STUDIES ON THE SYNGENEIC MIXED LYMPHOCYTE REACTION

III. Development of a Monoclonal Antibody with Specificity for Autoreactive T Cells*

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The autologous mixed lymphocyte reaction (AMLR), is the proliferative response of T lymphocytes cultured with autologous non-T cells (1, 2). T cells, activated in the human AMLR, exhibit both immunological memory and specificity (3). The interaction of T lymphocytes with autologous or syngeneic cells in vitro results in the expression of helper, suppressor, and cytotoxic activity, as well as in the secretion of immunoregulatory soluble factors (reviewed in references 4 and 5).

T cells activated by autologous macrophages participate in the immune response to antigen (6, 7). The activation of T cells in the syngeneic mixed lymphocyte reaction (SMLR) is governed by the same genetic rules that regulate antigen-specific T cells (8–10). Finally, the SMLR is compromised in autoimmune-prone strains of mice, BXSB, MRL, and NZB, as is the AMLR in humans with diseases associated with immunological abnormalities such as systemic lupus erythematosus, Sjogren’s syndrome, infectious mononucleosis, and rheumatoid arthritis (4, 11–13). These data suggest the possibility that autoreactive T cells may be important regulatory elements in the normal immune response.

While a monoclonal antibody to a subpopulation of autoreactive T cells has been developed for human cells, this antibody did not permit a direct assessment of the biological significance of the autologous mixed lymphocyte reaction (14). For this reason we developed antibodies specific for autoreactive T cells in the mouse with the purpose of defining the relevance of the SMLR by direct in vivo manipulations. These monoclonal antibodies blocked the SMLR but did not affect the allogeneic MLR or the proliferative response of T cells to phytohemagglutinin (PHA). Similarly, splenic T cells from mice treated with these anti-autoreactive T cell antibodies exhibited normal response to antigens.

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Abbreviations used in this paper: AMLR, autologous mixed lymphocyte reaction; C, complement; ELISA, enzyme-linked immunoabsorbent assay; FCS, fetal calf serum; HAT, hypoxanthine, aminopterin, and thymidine; [3H]Tdr, methyl-tritiated thymidine; MHC, major histocompatibility complex; NMS, normal mouse serum; NWNA, nylon wool nonadherent; PHA, phytohemagglutinin; SMLR, syngeneic mixed lymphocyte reaction; TNP, trinitrophenyl.
antibodies were impaired in their capacity to proliferate in an SMLR, but had normal responses to allogeneic spleen cells and to PHA.

Materials and Methods

Animals. 2-mo-old BALB/c and C57Bl/6 male mice were obtained from Charles River Laboratories, Inc., Wilmington, MA.

Lymphocyte Preparation. Mice were sacrificed by cervical dislocation. Spleens were removed aseptically and teased apart in RPMI 1630 (Gibco Laboratories, Grand Island, NY). Single-cell suspensions were prepared by filtration through cotton gauze. Cells were collected by centrifugation and treated with 0.83% ammonium chloride-0.17 M Tris buffer, pH 7.2 to lyse erythrocytes. The cells were washed twice with RPMI 1630, resuspended, counted, and divided into stimulating and responding cell populations.

Purified responding T cell populations were prepared as described by Julius et al. (15). Briefly, nylon wool columns were prepared and washed with RPMI 1640 (Flow Laboratories, Inc., McLean, VA) supplemented with 20 mM Hepes buffer (Gibco Laboratories) and 1% fresh normal syngeneic mouse serum (NMS). The column was drained thoroughly and equilibrated in a 37°C incubator for 45–60 min. 80–100 × 10^6 spleen cells in 1–2 ml of warm media were added to the column dropwise and washed into the column with an additional 1 ml of warm media. Columns were then incubated at 37°C for 45 min in a 5% CO₂/95% air humidified atmosphere. Nylon wool nonadherent (NWNA) cells were eluted with 10–15 ml of warm media at a flow rate of one drop per second. These cells were collected by centrifugation and adjusted to 5 × 10^6/ml in culture media. The culture media consisted of RPMI 1640 supplemented with 0.5%–1.0% NMS, 20 mM Hepes, 2 mM L-glutamine (Gibco Laboratories), 5 × 10^{-3} M 2-mercaptoethanol (Eastman Organic Chemicals, Rochester, NY), 2.5% sodium pyruvate (Microbiological Associates, Walkersville, MD), 100 U/ml penicillin (Gibco), and 100 μg/ml streptomycin (Gibco). The NWNA cells contained 5% splenic cells and <2% macrophages. This preparation will be referred to as “T cells.” Stimulating cells were prepared by resuspending irradiated (5,000 rad from a cesium source) spleen cells at a concentration of 5 × 10^6/ml in the above culture media.

