# TOLERANCE FOR SELF IG AT THE LEVEL OF THE LY1$^+$ T CELL*

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Current thinking about immunoregulation has been greatly influenced by Jerne's concept of the immune system as a network of lymphocytes and antibody molecules (1). Evidence for T cell participation in the network comes from two types of experiments. First, it has been established that T cells are targets of antibody-mediated regulation (2–10). For example, passive administration of guinea pig antibodies directed against the A5A idiotype was shown to induce T helper activity for A5A$^+$ B cells (2). Treatment at birth with A48 idiotype similarly leads to the activation of A48$^+$ B cells and the expansion of A48-specific helper cells (7), whereas idiotype-specific suppression is associated with the generation of idiotype-specific suppressor T cells (5, 8–10). Second, it has been demonstrated that the ability of T cells to communicate with other cells is controlled by immunoglobulin heavy gene (Igh)-linked genes (11–16). For example, homology at the Igh-V gene complex is required for optimal induction of feedback suppression (11). Igh-V-restricted T-B interactions have also been described (16). Thus, considerable data indicate that T cells function as legitimate members of the network.

On the other hand, molecular immunologists have determined that $V_H$ genes are neither rearranged (17, 18) nor transcribed (19) in T cells. These conclusions seriously challenge the idea that T cells are connected to other cells via idiotype-antiidiotype linkages. Moreover, several investigators have described T cells that participate in immunoregulatory circuits via recognition of allotypic determinants (20–24). Thus, there is evidence that T cells can communicate with other cells through recognition sites on immunoglobulin constant regions. Finally, network theory does not take into account the requirement for recognition of self major histocompatibility complex (MHC)-encoded molecules for T cell interactions with other cells. Thus, it remains unclear how T cells acquire specificity for self Ig.

We recently described an association between Igh haplotype and the capacity of Ly1$^+$ T cells to proliferate in response to cross-reactive determinants on 4-(hydroxy-3-nitrophenyl)acetyl (NP)- and 2,4,6-trinitrophenyl (TNP)-modified Ig (25). The present study analyzes the mechanism whereby Igh-encoded molecules influence Lyl$^+$ T cell specificity. We show that Igh-linked control of T cell

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[1] *Abbreviations used in this paper*: B6, C57BL/6; CGG, chicken gamma globulin; Ig, immunoglobulin; Igh, immunoglobulin heavy chain; MHC, major histocompatibility complex; NMS, normal mouse serum; NP, (4-hydroxy-3-nitrophenyl) acetyl; TNP, 2,4,6-trinitrophenyl.

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responses to NP-modified Ig is a secondary consequence of naturally acquired tolerance for self Ig. Unresponsiveness to self Ig is not due to a defect expressed functionally at the level of the antigen-presenting cell, nor is it associated with active suppression. Thus, the lack of responsiveness to self Ig probably reflects a functional deletion of $Lyl^+$ T cell clones specific for self Ig. It is proposed that regulatory effects mediated by passively administered antibodies may in part be due to induction of $Lyl^+$ T cell tolerance for self Ig.

## Materials and Methods

*Animals.* BALB/cAn mice were the progeny of animals obtained from the Kingston facility of Charles River Breeding Laboratories, Inc. (Wilmington, MA) in April, 1980. Because there was reason to doubt the genetic integrity of BALB/c mice provided by that supplier at that time (26), all results were confirmed in BALB/cAn mice provided by Dr. Frank Lilly, Albert Einstein College of Medicine, Bronx, NY. C.B-20 mice were derived from breeding pairs obtained from Dr. Michael Potter, National Cancer Institute, Bethesda, MD. BAB/14 and C.B/R4 mice were the progeny of breeding pairs given to us by Dr. Roy Riblet, the Institute for Cancer Research, Philadelphia, PA. (C.B-20 × BALB/c)$F_1$, (BALB/c × BAB/14)$F_1$, and (BALB/c × C.B/R4)$F_1$ mice were produced in our breeding colony. All mice were used at 6–12 wk of age.

