# REGULATION OF THE ANTI-INULIN ANTIBODY RESPONSE BY A NONALLOTYPE-LINKED GENE

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Antibody responses to soluble polysaccharides, such as streptococcus A carbohydrate, levan, galactan, and dextran are often of very limited heterogeneity, and the capacity to produce a given form of these anti-polysaccharide antibodies is frequently determined by genes that map to the *Igh-V* region of the *Igh* gene complex (1–5). The actual repertoire of antibodies that potentially can be produced in response to immunization with such polysaccharide antigens is, however, very much greater, as revealed by the appearance of new clonotypes in mice previously suppressed by anti-idiotype antibody or in mice that have been immunized with polysaccharide-protein complexes or with polysaccharide-containing microorganisms. Relatively little is known about the genetic or cellular events that determine which of the potential antibodies that an animal is capable of synthesizing in response to a given antigen is actually produced.

In this communication, we report a study of the genetic regulation of the isoelectric focusing (IEF)[1] pattern of the IgG antibody produced in response to immunization with a soluble polysaccharide, bacterial levan (BL). BL is a $\beta$-(2 → 6) polyfructosan with $\beta$-(2 → 1) branch points. BALB/c mice immunized with BL produce antibodies that bind inulin (In), a $\beta$-(2 → 1) polyfructosan, as well as antibodies that are specific for the $\beta$-(2 → 6) levan determinants. The anti-In antibodies express a family of cross-reactive idiotypes (IdX) that are also found on a series of BALB/c In-binding myeloma proteins. In contrast, C57BL/6 and C.B20 strains, the latter of which is a BALB/c congenic strain that possesses an *Igh* complex of *b* (C57BL/6) type, fail to produce significant levels of anti-In antibody or of IdX upon BL immunization.

We have examined the regulation of IgG anti-In antibodies by comparing the IEF patterns of anti-In antibodies of BALB/c mice and those of B.C8 mice, which are congenic to C57BL/Ka and possess the *Igh$^a$* complex of BALB/c, of (BALB/c × C57BL/6)F₁ mice, and of the (BALB/c × C57BL/6) recombinant inbred (RI) lines, CXBG and CXBJ, both of which are *Igh$^a$* homozygous. Our results show that although the production of IgG anti-In requires genes that are found in the *a* (BALB/

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TABLE I
*H-2 and Ig Haplotypes of Mice Used in These Studies*

| Strain | H-2 | Igh |
|--------|-----|-----|
| BALB/c AnN | d | a |
| C57BL/6N | b | b |
| B.C8 | b | a |
| C.B20 | d | b |
| CXBG | b | a |
| CXBJ | b | a |
| CXBI | b | b |

c) type of the *Igh* gene complex, the complexity of the IEF pattern is regulated by at least one C57BL gene. This regulatory gene, here designated as spectrotype regulation gene 1 (*Sr-1*), is not linked to either the *Igh* gene complex, the major histocompatibility complex (*MHC*), or to the genes that control coat color.

## Materials and Methods

*Mice.*   The strains used in these studies are listed in Table I, which indicates their *MHC* types and *Igh* types. They were obtained from the Small Animal Section, Division of Research Services, National Institutes of Health (NIH) (Bethesda, Md.) or The Jackson Laboratory (Bar Harbor, Maine), or were the kind gift of Dr. Michael Potter, NIH. $F_1$ hybrids were bred in the Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, NIH.

*Antigens.*   BL (2 × $10^7$ daltons), from *Aerobacter levanicum* (ATCC 1,552), was obtained as previously described (6). In coupled to *Brucella abortus* (In-BA) and In coupled to keyhole limpet hemocyanin (KLH) (In-KLH) were prepared as described by Chien et al. (7).

*Immunization of Mice.*   Adult mice (8–12 wk of age) were immunized intravenously with 10–50 μg of BL in saline and were bled 5, 10, 20, 40, and 60 d after immunization. Other mice were immunized with 0.1 ml of a 1% suspension of In-BA, emulsified in complete Freund's adjuvant (CFA) (Difco Laboratories, Detroit, Mich.), and bled 10 d later. Mice immunized with In-KLH or KLH were given 100 μg of antigen in CFA, intraperitoneally, and were boosted at 2 and 3 wk with 50 μg of antigen in incomplete Freund's adjuvant. They were bled 1 wk after the last injection.

*Determination of Immunoglobulin Allotypes.*   Allotype analysis was performed as described by Lieberman (8).

*Determination of H-2 Types.*   H-2 typing was performed by a modification of the microcytotoxicity method described by Sachs et al. (9). Typing was performed on peripheral blood lymphocytes isolated by Ficoll (Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Piscataway, N. J.)-Hypaque (Winthrop Laboratories, New York) centrifugation (D. Sachs. Personal communication.) with anti-H-2$^d$ and H-2$^b$ sera provided by Dr. David Sachs, National Cancer Institute, NIH.

*IEF.*   IEF was performed by a modification of the method described by Briles and Davie (10) and refined by Nicolotti et al. (11). 3-Methacryloxypropyltrimethoxysilane (methacrylsilane) and *N,N'*-diallyltartardiamide (DATD) were purchased from Polysciences, Inc., Warrington, Pa. Acrylamide, *N,N'*-methylene-bis-acrylamide (Bis), and *N,N,N',N'*-tetramethylethylenediamine (TEMED) were purchased from Bio-Rad Laboratories, Richmond, Calif. Riboflavin, taurine, and lysine (free base) were purchased from Sigma Chemical Co., St. Louis, Mo., and ampholines were purchased from LKB Instruments, Inc., Rockville, Md. Ultrapure urea was purchased from BRL, Inc., Rockville, Md., and stock solutions were deionized on a mixed-bed ion-exchange resin column of Amberlite, MB-3 (Mallinckrodt Inc., St. Louis, Mo.). Glutaraldehyde was purchased from Eastman Kodak Co., Rochester, N. Y.