T-depleted populations were prepared by lysis with anti-Thy-1.2 and complement. Anti-Thy-1.2 was produced by the hybridoma cell line 30-H.12 obtained from Dr. N. L. Warner (University of New Mexico), and grown intraperitoneally in pristane-pretreated, 350 rad-irradiated, BALB/c mice. The antibody containing ascites fluid was made 50% saturated (cold) in (NH₄)₂SO₄. The precipitate was collected, washed, dissolved, dialyzed, and applied to a DE-52 column, and the rate IgG2b anti-mouse Thy-1.2 was eluted with a Tris-phosphate buffer gradient.

Splenocytes to be treated were suspended to 10^7/ml in RPMI 1640 supplemented with 5% fetal bovine serum and a 1:400 dilution of anti-Thy-1.2 for 45 min at 4°C. Cells were washed twice and resuspended to 10^7/ml in media containing a 1:12 dilution of Low Tox rabbit complement (C) (Cederlane Laboratories, Hornby, Ontario, Canada) for 45 min at 37°C. The cells were washed twice, resuspended to 10^7/ml in RPMI 1640 and cultured in 100 × 13-mm Falcon petri dishes (Falcon Labware, Oxnard, CA) at 37°C for 45 min. The nonadherent cells, referred to as “B cells,” were removed by gentle pipetting. The remaining adherent cells were removed by a 10-min exposure to a 1:5 dilution of 2% Xylocaine (Astra Pharmaceutical Products, Inc., Worcester, MA) in RPMI 1640. The adherent monocytes and the B cells were washed twice and resuspended to 10^7/ml in RPMI 1640 for incubation with the monoclonal antibody and C.

Cell Culture. SMLR or allogeneic MLR cultures were performed in triplicate in 96-well round-bottom microtiter plates (Linbro Scientific, Hamden, CT). 5 × 10^5 T cells were cultured with medium alone or mixed with an equal number of irradiated syngeneic or allogeneic spleen cells or with PHA (Burroughs-Wellcome Co., Research Triangle Park, NC) at a concentration of 2.5 μg/ml in a total volume of 0.2 ml/well. SMLR and allogeneic MLR cultures were incubated for 5 d and 4 d respectively, and PHA-stimulated cultures were incubated for 3 d in a 37°C 5% CO₂/95% air humidified atmosphere. Proliferation was measured by the incorporation of methyl tritiated thymidine ([³H]Tdr)
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during the last 8 h of culture. Cultures were harvested onto glass fiber paper (Reeve Angle; Whatman, Inc., Clifton, NJ) by a multi-well automated sample harvester (Titertek, Flow Laboratories). The glass fiber disks were placed in mini-vials with 3 ml of scintillation fluid (Liquiscint; National Diagnostics, Parsippany, NJ) and counted in a Searle Delta 300 liquid scintillation counter. Data are given as the average of triplicate samples or as the Δ cpm (cpm of T cells plus stimulators – cpm of T cells cultured alone).

Separation of Activated and Nonactivated Cells. SMLR cultures were established as described above in 25-cm² tissue culture flasks (Falcon Labware) containing equal numbers of responding and stimulating cells at 5 × 10⁶/ml. Culture flasks were maintained at 37°C in 5% CO₂/95% humidified air environment for varying lengths of time. The kinetics of the SMLR cultures were observed to peak on the same day when cultured in either microtiter plates or flasks. Cultures were separated into activated and nonactivated cells by separation over a six step Percoll (Pharmacia Fine Chemicals, Uppsala, Sweden) gradient as previously described (16). Lymphocytes from each interface were removed with a pasteur pipette, washed twice with Hanks' balanced salts solution (HBSS) (Flow Laboratories), counted, and resuspended in culture medium.

