Induction of Allotype Suppression with Monoclonal Antibodies

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INTRODUCTION

Perinatal exposure of (BALB/c x SJL)F1 mice to antibody specific for the paternal IgGa (Ig-1b) allotype induces long-lived populations of suppressor T cells (Ts) that specifically suppress Ig-1b immunoglobulin production (1). The Ts suppress Ig-1b production indirectly by preventing the differentiation of Ig-1b B cells to Ig-1b antibody-forming cells (ac) rather than directly by removing Ig-1b B cells (2). The target of the Ts is a helper T cell (Ig-1b Th) population specifically required for Ig-1b B cell differentiation; and the extent of suppression appears to be a function of the balance between Ts and Ig-1b Th (2). A similar although substantially less severe chronic suppression of Ig-1a production can be induced by perinatally exposing reciprocal hybrids to anti-Ig-1a antibody; however, exposure to antibodies against IgG1 (Ig-4) allotype does not induce detectable chronic suppression (1).

Recently, Black and Herzenberg (3) showed that perinatal exposure to antibodies reactive with Ig on precursors of Ig-1 (IgG1a) producing cells induces chronic suppression of Ig-1 production in SJL-related Ig*/Ig* heterozygotes. Antibodies to IgM (Ig-6) and IgD (Ig-5) allotypes induce specific suppression for the corresponding Ig-1 allotype,
while heterologous (goat) anti-IgM antibodies induce suppression for both Ig-1a and Ig-1b in the exposed heterozygote. The suppressed mice obtained in this manner have allotype Ts and show the same characteristics as chronically suppressed mice obtained by perinatal exposure to anti-Ig-1 allotype antibodies. Thus, in genetically susceptible mice, antibody reaction with precursor Ig during the neonatal period leads to the establishment of Ts populations capable of suppressing Ig production by progeny B cells throughout the life of the animal.

In other mice (e.g., BALB/c), neonatal antibody exposures such as these have been shown to delay onset of Ig production, to temporarily deplete B cells (4), and to temporarily deplete what appears to be an Ig-specific Th population required for antibody production (see Jane-way et al., in this volume). These findings suggest that chronic suppression in genetically susceptible mice results from early depletion of B cell and Th populations which, in these mice, alters the balance between Ts and Th and allows Ts to dominate in the adult (3). Results of studies presented here on the induction of allotype suppression with monoclonal antibodies are consistent with this hypothesis.

Two questions difficult to resolve with conventional antisera are addressed in the studies presented here. The first concerns the target specificity of the inducing antiserum. The demonstration that Ig-1b suppression is induced by exposure to antibodies that react with IgM and IgD rather than with Ig-1b recalled a spectre that has haunted us for many years. The anti-Ig-1b antisera (maternal or injected) “classically” used to induce allotype Ts contain no detectable antibody to other Ig determinants; however, a cryptic antibody responsible for suppression induction could always be present, e.g., anti-idiotypic antibody or anti-IgD or -IgM. Thus, when monoclonal anti-Ig-1b antibodies were prepared in our laboratory, one of our first priorities was to use these to test directly for the ability of anti-Ig-1b antibody itself to induce Ig-1b specific suppression.

Next, the availability of monoclonal anti-Ig-1b antibodies also allowed us to investigate the importance of isotype to the effectiveness of the inducing antibody. Hetzelberger et al. (5) have shown that the injection of guinea pig IgG, anti-idiotypic antibody can induce idiotype-specific Ts, but that guinea pig I gGf anti-idiotypic antibody cannot. In allotype suppression, we could not evaluate isotype influence on Ts inductions for a variety of technical reasons. Now, however, with series of monoclonal anti-Ig-1b antibodies of different isotypes, we have been able to attack this problem. Furthermore, because the monoclonal anti Ig-1b antibodies in our series detect physically separated
allootypic determinants, we have also been able to investigate the importance of determinant location (on the Ig-1b Fc) for suppression induction.