*Antigens and Immunoglobulins.* Normal mouse and chicken gamma globulins (CGG) were purified by ammonium sulfate precipitation and chromatography on DEAE-cellulose. NP-OSU (succinimide ester) and trinitrobenzene sulfonate were purchased from Bio-Search, San Rafael, CA. NP- and TNP-Ig conjugates were synthesized as described previously (27). The plasmacytomas CBPC101 and LPC1 were obtained from Dr. Michael Potter, National Cancer Institute, Bethesda, MD. Myeloma proteins were isolated from ascitic fluid by ammonium sulfate precipitation and chromatography on DEAE-cellulose, and were further purified by affinity chromatography on Protein A-Sepharose according to method described by Ey et al. (28).

*Immunization.* Mice were injected with 100 μg NP-modified Ig emulsified in complete Freund's adjuvant (H37Ra; Difco Laboratories, Detroit, MI) subcutaneously at the base of the tail (29).

*Tolerance Induction.* Neonates were injected within 24 h of birth i.p. with 1 mg myeloma protein or with 0.1 ml normal mouse serum (NMS). Mice tolerized with serum received an additional 0.1 ml NMS at 1 wk of age.

*Cell Cultures.* Lymph node proliferation assays were carried out by a slight modification of the method of Corradin et al. (29). Briefly, 7 d after immunization, inguinal and para-aortic lymph nodes were removed and teased into single cell suspension. Cells were placed into 96-well flat-bottomed Linbro microtiter plates (Linbro Chemical Co., Hamden, CT) at a density of $5 \times 10^5$ cells in 200 μl of RPMI 1640 supplemented with 5% fetal calf serum, 2 mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 15 mM Hepes pH 7.2, 1 mM sodium pyruvate, $5 \times 10^{-5}$ M 2-mercaptoethanol per well. Following a 3-d period in culture, proliferation was assessed by a 16–18-h exposure to 1 μCi of [$^3$H]-thymidine (Amersham Corp., Arlington Heights, IL). Results are expressed as means of triplicate cultures.

*Cell Preparation.* T cells were purified from lymph node populations by passage over nylon wool columns (30). Irradiated spleen cells were prepared from unprimed control or tolerant mice. Spleens were teased into single cell suspension with forceps. Erythrocytes were lysed by incubation for 2 min at 37°C in 0.17 M ammonium chloride solution containing 10 mM Tris-HCl (pH 7.2). Spleen cell populations were then irradiated (2,000 rads).

## Results

BALB/c $Lyl^+$ T cells directed against NP-BALB/c Ig are specific for the immunizing hapten NP because they fail to respond to BALB/c Ig modified by

a different hapten, TNP (reference 25 and Fig. 1 *A*). They also have specificity for the self Ig carrier because there is no cross-reactivity between NP-BALB/c Ig and NP coupled to a heterologous carrier CGG (reference 25 and Fig. 1 *A*). Thus, BALB/c Lyl+ T cells that proliferate in response to NP-BALB/c Ig recognize exclusively conjugate-specific determinants—i.e., determinants created by attaching the hapten NP to the carrier self Ig. By contrast, when NP-BALB/c Ig is used as the immunogen in C.B-20 mice, a strain congenic with BALB/c but expressing the $Igh^b$ allotype of C57BL/6 (B6) mice, responding Lyl+ T cells recognize common determinant(s) expressed on NP- and TNP-BALB/c Ig (Fig. 1 *B*). Similarly, in the reciprocal experiment shown in Fig. 1 *C* and *D*, with NP-B6 Ig as the immunogen, a partial cross-reaction between NP- and TNP-B6 is observed in BALB/c but not in Igh-identical C.B-20 mice. Thus, as previously reported, specificity of the proliferative response to NP-modified Ig is regulated by Igh-linked genes.

