T LYMPHOCYTE-MEDIATED SUPPRESSION OF MYELOMA
FUNCTION IN VITRO

II. Evidence for Regulation of Hapten-binding Myelomas by
Syngeneic Hapten-specific Cytolytic T Lymphocytes*

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It is now well established that regulatory helper and suppressor T lymphocytes play a critical role in determining the magnitude and nature of a variety of immune responses. Different lines of experiments indicate that T cells recognize not only surface-bound antigens, but also idiotypic (1, 2) and allotypic (3) determinants on helper T cells and/or B lymphocytes. In addition, it is clear that the antigen-recognition function of several classes of T lymphocytes, including helper cells, cytolytic T lymphocytes (CTL)1, and the effector cells of delayed hypersensitivity (4), is restricted by the major histocompatibility gene complex (MHC). The MHC-restricted collaboration between antigen-specific helper T and B lymphocytes (5, 6) implies that antigen-specific T cells recognize receptor-bound antigen in some form of an association with MHC gene products, although it is difficult to demonstrate directly such an associative recognition mechanism in normal immune-cell populations.

The possibility that hapten-specific, MHC-restricted CTL (7) can function as regulators of anti-hapten immune responses has been raised by several investigators, although this has not been experimentally verified to date (8). Cytolytic T lymphocytes might specifically regulate humoral immune responses by recognizing receptor-bound antigen on B cells in association with self MHC determinants in a manner that may be analogous to the MHC-restricted interaction between antigen-specific helper T and B lymphocytes. Schmitt-Verhulst et al. (9) and Ballas and Henney (10) have shown that 2,4,6-trinitrophenyl (TNP)-protein conjugates that are noncovalently bound to target cell surfaces can be recognized by syngeneic TNP-specific CTL. This phenomenon, however, occurs only with cells from mice of the H-2k haplotype, and does not involve binding of TNP-proteins to specific anti-TNP receptors on the target cells. As one approach to directly studying the interactions between receptor-bound antigen and H-2-restricted T lymphocytes, we have examined the effects of lympho-

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1 Abbreviations used in this paper: ABA, p-azobenzene arsonate, BGG, bovine y-globulin, BSA, bovine serum albumin, CTL, cytolytic T lymphocyte(s), Cyclo, cyclophosphamide; DNP, 2,4-dinitrophenyl(ated), E T, effector target ratio(s), FCS fetal calf serum, KLH, keyhole limpet hemocyanin, MHC, major histocompatibility complex, PFC, plaque-forming cell(s); TNBS, 2,4,6-trinitrobenzene sulfonic acid, TNP, 2,4,6-trinitrophenyl(ated).
cyte populations containing hapten-specific CTL on the function of hapten-binding myelomas in the presence of soluble hapten-protein conjugates. In this system, myeloma cells are used as a homogeneous target cell population analogous in function and receptor expression to normal B lymphocytes or to antibody-secreting cells. In fact, recent experiments strongly suggest that myelomas are physiologically valid model systems for analyzing the regulation of normal immunocompetent lymphocytes (11, 12). Thus, tolerogenic antigens (13), antigen-antibody complexes (14), carrier-specific helper and suppressor T cells (15, 16), idiotype-specific T cells (17, 18), and alloreactive T cells (19) have all been shown to be capable of modulating myeloma cell function. The specific experimental protocol we have used is to stimulate BALB/c lymphocytes with syngeneic TNP-modified cells, and then to examine the effect of the TNP-reactive T cells generated in this manner on the TNP-binding BALB/c myeloma, MOPC 315, in the presence of TNP-protein conjugates. We have used assays of antibody secretion by the myeloma as a readout system for measuring the effect of CTL. The rationale for this approach is that the inhibition of target-cell function seems to represent a pre-lytic effect of CTL, and is a far more sensitive assay for CTL activity than $^{51}$Cr release or trypan blue dye exclusion methods (19, 20). The basic observations that have been made are that TNP-reactive T cells markedly inhibit antibody secretion by the myeloma in the presence of TNP-protein conjugates, although by $^{51}$Cr-release assays, the lysis of myeloma targets can be detected only if the targets are TNP modified and can not be detected in the presence of soluble antigen. The T cells responsible for this functional inhibition possess the phenotypic characteristics of CTL, are hapten specific and H-2 restricted in their function, and suppress only myeloma cells that bear specific receptors for the hapten. In addition, preliminary experiments indicate that TNP-specific CTL also inhibit primary antibody response to TNP-proteins in vitro. These findings are discussed in relation to the mechanisms of T lymphocyte-target cell interactions and the possible physiologic immunoregulatory role of hapten-reactive T lymphocytes.

**Materials and Methods**

**Myeloma Cell Lines, Antigens** The myeloma cell lines used in this study are listed in Table I. The majority of the experiments have been done with tissue-culture-adapted MOPC 315 cells, which were used in previous studies and whose characteristics have been described in detail (13). Briefly, the cells secrete an IgA, $\lambda_2$ antibody specific for TNP and 2,4-dinitrophenyl.

| Cell line | Form | Antibody product |
|-----------|------|------------------|
| MOPC 315  | Tissue culture, ascites | IgA, $\lambda_2$, anti-DNP and -TNP |
| TEP 15    | Ascites | IgA, $\kappa$, anti-phosphorylcholine |
| S107      | Tissue culture | IgA, $\kappa$, anti-phosphorylcholine |
| MPC 11    | Tissue culture | IgG3, $\kappa$, unknown specificity |
| MOPC 104E | Ascites | IgM, $\lambda$, anti-\(\alpha\), dextran |
| X5563     | Tissue culture | IgG3, $\kappa$, unknown specificity |

All are BALB/c (H-2b) myeloma lines except for X5563 (C3H, H-2b). Tissue-culture-adapted MOPC 315 and X5563 cells were maintained at 37°C in Lebovitz (L-15) medium with 2 mM l-glutamine, penicillin, streptomycin, and 15% FCS, whereas S107 and MPC 11 cells were cultured at 37°C in 5% CO2 in Dulbecco's modified Eagle's medium with penicillin, streptomycin, and 20% FCS.
(DNP), and, at any time, 50–75% of the cells express membrane IgA receptors for TNP as detected by rosette assays. The other tumor lines were obtained from the following sources: TEPC 15 from Dr. R. Germain, Harvard Medical School, Boston, Mass.; S107 from Dr. M. D. Scharff, Albert Einstein College of Medicine, Bronx, N.Y.; and MPC 11 from Dr. M. Gefter, Massachusetts Institute of Technology, Cambridge, Mass.

TNP conjugates of keyhole limpet hemocyanin (KLH), bovine γ-globulin (BGG), and bovine serum albumin (BSA) were prepared by the method of Ballas and Henney (10) using 2,4,6-trinitrobenzene sulfonic acid (TNBS) in 0.4 M borate buffer, pH 9. The conjugates used in these experiments were TNP2KLH, TNP3KLH, and TNP4KLH (17, 28, and 30 mol TNP, respectively, per 100,000 mol wt of KLH), TNP2BGG (52 mol TNP/mol of BGG), and TNP2BSA (24 mol TNP/mol of BSA). DNP20KLH was prepared by incubating KLH with 2,4-dinitro-fluorobenzene in 1% sodium carbonate. p-Azobenzene arsonate (ABA) conjugates of KLH were prepared as described by Nisonoff (21). The double conjugate TNP-ABA-KLH was prepared by incubating ABA-KLH with TNBS following the method of Ballas and Henney (10) as described above.