Our studies are still in progress; however, as we shall show here, we have already been able to establish that (a) anti-Ig-1b antibody is sufficient to induce chronic suppression; (b) both IgG₁ and IgG₂a anti-Ig-1b antibodies independently induce suppression; and (c) some monoclonal anti-Ig-1b antibodies do not induce suppression, most likely because these react with CH₃ domain determinants hidden on cell surface Ig-1b molecules.

RESULTS

The characteristics of four monoclonal anti-Ig-1b antibodies used in these studies are summarized in Table I. Three isotypes are represented: IgG₂a, IgG₁, and IgG₂. Each of the antibodies detects a distinct Ig-1b Fc determinant (see Fig. 6, p. 326). Two of these determinants are in the CH₃ domain.

As indicated in Table I all of the antibodies decay in vivo at roughly the same rate. We measured antibody half-life by injecting either ¹²⁵I-labeled monoclonal antibodies or unlabeled monoclonal antibodies into normal adult BALB/c mice intravenously and by following disappearance of labeled material or antibody activity. Results with both

<table>
<thead>
<tr>
<th>Hybridoma line (Mab)</th>
<th>Heavy chain isotype</th>
<th>Reaction</th>
<th>Chain composition</th>
<th>Location of allotype</th>
<th>Half-life (days)</th>
<th>Induces allotype suppression</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.9 IgG₂a</td>
<td>Ig-1b</td>
<td>HL</td>
<td>CH₃</td>
<td>7-8</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>4.7 IgG₁</td>
<td>Ig-1b</td>
<td>HL</td>
<td>Hinge</td>
<td>N.D.</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>3.1 IgG₂</td>
<td>Ig-1b</td>
<td>HLK</td>
<td>CH₃</td>
<td>7-8</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>5.7 IgG₂</td>
<td>Ig-1b</td>
<td>HL</td>
<td>CH₃</td>
<td>7</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>2B-81 IgG₂a</td>
<td>DNP</td>
<td>HL</td>
<td>—</td>
<td>N.D.</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>

* Mab is used as an abbreviation for the monoclonal antibodies.

⁺ HL is the monoclonal antibody whose heavy and light chains come from parental spleen cell immunized with antigen. HLK are the mixed molecules that contain one light chain from the myeloma parent of the hybridoma line.

⁺⁺ Half-lives of antibodies were measured by injecting ¹²⁵I-labeled antibodies into normal adult BALB/c mice intravenously.

⁺⁺⁺ N.D., not determined.
### TABLE II

**Monoclonal Anti-Ig-1b (Mab 2.9) Induces Allotype Suppression: Ig-1b Serum Levels in Representative Mice**

<table>
<thead>
<tr>
<th>Mab isotype</th>
<th>Mab reactivity</th>
<th>Amount (μg)</th>
<th>12 weeks old</th>
<th>16 weeks old</th>
<th>20 weeks old</th>
<th>24 weeks old</th>
<th>28 weeks old</th>
<th>Chronic suppression</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG&lt;sub&gt;4&lt;/sub&gt; Ig-1b</td>
<td>250</td>
<td>&lt;0.05</td>
<td>0.05</td>
<td>0.15</td>
<td>0.08</td>
<td>&lt;0.05</td>
<td>Complete</td>
<td></td>
</tr>
<tr>
<td>IgG&lt;sub&gt;4&lt;/sub&gt; Ig-1b</td>
<td>250</td>
<td>&lt;0.05</td>
<td>0.7</td>
<td>&gt;1.0</td>
<td>0.1</td>
<td>&lt;0.05</td>
<td>Complete</td>
<td></td>
</tr>
<tr>
<td>IgG&lt;sub&gt;4&lt;/sub&gt; Ig-1b</td>
<td>250</td>
<td>0.5</td>
<td>0.6</td>
<td>0.5</td>
<td>0.8</td>
<td>0.1</td>
<td>Partial</td>
<td></td>
</tr>
<tr>
<td>IgG&lt;sub&gt;4&lt;/sub&gt; Ig-1b</td>
<td>250</td>
<td>&lt;0.05</td>
<td>0.7</td>
<td>0.5</td>
<td>0.6</td>
<td>0.1</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>IgG&lt;sub&gt;4&lt;/sub&gt; Ig-1b</td>
<td>200</td>
<td>0.3</td>
<td>0.6</td>
<td>0.7</td>
<td>0.8</td>
<td>0.7</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>IgG&lt;sub&gt;4&lt;/sub&gt; DNP</td>
<td>200</td>
<td>0.5</td>
<td>0.9</td>
<td>0.8</td>
<td>&gt;1.0</td>
<td>&gt;1.0</td>
<td>No</td>
<td></td>
</tr>
</tbody>
</table>

