A SUBSET OF Ly-1 INDUCER T CELL CLONES ACTIVATES B CELL PROLIFERATION BUT DIRECTLY INHIBITS SUBSEQUENT IgG SECRETION

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Ly-1 inducer T cells play a critical role in the regulation of antibody synthesis. One subset of Ly-1 T inducer cells (Qa-1-') can activate B lymphocytes to secrete IgM (1). A second (Qa-1+) stimulates Ly-1,2+ T cells to differentiate into Ly-2+ T suppressor cells (1–3) that inactivate both subsets of inducer T cells, resulting in reduced levels of antibody formation and suppression.

Recent reports (4–7) have indicated that T cells can also inhibit antibody responses without induction of Ly-2 T suppressors. Heterogeneous or cloned Ly-1 T cells failed to function as T helper (Th) cells and inhibited primary IgM responses. This reflected direct inhibition of B cell maturation into IgM-secreting cells by the Ly-1:Qa-1+ fraction of inducer cells (4, 5). The nonhelper Ly-1 T cell induced B cell proliferation but inhibited primary IgM response to phosphorylcholine-ovalbumin (PC-OVA): suppression was I-A-restricted and required a hapten-carrier bridge between the B cell and the Ly-1 T cell clone (5). Inhibition of secondary responses with some nonhelper Ly-1+ T cells (6, 7) has also been observed. In this case, T cell clones prevent an antigen-specific, major histocompatibility complex (MHC)-restricted interaction between carrier-specific Th cells and B cells.

In the present study, we further analyzed the mechanism of inhibition of B cell responses in vitro, using Ly-1 T cell clones. We found that two Ly-1 T cell clones did not induce B cells to secrete antigen-specific IgG. Instead, these clones inhibited secretion of IgG antibody to T-dependent soluble antigens. Inactivation of the B cell occurred early and required cell-cell contact that is both I region restricted and antigen specific, resulting in an early proliferative response by antigen-specific memory B cells, without subsequent differentiation into antibody-secreting cells.

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Abbreviations used in this paper: ARS, arsonate, arsanyl; BCDF, B cell differentiation factor; BCGF, B cell growth factor; BSA, bovine serum albumin; Con A, concanavalin A; DME, Dulbecco's modified Eagle's medium; DNP, dinitrophenyl; FCS, fetal calf serum; IL-2, interleukin 2; IL-3, interleukin 3; KLH, keyhole limpet hemocyanin; LPS, lipopolysaccharide; MHC, major histocompatibility complex; OVA, ovalbumin; PC, phosphorylcholine; PFC, plaque-forming cell(s); SRBC, sheep red blood cell(s); Th, T helper; TNP, trinitrophenyl.
Materials and Methods

Animals

Female BALB/cByJ, A/J, DBA/2J, B10.A/SgSnJ, B10.D2nSnJ, (BALB/c x A/J)F1 (CAF1), and (BALB/c x DBA/2)F1 (CD2F1) mice were obtained from The Jackson Laboratory, Bar Harbor, ME. B10.GD mice were kindly provided by Dr. Martin Dorf (Harvard Medical School, Boston, MA) and (B10.A x B10.D2)F1, mice were bred in our colony at the Dana-Farber Cancer Institute. All mice were between the age of 2–6 mo.

Antigens

Dinitrophenylated (DNP) proteins were prepared using conditions favoring a low coupling ratio: DNP-OVA and DNP-KLH (Keyhole limpet hemocyanin) (per 100 kilodaltons) (8). Arsonylated (ARS) proteins were prepared as described previously (9); haptenation ratios (moles [Ars-Tyr + Ars-His]/mole protein) were estimated using an extinction coefficient of 8.15 mM⁻¹ in 0.1 N NaOH at the isobestic point of 350 nm (9, 10). The haptenation ratios of the antigens used were ARS-OVA, DNP-OVA ARS₄, and ARS-KLH (per 100 kD).

Immunization

Mice were primed to DNP-KLH, DNP-OVA, or ARS-KLH by intraperitoneal injection of 10 μg of antigen and 4 mg of alum. 3–4 wk later the mice were boosted with 10 μg of antigen in saline and used at 1–12 wk after the second immunization.

Antisera

Murine monoclonal anti-Thy-1.2 was kindly donated by Dr. Ed Clark (University of Washington, Seattle, WA). Murine monoclonal anti-Ly-1.2 and -Ly-2.2 were kindly donated by Dr. F. W. Shen (Memorial Sloan-Kettering Cancer Center). Rat monoclonal anti-Ly-1, anti-Ly-2, and anti-Thy-1 antibodies were purchased from Becton, Dickinson & Co. (Mountain View, CA). Antibodies from hybridomas 14-4-4S (I-Ed'k'PX:Ia.7) and MK-D6 (I-Ad'q:Ia.16) were purified from culture supernatants using protein A-Sepharose or DEAE cellulose. Purified monoclonal antibody H7-8.26 (7A) [I-Ekk'k'k':Ia.7(2)] was obtained from Dr. Chella David (Mayo Medical School, Rochester, MN).

Derivation of Ly-1 T Cell Clones

Ly-1-O3. After BALB/cByJ mice were injected with 50 μg of OVA in complete Freund’s adjuvant H37RA (Difco Laboratories, Inc., Detroit, MI) in the hind footpads and tail base; clones were derived as described previously (10). Briefly, wells were seeded at 1 and 0.3 cells/well, expanded, and tested for OVA specificity by a proliferation assay. OVA-specific T cell lines were recloned and expanded from wells seeded at 0.3 cells/well. The cloned T cell line has been maintained as follows: 2 x 10⁵ Ly-1-03 are stimulated, every 5 wk, with adherent cells from 5 x 10⁵ irradiated (2,500 rad) syngeneic BALB/cByJ spleen cells pulsed with 1.0 μg/ml OVA (see below), followed by expansion in Dulbecco’s modified Eagle’s medium (DME) containing 10% fetal calf serum (FCS), 20% interleukin 1 (IL-1), and 1% IL-2.

Ly-1-Ar-5. The derivation and maintenance of the ARS-specific T cell line Ly-1-Ar-5 has been described previously (10). Briefly, spleens were removed 18 d after intraperitoneal immunization of CAF1 mice with 100 μg ARS-KLH in complete Freund’s adjuvant. The spleen cell cultures were enriched for ARS-reactive T cells by stimulating weekly with 50 μg/ml ARS-BGG (bovine gamma globulin) and irradiated CAF1 spleen cells in complete medium. At 4 wk the cultures were cloned as described above.

