IMMUNOREGULATORY CHANGES INDUCED BY TOTAL LYMPHOID IRRADIATION

II. Development of Thymus-Leukemia Antigen-Positive and -Negative Suppressor T Cells That Differ in Their Regulatory Function*

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Studies of adult BALB/c mice given total lymphoid irradiation (TLI; 3,400 rad) have shown that both heterologous proteins and allogeneic bone marrow (BM) cells injected into irradiated mice evoke a state of tolerance rather than immunity (1). The ease of induction of tolerance to protein antigens appears to be related to the development of a population of T cells that can nonspecifically suppress the in vivo adoptive antibody response to the dinitrophenyl (DNP) hapten coupled to heterologous proteins such as bovine serum albumin (BSA) and bovine gammaglobulin (2). Similarly, the initial lack of responsiveness to allogeneic BM cells in TLI-treated mice is related to the presence of T cells that can nonspecifically suppress the mixed leukocyte reaction (MLR) in vitro and graft-vs.-host disease in vivo (3).

Our recent work demonstrated the presence of a population of spleen and lymph node cells bearing the thymus-leukemia (TL) surface antigen after TLI (1). The object of the present work was to determine whether these TL* cells are able to suppress the in vivo adoptive antibody response to DNP-BSA, and the in vitro proliferative response to allogeneic stimulator cells (MLR). The results suggest that two subpopulations of suppressor T cells are present in TLI-treated mice: a TL* subpopulation that mediates the suppression of the adoptive antibody response, and a TL* subpopulation that mediates the suppression of the MLR.

Materials and Methods

Animals. BALB/c (H-2b) and C57BL/Ka (H-2b) mice were obtained from the specific pathogen-free colony of Dr. Robert Kallman, Department of Radiology, Stanford University Medical School. Animals were housed in conventional animal rooms during administration of

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Abbreviations used in this paper: ABC, antigen-binding capacity; BM, bone marrow; BSA, bovine serum albumin; CFA, complete Freund’s adjuvant; DNP, dinitrophenyl; Iso-MEM, modified minimal essential medium; MLR, mixed leukocyte reaction; NMS, normal mouse serum; SRBC, sheep erythrocytes; [3H]Tdr, tritiated thymidine; TL, thymus-leukemia; TLI, total lymphoid irradiation; WBI, whole body irradiation.

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irradiation. Tetracycline (0.5 μg/ml) was added to the drinking water during TLI and for 1 wk after completion of irradiation.

Radiotherapy. TLI (3,400 rad) or WBI (500–550 rad) was administered to 4–6-mo-old BALB/c mice as described previously (3). TLI was given to ports including the cervical, axillary, mediastinal, para-aortic, and inguinal lymph nodes, as well as to the spleen and thymus (3). 17 fractions of 200 rad each were administered 5 d/wk during a period of 3–4 wk.

Preparation of Cells. Spleen cell suspensions were prepared by gently pressing spleen fragments through a nylon fiber mesh (Tetko, Inc., Elmsford, N. Y.) into cold modified minimal essential medium (Iso-MEM; Grand Island Biological Co., Grand Island, N. Y.), as described before (3).

Thymectomy. Thymectomy was performed on 2–3-mo-old BALB/c male mice using ether or sodium pentobarbital for anesthesia. The incision of the neck was closed with surgical clips. At 7–8 mo of age, half the mice were given TLI (total dose 3,400 rad), and the remainder served as thymectomized, nonirradiated controls. Each animal was carefully checked for the presence of residual thymic tissue in the mediastinum at the time of killing, and individual mice were eliminated from the study if thymectomy was incomplete.

Preparation of Antigens. BSA (Cohn fraction V; Sigma Chemical Co., St. Louis, Mo.) was dissolved in saline at a final concentration of 20 mg/ml before mixing with complete Freund's adjuvant (CFA). BSA was dimethylated using a modification of the procedure of Eisen (4). Readings of the absorbance of the conjugated protein at 280 and 360 nm yielded a value of 8.5 mg protein/ml and 10 DNP groups per molecule of BSA.

Immunization Procedures. Donors of carrier (BSA)-primed T cells were immunized 8–12 wk before cell transfer with 500 μg BSA, by an intraperitoneal injection of an emulsion of equal volumes of BSA in saline and CFA (Difco Laboratories, Detroit, Mich). Donors of hapten (DNP)-primed B cells were immunized with 500 μg DNP-BSA in CFA as described above.

