

Isoelectric focusing spectra of anti-bacterial α -amylase antibody unique for antigen-induced suppression

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Accepted for publication 18 September 1985

SUMMARY

The effect of intravenous (i.v.) administration of bacterial α -amylase (B α A) on the IgG antibody response to a subsequent challenge with B α A in incomplete Freund's adjuvant (IFA) varied with the difference in responsiveness of the parental strains. High-responder C3H/He (C3) mice given injections of either 200 or 4 μ g of B α A, which alone were unable to trigger a detectable IgG antibody response, generated an enhanced response to an immunogenic challenge given 25 days after the last i.v. injection. The response of low-responder C57BL/6 (B6) mice previously exposed to B α A, following a different kinetic course depending on the exposing dose, reached a plateau lower than the levels of control responses (tentatively designated as high- and low-zone suppression). Prior exposure of (B6 \times C3)F₁ hybrids to 200 μ g led to the enhanced response, whereas pretreatment with 4 μ g rendered them partially tolerant to a subsequent challenge. These results suggest that the capacity to achieve low-zone suppression is inherited as a dominant trait. Isoelectric focusing (IEF) analysis revealed that these enhanced responses expanded antibody heterogeneity in a strictly restricted, strain-specific manner as observed during the normal antibody response, although the rate of expansion was accelerated. The specific antibodies produced by individual high-zone suppressed B6 mice were focused as a limited set of bands in a narrow pH range where the specific antibodies produced early in the normal response were focused. In contrast, the response of low-zone suppressed B6 and F₁ hybrid mice was characterized by a unique process of heterogeneity expansion.

INTRODUCTION

Antigen (Borel & Stollar, 1979; Golub & Weigle, 1969; Debré *et al.*, 1975) or antibody (Voisin, 1980), as well as anti-idiotypic antibody (Augustin & Consenza, 1976; Nisonoff, Ju & Owen, 1977; Hetzelberger & Eichmann, 1978a, b; Takemori & Rajewsky, 1984), has been used to modulate the immune response in a variety of genetic studies of responsiveness in inbred mouse strains. These modulators are known to be able to enhance or suppress the specific immune response, depending on the experimental conditions employed. By following changes in isoelectric focusing (IEF) spectra of IgG antibody during the response to bacterial α -amylase (B α A), it was shown previously (Nakashima & Kamikawa, 1984a) that the antibody response sequentially expanded spectra in a strictly restricted manner, irrespective of whether mice were immunized with B α A in incomplete or complete Freund's adjuvant (IFA or CFA).

In the present study, an attempt has been made to explore the influence of modulation of the antibody response by prior exposure to high or low doses of B α A on the general manner of

sequential expansion of antibody heterogeneity. The effect of prior exposure varied depending on responsiveness of parental C57BL/6 (B6) and C3H/He (C3) mouse strains. The (B6 \times C3)F₁ hybrids behaved like high-responder C3 mice for the modulating effect of high dose exposure, whereas they behaved like low-responder B6 mice for the low-zone suppression. In addition, the low-zone suppressed response of B6 and the F₁ hybrid mice was initiated with the production of antibodies focusing in a more alkaline part of the pH gradient than those of initial antibodies produced in the normal immune response.

MATERIALS AND METHODS

Materials

Crystalline B α A (EC 3.2.1.1, α -amylase, *Bacillus subtilis*) was a product of Nagase Sangyo Co., Japan. Ampholine carrier ampholites were from LKB, Bromma, Sweden.

Animals

Parental C3H/He (C3), C57BL/6 (B6) and the offspring of mating between B6 mothers and C3 males, originally obtained from Shizuoka Agricultural Co-operative Association for Laboratory Animals and bred in the conventional condition,

Abbreviations: B α A, bacterial α -amylase; IEF, isoelectric focusing.

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were used at the age of 9–13 weeks. In the IgG anti-B α A antibody response, it has been shown that C3 mice were high and B6 mice were low responders, and that the high responsiveness was inherited as a dominant trait (Nakashima & Kamikawa, 1984a).

Experimental design

Mice were given two intravenous (i.v.) injections of 100 or 2 μ g each of B α A in 0.4 ml of saline at a 1-day interval. Controls received saline. All mice were immunized with B α A in 0.2 ml of IFA emulsion 25 days after the last i.v. injection. Responses to 30 μ g of B α A were compared between high-responder C3 and F₁ hybrid mice. For comparison of the response between parental strains, they were immunized with 100 μ g of B α A because of the variability of low responses of B6 mice to 30 μ g of B α A.