Both T cell clones expressed surface glycoproteins Thy-1, Ly-1, but not Ly-2 when assayed by fluorescence staining with antibody. In all assays, the T cell clones were used at least 2 wk after activation with antigen-pulsed adherent cells.

Antigen-specific Proliferation Assay

Cloned Ly-1 T cells were added to microwells (3040; Falcon Labware, Oxnard CA) containing irradiated syngeneic BALB/cByJ spleen cells in complete DME with 5% FCS
and the indicated antigen. \[^{3}\text{H}]\text{Thymidine incorporation was assayed on day 2 by pulse labeling cells at 24 h with 1 \mu\text{Ci} \[^{3}\text{H}]\text{thymidine (New England Nuclear, Boston, MA) and culturing for an additional 18 h. Results are expressed as mean counts per minute (cpm) of triplicate cultures.}

\textit{Preparation of Enriched B or Ly-1 Cells}

B cells plus accessory cells were purified by adherence of whole spleen cells \((3 \times 10^7)\) to petri dishes \((1029; \text{Falcon Labware})\) coated with goat anti-mouse IgM \((11)\). Nonadherent cells were removed by several washes in Cantor’s balanced salts \((\text{Irvine Scientific, Santa Ana, CA})\) containing 0.1% bovine serum albumin \((\text{BSA})\). The adherent cells were dissociated from the dishes by vigorous pipetting. To eliminate contaminating T cells, the eluted cells were incubated with monoclonal anti-Thy-1.2, anti-Ly-1.2, and anti-Ly-2.2 antibodies, plus rabbit complement, for 45 min at 37°C. To prepare Ly-1-enriched cells, the nonadherent cells from the goat anti-mouse IgM-coated petri dishes were incubated on rat anti-Ly-1-coated petri dishes \((12)\), eluted, and treated with monoclonal anti-Ly-2.2 and rabbit complement. Populations of both enriched B cells \((92\% \text{ Ig}^+\)) and Ly-1 cells \((93\% \text{ Ly-1}^+\)) still contained accessory cells.

\textit{Purified B Cells}

B cells plus accessory cells (purification described above) were depleted of adherent cells by passage over Sephadex G10 columns \((13)\).

\textit{Adherent and Antigen-pulsed Adherent Cells}

To prepare adherent, antigen-presenting cells, \(2.5 \times 10^9\) nonimmune whole spleen cells were incubated for 60–90 min in microtiter wells \((3040; \text{Falcon Labware})\) in complete DME medium containing 10% FCS at 37°C. To prepare antigen-pulsed adherent cells, antigen was included during the 60–90 min incubation period. To deplete cultures of nonadherent cells, the microtiter wells were washed by vigorous pipetting with fresh medium at least five times.

\textit{Miniature Mishell-Dutton Cultures}

Immune spleen cells \((10^6)\) or B cells \((6 \times 10^6)\) with or without immune Ly-1 T cells \((2.5 \times 10^5)\) were cultured in microtiter wells \((3040; \text{Falcon Labware})\) in complete DME supplemented with 10% FCS, 100 U/ml penicillin, 100 \mu g/ml streptomycin, 0.3% Funigzone \((\text{Gibco Laboratories, Grand Island, NY})\) \((14)\). A pool of triplicate cultures was assayed for hemolytic plaque-forming cells \((\text{PFC})\) by modification of the Jerne plaque technique \((15)\), using trinitrophylated sheep red blood cells \((\text{TNP-SRBC})\) \((16)\) or arsanylated SRBC \((\text{ARS-SRBC})\) \((17)\). In response to DNP-proteins, the PFC assay detects antibody that is crossreactive with TNP. To develop total PFC, rabbit anti-mouse Fc of IgG was added to the complement; IgG PFC were calculated as total PFC minus direct PFC \((\text{IgM})\) \((18)\). The avidity of the IgM or IgG PFC was determined by hapten inhibition \((19)\). In response to DNP-KLH or DNP-OVA, IgG anti-DNP PFC were 90–95% inhibited by \(10^{-6}\) M DNP-lysine.

\textit{Results}

\textit{Activation of Ly-1 T Cell Clones}

\textit{Antigen specificity.} The response of the OVA-specific clone Ly-1-03 to OVA was detectable at 0.01 \mu g/ml and maximal at 1 \mu g/ml. The clone was also activated by hapten conjugates of OVA such as DNP-OVA, ARS-OVA, or DNP-OVA-ARS, and maximal responses to the latter required 10-fold lower concentrations than to conjugated OVA. Clone Ly-1-03 recognized OVA and not hapten determinants on the conjugates, since DNP-KLH and ARS-KLH did not stimulate responses even at high concentrations \((\text{Fig. 1A and data not shown})\).
Antigen dose curve and specificity of the activation of the T cell clones. (A) To
5 × 10⁵ irradiated (2,500 rad) BALB/cByJ spleen cells, 10⁴ clone Ly-1-03 cells were added in
the presence of increasing concentrations of DNP-OVA (DO), ARS-OVA (AO), DNP-OVA-
ARS (DOA), OVA, or DNP-KLH (DK). (B) To 5 × 10⁶ irradiated CAF1 spleen cells, 10⁴ clone
Ar-5 cells were added in the presence of increasing concentrations of DNP-OVA-ARS, ARS-
KLH (AK), ARS-OVA, DNP-KLH, or DNP-OVA. [³H]Thymidine incorporation was assayed
on day 2, as described in Materials and Methods.

The ARS-specific clone, Ly-1-Ar-5, responded to ARS-OVA, ARS-KLH, and
DNP-OVA-ARS, and did not respond to DNP-KLH and DNP-OVA. [³H]-Thymidine incorporation was detectable after stimulation by 0.10 µg/ml ARS-
KLH and DNP-OVA-ARS or 1.0 µg/ml ARS-OVA; it was maximal at 1.0 µg/
ml ARS-KLH and DNP-OVA-ARS, and 10 µg/ml ARS-OVA (Fig. 1B).