Generation of Hapten-reactive T Cells and Assay for Cytolysis

Normal BALB/c spleen lymphocytes (7 × 10⁶) were stimulated in vitro with 6 × 10⁶ syngeneic, 1,500-rad x-irradiated, TNBS-modified, erythrocyte-free splenocytes as described previously (22). Cultures were carried out for 5 d in 2 ml RPMI-1640 supplemented with penicillin, streptomycin, and 10% fetal calf serum (FCS) (Grand Island Biological Co., Grand Island, N.Y.) in 16-mm Linbro wells (Linbro Chemical Co., Hamden, Conn.) at 37°C in a humidified atmosphere of 5% CO₂. In some experiments, animals were primed subcutaneously 6–7 d previously with 3 × 10⁷ TNBS-modified syngeneic cells, and their spleen lymphocytes boosted in vitro as described above. For generating DNP- and ABA-reactive T lymphocytes, animals were always primed subcutaneously with hapten-modified cells and spleen cells boosted in vitro, as described previously (23, 24). Such stimulated cells are referred to as anti-hapten effector lymphocytes or hapten-specific CTL, their detailed characterization is described in Results. In addition, in all experiments spleen cells were cultured in parallel without hapten-modified stimulators (referred to as unstimulated lymphocytes). In some experiments, responder spleen cells were obtained from BALB/c mice that had been injected with 100 mg/kg cyclophosphamide intraperitoneally 2 d earlier. TNP-specific CTL were also induced in vivo by the method of Rollinghoff et al (25). Briefly, BALB/c mice were injected with 100 mg/kg cyclophosphamide intraperitoneally, and 3 d later, were immunized in the hind footpads with 3 × 10⁷ TNP-modified, 1,500-rad x-irradiated syngeneic spleen cells. Draining lymph nodes harvested 4 d later were used as the source of TNP-specific CTL. Unstimulated lymphocytes were obtained from lymph nodes of cyclophosphamide-pretreated mice that were either not immunized or were injected in footpads with 3 × 10⁷ unmodified syngeneic spleen cells.

Cytolytic activity of cultured cells was measured by ⁵¹Cr-release assays, as described previously (22). For BALB/c (H-2b) cells, the targets used were syngeneic ⁵¹Cr-labeled MOPC 315 or P 815 (DBA/2) mastocytoma cells, modified with 10 mM TNBS (for assessing TNP-reactive CTL), 10 mM DNBS (for anti-DNP CTL), or p-azobenzene arsanilate (for anti-ABA CTL), all of which have been previously described (22–24). Anti-TNP CTL gave comparable levels of lysis using TNP-modified MOPC 315 or P 815 cells as targets. For B10 BR (H-2b) effectors, the targets were ⁵¹Cr-labeled TNBS-modified RDM-4 (AKR) tumor cells. Assays were done at effector:target (E:T) ratios ranging from 4:1 to 100:1, for 4 h at 37°C in 10- × 75-mm glass tubes, after which the ⁵¹Cr released into the supernate was counted. Results are expressed as percent specific lysis = (E - C)/(FT - C), where E = release in experimental groups with CTL, C = background release from targets with normal spleen cells, and FT = maximal release after four cycles of freezing and thawing.

Myeloma-Cell Cultures and Assays for Antibody Secretion

Viable tissue-culture-adapted myeloma cells (>90% viable by trypsin blue dye exclusion) were purified by centrifugation over Ficoll-Isoopaque (Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Piscataway, N.J.) (13), and ascitic cell lines were freed of erythrocytes by lysis with Tris-buffered 0.83% ammonium chloride, pH 7.4. In all experiments, 10⁶ myeloma cells in triplicate were first incubated with or without the appropriate concentration of antigen (hapten conjugates of KLH) in RPMI-1640 with penicillin, streptomycin, 10% FCS, 5 × 10⁻⁴ M 2-mercaptoethanol, and 10 mM Hepes. These
incubations were done in round-bottomed microculture plates (Linbro Chemical Co.) at 37°C in 5% CO2. At the end of 60 min, lymphocytes generated by in vitro culture as described above were added to a final vol of 0.2 ml, and the incubations continued for a further 4–48 h. At the end of this coculture, cells were harvested, washed three times, and assayed for antibody secretion as described below. In all experiments, control groups of myeloma cells alone and myeloma cells with antigen alone were included; the antigens chosen have been shown to have no significant effect on myeloma growth or function ([13]; and these experiments).

Antibody secretion was measured by a reverse hemolytic plaque assay (26), the details of which have been described previously (13, 19). Briefly, 1/50ths or 1/100ths of each washed culture were mixed with 20 μl of 30% sheep erythrocytes coated with staphylococcal protein A, 20 μl of an optimal concentration of developing serum, 20 μl of a 1:3 dilution of guinea pig complement (Pel-Freeze Biologicals Inc., Rogers, Ark.), and 300 μl of 0.5% agarose in Hanks' balanced salt solution. The mixture was smeared on a 10-cm plastic dish, incubated at 37°C in a humidified atmosphere for 5–6 h, and the plaques counted. The developing sera used were: 1:50 dilution of rabbit anti-M315 IgA for MOPC 315, S107, and TEPC 15 cells (all IgA secretors), 1:50 rabbit anti-MPC 11 for MPC 11 cells, and 1:50 rabbit anti-104E for MOPC 104E cells. Each myeloma cell line produced plaques only with the appropriate developing serum; moreover, cultured lymphocytes by themselves did not produce significant numbers of plaques under these conditions. All results are expressed as plaque-forming cells (PFC)/culture. Statistical analyses and determinations of the percent inhibition caused by hapten-reactive cells are calculated using groups lacking the effector lymphocytes as controls.

Binding of 125I-labeled Antigens to Myeloma Cells. The techniques have been described previously (27). Briefly, TNP-KLH was trace-labeled with 125I by the chloramine T method. Free 125I was removed by exhaustive dialysis, and >90% of the radioactivity was precipitable in 10% trichloroacetic acid. 10^7 myeloma cells were incubated in duplicate with various concentrations of 125I-labeled antigen in 0.2 ml of RPMI-1640 with supplements in 12- × 75-mm plastic tubes at 37°C in 5% CO2. After 1–2 h, cells were washed four times, and the cell-associated radioactivity measured in a Beckman Biogamma II γ-counter (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif). Standards with known amounts of 125I-labeled antigen were included, and results are expressed as nanograms of antigen bound/10^6 cells. For ascitic lines, macrophages were depleted by two cycles of adherence to plastic dishes, each for 45 min at 37°C, before antigen incubations.

Induction of Anti-TNP Antibody Responses In Vitro. A microculture system was used for generating primary anti-TNP antibody responses (28). 5 × 10^6 normal BALB/c spleen cells were cultured with TNP-KLH in 0.2 ml of RPMI-1640 with supplements as described above. Cultures were done in triplicate in flat-bottomed microculture plates (Linbro Chemical Co.) in 5% CO2 at 37°C for 4 d, after which each well was harvested, washed twice, and anti-TNP PFC assayed by the slide modification of the Jerne method using TNP-coated sheep erythrocytes as indicator cells. Preliminary experiments established that, in our hands, the optimal concentration of TNP-KLH was 20–25 μg/ml; unconjugated KLH elicited no response; the PFC response was T cell dependent; only IgM PFC were observed; and the response was TNP specific (>95% inhibition with 3 × 10^-4 M TNP in the form of TNP=BSA).

