CARRIER-SPECIFIC SUPPRESSION OPERATES THROUGH THE HAPten-SPECIFIC SYSTEM

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INTRODUCTION

Although Ig-oriented regulatory systems such as allotype and idotype suppression have been well studied in their own right, they are usually treated as peripheral to regulation of the broadly heterogeneous antibody responses normally produced to hapten-carrier conjugates. Nevertheless, our recent studies show that a "hapten-specific" regulatory system which combines the properties of the allotype and idotype systems plays a central role in controlling the amount, affinity and isotype representation in these kinds of antibody responses (1). In essence, the T cells and other cell populations active in this system selectively prevent or permit the expression of individual memory B cells according to the antibody combining site (variable region) and isotype (light constant region) commitment such B cells display.

Although the hapten-specific system has remained cryptic through roughly 10 years of intensive study of the carrier-specific regulatory systems commonly considered to be the principle mechanisms controlling heterogeneous antibody responses, its presence is clearly discernable in retrospect. That is, once the properties of the hapten-specific system are recognized, the data from these earlier studies become interpretable in terms of carrier-specific mechanisms which induce the hapten-specific system to support or suppress antibody production. Thus, data presented here, which directly demonstrate that carrier-specific suppressor T cells (CTs) regulate antibody production in this way, introduce a substantially new perspective on how the immune system is organized and how "ordinary" antibody responses are controlled.

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We cannot present a full description of our studies within the space allotted in this volume. Thus we have chosen instead to outline our symposium presentation and hope that the reader will seek more detailed information from our recent publications (1-5).

I. OVERVIEW

Antigenic molecules have a series of structural determinants (haptens) to which B cells produce antibody (see Fig. 1). In addition, these molecules must have at least one (carrier) determinant used by "carrier-specific" regulatory T cells (CTs and CTh) to present the haptens on the antigen to B cells and other regulatory T cells.

The "hapten-specific" regulatory system (see Fig. 2) is composed of parallel elements that directly regulate the expression of memory B cells according to the structure of the antibodies such cells are committed to produce, i.e., according to the specificity and affinity the antibody displays for haptens on the antigenic molecule and the isotype and allotype of the antibody heavy chain constant region.

Each of the elements in the system can be independently induced to either suppress or support antibody production (depending on conditions under which "its" hapten is first introduced). Once induced to suppress antibody production, however, an element will resist subsequent induction (shifting) into a supportive (help) configuration and vice versa. Thus these elements provide the system with a selective bistable regulatory capability that allows the spectrum of antibodies produced in a given response to be defined initially and then maintained through subsequent antigen stimulations unless conditions change dramatically.

Regulatory systems that more directly "sense" the immunologic environment (e.g., the carrier-specific suppression and allotype suppression systems) induce the hapten-specific elements to suppress or support antibody production. Signals from these systems appear to be independently integrated within each of the elements to yield a final set of decisions defining the character of the antibody response generated under a given set of conditions. Thus the hapten-specific system offers a common channel through which a combination of diverse regulatory influences can be expressed in an orderly fashion.

Carrier-specific suppressor T cells (our primary concern here) induce hapten-specific elements to suppress production of the antibodies they regulate. Carrier-specific helper
Figure 1. Schematic of an Antigenic Molecule. Haptenic and carrier determinants are functionally distinct; however, the same structure could, in principle, serve as a haptenic determinant in one instance and a carrier determinant in another.

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T cells appear to provide pressure in the opposite direction, i.e., to induce hapten-specific elements to support antibody production. Which of these activities prevails depends largely on whether the more slowly maturing CTs population has reached full functionality before or after a given hapten is presented on the priming carrier, i.e., whether antibody production to the hapten become established before the CTs population becomes capable of inducing suppression for it.
Figure 2. Simplified Schematic for Antibody Response Regulation.
CTs = carrier-specific suppressor T cells
CTh = carrier-specific helper T cells
See Overview in text for the rationale.

Contrary to previous belief, carrier-specific populations show no evidence of regulating each other's activity in the KLH-specific systems where they have been most extensively studied (e.g., 6-10). That is, CTs do not interfere with delivery or maintenance of carrier-specific help and CTh do not interfere with CTs activity. Thus the precedent for "feedback" suppression loops and other regulatory mechanisms that ostensibly rely on interactions between carrier-specific populations is severely undermined. The mechanism operant in these systems therefore requires re-examination with appropri-
ate diagnostic methods to determine whether they also operate by inducing hapten-specific suppression.