Antigen-presenting cells. Clone Ly-1-03 responded to OVA presented by ir-
radiated whole spleen cells, purified B cells, and pulsed or unpulsed splenic
adherent cells (Fig. 2A) (20). At 1.0 µg/ml OVA, spleen cells were twice as
efficient as either purified B cells or adherent cells in presenting antigen; at
higher concentrations of antigen (e.g., 10 µg/ml), there was no difference
between the three cell populations (data not shown). Similarly, we found that all
of the above cell types can present arsanylated proteins to clone Ly-1-Ar-5.

Genetic restriction. The response to OVA by clone Ly-1-03 required recogni-
tion of I-A(d), B10.D2 and B10.GD but not B10.A spleen cells presented OVA to
close Ly-1-03 (Fig. 2B). The resulting proliferative response was inhibited only
by monoclonal anti-I-A(d) (MK-D6) and not by anti-I-E(d) [H7-8.26(7A), 14-4-4S]
(not shown). Clone 03 also responded to OVA presented by F1 strains like
(BALB/c × A/J)F1 (CAF1) and (B10.A × B10.D2) (Fig. 2B). Similarly, the
CAF1-derived clone Ly-1-Ar-5 responds to arsanylated protein in association
with I-A(d) on antigen-presenting cells from CAF1, parent BALB/c, but not A/J
(10, 21).

Both T cell clones produce factors typical of Ly-1+ cells. Both T cell clones are
Thy-1+, Ly-1+, Ly-2- and, after stimulation with antigen-pulsed adherent cells,
produce several proliferation and differentiation factors: IL-2, IL-3, and B cell
growth factors, BCGF-1 and BCGF-2, (data not shown; and 22, 23). Ly-1-03
produces B cell differentiation factors (BCDF) required for IgM anti-SRBC response, anti-\( \mu \) polyclonal stimulation of IgM reverse plaques, and the induction of \( \kappa \) light chain synthesis in the pre-B cell line 70Z/3 (Ly-1-03) (data not shown; and 24–26). Ly-1-Ar-5 produces macrophage-activating factor (10).

**Ly-1 T Cell Clones Specifically Inhibit Ig Secretion of B Cells**

Despite their production of B cell growth factors and B cell differentiation factors, clones Ly-1-03 and Ar-5 do not induce B cells to secrete antibody to protein antigens: Ly-1-03 did not stimulate primary or secondary DNP-specific B cells to secrete Ig in cultures activated with DNP-OVA. Clone Ly-1-Ar-5 did not stimulate primary or secondary DNP- or ARS-specific B cells to secrete Ig to either hapten in cultures activated by DNP-OVA-ARS or ARS-OVA, respectively. Varying the activation state of both the immune B cells and the Ly-1 T cell clones, and varying the time of addition of Ly-1 T cells did not induce helper function in these Ly-1 T cell clones. The addition of second signals: lipopolysaccharide (LPS) (27), virgin or immune T cells (28), lymphokine-containing supernatants from concanavalin A (Con A)-stimulated spleen cells (29), or antigen- or Con A-stimulated T cell clones (30), to cultures containing immune B cells plus Ly-1 T cells, failed to induce antigen-specific Ig secretion (data not shown).

Therefore, we tested the possibility that these Ly-1 cloned T cells inhibited differentiation of antibody-secreting B cells. Addition of \( 10^4 \) Ly-1-03 cells to DNP-OVA-immune spleen cells incubated with DNP-OVA completely inhibited the secondary IgG anti-DNP PFC response. Inhibition was antigen specific, since Ly-1-03 did not significantly affect the IgG PFC response of DNP-KLH-primed spleen cells in DNP-KLH-stimulated cultures (Table I). Similarly, the ARS-specific clone Ar-5 inhibited secondary IgG anti-ARS PFC response in ARS-
TABLE I
Inhibition of IgG PFC Response by Ly-1 T Cell Clones Is Antigen Specific

<table>
<thead>
<tr>
<th>Group</th>
<th>Anti-hapten IgG PFC</th>
<th>Percent inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNP-OVA</td>
<td>3,791</td>
<td></td>
</tr>
<tr>
<td>DNP-OVA + Ly-1-03</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>DNP-KLH</td>
<td>3,248</td>
<td></td>
</tr>
<tr>
<td>DNP-KLH + Ly-1-03</td>
<td>3,106</td>
<td>4</td>
</tr>
<tr>
<td>ARS-KLH</td>
<td>1,245</td>
<td></td>
</tr>
<tr>
<td>ARS-KLH + Ly-1-Ar-5</td>
<td>300</td>
<td>76</td>
</tr>
<tr>
<td>DNP-KLH</td>
<td>1,178</td>
<td></td>
</tr>
<tr>
<td>DNP-KLH + Ly-1-Ar-5</td>
<td>1,238</td>
<td>0 (+5)</td>
</tr>
</tbody>
</table>

10^4 irradiated clone 03 or Ar-5 cells were added to 10^6 spleen cells from immune mice and the cultures were stimulated with the immunizing antigen as indicated. IgG anti-hapten (DNP or ARS) PFC response was quantitated on day 5.

Figure 3. Cell number and antigen concentration required for inhibition. (A) Increasing numbers of clone Ly-1-03 T cells, either nonirradiated (—) or irradiated (2,500 rad) (— — —), were added to cultures of 10^6 DNP-OVA immune BALB/cByJ spleen cells restimulated with 0.01 μg/ml of DNP-OVA. (B) In the same experiment, 10^6 DNP-OVA-immune spleen cells were restimulated with increasing concentrations of DNP-OVA, in the presence (●—●) or absence of (○—○) 10^4 irradiated Ly-1-03. Both sets of cultures were assayed for IgG anti-DNP PFC response on day 6.

KLH-stimulated cultures, and did not inhibit IgG anti-DNP PFC response in DNP-KLH-stimulated cultures (Table I).

Conditions for inhibition. To determine the number of inducer cells required for inhibition, increasing numbers of Ly-1-03 (10 to 10^4 cells) were added to cultures stimulated with 0.01 μg/ml DNP-OVA. Significant inhibition was seen with as few as 10-100 Ly-1-03 cells; maximal inhibition (95-100%) occurred when 5 × 10^3 to 10^4 cells were added. Irradiation of the T cell clone slightly decreased its ability to inhibit the PFC response to DNP-OVA (Fig. 3A).

DNP-OVA-immune spleen cell cultures were stimulated in vitro with increasing concentrations of DNP-OVA, from 0.001 to 10 μg/ml. The addition of 10^4
irradiated (Fig. 3B) or nonirradiated (data not shown) clone Ly-1-03 cells caused 75–100% inhibition of the IgG anti-DNP response at all concentrations tested. Inhibition was maximal (92%) at 0.001 μg/ml DNP-OVA, 100-fold less than that required to induce optimal DNA synthesis (Figs. 3B and 1A).