A 10- × 15-cm gel (0.6-mm thick) was cast in a vertical apparatus (10) between a plexiglass backing plate and a glass plate that had been previously treated with methacrylsilane (11) to produce covalent attachment of the acrylamide gel to the glass surface. The gel solution was prepared from the following components: 5.1 ml DATD-acrylamide (3% [wt/vol] DATD +

17% [wt/vol] acrylamide); 7.75 ml 8 M urea; 5.1 g glycerol; 0.7 ml pH 5-8 ampholines; 0.4 ml pH 8-9.5 ampholines (the mixture was degassed for 1-2 min before adding catalysts); 1 ml riboflavin (4 mg/100 ml); 100 $\mu$l TEMED (20%); and 100 $\mu$l ammonium persulfate (1%). The gel mixture was immediately poured into a 20-ml syringe and was applied between the plates of the apparatus with a 22-g (1.5-in.) needle. A 2- $\times$ 15-cm Bis gel overlay, into which a Teflon (E. I. DuPont de Nemours & Co., Inc., Wilmington, Del.) comb that contained 20 sample slots was imbedded during polymerization, was immediately applied over the DATD-acrylamide mixture with a 3-ml syringe that was fitted with a 22-g needle. The Bis gel was prepared from the following mixture: 1.2 ml 8 M urea; 0.49 ml Bis (33% acrylamide [wt/vol] + 0.81% Bis [wt/vol]); 1.05 ml $H_2O$; 0.16 $\mu$l pH 5-8 ampholines (the mixture was degassed for 30 s before adding the catalysts); 156 $\mu$l riboflavin (4 mg/100 ml); 15.6 $\mu$l TEMED (20%); and 1 $\mu$l ammonium persulfate. The gels were polymerized for 3 h at room temperature under fluorescent illumination. The Teflon comb was removed from the gel and any unpolymerized gel was aspirated from the sample slots. Serum (10 $\mu$l) was mixed with 10 $\mu$l 8 M urea and loaded into the sample slots. Samples were then overlaid with ~5 $\mu$l 1 M urea that contained 3% pH 3.5-5 ampholines. Gels were run at 2 mA at constant current for 14-15 h at 4°C. The positive electrode compartment (top) was filled with 0.1 M taurine, which contained five drops of phosphoric acid per liter, and the negative electrode compartment (bottom) was filled with 0.1 M lysine (free base), which contained 2 ml of ethanolamine per liter. After completion of IEF, the gels were rinsed briefly under running distilled water, and the pH was measured over the entire length of the gel with a flat-bed electrode. Immunoglobulins were precipitated by three changes of 18% $Na_2SO_4$ over a period of 6 h. The gels were overlaid for 24 h with 15-20 $\times$ 10$^6$ cpm/gel (or 7-10 $\times$ 10$^6$ cpm/half gel) of $^{125}$I-labeled antigen that was diluted in 18% $Na_2SO_4$ that contained 1% bovine serum albumin (BSA). After exposure to labeled antigen, the gels were washed for 24 h with at least three changes of 18% $Na_2SO_4$ and were fixed for 30 min in 18% $Na_2SO_4$ that contained 0.5% glutaraldehyde. After fixation, the gels were washed overnight in phosphate-buffered saline (0.02 M $PO_4$ and 0.15 M NaCl, pH 7.4), followed by 6 h in distilled water (three changes), and finally for 1 h in 40% ethanol. The gels were then dried (either overnight in air or for 30 min at 100°C) and exposed to x-ray film. Either Kodak single emulsion SB-5 or double emulsion XR5 film was used. Films were exposed for 7 d. When XR5 film was used, the emulsion on the side of the film not exposed directly to the gel was removed after development of the film by gently rubbing off the emulsion with gauze soaked in a 5.25% solution of NaClO (commerical laundry bleach) followed by rinsing under running tap water.

*Iodinated Antigens.*    In-BSA was prepared by the method of Chien et al. (7) and iodinated by the chloramine T method (12) to a 30 $\mu$Ci/$\mu$g sp act. Levan was tyraminated by the method of Keck (13) and iodinated by the chloramine T method to a 10 $\mu$Ci/$\mu$g sp act. Labeled antigens were stored at −20°C and used for 4-6 wk.

## Results

*IEF Pattern of Antibodies Produced by BALB/c Mice Immunized with BL.*    BALB/c mice immunized with BL produce antibodies that react with BL and cross-reacting antibodies that bind In. The anti-In antibodies bear the IdX found on many In-binding myeloma proteins (4). These BALB/c anti-In antibodies display a characteristic IEF pattern (Fig. 1) that is essentially identical in all individuals, although 1 of the 18 mice shown in Fig. 1 failed to respond to BL. This pattern consists of a single spectrotype comprised of five bands, of which three predominate, and which focus on IEF gels in the pH 6.3-6.8 range (Fig. 1). The anti-BL antibodies from the same 18 individuals are shown in the lower half of Fig. 1. As can be seen, the anti-In antibodies react with BL, although weakly, probably a result of the paucity of $\beta$-(2 → 1) determinants in BL. Most individuals produced IgG anti-BL in addition to anti-In, and these antibodies are specific for the $\beta$-(2 → 6) levan determinant because they do not cross-react with In. Unlike the IEF pattern of anti-In antibodies, which is shared by all individual BALB/c mice, the anti-BL antibodies of these 18 individuals consist
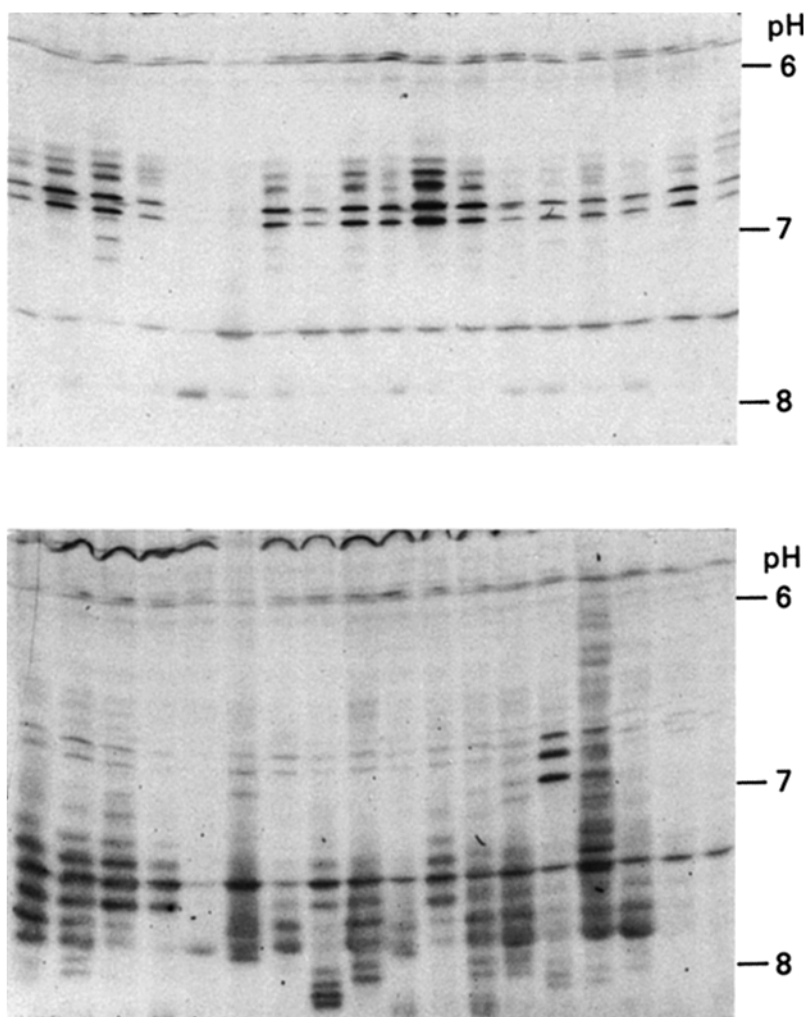
FIG. 1.  IEF pattern of BALB/c anti-BL antibodies. Sera obtained from 18 individual BALB/c mice at 10 d after immunization with BL were focused in duplicate gels in the same sample slot in both gels. The upper panel shows the antibodies reactive with $^{125}$I-In-BSA, and the lower panel shows the antibodies reactive with $^{125}$I-BL.