Antibody assay, IEF and straining of focused antibody

These procedures were identical to those described in our previous paper (Nakashima & Kamikawa, 1984a).

RESULTS

Effect of prior exposure to B α A on the IgG anti-B α A antibody response in parental strains

It is widely accepted that i.v. administration of an appropriate dose of soluble antigen favours the induction of unresponsiveness in adult animals (Weigle, 1973). By using the i.v. route, the modulating effect of prior exposure to B α A was examined in parental strains differing in their responsiveness to B α A. High-responder C3 and low-responder B6 mice received two i.v. injections of 100 or 2 μ g each of B α A at a 1-day interval. This treatment alone was incapable of generating a detectable IgG antibody response. They were challenged by an intraperitoneal injection of 100 μ g of B α A in IFA 25 days after the last i.v. injection, and their responses were followed by estimating antibody titres with the enzymatic procedure (Nakashima & Kamikawa, 1984a). The results are shown in Fig. 1. Regardless

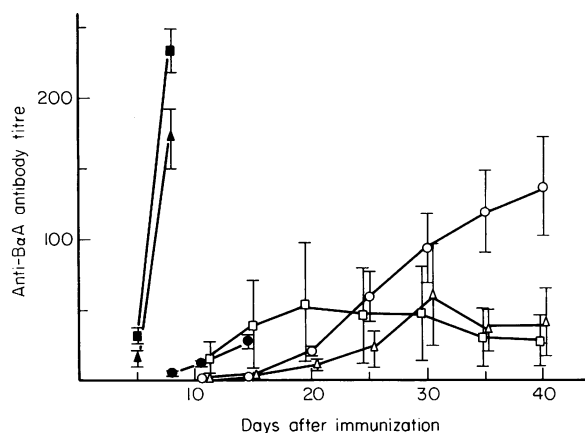


Figure 1. Modulated responses in the parental strains. Female C3 (closed symbols) and B6 (open symbols) mice were exposed to 200 μ g (■, □) or 4 μ g (▲, △) of B α A by the i.v. route. Controls received saline (●, ○). All mice were immunized with 100 μ g of B α A in IFA 25 days after the last i.v. injection. IgG antibody titres were estimated by the enzymatic procedure. Values are the means of four mice with standard errors.

of the exposing dose, pretreatment of C3 mice led to the development of enhanced responses, accompanied by the earlier appearance of antibody and by the rapid rise in antibody titre as compared with the controls. High-dose exposure of B6 mice exerted an enhancing effect at an early stage of the response. The enhanced response, however, reached a plateau on Day 20, and thereafter the titres gradually declined. Low-dose exposure of B6 mice had a suppressive effect over the experimental period. The response of control B6 mice showed a steady rise and reached levels higher than those of pretreated mice on Day 35. Although the response of B6 mice exposed to high or low doses followed a different kinetic course at the early stage of the response, they were rendered tolerant in the later maturational phase, tentatively designated as high- and low-zone suppression. The results demonstrate that the modulating effect of prior exposure is distinct in the parental strains.

Effect of prior exposure in the F₁ hybrids

The effect of prior exposure was compared between C3 and the F₁ mice under the same conditions as used in Fig. 1 except the challenging dose (30 μ g). The results are illustrated in Fig. 2.

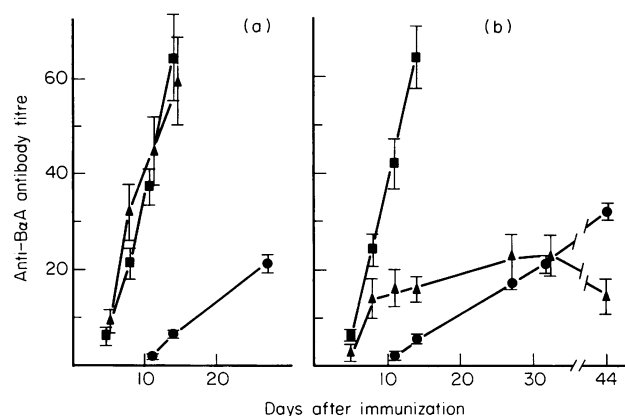


Figure 2. Modulated responses in (a) C3 and (b) (B6 \times C3)F₁ hybrid mice. These females were exposed to high (■) or low doses (▲) of B α A. Controls received saline (●). All mice were immunized with 30 μ g of B α A in IFA 25 days after the last i.v. injection. Values are the means of four mice with standard errors.