Other Techniques. Effector lymphocytes were treated with anti-Thy-1 serum and complement as follows: in vitro generated effector lymphocytes were treated with a monoclonal anti-Thy-1.2 antibody (a gift of Dr. Ann Rothstein, Massachusetts Institute of Technology), followed by rabbit complement (Pel-Freeze Biologicals Inc.). Cells were washed, counted, and trypan blue, and used in cocultures. Effector cells were also treated with a monoclonal anti-Ly-2.2 antibody (a gift of Dr. Paul Gottlieb, Massachusetts Institute of Technology) plus complement. Controls consisted of complement treatment alone.

Results

Effect of TNP-reactive Lymphocytes on MOPC 315 Cells. In the initial series of experiments, BALB/c spleen cells stimulated in vitro with syngeneic TNP-modified splenocytes were tested for their ability to lyse 51Cr-labeled MOPC 315 cells in the presence of 50–100 μg/ml of TNP-KLH or TNP-BGG. Although such stimulated
lymphocytes efficiently lysed TNP-modified MOPC 315 cells, we were unable to
detect any cytolysis of MOPC 315 targets incubated with soluble hapten-protein
conjugates in a 4-h assay. In addition, MOPC 315 cells incubated with soluble antigen
could not serve as cold targets to block the lysis of $^{51}$Cr-labeled TNP-modified MOPC
315 targets (data not shown). We then tested the effect of this anti-TNP CTL-
containing lymphocyte population on antibody secretion by MOPC 315 cells in the
presence of TNP-KLH, an antigen which by itself does not affect MOPC 315 cell
function. As shown in Table II, TNP-reactive lymphocytes (anti-TNP effector cells)
significantly suppress IgA secretion by the myeloma cells in the presence of 10 or 50
µg/ml of TNP-KLH, whereas BALB/c splenocytes cultured in parallel, but without
TNP-modified stimulators (unstimulated effector cells), are not suppressive. In addi-
tion, the TNP-reactive cells capable of inhibiting myeloma function are radioresistant,
and can also be generated by in vitro stimulation of spleen cells from mice pretreated
with 100 mg/kg of cyclophosphamide (Cyclo) (Table II). Moreover, TNP-reactive
cells are incapable of suppressing MOPC 315 cells in the absence of soluble antigen
(Table III). In most of the experiments, the degree of suppression observed is 40–65%.
This may be because only 50–75% of MOPC 315 cells express TNP-specific surface
receptors by rosette assays. Attempts to determine if PFC are present only in the
receptor-bearing fraction or if they are distributed among receptor-positive and
-receptor-negative myeloma cells have produced inconclusive results so far. In some experi-
ments, we have found that BALB/c spleen cells cultured in FCS-containing media
without additional stimulators are suppressive for MOPC 315 cells, but this suppres-
sion is seen with or without the presence of TNP-proteins. This nonspecific suppression
is eliminated by irradiating the effector cells, a procedure that has no effect on the
lytic activity of TNP-reactive cells. Moreover, the consistent inability of anti-TNP
effectors to suppress MOPC 315 cells in the absence of soluble antigen provides an
additional specificity control.

In addition to the inhibition of IgA secretion described above, cocultures of MOPC
315 cells with TNP-KLH and anti-TNP effector lymphocytes show a 15–25% reduc-
tion in viable myeloma-cell recovery after 24 h. After washing and reculture in fresh
medium for 2–3 d, viable MOPC 315 cell recovery in these groups is reduced by 40–
50% compared to groups with myeloma cells alone or myeloma cells and anti-TNP
effectors without antigen. Taken together, these results suggest that the functional
suppression of myeloma targets is a pre-lytic event induced by the CTL, eventually
resulting in reduced viable myeloma-cell recovery. Because, in this system, measure-
ment of myeloma-cell function gives the most consistent and reproducible results, and
because a similar assay has been used by other investigators to study CTL-target cell
interactions (19, 20), we have used PFC assays to analyze the nature of the interaction
between anti-TNP effector cells and myeloma targets, in all subsequent experiments.

Properties of TNP-reactive Effector Cells. The data in Table II have shown that the
TNP-reactive lymphocytes that suppress MOPC 315 cell function are radioresistant,
and their precursors are insensitive to Cyclo. These cells are T lymphocytes, as judged
by their sensitivity to treatment with anti-Thy-1.2 serum and complement (Table
IV) and their nonadherence to nylon wool (data not shown). Moreover, the cells bear
Ly-2.2 antigens, as shown by antiserum and complement treatment (Table IV). In a
series of kinetics and dose-response experiments, we have found that significant
suppression requires >4 h coculture of $10^5–3 \times 10^5$ effector lymphocytes with $10^5$
TABLE II
Inhibition of MOPC 315 Cells by TNP-reactive Lymphocytes

| Effector cells | TNP17KLH IgA PFC/culture (mean ± SE) | P* | Inhibition† |
|----------------|-------------------------------------|----|-------------|
|                | × 10⁻³ %                            |    |             |
| None           | None                                | 67 5 ± 6 9 | —            | —            |
| None           | 10 μg/ml                             | 60 1 ± 2 8 | —            | —            |
| None           | 50 μg/ml                             | 55 3 ± 6 9 | —            | —            |
| Anti-TNP       | 10 μg/ml                             | 28 4 ± 1 2 | 0 005        | 52 7         |
| Anti-TNP       | 50 μg/ml                             | 23 0 ± 5 2 | 0 007        | 58 4         |
| Unstimulated   | 50 μg/ml                             | 48 7 ± 3 5 | NS§          | 11 9         |
| Anti-TNP, Cyclo pretreated | 10 μg/ml                             | 33 2 ± 4 5 | 0 01         | 44 8         |
|                | 50 μg/ml                             | 32 1 ± 2 1 | 0 008        | 42 0         |
| Unstimulated, Cyclo pretreated | 50 μg/ml                             | 59 7 ± 7 7 | NS           | 0            |
| Anti-TNP, 1,500 rad x-irradiated | 10 μg/ml                             | 28 8 ± 1 2 | 0 005        | 52 1         |
|                | 50 μg/ml                             | 24 3 ± 4 2 | 0 006        | 56 1         |
| Unstimulated, 1,500 rad x-irradiated | 50 μg/ml                             | 45 2 ± 4 7 | NS           | 18 3         |

Effector cells Assay for cytolysis of ⁵¹Cr-labeled, TNP-modified P 815 targets specific lysis at E T

| Effector cells | Assay for cytolysis of ⁵¹Cr-labeled, TNP-modified P 815 targets specific lysis at E T |
|----------------|-------------------------------------------------------------------------------------|
|                | 11 1                                                                                 |
|                | 33 1                                                                                 |
|                | 100 1                                                                                |

| Effector cells | Assay for cytolysis of ⁵¹Cr-labeled, TNP-modified P 815 targets specific lysis at E T |
|----------------|-------------------------------------------------------------------------------------|
| Anti-TNP       | 47 5                                                                                |
| Unstimulated   | 16 0                                                                                |
| Anti-TNP, Cyclo pretreated | 14 6                                 |
| Unstimulated, Cyclo pretreated | 1 4                                  |

10⁶ MOPC 315 cells were cultured for 24 h in triplicate alone, with TNP17KLH, or with TNP17KLH and 3 × 10⁶ viable lymphocytes generated by in vitro stimulation of BALB/c spleen cells with syngeneic TNP-modified cells (anti-TNP), or control cells cultured by themselves (unstimulated). Effector cells were also (a) generated with spleen cells from mice pretreated with Cyclo, or (b) x-irradiated (1,500 rad) before adding to myeloma targets.

