# CHRONIC ALLOTYPE SUPPRESSION IN MICE: AN ACTIVE REGULATORY PROCESS

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# CHRONIC ALLOTYPE SUPPRESSION IN MICE: AN ACTIVE REGULATORY PROCESS

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The normal process of differentiation to specific immunoglobulin (i.e., antibody) production is the commitment of an originally totipotent cell to the production of a large amount of a unique immunoglobulin molecule. During this process, one of the two autosomal chromosomes carrying H chain variable and constant region genes is in some way selected, the variable region fixed and one of the four linked genes for H chain constant regions (in the mouse Ig-1 to Ig-4, specifying $\gamma G_{2a}$, $\gamma A$, $\gamma G_{2b}$ and $\gamma G_1$ respectively) paired in some manner which allows for the transcription of the information for the entire H chain as a single messenger. In a similar fashion, one of the two light chain chromosomes is chosen, a variable region determined, paired to the constant region, and information for a unique light chain is produced. Light and heavy chains are then produced, assembled and exported while the cell differentiates further to a mature plasma cell. Although recent evidence [1, 2] indicates that during this process the variable region gene in some or all cells may go through a stage of pairing with the $\mu$ chain ($\gamma M$) constant region gene prior to pairing with the final $\gamma G$ or $\gamma A$ H chain gene, this does not substantially alter the above description.

Almost nothing is known of the mechanisms by which one autosomal chromosome is chosen and the other excluded, the variable region fixed and joined, or the H chain class selected. If the intermediate of a $\gamma M$ step proves correct, however, then chromosome exclusion and variable region choice are earlier events than selection of H chain class. In any event, at the end of the process, a single cell produces an immunoglobulin with an H chain determined by a gene (or genes) on only one of the two parental chromosomes. No other such system for exclusion of autosomal genes is known in mammalian cells.

In an allotype heterozygote, such chromosomal (or allelic) exclusion leads to two populations of immunoglobulin-producing cells,[3] those making immunoglobulins of paternal allotype and those making immunoglobulins of maternal allotype for any given class. Usually, an animal with a given allele combination has a characteristic ratio of paternal and maternal type cells. Circulating immunoglobulins reflect this ratio.

Allotype suppression upsets the normal balance.[4–6] In young heterozygotes exposed to maternal antibody directed against a paternal allotype, the appearance of circulating paternal allotype is significantly retarded. Although in rabbits the suppression is severe and long-term, enduring in some cases for over a year, in most mouse hybrids only a short repriod of suppression occurs, and by 12–14 weeks the normal balance is restored. However, many progeny

of the strain cross, SJL males × BALB/c females, show a chronic suppression somewhat similar to that reported for rabbits [5] following initial exposure to maternal anti paternal antibody.

We have shown, using cell transfer experiments,[7] that there is a cell population resident in the spleens of chronically suppressed (SJL × BALB/c)$F_1$ mice that is capable of preventing differentiation to Ig-1b (paternal $\gamma G_{2a}$ allotype) production. The evidence leading to this inclusion may be summarized as follows:

1. In the intact animal, the existence of this population leads to the chronic (long-term) suppression of Ig-1b synthesis by an animal genetically capable of Ig-1b production. Half the animals of the cross (SJL × BALB/c)$F_1$ exposed when young to maternal anti Ig-1b are negative for Ig-1b at 6 months of age (FIGURE 1).
2. When transferred to irradiated Ig-1b (BAB/14) homozygous hosts, the suppressing cell population is able to prevent recovery of host Ig-1b synthesis where such recovery is expected. BAB/14 is congenic to BALB/c but carries the Igb chromosome segment including the Ig-1b allele. These experiments were preformed originally as transplantation controls to show persistence of $F_1$ (Ig-1a/Ig-1b) cells in irradiated parents. As expected, using production of Ig-1a as marker, $F_1$ cells of either suppressed or normal origin persisted equally well. Many of the recipients of suppressed spleen cells, however, lost their ability to produce Ig-1b (TABLE 1).