Preparation of Monoclonal Antibodies. Antibodies to T cells responding in the SMLR were prepared utilizing somatic cell hybridization as described by Kohler and Milstein (17). SMLR-activated lymphocytes were isolated as described above and mixed with complete Freund’s adjuvant and injected into the peritoneum of male BALB/c mice. This injection was followed by two intraperitoneal injections of activated cells in incomplete Freund’s adjuvant at weekly intervals and one intravenous injection of activated cells. 3 d after the last injection, spleens were removed aseptically and single-cell suspensions prepared and fused to the nonsecreting murine myeloma cell line SP 2/0-Ag14 with polyethylene glycol (PEG 1000; J.T. Baker Chemical Co., Phillipsburg, NJ). The cells were plated into multiwell plates and cultured initially in DME (Gibco) with 10% NCTC 109 (Microbiological Associates), 10% horse serum (Gibco), 1 mM oxalacetic acid (Sigma Chemical Co., St. Louis, MO), 0.45 mM pyruvic acid (Gibco), and 10⁻² M hypoxanthine, 5 × 10⁻⁵ M aminopterin, and 4 × 10⁻⁴ M thymidine (HAT) (Sigma Chemical Co.). Detection of lines producing antibodies and the classes of antibodies produced was performed using an enzyme-linked immunosorbent assay (ELISA). Specificity for the responding cells in the SMLR was initially determined by an indirect immunofluorescent assay with fluorescein-conjugated IgG goat anti-mouse Ig (N. L. Cappel Laboratories, Cochrannville, PA). Fluorescent lymphocytes were enumerated using a Zeiss fluorescent microscope. Control T cell samples were incubated with fluorescent label alone. The percent binding detected in these control populations was subtracted from the experimental results and the data expressed as the net percent fluorescence. Four clones were detected producing antibody with specificity for the responding cell in the SMLR. These have been designated SMLR-2-1, 2-2, 2-5, and 2-6. They have since been subcloned and have been stable in culture for over one year.

The IgM monoclonal antitrinitrophenyl (TNP) antibody B-22 was made by Dr. J. Gibbons. Briefly, C57BL/6 mice were immunized with 10 μg TNP-Ficoll intravenously and spleens were removed 4 d later and fused to the nonproducing X-68 Ag 8.653 cell line. Specificity for the TNP determinant was assessed by ELISA and by a direct plaque-forming cell response against TNP-SRBC target cells.

Purification of Monoclonal Antibodies. One antibody, SMLR-2-1, was purified by affinity chromatography using a Sepharose 4B-coupled goat anti-mouse IgM column. Hybridoma supernatant, concentrated three- to fivefold by ultrafiltration, was passed through the column, and bound material eluted with 3 M MgCl₂. This column did not retain any horse serum protein. Fractions that contained antibody were pooled and dialyzed against multiple changes of phosphate-buffered saline (PBS). The amount of protein present was determined from the absorbance at 280 nm.

In Vitro Use of Monoclonal Antibodies. The purified antibody was used in vitro in blocking studies as well as in complement-dependent cytotoxicity assays. For the blocking studies, T cells and irradiated syngeneic and allogeneic spleen cells as well as PHA were cultured.
as described above with 50 μg/ml, 5 μg/ml, and 0.5 μg/ml purified monoclonal antibody present for the entire culture period. For the cytotoxicity assays, 10⁷ T cells in 1 ml of RPMI 1640 buffered with 10 mM Hepes and 10 μg of antibody were incubated for 45 min at 4°C. After incubation the cells were washed twice and resuspended in 1 ml of a 1:6 dilution of complement and incubated for an additional 60 min at 37°C. Cells were washed three times and resuspended at 5 × 10⁵/ml in culture media containing 0.5–1.0% NMS.

Treatment of Mice with Monoclonal Antibody to Autoreactive T Cells. Two treatment schedules were used to deplete the autoreactive T cell population in vivo. In the first, BALB/c mice were injected intravenously with 20 μg of affinity-purified SMLR-2-1 for 5 d. Control mice received affinity-purified monoclonal anti-TNP antibody for the same time period. In the second, BALB/c mice were injected with 0.4 cm³ of threefold concentrated SMLR-2-1 culture supernatant three times weekly for 4 wk (calculated to be 20–25 μg/injection). Control animals received the same quantity of concentrated SP 2/0-Ag 14 supernatant. 3 d after the final injection, the animals were sacrificed and their T cells cultured with syngeneic spleen cells, allogeneic spleen cells, or PHA.