of at least five distinct sets of bands, each of which may be considered to be a spectrotype. Moreover, individual sera exhibit different combinations of these spectrotypes. The levan-specific antibodies focus between pH 7 and 8.5 and are thus quite distinct from the anti-In antibodies. Neither the anti-In nor the anti-BL spectrotypes change over a period of 40 d after immunization (Fig. 2).

To investigate whether the anti-In spectrotype represented the entire clonal repertoire of IgG anti-In antibodies of BALB/c mice, other BALB/c mice were immunized with In coupled to various carriers such as KLH and *B. abortus*. Immunizations with In-BA and In-KLH were performed in CFA. The IEF pattern of anti-In antibodies elicited by In-BA is considerably more heterogeneous than that elicited by BL (Fig.
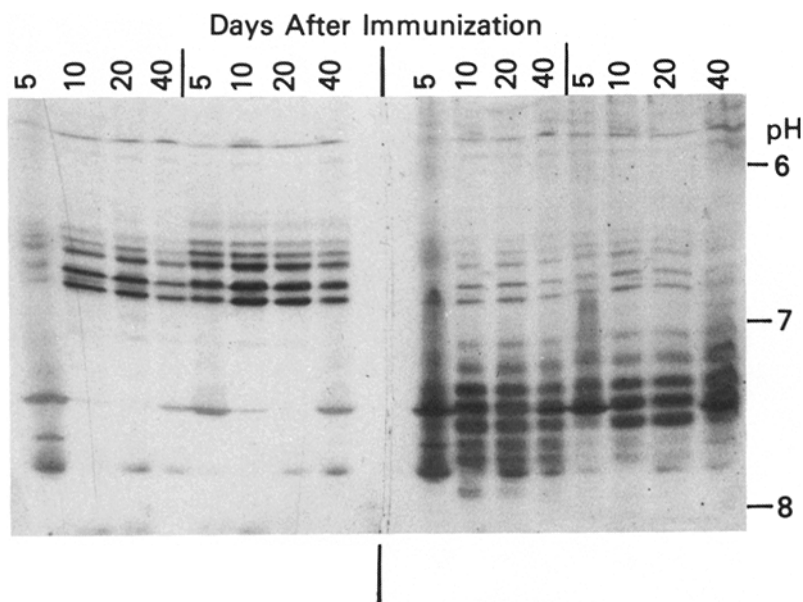
Days After Immunization



Fig. 2. IEF pattern of BALB/c anti-BL antibodies at various times after immunization. Sera obtained from two representative individual BALB/c mice at 5, 10, 20, and 40 d after immunization with BL were focused in duplicate in the same gel. The gel was cut and one-half was exposed to $^{125}$I-In-BSA (left panel) and the other to $^{125}$I-BL (right panel).

3). The IEF pattern of antibodies elicited by In-KLH shows even greater complexity (Fig. 3). We conclude from these data that BALB/c mice possess an extensive repertoire of anti-In antibodies that, nevertheless, is not fully expressed after immunization with soluble BL.

*IEF Pattern of Antibodies Produced by C57BL/6 Mice Immunized with BL.* In contrast to BALB/c mice, C57BL/6 mice immunized with BL produce high titers of anti-BL antibodies by hemagglutination analysis (HA) but only little or no hemagglutinating anti-In antibodies. They also fail to produce the IdX characteristic of BALB/c mice (4). Examination of such antisera by IEF shows that C57BL/6 mice produce IgG anti-BL antibodies but no IgG anti-In antibodies (Fig. 4). The population of C57BL/6 mice expresses two principal anti-BL spectrotypes. An occasional mouse (Fig. 4, animal 9) produces a more complex pattern that appears to consist of the antibodies present in both of the index spectrotypes.