It was previously shown that cross-reactivity between NP- and TNP-nonself Ig is a consequence of T cell responsiveness directed towards allotypic determinants expressed on the foreign Ig (25). The objective of the present study was to determine whether the inability of responding Lyl+ T cells to recognize common determinant(s) on NP- and TNP-self Ig was due to naturally acquired tolerance for self Ig. It was therefore of interest to describe the phenotype of (responder × nonresponder)$F_1$ mice. As shown in Fig. 2 *A*, when NP-BALB/c Ig is used as the immunogen in (C.B-20 × BALB/c)$F_1$ mice, there is no cross-reactivity directed towards TNP-BALB/c Ig. Similarly, with NP-B6 Ig as the immunogen,

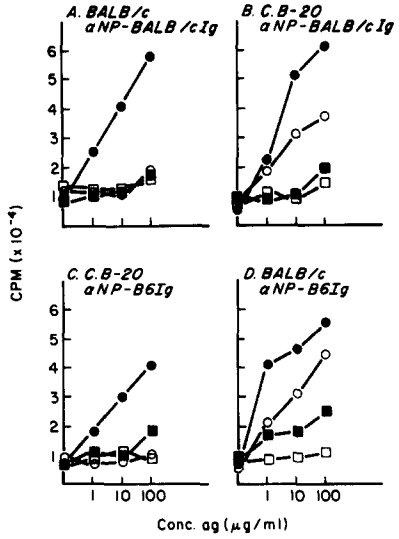


FIGURE 1. Specificity of the proliferative response to NP-modified Ig is regulated by Igh-linked genes. In panels A and B, increasing concentrations of NP-BALB/c Ig (●), TNP-BALB/c Ig (○), NP-CGG (■), or TNP-CGG (□) were added to cultures containing 5 × $10^5$ NP-BALB/c Ig-immune BALB/c (panel *A*) or C.B-20 (panel *B*) lymph node cells. In panels *C* and *D*, increasing concentrations of NP-B6 Ig (●), TNP-B6 Ig (○), NP-CGG (■), or TNP-CGG (□) were added to cultures containing 5 × $10^5$ NP-B6 Ig-immune C.B-20 (panel *C*) or BALB/c (panel *D*) lymph node cells.

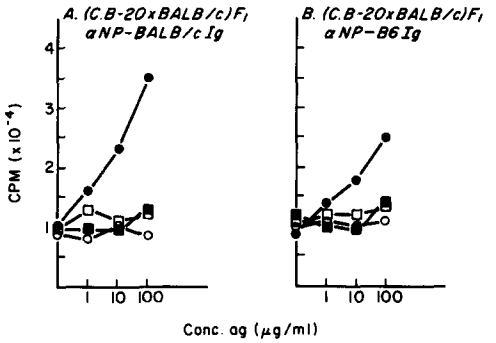


FIGURE 2. Dominant nonresponsiveness in (responder × nonresponder)$F_1$ mice. In panel *A*, increasing concentrations of NP-BALB/c Ig (●), TNP-BALB/c Ig (○), NP-CGG (■), or TNP-CGG (□) were added to cultures containing $5 \times 10^5$ NP-BALB/c Ig-immune (C.B-20 × BALB/c)$F_1$ lymph node cells. In panel *B*, increasing concentrations of NP-B6 Ig (●), TNP-B6-Ig (○), NP-CGG (■), or TNP-CGG (□) were added to cultures containing $5 \times 10^5$ NP-B6 Ig-immune (C.B-20 × BALB/c)$F_1$ lymph node cells.