Results

SMLR in the Presence of Syngeneic Sera. Recently, it has been reported that the proliferative response observed in the human AMLR may be a major histocompatibility (MHC)-restricted response to xenoantigens, e.g., fetal calf serum (FCS) or sheep erythrocytes (17). Lymphocytes are frequently exposed to these antigens during the separation of T and non-T cells. Methods used to separate and culture mouse lymphocytes in the SMLR also frequently used FCS. To eliminate the possibility that the T cells activated in the SMLR had specificity for FCS, we have routinely used fresh syngeneic NMS in place of FCS during the nylon wool separation of T and non-T cells in culture.

The data presented in Table I demonstrate that there is no difference in the amount of [³H]Tdr incorporated at the peak of the SMLR in the presence of NMS or FCS. The peak proliferative response occurred on the fifth day of the SMLR in the presence of either serum (data not shown). It should be noted that the background response of T cells cultured alone for 5 d was lower (0.2–2.3 × 10³ cpm) in the presence of NMS than in the presence of FCS (0.2–5.8 × 10³ cpm). Unless stated otherwise, all experiments were carried out with 1.0% NMS as a serum supplement.

| Table I |
| --- |
| **Proliferation in the SMLR in the Presence of Syngeneic or Xenogeneic Sera** |
| **Sera** |
| **Serum source** | Mouse (15) | Fetal calf (4) |
| **Responding cell** | Syngeneic spleen cell | Cell | Serum source |
| T cells | Absent | 0.7 ± 0.2 | 1.9 ± 1.3 |
| T cells | Present | 7.9 ± 1.8 | 8.5 ± 1.1 |

* 5 × 10⁶ splenic T cells from BALB/c mice were cultured for 5 d with medium alone or with an equal number of irradiated syngeneic spleen cells. Culture medium was supplemented with either 1% NMS or 10% FCS. Cultures were pulsed with [³H]Tdr for the last 8 h of culture. The data are presented as the mean ± SEM.

† The number of experiments is indicated in parentheses.
Separation of Activated from Nonactivated Cells on Percoll Gradients After an SMLR. The densities of activated and nonactivated lymphocytes differ, and this parameter was used to separate activated from resting lymphocytes. Lymphocytes from a primary SMLR were fractionated on discontinuous Percoll gradients as previously described (15). >90% of the cells at the 50–55% Percoll interface were large lymphoblasts. These cells incorporated 75% (25,846 cpm) of the total (34,168 cpm) [3H]Tdr. Cells at the 55–60% and 60–70% Percoll interfaces were a mixture of large and small lymphocytes. The cells from these interfaces each incorporated <15% of the [3H]Tdr incorporated by cells from the 50–55% interface (55%/60% = 11.7%; 60%/70% = 11.6%). Virtually all of the cells were viable cells.

Production of Monoclonal Antibodies with Specificity for T Cells Activated in the SMLR. 4–6-wk-old male BALB/c mice were immunized and subsequently boosted three times with activated cells (50%/55% Percoll interface) from an SMLR. After fusion with the HAT-sensitive SP2/0-Ag 14 cell line, supernatants from wells containing growing cell lines were tested for the presence of immunoglobulin by ELISA and for binding of lymphocytes by indirect immunofluorescence (Table II). Supernatant from four lines contained Ig. Three antibodies were of the IgM class and one of the IgG class. Supernatant medium from one actively growing clone (SMLR-2-3) had no detectable IgG or IgM.

The monoclonal antibodies raised to SMLR-activated T cells stained the majority (57–81%) of BALB/c lymphoblasts from an SMLR, but only a small percentage (6–8%) of fresh BALB/c splenic T cells (Table II). The reactivity of SMLR-2-1 was tested on splenic T cell preparations from C57BL/6 and LAF1 mice. As in BALB/c, we found, in two experiments, that 6–8% of the T cells reacted with this antibody. These fresh T cells were small lymphocytes as determined by light microscopy. Of particular importance is that these antibodies did not react with BALB/c lymphoblasts from an allogeneic MLR. Supernatant from one actively growing clone that produced no detectable IgG or IgM (SMLR-2-3) had no detectable IgG or IgM.

