NORMAL Lyt-1+2- T CELLS HAVE THE UNIQUE CAPACITY TO RESPOND TO SYNGENEIC AUTOREACTIVE T CELLS
Demonstration of a T Cell Network

By PRAKASH S. NAGARKATTI, MITZI NAGARKATTI, AND ALAN M. KAPLAN

From the Department of Medical Microbiology and Immunology, College of Medicine, University of Kentucky, Lexington, Kentucky 40536-0084

T helper cells (Lyt-1+/L3T4+) have been shown to interact with macrophages and B cells in a major histocompatibility complex (MHC)-restricted manner as well as with other T cells in a receptor-directed regulatory idiotype network (reviewed in 1–4). Recently (5), we isolated and characterized several self-Ia-reactive (autoreactive), Lyt-1+2-, Ia- T cell clones from normal, unimmunized DBA/2 mice. These autoreactive T cell clones, isolated from nonimmune animals, were used to probe the T-T network concept postulated to exist in the normal immune system. We describe in this report the unique finding that Lyt-1+ but not Lyt-2+ T cells isolated from normal DBA/2 mice proliferate strongly and directly in response to an autoreactive T cell line and a clone. We suggest that autoreactive T cells may function as an important feature of the regulatory network and/or lead to the expansion of the T cell repertoire.

Materials and Methods

Establishment of Autoreactive and Antigen-specific T Cell Lines/Clones. Autoreactive T cell lines and clones designated PK were established from normal DBA/2 mice and purified as described in detail elsewhere (5). These cell lines were Thy-1+, Lyt-1+2+, L3T4+, and Ia-. The uncloned PK 4 cell line responded to Iaα and the clone PK 4.18 responded to I-Eα determinants. Antigen-specific T cell lines were established as described previously (5). TGG(T) and HGG(T) are DBA/2 long-term T cell lines, cultured for over a year, and respond to turkey gamma globulin (TGG) and human gamma globulin (HGG), respectively, in association with syngeneic Iaα molecules.

Separation of Lyt Subsets of T Cells from Normal DBA/2 Mice. Lyt subsets of T cells were separated by either positive or negative selection techniques (6–8). Briefly, spleen cells from normal DBA/2 mice (Harlan Sprague-Dawley, Inc., Indianapolis, IN) were enriched for T cells by passage through nylon wool columns and incubated on 100-mm plastic petri dishes (Corning Glass Works, Corning, NY) at 37°C for 2 h to remove contaminating macrophages. These cells were treated with anti-Iaα and complement (C'') to further deplete any contaminating Ia+ B cells and macrophages. Such T cells, with >98% purity, were then separated into Lyt-1+2-, Lyt-1-2+, and Lyt-1+2+ T cells by double-positive selection with a panning technique (7) as described in detail elsewhere (8). The purity of the subsets was >95% as assessed by indirect immunofluorescence. In some experiments Lyt-1+2+ T cells were prepared by treatment of purified T cells with anti-Lyt-2.2 + C'".

Antisera and Other Reagents. The monoclonal antibodies, MK-D6 (anti-I-Aα), 14-4-4...
Figure 1. Splenic B cells (A), T cells (O), or Lyt-1+2−T cells (●) isolated from normal DBA/2 mice were stimulated with the irradiated, autoreactive T cell clone PK 4.18. The B cells were obtained by treating spleen cells with anti-Thy-1.2 + C'. The cultures were incubated at 37°C for 1–6 d and cell proliferation studied as described in the footnote to Table I. Vertical bars represent the SE of the mean cpm. The cpm in cultures containing irradiated PK 4.18 cells alone was <1,000.

376  NAGARKATTI ET AL. BRIEF DEFINITIVE REPORT

Results

Kinetics of T-T Interaction. The kinetics of the proliferative response of normal DBA/2 splenic B lymphocytes, T lymphocytes, and Lyt-1+2−T cells stimulated with the irradiated, autoreactive T cell clone PK 4.18 is shown in Fig. 1. B cell proliferation, described at length elsewhere (5), peaked on day 2, followed by a gradual decline, whereas the response of unseparated T cells or Lyt-1+2−T cells peaked on days 4 or 5. Thus, in all subsequent experiments, the T-T interaction was studied on day 4. The Lyt-1+2−T cells responded to irradiated PK 4.18 cells in a dose-dependent fashion (data not shown).