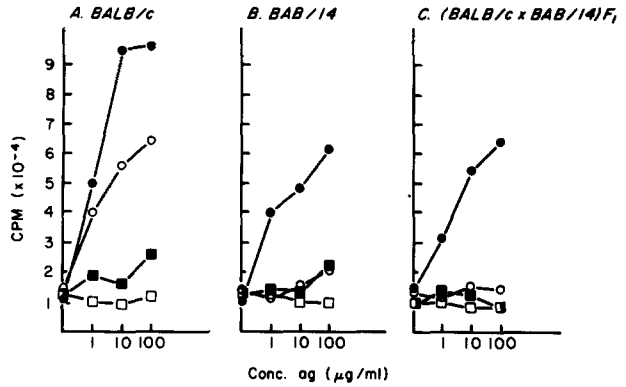


FIGURE 3. Nonresponsiveness to TNP-B6 Ig is associated with expression of the $Igh^b$ allotype. Increasing concentrations of NP-B6 Ig (●), TNP-B6 Ig (○), NP-CGG (■), or TNP-CGG (□) were added to cultures containing $5 \times 10^5$ NP-B6 Ig-immune BALB/c (panel *A*), BAB/14 (panel *B*), or (BALB/c × BAB/14)$F_1$ (panel *C*) lymph node cells.

responding $Lyl^+$ T cells fail to recognize cross-reactive determinant(s) expressed on TNP-B6 Ig (Fig. 2*B*). Thus, nonresponsiveness is dominant in (responder × nonresponder) $F_1$ mice. These data suggest that BALB/c mice express an Igh-encoded molecule that prevents the response to TNP-BALB/c Ig, that C.B-20 mice express an Igh-encoded molecule that prevents the response to TNP-B6 Ig, and that $F_1$ mice expressing both Igh-encoded molecules respond to neither TNP-BALB/c Ig nor TNP-B6 Ig.

We next asked whether this putative repressor molecule was encoded by Igh-V and/or Igh-C-linked genes. The BAB/14 mouse strain, like C.B-20, expresses the $Igh^b$ allotype of C57BL/6, but most of its Igh-V genes are from BALB/c. As shown in Fig. 3*B*, when NP-B6 Ig is used as the immunogen in BAB/14 mice, there is no significant cross-reactivity directed towards TNP-B6 Ig. Moreover, (BALB/c × BAB/14)$F_1$ mice that are homozygous at Igh-V and heterozygous within Igh-C also display a nonresponder phenotype (Fig. 3*C*). Thus, the inability

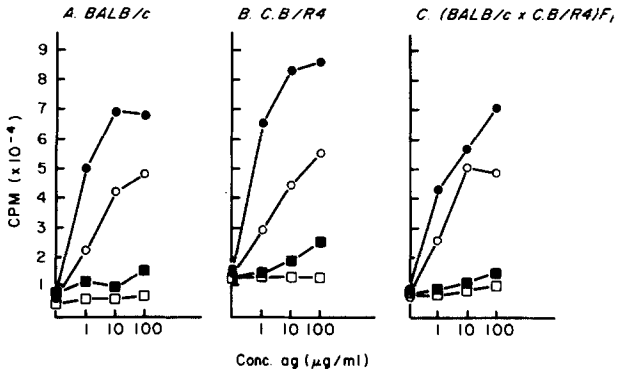


FIGURE 4. Mice that are heterozygous at Igh-V and homozygous within Igh-C display a responder phenotype. Increasing concentrations of NP-B6 Ig (●), TNP-B6 Ig (○), NP-CGG (■), or TNP-CGG (□) were added to cultures containing $5 \times 10^5$ NP-B6 Ig-immune BALB/c (panel *A*), C.B/R4 (panel *B*), or (BALB/c × C.B/R4)$F_1$ (panel *C*) lymph node cells.