   BAB/14 recipients of bone marrow suspensions did not become suppressed, although suppressed bone marrow transplants to Ig-1a (BALB/c indicator) hosts did suppress themselves, thus suggesting that there may be a larger or more effective suppressing population in spleen. Such failure of the bone marrow transplant to suppress host Ig-1b production, however, demonstrates the ability of the irradiated BAB/14 host to recover produc- of Ig-1b, thus strengthening the conclusion that absence of Ig-1b in BAB/14 animals receiving suppressed spleen cells is due to allotype suppression.
3. Transferred with its own complement of stem cells or in a mixture with syngeneic cells from a normal (SJL × BALB/c)$F_1$ donor, the suppressing population is able to reverse the initial establishment of Ig-1b production and shut off Ig-1b synthesis by the transferred normal cells (TABLE 2). In the first experiments, pooled spleen and bone marrow cells from chronically suppressed or normal (SJL × BALB/c)$F_1$ animals were injected into irradi-

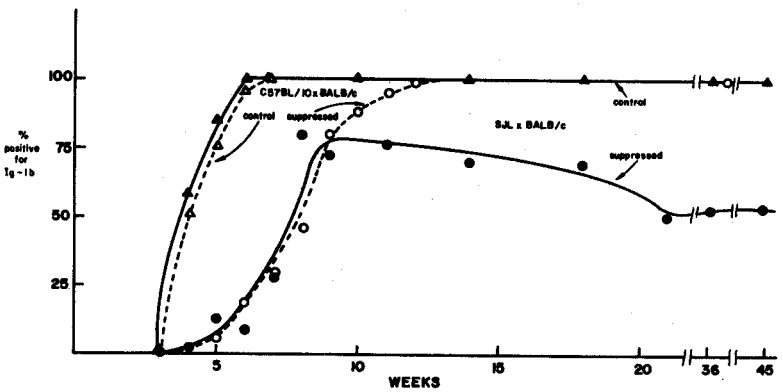


FIGURE 1.

TABLE 1

IG-1B PRODUCTION IN IG-1B RECIPIENTS OF NORMAL AND SUPPRESSED CELLS

| Donor Cells* [(SJL × BALB/c)$F_1$] (No. of cells transferred per recipient) | Experiment No. | BAB/14 (Ig-1b/Ig-1b) Recipients No. Positive for Ig-1b/Total Week after Transfer | | | |
|---|---|---|---|---|---|
| | | 2 | 3 | 5 | 7 |
| Suppressed spleen | 3 | 10/10 | 10/10 | 1/10† | 1/10 |
| ($1.4 \times 10^7$) | 7 | 2/2 | 2/2 | 2/2 | 1/1 |
| | 4 | 5/5 | 5/5 | 4/5 | 3/5‡ |
| | Total | 17/17 | 17/17 | 7/17 | 5/16 |
| Normal spleen | 3 | 2/2 | 2/2 | 2/2 | 2/2 |
| ($1.4 \times 10^7$) | 7 | 1/1 | 1/1 | 1/1 | 1/1 |
| | Total | 3/3 | 3/3 | 3/3 | 3/3 |
| Suppressed bone marrow | 3 | 6/6 | 6/6 | 6/6 | 6/6 |
| ($5 \times 10^6$) | 7 | 2/2 | 2/2 | 2/2 | 2/2 |
| | 4 | 6/6 | 5/5 | 5/5 | 5/5 |
| | Total | 14/14 | 13/13 | 13/13 | 13/13 |
| Normal bone marrow | 3 | 2/2 | 1/1 | 1/1 | 1/1 |
| ($5 \times 10^6$) | 7 | 3/3 | 2/2 | 2/2 | 2/2 |
| | Total | 3/3 | 2/2 | 2/2 | 2/2 |

* The suppressed spleen and bone marrow cell suspensions used in each experiment were pooled from 3 mice. Normal bone marrow and spleen cells were obtained from individual animals. All of the mice were positive for Ig-1a.

† Low levels of Ig-1b were still detectable in the sera of 5 of the mice at week 4 (not shown in Table).