*IEF Pattern of Antibodies Produced by (BALB/c × C57BL/6)F₁ and Backcross Mice.* (BALB/c × C57BL/6)F₁ hybrid mice were immunized with BL, and all developed a vigorous IdX-positive anti-In antibody response. IEF analysis revealed that the pattern of their IgG anti-In antibodies was considerably more heterogeneous than that of anti-In antibodies produced by BALB/c mice in response to BL (Fig. 5). The increased heterogeneity of the $F_1$ response is almost certainly not a result of the expression of a latent anti-In response of the $Igh^b$ type, because B.C8 anti-In antibodies express a degree of antibody heterogeneity equivalent to, or greater than, that of the $F_1$ anti-In antibodies (Fig. 6). B.C8 mice are $Igh^a$-homozygous mice that derive the remainder of their genome from C57BL/Ka. Thus, a gene or genes of the C57BL
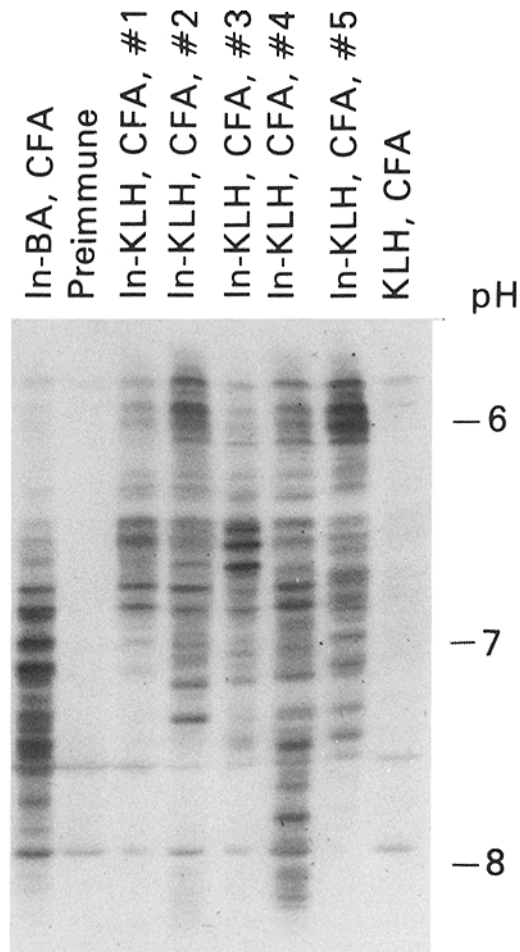
Fig. 3. IEF pattern of BALB/c anti-In antibodies raised against In conjugates. The anti-In-BA serum is a pool prepared from the sera obtained from three BALB/c mice at 10 d after immunization with In-BA. The preimmune and anti-KLH sera were from pools prepared from sera obtained from 10 BALB/c mice before immunization or 10 d after immunization with KLH. Anti-In-KLH sera were obtained from five individual BALB/c mice 1 wk after the third immunization with In-KLH. Sera were focused, and the gel was exposed to $^{125}$I-In-BSA.

mouse can lead to increases in the amount of diversity of the $Igh^a$ anti-In antibodies that are produced in response to immunization with BL.

*Backcross Analysis of the Anti-In Response.*    To estimate the number of C57BL/6 genes involved in this regulation of $Igh^a$-determined antibody expression, the anti-In antibody response to BL of (BALB/C × C57BL/6) × BALB/c backcross mice was examined (Fig. 7). Of 25 such progeny, 10 made antibody responses comparable to those of BALB/c mice and 15 displayed more heterogeneous responses. This suggests that one C57BL6 gene plays a dominant role in regulating the expression of $Igh^a$ anti-In antibodies. We found that BALB/c-like and non-BALB/c-like responses were essentially equally distributed among mice that were homozygous for the $a$ allotypic form of Ig H-chain constant regions ($Igh$-$C$) and those that were $a/b$ heterozygotes

Animal
1 2   3   4   5 6 7 8 9 10 1   2   3   4   5   6   7   8   9   10   pH
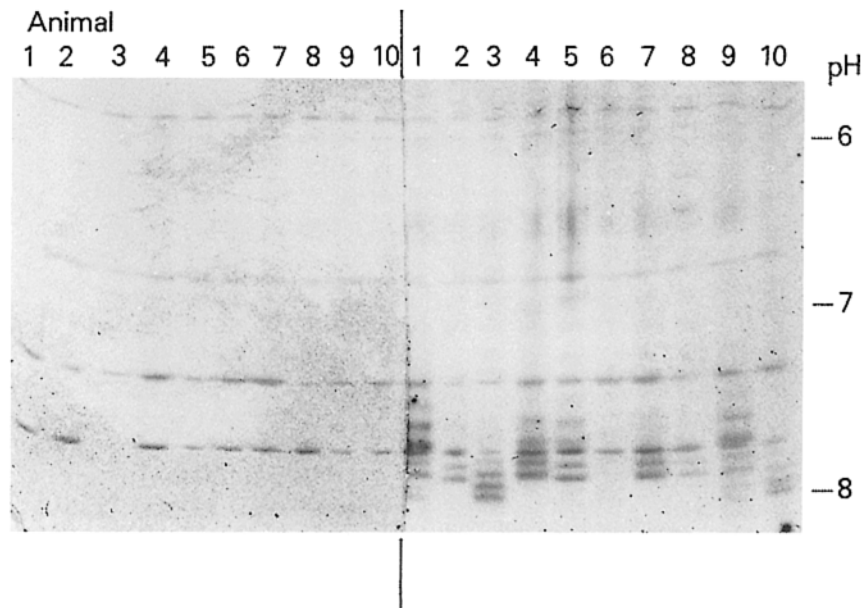
— 6

— 7

— 8

Fig. 4.   IEF pattern of C57BL/6 anti-BL antibodies. Sera obtained from 10 individual C57BL/6 mice at 10 d after immunization with BL were focused in duplicate in the same gel. The gel was cut and one-half was exposed to $^{125}$I-In-BSA (left panel) and the other half to $^{125}$I-BL (right panel). The bands seen on the left half of the gel (In overlay) are bands that we consider to represent nonspecific binding because the identical pattern can be seen with sera from unimmunized mice, as well as with immune sera, and with virtually any iodinated antigen that we have examined. No effort has been made to eliminate these bands as they serve as a useful internal marker.