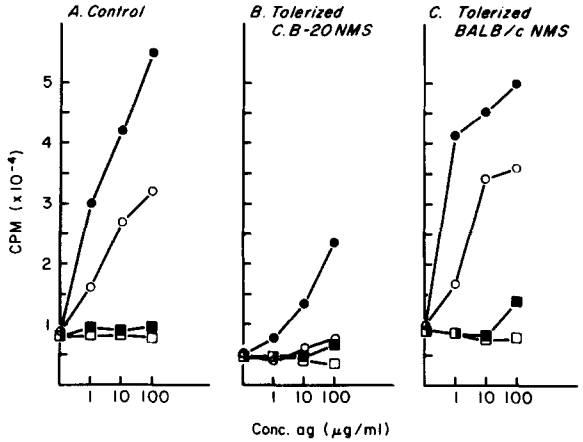


FIGURE 5. BALB/c mice made tolerant to C.B-20 serum behave phenotypically like (C.B-20 × BALB/c)$F_1$ mice. Increasing concentrations of NP-B6 Ig (●), TNP-B6 Ig (○), NP-CGG (■), or TNP-CGG (□) were added to cultures containing $5 \times 10^5$ NP-B6 Ig-immune lymph node cells from control BALB/c mice (panel *A*) or BALB/c tolerized with C.B-20 (panel *B*), or BALB/c (panel *C*) NMS.

of Lyl+ T cells to respond to common determinant(s) on NP- and TNP-B6 Ig is associated with expression of the $Igh^b$ allotype. As a control, a significant response to TNP-B6 Ig was generated in C.B/R4 and (BALB/c × C.B/R4)$F_1$ mice (Fig. 4). C.B/R4 is a new Igh recombinant mouse strain that carries the $Igh^a$ allotype of BALB/c and Igh-V genes from C57BL/6. Thus, mice that are heterozygous at Igh-V and homozygous within Igh-C display a responder phenotype. In sum, experiments presented in Fig. 3 and Fig. 4 indicate that the repressor is a product of Igh-C-linked genes.

The finding that strains possessing BALB/c Igh-C genes were responsive to TNP-B6 Ig, whereas strains whose serum immunoglobulins bear allotypic determinants in common with B6 were nonresponders suggested that the Igh-encoded repressor molecule was serum Ig. To test this hypothesis, we analyzed responses

of BALB/c mice injected with C.B-20 serum within 24 h of birth. As shown in Fig. 5*B*, this treatment resulted in a selective loss of cross-reactivity directed towards TNP-B6 Ig. Thus, BALB/c mice made tolerant to C.B-20 serum behave phenotypically like (C.B-20 × BALB/)$F_1$ mice. By contrast, responses of BALB/c mice injected with autologous BALB/c serum within 24 h of birth were not significantly different from those of untreated control BALB/c mice (Fig. 5*C*). These data strongly suggest that Igh-linked control of T cell response is a secondary consequence of naturally acquired tolerance for self Ig. However, many molecules in serum have immunoregulatory potential. In particular, T cell–derived suppressor factors have been isolated from hyperimmune sera (31, 32). It was therefore important to demonstrate that $Lyl^+$ T cell tolerance was induced by immunoglobulin and not any other serum component. These obser-

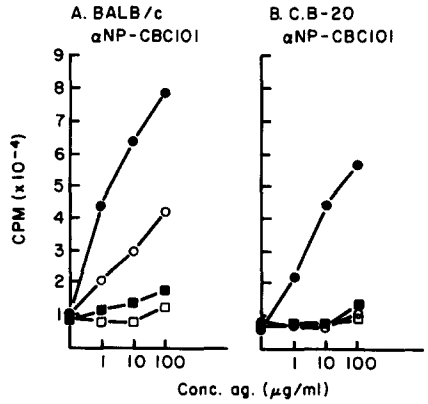


FIGURE 6. Specificity of the proliferative response to NP-CBPC101 is regulated by Igh-linked genes. Increasing concentrations of NP-CBPC101 (●), TNP-CBPC101 (○), NP-CGG (■), or TNP-CGG (□) were added to cultures containing 5 × $10^5$ NP-CBPC101-immune BALB/c (panel *A*) or C.B-20 (panel *B*) lymph node cells.

