-CHO is associated with preferential propagation of monoclonal antibodies with a similar net charge.

## DISCUSSION

The influence of genetic factors on the magnitude of the immune response to a variety of antigens is well documented in inbred strains of animals (16). When synthetic polypeptides of structurally restricted properties are used as antigens, the alleles coding for the response behave as autosomal dominant Mendelian factors (17, 18).

If similar studies employed experimental animals from an outbred source, segregation into low and high responders was readily demonstrable after several generations of selective breeding (19, 20). It appears that the number of generations required to achieve such segregation is dependent on the number of distinct determinants involved in immune recognition (16).

A substantially different approach was introduced to this problem when selective breeding not only involved the traits of high and low responsiveness but selection of antibodies of limited clonal origin. For the streptococcal Groups A and C polysaccharides, such selection was successfully made (3). Both of these carbohydrate antigens are distinctive by their terminal *N*-acetylated hexosamines that constitute the immunodominant determinants (5, 6). As a result of this, such antibodies if analyzed for antigen binding on the level of monoclonal antibodies, which is now possible by analytical isoelectric focusing, show no cross-reaction with the Av-CHO (e.g., K23 C-CHO-specific antibodies). A similar situation with a lack of cross-reactivity was found in mouse A-CHO-specific antibodies.[5] The absence of cross-reactivity is remarkable since both the A-CHO and C-CHO share a branched backbone of rhamnose chemically and serologically indistinguishable from the Av-CHO (5, 6, 21–23), which has its serological correlates in di- and tri-saccharide units of rhamnose (21).

Earlier studies reported the identification of rabbits with high levels of restricted antibodies in response to the Group Av vaccine (9, 15, 24). As mortality of rabbits immunized with Av vaccines was significantly higher than during immunization with Groups A and C streptococcal vaccines, these studies were not pursued to select low from high responders by breeding ex-

periments. However, the better survival rates of rabbits from the closed colony employed here prompted the attempt to breed for offspring from the monoclonal high responder K4820. This approach on the one hand yielded progeny that on the average produced significantly greater amounts of Av-CHO antibodies than rabbits from the random group. It also made possible the selection of rabbits with restricted or monoclonal antibody populations. For comparison, only a very small group of low-responder rabbits was bred to yield F-1 generation progeny with the suggestive evidence that this trait can be separated from high responsiveness similar to responder types identified to the A-CHO and C-CHO (3). Clearly, with every generation of selective breeding for high responsiveness over two generations, the average antibody level of Av-CHO antibodies increased by as much as 10 mg/ml. These data suggest a control of the magnitude of the Av-specific response by heritable factors, which is in agreement with earlier studies done for the related A-CHO and C-CHO antigens (3).

From these studies, a further piece of information emerged by the use of progeny from the high-response rabbit colony to the streptococcal Group C polysaccharide (3). Progeny of this genetic background were found on the average to produce three times as much Av-CHO-specific antibodies as the average rabbit from the random experimental group. Their levels of specific antibody were thus comparable to the selected F-2 generation progeny of the Copenhagen colony. Further substantiation for these data was furnished by the magnitude of the immune responses in progeny from the intercolony mating. These results would therefore suggest that high responsiveness to these two distinct polysaccharide antigens may be inherited as genetically linked traits. Genes controlling this trait may be considered as regulatory during the phase of immune recognition (16), and thereby influence the extent of propagation of certain antibody-forming clones.

One of the basic questions in this context relates to the molecular properties of specifically propagated antibody molecules triggered to high levels in response to either of the three streptococcal polysaccharide antigens. The argument has been raised that such extreme antibody levels of monoclonal origin may be only explainable on the basis of low affinity to the polysaccharide antigen that would allow escape from control mechanisms (G. F. Mitchell, personal communication). This argument may be reasonable for an anamnestic encounter of the rabbit with a closely related antigen, as was recently shown for homogeneous mouse antibodies.[5]

The fact that the homogeneous antibodies reported in this study all bind to the Av-CHO would favor an alternative explanation. This rests on the assumption that preferential propagation of certain antibody-forming clones is a reflection of a relatively smaller number of genes present in the repertoire of such rabbits in comparison with heterogeneous responders (3). If this were so, selective breeding should enable the identification of similar or even identical monoclonal antibodies in response to the same antigen. Idiotypic variability

of specific C-CHO antibodies was reported to be limited in the selectively bred rabbit family of restricted high responders (4), members of which were crossed with progeny from the Copenhagen colony. Idiotypic identity of such antibodies, however, was not apparent in F-1 generation progeny of the family reported here, although an unexpectedly high proportion of F-1 generation offspring had expressed similar antibodies to the antibody of the paternal parent present at rather low levels.[1]

It appears that idiotypic determinants are only representative for a limited portion of the variable part of antibody molecules and may therefore not necessarily reflect the overall similarity of specific antibodies in related rabbits.[1] Such features may be better demonstrable by the net charge properties of monoclonal antibody molecules expressed by their isoelectric points. This reasoning still holds, even though similar isoelectric focusing patterns of antibodies from two related rabbits are not necessarily associated with idiotypic cross-reactivity.[1] Conversely, indistinguishable idiotypic specificities were recently described for antibody molecules that differed by their isoelectric points and by their heavy chain allotypic markers (25).

If comparative analytical isoelectric focusing is used on this background of interpretation, it is a useful tool for the analysis of charge properties of monoclonal antibodies raised in related rabbits followed over several generations. The data of such comparative analysis for the biclonal antibody pattern of the stem parent K4820 would suggest that these two clones were basically maintained through three generations of offspring as germline gene products and then preferentially expressed in that generation that arose through selective brother-sister matings. Although slight charge differences are apparent, suggesting alterations of the product, e.g. by somatic mutation process, the principal overall charge remained constant. Such a finding might indicate that basic charge properties of biologically useful antibody molecules are preserved, on top of which mutation events may occur.

## SUMMARY

Immunization of rabbits from a closed colony with streptococcal Group A-variant vaccines identified about two-thirds of them as low and heterogeneous responders. One-third of the rabbits showed a restriction of the response independent from the magnitude. Selective breeding from one monoclonal high-responder male and two restricted high-responder female rabbits succeeded in segregation of high-responder progeny after two generations. Their antibody levels were on the average 2.5 times higher than those of the random group of rabbits and a small group of low-responder offspring.

Immunization of 13 offspring originating from rabbits bred for restricted high response to the streptococcal Group C polysaccharide revealed that 11 progeny were restricted high responders and 2 progeny monoclonal high responders. This finding suggests that high responsiveness to the Groups A-variant and C polysaccharides is inherited as genetically linked traits.

Selective breeding combinations between restricted and monoclonal high-responder rabbits by brother-sister matings succeeded in narrowing the isoelectric focusing spectra of Group A-variant-specific antibodies in the offspring. It furthermore revealed a preferential expression of monoclonal antibodies after three generations with a similar net charge as those identified first in the original monoclonal paternal parent. These data suggest that similar copies of structural genes for the variable regions are transmitted from the parent to the progeny.

The competent technical assistance of Mrs. E. Hillebrand, Miss D. Wieghardt, and Miss K. Hild is gratefully acknowledged.

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