Lyt-1+2− Cells But Not the Other Subsets of T Cells Proliferate in Response to Autoreactive T Cells. Lyt-1+2−T cells from normal DBA/2 mice proliferated strongly when stimulated with the irradiated, syngeneic autoreactive T cell line (PK 4) or clone PK 4.18 (Table I). In contrast, Lyt-1−2+ or Lyt-1+2+ T cells showed only a minor response, probably due to only few Lyt-1+2− cells contaminating the cultures. Treatment of responder or stimulator cells with anti-Thy-1.2 plus C' or anti-Lyt-1 plus C', but not by anti-Ig plus C', abolished the proliferative response (data not shown), suggesting that both the responder and
stimulator cells were Lyt-1\(^{+}2^{-}\) T cells.

**Lyt-1\(^{+}2^{-}\)** T Cells Proliferate Directly in Response to Autoreactive T Cells, Independently of Class II MHC Molecules. Experiments were carried out to substantiate that the Lyt-1\(^{+}2^{-}\) T cells responded directly to autoreactive T cells and not to contaminating Ia\(^{+}\) cells. Treatment of PK 4.18 cells with anti-Ia\(^{d}\) plus C' did not inhibit their stimulatory activity (data not shown). Furthermore, PK 4 cells did not proliferate when stimulated by irradiated Lyt-1\(^{+}2^{-}\) cells, suggesting that there was not a significant number of contaminating Ia\(^{+}\) cells in the lymphocyte cultures (Table I).

Anti-Ia\(^{d}\) antibodies or monoclonal antibodies directed against I-E\(^{d}\) or I-A\(^{d}\) failed to inhibit the response to Lyt-1\(^{+}2^{-}\) T cells to the autoreactive PK 4.18 clone (Table II). In contrast, antibodies to Ia\(^{d}\) and I-E\(^{d}\) suppressed the autoreactivity of the PK 4.18 clone. Neither anti-H-2\(^{d}\) serum nor anti-Lyt 2 antibodies inhibited the T-T interaction, thereby also excluding the involvement of a class I-restricted interaction (data not shown). It was interesting to note that anti-L3T4 antibodies blocked the T-T cell interaction (Table II).

**Lyt-1\(^{+}2^{-}\)** T Cells Respond Only to an Autoreactive T Cell Clone/Line But Not to Antigen-specific T Cell Lines or Other T Cells Isolated from DBA/2 Mice. Long-term cultured TGG- or HGG-specific T cell lines, Con A-activated T cells from normal DBA/2 mice, and Lyt-1\(^{+}2^{+}\) or Lyt-1\(^{-}2^{+}\) T cells, all failed to stimulate syngeneic Lyt-1\(^{-}2^{-}\) T cells (Table III). Also, recombinant IL-2 or supernatants obtained from the cultures of autoreactive PK 4.18 T cells and irradiated syngeneic spleen cells, failed to induce the proliferation of Lyt-1\(^{-}2^{-}\) T cells, suggesting that cell-cell interaction was essential for the growth of Lyt-1\(^{-}2^{-}\) T cells.

### Table I

| Exp. | Responder cells* (H-2\(^{d}\)) | Stimulator cells (H-2\(^{d}\)) | Cell proliferation (cpm ± SE) |
|------|-------------------------------|-------------------------------|-----------------------------|
| 1    | Lyt-1\(^{+}2^{-}\)            | PK 4                          | 3,081 ± 525                 |
| 1    | Lyt-1\(^{+}2^{-}\)            | PK 4                          | 95,908 ± 3,544              |
| 1    | Lyt-1\(^{-}2^{-}\)            | PK 4                          | 507 ± 31                    |
| 1    | Lyt-1\(^{-}2^{-}\)            | PK 4                          | 5,142 ± 2,097               |
| 1    | Lyt-1\(^{-}2^{-}\)            | PK 4                          | 537 ± 35                    |
| 1    | Lyt-1\(^{-}2^{+}\) + Lyt-1\(^{-}2^{-}\) | PK 4                          | 1,427 ± 304                 |
| 1    | Lyt-1\(^{-}2^{+}\)            | PK 4                          | 5,747 ± 1,928               |
| 1    | PK 4                          | Lyt-1\(^{-}2^{-}\)            | 665 ± 112                   |
| 1    | PK 4                          | Spleen cells                  | 34,888 ± 3,842              |
| 1    | PK 4                          | PK 4                          | 502 ± 54                    |
| 2    | Lyt-1\(^{-}2^{-}\)            | PK 4.18                       | 646 ± 310                   |
| 2    | Lyt-1\(^{-}2^{+}\)            | PK 4.18                       | 61,202 ± 3,185              |
| 2    | Lyt-1\(^{-}2^{-}\)            | PK 4.18                       | 1,321 ± 163                 |
| 2    | Lyt-1\(^{-}2^{+}\)            | PK 4.18                       | 7,407 ± 644                 |
| 2    | Lyt-1\(^{-}2^{-}\)            | PK 4.18                       | 219 ± 34                    |
| 2    | Lyt-1\(^{-}2^{+}\)            | PK 4.18                       | 2,957 ± 23                  |
| 2    | PK 4.18                       | PK 4.18                       | 483 ± 82                    |