(Table II). Similarly, no linkage of the IEF pattern of anti-In antibodies to the presence of the *b* MHC haplotype was noted (Table II). Both patterns were found in both males and females and in mice of the three distinct coat colors found among the backcross progeny (Table II). Thus, the C57BL/6 gene that was involved in regulating the *Igh*$^a$ anti-In response appears not to be linked to the *Igh* complex, to the *MHC*, or to the genes specifying coat color. As noted earlier (Introduction), we define this gene as *Sr-1*. The equal sex distribution is not informative for X chromosomal linkage of *Sr-1* because the (BALB/c × C57BL/6)F$_1$ was the mother of the backcross progeny and thus transmitted a C57BL/6 X chromosome to one-half of both the male and female offspring.

*IEF Patterns of Antibodies of RI Mice Immunized with BL.*   To further investigate the contribution of C57BL genes to the regulation of the anti-In antibody response, we studied the anti-In antibodies produced in response to BL immunization in CXB RI mice. These RI lines were derived from separated F$_2$ lines of BALB/c × C57BL/6 mice. Two such lines possess the *a* haplotype of the *Igh* gene complex, CXBJ, and CXBG; both produce IdX-positive anti-In antibodies after immunization with BL (14). In contrast, CXBI mice, which possess the *b* haplotype of the *Igh-C* gene complex, do not develop an IdX-positive anti-In response. The IEF patterns of anti-In antibodies of these strains are shown in Fig. 8. CXBI mice fail to produce IgG anti-In antibodies. CXBJ mice produce anti-In antibodies with the spectrotype characteristic of that of BALB/c mice, although the bands are of greater intensity than those
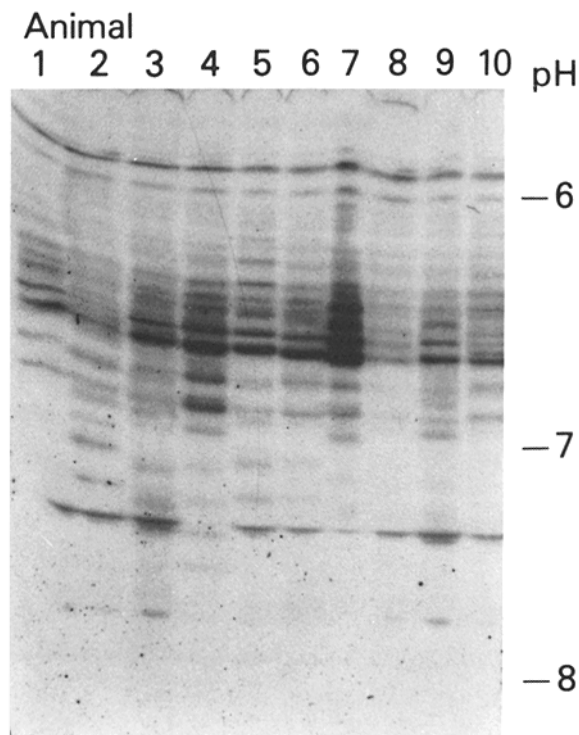
Fig. 5. IEF pattern of (BALB/c × C57BL/6)F₁ anti-BL antibodies. Sera obtained from 10 individual (BALB/c × C57BL/6)F₁ mice at 10 d after immunization with BL were focused and exposed to ¹²⁵I-In-BSA.

observed in BALB/c anti-BL antisera. CXBG mice produce a more heterogeneous pattern than do BALB/c. These data suggest that $Sr-1$, or some other C57BL/6 gene that regulates heterogeneity of the anti-In response, is present in CXBG but not CXBJ mice. In further experiments, we tested this possibility by examining the anti-In antibodies produced in response to BL immunization of $F_1$ hybrids of CXBJ and CXBG with BALB/c and C.B20 mice.

*IEF Patterns of Antibodies of $F_1$ Hybrids Between RI Mice and BALB/c and C.B20 Mice Immunized with BL.* $F_1$ hybrids from (CXBI × BALB/c), (CXBJ × BALB/c), and (CXBG × BALB/c) matings all develop IdX-positive anti-In antibodies in response to BL immunization (data not shown). The IEF pattern of (CXBG × BALB/c)$F_1$ anti-In antibodies resembles that of CXBG (Fig. 8), again supporting the conclusion that CXBG mice possess $Sr-1$ or another gene that regulates heterogeneity of anti-In antibodies. Similarly, the finding that (CXBI × BALB/c)$F_1$ mice express an anti-In IEF pattern (Fig. 8) that is more complex than that of BALB/c indicates that CXBI, although itself unable to produce anti-In antibodies, possesses an anti-In regulatory gene or genes. Quite surprisingly, the (CXBJ × BALB/c)$F_1$ mice produced anti-In antibodies that were more complex than either parent (Fig. 8). This suggests that CXBJ does possess a regulatory gene, although it is not expressed in the CXBJ itself.

Certain of these regulatory effects also appear to be dependent upon homozygosity at the *Igh* gene complex. Thus, (CXBG × C.B20)$F_1$ mice make an anti-In response to
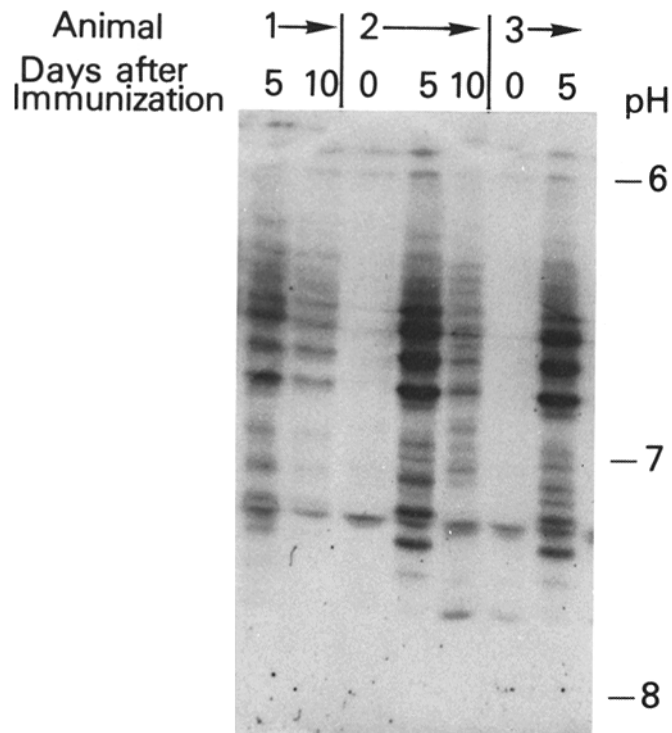
Fig. 6. IEF pattern of B.C8 anti-BL antibodies. Sera obtained from three individual B.C8 mice at various times (as indicated) before or after immunization with BL were focused and exposed to ^{125}I-In-BSA.