Antiserum. Anti-Thy-1.2 antiserum was obtained by multiple immunizations of AKR/J mice with C3H/Km thymocytes, according to the method of Reif and Allen (5). The first immunization included 1 x 10⁸ Bordetella pertussis organisms (Public Health Dept., Commonwealth of Massachusetts) as adjuvant. Anti-TL antiserum [(C57 × A/Tla)F₁ anti-ASL₁] was kindly provided by Dr. E. S. Vitetta, University of Texas, Dallas.

Preparation of Purified T Cells. Cell suspensions of mesenteric lymph nodes taken from BSA-primed donors 8 wk after immunization were applied to columns of nylon wool (LP-1 Leuko-Pak filter; Fenwal Laboratories, Morton Grove, Ill.), as described by Julius et al. (6). Nylon wool was washed before use by boiling in distilled water, and dried in an oven. Approximately 3.6 g of nylon wool was gently picked free of clumps, and the wool was packed to the 50-ml mark in 60-ml syringes. The column was wet with Iso-MEM containing 5% heat-inactivated fetal calf serum (Grand Island Biological Co.), and allowed to equilibrate for 1 h at 37°C. Approximately 65 x 10⁶ cells in 1.5 ml were loaded in each column, and the columns were reincubated at 37°C for 45 min. Nonadherent cells were removed from the column with an excess (120 ml) of medium by gently flushing the column. Direct cytotoxicity of the effluent cells using the anti-Thy-1.2 alloantiserum and complement resulted in lysis of 85% or more of the nonadherent population.

Preparation of DNP-primed B Cells. T cells were depleted from spleen cell suspensions prepared from DNP-BSA-primed donors by a two-stage in vitro cytotoxicity procedure. Cells at 10 x 10⁶/ml were incubated with anti-Thy-1.2 serum (final dilution, 1:5 in Iso-MEM) for 45 min at 4°C. The suspension was spun down and the cells were resuspended in an equal volume of agarose-absorbed rabbit serum, diluted 1:12 (complement source). The reaction mixture was incubated for 45 min at 37°C. The remaining cells were spun down, washed twice, and resuspended in Iso-MEM before injection into adoptive hosts. Approximately 40–55% of the spleen cells were lysed by this procedure.

Treatment of Cells from TLI-treated Donors with Antiserum and Complement. Spleen cell suspensions were prepared from mice after the completion of TLI. Cells (10 x 10⁶/ml) were incubated for 45 min at 4°C with anti-Thy-1.2 or anti-TL antiserum (final dilution of anti-Thy-1.2 antiserum, 1:5; final dilution of anti-TL antiserum, 1:15). Cells were collected by centrifugation, resuspended in an equal volume of a 1:12 dilution of agarose-absorbed rabbit complement, and incubated for 45 min at 37°C. Residual spleen cells were spun down and resuspended in Iso-MEM for injection, or resuspended in complete medium for assay in the MLR. In the case of
cells to be used in the MLR assay, complement treatment was repeated twice to ensure complete lysis of all target cells.

**Cell Transfer System.** 3-4-mo-old BALB/c mice were given 500 rad WBI. 1 d later, they were injected intravenously with limiting numbers ($5 \times 10^6$) of BSA-primed, nylon-wool-purified T cells and an excess ($5 \times 10^6$) of DNP-primed, anti-Thy-1.2-treated B cells. The B cells were determined to be in excess in a series of experiments in which animals reconstituted with $10 \times 10^6$ T cells and graded numbers of purified B cells showed identical, maximal levels of the anti-DNP antibody response with cell doses between $5 \times 10^6$ and $25 \times 10^6$ B cells. The dose of $5 \times 10^6$ BSA-primed T cells was determined to be on the linear portion of the dose response curve in a similar experiment (data not shown). Adoptive hosts were challenged with 200 μg DNP-BSA in saline intraperitoneally 1 d after cell transfer. The animals had a combination of antibiotics consisting of 10 mg/ml polymyxin B (Sigma Chemical Co.) and 50 mg/ml neomycin sulfate (Pharma-Tek, Inc., Huntington, N. Y.) added to their drinking water from 1 wk before irradiation to 2 wk after cell transfer. The recipients were housed in conventional animal rooms with food and water ad libitum.