‡ The level of Ig-1b detected in these 3 mice was very low.

ated (600R) BALB/c (non Ig-1b) hosts, and recipients scored weekly for Ig-1b. Although transferred spleen cells from normal donors were able permanently to establish Ig-1b production, cells from suppressed donors generally produced only a short, initial burst of Ig-1b. In two of the three experiments shown in the Table, all recipients of suppressed cells were negative for Ig-1b by 7 weeks after transfer. In the third experiment, although several of the animals were still positive at 7 weeks, the individual test records (not shown) [7] show a pattern of fluctuation of Ig-1b levels similar to untransferred chronic suppressed animals. The results with bone marrow transfers into BALB/c hosts show the same pattern as spleen, i.e., initial burst and subsequent suppression of Ig-1b synthesis.

For the mixture experiments, cell suspensions from normal and suppressed animals were mixed *in vitro*, injected into BALB/c (Ig-1a) hosts, and recipients followed for Ig-1b production. The data are presented in TABLE 3. $1.6 \times 10^7$ spleen cells from a normal $F_1$ donor promptly established Ig-1b production and maintained it throughout the experiment. The same number of suppressed cells followed the usual suppression pattern of establishing synthesis early after transfer but becoming suppressed after 5 weeks. A mixture of $3 \times 10^6$ suppressed cells and $12 \times 10^6$ normal cells established

TABLE 2

IG-1B PRODUCTION IN IG-1A RECIPIENTS OF NORMAL AND SUPPRESSED CELLS

| Donor Cells* [(SJL × BALB/c)$F_1$] (No. of cells transferred per recipient) | Experiment No. | BALB/c (Ig-1a/Ig-1a) Recipients No. Positive for Ig-1b/Total† Week after Transfer | | | |
|---|---|---|---|---|---|
| | | 2 | 3 | 5 | 7 |
| Suppressed spleen | 3 | 5/10 | 5/9 | 1/9 | 0/9 |
| ($1.4 \times 10^7$) | 7 | 4/6 | 4/5 | 0/5 | 0/5 |
| | 4 | 10/10 | 8/9 | 7/9 | 4/9 |
| | Total | 19/25 | 18/23 | 8/23 | 4/23 |
| Normal spleen | 3 | 2/2 | 2/2 | 2/2 | 2/2 |
| ($1.4 \times 10^7$) | 7 | 1/1 | 1/1 | 1/1 | 1/1 |
| | Total | 3/8 | 3/9 | 3/3 | 3/3 |
| Suppressed bone marrow | 3 | 5/6 | 4/5 | 1/5 | 0/5 |
| ($5 \times 10^6$) | 7 | 4/8 | 3/7 | 4/7 | 4/7 |
| | 4 | 1/6 | 5/6 | 5/6 | 4/5 |
| | Total | 10/20 | 12/18 | 10/18 | 8/18 |
| Normal bone marrow | 3 | 2/2 | 2/2 | 2/2 | 2/2 |
| ($5 \times 10^6$) | 7 | 2/2 | 2/2 | 2/2 | 2/2 |
| | Total | 4/4 | 4/4 | 4/4 | 4/4 |

* The suppressed spleen and bone marrow cell suspensions used in each experiment were pooled from 3 mice. Normal bone marrow and spleen cells were obtained from individual animals.

† All of the mice were positive for Ig-1a.

weaker but stable Ig-1b production in four out of five recipients, but after 9 weeks Ig-1b levels fell either to negative (i.e., below detection) or to a stable, considerably lower level. At 22 weeks after transfer, three animals were negative for Ig-1b, one was weak positive ($\sim$ 0.03 mg Ig-1b/ml) and one was still a 1 + reaction, approximately $\sim$ 0.08 mg/ml serum.

The suppression of Ig-1b production may also be demonstrated by the inability of suppressed animals to produce Ig-1b antibody to sheep erythrocytes (SRC). FIGURE 2 shows the results obtained after transfer of SRC primed cells from suppressed and normal mice into irradiated, normal syngeneic recipients. A challenge dose of SRC was given at time of transfer. The values given are for $\gamma$G PFC only. The anti-*b* allotype antiserum used develops plaques only from Ig-1b producing antibody-forming cells (i.e., $\gamma G_{2a}$PFC of *b* allotype). The antiserum used to develop plaques from *a* allotype cells reacts with both Ig-1a and Ig-4a and therefore develops both $\gamma G_{2a}$ and $\gamma G_1$ plaques, which probably accounts for the high a/b ratio of PFC in cell suspensions from normal heterozygotes. Comparison of suppressed and nonsuppressed donors shows that although the *a* allotype response is essentially the same in both groups, suppressed mice were unable to match their *a* response with a proportional *b* response. For example, while a strongly responding normal donor male made *a* and *b* plaques, a comparable suppressed donor male made *a* but no detectable *b*. Our studies show that the suppression by the suppressing