* Calculated by Student’s t tests, using groups that contained myeloma cells and equivalent concentration of TNP17KLH without effector cells as controls.
† Determined using groups that contained myeloma cells and antigen only as controls (i.e., 100% response).
§ NS, not significant (P > 0.05)
‖ Spontaneous release was 8 4%, unmodified ⁵¹Cr-labeled targets showed no significant lysis.

myeloma targets, and is consistently seen after 24 or 48 h (data not shown). Moreover, the anti-TNP effector cells can be generated by in vitro stimulation in 0.5% syngeneic mouse serum or 2% rat serum, indicating that their activity is not related to the FCS in the culture medium.

**H-2 Restriction of Anti-TNP Effector Cells.** To determine if the anti-TNP effector...
TABLE III

TNP-reactive Cells Suppress MOPC 315 Cells Only in the Presence of TNP-KLH

| Effector cells (3 × 10⁶) | TNP₃KLH | IgA PFC/culture (mean ± SE) | p* | Inhibition |
|-------------------------|---------|-----------------------------|----|-----------|
| None None               | none    | 42.3 ± 1.9                  |    | —         |
| None 50 μg/ml           | None    | 42.7 ± 1.8                  |    | —         |
| Anti-TNP 50 μg/ml       | TNP-KLH | <0.001                      | 43 1 |
| Anti-TNP None           | 42.0 ± 3 7 | >0.9                      | 0 7 |

10⁶ MOPC 315 cells were cultured for 24 h with or without TNP₃KLH and 1,500-rad x-irradiated BALB/c anti-TNP effector lymphocytes that were generated in vitro. Data are pooled from five experiments, each done in triplicate; the group showing significant inhibition is italicized.

* Calculated as in Table II

TABLE IV

Surface Properties of TNP-reactive Lymphocytes

| Effector cells (10⁶) | Treatment | TNP₃KLH | IgA PFC/culture (mean ± SE) | p* | Inhibition |
|---------------------|-----------|---------|-----------------------------|----|-----------|
| None                | None      | none    | 76.7 ± 3.7                  |    | —         |
| None                | None      | 50 μg/ml | 73.1 ± 2.1                  |    | —         |
| Anti-TNP            | None      | 50 μg/ml | 44.0 ± 5.7                  | <0.01 | 39.8 |
| Anti-TNP            | Anti-Thy-1 2 + C | 64.4 ± 5.2 | NS§ | 11.9 |
| Anti-TNP            | Anti-Ly-2 2 + C | 76.5 ± 4.4 | NS | 0 |
| Anti-TNP            | C         | 50 μg/ml | 40.3 ± 1.9                  | <0.001 | 44.9 |

10⁶ MOPC 315 cells were cultured for 24 h with or without TNP-KLH and 1,500-rad x-irradiated BALB/c anti-TNP effector cells which were generated in vitro, and treated as shown C, complement

* Calculated as in Table II

§ NS, not significant

§ Spontaneous ⁶Cr release 9.3%

Lymphocytes are H-2 restricted (similar to hapten-specific CTL described in other systems [7, 9, 10, 22-24]), we tested the effects of CTL generated with spleen lymphocytes of different mouse strains on the myeloma targets. As shown in Table V, B10.BR (H-2b) anti-TNP CTL do not affect the function of MOPC 315 (H-2a) targets, whereas syngeneic BALB/c anti-TNP CTL are suppressive in the presence of TNP-KLH. Because no TNP-binding myeloma of an H-2 type other than H-2a is available, the reciprocal coculture is not feasible. As a further verification of H-2


Table V

| Effector cells (3 × 10^6) | TNP-P-KLH | IgA PFC/culture (mean ± SE) | P | Inhibition |
|--------------------------|-----------|-----------------------------|---|-----------|
| None                     | None      | 40.6 ± 2.4                  | ---| ---       |
| None                     | 50 µg/ml  | 44.1 ± 2.5                  | ---| ---       |
| BALB/c anti-TNP          | 50 µg/ml  | 25.4 ± 2.4                  | <0.001 | 42.4     |
| BALB/c anti-TNP          | None      | 48.8 ± 3.3                  | NS* | 0         |
| B10 BR anti-TNP          | 50 µg/ml  | 48.4 ± 5.7                  | NS  | 0         |
| B10.BR anti-TNP          | None      | 48.6 ± 5.7                  | NS  | 0         |

Assay for cytolysis of ^51Cr-labeled, TNP-modified P 815 (H-2^d) and RDM4 (H-2^k) targets; specific lysis of:

| Effector cells | TNP-P 815 at E:T | TNP-RDM4 at E:T |
|---------------|------------------|------------------|
|               | 11:1             | 33:1             | 100:1           |
| BALB/c anti-TNP | 8,1              | 15.4             | 32.8            | 2.8 | 11.5 | 21.8 |
| B10 BR anti-TNP | 2,1              | 4.2              | 9.4             | 23.3 | 41.8 | 57.6 |

10^6 MOPC 315 cells were cultured for 24 h with or without TNP-KLH and 1,500-rad x-irradiated BALB/c or B10.BR anti-TNP effector lymphocytes that were generated in vitro. Data are pooled from two experiments and expressed as in Table II

* NS, not significant (P > 0.1)

† Spontaneous ^51Cr release was <20%

restriction, we attempted to block the effect of BALB/c anti-TNP effector lymphocytes on MOPC 315 cells with cold targets; in this system, cold targets refer to tumor cells that do not secrete IgA and are, therefore, not detected in the PFC assay. As shown in Table VI, TNP-modified MPC 11 and P815 (H-2^a) cells block the suppression of MOPC 315 cells by BALB/c anti-TNP CTL in the presence of TNP-KLH, whereas TNP-modified X5563 (H-2^k) or unmodified "cold targets" have no effect. These experiments establish that the anti-TNP effector cells detected in this system are H-2 restricted, and, taken together with the phenotypic characteristics detailed above, strongly suggest that the effector cells are, indeed, hapten-reactive CTL.

**Hapten Specificity of CTL.** The experiments described above have demonstrated that inhibition of MOPC 315 cell function is seen with TNP-reactive but not with unstimulated cells, and only in the presence of appropriate TNP-protein conjugates, suggesting that the phenomenon is hapten-specific. As a more stringent control for specificity, T cells were generated by priming in vivo followed by in vitro stimulation with DNP- and TNP-modified syngeneic splenocytes. These hapten-reactive CTL were tested for cytolysis of hapten-modified ^51Cr-labeled targets and for their effects on MOPC 315 cells in the presence of DNP-KLH or TNP-KLH. In our hands, anti-DNP CTL lyse both DNP- and TNP-modified syngeneic targets, whereas anti-TNP CTL lyse only TNP-modified targets (Table VII) (R. Finberg, M.-S. Sy, M. I. Greene, and S. J. Burakoff. Manuscript in preparation.) Consistent with this pattern, TNP-specific CTL inhibit MOPC 315 cells only in the presence of TNP-KLH, whereas DNP-specific CTL cause significant suppression in the presence of both hapten-protein conjugates, TNP-KLH and DNP-KLH (Table VII). Using antigens trace-
labeled with $^{125}$I, we have found that at equivalent concentrations comparable amounts of DNP$_{20}$KLH and TNP$_{20}$KLH are bound to MOPC 315 cells (data not shown). Therefore, the differences in the effects of DNP- and TNP-specific CTL cannot be attributed to differences in the amount of antigen bound to the myeloma targets and presumably reflect inherent differences in the recognition or reactivity patterns of the T cells themselves.