**Antibody Titration.** Mice were bled from the retro-orbital plexus at weekly intervals after antigenic challenge. The serum was separated by centrifugation and stored at $-20^\circ$C. The anti-DNP content of the sera was calculated in terms of antigen-binding capacity (ABC), using $^3$H-DNP-lysine (New England Nuclear Corp., Boston, Mass.) as the ligand in a modification of the Farr assay (7). Each serum sample was assayed in triplicate. The mean ABC determination from groups of four animals was calculated as the mean of individual serum ABC within that group.

**Assay for Suppression of the Adoptive Antibody Response.** In all experiments designed to determine the suppressor cell activity of spleen cells from TLI-treated donors, adoptive hosts received 5 x $10^6$ DNP-primed B cells, $5 \times 10^6$ BSA-primed T cells, and $25 \times 10^6$ cells from donors given TLI. Recipients were challenged with DNP-BSA, and serum antibodies were measured as described above.

**MLR.** Responder ($1 \times 10^6$) and stimulator ($1 \times 10^6$) cells were cultured in a final volume of 0.2 ml/well, using flat-bottomed microculture plates (3596; Costar, Data Packaging, Cambridge, Mass.). The cells were incubated in RPMI-1640 medium supplemented with 10% human AB serum (batch A481917), 2 mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 10 mM Hepes buffer (all from Grand Island Biological Co.). Cultures were incubated for 96 h at 37°C in an atmosphere of 5% CO$_2$ in air. Stimulator cells were treated with 3,400 rad from a 137Cesium source (Mark 1 model 25 irradiator, J. L. Shepherd and Associates, Glendale, Calif.) just before culture.

DNA synthesis was assayed by the addition of 1.0 μCi tritiated thymidine ($^3$H]Tdr, sp ac 6.7 Ci/mM; New England Nuclear Corp.) to each culture during the final 18 h of the incubation period. Cultures were harvested onto glass fiber filter paper (Whatman, Inc., Clifton, N. J.) by means of an automated sample harvester (Bio-Plastics, Redwood City, Calif.). The filters were washed with saline, dried, and placed into a scintillation cocktail composed of spectrofluor (Amersham Corp., Arlington Heights, Ill.) and toluene. Isotope incorporation was measured in a liquid scintillation counter (Beckman Instruments, Inc., Fullerton, Calif.).

**MLR-Suppressor Assay.** Culture conditions were as described for the MLR except that $1 \times 10^6$ responder, stimulator, and putative suppressor cells were incubated in a final volume of 0.3 ml/well. Stimulator and putative suppressor cells were treated in vitro with 3,400 and 1,500 rad, respectively, before assay. Data shown for all MLR cultures are the mean cpm and the SE of triplicate cultures. The stimulation index was calculated as the ratio of cpm from cultures containing allogeneic responder and stimulator cells to cpm from cultures containing syngeneic responder and stimulator cells. The following formula was used to calculate suppression:

$$\text{percent suppression} = 1 - \frac{\text{cpm with putative suppressor cells}}{\text{cpm with control cells}} \times 100.$$
transferred to adoptive hosts along with BSA-primed T cells (5 × 10^6) and DNP-primed B cells (5 × 10^6). In both cases, putative suppressor cells were obtained \sim 1\text{ mo} after irradiation. Recipient mice were challenged intraperitoneally with DNP-BSA in saline, and anti-DNP serum antibodies were determined. Table I shows that spleen cells from TLI-treated donors, but not from donors treated with sublethal WBI, suppressed the adoptive anti-DNP response on days 14 and 21 by 99 and 92% as compared with that observed with 25 × 10^6 cells from normal (nonirradiated) BALB/c mice. Suppression of the response was evident at all times measured up to 35 d after challenge (data not shown). In addition, the level of suppression in different experiments varied somewhat. The mean percent suppression from five experiments was 89% at day 14 and 86% at day 21 after challenge. The injection of 25 × 10^6 normal cells appeared to have no substantial effect on the magnitude of the response exhibited by primed T and B cells alone. The responses of adoptive hosts receiving 25 × 10^6 cells from normal or WBI-treated donors were similar (Table I).