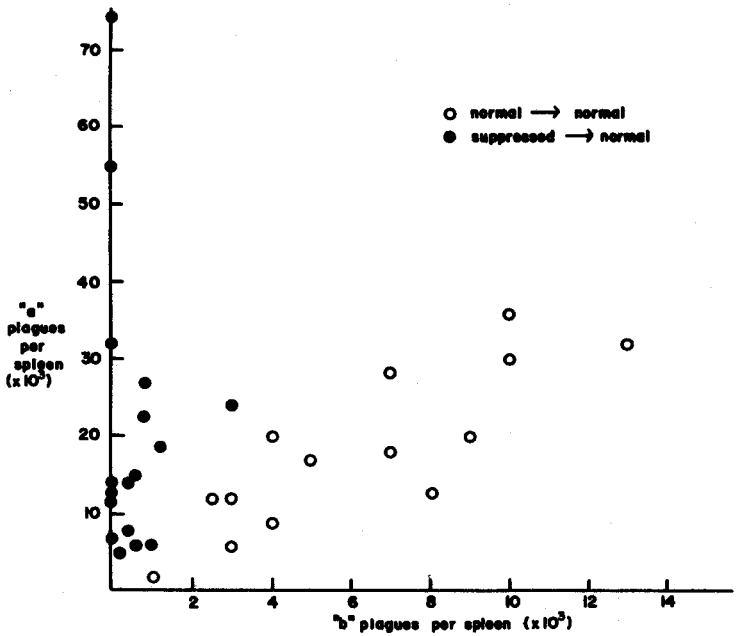


FIGURE 2.

cell population is specific for Ig-1b, i.e., immunoglobulins of the $\gamma G_{2a}$ class, probably due to the fact that the original exposure to maternal antibody was to antibody specific for the paternal $\gamma G_{2a}$ globulins (i.e., Ig-1b). Ig-4b (of $\gamma G_1$ class) synthesis is not suppressed in any of the animals in these experiments.

Suppression cannot be overcome or reversed by flooding either the intact suppressed or transferred suppressed animals with Ig-1b globulins, suggesting either that Ig-1b on the cell surface may have a different conformation, or that active cells may not be blocked by soluble Ig-1b even though the cell reacts with Ig-1b determinants.

Considering the paucity of knowledge of the normal differentiation process which results in commitment to immunoglobulin production, it is not surprising that no simple hypothesis presents itself to explain allotype suppression. One possibility which is reasonably consistent with the currently available data is that the initial antibody exposure rids the animal of all Ig-1b committed cells. If we then postulate an antigen unique to Ig-1b precursors, e.g., cell-bound Ig-1b, perhaps with a different configuration from circulating Ig-1b, it is possible that by elimination of the cells with that antigen we have destroyed tolerance to it, so that when newly committed Ig-1b producers appear after the disappearance of maternal anti Ig-1b, they are met with a destructive, cellular immune response. The fluctuation of Ig-1b levels would, under this hypothesis, be explained by a balance being maintained between new differentiation to Ig-1b and immune reaction to the differentiating cells. At present there is little direct evidence to support or deny the validity of this hypothesis; however, it has the virtue of being testable, and experiments toward this end are in progress.

