GENERATION OF CYTOTOXIC EFFECTOR CELLS BY IMMUNOCOMPETENT THYMUS CELL SUBPOPULATIONS*

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Purified subpopulations of immunocompetent thymus cells (TH-2) do not proliferate in response to immunocompetent allogeneic thymus cells. This TH-2a/TH-2b incubation mixture requires a peripheral lymphoid helper cell in order to become a proliferative mixed lymphocyte reaction (MLR) (1). The peripheral cell subpopulation that acts as helper cell has the identified attributes of a B cell.2 Apparently equivalent helper effects were provided by critical concentrations of IgG. This report shows that a B-helper cell, or IgG is required by responder TH-2 cells in order to generate specific effector cells in cell-mediated lympholysis (CML), which have cytotoxicity toward the alloantigens of the stimulator cell.

Materials and Methods

Mice. Female, 6-wk-old mice of the congenic strains: C57BL/10Sn, B10.BR, B10.A, B10.A(2R), B10.A(5R), B10.AKM, and B10.D2 were obtained from The Jackson Laboratory, Bar Harbor, Maine, and kept in the animal rooms for at least 2 wk before use.

Isolation and Separation of Cell Populations. Lymphoid cell suspensions were prepared as previously outlined (2). The modified five-step discontinuous gradient method was used to obtain subpopulations of thymus cells (3). Thymus cell suspension separated in density gradients yielded one population of low density cells (fraction B) that contains most of the immunocompetent cells (TH-2) found in the thymus (1, 2, 4). The majority of thymus cells (TH-1) sediment in the denser portions of the gradient (fraction D). Cells were also separated on the basis of properties of adherence to glass, plastic, or nylon. This procedure has been described in detail previously (1). Proliferation by stimulator and helper cells was inhibited either by mitomycin-C at a concentra-

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1 Abbreviations used in this paper: B cell, nonthymus-processed lymphoid cell; CML, cell-mediated lympholysis; CTL, cytotoxic T cells; MLR, mixed lymphocyte reaction; PBS, phosphate-buffered saline; T cell, thymus-processed lymphoid cell; TH-1, a major population of mouse thymocytes, immunoincompetent by known criteria; TH-2, a minor subpopulation of mouse thymocytes, immunoincompetent by known criteria; TH-2a/TH-2b, general expression for one-way allogeneic TH-2 MLR (specific strain designations may be substituted in the text).

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tion of 50 mg/ml as described elsewhere (5), or by irradiation. Irradiation was carried out in a "Cs source (Gammeter M; Radiation Machinery Corp., Parsippany, N. J.) at a dose rate of 100 rads/0.1 min.

Antisera. AKR anti-Thy-1.2 was prepared by procedures previously described (2). Goat anti-mouse immunoglobulin sera were purchased from Meloy Laboratories, Springfield, Va. Rabbit anti-mouse immunoglobulin antisera were prepared by two injections of 1.0 mg of the mouse immunoglobulin 14 days apart. The first injection was given at multiple sites subcutaneously in complete Freund's adjuvant. The second injection was given intravenously. The rabbits were bled 7 days after the second injection and every other day thereafter for 2 wk. Samples of comparable titer were pooled. The specificity of anti-Ig sera were affirmed by precipitin analysis in gel. Anti sera used in cytotoxicity assays were diluted to the highest titer giving maximum cytotoxicity.

Column Separation of Lymphoid Cells. Antibody columns of the Wigzell type (5, 6) were employed in several experiments to segregate lymphoid cell subpopulations based on surface representation of Fc receptors. The columns were prepared with Degalon V-26 plastic beads (Degussa, Inc., Teterboro, N. J.) which had been extensively washed and soaked in phosphate-buffered saline (PBS; pH 7.3) before use. 10 g of beads in PBS were incubated with 10 mg mouse immunoglobulin (Cappel Laboratories, Inc., Downingtown, Pa.) for 60 min at 37°C, followed by an overnight incubation at 4°C. The beads were then poured into a glass column (10 x 1.5 cm; Bio-Rad Laboratories, Richmond, Calif.). The column was washed through with 50 ml saline. 5 ml of a 1:5 dilution of rabbit anti-mouse immunoglobulin serum (Meloy Laboratories) was then added. The complexes formed were thus in antibody excess. The columns were then incubated for 2 h at 4°C and washed through with 100 ml cold RPMI-1640. Lymphoid cell suspensions were added to the column and the cells allowed to incubate for 30 min at 37°C. The column was then eluted with RPMI-1640 at a rate of 2 ml/min. Recovery of cells ranged from 30 to 90% depending on the rabbit antibody employed. Viability of recovered cells by the trypan blue staining technique was greater than 85%.