Again, the similar enhancing effect was noted in C3 mice (Fig. 2a). The control F₁ hybrids made the levels of antibody close to those in control C3 responses, and prior exposure of F₁ and C3 mice to high doses led to the comparable level of enhancement (Fig. 2b). There was a marked difference in the modulating effect of low doses between C3 and the F₁ hybrid mice. The response of F₁ hybrids given 4 μ g was accompanied by the early production of antibody, but not by the subsequent brisk rise in titre as noted in C3 mice. The titres reached on Day 32 were comparable to the level of control F₁ hybrids. When they were boosted with 30 μ g of B α A in IFA on Day 34, a positive secondary response was seen in control, but not in the pretreated F₁ mice. Both B6 and F₁ mice could be rendered tolerant by the exposure to low doses in terms of suppression at the late maturational stage.

Changes in IEF spectra during the modulated response of the parental strains

Our previous papers (Nakashima & Kamikawa, 1984a, b) demonstrated the strictly restricted manner in which the antibody response to B α A sequentially expanded IgG antibody heterogeneity in the course of primary response: in spite of the fact that these mice were able to make specific antibodies focusing over the entire range of the pH gradient, the response was initiated with the production of antibodies with a limited range of isoelectric point (pI) values, overlapping among different mice, and a further rise in titre was associated with the contiguous expansion of spectra involving the appearance of new bands in the area of the gradient adjacent to the initial bands.

In order to see what limitations are imposed on the general manner of heterogeneity expansion, these modulated primary responses were analysed for antibody heterogeneity by IEF combined with the enzymatic staining (Nakashima & Kamikawa, 1984a). Serum samples were taken from parental strains developing the responses as shown in Fig. 1. The spectra of C3 antisera taken on Day 11 are shown in Fig. 3a, and the results obtained with sera taken Day 30 from responding B6 mice are in Fig. 3b. The enhanced response of C3 mice was accompanied by the rapid expansion and by intensified staining of focused antibody, although each serum from different mice displayed an individual banding pattern. Main bands of antibody from high- or low-dose exposed mice (Fig. 3a, lanes E–H and I–L, respectively) were localized in a limited pH range where the specific antibodies produced early in the normal response were focused (A–D), suggesting that prior exposure to B α A did not significantly affect the general C3 strain-specific manner of spectra expansion. Although some, but not all, B6 mice exposed to 200 μ g of B α A responded with accelerated production of antibodies with a limited range of pI values at an early maturational stage, these sera displayed discrete bandings more widely spaced (Fig. 3b, E–G) than those of immune sera of similar antibody titres obtained during the normal response (A–D). B6 mice exposed to 4 μ g produced low levels of antibodies which were focused as unique spectra consisting of two major sets of bands (H–J). The more acidic set was localized in a pH area where B6 antibodies produced early in the course of normal response were focused. Another set, characteristic of low-zone suppression, was found in a limited range of higher pI values.

The sequential changes in IEF spectra during the enhanced response of C3 mice were similar to the strictly restricted expansion during the enhanced response by employing CFA emulsion (Nakashima & Kamikawa, 1984a) (not shown). Typical changes during high- and low-zone suppressed responses of B6 mice (Days 15, 20, 25, 30 and 40) are shown in Fig. 4 (F–J and K–O, respectively). Two out of four mice in each group (Fig. 4a, b) shared similar spectrotypic properties unique for high- and low-zone suppression. Modulation of B6 responses by high doses did not critically affect the manner of expansion seen during the normal response (A–E). The response, however, was distinguished from the normal response by the widely spaced banding at either maturational stage tested. By contrast, the low-zone suppression in B6 mice (K–O) was accompanied by the initial production of antibodies with higher pI values than those of antibodies produced early in the normal response. The further rise in antibody titre was asso-