TABLE 3

SUPPRESSION OF IG-1B PRODUCTION IN TRANSFERRED MIXTURE OF SUPPRESSED AND NORMAL CELLS

| Irradiated Recipient (Ig-1a)+ | (SJL × BALB/c) $F_1$ Spleen Cells | | Ig-1b Level* Weeks After Transfer | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Suppressed | Normal | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 |
| 1 | $12 \times 10^6$ | 0 | 2 | 1 | +w | +w | vw | – | – | – | – | – | X | | | | | | | | | | | |
| 2 | | | 1 | 1 | +w | +w | – | – | – | – | – | – | ** | – | – | | | | | | | | | – |
| 3 | | | 1 | 1 | +w | +w | vw | – | – | – | – | – | | – | – | | | | | | | | | – |
| 4 | | | 1 | +w | +w | +w | ? | – | – | – | – | – | | – | – | | | | | | | | | – |
| 5 | | | 1 | 1 | 1 | 1 | vw | – | – | – | – | – | | – | – | – | – | – | – | – | – | – | – | |
| 6 | $3 \times 10^6$ | $12 \times 10^6$ | 2 | 2 | 2 | 1 | 1 | – | – | vw | – | – | – | – | – | | | | | | | | | – |
| 7 | | | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | +w | +w | +w | 1 | +w | | | | | | | | – |
| 8 | | | 2 | 2 | 2 | 2 | 1 | 1 | +w | 2 | +w | +w | +w | +w | 1 | +w | +w | 1 | +w | 1 | | 1 | | 1 |
| 9 | | | +w | – | – | – | vw | +w | – | – | 1 | – | – | – | – | – | – | – | – | – | – | – | | – |
| 10 | | | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | | | | +w |
| 11 | 0 | $16 \times 10^6$ | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | | 3 | 3 | X | | | | | | | | |
| 12 | | | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | | 3 | 3 | | | died | | | | | | |
| 13 | | | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | | 3 | 3 | | | died | | | | | | |
| 14 | | | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | | 3 | 3 | | | died | | | | | | |
| 15 | | | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | | 3 | 3 | | | | | | | | | 3 |

* Numbers = Ig-1b level as scored on double diffusion (Ouchterlony) plates: 3 = 0.4 mg/ml, 2 = 0.25 mg/ml, 1 = 0.08 mg/ml, +w = 0.03 mg/ml, – = .008 mg/ml.

** No entry means animals not bled on that date. X: Animal sacrificed. + BALB/c NHz (Ig-1a) recipients received 600 R total body irradiation.

Two other facts deserve mention as potentially relevant to construction of hypotheses on chronic allotype suppression. One is that in homozygous suppressed rabbits there is a compensatory increase in a different class (so-called allotype blank) of immunoglobulin, suggesting a possible interference at the point of selection of the H chain constant region. Alternately, of course, this compensation could be a secondary manifestation of the absence of cells producing the allotype, making room, as it were, for the compensating cells to expand and produce.

Secondly, there is the question of the relevance of the SJL genome to the chronic suppression found when SJL is the paternal strain. Since virtually all SJL mice suffer from severe fluctuating disgammaglobulinemias (hyper, hypo, multiple class elevations,[8–10] single class elevations, restricted mobility elevations), and ultimately die of reticulum cell sarcoma,[11, 12] it is possible that the heterozygote, too, has a faulty immunoglobulin regulation system. Electrophoretic studies of heterozygote sera do in fact show extraordinary elevations and depressions, although these differences do not approach the severity of the homozygous disgammaglobulinemias.[10] If there is a heritable immunoglobulin control mechanism defect in this hybrid, suppression could be the result of creation of an overreactive regulating mechanism due to perturbation by the initial exposure to maternal anti-Ig-1b.

### *References*


1. PERNIS, NISONOFF, WANG. This monograph.
2. WANG, A. C., S. K. WILSON, J. E. HOPPER, H. H. FUDENBERG & A. NISONOFF. 1970. Proc. Nat. Acad. Sci. **66:** 337–343.
3. HERZENBERG, L. A. & N. L. WARNER. 1966. J. Exp. Med. **123:** 707–721.
4. DRAY, S. 1962. Nature **195:** 677.
5. MAGE, R. G., G. O. YOUNG & S. J. DRAY. 1967. J. Immunol. **98:** 502.
6. HERZENBERG, L. A., L. A. HERZENBERG, R. C. GOODLIN & E. C. RIVERA. 1967. J. Exp. Med. **126:** 701–713.
7. ABSTRACTS. 1971. (Detailed publications are in preparation.) Fed. Proc. **30:** 651.
8. WANEBO, H. J., W. M. GALLMEIR, E. A. BOYSE & L. J. OLD. 1966. Science **154:** 901–903.
9. MCINTIRE, K. R. & L. W. LAW. 1967. J. Nat. Cancer Inst. **39:** 1197–1211.
10. HERZENBERG, L. A. Unpublished data.
11. MURPHY, J. D. 1969. J. Nat. Cancer Inst. **42:** 797–807.
12. CARSWELL, E. 1970. J. Nat. Cancer Inst. **44:** 1281–1288.