* Responder T cell subsets were obtained from normal DBA/2 mice and purified by positive selection. The proliferative response of Lyt subsets and PK 4 cells to irradiated stimulators was assayed on days 4 and 2, respectively, by adding [\(\text{H}\)]-thymidine (2 μCi/well) during the last 18 h of culture.
TABLE II
Anti-La\(^d\) But Not Anti-L3T4 Antibodies Inhibit the T-T Cell Interaction

| Exp. | Dilution of antibody added to cultures | Proliferative response in cultures* (cpm ± SE) |
|------|---------------------------------------|-----------------------------------------------|
|      |                                       | PK 4.18 + syngeneic spleen cells                |
|      |                                       | PK 4.18 + syngeneic spleen cells                |
|      |                                       | Lyt-1*2* cells + PK 4.18                        |
| 1    | Anti-La\(^a\)                          | 80,116 ± 5046                                  |
|      | 1:40                                  | 118,920 ± 8786 (0)                            |
|      | 1:80                                  | 115,105 ± 419 (0)                             |
|      | 1:40                                  | 135,841 ± 7522 (0)                            |
|      | Anti-La\(^a\)                          | 16,684 ± 700                                  |
|      | 1:40                                  | 5,126 ± 385 (70)                              |
|      | 1:80                                  | 5,464 ± 1120 (63)                             |
|      | 1:40                                  | 17,096 ± 519 (0)                              |
| 2    | Anti-L1\(^d\)                          | 16,289 ± 108                                  |
|      | 1:40                                  | 16,488 ± 2770 (0)                            |
|      | 1:50                                  | 18,377 ± 675 (0)                             |
|      | Anti-L1\(^d\)                          | 95,322 ± 3106                                 |
|      | 1:40                                  | 53,724 ± 2529 (44)                            |
|      | 1:50                                  | 100,077 ± 655 (0)                             |
| 3    | Anti-L3T4                              | 49,373 ± 5924                                 |
|      | 1:40                                  | 9,816 ± 3117 (80)                             |
|      | 1:10                                  | 1,286 ± 133                                  |
|      | 1:10                                  | 637 ± 89                                      |
|      | 1:50                                  | 3,119 ± 1100                                  |
|      | 1:10                                  | 42,000 ± 8031 (15)                            |
|      | 1:10                                  | 55,175 ± 2905 (5)                            |
|      | 1:50                                  | 16,289 ± 108                                  |
|      | 1:50                                  | 34,895 ± 1546                                 |
|      | 1:40                                  | 17,096 ± 519 (0)                              |
|      | 1:40                                  | 5,126 ± 385 (70)                              |
|      | 1:80                                  | 5,464 ± 1120 (63)                             |
|      | 1:40                                  | 17,096 ± 519 (0)                              |

* The cultures consisted of either Lyt-1*2* T cells, obtained by negative selection and stimulated with irradiated PK 4.18, or responder PK 4.18 cells (10^6) stimulated with irradiated syngeneic spleen cells (2 x 10^5). The proliferative response was measured as described in the footnote to Table I.