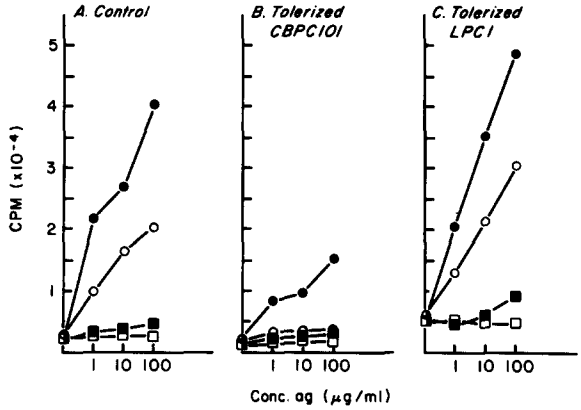


FIGURE 7. BALB/c mice made tolerant to the CBPC101 myeloma protein behave phenotypically like C.B-20 mice. Increasing concentrations of NP-CBPC101 (●), TNP-CBPC101 (○), NP-CGG (■), or TNP-CGG (□) were added to cultures containing 5 × $10^5$ NP-CBPC101-immune lymph node cells from control BALB/c mice (panel *A*), or BALB/c mice tolerized with CBPC101 (panel *B*), or LPC1 (panel *C*) myeloma protein.

vations were therefore extended in similar experiments with NP-modified myeloma proteins.

As shown in Fig. 6, Lyl+ T cells from BALB/c but not syngeneic C.B-20 mice recognize common determinant(s) on NP- and TNP-CBPC101. CBPC101 is a C.B-20 IgG2a, κ-bearing myeloma protein. There is similarly no cross-reactivity directed toward TNP-CBPC101 in BALB/c mice injected with 1 mg CBPC101 myeloma protein within 24 h of birth (Fig. 7*B*). Thus, BALB/c mice made tolerant to the CBPC101 myeloma protein behave phenotypically like C.B-20 mice. By contrast, responses of BALB/c mice injected with 1 mg LPC1 were indistinguishable from those of untreated control BALB/c mice (Fig. 7*C*). LPC1 is a BALB/c IgG2a, κ-bearing myeloma protein. Thus, the ability of Lyl+ T cells to recognize potentially immunogenic determinants on antibody molecules later in life was influenced by immunoglobulin seen during development. Taken together, experiments presented in Fig. 5 and Fig. 7 demonstrate that as for any other self component, there is naturally acquired tolerance for self Ig.

One mechanism that has been proposed to explain self tolerance is that suppressor cells may be responsible for the induction and/or maintenance of unresponsiveness to self. We therefore decided to test whether the loss of responsiveness to TNP-B6 Ig in BALB/c mice given C.B-20 serum was associated with active suppression. As shown in Fig. 8, vigorous responses to NP- and TNP-B6 Ig are observed in cultures containing a mixture of equivalent numbers of tolerant and normal NP-B6 Ig-immune BALB/c lymph node cells. This lack of evidence for "infectious tolerance" suggests that nonresponsiveness to TNP-B6 Ig is not due to the activity of suppressor T cells.

Another possibility is that self tolerance may be expressed at the level of the antigen-presenting cell. Indeed, it has been reported that macrophages from HGG-tolerant mice are defective in their ability to initiate HGG-specific responses (33). We therefore decided to test whether the loss of responsiveness to TNP-B6 Ig in BALB/c mice given C.B-20 serum was due to a lack of accessory cell function. As shown in Fig. 8, nylon-purified T cells from NP-B6 Ig-immune-BALB/c mice gave comparable responses in the presence of accessory cells from