**Cellular Mechanisms of Inhibition**

*Ly-2 T suppressors are not required for inhibition.* Previous analysis (1–3) of Ly-1 induction of suppression showed that these cells activate Ly-2 T suppressors in vitro which, in turn, inactivate Ly-1 T helper activity. We therefore tested whether inhibition by clones 03 and Ar-5 required antigen-specific Ly-2 T suppressors.

B cells from DNP-immune donors were incubated with OVA-specific Ly-1 Th cells, and DNP-OVA or DNP-OVA-ARS. When Ly-1-03 was added to these cultures, the IgG PFC response was completely inhibited (Fig. 4 and data not shown). The ARS-specific Ly-1 T cell clone Ar-5 slightly enhanced the IgG anti-DNP PFC response in B plus Ly-1 cultures stimulated with DNP-OVA, while completely inhibiting PFC responses in cultures stimulated with DNP-OVA-ARS (not shown). Since these cultures did not contain detectable numbers of Ly-2 T cells, we conclude that the induction of Ly-2 T suppressors is not the predominant mechanism of inhibition by these two Ly-1 T cell clones.

*Direct inactivation of B cells.* There are at least two remaining mechanisms by which an Ly-1 T cell clone can inhibit IgG responses: the clone may directly inactivate B cells or functionally delete Ly-1 Th cells. To differentiate between these two mechanisms we used antigens that had activating determinants both for the clones (i.e., OVA or ARS) and for either the DNP-specific B cells or the appropriate carrier-specific Th cells also present in the cultures. This tests the probable cellular target of inhibition, by focusing the Ly-1 T cell clones onto either B cells or Th cells, respectively.

The relative role of the above mechanisms was analyzed in the T cell-

![Figure 4](https://www.jem.org/article/friedman-et-al/article-fig4.png)

**Figure 4.** Inhibition of B plus Ly-1 cultures by Ly-1-03 in response to DNP-OVA. Increasing numbers of irradiated cloned T cells 03 (○) and Ar-5 (□) were added to cultures containing purified B cells, Ly-1 cells, accessory cells, and 0.01 μg/ml DNP-OVA. IgG anti-DNP PFC were assayed on day 6.
dependent and MHC-restricted IgG PFC response to DNP-KLH in BALB/c and CAF1 mice. The IgG response was MHC restricted rather than dependent on nonspecific factors. The PFC response was inhibited by anti-class II (I-A, I-E) antibodies, and supernatants from Con A-stimulated cells did not induce significant IgG secretion by purified DNP-KLH-immune B cells (29). To test whether inhibition was effective when the Ly-1 clone was focused onto B cells, we compared the ability of Ly-1-03 to inhibit DNP-KLH responses in the presence of DNP-OVA and OVA. These two antigens were almost equivalent in their ability to activate the clone (Fig. 1A), but only DNP-OVA was capable of also binding to DNP-specific B cells from DNP-KLH-immune mice.

DNP-KLH-immune spleen cells were stimulated with increasing concentrations of OVA or DNP-OVA (0.001–10 μg/ml) in the presence or absence of 10^4 Ly-1-03 T cells. The IgG anti-DNP response was 75% inhibited in cultures containing 0.001 μg/ml DNP-OVA plus 10^4 Ly-1-03, and completely inhibited by 0.01–0.1 μg/ml DNP-OVA plus 10^4 Ly-1-03. In contrast, inhibition in cultures containing OVA was significant (86%) only at 1 μg/ml (Fig. 5A). Thus, although DNP-OVA was only 10-fold more efficient than OVA in activation of Ly-1-03 (0.1 vs. 1.0 μg/ml, respectively; Fig. 1A), DNP-OVA was 1,000-fold more efficient than OVA in inhibiting IgG anti-DNP responses (Fig. 5A).

This result suggests that inhibition by clone Ly-1-03, like help mediated by
Ly-1 T cells, is most effective if the clone makes contact with B cells through an antigen bridge. Again, as in Th and B cell interactions, the antigen bridge can be mediated by antigen-pulsed adherent cells (31). Spleen cells (depleted of adherent cells) from DNP-KLH-immune BALB/c mice were added to cultures containing adherent cells from nonimmune BALB/c mice pulsed with increasing concentrations of DNP-OVA. The cultures that contained adherent cells pulsed with 0.10 μg/ml DNP-OVA and 10⁴ Ly-1-03 were 99% inhibited. The inhibition was equivalent to cultures that had soluble DNP-OVA and Ly-1-03 added in the beginning of the culture period (Fig. 5B).

**Ly-1 T cell clone and Ly-1 Th interaction.** In addition to the direct inactivation of B cells described above, it was also possible that the Ly-1 T cell clones could interact with and functionally inactivate the Ly-1 Th cells required for B cell maturation (32). This possibility was directly tested: Ly-1-Ar-5 cells were added to cultures containing DNP-KLH-immune spleen cells, and were stimulated with DNP-KLH as well as either DNP-OVA-ARS, to focus the clone onto B cells (controls DNP-OVA and ARS-OVA), or ARS-KLH (control ARS-OVA), to focus the clone onto carrier-specific Th cells.

Clone Ly-1-Ar-5 behaved similarly to clone Ly-1-03, in that B cell-directed antigens were more effective at inhibiting a DNP-KLH response. DNP-OVA-ARS elicited 50% inhibition at 0.001 μg/ml and 95% inhibition at 0.01-0.10 μg/ml, in cultures containing 10⁴ Ly-1-Ar-5 (Fig. 6A). The non-B-cell-directed antigen control, ARS-OVA, was 1,000-fold less effective, since it elicited only 50% inhibition at 1 μg/ml (Fig. 6B).