### Table II

| Hybridoma supernatant | Antibody class | NWNA spleen cells | Autoactivated blasts | Alloactivated blasts |
|-----------------------|---------------|------------------|---------------------|---------------------|
| SMLR-2-1              | IgM           | 6                | 65                  | 7                   |
| SMLR-2-2              | IgM           | 7                | 81                  | 3                   |
| SMLR-2-3              | IgG           | 1                | <1                  | ND                  |
| SMLR-2-5              | IgG           | 8                | 57                  | <1                  |
| SMLR-2-6              | IgM           | 7                | 81                  | 4                   |
| B-22                  | IgG           | ND               | <1                  | 1                   |

*Activated cells from a syngeneic or allogeneic MLR or fresh NWNA spleen cells were separated and prepared for indirect immunofluorescence as described in Materials and Methods. Hybridoma supernatant (100 µl of a 1:5 dilution) was added to 10⁶ cells in 100 µl and incubated for 30 min on ice followed by an additional incubation with the fluorescent antibody. 200 cells were counted per slide. B-22 is a monoclonal IgM anti-TNP antibody.
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2-3) and B-22, an IgM antibody with specificity for the TNP determinant, did not react with any of the cell populations tested.

The monoclonal antibody SMLR-2-1 did not react with B lymphocytes or monocytes. The data shown in Table III demonstrates that 7.5% of T cells incubated with this antibody and C were killed. There was no significant killing of B lymphocytes or monocytes.

Inhibition of the SMLR by Monoclonal Antibodies. Supernatants from SMLR-2-1, SMLR-2-6, and the anti-TNP B-22 cell lines as well as fresh hybridoma medium were added to wells containing T lymphocytes and syngeneic spleen cells, allogeneic spleen cells, or PHA at the initiation of culture. Supernatants from SMLR-2-1 and SMLR-2-6 markedly inhibited the SMLR by >80% but had little effect on the proliferative response induced by allogeneic (C57Bl/6) spleen cells or PHA (Table IV). This selective inhibition could be due to a non-Ig-suppressive

| Table III |
| Specificity of SMLR-2-1 for Responder T Cells |

| Cells    | Treatment | Dead cells (%) |
|----------|-----------|---------------|
| T cells  | SMLR-2-1 + C | 15.5          |
|          | NMS + C   | 8.0           |
| B cells  | SMLR-2-1 + C | 7.1          |
|          | NMS + C   | 9.0           |
| Macrophages | SMLR-2-1 + C | 8.2 |
|          | NMS + C   | 7.5           |

T cells, B cells, and adherent macrophages were incubated with 10 μg of affinity-purified monoclonal SMLR-2-1 or NMS for 45 min at 4°C. The cell populations were then washed twice and incubated for 1 h at 37°C with a 1/4 dilution Low Tox rabbit C. The viability of the recovered cells was determined by trypan blue exclusion. The percentage of dead cells was determined by dividing the number of blue cells by the total number of recovered cells.

| Table IV |
| Effect of Hybridoma Supernatants on the SMLR |

| Stimulus | Hybridoma supernatant | Media | Spleen cells | PHA (cpm × 10^-3) |
|----------|-----------------------|-------|--------------|------------------|
|          | Culture media         | 1.6   | 11.2 39.8    | 97.2             |
|          | B-22                  | 1.4   | 10.1 41.3    | 91.1             |
|          | SMLR-2-1              | 1.4   | 2.2 44.4     | 71.1             |
|          | SMLR-2-6              | 1.5   | 1.9 37.8     | 72.4             |

Supernatants from actively growing hybridoma cell lines were added to BALB/c T cells cultured with syngeneic non-T cells, allogeneic non-T cells, or 2.5 μg/ml PHA at a final dilution of 1:5. Proliferation was measured at day 5 for the SMLR, on day 4 for the allogeneic on MLR, and at day 3 for the PHA response. B-22 is an IgM anti-TNP monoclonal antibody made in our laboratory. Data are the average of triplicate samples.
material present in the supernatants. In order to eliminate this possibility, the monoclonal antibody produced by clone SMLR-2-1 was purified on a goat antimouse IgM affinity column. This purified antibody was added at the initiation of cultures containing BALB/c T cells incubated with syngeneic spleen cells or allogeneic (C57BL/6) spleen cells. The results presented in Table V demonstrate that affinity-purified SMLR-2-1 antibody at a final concentration of 5 µg/ml totally inhibits the SMLR of lymphocytes from BALB mice. In contrast, the allogeneic MLR and the PHA response were not altered by the presence of a 10-fold excess of this antibody. This same concentration of SMLR-2-1 also markedly inhibited the SMLR of lymphocytes from C57BL/6 mice, without diminishing the allogeneic MLR to BALB/c stimulators. Incorporation of \([^{3}\text{H}]\text{TdR}\) by SMLR cultures was decreased from 7,000 to 1,000 cpm by the addition of 5 µg/ml of SMLR-2-1. In contrast, thymidine incorporation during an allogeneic MLR increased from 16,000 to 27,000 cpm in the presence of SMLR-2-1. It should be pointed out that the addition of SMLR-2-1 to T cells cultured alone did not alter the background \([^{3}\text{H}]\text{TdR}\) incorporation (data not shown). These data demonstrate that IgM antibody in the hybridoma supernatant from the SMLR-2-1 hybridoma specifically blocks the SMLR of two H-2 disparate strains.