BL that resembles that of BALB/c mice rather than that of either CXBG or (CXBG × BALB/c)F₁ mice. (CXBG × C.B20)F₁ and (CXBG × BALB/c)F₁ should differ only in that the former is heterozygous at the *Igh* gene complex, whereas the latter is homozygous. This effect of *Igh* gene complex heterozygosity is seen even more strikingly in (CXBJ × C.B20)F₁ mice, which fail to produce an IdX-positive anti-In response to BL and lack IgG anti-In antibodies. This failure to produce IgG anti-In antibodies has been observed in analysis of three separate groups of (CXBJ × C.B20)F₁ mice immunized on different occasions. To confirm the role of *Igh* gene complex heterozygosity in the regulation of the anti-In response, (CXBJ × C.B20)F₂ mice were bred and immunized with BL. Among 11 mice of this type, 4 were homozygous for the *a* allotype. Analysis of the anti-In and anti-BL antibodies of eight of these mice by IEF is shown in Fig. 9. Mice 1, 5, 7, and 11 were found to be homozygous for the *a* haplotype of the *Igh* gene complex, whereas mice 2–4 and 10 were found to be *a/b* heterozygous. One of the *a* allotype homozygous mice (No. 11) and all of the *a/b* heterozygous mice produced no or very little IgG anti-In. In contrast, one of the *a/a* homozygotes (No. 5) produced a weak IgG response characteristic of BALB/c mice, whereas the two others (1 and 7) produced IgG anti-In antibodies substantially more heterogeneous than either CXBJ or BALB/c. This suggests that a heterogeneous anti-In response to BL is favored by the presence of a C57BL background gene and by *Igh*$^a$ homozygosity. We should emphasize that *Sr-1*,
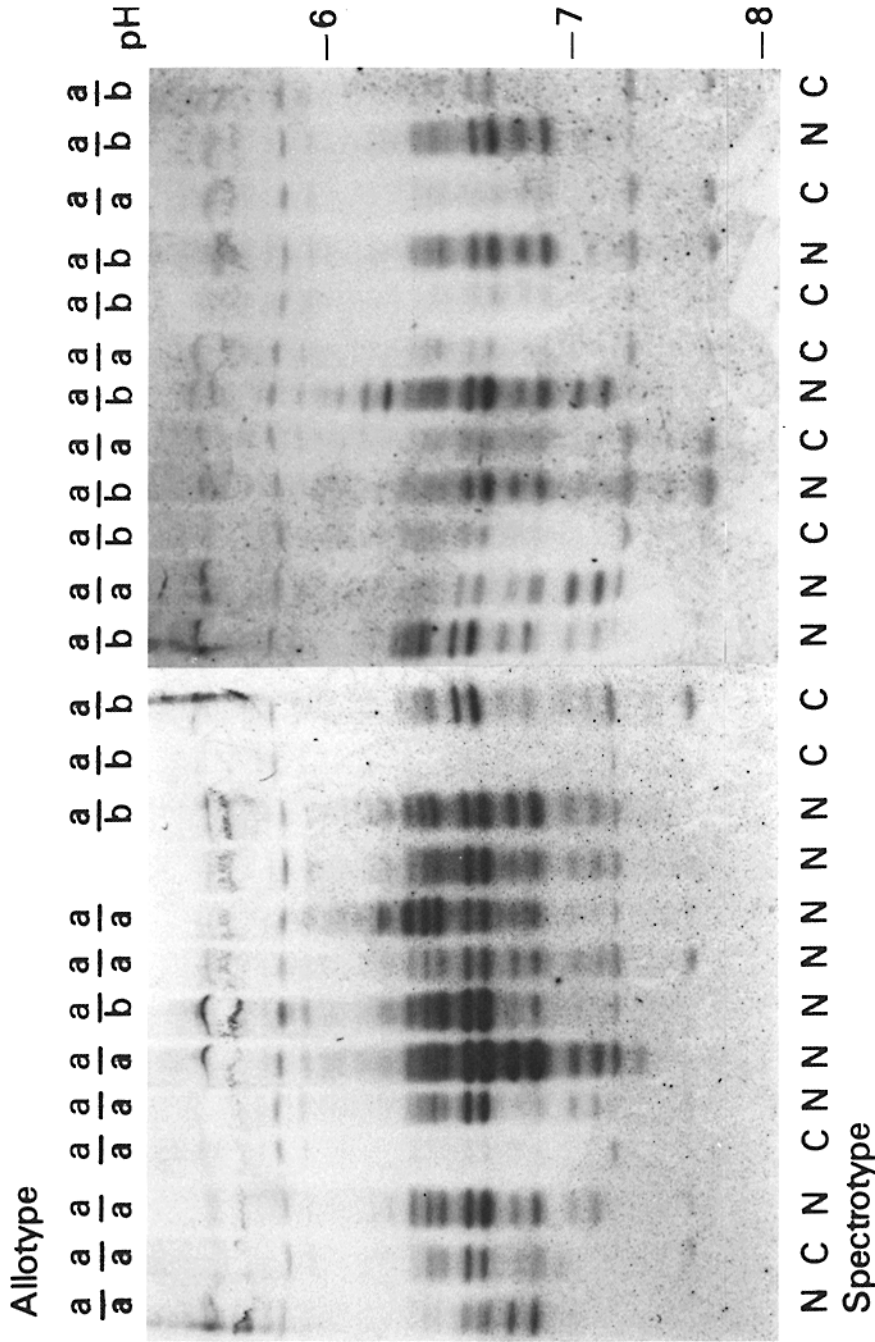
FIG. 7. IEF pattern of anti-BL antibodies of backcross mice. Sera obtained from 25 (BALB/c × C57BL/6)F₁ × BALB/c backcross mice at 10 d after immunization with BL were focused and exposed to ¹²⁵I-In-BSA. 24 mice were analyzed for Igh allotype, and the results are indicated above the sera. The IEF patterns were classified as either (C) BALB/c-like or (N) non-BALB/c-like, as indicated below the sera.