To focus Ly-1-Ar-5 T cells onto KLH-specific Ly-1 Th cells, increasing

![Figure 6](https://jem.rupress.org/content/jem/79/4/793/f6.large.jpg)

**Figure 6.** The interaction of clone Ar-5 with B cells, but not with Ly-1 Th cells, inhibits IgG secretion. (A) 10⁶ CAF₁ immune spleen cells were stimulated with 0.10 μg/ml DNP-KLH in the presence or absence of 10⁴ clone Ly-1-Ar-5 cells and increasing concentrations of DNP-OVA or DNP-OVA-ARS. (B) 10⁶ immune CAF₁ spleen cells, treated twice with anti-Ly-2, were activated with 0.10 μg/ml DNP-KLH. To the cultures were added increasing concentrations of DNP-OVA-ARS, ARS-OVA, or ARS-KLH, with 10⁴ Lys-1-Ar-5. IgG anti-DNP PFC were assayed on day 6. The addition of antigens alone had no effect on the anti-DNP PFC response (not shown).
concentrations of ARS-KLH were added to anti-Ly-2.2-treated DNP-KLH-immune spleen cells. If one component of inhibition requires an antigen-specific interaction between the Ly-1 T cell clone Ar-5 and KLH-specific Th cells, then ARS-KLH should be comparatively more efficient at eliciting inhibition than ARS-OVA. However, we detected no difference between ARS-KLH and ARS-OVA, even though cultures contained KLH-specific Th cells. PFC responses in cultures containing ARS-KLH and ARS-OVA were reduced 50% at 1 μg/ml, in the presence of 10⁴ clone Ly-1-Ar-5 cells (Fig. 6B). In the same experiment, the antigen DNP-OVA-ARS, which focuses the clone onto DNP-specific B cells, was 1000-fold more efficient than either ARS-KLH or ARS-OVA, as described above (Fig. 6B). The more potent inhibition by DNP-OVA-ARS vs. ARS-KLH or ARS-OVA cannot be explained by differential activation of the clone, since dose response curves for all the antigens are very similar (Fig. 1B). We conclude that Ly-1-Ar-5, like clone Ly-1-03, may inhibit by directly inactivating B cells, and that inactivation of KLH-specific Th cells by clone Ly-1-Ar-5 is unlikely to be the predominant mechanism of inhibition.

Antigen-specific Interaction Between the Cloned T Cells and B Cells Is I-A<sup>d</sup> Restricted

Next we determined the genetic restriction of the antigen-specific interaction between the cloned T cells and B cells. 10⁴ irradiated Ly-1-03 T cells were added with DNP-OVA to DNP-KLH-stimulated immune spleen cells of the relevant strains of mice. When necessary, the clone was activated with the appropriate (BALB/c × A/J)F<sub>1</sub> or (B10.A × B10.D2)F<sub>1</sub> antigen-presenting adherent cells. The clone (which is I-A<sup>d</sup> restricted) inhibited IgG anti-DNP responses of BALB/c, B10.D2, and B10.GD immune spleen cells, but did not inhibit IgG anti-DNP responses of A/J or B10.A immune spleen cells. We conclude that the inhibition of B cell secretion by Ly-1-03 is I-A<sup>d</sup> restricted (Table II). Similarly, we have shown that the inhibition of B cell secretion by the hapten-specific T cell clone Ar-5 is I-A<sup>d</sup> restricted (data not shown).

It is noteworthy that recognition of one parental MHC molecule (I-A<sup>d</sup>) by both the BALB/c-derived clone Ly-1-03 and the CAF<sub>1</sub>-derived clone Ar-5 was sufficient to inhibit the entire response of CAF<sub>1</sub> mice, provided that the appropriate B cell-directed antigens (DNP-OVA or DNP-OVA-ARS for clone 03, DNP-OVA-ARS for clone Ar-5) were also present in the cultures (data not shown and Fig. 6A). We have determined by inhibition with specific anti-Ia antibodies that the DNP-KLH-immune spleen cells from CAF<sub>1</sub> mice contain similar levels of helper activity for B cells bearing any of the four I region-coded molecules: I-A<sup>k</sup>, I-E<sup>k</sup>, I-A<sup>d</sup>, and I-E<sup>d</sup> (data not shown).

Unlike the T-T interaction that generates suppression (3), interaction between the cloned T cells and B cells is not Ig-H restricted; the addition of either T cell clone inhibited IgG anti-DNP PFC in both BALB/c allotype-congenic strains, C.AL/20 (Ig-I<sup>a</sup>) and C.B20 (Ig-I<sup>b</sup>) (data not shown).

Kinetics of Inhibition

To test if the clone inhibits the early phase of the PFC response, Ly-1-03 was added at time zero with DNP-OVA to DNP-KLH-primed immune spleen cells
TABLE II

Interaction Between Ly-1-03 and B Cells Is I-A<sup>d</sup> Restricted

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Genetic restriction</th>
<th>IgG anti-DNP PFC/culture of DNP-KLH-immune spleen cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ly-1-03</td>
<td>BALB/cByJ*</td>
</tr>
<tr>
<td></td>
<td>DNP-OVA</td>
<td>A/J*</td>
</tr>
<tr>
<td></td>
<td>DNP-OVA + Ly-1-03</td>
<td>971</td>
</tr>
<tr>
<td></td>
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<td>2</td>
<td>H-2&lt;sup&gt;d&lt;/sup&gt;</td>
<td>B10.D2nSnJ&lt;sup&gt;δ&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Ly-1-03</td>
<td>826</td>
</tr>
<tr>
<td></td>
<td>DNP-OVA</td>
<td>1,762</td>
</tr>
<tr>
<td></td>
<td>DNP-OVA + Ly-1-03</td>
<td>667</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1,428</td>
</tr>
<tr>
<td>3</td>
<td>I-A&lt;sup&gt;d&lt;/sup&gt;</td>
<td>BALB/cByJ</td>
</tr>
<tr>
<td></td>
<td>Ly-1-03</td>
<td>B10.GD</td>
</tr>
<tr>
<td></td>
<td>DNP-OVA</td>
<td>2,256</td>
</tr>
<tr>
<td></td>
<td>DNP-OVA + Ly-1-03</td>
<td>2,616</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2,400</td>
</tr>
</tbody>
</table>

In experiments 1 and 2, DNP-KLH-immune spleen cells of BALB/c, A/J, B10.D2, and B10.A were depleted of most adherent cells by two 90-min incubations of 5 x 10<sup>6</sup> cells/ml on petri dishes. The nonadherent immune spleen cells (10<sup>6</sup>) were added to microwells containing adherent cells from 2.5 x 10<sup>5</sup> (BALB/c x A/J)<sub>F1</sub> or (B10.D2 x B10.A)<sub>F1</sub> nonimmune spleen cells. In experiment 3, the cultures contained 10<sup>6</sup> immune spleen cells of strain BALB/c or B10.GD. In all three experiments, the cultures contained 0.10 µg/ml DNP-KLH, 0.01 µg/ml DNP-OVA, and 10<sup>4</sup> irradiated Ly-1-03. IgG anti-DNP PFC were assayed on day 6.