**Persistence of Suppressor Cells of the Adoptive Anti-DNP Responses after TLI.** To determine the kinetics of suppressor cell activity after completion of TLI, spleen cells were obtained from mice at various times after radiotherapy. Cells obtained from mice at similar times after a single dose of WBI served as controls. As seen in Table II, transfer of 25 × 10^6 spleen cells obtained from TLI-treated donors 1 mo after irradiation resulted in suppression of the adoptive anti-DNP response by \geq 90% as compared with transfer of a similar number of cells obtained from WBI donors at the same time after irradiation. Similar results were obtained at 3 and 5 mo after TLI (Table II). Although cells taken from TLI-treated donors within 1 mo after irradiation appeared to show greater suppression than spleen cells taken at later times after TLI (~90% suppression vs. ~80% suppression, Table II), variability in the level of suppression between similar experimental groups could account for these differences.

**Surface Markers of TLI-induced Suppressor Cells of the Adoptive Anti-DNP Response.** The surface markers of the suppressor cells of the adoptive anti-DNP response were examined by incubating the spleen cells from TLI donors in vitro with anti-Thy-1.2 or anti-TL antisera and complement before transfer to adoptive hosts. In each case,

### Table I

| BSA-primed T cells* | DNP-primed B cells | Putative suppressor cells | Treatment of donor of suppressor cells‡ | ABC§ |
|---------------------|---------------------|--------------------------|-----------------------------------------|------|
| +                   | +                   | −                        | None (normal)                           | 0.14 ± 0.02 |
| +                   | +                   | +                        | WBI (550 rad)                           | 0.12 ± 0.01 |
| +                   | +                   | +                        | TLI (3,400 rad)                         | 0.001 ± 0.001 |
| +                   | −                   | −                        |                                         | <0.001 |
| +                   | −                   | −                        |                                         | <0.001 |

* Sublethally irradiated (500 rad) mice were injected with 5 × 10^6 BSA-primed T cells and 5 × 10^6 DNP-primed B cells.
‡ Adoptive hosts also received 25 × 10^6 cells taken from TLI-treated or WBI donors 30 d after completion of irradiation. Adoptive hosts were challenged with DNP-BSA 1 d after cell transfer.
§ ABC of serum was measured at days 14 and 21 after antigenic challenge. The mean of four mice ± SD is shown for each group. One of five experiments is shown.
TABLE II
Persistence of Suppressor Cells of the Adoptive Anti-DNP Response after Completion of TLI

| Treatment of donor of suppressor cell | Mo after irradiation | ABC§ | | | |
|-------------------------------------|----------------------|------| | | |
|                                     |                      | Day 14 | Day 21 |
| WBI (550 rad)                       | 1                    | 0.12 ± .01 | 0.22 ± .02 |
| TLI (3,400 rad)                     | 1                    | 0.001 ± .001 | 0.01 ± .01 |
| WBI (550 rad)                       | 3                    | 0.07 ± .01 | 0.10 ± .02 |
| TLI (3,400 rad)                     | 3                    | 0.01 ± .01 | 0.01 ± .01 |
| WBI (550 rad)                       | 5                    | 0.12 ± .02 | 0.25 ± .03 |
| TLI (3,400 rad)                     | 5                    | 0.03 ± .03 | 0.02 ± .02 |

* Sublethally irradiated mice received 5 × 10⁶ primed T cells and 5 × 10⁶ primed B cells. Adoptive hosts were challenged with DNP-BSA 1 d after cell transfer.
‡ Adoptive hosts received 25 × 10⁶ cells taken from TLI-treated or WBI-treated donors at various times after completion of irradiation.
§ ABC of serum was measured at days 14 and 21 after antigenic challenge. The mean response of four mice ± SD is shown. One of two experiments is shown in each case.

TABLE III
Nature of the Suppressor Cell Found in TLI-treated Mice

| Treatment of donor of suppressor cell | Treatment of suppressor cell§ | ABC|| |
|--------------------------------------|--------------------------------|------|------| |
|                                     |                                | Day 14 | Day 21 |
| WBI (550 rad)                       |                                | 0.16 ± .01 | 0.16 ± .01 |
| TLI (3,400 rad)                     |                                | 0.03 ± .01 | 0.06 ± .01 |
| TLI NMS                             |                                | 0.03 ± .02 | 0.06 ± .02 |
| TLI Anti-Thy-1.2                    |                                | 0.08 ± .02 | 0.11 ± .03 |
| TLI Anti-TL                         |                                | 0.15 ± .02 | 0.20 ± .01 |

* Sublethally irradiated mice received 5 × 10⁶ primed T cells and 5 × 10⁶ primed B cells. Adoptive hosts were challenged with DNP-BSA 1 d after cell transfer.
‡ Adoptive host received 25 × 10⁶ spleen cells from mice given TLI or WBI. Spleens were obtained ~90 d after irradiation.
§ Spleen cells were treated in vitro with antisera and complement before cell transfer (described in Materials and Methods).
ABC of serum was measured 14 and 21 d after antigenic challenge. Mean of response of four mice ± SD is shown. One of four experiments is shown.