Generation of Cytotoxic Responder Cells. Lymphoid cells were sensitized in Linbro tissue culture plates (no. FB-24TC; Linbro Chemical Company, New Haven, Conn.). The incubation mixtures consisted of 10 x 10^6 responder cells and 2.5 x 10^6 mitomycin-blocked stimulator cells suspended in 1.5 ml RPMI-1640 supplemented with 5% human serum and antibiotics. Mitomycin-blocked spleen helper cells were added as indicated. The mixture was incubated on a rocker platform for 5 days at 37°C, in 5% CO2. Each test mixture of responder and stimulator with or without helper cells was performed in triplicate. At 5 days the contents of each well were gently resuspended in tubes, centrifuged, and washed twice before use in the cytotoxicity assay.

Cell-Mediated Lymphocytotoxicity. This is a modified 4-h cytotoxicity assay in which 51Cr-labeled target cells of various origins are added to responder cell subsets generated after 5 days culture as described above. The time- and dose-response kinetics of this assay are illustrated in Fig. 1 a-d. The generating incubation mixture consisted of 2 x 10^6 lymphoid cells, 2 x 10^6 washed sheep erythrocytes (SRBC), and 2 x 10^4 51Cr-labeled target cells in 0.2 ml RPMI-1640 supplemented with 5% human serum and antibiotics. SRBC were added based upon the observation (Fig. 2) that spontaneous release of 51Cr by the labeled target cells could be substantially reduced if "cold" SRBC were present in the incubation mixture. This procedure was suggested by similar observation reported in antibody-dependent lymphocytotoxicity using RBC for assays, augmenting cell density (7, 8).

Target cells for the cytotoxicity assay were labeled with 51Cr (sodium chromate; Amersham/Searle Corp., Arlington Heights, Ill.) at a ratio of 10 μCi/10^6 cells. Labeling was for 30 min at 37°C followed by 30 min at 4°C. Labeled cells were washed five times before use. The assay was performed in triplicate. The final assay mixture was incubated in the Microtest II plates on a rocking platform at 37°C for 4 h; at the end of incubation, the plates were centrifuged and 0.1-ml supernate was removed from each well. Aliquots of the supernates were counted to determine 51Cr release. Maximum 51Cr release was determined by freeze-thawing an aliquot of labeled target cells three times. Results are expressed as "specific 51Cr release" according to the following formula:

% specific 51Cr release = \( \frac{\% \text{ release (experimental)} - \% \text{ release (control)}}{\% \text{ release (freeze-thaw)}} \times 100. \)
Fig. 1. (a) Time-course kinetics for the generation of cytotoxic effector cells from cultures of $10 \times 10^6$ B10.BR TH-2 responder cells with $2.5 \times 10^6$ mitomycin-blocked B10 TH-2 stimulator cells in presence (●) or absence (○) of $1 \times 10^6$ mitomycin-blocked B10.BR spleen helper cells. (b) Dose-response kinetics for the generation of B10.BR TH-2 effector cells. The indicated number of B10.BR TH-2 responder cells were cultured with a fixed number ($2.5 \times 10^6$) of mitomycin-blocked B10 TH-2 stimulator cells in the presence (●) or absence (○) of mitomycin-blocked B10.BR spleen helper cell. The number of B10.BR spleen cells present was adjusted to 10% of the number of B10.BR TH-2 responder cells present. Target cells were $^{51}$Cr-labeled B10 spleen cells. Ratio of effector:target cells was 100:1; $2 \times 10^6$ cold SRBC were present in each assay mixture. (c) Time-course kinetics for cytotoxicity assay. Each assay mixture contained $2 \times 10^6$ B10.BR TH-2 effector cells, $2 \times 10^4$ $^{51}$Cr-labeled B10 spleen cells, and $2 \times 10^6$ SRBC. Incubation of mixtures was for the indicated length of time. (d) Dose-response kinetics to effect maximum cytolysis. 10 million B10.BR TH-2 responder cells were cultured with $2.5 \times 10^6$ mitomycin-blocked B10 TH-2 stimulator cells in presence (●) or absence (○) of $1 \times 10^6$ mitomycin-blocked B10.BR spleen helper cells as helpers. Number of B10.BR TH-2 effector cells per assay ranged from $2 \times 10^4$ to $4 \times 10^6$; number of $^{51}$Cr-labeled B10 spleen cells remained constant at $2 \times 10^6$ per culture. $2 \times 10^6$ per well were present in each assay mixture.