Since the magnitude of the proliferative response induced by PHA was so much greater than that of the SMLR, an effect of SMLR-2-1 on this reaction might be obscured. For this reason we have measured the effect of SMLR-2-1 on the proliferative response induced by a wide range of PHA concentrations. At the lowest concentration of PHA tested, thymidine incorporation was comparable in magnitude to that of the SMLR. The monoclonal antibody had no effect on \([^{3}\text{H}]\text{TdR}\) incorporation at any dose of PHA tested (Table VI).

### Table V

**Specific Inhibition of the SMLR by Affinity-purified Monoclonal Antibody**

| Stimulus          | \([^{3}\text{H}]\text{TdR}\) incorporation | Amount of SMLR-2-1 antibody added to cultures |
|-------------------|-------------------------------------------|----------------------------------------------|
|                   |                                           | None  | 0.5 µg/ml | 5 µg/ml | 50 µg/ml |
|                   |                                           | cpm × 10^-3 |   |   |   |
| Exp. 1            |                                           |       | 50      |       |       |
| PHA               | 16.1                                      | 16.0  | 16.3    |       |       |
| Allogeneic SCx    | 28.4                                      | 28.7  | 28.7    | 29.0  |       |
| Syngeneic SCx     | 6.9                                       | 6.8   | 0       | .5    |       |
| Exp. 2            |                                           |       |         |       |       |
| PHA               | 75.5                                      | 77.0  | 76.3    | 74.0  |       |
| Allogeneic SCx    | 22.6                                      | 24.2  | 23.1    | 23.3  |       |
| Syngeneic SCx     | 12.9                                      | 6.3   | 0.1     | 0     |       |

\(5 \times 10^3\) T cells were incubated for 5 d with PHA (2.5 µg/ml) or 5 d with an equal number of irradiated allogeneic or syngeneic spleen cells (SCx). Cultures were pulsed with \([^{3}\text{H}]\text{TdR}\) during the last 8 h of culture. Where indicated, SMLR-2-1 antibody was added in the amounts specified for the entire culture period.
**TABLE VI**

*Failure of SMLR-2-1 to Inhibit Mitogenicity by PHA*

| SMLR-2-1 | Amount of PHA added to culture | [\(^3\)H]Tdr incorporation $\times 10^3$ |
|----------|-------------------------------|----------------------------------------|
|          | None                          | -                                      |
|          | 0.25 µg/ml                    | 0.3                                    |
|          | 0.5 µg/ml                     | 7.3                                    |
|          | 1.0 µg/ml                     | 20.0                                   |
|          | 2.5 µg/ml                     | 44.5                                   |
|          | 2.0 µg/ml                     | 43.8                                   |

5 x 10^5 T cells were incubated with four different concentrations of PHA for 3 d in the presence or absence of 5 µg/ml affinity-purified SMLR-2-1. Reactivity was determined by the incorporation of [\(^3\)H]Tdr on the third day of culture.

**TABLE VII**

*Deletion of the Autoresponsive Cell in the SMLR by Monoclonal SMLR-2-1 and Complement*

| Treatment of cells | [\(^3\)H]Tdr incorporated $\times 10^3$ |
|--------------------|----------------------------------------|
|                    | Syngeneic spleen  | Allogeneic spleen  | PHA               |
| None               | 15.0                 | 38.2                 | 110.1             |
| C only             | 12.1                 | 49.5                 | 115.3             |
| SMLR-2-1 + C       | 4.4                 | 45.5                 | 134.9             |

10^7 BALB/c T cells in 1 ml of RPMI 1640 with 5% FCS and 10 µg of antibody were incubated for 45 min at room temperature. After incubation the cells were washed twice and resuspended in 1 ml of a 1:6 dilution of complement and incubated for 45 min at 37°C. Cells were again washed and resuspended in culture media at 5 x 10^6 viable cells/ml. The results are the mean of two experiments.