The antiserum was used at a concentration known to be on the plateau portion of the dilution curve. Control responses were those of adoptive hosts receiving 25 × 10⁶ untreated cells from WBI-treated animals or 25 × 10⁶ cells from TLI-treated animals treated with normal mouse serum (NMS) and complement before transfer. Cells were obtained 1 mo after either TLI or WBI.

Table III shows that both anti-Thy-1.2 and anti-TL treatment of spleen cells from TLI-treated donors markedly reduced their suppressive activity. In the case of treatment with the anti-TL serum, suppression was completely eliminated (Table III). The same pattern was seen in four out of four experiments (data not shown). Spleen cells from TLI-treated mice that were treated with NMS and complement before transfer still exhibited potent suppressive activity (Table III). These results suggest that the suppressor cells of the adoptive anti-DNP response are a population of T cells that bear the TL surface antigen.
Effect of Thymectomy on Anti-DNP Suppressor Cell Activity. Our recent work shows that adult thymectomy before TLI will prevent the appearance of cells bearing the TL surface antigens (TL+ cells) in the peripheral tissues of irradiated mice. It was therefore of interest to determine the effect of thymectomy on the generation of cells capable of suppressing the adoptive anti-DNP response. Adult BALB/c mice were thymectomized as described in Materials and Methods. 5–6 mo after surgery, one half of the thymectomized group was given TLI (total dose, 3,400 rad). The remainder of the mice served as thymectomized, nonirradiated control animals. Cell suspensions were prepared from spleens obtained from mice given a single dose of WBI, from non-irradiated, thymectomized mice, and from TLI-treated mice both with and without thymectomy before irradiation. Cells were obtained from all irradiated donors within 3 mo after completion of irradiation and transferred with primed T and B cells into adoptive hosts.

As seen before, spleen cells from TLI-treated animals markedly reduced the anti-DNP response of adoptive hosts, as compared with cells from WBI-treated control mice (Table IV). However, thymectomy of donor mice before administration of TLI completely eliminated the suppressor activity of the spleen cells (Table IV). Spleen cells from thymectomized, nonirradiated mice did not suppress the adoptive anti-DNP response (Table IV).

Surface Markers of TLI-induced Suppressor Cells of the MLR. It has previously been established that spleen cells obtained from mice within 40 d of completion of TLI will markedly suppress the MLR of BALB/c responders against C57BL/Ka stimulators and vice versa (3). MLR suppressor activity can be eliminated by treatment of the spleen cells with anti-Thy-1.2 antibodies and complement before assay (3). To determine whether the MLR suppressor T cells are also TL+, spleen cells obtained from mice within 15 d of completion of TLI were treated in vitro with anti-TL antiserum and complement before assay in the MLR. In each experiment, separate aliquots of the spleen cells from irradiated mice were treated either with NMS from C57BL/Ka mice or with anti-Thy-1.2 serum and complement to serve as negative controls.

### Table IV

**Effect of Thymectomy on Suppression of the Adoptive Anti-DNP Response by Spleen Cells from TLI-treated Mice**

| BSA-primed T cells* | DNP-primed B cells | Treatment of donor of suppressor cells‡ | ABC§ |
|---------------------|-------------------|---------------------------------------|------|
|                     |                   | Thymectomy Irradiation               | Day 14 | Day 21 |
| +                   | +                 | –                                    | WBI (550 rad) | 0.19 ± .01 | 0.18 ± .01 |
| +                   | –                 | +                                    | TLI (3,400 rad) | 0.004 ± .003 | 0.01 ± .01 |
| +                   | +                 | –                                    | –         | 0.14 ± .03 | 0.12 ± .01 |
| +                   | +                 | +                                    | TLI (3,400 rad) | 0.26 ± .01 | 0.23 ± .01 |

* Sublethally irradiated mice received 5 × 10⁶ primed T cells and 5 × 10⁶ primed B cells. Adoptive hosts were challenged with DNP-BSA 1 d after cell transfer.
‡ Adoptive hosts received 25 × 10⁶ spleen cells from one of the following: normal BALB/c mice, mice given WBI, or mice given TLI, with or without prior thymectomy (see text for details).
§ ABC of serum was measured 14 and 21 d after antigenic challenge. Mean of response of four mice ± SD is shown. One of two experiments is shown.
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and positive controls, respectively. All cells received 1,500 rad in vitro before addition to the MLR.