TABLE III
Lyt-1*2* Cells from DBA/2 Mice Respond Only to Autoreactive But Not to Antigen-specific T Cells*

| Responder cells (H-2a) | Stimulator cells (H-2a) | Cell proliferation (cpm ± SE) |
|------------------------|------------------------|-------------------------------|
| Lyt-1*2*               | PK-4                   | 646 ± 510                     |
|                        | PK 4.18                | 61,202 ± 3185                 |
|                        | PK 4                   | 1,286 ± 133                   |
|                        | TGG(T)                 | 938 ± 171                     |
|                        | HGG(T)                 | 3,483 ± 1682                  |
|                        | Con A(T)               | 1,089 ± 164                   |
|                        | Lyt-1*2*               | 1,286 ± 133                   |
|                        | Lyt-1*2*               | 637 ± 89                      |
|                        | IL-2                   | 3,119 ± 1100                  |
|                        | Culture supernatant    | 2,024 ± 797                   |
|                        | PK 4                   | 332 ± 85                      |
|                        | PK 4.18                | 736 ± 35                      |

* Lyt subsets were prepared by positive selection. Con A-activated T cells were prepared by culturing DBA/2 spleen cells with Con A for 48 h at 37°C, at which time the cultures were enriched for T cells by removing macrophages and B cells. Recombinant IL-2 was used at a final dilution of 200 U/ml. The culture supernatant was obtained from the cultures of autoreactive clone PK 4.18 stimulated with irradiated syngeneic spleen cells for 24 h at 37°C and used at a final concentration of 25%.

Discussion
In the present study we observed that Lyt-1*2* T cells from normal DBA/2 spleen cells responded directly to a syngeneic, irradiated, autoreactive T cell line/clone. Since this T-T interaction was independent of antigen-presenting cells and Ia molecules, we suggest that the Lyt-1*2* T cells recognized and proliferated directly in response to the autoreactive T cell receptor. From the present study, it was difficult to conclude whether the T-T interaction observed was uni- or bidirectional. Our findings of a unidirectional response could result from a limited frequency of Lyt-1*2* T cells for the receptor on autoreactive T cells (as observed for the number of blasts in the cultures) and the unselected
Lyt-1\(^{+2}\) T cells, when used as stimulators, failing to provide a sufficient number of receptor targets for the autoreactive T cells to recognize and respond to. A bidirectional T-T interaction, independent of antigen-presenting cells, was demonstrated at the clonal level by Lamb and Feldman (10) who isolated a human suppressor T cell clone responding to a helper T cell clone.

Although the nature and significance of the T-T interaction observed in the present study has yet to be delineated, several possibilities exist. One possibility is that the autoreactive cell lines expressed variant class II MHC molecules to which the Lyt-1\(^{+2}\) T cells responded. This is, however, unlikely, since Ia-restricted T cell stimulation generally requires accessory cell function. Second, if the T-T interaction is considered to be unidirectional, the autoreactive T cells may positively select a large pool of Lyt-1\(^{+2}\) T cells which would then undergo somatic diversification to expand the T cell repertoire. Third, the Lyt-1\(^{+2}\) response to autoreactive T cells may be part of a regulatory network controlling the activity of autoreactive T cells. A fourth, and perhaps most exciting, possibility is based on a model by Sim and Augustin (11) that hypothesizes that internal images of MHC antigens on T cell receptors play a role in the generation of the T helper cell repertoire. Based on this model and our data, it appears that autoreactive T cells may represent the first set of T cells (T\(_1\)) selected to proliferate by thymic self-Ia molecules. These cells, in turn, could induce proliferation of cells, like the responding Lyt-1\(^{+2}\) T cells (T\(_1\)), that have receptors resembling internal images of self-Ia. This T-T interaction could result in the expansion and somatic diversification of both T cell types. Due to this diversification, antigen-specific T cells that might arise from the autoreactive T cell pool would no longer interact strongly and directly to self-Ia but could see antigen in association with Ia.

The internal image concept is supported by the demonstration of T cell hybrids reacting specifically to an anti-allo-Ia–specific T cell hybrid (11) and by a similar reactivity reported in the present investigation. If the Lyt-1\(^{+2}\) T cells express internal images of Ia\(^d\) molecules, the failure of anti-Ia\(^d\) antibodies to interact with the receptors on Lyt-1\(^{+2}\) T cells remains unresolved, although this can be explained if an internal image of an Ia epitope is not strictly identical to the Ia epitope it mimics (11). Questions concerning the directionality and other facets of the T-T interaction are currently being approached through the isolation of Lyt-1\(^{+2}\) T cell clones directed to autoreactive T cells and development of monoclonal antibodies to the autoreactive T cell receptor.