* Plus (BALB/c x A/J)<sub>F1</sub> adherent cells.

† Plus (B10.D2 x B10.A)<sub>F1</sub> adherent cells.

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* Plus (BALB/c x A/J)<sub>F1</sub> adherent cells.

† Plus (B10.D2 x B10.A)<sub>F1</sub> adherent cells.

FIGURE 7. The kinetics of inhibition of IgM and IgG PFC responses. Cultures contained 10<sup>6</sup> immune spleen cells, 0.10 µg/ml DNP-KLH, 0.01 µg/ml DNP-OVA, and 10<sup>4</sup> Ly-1-03. The groups are DNP-KLH + Ly-1-03 (O), DNP-KLH + DNP-OVA (O), and DNP-KLH + DNP-OVA + Ly-1-03 (O). IgM and IgG PFC anti-DNP were assayed daily from day 3 to 6.

and the cultures were assayed for IgM and IgG anti-DNP PFC daily from day 3 to day 6. IgM antibody synthesis was detected from day 3 to 5, and was 60–75% inhibited in the presence of both DNP-OVA and clone Ly-1-03, (Fig. 7A).
Significant number of IgG PFC were detected beginning on day 4; complete inhibition (95–100%) was seen in the presence of both DNP-OVA and clone Ly-1-03 on all days tested (days 3 to 6, Fig. 7B).

We also tested whether the time of addition of the clone was important for inhibition. We added $10^4$ irradiated Ly-1-03 at 0, 24, 48, and 72 h to separate cultures of immune spleen cells stimulated with DNP-KLH or DNP-OVA. When the clone was added at time 0, IgG PFC responses of DNP-OVA- but not DNP-KLH-stimulated spleen cells were completely inhibited, as tested at days 4–6. The clone inhibited 95% of PFC response when added 24 h after initiation of culture, but did not inhibit when added at later times (Fig. 8). These data indicate that the Ly-1–B interaction that results in B cell inactivation must occur early (within the first 24 h) of the response.

Short-term Incubation of B Cells with Clone Ly-1-03 Impairs their Ability to Differentiate Into IgG-secreting Plasma Cells

To test whether the clones inactivated B cells before differentiation into IgG-secreting plasma cells, purified B cells from DNP-KLH-immunized mice were incubated with antigens and Ly-1-03 as indicated in Table III. At 48 h, Th cells and cloned Ly-1-03 T cells were eliminated with anti-Thy-1.2, -Ly-1.2, -Ly-2.2, and rabbit complement. After extensive washing, the B cells were cultured with KLH-specific Ly-1 Th cells and DNP-KLH for an additional 5 d, before assaying for an anti-DNP PFC response. Purified B cells that were preincubated with DNP-OVA and Ly-1-03 were irreversibly inhibited from secreting DNP-specific Ig. The inhibition of secretion by DNP-OVA and Ly-1-03 is antigen specific and dose dependent; maximum inhibition (89–95%) occurred when B cells were cultured with 0.01–0.10 μg/ml DNP-OVA (Table III). Inactivation was not caused by cell lysis; on the contrary, Ly-1-03 delivered an early proliferative signal to the antigen-specific B memory cells. Hapten-purified or DNP-binding B cells were activated by Ly-1-03 T cells in the presence of the same low doses of DNP-OVA (data not shown).
TABLE III
*T Cell Clone Ly-1-03 Irreversibly Inhibits B Cell Secretion*

<table>
<thead>
<tr>
<th>Incubation of B cells for 1st 48 h</th>
<th>Total anti-DNP PFC/culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium</td>
<td>816</td>
</tr>
<tr>
<td>Medium + Ly-1-03 (10^6)</td>
<td>624</td>
</tr>
<tr>
<td>DNP-KLH, 0.10 µg/ml</td>
<td>576</td>
</tr>
<tr>
<td>DNP-KLH + Ly-1-03</td>
<td>1260</td>
</tr>
<tr>
<td>DNP-OVA, 0.001 µg/ml</td>
<td>864</td>
</tr>
<tr>
<td>DNP-OVA + Ly-1-03</td>
<td>348</td>
</tr>
<tr>
<td>DNP-OVA, 0.01 µg/ml</td>
<td>648</td>
</tr>
<tr>
<td>DNP-OVA + Ly-1-03</td>
<td>72</td>
</tr>
<tr>
<td>DNP-OVA, 0.10 µg/ml</td>
<td>816</td>
</tr>
<tr>
<td>DNP-OVA + Ly-1-03</td>
<td>48</td>
</tr>
</tbody>
</table>

B plus accessory cells from DNP-KLH-immune mice were incubated at time 0 with DNP-KLH, DNP-OVA, and 10^6 irradiated Ly-1-03, as indicated. At 48 h, the cultures were treated with anti-Thy-1.2 and anti-Ly-1.2 plus rabbit complement to remove the cloned T cells. On day 2, 2.5 × 10^5 purified KLH-immune Ly-1 spleen cells were added with 0.01 µg/ml DNP-KLH to the precultured B plus accessory cells; 5 d later cultures were plaqued for total anti-DNP responses.

Co-recognition of an MHC (I-E) and a Non-MHC Molecule on B Cells by Ly-1-03 Inhibits Secretion of Anti-DNP Ig

The Ly-1-03 T cell clone was also activated by I-E^d and a non-MHC-encoded antigen (X) present on DBA/2 B cells but not on (BALB/cByJ × DBA/2)F_1 B cells (Friedman, S., D. Sillcocks, and H. Cantor, manuscript in preparation). The non-MHC-encoded molecule is a B cell-specific surface antigen not found on adherent cells (Fig. 9A). Parenthetically, stimulation by I-E and the B cell membrane determinant was less sensitive to irradiation than B cell presentation of soluble antigen. (Figs. 9A and 2A; 2,500 vs. 1,000 rad). The recognition of I-E^d + X on DBA/2 B cells resulted in proliferation (Fig. 9A) and production of factors that induce proliferation of DBA/2 B cells (Fig. 9B), but did not induce polyclonal Ig secretion (data not shown). As few as 10^3 Ly-1-03 inhibited IgG anti-DNP secretion by DBA/2 B cells to DNP-KLH (Fig. 9C), and 10^4 cells completely suppressed the response. The efficiency of suppression was unchanged despite the absence of an antigen bridge to focus Ly-1-03 cells onto the correct (DNP specific) B memory cell. Finally, (BALB/c × DBA/2)F_1 B cells, which do not express X and therefore do not activate Ly-1-03, were not inhibited to secrete IgG in the presence of Ly-1-03.