As shown in Table V, treatment of the spleen cells from TLI-treated mice with anti-Thy-1.2 antibodies and complement substantially reduced their suppressive activity. However, treatment of the same cells with an anti-TL serum and complement was ineffective in removing suppressor cell activity. Suppression mediated by these cells remained equal to that of NMS-treated cells (Table V). Thus, the MLR suppressor cells express the Thy-1+, TL− surface antigen phenotype.

**Effect of Adult Thymectomy on the Appearance of MLR Suppressor Cells after TLI.** The effect of adult thymectomy before TLI on the subsequent appearance of the MLR suppressor cells was also investigated. BALB/c mice were thymectomized as previously described, and 5–6 mo later, one half of the group of thymectomized animals received TLI (total dose, 3,400 rad). At various times after completion of TLI, spleen cells obtained from mice in both the nonirradiated, thymectomized group and the thymectomized group given TLI were assayed for suppressor cell activity in the MLR. Cells from normal (nonirradiated) BALB/c mice served as additional coculture control cells.

As shown in Table VI, cells obtained from thymectomized TLI-treated animals 7 d after completion of TLI markedly suppressed (73%) the MLR. In most cases, the addition of cells from either normal or adult thymectomized, nonirradiated mice did not affect the magnitude of the normal two-party MLR, although the addition of cells from thymectomized animals occasionally resulted in a slight reduction of the response as compared with the addition of cells from normal (untreated) mice (data not shown). In assessing suppressor activity, comparisons were made between cells from thymectomized, nonirradiated, and thymectomized, TLI-treated animals.

The pattern of persistence of the suppressor cell after TLI was also monitored in these experiments, using cells obtained from irradiated mice at various times after completion of TLI. Thymectomy before TLI appeared to have no effect on the previously demonstrated pattern of persistence of the suppressor cells (3). Suppressor activity gradually decreased with increasing time between irradiation and suppressor

### Table V

| Source of responder cell | Source of stimulator cell* | Source of cocultured cell† | Treatment of cocultured cell | Mean [3H]TdR incorporation | Percent suppression |
|-------------------------|---------------------------|----------------------------|------------------------------|---------------------------|--------------------|
| BALB/c                  | C57BL/Ka                  | BALB/c                    | 1:5 NMS + C§                 | 32,241 ± 1,071            | —                  |
| BALB/c                  | C57BL/Ka                  | TLI                       | 1:5 NMS + C'                 | 5,503 ± 659              | 83                 |
| BALB/c                  | C57BL/Ka                  | TLI                       | 1:5 anti-Thy-1.2 + C'        | 22,829 ± 971             | 29                 |
| BALB/c                  | C57BL/Ka                  | TLI                       | 1:5 anti-TL + C'             | 7,893 ± 798              | 76                 |
| BALB/c                  | C57BL/Ka                  | BALB/c                    | 1:5 NMS + C'                 | 21,129 ± 3,748           | —                  |
| BALB/c                  | C57BL/Ka                  | TLI                       | 1:5 NMS + C'                 | 6,582 ± 810             | 69                 |
| BALB/c                  | C57BL/Ka                  | TLI                       | 1:5 anti-Thy-1.2 + C'        | 18,443 ± 1,273           | 13                 |
| BALB/c                  | C57BL/Ka                  | TLI                       | 1:5 anti-TL + C'             | 7,688 ± 1,148           | 64                 |

* 1,500 rad in vitro.
† 1,500 rad in vitro.
§ C', complement.
Two representative experiments are shown.
### Table VI