TABLE II
*Analysis of Backcross Mice*

| Trait | | Spectrotype | |
|---|---|---|---|
| | | BALB/c like | Non-BALB/c like |
| Allotype | a/a | 5 | 7 |
| | a/b | 5 | 7 |
| H-2 type | d/d | 7 | 6 |
| | b/d | 2 | 5 |
| Sex | Male | 4 | 6 |
| | Female | 6 | 9 |
| Coat color | Albino | 3 | 9 |
| | Agouti | 3 | 2 |
| | Brown | 4 | 4 |

as defined by the backcross analysis, is expressed in both *Igh* homozygous and heterozygous individuals. This suggests that the regulatory gene observed in CXBG and CXBJ mice may be different from *Sr-1*. In contrast, CXBI mice possess a regulatory gene that functions in *Igh* heterozygous individuals and, thus, may be *Sr-1*.

## Discussion

We have demonstrated previously (4, 14) and confirmed in this study that the ability to produce IdX-positive anti-In antibodies in response to immunization with BL is linked to the *a* haplotype of the *Igh* gene complex. We have demonstrated here that this is also correlated with the presence of IgG anti-In antibodies, as demonstrated by IEF. The IEF data show that BALB/c mice develop a very restricted IgG anti-In response after immunization with BL. This consists of a single spectrotype that is shared by all individuals and that does not change when examined at various intervals from 5 to 40 d after immunization. Nonetheless, a dramatic increase in the heterogeneity of the response was observed after immunization with In-BA in CFA or In-KLH in CFA, which indicates that the repertoire of anti-In antibodies in BALB/c mice is much greater than that normally expressed after immunization with BL. The increased heterogeneity was also observed after the introduction of at least one C57BL background gene (*Sr-1*) into BALB/c mice. Indeed, the spectrotypes of IgG anti-In antibodies in (BALB/c × C57BL/6)$F_1$ mice and in B.C8 mice are both significantly more heterogeneous than those of BALB/c anti-In antibodies. The *Sr-1* gene that is expressed in $F_1$ and B.C8 mice appears to be present in CXBI mice. The data suggest, furthermore, that other regulatory genes, the expression of which may differ in mice that are heterozygous for the *a* allotype, may exist. This is shown by the expression of a strong IdX-positive anti-In response and a heterogeneous IgG anti-In IEF pattern in the $F_1$ combination of CXBG crossed to BALB/c mice. In contrast, (CXBG × C.B20)$F_1$ mice produce the spectrotype characteristic of BALB/c mice.

The responsiveness of CXBJ mice is more difficult to interpret. The IEF pattern of anti-In antibodies of CXBJ mice is similar to BALB/c, although the magnitude is greater. When $F_1$ hybrids of (CXBJ × BALB/c) were immunized with BL, the IEF pattern of their anti-In antibodies was considerably more heterogeneous than that of
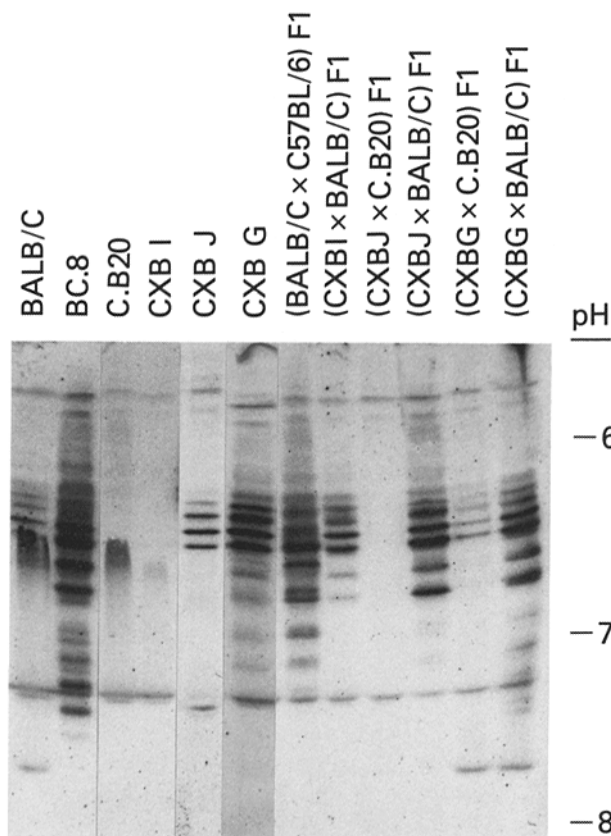
Fig. 8. IEF pattern of anti-BL antibodies of different strains of mice. Sera obtained from representative individual mice (at least 10 individuals of each strain and 4 of each $F_1$ combination were examined) of various strains at 5 or 10 d after immunization with BL were focused and overlaid with ^{125}I-In-BSA.

either parent. This IEF regulatory effect, whether caused by *Sr-1* or not, is also subject to the influence of allotype heterozygosity. Thus, (CXBJ × C.B20)$F_1$ mice fail to produce anti-In antibodies after immunization with BL. In addition, *Igh* heterozygous (CXBJ × C.B20)$F_2$ mice made little or no IgG anti-In antibody, whereas two of four *a* allotype homozygous $F_2$ mice produced heterogeneous IgG anti-In antibodies. These experiments strongly support the concept that the regulation of the anti-In response by certain C57BL genes is influenced by heterozygosity at the *Igh* gene complex.