* Monoclonal antibody was injected on 5 days of age.

* Measured by the solid phase radioimmunoassay.

Assay systems were comparable, indicating that the antibodies have a half-life of about 7–8 days in adult mice.

Injection of two of the four monoclonal antibodies into (BALB/c × SJL)F<sub>1</sub> neonates induced chronic suppression of Ig-1b production. Detailed time course and dose–response data for one of the inducing antibodies (Mab 2.9) are presented in the tables and figures that follow. These show that the suppression induced is similar in all respects to allotype suppression as induced by neonatal exposure to conventional anti-Ig-1b antisera (1).

The serum Ig-1b levels for several representative (BALB/c × SJL)F<sub>1</sub> injected with Mab 2.9 at 4–7 days old are shown as a function of age in Table II. Representative controls injected with IgG<sub>4</sub> anti-DNP monoclonal antibody are shown for comparison. There is considerable variability in the degree of suppression seen, from animals that are suppressed throughout life to those that show only slightly decreased serum Ig-1b levels. These variations are similar to the variations seen in mice exposed to maternal anti-Ig-1b and are a characteristic feature of the system.

The variability among suppressed mice and the tendency for suppressed mice to escape from suppression for shorter or longer periods between 12 and 20 weeks of age makes it necessary to compare a large series of antibody exposed and nonexposed (control) mice in order to obtain a comprehensive picture of the effect of neonatal antibody exposure on Ig-1b production prior to the onset of stable, essentially complete suppression (at approximately 6 months of age). The rising serum allotype (Ig-1b) levels in controls during the first 16 weeks of
life, however, complicates this comparison. Thus we have adopted a statistical method for presentation of the data in which we classify mice at a given age according to the number of standard deviations that separate them from the mean of the Ig-1b levels in controls. We are then able to draw a series of comparable histograms for each age showing the relative production of Ig-1b in normal and suppressed mice.

Data from these analyses are shown in Fig. I. They demonstrate

![Graph showing Ig-1b levels across different ages and conditions.]

**Fig. I.** Ig-1b suppression as a function of age. Distribution of anti-Ig-1b exposed and normal (BALB/c × SJL)F1 according to serum Ig-1b level. Mab 2.9 injected mice are least suppressed between 16 and 20 weeks of age.
that as a group, mice exposed neonatally to anti-Ig-1b (Mab 2.9) produce lower serum Ig-1b levels than controls. The suppressive effects of anti-Ig-1b exposure are most marked early in life (until 12 weeks of age) and in aging mice (after 24 weeks of age). Between 12 and 20 weeks, the distributions of Ig-1b levels in normal and suppressed mice overlap so that some mice become indistinguishable from controls; however, the differences in the overall distributions of suppressed and normal mice indicate that suppression is maintained (more or less strongly) throughout this period in all antibody-exposed mice.