II. The Hapten-specific Regulatory System Controls the Expression of IgG Anti-hapten Memory B cells Without Reference to the Carrier on which the Hapten is Presented

Carrier-primed animals subsequently presented with a new hapten on the priming carrier (e.g., KLH) produce very little IgG anti-hapten antibody (11,12). Earlier studies (13) attributed this response failure to interference with anti-hapten memory B cell development; however, our studies demonstrate that it is due to the specific suppression of anti-hapten antibody production (1-5). That is, anti-hapten memory B cells develop and persist normally after this "carrier/hapten-carrier" immunization sequence but are not expressed either initially or following restimulation with the hapten on the priming carrier (e.g., DNP-KLH). These memory cells also remain cryptic when suppressed animals are restimulated with the hapten on an unrelated carrier (e.g., DNP-CGG) (see Table 1 and Figs. 3 and 4).

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Table 1
HAPten-CARRIER STIMULATION OF Anti-HAPten MEMORY B CELLS IS NORMAL IN CARRIER-PRIMED MICE

<table>
<thead>
<tr>
<th>Memory B Cell Donors</th>
<th>Adoptive IgG Anti-DNP Response</th>
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<tbody>
<tr>
<td></td>
<td>Form of Antigenic Stimulation</td>
</tr>
<tr>
<td></td>
<td>KLH</td>
</tr>
<tr>
<td>I</td>
<td>*</td>
</tr>
<tr>
<td>II</td>
<td>Alum</td>
</tr>
<tr>
<td>III</td>
<td>*</td>
</tr>
<tr>
<td>IV</td>
<td>Alum</td>
</tr>
</tbody>
</table>

Donors (BALB/c x SJL) were killed 3 weeks after the first indicated stimulation with DNP-KLH. Splenic B cells were prepared by cytotoxic depletion of T cells using monoclonal anti-Thy-1.2 (30-H12, J. Ledbetter). Cells obtained from 10^7 spleen cells were co-transferred with 2 x 10^6 nylon-passed syngeneic T cells from KLH-primed donors (100 μg on alum + 10^9 Bordetella pertussis at least 6 weeks before transfer).
Recipients (650 rad irradiated BALB/c) were stimulated with 1 μg aqueous DNP-KLH (intravenous) at time of transfer and bled 1 week later for assay. Assay details are as described in Ref. 14. IgM-1b (IgG₂a) response data are shown here. IgM-1a (IgG₂a) and IgM-1 response data were similar.

** Figure 3. Anti-DNP Responses are Suppressed in KLH-Primed Mice Exposed to DNP-KLH. **

Each indicated antigenic stimulation was given as 100 μg of antigen on alum, i.p., at approximately 6 week intervals. Responses were measured 2 weeks after last indicated stimulation; radioimmunoassay (RIA) units shown are relative to "standard" secondary response antiserum pools. Mean Kₙ (in parentheses, x 10⁹) was determined from RIA binding. Assay details are described in Ref. 14.

** This suppression does not interfere with antibody production to either the priming carrier or the unrelated (second) carrier molecule. Such interference should certainly have been detectable in the primary response mounted to the unrelated carrier (even if the secondary and tertiary responses to the initial priming were sufficiently strong to mask... **
Figure 4. The carrier/hapten-carrier exposure sequence induces hapten-specific suppression.
See Legend to Figure 3.

* * *

adverse effects). Thus data from these experiments clearly demonstrate that the suppressive mechanism is "hapten-specific" in that it suppresses responses to one haptenic determinant on an antigenic molecule without impairing antibody production to other determinants on the same molecule (See Fig. 4).

These data also demonstrate that the suppressive mechanism does not deplete carrier-specific help (since depletion of such help would impair anti-carrier as well as anti-hapten responses). Therefore, the failure of the IgG anti-hapten antibody response in carrier/hapten-carrier immunized animals is clearly ascribable to a suppression-effector mechanism that operates independently of carrier-specific interactions.