The findings presented here indicate that the expression of *Igh-V* structural genes of anti-In antibodies is under the influence of nonallotype-linked background genes, such as *Sr-1*, which are present even in strains that lack the appropriate *Igh-V* genes themselves. Another example of this type of regulation is found in the response to BL of 129/Sv (*Igh^a*), C57BL/6 (*Igh^b*), and their RI lines. The results of these studies also indicate the existence of a C57BL gene(s) that regulates spectrotype expression (K. E. Stein, C. Bona, P.-A. Cazenave, J.-L. Guénet, and W. E. Paul. Unpublished observations.). Other studies of the BL response in (BALB/c × DBA/2) (BXD) RI lines and in AL/N and C.AL20 strains strongly suggest that such regulatory genes are not
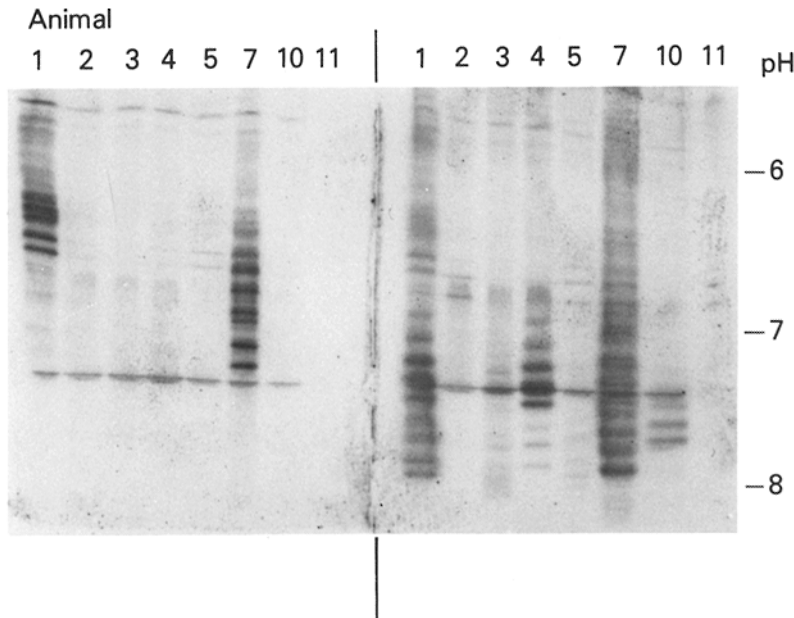
Fig. 9. IEF pattern of anti-BL antibodies of (CXBJ × C.B20)F₂ mice. Sera obtained from 8 (CXBJ × C.B20)F₂ mice at 10 d after immunization with BL were focused in duplicate in the same gel. The gel was cut, and one-half was exposed to $^{125}$I-In-BSA (left panel) and the other half to $^{125}$I-BL (right panel).

found exclusively in C57BL/6 mice (K. E. Stein, C. Bona, R. Lieberman, C. C. Chien, and W. E. Paul. Unpublished observations.).

There are several mechanisms by which regulatory genes could influence antibody expression. A trivial explanation is that the C57BL background contributes a gene that results in an increased amount of anti-In antibodies in mice of the $Igh^a$ type, thus allowing spectrotypes normally present in BALB/c mice in nondetectable amounts to now be detected. Our quantitative data on the amount of anti-In antibodies as detected by HA (14) or by radioimmunoassay (K. E. Stein, C. Bona, R. Lieberman, C. C. Chien, and W. E. Paul. Unpublished observations.) as well as the radioimmunoassay data of Slack et al. (15) indicate that there are no significant differences in the total amount of anti-In antibodies in BALB/c, B.C8, CXBJ, and CXBG mice. We do not believe, therefore, that the IEF differences observed here are entirely a result of quantitative differences in the anti-In antibodies.

A second explanation for the observed differences in spectrotype is that C57BL mice contribute a different light chain to the anti-In antibodies than is present in BALB/c mice and that this results in new clonotype expression. Because there are no markers to distinguish BALB/c and C57BL light chains, this explanation cannot be directly tested. We do not favor this explanation, however, because we know that BALB/c mice can produce heterogeneous anti-In antibodies if they are immunized with stronger immunogens than soluble polysaccharides, such as In-BA or In-KLH in CFA (Fig. 3). We conclude from these studies that BALB/c mice possess light chains that can participate in the formation of antibodies that express heterogeneous IEF patterns.

A third explanation for the observed regulation is that the increased diversity seen upon introduction of Sr-1 or other C57BL regulatory genes into BALB/c mice results from the expression of additional IgG subclasses and perhaps greater switching from IgM to IgG. Our preliminary data suggest that whereas the IgG anti-In antibodies in BALB/c mice are all of the IgG$_1$ subclass, B.C8 mice produce anti-In antibodies of the IgG$_{2a}$, IgG$_{2b}$, and IgG$_3$ subclasses in addition to IgG$_1$. We are currently extending these studies to determine if regulation of Ig class expression is indeed the mechanism through which Sr-1 and other regulatory genes exert their effect and if this is mediated via the T cell.

## Summary

The antibody response to the inulin [(In), $\beta$-(2 → 1) fructosan] determinant of bacterial levan [(BL), a $\beta$-(2 → 6) polyfructosan that contains $\beta$-(2 → 1) branch points] requires the presence of the a haplotype of the Igh gene complex. BALB/c (Igh$^a$) mice immunized with BL produce IgG anti-In antibodies of a single spectrotype by isoelectric focusing analysis. C57BL/6 mice, which possess the b haplotype of the Igh gene complex and which fail to produce anti-In antibodies, nevertheless possess a gene, spectrotype regulation gene 1 (Sr-1), that regulates the isoelectric focusing (IEF) pattern of anti-In antibodies in mice of the a haplotype. Thus, the IEF patterns of anti-In antibodies of (BALB/c × C57BL/6)F$_1$ mice and of B.C8 mice (C57BL/Ka · Igh-C$^a$) are considerably more complex than those of BALB/c. Backcross analysis indicates that Sr-1 is not linked to the Igh complex, the major histocompatibility complex, or to the genes that code for coat color. Studies of the heterogeneity of anti-In antibodies in recombinant inbred lines and their progeny from matings to BALB/c and C.B20 (BALB/c · Igh-C$^b$) suggest the existence of other regulatory genes.

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