To create a simplified measure of the frequency of suppressed mice
at a given age, so that various parameters of suppression induction could be examined [e.g., dose of monoclonal antibody (Mab) and time of injection], we arbitrarily define a suppressed mouse as one with a serum Ig-1b level lower than 3 standard deviations from the mean of Ig-1b level in age-matched controls. The upper curves in Fig. 2 shows the percentage of suppressed mice (thus defined) as a function of age for the experimental group (Mab 2.9 exposed) represented in the histograms in Fig. 1. Reduction of the data in this way loses some clarity, since, as we have indicated, all mice appear to be somewhat suppressed; however, for comparative purposes, this method of presentation offers substantial advantages.

The effects of the dose of Mab on suppression induction are shown in Fig. 2. Within the dose range used (125–1000 μg), there are no differences either in the frequency of suppressed mice or the length of the initial delay in onset of Ig-1b production. The time course at these doses is also indistinguishable from the time course for exposure to maternal anti-Ig-1b (which provides an antibody dose of approximately 250 μg). Thus, at least within this dose range, the amount of antibody to which the neonates is exposed does not influence the pattern of suppression induction.

The persistence of the injected antibody as a function of time is shown in Fig. 3. Higher doses persist somewhat longer; however, detectable anti-Ig-1b activity is lost in all cases by 7 weeks of age. The rate of decay of the antibody is similar to the measured half-life for these antibodies in adults (7–8 days), suggesting that production of Ig-1b by the neonate and subsequent complexing with antibody does not contribute to the loss of anti-Ig-1b activity. This is consistent with observations indicating that onset of detectable Ig-1b production in antibody-exposed mice rarely occurs earlier than 8 weeks of age (data not shown) and often not before 12 to 16 weeks of age (see Fig. 2).

The gap between loss of detectable antibody and onset of detectable Ig-1b production appears to be due to regulation by suppressor T cells rather than to a direct effect of the anti-Ig-1b on Ig-1b B cells.

Data in Tables III and IV show that such Ts are indeed demonstrable as early as 3 weeks of age in neonates exposed to maternal anti-Ig-1b. These Ts generally become eclipsed in older mice (between 8 and 20 weeks of age) until chronic suppression "sets in" (data not shown) and Ts are again demonstrable. As in chronically suppressed mice, Ig-1b memory B cells can be generated in antibody-exposed neonates, even as early as 4 weeks of age (data not shown), although the expression of these memory cells is prevented in situ because Ig-1b production is suppressed. Taken in concert, these data indicate that Ts are induced
Fig. 3. Decay of maternally transmitted and injected (Mab 2.9) anti-Ig-lb antibodies in (BALB/c × SJL)/F₁ neonates.

early after exposure to anti-Ig-lb and persist at greater or lesser effectiveness throughout the life of the animal; finally coming to dominance in a majority of mice over the age of 24 weeks.

The time during which mice are sensitive to Ts induction appears to be limited. Exposure to Mab 2.9 at 2 weeks of age yields the same result as earlier exposures; but exposure at 4 weeks of age generally does not induce detectable suppression (Fig. 4), even though serum
### TABLE III
Allotype Suppressor T Cells in Young Mice

<table>
<thead>
<tr>
<th>DNP-KLH primed spleen cells (×10⁶)</th>
<th>Treatment</th>
<th>No. of cells (×10⁶)</th>
<th>IgG anti-DNP response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ig-1a (%)</td>
</tr>
<tr>
<td>10</td>
<td>C' only</td>
<td>5</td>
<td>80</td>
</tr>
<tr>
<td>10</td>
<td>Nylon passed</td>
<td>5</td>
<td>70</td>
</tr>
<tr>
<td>10</td>
<td>Nylon passed</td>
<td>2.5</td>
<td>72</td>
</tr>
<tr>
<td>10</td>
<td>Anti-Thy-1-2 + C'</td>
<td>5</td>
<td>80</td>
</tr>
<tr>
<td>10</td>
<td>Anti-I-J⁺ + C'</td>
<td>5</td>
<td>72</td>
</tr>
</tbody>
</table>

* Spleen cells obtained from BALB/c × SJL mice exposed to maternal anti-Ig-1b antibodies.
* Percentage of standard BALB/c × SJL anti-DNP adoptive secondary antiserum response measured by radioimmunoassay 14 days after transfer. X-irradiated (650R) BALB/c recipients challenged with DNP-KLH.
* Monoclonal rat anti-Thy-1-2 antibody.
* B10A(3R) anti-B10A(5R) antibody.