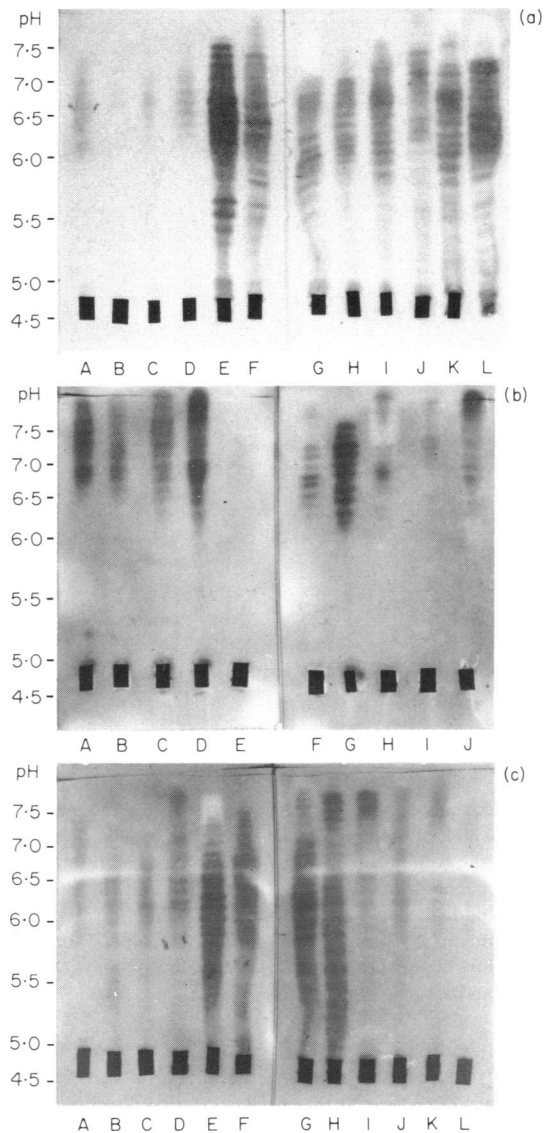


Figure 3. Isoelectric focusing (IEF) spectra of antisera from (a) C3, (b) B6 and (c) their F₁ hybrid mice. Serum samples (a) were taken on Day 11 from C3 mice developing the responses shown in Fig. 1. Lanes A–D, control mice; E–H, high-dose exposed mice; I–L, low-dose exposed mice. Samples (b) were taken on Day 30 from B6 mice developing the responses in Fig. 1. Lanes A–D, control mice; E–G, responding B6 mice exposed to high doses; H–J, responding mice exposed to low doses. Samples (c) were taken on Day 27 from the F₁ hybrids developing the responses in Fig. 2b. Lanes A–D, control F₁ hybrids; E–H, high-dose exposed mice; I–L, low-dose exposed mice.

ciated with intensified staining and expansion of spectra to form another set of acidic bands. Thus, the present high- and low-zone suppression was shown to be distinguishable on the basis of IEF spectra unique for each type of suppression. A similar spectrotypic restriction of antibody heterogeneity has been reported for several suppressed responses (Doenhoff *et al.*, 1979; Cecka *et al.*, 1976; Appleby & Catty, 1985).

Changes in IEF spectra during the modulated F₁ response

Sera were taken on Day 27 from C3 and the F₁ hybrid mice

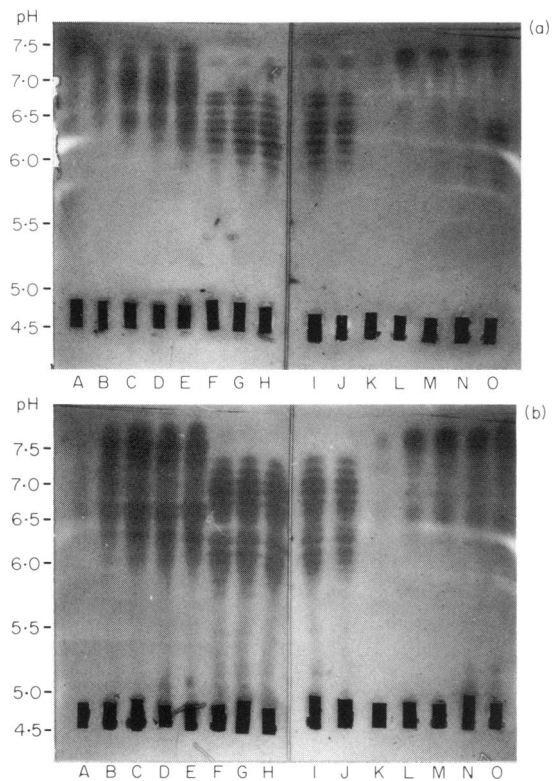


Figure 4. Sequential changes in IEF spectra during the modulated B6 response. Samples were taken on Days 15, 20, 25, 30 and 40 from mice developing the responses shown in Fig. 1. Lanes A–E in (a) and (b), sequential changes in control mice whose spectra on Day 30 are seen in Fig. 3b, A and D; F–J in (a) and (b), changes in high-dose exposed mice whose spectra on Day 30 are seen in Fig. 3b, F and G; K–O in (a) and (b), changes in low-dose exposed mice whose spectra on Day 30 are seen in Fig. 3b, H and J.