### *Discussion*

DR. POTTER: Do the suppressed mice compensate by making different levels of the other immunoglobulin classes?

DR. HERZENBERG: I knew somebody was going to ask me that question. They don't compensate by making the other Ig-1 allotype. I don't know yet whether they compensate with the other classes.

DR. BENACERRAF: Do you think that the suppression which is observed

may be explained by antibody exchange from the surface of cells which are suppressed to others or by another unsuspected mechanism?

DR. HERZENBERG: I left out, of course, a number of experiments that we've done just directed to this point. We have tried to break suppression by infusing large amounts of Ig-1b and then irradiating the animal and allowing it to come out of the irradiation and to repopulate itself in the presence of Ig-1b. If it was anti-Ig-1b antibody, I feel that the circulating Ig-1b would block it and be able to overcome suppression. So I have a strong prejudice which says that it's not anti-Ig-1b (as the b antigen exists on circulating globulins), which is the effector agent. We've tried all sorts of things, looking for sequestered antibody but I would agree that perhaps antibody sitting on the surface of a cell, if it had become fixed in some way, could do this job. I would expect, however, that we'd have to postulate that the b antigen of the b allotype on the cell before it is secreted and perhaps on the stem cell, would have to be in a form which was different from circulating b globulin, so that the b globulin could not block.

DR. WILLIAMS: With respect to your answer to Dr. Benacerraf's question, there are some patients that have very low levels of gamma A and that apparently have antibody to gamma A. Have you looked for instance at your animals to see if they have antibody? I'm sure you have by your answer, but have you also looked for cell-bound anti-gammaglobulin with rosettes or any other technique? Since you can transfer this suppression with cells, do you have anti-gammaglobulin on your cells that you transfer? Have you looked at this specifically with rosettes or any other sensitive technique?

DR. HERZENBERG: I haven't looked specifically with rosettes but we have looked with rather sensitive technique for an antiglobulin. What we did was to inject radioactive labeled Ig-1b globulin into the suppressed animal and follow its disappearance with time and there was no difference between the normal animal and the suppressed animal in the rate of disappearance of the globulin. So I don't know whether one can argue that the cell-bound antibody wouldn't remove it from circulation; I should think it would. We also did look by a number of other sensitive techniques for antibody production because we thought that perhaps what had happened was that the animals made a small amount of b globulin late in life, that b globulin was immunogenic and, therefore, caused the animal to make anti-b globulin, and that the animal was suppressing itself. I guess I could just add that probably the most appealing hypothesis right now, although it may not be terribly appealing, is that somehow by suppressing these animals early in life, immunization occurs due to the appearance of a little bit of b globulin on the surface of their cells, perhaps in some other configuration than that which is in the circulation. The animal is essentially showing a low-level graft rejection. It is a hard hypothesis to justify, but it is one which we are testing because otherwise we are still completely out in the cold.

DR. MAGE: Is it possible that what you are calling anti-b in this particular strain combination has a specificity for some other heavy chain markers, perhaps V region?

DR. HERZENBERG: I don't think so but this antiserum is an anti-C57 black antiserum, essentially it's the classical one that we have used originally to define the Fc allotype markers, and at that time we never found an example of any animal having anything other than the Fc marker. I guess it's possible though that the SJL b allotype transfers one of these markers into the variable

region and that is why we're getting into what we're getting into. I think probably we ought to check that out. I might add that probably the most appealing hypothesis right now is that, somehow by suppressing these animals early in life, immunization occurs due to the appearance of a little bit of b globulin on the surface of their cells, perhaps in some other configuration than that which is in the circulation.