### TABLE IV
Young Mice Exposed to Maternal Anti-Ig-1b Have Allotype Suppressor T Cells

<table>
<thead>
<tr>
<th>DNP-KLH primed spleen cells* (×10⁶)</th>
<th>Age at transfer (weeks)</th>
<th>Perinatal antibody exposure*</th>
<th>Spleen cells transferred (×10⁶)</th>
<th>IgG anti-DNP response*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ig-1a</td>
</tr>
<tr>
<td>10</td>
<td>3</td>
<td>Anti-Ig-1b</td>
<td>10</td>
<td>40</td>
</tr>
<tr>
<td>10</td>
<td>4</td>
<td>Anti-Ig-1b</td>
<td>10</td>
<td>40</td>
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<tr>
<td>10</td>
<td>6</td>
<td>Anti-Ig-1b</td>
<td>10</td>
<td>55</td>
</tr>
<tr>
<td>10</td>
<td>7</td>
<td>Anti-Ig-1b</td>
<td>10</td>
<td>60</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>Anti-Ig-1b</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>10</td>
<td>28</td>
<td>Anti-Ig-1b</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>10</td>
<td>4</td>
<td>None</td>
<td>10</td>
<td>80</td>
</tr>
</tbody>
</table>

* BALB/c × SJL mice used as donors in all cases.
* Mice perinatally exposed to anti-Ig-1b were born to BALB/c mothers immunized with Ig-1b.
* Measured 14 days after transfer into X-irradiated (650R) BALB/c recipients challenged with DNP-KLH. Units = percentage of standard BALB/c × SJL anti-DNP adoptive 2nd antiserum.
anti-Ig-1b levels in the 4-week-old mice when injected are equivalent to levels that induce suppression in younger mice. Therefore, somewhere around 3 weeks of age, neonates lose the ability to develop persistent suppression when exposed to anti-allotype antibody.

The isotype of the anti-Ig-1b antibody in contrast, does not appear to affect its ability to induce suppression. Initially, we had tested one IgG_2a anti-Ig-1b (Mab 2.9) and one IgG_1 anti-Ig-1b (Mab 3.1) and thought our data would show the same isotype effects that Hetzelberger et al. (5) found, i.e., the IgG_2a antibody (Mab 2.9) induced suppression but the IgG_1 antibody (Mab 3.1) did not. Studies with Mab 4.7, however, which is also IgG_1, showed that both IgG_1 anti-Ig-1b
antibodies can induce suppression (see Fig. 5). Thus some property of Mab 3.1 other than isotype must be responsible for its inability to induce suppression.

Recent studies in our laboratory (6), taken in conjunction with some other observations by Froiland et al. (7) and Jones and Cebra (8), suggest that Mab 3.1 does not induce suppression because the allotype determinants it detects are hidden on cell-surface IgG1b. Mab 3.1 detects determinants in the CH2 domain of the IgG1b heavy chain (see Fig. 6) (6). The studies cited above (7,8) indicate that part or all this domain is hidden on human IgG-bearing cells and rabbit IgA-bearing

![Graph showing percentage suppression over weeks for different isotypes of Mab](image)

**Fig. 5.** Differences in ability to induce suppression appear independent of Mab isotype. Mabs were injected into (BALB/c x SJL)F1 mice at 4-7 days of age. Suppressed mice defined as having IgG1b serum levels lower than 3 S.D. from control levels.