**Summary**

In the present communication, we describe the unique observation that Lyt-1\(^{+2}\), L3T4\(^+\) T cells but not Lyt-2\(^+\) T cells isolated from the spleens of normal, unimmunized H-2\(^d\) mice proliferate strongly and directly to an irradiated, syngeneic, Lyt-1\(^{+2}\), L3T4\(^+\), Ia\(^+\) autoreactive T cell line/clone. In contrast, Lyt-1\(^{+2}\) T cells failed to proliferate when stimulated by long-term, antigen-specific, H-2\(^d\)-restricted T cell lines. Supernatants from the cultures of autoreactive T cells or recombinant interleukin 2 (IL-2) alone, failed to induce proliferation of the Lyt-1\(^{+2}\) T cells, suggesting that cell-cell interaction is essential for growth. In addition, the proliferative response of Lyt-1\(^{+2}\) T cells was independent of
Ia\(^{+}\) antigen-presenting cells and was not blocked by either anti-Ia\(^{d}\) or anti-H-2\(^{d}\) antibodies, but was inhibited by anti-L3T4 antibodies. All these observations suggested that the responding Lyt-1\(^{+}\)2\(^{-}\) T cells were recognizing the anti-self-Ia receptor expressed on autoreactive T cells and that such T cells might therefore express the internal image of self-Ia determinants. We suggest that the T-T interactions observed may represent interactions between two helper T cell subpopulations at the idiotope level and that autoreactive T cells may function as an important feature of the regulatory network and/or lead to the expansion of the T cell repertoire.

We wish to thank Ms. Lisana Mann for excellent technical assistance, and Ms. Nancy Gay and Mr. Bradley Moore for secretarial assistance.

Received for publication 25 March 1985 and in revised form 6 May 1985.

References

1. Jerne, N. K. 1984. Idiotypic networks and other preconceived ideas. *Immunol. Rev.* 79:5.
2. Bona, C. A., C. Victor-Kobrin, Q. J. Manheimer, B. Bellon, and L. J. Rubinstein. 1984. Regulatory arms of the immune network. *Immunol. Rev.* 79:25.
3. Cooper, J., K. Eichmann, K. Fey, I. Melchers, M. M. Simon, and H. U. Weltzien. 1984. Network regulation among T cells: qualitative and quantitative studies on suppression in the non-immune state. *Immunol. Rev.* 79:63.
4. McNamara, M., K. Gleason, and H. Kohler. 1984. T-cell helper circuits. *Immunol. Rev.* 79:87.
5. Nagarkatti, P. S., E. C. Snow, and A. M. Kaplan. 1985. Characterization and function of autoreactive T lymphocyte clones isolated from normal, unprimed mice. *Cell. Immunol.* In press.
6. Nagarkatti, P. S., and D. A. Clark. 1983. In vitro activity and in vivo correlates of alloantigen-specific murine suppressor T cells induced by allogeneic pregnancy. *J. Immunol.* 131:638.
7. Vidovic, D., J. Klein, and Z. A. Nagy. 1984. The role of T cell subsets in the generation of secondary cytolytic responses in vitro against class I and class II major histocompatibility complex antigens. *J. Immunol.* 132:1113.
8. Nagarkatti, M., and A. Kaplan. 1985. The role of suppressor T cells in BCNU-mediated rejection of a syngeneic tumor. *J. Immunol.* In press.
9. Cohen, D. A., and A. M. Kaplan. 1981. Adherent Ia\(^{+}\) murine tumor lines with characteristics of dendritic cells. I. Morphology, surface phenotype and induction of syngeneic mixed lymphocyte reactions. *J. Exp. Med.* 154:1581.
10. Lamb, J. R., and M. Feldmann. 1982. A human suppressor T cell clone which recognizes an autologous helper T cell clone. *Nature (Lond.)*, 300:456.
11. Sim, G. K., and A. A. Augustin. 1983. Internal images of major histocompatibility complex antigens on the T-cell receptors and their role in the generation of the T-helper cell repertoire. *NY Acad Sci.* NY 272–281.