**Deletion of the Autoresponsive T Cell in the SMLR.** In order to demonstrate that these antibodies were inhibiting the functional capability of the responding cell and not the stimulating cell population, we treated the responding T cell population with the monoclonal antibody SMLR-2-1 and complement before culture. Control populations were treated with media only or with complement alone. In three experiments, treatment of the responding T cell population with affinity-purified antibody SMLR-2-1 and complement decreased the response to syngeneic spleen cells by an average of 59% but did not alter the response to allogeneic spleen cells or PHA (Table VII). Treatment with complement alone did not substantially alter the SMLR, the allogeneic MLR, or the PHA response. In addition, treatment of stimulator spleen populations with the monoclonal antibody and C did not alter their ability to stimulate an SMLR. T cells stimulated with splenocytes treated with C alone incorporated a similar amount of [\(^3\)H]Tdr as T cells responding to stimulators treated with SMLR-2-1 and C before culture (26,000 cpm vs. 22,000 cpm). Thus, the results presented in Tables IV–VII demonstrate that the monoclonal antibody SMLR-2-1 specifically inhibits the SMLR by recognizing a determinant on T cells that responds in the SMLR.

**In Vivo Depletion of T Cell Activated in the SMLR.** We next tested the capacity...
Table VIII

Treatment of BALB/c Mice with Monoclonal Antibody

| Stimulus in vitro | Treatment in vivo | [3H]Tdr incorporation \( \text{cpm} \times 10^{-3} \) | Inhibition | % |
|------------------|------------------|---------------------------------|------------|---|
| Protocol 1        |                  |                                 |            |   |
| Syngeneic spleen  | None             | 37.0                            | -          |   |
| Syngeneic spleen  | B-22             | 33.9                            | 8          |   |
| Syngeneic spleen  | SMLR-2-1         | 17.8                            | 52         |   |
| Allogeneic spleen | None             | 26.7                            | -          |   |
| Allogeneic spleen | SMLR-2-1         | 38.4                            | 0          |   |
| Protocol 2        |                  |                                 |            |   |
| Syngeneic spleen  | None             | 10.0                            | -          |   |
| Syngeneic spleen  | SP2/0            | 9.0                             | 10         |   |
| Syngeneic spleen  | SMLR-2-1         | 1.7                             | 83         |   |
| Allogeneic spleen | None             | 26.5                            | -          |   |
| Allogeneic spleen | SP2/0            | 25.4                            | 4          |   |
| Allogeneic spleen | SMLR-2-1         | 27.1                            | 0          |   |
| PHA               | None             | 109.5                           | -          |   |
| PHA               | SP2/0            | 102.8                           | 6          |   |
| PHA               | SMLR-2-1         | 114.0                           | 0          |   |

BALB/c mice received five daily intravenous injections of either 20 μg affinity-purified SMLR-2-1 or B-22 (protocol 1) or 0.4 cm³ of supernatant medium from the hybridoma or SP2/0 concentrated threefold and injected three times weekly for 4 wk (protocol 2). Splenic T cells from treated mice were cultured with syngeneic or allogeneic spleen cells or with PHA. The data in protocol 1 represents the results of two experiments; the data in protocol 2 represents the results of four experiments.

Discussion

We have raised monoclonal antibodies to T lymphocytes from BALB/c mice activated in the SMLR. The SMLR was carried out in culture medium that contained fresh syngeneic serum but no xenogeneic serum. Thus, the proliferative response in the SMLR did not depend upon exposure of lymphocytes to known xenogeneic antigens. The human and guinea pig autologous mixed lymphocyte reaction can also be generated in the absence of foreign serum during the isolation or culture of the lymphocytes (9, 10, 21). Glimcher and her associates (8) showed that the SMLR did not depend upon exposure to foreign antigens during the maturation of T cells in vivo or during the preparation of these antibodies to regulate autoreactive T cells in vivo. BALB/c mice were treated with monoclonal antibody. The SMLR of splenic T cells from mice that received intravenous injections of SMLR-2-1 were inhibited between 52% and 83%. The allogeneic MLR and the PHA response were unaltered (Table VIII).