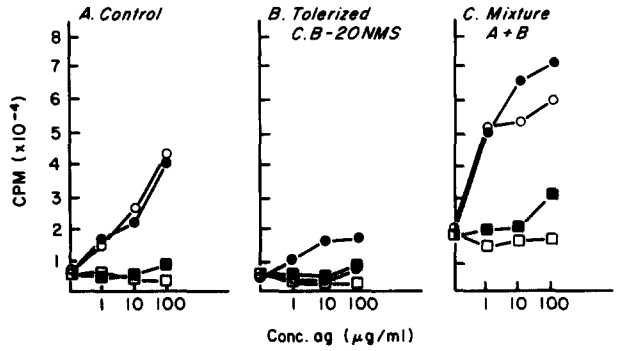


FIGURE 8. Lack of active suppression in tolerant lymph node cells. Increasing concentrations of NP-B6 Ig (●), TNP-B6 Ig (○), NP-CGG (■), or TNP-CGG (□) were added to cultures containing $5 \times 10^5$ NP-B6 Ig-immune lymph node cells from control mice (panel *A*), BALB/c mice made tolerant to C.B-20 serum (panel *B*), or a 1:1 mixture of control and tolerant lymph node cells (panel *C*).

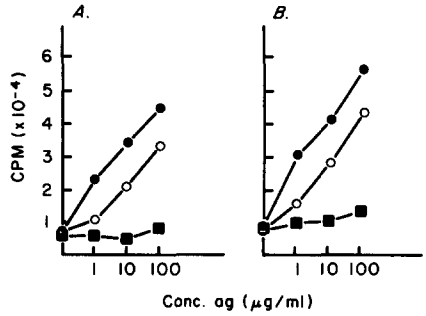


FIGURE 9. Loss of responsiveness to TNP-B6 Ig is not due to a defect expressed functionally at the level of the antigen-presenting cell. Increasing concentrations of NP-B6 Ig (●), TNP-B6 Ig (○), or NP-CGG (■) were added to cultures containing $5 \times 10^5$ NP-B6 Ig-immune nylon-purified BALB/c T cells and $5 \times 10^5$ irradiated spleen cells from normal BALB/c mice (panel *A*) or BALB/c mice made tolerant to C.B-20 serum (panel *B*).

normal or tolerant mice. Thus, unresponsiveness to TNP-B6 Ig is not due to a defect expressed functionally at the level of the antigen-presenting cell. In sum, experiments presented in Fig. 8 and Fig. 9 lead to the conclusion that the lack of responsiveness to self Ig probably reflects a functional deletion of $Lyl^+$ T cell clones specific for self Ig.

## Discussion

Experiments presented in this report demonstrate that specificity of the $Lyl^+$ T cell proliferative response to NP-modified Ig is controlled by Igh-C linked genes. There is overwhelming evidence (*a*) that Igh genes are structural genes coding for immunoglobulin heavy chains and (*b*) that T cells do not express conventional immunoglobulin. On the other hand, it has been demonstrated that T cell interactions with other cells are restricted by Igh-C-linked genes (21, 22, 24), and products of genes adjacent to the Igh-C locus have been serologically detected on T cells (34–36). It was therefore of interest to describe the mechanism whereby Igh-C-encoded molecules influence $Lyl^+$ T cell activity. In particular, we wanted to know whether Igh-linked control of T cell responses to NP-modified Ig was a secondary consequence of naturally acquired tolerance for self Ig. Consistent with this idea, we found that nonresponsiveness was dominant in (responder × nonresponder)$F_1$ mice. Moreover, BALB/c mice injected with C.B-20 serum within 24 h of birth gave responses that were indistinguishable from those of (C.B-20 × BALB/c)$F_1$ mice. Similar experiments with myeloma proteins established that these regulatory effects were mediated by immunoglobulin and not any other Igh-encoded serum protein.

Unresponsiveness to self Ig was not expressed functionally at the level of the antigen-presenting cell, nor was it mediated via the activity of suppressor T cells. Our interpretation of these results is that tolerance is due to functional deletion of $Lyl^+$ T cell clones specific for self Ig. This viewpoint is consistent with conclusions previously reached by several investigators studying experimentally induced tolerance to foreign protein antigens. In particular, it was shown that mice rendered tolerant to human gamma globulins (HGG) often do not contain demonstrable suppressive activity (37–40). Thus, a strong argument can be made

that suppressor T cells are not responsible for induction or maintenance of the tolerant state. Unequivocal evidence for T cell tolerance in the absence of suppression was recently reported by Lamb et al. (41). These investigators demonstrated that the ability of isolated human T cell clones specific for defined peptides of the influenza A hemagglutinin to proliferate in response to antigen was selectively eliminated following exposure to high concentrations of antigen. These data and our own support the idea that developing T cells can be inactivated through a direct interaction with self antigens.