*Effect of Adult Thymectomy on the Appearance of the Suppressor Cell after TLI*

| Days after TLI | Source of responder cells | Source of stimulator cells* | Source of cocultured cells‡ | Mean [¹⁴C]TdR incorporation | Percent suppression |
|---------------|---------------------------|----------------------------|------------------------------|-----------------------------|---------------------|
| 7 BALB/c      | BALB/c                    | BALB/c                     | 1,243 ± 27                   |                             |                     |
|               | BALB/c                    | C57BL/Ka                   | 35,729 ± 2,641               |                             |                     |
|               | BALB/c                    | C57BL/Ka                   | 9,684 ± 464                  |                             |                     |
| 14 BALB/c     | BALB/c                    | BALB/c                     | 2,397 ± 254                  |                             |                     |
|               | BALB/c                    | C57BL/Ka                   | 61,878 ± 2,964               |                             |                     |
|               | BALB/c                    | TLI                        | 25,898 ± 701                 |                             | 58                  |
| 21 BALB/c     | BALB/c                    | BALB/c                     | 291 ± 51                     |                             |                     |
|               | BALB/c                    | C57BL/Ka                   | 17,025 ± 750                 |                             |                     |
|               | BALB/c                    | TLI                        | 7,404 ± 1,721                |                             | 57                  |
| 35 BALB/c     | BALB/c                    | BALB/c                     | 1,228 ± 62                   |                             |                     |
|               | BALB/c                    | C57BL/Ka                   | 60,863 ± 1,861               |                             |                     |
|               | BALB/c                    | TLI                        | 36,389 ± 1,557               |                             | 40                  |
| 45 BALB/c     | BALB/c                    | BALB/c                     | 444 ± 231                    |                             |                     |
|               | BALB/c                    | C57BL/Ka                   | 23,470 ± 1,257               |                             |                     |
|               | BALB/c                    | TLI                        | 19,097 ± 1,243               |                             | 19                  |
| 55 BALB/c     | BALB/c                    | BALB/c                     | 1,196 ± 76                   |                             |                     |
|               | BALB/c                    | C57BL/Ka                   | 16,930 ± 1,237               |                             |                     |
|               | BALB/c                    | TLI                        | 18,032 ± 1,143               |                             |                     |

* 3,400 rad in vitro.
‡ 1,500 rad in vitro.
§ Tx, cells obtained from thymectomized BALB/c mice; TLI, cells obtained from thymectomized BALB/c mice given TLI.

One of two experiments at each time-point is shown.

**cell assay (Table VI). By 55 d after TLI, no suppressor activity could be demonstrated using spleen cells from TLI-treated animals (Table VI). Throughout this time course, the responses of both BALB/c and C57BL/Ka responder cells were equally suppressed (data not shown).**

### Discussion

Our previous studies have shown that the spleens of BALB/c mice given TLI contain suppressor cells of the adoptive anti-DNP antibody response and of the MLR (2, 3). Recently, we described a large subpopulation of TL⁺ cells in the spleen and lymph nodes of similarly treated mice. The object of the present study was to determine whether the suppressor cells express the TL antigen on their cell surface.

To study the suppression of humoral immune responses by spleen cells from TLI-treated mice, irradiated (500 rad) BALB/c mice were given limiting numbers of BSA-primed T cells, an excess of DNP-primed B cells, and an intraperitoneal injection of DNP-BSA in saline. Co-transfer of 25 × 10⁶ spleen cells from TLI-treated donors reduced adoptive secondary responses >80%, as compared with that observed with 25 × 10⁶ cells from animals given a single dose of sublethal (550 rad) WBI. Because the TLI-treated donors had not received any stimulating antigen before cell transfer, inhibition of the adoptive response was considered non-antigen-specific. Suppressive activity was dependent upon T cells that bear the TL surface antigen, because treatment of the spleen cells with anti-Thy-1.2 or anti-TL antiserum and complement eliminated this activity. The suppressor cells persisted in the spleen for at least 5 mo
after completion of TLI. However, no suppressor cells developed in adult mice thymectomized before the administration of radiotherapy.

Although the suppressor cells of the MLR in TLI-treated mice were removed after incubation with anti-Thy-1.2 antibodies and complement (see also ref. 3), these cells appear to differ from those that suppress the adoptive anti-DNP response. The activity of the MLR suppressors was neither eliminated by treatment with anti-TL serum and complement, nor by adult thymectomy before TLI. In addition, previous work has shown that these suppressor cells are detectable for only 30–40 d after completion of irradiation (3). These studies indicate that at least two distinct suppressor T cell populations that differ both in their cell surface antigen phenotype and in their functional capabilities are present in the peripheral lymphoid tissues after TLI (Table VII).