**Requirement for Hapten-binding Receptors on Myeloma Targets.** To determine if non-TNP binding H-2$^{d}$ myelomas incubated with TNP-KLH could also be inhibited by TNP-specific CTL, we tested a battery of tissue-culture-adapted and ascitic-myeloma-cell lines in this system. The data in Table VIII show that tissue-culture-adapted or ascitic MOPC 315 cells can be consistently suppressed when incubated with TNP$_{20}$KLH and TNP-specific CTL. However, under identical conditions, S107 (IgA, $\kappa$; anti-phosphorylcholine) and MPC 11 (IgG$_{2b}$, $\kappa$; of unknown specificity) tissue-culture-adapted lines, and TEPC 15 (IgA, $\kappa$; anti-phosphorylcholine) and MOPC 104E (IgM, $\lambda$, anti-dextran) ascitic cells are not significantly inhibited. In all these experiments, antigen alone or TNP-specific CTL alone are nonsuppressive. Moreover, all the myeloma cell lines tested, when directly haptenated, can be lysed by syngeneic TNP-specific CTL in $^{51}$Cr-release assays. Finally, when mixtures of MOPC 315 cells
TABLE VII
Specificity of TNP- and DNP-reactive CTL

| Effector cells | Antigen (50 µg/ml) | IgA PFC/culture (mean ± SE) | P    | Inhibition |
|----------------|-------------------|---------------------------|------|------------|
| 10^3 MOPC 315 cells were cultured for 24 h with or without antigens and 1,500-rad x-irradiated BALB/c effector cells that were generated by subcutaneous priming and in vitro boosting. Data are expressed as in Table II, groups showing significant inhibition are italicized
* NS, not significant (P > 0.1)
† Spontaneous ^51Cr-release was <15%.

and MPC 11 cells are cultured with TNP-KLH and syngeneic TNP-specific CTL, only IgA secretion is suppressed (data not shown), indicating that there is no bystander effect on the non-TNP-receptor-bearing, IgG-producing MPC 11 targets. The inability to suppress non-TNP-binding myelomas in the presence of TNP-KLH cannot be simply attributed to differences in total binding of antigen to the myeloma cells, because, at least over 1–2 h, approximately equivalent amounts of antigen are bound to all the tissue-culture-adapted lines or the ascitic lines tested at the same concentration (Table VIII B). In cold-antigen-competition experiments, we have found that the binding of ^125I-TNP-KLH to MOPC 315 cells can be inhibited by 34–48% in the presence of a 10-fold higher concentration of unlabeled TNP32BGG, which is similar to previously published results (27). In contrast, the binding of ^125I-TNP-KLH to non-TNP-binding myelomas can only be blocked by 10–20% with cold TNP32BGG, implying that much of this binding is not to hapten-specific surface receptors. Nonspecific binding of ^125I-labeled hapten proteins to tumor targets has been observed by other investigators, although the biochemical explanation for this is unknown. For instance, Bystryn et al. (29) found comparable binding of ^125I-DNP-BSA to MOPC
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**Table VIII**

**TNP-specific CTL Fail to Suppress Non-TNP-binding Myelomas**

| Myeloma cells incubated with | MOPC 315 (culture) | MPC 11 (culture) | S107 (culture) | MOPC 315 (ascites) | TEPC 15 (ascites) | MOPC 104E (ascites) |
|-----------------------------|--------------------|-----------------|---------------|-------------------|------------------|-------------------|
| A. Percent suppression of antibody secretion | mean ± SE | | |
| TNP-KLH 10 µg/ml + 3 × 10^5 anti-TNP cells | 46.7 ± 5.7 | ND* | ND | ND | ND | ND |
| TNP-KLH 50 µg/ml + 3 × 10^5 anti-TNP cells | 45.2 ± 2.0 | 42 ± 2.6 | 99 ± 99 | 55.2 ± 8.4 | 11.5 ± 6.7 | 66 ± 2.1 |
| 3 × 10^5 anti-TNP cells | 10.0 ± 3.3 | 23 ± 2.3 | 0 | 10.1 ± 10.1 | 7.3 ± 7.3 | 0 |

**B. Nanograms TNP-KLH bound/10^5 cells**

| Myeloma cells (culture) | MOPC 315 | MPC 11 | S107 | MOPC 315 | TEPC 15 | MOPC 104E |
|-------------------------|----------|--------|------|----------|---------|-----------|
| ^125I-TNP-AKLH 10 µg/ml | 86.8     | 72.7   | 73.6 | 29.5     | 23.5    |           |
| ^125I-TNP-AKLH 50 µg/ml | 325      | 252.8  | 249.4| 93.7     | 115.8   |           |

In A, 10^6 BALB/c myeloma cells were cultured in triplicate with TNP-KLH and 3 × 10^5 1,500-rad x-irradiated BALB/c anti-TNP effector lymphocytes for 24 h and then assayed for antibody secretion. Data are normalized and expressed as the percent suppression of PFC compared to groups lacking effector cells. In all experiments, TNP-KLH alone had no significant effect on PFC. All the myeloma lines have been tested at least three times. Statistically significant results (P < 0.05 in each experiment) are italicized. In B, the same tumor lines were incubated with ^125I-TNP-AKLH for 1 h (ascites cells) or 2 h (tissue-culture-adapted lines) at 37°C, washed, and the cell-bound radioactivity measured. Each cell line has been assayed two to six times.

* ND, not done

315 cells and control cells that lack receptors for DNP in the absence of unlabeled carrier protein. The important result relevant to the present experiments is that such nonspecific binding does not permit TNP-specific CTL to interact with non-TNP-binding myeloma targets. In fact, TNP-specific CTL suppress MOPC 315 cell function when soluble-antigen concentrations are as low as 10 µg/ml (Table II and Table VIII), but fail to suppress the non-TNP binding myelomas even at a fivefold-higher antigen concentration. These results indicate that TNP-KLH bound only to TNP-specific receptors sensitizes target cells so as to render them suppressible by TNP-reactive CTL.