Considering that during development, Lyl+ T cells become tolerant to products of Igh-C-linked genes, an important question is whether Lyl+ T cell tolerance is similarly induced to immunoglobulin variable region sequences. On the one hand, induction of autologous antiidiotypic antibodies provides clear evidence that products of Igh-V–linked genes can be recognized as foreign (42–45). On the other hand, it is in general exceedingly difficult to raise autologous antiidiotypic antibodies in response to unmodified soluble immunoglobulin as the immunogen. Production of antiidiotypes usually involves chemical modification of the immunoglobulin—for example, by glutaraldehyde cross-linking, by attaching a haptenic moiety (46), by formation of antigen-antibody complexes (47), or by coupling the immunoglobulin to an immunogenic carrier such as KLH (48). All these protocols would successfully bypass Lyl+ T cell tolerance by providing new helper determinants. Moreover, it is well known that immunoglobulin variable regions represent an extended family of molecules whose members frequently share extensive sequence homology. Thus, the actual concentration in serum of determinants associated with a highly conserved framework sequence and those expressed on the constant region of a rare class of Ig may in fact be very similar. There is no a priori reason why cellular events involved in induction of tolerance to the one should be different from those used for maintenance of unresponsiveness to the other. Consistent with this way of thinking, Ortiz-Ortiz, Weigle, and Parks (49) recently described induction of tolerance in an antiidiotypic response. IgE-class-restricted tolerance induced by neonatal administration of IgE was recently described by Chen and Katz (50). It will be interesting to learn whether idiotype- and/or IgE-specific tolerance is due to functional deletion of idiotype- and/or IgE-specific Lyl+ helper T cells.

Passively administered antibodies have been shown to influence T cell activity in a variety of ways. Regulatory effects may in part be due to a disturbance of idiotype-antiidiotype linkages that connect T cells and other cells of the immune systems into a functional network. Particularly in the case of regulatory effects induced by anti-allotype (20) and anti-$\mu$ antibodies (51–53) however, it is extremely unlikely that T cells are direct targets of antibody-mediated regulation since it is known that T cells do not express conventional Ig. If we accept (*a*) that as for any other self component, there is naturally acquired tolerance for self Ig and (*b*) that induction of self tolerance is influenced by the concentration and form of antigen seen during development, then it follows that treatments affecting serum Ig expression would also have an impact on induction of Lyl+ T cell tolerance for self Ig. Removal (or addition) of specific antibody molecules in the form of soluble and/or membrane-bound Ig may therefore indirectly influ-

ence clonotype representation within the T cell compartment. Future studies will be directed towards understanding this process of self learning.

## Summary

Experiments presented in this report demonstrate that specificity of the Lyl$^+$ T cell proliferative response to NP-modified Ig is controlled by Igh-C–linked genes. In addition, we describe the mechanism whereby Igh-C–encoded molecules influence Lyl$^+$ T cell activity. We show that Igh-C–linked control of T cell responses to NP-modified Ig is a secondary consequence of naturally acquired tolerance for self Ig. Unresponsiveness to self Ig is not due to a defect expressed functionally at the level of the antigen-presenting cell, nor is it associated with active suppression. These results suggest that tolerance for self Ig at the level of the Lyl$^+$ T cell is due to functional deletion of Lyl$^+$ T cell clones specific for self Ig. The possibility is considered that regulatory effects mediated by passively administered antibodies may in part be due to induction of Lyl$^+$ T cell tolerance for self Ig.

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