Discussion

Inhibition by Ly-1 T Cell Clones. These data provide the first direct evidence that at least some clones of inducer cells directly inhibit antibody secretion by B memory cells. The inhibitory function of these Ly-1 T cell clones has been stable.
B CELL-LY-1 T CELL INTERACTION INHIBITS IgG RESPONSES

Figure 9. Though allo B cells activate Ly-1-03 and respond to growth factors produced by Ly-1-03, the B cells are inhibited to differentiate into IgG PFC by Ly-1-03. (A) B cells and not adherent cells purified from normal DBA/2 spleen cells activate Ly-1-03 to proliferate. Purified B cells were prepared from normal DBA/2 spleen cells as described in Materials and Methods. Adherent cells were prepared by incubation of normal DBA/2 spleen cells for 60 min at 37°C. 2.5 × 10^4 Ly-1-03 were added to increasing number of irradiated (2,500 rad) B cells (Δ) and adherent cells (Ο). (B) Activated Ly-1-03 induces the proliferation of DBA/2 B cells. 10^4 irradiated Ly-1-03 were activated with 5 × 10^5 irradiated DBA/2 spleen cells, and increasing number of purified splenic DBA/2 B cells (△) were added. [3H]thymidine incorporation was assayed on day 2. (C) Ly-1-03 inhibits immune stimulated DBA/2 spleen cells from secreting IgG PFC anti-DNP in response to DNP-KLH. 10^6 immune spleen cells from DBA/2J (♂) and (BALB/cByJ × DBA/2J)F1 (♀) mice were stimulated with 0.10 μg/ml DNP-KLH and an increasing number of nonirradiated (——) or irradiated (-----) clone Ly-1-03 cells were added at time 0. IgG anti-DNP PFC were assayed on day 6.

over a 3 yr test period. The inducer cell must be focused onto the B cell by either a soluble antigen bridge or a B cell surface antigen, and requires recognition of B cell Ia (Tables I and II and Fig. 9 C). Within 48 h or less, incubation of B cells with Ly-1 T cell clones and a bridging antigen inhibited B cells from receiving helper signals from immune Ly-1 Th cells (Table III). Inhibition of IgG synthesis or secretion does not require that the Ly-1 T cell clones interact via antigen with antigen-specific Ig on the surface of B cells: Ly-1-03 inhibited allogeneic DBA/2 B cells from responding to DNP-KLH by interacting with non-Ig B cell surface molecules (Fig. 9 C). Inhibition of the secondary antibody responses in vitro does not require induction of T suppressors. Removal of Ly-2^+ cells from the cultures by antibody and complement had no effect on inhibition (Fig. 4). Inhibition was not influenced by Ig-H-linked genes (data not shown), unlike the induction of Ly-2^+ suppressors (3, 33).

Inhibition was seen at concentrations of antigen that do not induce proliferation of the T cell clone (compare Fig. 1 with 5 and 6). Irradiation had little effect on their suppressor function (Figs. 3 and 9 C), unlike Ly1:Qa^+ cells that paralyze B cells in the primary response to SRBC (4).

There has been only one report of suppression of IgG antibody responses to soluble T-dependent antigens in vitro by Ly-1 T cell clones (6). Asano and Hodes (6) postulated that the Ly-1 T cell clone acts by interfering with the MHC-restricted interaction between Ly-1 T helpers (in a heterogeneous population) and B cells. Thus, if the clone recognizes one MHC-encoded molecule on F1 B cells, only 25–50% of helper activity (restricted to that Ia molecule) would be inhibited. This is not the case: Clones restricted to I-A^d completely inhibit the IgG PFC response to DNP-KLH by (BALB/cJ × A/J)F1 spleen cells, although DNP-KLH was presented on I-A^d, I-E^d, I-A^k, and I-E^k-encoded molecules (Fig.
Moreover, we failed to detect inhibition between cloned T cells and Ly-1 Th cells in vitro, even when we attempted to link these two cells by an antigen bridge (Fig. 6B). These data strongly suggest that the target cell is the B cell rather than Ly-1 Th cells and that the inhibition is not mediated by interference with the positive interaction between B cells and Th cells.

**Lack of Helper Function.** Although these Ly-1 T cell clones fail to induce antigen-specific IgG secretion, they have some properties of inducer Ly-1+ T cells, including production of BCGF-1, BCGF-2, and BCDF required for both primary anti-SRBC response and polyclonal stimulation of IgM secretion in anti-μ-stimulated spleen cell cultures. The failure to help may be due to (a) lack of a specific BCDF required for IgG secretion (34) and/or (b) inhibition of secretion of B cells. Since the DNP-KLH-immune spleen cells contain all the factors required for the differentiation of IgG anti-DNP PFC, the second possibility is more likely. ~50% of antigen-specific inducer clones analyzed in this laboratory induce IgM but not IgG responses to proteins. Several of our investigators have derived T cell clones that do not induce IgG and have inhibitory activity as described. The frequency of these inhibitory clones among our nonhelper Ly-1 T cell lines is to be determined.

**Characteristics of Inhibition.** Effective inhibition at low antigen concentrations (0.001–0.10 μg/ml) requires cell contact between the Ly-1 T cell clones and B cells. Supernatants derived from T cell clones activated by antigen-pulsed adherent cells, or the supernatants enriched for antigen-binding material by affinity chromatography on antigen-coupled Sepharose columns, did not specifically inhibit DNP-immune B cells. If a population of 10^6 DNP-immune spleen cells contains ~10^5 DNP-specific B cells, then anywhere from 1- to 10 Ly-1 cloned T cells per immune B cell is sufficient for inhibition. This is also true for inhibition of PFC responses of allogeneic (DBA/2) B cells. One would have expected a requirement for more cloned T cells in the latter case, since all nonimmune DBA/2 B cells would be expected to display determinants recognized by the cloned T cells, thus preventing efficient contact with DNP-specific B cells. Perhaps the DNP-KLH-activated B cells bind more avidly to Ly-1 T cell clones because they express higher concentrations of (I-E^d + X) on their surface (35).