As an additional approach to this question, we tested the ability of ABA-KLH and the double conjugate TNP-ABA-KLH to sensitize tissue-culture-adapted MOPC 315 cells for suppression by ABA-specific CTL. As shown in Table IX, TNP-specific CTL in the presence of TNP-KLH, but not ABA-KLH, suppress MOPC 315 cells, whereas ABA-reactive CTL are ineffective even in the presence of 100 µg/ml of ABA-KLH or TNP-KLH. This phenomenon is also not evidently attributable to the total amount of antigen bound to myeloma targets, because at 50 and 100 µg/ml, the amount of ABA-KLH bound/10^5 MOPC 315 cells in 1 h is 183.6 and 245.1 ng, respectively. Thus, the total amount of ABA-KLH bound to the cells at 100 µg/ml is greater than the amount of TNP-KLH bound at 10 µg/ml and roughly comparable to the amount of TNP-KLH bound at 50 µg/ml. However, when MOPC 315 cells are incubated with the double conjugate TNP-ABA-KLH so that the ABA determinant is bound to
TABLE IX

Effect on ABA-specific CTL on MOPC 315 Cells

| Effector cells | Antigen (100 µg/ml) | IgA PFC/culture (mean ± SE) | P | Inhibition |
|----------------|---------------------|----------------------------|----|------------|
| None           | None                | 75.9 ± 4.9                 |    |            |
| None           | TNP-KLH             | 72.7 ± 5.2                 |    |            |
| None           | ABA-KLH             | 68.1 ± 5.6                 |    |            |
| None           | TNP-ABA-KLH         | 75.4 ± 3.7                 |    |            |
| Anti-TNP       | TNP-ABA-KLH         | 44.1 ± 3.5                 | <0.001 | 39.3 |
| Anti-ABA       | TNP-ABA-KLH         | 69.0 ± 7.3                 | NS* | 5.1 |
| Anti-TNP       | ABA-KLH             | 55.9 ± 6.4                 | NS  | 17.9 |
| Anti-ABA       | ABA-KLH             | 64.8 ± 3.5                 | NS  | 48  |
| Anti-TNP       | TNP-ABA-KLH         | 42.1 ± 1.9                 | <0.001 | 43.8 |
| Anti-ABA       | TNP-ABA-KLH         | 45.0 ± 3.1                 | <0.001 | 40.3 |
| Anti-TNP       | None                | 68.3 ± 5.9                 | NS  | 10.0 |
| Anti-ABA       | None                | 71.6 ± 5.9                 | NS  | 5.7 |

Assays for cytolyis of 51Cr-labeled, TNP- and ABA-modified P 815 targets specific lysis of:

| Effector cells | TNP-P 815 at E.T | ABA-P 815 at E.T |
|----------------|------------------|------------------|
|                | 10 1 33 1 100 1  | 11 1 33 1 100 1  |
| Anti-TNP       | 13 3 30 0 64 0  | 14 3 9 7 3       |
| Anti-ABA       | 2.7 7 1 22 4   | 45 8 70 1 87 6   |
| Unstimulated   | -0 1 -0 1 5.9  | 0 3 1 3 1.2      |

10^6 MOPC 315 cells were cultured for 24 h with 100 µg/ml of the antigens as shown, with or without 3 × 10^5 (in two experiments) or 5 × 10^5 (in one experiment) 1,500-rad x-irradiated TNP-reactive or ABA-reactive BALB/c lymphocytes generated by subcutaneous priming and in vitro boosting. Groups showing significant suppression of IgA secretion by MOPC 315 cells are italicized. Data are pooled from three experiments, using two batches of TNP-ABA-KLH in each, statistical analyses and percent inhibition are calculated as in Table II.

* NS, not significant (P > 0.1)
‡ Spontaneous 51Cr-release was <20%.

TNP-specific receptors, they become susceptible to suppression by both TNP-specific and ABA-specific CTL (Table IX). This experiment provides strong confirmation for the central role of binding of antigens to specific receptors in this phenomenon.

Effect of TNP-specific CTL on Primary Anti-TNP Antibody Responses. Preliminary experiments have shown that TNP-specific CTL generated in vitro and in vivo markedly suppress the primary antibody response of normal syngeneic spleen cells to TNP-KLH in vitro (Table X). The effector cells are radiation insensitive, and their precursors are resistant to treatment with Cyclo; and unstimulated lymphocytes from Cyclo-pretreated mice have no effect on anti-TNP PFC. In other experiments, we have found that the effector cells are Thy-1.2-bearing T cells, and they cannot be bound to plastic dishes coated with TNP_{58}BGG (data not shown). Studies are now in
Inhibition of Primary Anti-TNP Antibody Responses by TNP-reactive CTL

Table X

| Experiment | Effector cells | IgM anti-TNP PFC/culture | P     |
|------------|----------------|--------------------------|-------|
|            |                | mean ± SE                |       |
| 1          | None           | 69 ± 10                  | —     |
|            | Anti-TNP (5 × 10⁵) | 21 ± 9               | <0.05 |
|            | Anti-TNP (5 × 10⁶) | 10 ± 1                | 0.005 |
|            | Unstimulated (5 × 10⁵) | 57 ± 13            | NS*   |
|            | Unstimulated (5 × 10⁶) | 16 ± 8              | 0.03  |
|            | Anti-TNP, Cyclo pretreated (5 × 10⁵) | 35 ± 4         | 0.03  |
|            | Anti-TNP, Cyclo pretreated (5 × 10⁶) | 12 ± 5           | 0.008 |
|            | Unstimulated, Cyclo pretreated (5 × 10⁵) | 58 ± 4         | NS    |
|            | Unstimulated, Cyclo pretreated (5 × 10⁶) | 56 ± 4           | NS    |
|            | Anti-TNP, 1500 rad (5 × 10⁵) | 28 ± 4           | 0.02  |
|            | Anti-TNP, 1500 rad (5 × 10⁶) | 13 ± 2           | 0.007 |
| 2          | None           | 133 ± 7                  | —     |
|            | Anti-TNP, 1500 rad (10⁵) | 76 ± 8              | <0.01 |
|            | Anti-TNP, 1500 rad (4 × 10⁶) | 61 ± 1            | <0.001|
|            | Unstimulated, 1500 rad (10⁵) | 155 ± 12        | NS    |
|            | Unstimulated, 1500 rad (4 × 10⁶) | 138 ± 6          | NS    |

5 × 10⁶ BALB/c spleen cells were stimulated in vitro with TNP-KLH, with or without effector lymphocytes. IgM PFC were measured after 4 days with TNP-coated sheep erythrocytes as indicators. In experiment 1, effector lymphocytes were generated by in vitro stimulation and are the same cells as those used in the experiment shown in Table II. In experiment 2, the effector lymphocytes were induced in vivo as described in Materials and Methods. Background PFC responses (without TNP-KLH) were experiment 1, 18 ± 2; experiment 2, 24 ± 4.

* NS, not significant.
† Spontaneous ⁵¹Cr release was ≤20%.

Assays for cytolyis of ⁵¹Cr labeled TNP-modified P 815 targets, specific lysis at E/T:

| Experiment | Effector cells | Assays for cytolyis of ⁵¹Cr labeled TNP-modified P 815 targets, specific lysis at E/T: |
|------------|----------------|------------------------------------------------------------------|
| 1          | See Table II   | 30.1 100.1                                                        |
| 2          | Anti-TNP       | 6 25                                                             |
|            | Unstimulated   | 0 0                                                              |

Discussion

Our results demonstrate that BALB/c TNP-reactive effector cells interact with a syngeneic TNP-binding myeloma, MOPC 315, in the presence of a soluble TNP-protein conjugate, the interaction being manifested as a reduction in antibody progress to define the cellular target of these hapten-specific CTL; i.e., whether they act on antigen-presenting cells, helper T lymphocytes, or B cells; and the role of H-2-encoded determinants in this phenomenon.
secretion by the myeloma targets. The effector cells are hapten-specific and H-2-restricted, Thy-1-bearing, Ly-2-positive T cells, and they only recognize myeloma targets that bind the hapten-proteins to specific surface receptors.