developing the responses shown in Fig. 2 and were subjected to IEF. The enhanced response of C3 mice given high or low doses expanded spectra in a manner similar to the general expansion seen during the normal C3 response (data not shown). The results obtained with F₁ immune sera are shown in Fig. 3c. The enhanced response of F₁ hybrids exposed to high doses (Fig. 3c, E–H; Fig. 5, D–F) was also associated with the general heterogeneity expansion, initiating with the production of antibody with a limited range of pI values. In this respect, the F₁ hybrids behaved like C3 mice. On the other hand, the low-zone suppression of F₁ hybrids was characterized by the production of antibodies focusing as two major sets of bands at this maturational stage (Fig. 3c, I–L). Sequential changes in spectra were followed during the modulated F₁ response as shown in Fig. 5. Samples were taken on Days 5, 11 and 17 from individual mice given high (D–F) or low doses (G–I and J–L). The results were compared with the spectra taken on Days 11, 14 and 17 from F₁ mice developing the normal response (A–C). The sequential changes during the low-zone suppressed responses suggest that these responses are initiated with the production of a more alkaline set of antibodies, followed by the expansion of spectra to express another set of the acidic bands. The similar trend of sequential changes in IEF spectra was shared among the low-zone suppressed B6 and F₁ hybrid mice.

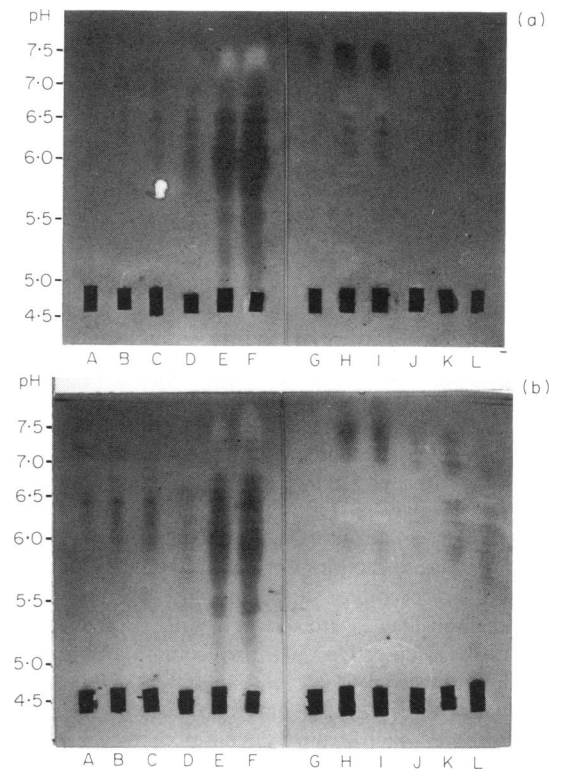


Figure 5. Sequential changes during the modulated F₁ response shown in Fig. 2b. Lanes A–C in (a) and (b), samples were taken on Days 11, 14 and 17 from control mice whose spectra on Day 27 are seen in Fig. 3c, A and C; D–F in (a) and (b), samples were taken on Days 5, 11 and 17 from high-dose exposed mice whose spectra on Day 27 are seen in Fig. 3c, E and G; G–I and J–L in (a) and (b), samples were taken on Days 5, 11 and 17 from four mice exposed to low doses.