The Ly-1 T cell clone may be brought into close contact with the B cells on the surface of antigen-presenting adherent cells (Fig. 5B) (20, 36), or via released vesicles and/or protein complexes containing Ia and antigen (37, 38). We favor direct interaction with B cells since recognition of I-E and a non-MHC surface component on B cells was sufficient for inhibition of the response (Fig. 9, A and C). Inhibition by the Ly-1 T cell clones is not restricted to the Lyb-5+ B cell subset (39); the responses in both (BALB/c × CBA/N)F1 normal (Lyb-5+) and Lyb-5− and (CBA/N × BALB/c)F1 X-linked immune-deficient (xid) (Lyb-5−) mice were equally inhibited (data not shown).

Both T cell clones inhibit IgG antibody responses to a greater extent than IgM responses. In >50 experiments, IgG-specific responses were inhibited by 80–100%, whereas IgM-specific responses were either inhibited, unaffected, or even enhanced. We feel that the data are best explained by the affinity differences between the two classes of antibody (IgG, K_a = 10^{-7} M; IgM, K_a ≥ 10^{-5} M) rather than by invoking isotype-restricted suppression (40). Thus, higher affinity
IgG antibodies would be more efficient in forming a B cell–Ly-1 cell bridge required for inhibition. It is also unlikely that the Ly-1 T cell clones caused the B cells to switch to a class of Ig not measured in the PFC assays (e.g., IgA); memory B cells committed to IgG synthesis apparently do not switch to another class of Ig (41, 42).

This inducer–B cell interaction occurs early in the immune response, before differentiation of B cells into Ig-secreting cells. First, as soon as IgG secretion was detected in control cultures, on days 3 or 4, IgG secretion was inhibited in cultures containing antigen-activated Ly-1 T cell clones (Fig. 7). Second, incubation of DNP-immune B cells with antigen and the Ly-1 T cell clone for 24–48 h prevented these B cells from receiving Th cell signals over the next 5 d (Fig. 8, Table III).

Mechanism of B Cell Suppression. T cell clones did not lyse or inhibit proliferation of antigen-specific B cells during the early phase of the response. Nor do these Ly-1 T cell clones lyse B cells, LPS blasts, or Ia-bearing tumor cells, when incubated with antigen (Fig. 9B and data not shown). A critical observation is that incubation of B cells with either clone resulted in antigen-dependent proliferation of antigen-specific B memory cells. Affinity-purified, DNP-specific B cells from DNP-KLH-immune spleen cells underwent a substantial proliferative response 24–48 h after incubation with Ly-1-03 in the presence of low concentrations of DNP-OVA; after stimulation with low doses of antigen, the clone does not produce nonspecific B cell proliferative responses (data not shown). Thus, Ly-1-03 induces an early proliferative response of DNP-specific B cells, but inhibits subsequent antibody secretion.

The negative signal imparted by the Ly-1 T cell clones in the presence of soluble antigens is unusual in that it inactivates adult B cells to conventional antigens like DNP-OVA or ARS-OVA. Tolerance of B cells usually requires contact with antigens that present a polyvalent matrix to B cells like DNP-SIII (pneumococcal polysaccharide) or unusual antigens like haptenated Ig vs. haptenated BSA (43–45). The gamma globulins are unique tolerogens in that they can impart negative signals to B cells via Fc receptor binding (46). We have used a conventional antigen (e.g., DNP-OVA) at concentrations (≤10^{-9} M) that are 100–1,000-fold less than usually required to inhibit adult B cells by equivalently haptenated Ig (45). The inactivating molecule is not the antigen-binding T cell receptor secreted by Ly-1 T cell clones. On the contrary, whereas antigen-binding material secreted by a TNP-specific Ly-1 T cell clone provides positive signals for B cell maturation and IgM secretion (47, 48), the same T cell clone inhibits the secretion of IgG when it makes an antigen-specific and I region–restricted contact with B cells (data not shown). If receptor blockade is the mechanism of inactivation by the clones, then the molecules causing the blockade are bound so tightly to the Ig receptor that they cannot be removed by extensive washing (Table III). If, instead, the T cell clones cause receptor modulation of B cells, then the T cell must present the antigen bound to its receptor in a bivalent or multivalent complex that leads to patching, capping, and endocytosis of membrane Ig receptor (43).

It is possible that the Ly-1 T cell clone releases factors (49) (other than antigen-binding factors) that impart a negative signal only to the B cell with which it has
previously made the antigen-specific and MHC-restricted contact. Whatever the negative signal may be, it cannot be neutralized by LPS, a polyclonal activator of B cells (data not shown; 50).

Relevance to the In Vivo Immune Response. These T cell clones can also inhibit secondary antibody responses in vivo. Secondary IgG anti-DNP responses of mice immunized with DNP-KLH were inhibited by simultaneous injection of clone Ly-1-03 and DNP-OVA, or to a lesser extent with OVA (Friedman, S., D. Sillcocks, and H. Cantor, manuscript in preparation). Ly-1 T cells with similar function to Ly-1-03 may regulate physiological antibody responses to DNP-OVA by one of two mechanisms. As the immune response progresses, more B cells with higher affinity antibody will be selected and thus more of these B cells may be inactivated by antigen-specific suppressor Ly-1 T cells. Alternatively, these Ly-1 T cells may expand a population of antigen-specific B cells to become memory B cells that are refractory to secretion until the next antigenic challenge.

Analysis of inducer T cell clones thus indicates that the set of Ly-1/OKT4+ T cells may include cells that differentially regulate B cell activation and maturation (51, 52). Further studies should help to clarify the role of each in the production of appropriate immune responses to foreign and autologous proteins.

Summary

We find that a fraction of Ly-1+2− inducer T cell clones inhibits differentiation of memory B cells into IgG-secreting plaque-forming cells. Inhibition of secondary antibody responses was not the result of induction of Ly-2+ T suppressors. Instead, inducer cells directly inactivated B cells, requiring an antigen bridge as well as identity at the major histocompatibility complex (I-A) locus. The interaction between the inducer T cell clone and hapten-specific B memory cells results in an early proliferative response and subsequent failure of B cells to secrete antibody in response to T helper cell signals. Possible mechanisms for this novel type of B cell inactivation are explored.

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