One of the initial questions raised by these experiments is the nature of the effector cells and precisely what effect they have on the myeloma targets. Although in vitro stimulation of lymphocytes with TNP-modified syngeneic cells generates not only CTL but hapten-specific suppressor T cells as well (8), the phenotypic characteristics of the effect cells detected in our system are typical of CTL. Thus, the cells are radioresistant, and their precursors are insensitive to Cyclo treatment (Table II). Secondly, the hapten specificity of DNP- and TNP-reactive CTL, as measured by 51Cr release, closely parallels the specificity of these same cells as far as interaction with MOPC 315 cells in the presence of DNP-KLH and TNP-KLH is concerned (Table VII). Thirdly, the effector cells cannot be bound to TNP-protein-coated plastic dishes (data not shown) under conditions that have been shown to bind a variety of hapten- and idiotype-specific suppressor T cells but not CTL (17, 30, 31). Finally, TNP-specific CTL generated in vivo (25) also inhibit MOPC 315 cells in the presence of TNP-KLH, albeit to a lesser degree than effectors induced in vitro, probably because the in vivo protocol induces relatively weak CTL (data not shown). Taken together, these data support the view that the effector cells that inhibit MOPC 315 targets in the presence of TNP-KLH are TNP-specific CTL. Our inability to detect significant lysis of myeloma targets after this coculture by 51Cr-release assays or by dye exclusion is probably because these latter techniques are relatively insensitive and measure late consequences of CTL-target interactions. In fact, functional inactivation of myeloma targets may be an early effect of CTL and has been used as a highly sensitive assay for classical, alloreactive CTL (19, 20). It is also noteworthy that the 51Cr-release assay we have utilized is only a 4-h assay, whereas cocultures that are undertaken to measure effects on myeloma cell function are done for 24 h. So far, we have been unable to do 24-h 51Cr-release assays because of high spontaneous release of 51Cr from the myeloma targets. In any event, it is likely that the myeloma targets that are incubated with soluble TNP-KLH and TNP-specific CTL, and that show inhibition of antibody secretion, are committed to lyse, and the reduced viable-cell recoveries after 2-3 d of reculture are consistent with this view.

Irrespective of the ultimate fate of myeloma cells, the ability of hapten-reactive CTL to inhibit myeloma function in the presence of soluble hapten-protein conjugates has several important implications. One central observation is that only myeloma cells that bind the hapten to specific surface receptors are susceptible to such regulation, implying that receptor-bound antigen forms an essential component of the target moiety recognized by the T cells. This is clearly different from the results of Schmitt-Verhulst et al. (9) and Ballas and Henney (10), who have shown that only with H-2a mice, soluble TNP-protein conjugates can generate TNP-reactive, H-2-restricted CTL and sensitize a variety of non-TNP-binding tumor targets for lysis by such CTL. In our studies, with H-2b effectors and targets, only the TNP-binding myeloma MOPC 315 serves as a target for TNP-specific CTL in the presence of TNP-KLH (Table VIII A). Moreover, ABA-specific CTL do not affect myeloma function in the presence of ABA-KLH (Table IX), and these findings cannot be attributed to significant quantitative differences in the binding of different antigens to the myeloma targets. Most strikingly, ABA-specific CTL do interact with MOPC 315 cells in the
presence of the double conjugate TNP-ABA-KLH, strongly indicating that binding of antigens to specific surface receptors is necessary to render the cells suppressible by hapten-reactive CTL. The second key observation is that the TNP-reactive CTL are H-2 restricted, in that only H-2\(^d\) effectors inhibit MOPC 315 cells (Table V) and only TNP-modified H-2\(^d\) cold targets are effective blockers (Table VI). This result is consistent with the view that H-2-encoded surface determinants constitute another component of the target structure recognized by hapten-reactive CTL, similar to the proposed interaction of virus- or hapten-reactive CTL with appropriate virus-infected or hapten-modified syngeneic targets (7, 32). Thus, it appears that TNP-specific CTL recognize TNP-KLH bound to specific receptors on MOPC 315 cells in association with H-2-encoded antigens, and this lymphocyte-target cell interaction leads to a detectable inhibition of antibody secretion by the myeloma cells. In B lymphocytes, it is clear that surface immunoglobulin and H-2 antigens are independent surface macromolecules (33), and it is likely that immunoglobulin receptors for antigen and H-2 determinants on myeloma cells are also not directly linked in any fashion. Our results, therefore, imply that two independent membrane moieties, viz., receptor-bound ligand and H-2 antigens, can be recognized as one functional unit by T cells. Because both antigen receptors and H-2 antigens are freely mobile in the plasma membrane, it is conceivable that they come into intimate association after antigen binding. These results, therefore, do not shed any light on whether the receptor on the T cell recognizes target structures as one or two entities, and, hence, whether the functional T cell receptor has a dual specificity for antigen (or hapten) and H-2 determinants or a single specificity for modified H-2 determinants.

Finally, these experiments provide the first direct demonstration of recognition of receptor-bound antigens by H-2-restricted T lymphocytes. To the extent that myeloma cells are analogous to normal B lymphocytes and antibody-secreting cells (11, 12), these results imply that associative recognition of H-2 determinants and of antigen on specific B cells may be an important mechanism whereby antigen-reactive T cells regulate immune responses specifically. Preliminary experiments (Table X) do indicate that TNP-specific CTL can regulate an anti-TNP antibody response in vitro, although we have not yet formally established the cellular targets of such CTL. Clearly a similar mechanism is likely to be operative in situations where H-2-restricted, antigen-specific T cells interact with other antigen-specific immunocompetent cells, such as the postulated I-region-restricted collaboration between antigen-specific helper T and B lymphocytes (5, 6). The myeloma system provides a model for exploring in greater depth the mechanisms of and requirements for regulatory interactions between T lymphocytes and other immunocompetent cells in which both cell types are H-2 restricted and antigen specific.

Summary

BALB/c splenocytes stimulated in vitro with trinitrophenyl (TNP)-modified syngeneic cells inhibit the secretion of antibody by the TNP-binding BALB/c myeloma MOPC 315 in the presence of soluble TNP-Keyhole limpet hemocyanin (KLH). The effector cells are hapten-specific, H-2-restricted, Thy-1.2-bearing, Ly-2-positive T lymphocytes whose precursors are resistant to pretreatment with cyclophosphamide. These phenotypic properties are typical of hapten-specific cytolytic T lymphocytes (CTL). The TNP-reactive CTL that inhibit MOPC 315 cells fail to suppress H-2"
myelomas that do not bear TNP-specific surface receptors, and this is not attributable
to differences in total binding of TNP-KLH to the different myeloma cells. Moreover,
azobenzene arsonate (ABA)-specific CTL inhibit MOPC 315 cells in the presence of
the double conjugate TNP-ABA-KLH, but not in the presence of soluble TNP-KLH
or ABA-KLH. These results show that H-2-restricted, hapten-specific lymphocytes
regulate the function of myeloma cells that bind the hapten only to specific surface
receptors, and provide a model for associative recognition of surface H-2 determinants
and receptor-bound antigen. The results are discussed with reference to the mecha-
nisms of T lymphocyte-target cell interactions, and the possible physiologic role of
hapten-reactive CTL in specifically regulating anti-hapten antibody responses.