The SMLR-2-1 antibody did not significantly alter the capacity of spleen cells to stimulate SMLR. Spleen cells from SMLR-2-1–treated mice and from control mice stimulated comparable amounts of [3H]Tdr by control T cell populations. A mean 87% of normal stimulatory capacity remained in three experiments. This contrasted with the total ablation of the capacity of T cells from SMLR-2-1–treated animals to respond to syngeneic spleen cells.
cells for culture or during the culture period. Thus, it appears reasonably certain that the SMLR does not depend upon exposure to foreign antigens.

Conversely, T cell responses to foreign antigens in vitro appear to require the participation of autoreactive T cells (6, 7, 9). Elimination of autoreactive T cells using bromodeoxyuridine and light abrogated the primary and secondary response of T cells to a number of soluble antigens (7, 9). Furthermore, the SMLR is capable of facilitating the proliferation of T cells to foreign determinants that themselves are unable to stimulate the proliferation of T cells or their differentiation into cytolytic T cells (19, 20). This mechanism probably explains the increased thymidine incorporation observed when lymphocytes are exposed to foreign antigens before or during the syngeneic or autologous MLR (18).

The monoclonal antibodies raised to BALB/c T cells activated in the SMLR inhibited the response of T cells from BALB/c to syngeneic non-T cells, but did not inhibit their response to allogeneic non-T cells or to PHA. Autoactivated T cells express Ia and other activation antigens (22). It was therefore possible that the monoclonal antibodies reacted with either the responding or stimulatory cells, or both. As these antibodies do not react with alloactivated T cells, it appeared that the antibodies are not directed at activation antigens per se. The fact that pretreatment of the responding T cells with the monoclonal antibody and complement as well as the finding that treatment of mice with monoclonal antibody in vivo selectively inhibited the capacity of splenic T cells to respond in the SMLR supports the conclusion that the antibody reacts with a population of T cells that includes autoreactive T cells.

The monoclonal antibodies react with a cell surface determinant on a small percentage (6–9%) of splenic T cells from H-2 disparate strains and isolated alloactivated lymphoblasts. They do not react with cells required to stimulate an SMLR. In contrast, 50–80% of isolated autoactivated lymphoblasts react with these antibodies. This suggests that this antibody does not react with an activation antigen, an MHC-restricted determinant or its receptor on T cells.

Autoreactive T cells are increased in animals immunized with protein antigens (23–25). Some antigen-induced T cells can be activated in the absence of antigen by self-Ia determinants, although their response is augmented in the presence of nominal antigen (23, 24). It is possible that these cells proliferate in the SMLR. Thus, those cells induced during an immune response may function in a nonspecific manner, as they can be restimulated by autologous accessory cells in the absence of nominal antigen. Recently, Zauberer and his colleagues (25) have recovered autoreactive clones from mice immunized with protein antigens in vivo after restimulation with syngeneic accessory cells in vitro. These cells express nonspecific helper activity, facilitating proliferation of antigen-specific B cells and their secretion of specific antibody. Autoreactive T cells were not found before immunization.

Immunization may stimulate T cells with a range of affinity for self-Ia determinants. Some T cells may have low affinity receptors for syngeneic self-Ia determinants and be totally dependent upon antigen-pulsed syngeneic accessory cells for activation. Other T cells may have higher affinity for self-Ia determinants and be stimulated by accessory cells alone, but are stimulated to a greater extent by antigen-pulsed syngeneic accessory cells. Finally, there may be a population
of T cells activated in the normal immune response with high affinity for self-Ia whose maximal response is induced by accessory cells alone. If autoreactive T cells are essential for the development of an immune response to antigens, as suggested by in vitro and in vivo studies, one would predict that mice depleted of autoreactive cells by treatment with these monoclonal antibodies would have immunological defects. We are now testing this hypothesis.

Summary

Monoclonal antibodies with specificity for autoreactive murine T cells have been developed. These antibodies inhibit proliferative response of splenic T cells activated by syngeneic spleen cells. These antibodies have no effect on the proliferative response of T cells activated by allogeneic spleen cells or PHA. The number of splenic T cells that react with these monoclonal antibodies is comparable in several normal mouse strains.

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