**Note in Proof:** Studies in mice exposed to IgG2a anti-IgG1b were completed through 27 weeks of age. No suppression developed in these mice.
cells. Studies with Mab 5.7 (not yet complete) are consistent with this hypothesis. Mab 5.7 also detects CH$_2$ domain determinants (6) and also does not appear to induce suppression (see Fig. 5).

Unfortunately, no firm conclusion can be drawn as yet in this matter because it is possible that (a) Mab 3.1 fails to induce because it has substantial properties of "mixed" molecules that contain one light chain from the myeloma parent of the hybridoma line producing the Mab (i.e., is an HLK-producing clone) and (b) Mab 5.7 does not induce because it is an IgG$_3$ antibody. We are currently attempting to isolate a clone from the Mab 3.1 line which has lost the myeloma parent light chain (i.e., is an HL-producing line) so that the question can be resolved.

DISCUSSION

Recent studies, including those presented here, have begun to provide the broad outlines of the mechanisms involved in the induction of allotype suppression. These studies, although still somewhat incomplete, indicate that combination of the inducing antibody with Ig on immature B cells initiates allotype Ts induction in the neonate. Since antibody interaction is known to temporarily deplete B cells (4)
and certain helper T cells (Janeway et al., this volume), these findings suggest that Ts emerge as a dominant regulatory population in mice genetically susceptible to allotype suppression when B cells are missing, perhaps because the B cells are required to induce or protect a population of Th that normally maintain the regulatory T cell balance in favor of help rather than suppression. The Th population in this case would be expected to be the allotype-specific Th population that helps Ig-1b B cells and is the target of the allotype Ts (2).

Black and Herzenberg proposed this hypothesis on the basis of their studies (3) showing that allotype suppression is induced not only by antibody reactive with Ig-1b (the suppressed allotype) but also by antibodies reactive with IgM (or IgD) present on precursors of Ig-1b memory cells and Ig-1b antibody-forming cells (afcs). These latter antibodies, they argued, do not react with Ig-1b and therefore must indirectly induce Ts, most likely by depleting precursors of Ig-1b B cells.

Our data support this hypothesis. First, we have shown that monoclonal antibodies that react with Ig-1b determinants expected to be exposed on B cell surfaces induce suppression, whereas monoclonal antibodies that react with CH3 domain Ig-1b determinants expected to be hidden on the cell surface (7,8) do not induce suppression. These data rule out the participation of complexes between inducing antibody and secreted Ig-1b, since all four monoclonal antibodies should be equally efficient in complex formation. Instead, they indicate again that direct interaction between inducing antibody and precursor B cells initiates Ts induction.

Second, we have shown that Ts are induced in the neonate and can be detected as early as 3 weeks of age in anti-Ig-1b exposed mice. Thus Ts first appear during the period when B cells are likely to be depleted due to the antibody exposure and then remain active to a greater or lesser extent throughout the life of the animal, eventually becoming the dominant regulatory population.

Finally, we have shown that sensitivity to Ts induction terminates at roughly 4 weeks of age, coincident with the emergence of a mature immune system capable of producing IgG antibodies. Whether this indicates that by this age the B cell population is no longer susceptible to depletion or that the balance between Ts and Th is no longer modifiable is unclear; but the fixation of this time point demonstrates that induction of suppression must occur while the immune system is still developing.

Thus, between Black and Herzenberg’s study (3) and our own we have amassed a fair amount of circumstantial evidence indicating that
interference in B cell maturation results in the induction of allotype suppression. Direct studies are clearly required at this point to determine whether B cell depletion occurs and is responsible for Ts induction, and, if so, whether the failure to induce Ig-1b Th is involved in the process. The clarification of the mechanism of Ts induction will then (hopefully) provide some clues as to how the genetic constitution of the SJL-related strains renders them peculiarly susceptible to induction of chronic suppression (9).

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