DISCUSSION

The aims of the present study were to explore the effect of prior i.v. exposure to B α A on the IgG antibody response to a subsequent challenge, and to analyse antibody heterogeneity in the course of the modulated response. All experiments were carried out with females, because a sex difference in the susceptibility to tolerance induction has been reported by Borel & Stollar (1979). Prior exposure alone was unable to stimulate the production of the specific IgG antibody in these naive mice employed. Although B α A is a naturally occurring, multi-determinant antigen (Nakashima & Kamikawa, 1984b), strain-dependent modulation of the response was noted. The susceptibility to an enhancing effect of high dose exposure was shown to be inherited as a dominant trait in the F₁ hybrids from high-responder C3 mice, while the F₁ mice behaved like low-responder B6 mice for low-zone suppression. The failure of high dose exposure of F₁ mice to induce suppression suggests that low-zone suppression is not due to a simple neutralization of the specific antibodies by B α A given for prior exposure. It has been demonstrated by Debré *et al.* (1975) that the capacity to generate suppressor cells specific for the copolymer Glu-Tyr is under dominant genetic control. Other instances of a similar dominant inheritance have been reported (Golub & Weigle, 1969; Borel & Stollar, 1979), although the cellular mechanisms remain unclear. It has been proposed that clonal inactivation of

specific T and/or B cells (Nossal & Pike, 1975; Cambier *et al.*, 1980) or regulatory circuits for stimulation of suppressor T cells (Tada & Okumura, 1979; Gershon *et al.*, 1981; Thomas & Kennedy, 1983; Loblay *et al.*, 1983) as well as macrophage processing (Golub & Weigle, 1969; Lukić, Cowing & Leskowitz, 1975) might be responsible for the induction and maintenance of a tolerant state. It has been established that helper or suppressor T cells are more susceptible to the modulating effect of low doses of antigen (Falkoff & Kettman, 1972; Weigle, 1973) or anti-idiotypic antibody (Hetzelberger & Eichmann, 1978b) than antibody-forming cell precursors. Thus, it seems likely that the present modulating effect of low dose exposure is mediated by regulatory T cells rather than by B cells.

The difference in pI values of antibodies are ascribed to the differences in the variable or the constant regions of antibody or to the post-translational modification as shown for monoclonal antibody (Awdeh, Williamson & Askonas, 1970). Conventionally, thymus-dependent antigens are considered to stimulate predominantly IgG1 antibodies of the IgG isotypes (Slack *et al.*, 1980). Actually, the same isotype preference has been shown by Hamaoka *et al.* (1969) to occur early in the primary response to the same antigen used in the present study. These results suggest that the expansion of IEF spectra in the normal anti-B α A antibody response mainly reflects the sequential production of additional antibodies substituted for charged amino acids in the variable regions of initial IgG antibodies, in which determinants-specific and idiotypic-specific regulatory pressures on a number of specific B-cell clones are involved. Since heterogeneity expansion during some of these modulated responses occurred in a manner of strictly restricted expansion seen in the course of normal primary responses, it is reasonable to assume that these modulated responses are accompanied by the predominant production of IgG1 antibodies. However, similarities of pI values do not directly relate to similarities in determinant specificities between the two antibody populations produced by these mice developing the normal and the modulated response.

It is generally assumed that antibody responses mature at the level of IgG antibody from low affinity to high affinity. High-affinity antibody production has been shown to be selectively inhibited by suppressor cells (Takemori & Tada, 1974; Warren, Murphy & Davie, 1976). The specific antibodies taken on Day 40 from high-zone suppressed B6 mice had a limited range of pI values similar to those of antibodies produced early in the normal response. These similarities may reflect predominant production of low-affinity antibodies in high-zone suppressed B6 mice, although it would be difficult to evaluate the precise affinities of antibodies for a multi-determinant antigen, and the specific antibodies bearing different idiotypic markers can be cofocused within the limited pH range.

The low-zone suppressed B6 and F₁ responses expanded spectra in a unique manner differing from the general expansion seen during the normal response. These suppressed responses were initiated with the production of antibodies focusing in the more alkaline part of the pH gradient than that of antibodies produced early in the normal response, followed by expansion of spectra involving the appearance of another set of acidic bands. There are accumulating data demonstrating that antigen-induced or anti-idiotypic antibody-induced suppression is mediated by the T cells bearing or recognizing the major cross-reactive idiotypic marker (Germain *et al.*, 1979; Weinberger *et al.*, 1979; Greene *et al.*, 1982), resulting in the

predominant production of the corresponding cross-reactive idiotypic-negative antibody (Takamori & Rajewsky, 1984; Hetzelberger & Eichmann, 1978b; Nisonoff *et al.*, 1977). The present unique spectrotypic properties shared among antibodies from low-zone suppressed B6 and F₁ hybrid mice might be interpreted as suggestive of the idiotypic shift in these suppressed mice; however, another possibility that they may be due to the differences in the IgG isotypes still remains.

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