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References
1. Eichmann, K., I. Falk, and K. Rajewsky. 1978. Recognition of idiotypes in lymphocyte
interactions. II Antigen-independent cooperation between T and B lymphocytes that
possess similar and complementary idiotypes. Eur. J. Immunol. 8:853
2. Nisonoff, A., S T. Ju, and F. L. Owens. 1977. Studies of structure and immunosuppression
of a cross-reactive idiotype in strain A mice. Immunol. Rev. 34:89.
3. Herzenberg, L. A., K. Okumura, and C. M. Metzler. 1975. Regulation of immunoglobulin
and antibody production by allotype suppressor T cells in mice. Transplant. Rev. 27:57.
4 Paul, W. E., and B. Benacerraf 1977. Functional specificity of thymus-dependent lympho-
cytes. Science (Wash. D. C). 195:1293.
5. Katz, D. H., and B. Benacerraf. 1976. Genetic control of lymphocyte interactions and
derdifferentiation. In The Role of Products of the Histocompatibility Gene Complex in
Immune Responses. D. H. Katz and B. Benacerraf, editors Academic Press, Inc., New
York. 355
6. Sprent, J., and H. von Boehmer 1976 Helper function of T cells depleted of alloantigen-
reactive lymphocytes by filtration through irradiated F1 hybrid recipients. I. Failure to
collaborate with allogeneic B cells in a secondary response to sheep erythrocytes measured
in vitro J. Exp Med. 144:617.
7 Shearer, G. M., and A.-M. Schmitt-Verhulst. 1977. Major histocompatibility complex
restricted cell-mediated immunity. Adv. Immunol. 25:55.
8 Scott, D W. 1978. Role of self carriers in the immune response and tolerance III. B cell
tolerance induced by hapten-modified self involves both active T cell mediated suppression
and direct blockade Cell. Immunol. 37:327.
9 Schmitt-Verhulst, A.-M., C. B. Pettinelli, P. A Henkart, J. K. Lunney, and G. M Shearer.
1978 H-2-restricted cytotoxic effectors generated in vitro by the addition of trinitrophenyl-
conjugated soluble proteins. J. Exp Med. 147:352.
10 Ballas, Z. K., and C. S. Henney. 1979. Generation of H-2 restricted cytotoxic T cells by
trinitrophenylated proteins in vitro: specificity and requirements. J. Immunol. 123:1696.
11 Abbas, A. K. 1979. Antigen and T lymphocyte mediated suppression of myeloma cells:
model systems for regulation of lymphocyte function. Immunol. Rev. 48:245
12 Lynch, R G., J. W. Rohrer, B. Odermatt, H. M Gebel, J R. Autry, and R. G Hoover.
1979 Immunoregulation of murine myeloma cell growth and differentiation: a monoclonal
model of B cell differentiation. Immunol. Rev. 48:45
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13. Abbas, A. K., and G. G. B. Klaus 1977 Inhibition of antibody production in mouse plasmacytoma cells by antigens. *Eur. J. Immunol.* 7:567.

14. Abbas, A. K., and G. G. B. Klaus. 1978. Antigen-antibody complexes suppress antibody production in mouse plasmacytoma cells *in vivo*. *Eur. J. Immunol.* 8:217.

15. Rohrer, J. W., and R. G. Lynch 1977. Specific immunologic regulation of differentiation of immunoglobulin expression in MOPC 315 cells during *in vivo* growth in diffusion chambers. *J. Immunol.* 119:2045.

16. Rohrer, J. W., and R. G. Lynch 1978. Antigen-specific regulation of myeloma cell differentiation in vivo by carrier-specific T cell factors and macrophages. *J. Immunol* 120:1066.

17. Abbas, A. K., L. L. Perry, B. A. Bach, and M. I. Greene. 1980. Idioype specific T cell immunity. I. Generation of effector and suppressor T lymphocytes reactive with myeloma idioypc determinants. *J. Immunol.* 124:1160.

18. Rohrer, J. W., B. O. Odermatt, and R. G. Lynch. 1979. Immunoregulation of murine myeloma isologuous immunization with M315 induces idioype-specific T cells that suppress IgA secretion by MOPC 315 cells *in vivo*. *J. Immunol.* 122:2011.

19. Abbas, A. K. 1979. T lymphocyte-mediated suppression of myeloma function *in vitro*. I. Suppression by allogeneically activated T lymphocytes. *J. Immunol.* 123:2011.

20. Watanabe, T., C. G. Fathman, and A. Coutinho. 1977. Clonal growth of T cells in vitro preliminary attempts to a quantitative approach. *Immunol. Rev.* 35:3.

21. Nisonoff, A. 1967. Coupling of diazonium compounds to proteins. *Methods Immunol. Immunochm.* 1:120.

22. Burakoff, S. J., R. N. Germain, M. E. Dorf, and B. Benacerraf. 1976. Inhibition of cell mediated cytolysis of trinitrophenyl derivatized target cells by alloantiserum directed at the products of the K and D loci of the H-2 complex. *Proc. Natl. Acad. Sci. U. S. A.* 73:625.

23. Finberg, R., M. I. Greene, B. Benacerraf, and S. J. Burakoff. 1979. The cytolytic T lymphocyte response to trinitrophenyl-modified syngeneic cells. I. Evidence for antigen-specific helper T cells. *J. Immunol.* 123:1205.

24. Sherman, L. A., S. J. Burakoff, and B. Benacerraf. 1978. The induction of cytolytic T lymphocytes with specificity for 4-azophenylarsonate-coupled syngeneic cells. *J. Immunol.* 121:1432.

25. Rollinghoff, M., A. Starzuski-Powitz, K. Pfizenmaier, and H. Wagner. 1977. Cyclophosphamide-sensitive T lymphocytes suppress the in vivo generation of antigen-specific cytotoxic T lymphocytes. *J. Exp. Med.* 145:455.

26. Gronowicz, E., A. Coutinho, and F. Melchers. 1976. A plaque assay for all cells secreting immunoglobulin of a given type or class. *Eur. J. Immunol.* 6:588.

27. Abbas, A. K. 1979. Inhibition of antibody production in plasmacytoma cells. III. Interactions of suppressive and non-suppressive DNP conjugates with MOPC 315 cells. *J. Immunol.* 122:1791.

28. Hodes, R. J., and A. Singer. 1977. Cellular and genetic control of antibody responses *in vitro*. 1. Cellular requirements for the generation of genetically controlled primary IgM responses to soluble antigens. *Eur. J. Immunol.* 7:892.

29. Bystryn, J. C., G. W. Sinkind, and J. W. Uhr. 1973. Binding of antigen by immunocytes. I. Effect of ligand valence on binding affinity of MOPC 315 cells for DNP conjugates. *J. Exp. Med.* 137:301.

30. Taniguchi, M., and J. F. A. P. Miller. 1977. Enrichment of specific suppressor T cells and characterization of their surface markers. *J. Exp. Med.* 146:1450.

31. Weinberger, J. Z., R. N. Germain, S.-T. Ju, M. I. Greene, B. Benacerraf, and M. E. Dorf. 1979. Hapten-specific T cell responses to 4-hydroxy-3-nitrophenyl acetyl II. Demonstration of idioypic determinants on suppressor T cells. *J. Exp. Med.* 150:761.

32. Zinkernagel, R. M., and P. C. Doherty. 1979. Virus-specific cytotoxic T cells. role of major
histocompatibility gene complex in defining specificity and responsiveness. *Adv. Immunol* 27:52

33. Unanue, E. R., M. E. Dorf, C. S. David, and B. Benacerraf. 1974 The presence of I-region-associated antigens on B cells in molecules distinct from immunoglobulin and H-2K and H-2D. *Proc. Natl Acad. Sci U. S. A.* 71:5014