Nonspecific suppressor T cells of humoral antibody responses in young mice have been reported previously by Mosier and Johnson (8). These in vitro studies shown that an excess of suppressor T cells in the neonatal spleen cell population inhibits the response of the functionally mature neonatal B cells. Other investigators (9) have shown that nylon wool nonadherent spleen cells of 1-wk-old animals nonspecifically suppress both the primary and secondary plaque-forming cell responses to sheep erythrocytes in vitro.

Recently, Mosier et al. (10) examined the phenotype of the neonatal suppressor cells found in the thymus. Suppressors of the antibody response are large, rapidly dividing cells located in the outer cortex. These cells constitute a minor proportion (<5%) of the total T cell population within the thymus, and have an Lyt-1+, Lyt-2,3+, TL+ surface antigen phenotype. Although the spleen cells with similar suppressor activity were contained in a fraction of cells too small to permit direct phenotypic analysis, the authors inferred from their data that the splenic and thymic suppressor cells had the identical phenotype (10). These neonatal cells are similar to the TL+ suppressor cells found in TLI-treated mice, although the Lyt antigen phenotype of the latter cells has not yet been established.

Nonspecific suppressor cells of the MLR have also been reported previously in the spleens of adult animals. Folch and Waksman (11) described the presence of a weakly, glass-adherent, thymus-dependent suppressor cell of the MLR in the spleens of unimmunized adult rats. Removal of this cell population resulted in an increase in

| Table VII |
| Characteristics of Suppressor Cells Found in Mice after TLI |
| Suppressor cells of Adoptive anti-DNP response MLR |
| Persist more than 30 d after TLI | + | – |
| Cells found in spleen | + | + |
| Cells found in lymph node | + | – |
| Radiosensitive (≥1,500 rad) | ND* | – |
| Surface markers: Thy-1.2 | + | + |
| TL | + | – |
| Susceptible to adult thymectomy | + | – |

* Not done.
the MLR response of the remaining cells. Similarly, a nylon wool column-adherent subpopulation of splenic murine T cells has been isolated after in vitro culture of spleen cells without antigen for 3–7 d (12, 13). These cultured cells nonspecifically suppress the MLR and the in vitro induction of cell-mediated cytotoxicity (13).

The unexpected finding that two distinct suppressor T cell subpopulations appear to regulate different aspects of immune responsiveness in TLI-treated mice adds an additional level of complexity to the analysis of the cellular mechanisms that underlie the unusual effects of TLI on the immune system. The role of these suppressor cells in the induction of tolerance to heterologous proteins or transplantation antigens in TLI-treated mice (2, 14) is not clear. It is possible that the TL+ nonspecific suppressor cells differentiate into antigen-specific suppressor cells after interaction with heterologous proteins in solution. This notion is consistent with the presence of antigen-specific suppressor T cells in the spleens of TLI-treated mice tolerized to BSA by the intraperitoneal injection of the protein in saline (2).

However, it is unlikely that the TL- nonspecific suppressors of the MLR differentiate into antigen-specific suppressors that mediate transplantation tolerance after stimulation with alloantigens. Our recent data suggest that the non-antigen-specific MLR suppressors facilitate tolerance induction in allogeneic BM chimeras by the preferential inhibition of the generation of cytolytic T cells from cytolytic T cell precursors. However, the nonspecific suppressors do not inhibit the generation of a new population of antigen-specific suppressor cells that appear to maintain tolerance in the chimeras (D. P. King and S. Strober, manuscript in preparation). Thus, the two subpopulations of suppressor cells in TLI-treated mice may not only act on different target cell populations, but may also function through different mechanisms of regulator-effector cell interactions.

Summary

BALB/c mice treated with total lymphoid irradiation (TLI) develop non-antigen-specific suppressor cells of the adoptive secondary antibody response and of the mixed leukocyte reaction. Suppressors of the adoptive anti-DNP response were eliminated by incubation of spleen cells with anti-Thy-1.2 or anti-thymus-leukemia (TL) antiserum and complement before cell transfer. Thymectomy before TLI prevented the appearance of the latter suppressor cells. On the other hand, suppressors of the MLR were eliminated by incubation of spleen cells with anti-Thy-1.2 but not anti-TL antiserum and complement. Thymectomy before TLI did not prevent their subsequent development. Thus, two subpopulations of suppressor T cells that differ in the expression of the TL surface antigen, dependence on the presence of the thymus, and in regulatory functions develop after TLI. The TL+, thymus-dependent cell suppresses the adoptive antibody response, and the TL-, thymus-independent cell suppresses the MLR.

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