In sum, suppression occurs in carrier/hapten-carrier immunized animals that have normal anti-hapten memory B cell populations and normal carrier-specific helper T cell populations. In this sense, it mimics a concerted idioype suppression directed at preventing the expression of memory B cells capable of producing antibodies with combining sites for the "new" hapten introduced in the carrier/hapten-carrier immunization sequence. However, the ability to selectively suppress production of individual idiotypes with such combining sites (see Table 2) distinguishes the hapten-specific system from currently known idioype-suppression systems. Thus these data
### TABLE 2
THE HAPTEN-SPECIFIC SYSTEM SELECTIVELY REGULATES ISOTYPE

<table>
<thead>
<tr>
<th>Immunizations (weeks)</th>
<th>Relative Anti-DNP Levels in Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgM</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>DK</td>
<td>1</td>
</tr>
<tr>
<td>K</td>
<td>2</td>
</tr>
<tr>
<td>DK</td>
<td>3</td>
</tr>
<tr>
<td>DK</td>
<td>4</td>
</tr>
</tbody>
</table>

* >10 BALB/c x SJL mice per group; 100 µg each antigen on alum
† Normalized means of responses measured by RIA two weeks after last immunization. See Fig. 2 for representative IgG₁ and IgG₂a responses in individual animals.
‡ K = KLH; DK = DNP-KLH
§ Broadly distributed

define a novel regulatory mechanism of prime importance for determining the composition of heterogeneous antibody responses.

### III. Carrier-specific Suppressor T Cells Induce Hapten-specific Suppression

As indicated above, DNP-KLH induces suppression in KLH-primed mice (see Figs. 3 and 4). Similarly, DNP-CGG induces suppression in CGG-primed mice. Nevertheless, KLH-primed mice produce normal anti-DNP responses to DNP-CGG and vice versa (except when suppression has already been induced by previous exposure to DNP on the initial carrier). Thus, hapten-specific suppression is induced only when carrier-primed animals are immunized with (a new) hapten on the priming carrier. In other words, although the suppression-effector mechanism operates independently of carrier-specific interactions, suppression is induced by a carrier-specific mechanism.
Several lines of evidence suggest that the "traditional" KLH-specific CTs (6) are responsible for inducing hapten-specific suppression: 1) the minimal, low affinity anti-DNP responses reported in CTs recipients challenged with DNP-KLH are similar to the anti-DNP responses obtained when hapten-specific suppression is induced; 2) both hapten-specific suppression and what is commonly called carrier-specific suppression affect IgG but not IgM anti-hapten responses; and, 3) immunization protocols that induce CTs (e.g., two stimulations with 100 μg aqueous KLH) prime animals for in situ induction of typical hapten-specific suppression by DNP-KLH.

Recent studies done at Chiba University in Japan in collaboration with Dr. Masaru Taniguchi directly confirm this hypothesis. These studies show that KLH-specific CTs and soluble suppressor factors (TsF) induce hapten-specific rather than carrier-specific suppression in "traditional" in vivo CTs assays. That is, IgG anti-DNP responses are specifically suppressed when unprimed (non-irradiated) recipients of these cells and factors are challenged with DNP-KLH and remain suppressed when recipients are subsequently challenged with DNP-CGG (see Tables 3 and 4). Thus although matching between carrier and hapten-carrier conjugate is clearly required for suppression induction, once suppression is induced it suppresses antibody production to the hapten on an unrelated carrier.

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**TABLE 3**

<table>
<thead>
<tr>
<th>KHL TsF</th>
<th>Antigenic Stimulation</th>
<th>IgG2a</th>
<th>Anti-DNP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>day 0</td>
<td>day 0</td>
<td>4 weeks</td>
</tr>
<tr>
<td>no</td>
<td>DK</td>
<td>--</td>
<td>100</td>
</tr>
<tr>
<td>yes</td>
<td>DK</td>
<td>--</td>
<td>38</td>
</tr>
<tr>
<td>no</td>
<td>DK</td>
<td>DC</td>
<td>295</td>
</tr>
<tr>
<td>yes</td>
<td>DK</td>
<td>DC</td>
<td>70</td>
</tr>
</tbody>
</table>

*Tada protocol: sonicated extract from 5 x 10⁶ KHL-primed spleen (aqueous, 2X) |

†100 μg each antigen on alum; responses measured 2 weeks after last stimulation.
<table>
<thead>
<tr>
<th>KLH 2° Cell Transferred</th>
<th>Recipient Immunizations</th>
<th>( \mu g_{\text{a}} ) Anti-DNP (µg/ml)</th>
<th>( \mu g_{\text{a}} ) Anti-KLH (Units)</th>
<th>( \mu g_{\text{a}} ) Anti-CGG (Units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spleen</td>
<td>DK DK</td>
<td>20</td>
<td>8</td>
<td>n.t.</td>
</tr>
<tr>
<td></td>
<td>DK DK DC</td>
<td>10</td>
<td>n.t.</td>
<td>35</td>
</tr>
<tr>
<td>Spleen (T-depleted)</td>
<td>DK DK</td>
<td>140</td>
<td>11</td>
<td>n.t.</td>
</tr>
<tr>
<td></td>
<td>DK DK DC</td>
<td>125</td>
<td>n.t.</td>
<td>20</td>
</tr>
<tr>
<td>None</td>
<td>DK DK</td>
<td>120</td>
<td>9</td>
<td>n.t.</td>
</tr>
<tr>
<td></td>
<td>DK DK DC</td>
<td>100</td>
<td>n.t.</td>
<td>39</td>
</tr>
</tbody>
</table>

*Tada protocol: 100 µg aqueous KLH at -4 and -2 weeks; 5 x 10^7 BALB/c spleen (or remainder after Thy-1^-C^-).

+100 µg each antigen on alum; response measured 2 weeks after last stimulation

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Anti-KLH and anti-CGG responses in CTs recipients proceed normally, again indicating that hapten-specific suppression has been induced (see Table 3). Similarly, anti-CGG responses proceed normally in TsF recipients subsequently immunized with DNP-CGG despite pronounced suppression of the anti-DNP response to this antigen. Thus, in both kinds of recipients, the data clearly demonstrate that hapten-specific suppression is induced by the initial exposure to DNP-KLH.

Anti-KLH responses, however, are impaired in TsF recipients immunized with DNP-KLH but not in similarly immunized CTs recipients. This impairment apparently occurs in these mice but not in recipients of KLH-primed spleen cell populations containing CTs because these populations also contain the hapten-specific cell populations that support antibody production to the native determinants on the KLH molecule and hence prevent suppression-induction for these determinants (but not for DNP on KLH). The soluble TsF preparation, in contrast, lacks
these cell populations. Thus when TsF recipients are challenged with DNP-KLH, all of the determinants on the KLH molecule are treated as "new" haptenic determinants and the bistable hapten-specific system is induced to suppress rather than support antibody production to these determinants.

Similar results are obtained by priming animals with KLH or DNP-KLH under conditions where the animal is prevented from producing an antibody response to the antigen. For example, when young allotype (Igh-1b) suppressed mice are primed with DNP-KLH, they fail to produce Igh-1b antibodies to either DNP or KLH. When these animals go into remission (about 12 weeks of age), they develop the ability to produce 1b antibodies to any subsequently introduced antigen but nonetheless fail to produce antibodies to KLH or to DNP introduced on either KLH or CGG. Since these DNP-KLH-primed animals show normal 1b anti-DNP and 1b anti-KLH memory B cells and mount a normal primary response to CGG, their inability to produce antibodies to DNP and KLH determinants clearly is ascribable to hapten-specific suppression. We have suggested that this suppression is induced because the cell populations that normally support 1b production and thereby prevent suppression induction are depleted (by allotype Ts). Thus CTs, once mature, can induce hapten-specific suppression for the 1b antibody responses.

The evidence we have obtained contradicts the common belief that CTs regulate antibody production by depleting CTH. That is, we have shown that CTs induce hapten-specific suppression, that the hapten-specific suppression mechanism operates independently of the carrier on which the hapten is presented, and that CTH are present and functional in suppressed animals. Thus earlier evidence based only on measurement of anti-hapten responses and only on measurement of responses following a stimulation with a single hapten-carrier conjugate (rather than sequential stimulation with two different conjugates) yielded ambiguous evidence with respect to whether CTs depleted CTH or induced hapten-specific suppression. (It is curious that although those of us interested in regulatory immunology reviewed this evidence extensively and repeatedly over the years, we nonetheless failed collectively to recognize its potential ambiguity.)

In sum, studies discussed here create a substantially different picture of the mechanisms involved in carrier-specific regulation. Our data indicate the following:

1) Carrier-priming generates both carrier-specific suppressor T cells (CTs) and carrier-specific helper (CTH) T cells in the same animal.
2) CTs do not deplete CTh.

3) CTs suppress by inducing specific suppression for IgG anti-hapten antibody production when confronted with "new" hapten on the priming carrier.

4) CTs do not generally induce suppression for determinants on the priming carrier because the bistable properties of the hapten-specific system premit the establishment and maintenance of antibody production to initially introduced determinants despite the subsequent maturation of the CTs population.

The generality of these conclusions requires testing since our analysis has mainly been restricted to antibody responses stimulated by hapten-carrier conjugates. The idea that CTs deplete CTh, however, derives largely from these kinds of studies. Thus, there is ample reason to suspect that many if not all antigen-induced suppressions currently under study are based on mechanisms similar to the carrier-specific induction of hapten-specific suppression described here.

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