Results

Generation of Alloantigen-Specific Cytotoxicity in Immunocompetent Thymus Cells. In order for specific recognition to trigger cell proliferation in one-way MLR between allogeneic TH-2 cells (TH-2a/TH-2t), a critical quantity of non-participating peripheral B cells, or IgG, must be added. Since proliferation is not
Fig. 2. Effect of nonlabeled SRBC on the spontaneous release of \(^{51}\)Cr from B10 spleen cells. \(^{51}\)Cr-labeled B10 spleen cells \((2 \times 10^5)\) were incubated for the indicated time with \(2 \times 10^6\) normal, nonsensitized B10.BR TH-2 cells to determine the rate of spontaneous \(^{51}\)Cr release from the labeled target cells in the presence (Q) or absence (©) of \(2 \times 10^6\) SRBC.

required to generate cytotoxic cells in certain circumstances (9, 10), it was of interest to determine whether the nonproliferative TH-2\(_a\)/TH-2\(_b\) MLR generated specific cytotoxic T cells (CTL) without helper being added. Experiments such as those summarized in Table I involving TH-2\(_{B10}\)/TH-2\(_{B10,BR}\) revealed that generation of cytotoxic cells in 5-day cultures also requires a peripheral helper source. This source could be supplied as a nonproliferating addition to the mixture in 10:1 responder:helper ratios, or be included in the responder, or stimulator cells of spleen, or LN origin. The data also show that TH-2 cells provided a target cell in the cytotoxicity assay equivalent to that of a spleen cell. This is consistent with the equivalent representation of serologically defined alloantigens in the cell membrane of spleen, or LN cells in this subclass of thymus cells (2).

The specificity of TH-2 plus helper cell cytotoxicity generation was examined for each element of the culture and assay system. Table II exemplifies experiments establishing that stimulator-specific cytotoxicity is generated. The TH-2 stimulator cell (B10) gave rise to anti-B10 CTL, but not CTL directed toward the syngeneic helper (B10.BR), or toward a third-party set of alloantigens (B10.D2).

The second type of experiment involved helper cells carrying alloantigens differing from the responder cell. In this case, the helper cells have potential stimulator activity even though the 10:1 ratios employed usually precludes detectable stimulation. Table III shows that at the ratios tested, mitomycin-blocked helper cells allogeneic to both stimulator and responder (B10.A, B10.D2) failed to stimulate detectable specific cytotoxicity. The data also show that when the stimulator was TH-2\(_{B10, D2}\), for example, maximal anti-B10.D2 killing occurred only when the helper was syngeneic with responder (B10.BR). This was also true in the TH-2\(_{B10,BR}\)/TH-2\(_{B10,A}\) combination in which the target provided only D-end histoincompatibility. In this respect, the helper effect for cytotoxicity had similar histocompatibility requirements to the proliferative effect previously reported.

In the third context (Table IV) the target cell specificity of killing was
### Table I

**Generation of B10.BR Lymphocytes Specifically Cytotoxic Toward B10 Target Cells**

| Exp. no. | B10.BR responder cell origin | [B10 stimulator] | [B10.BR spleen helper] | TH-2 target | Percent specific lysis of B10 target cells§ |
|----------|-----------------------------|------------------|------------------------|-------------|------------------------------------------|
|          |                             |                  |                        | Spleen      |                                          |
| 1        | TH-2                        | TH-2             | -                      | -           | 6                                        |
|          |                             |                  |                        | +           | 23                                       |
|          |                             |                  |                        | Spleen      | 28                                       |
|          |                             |                  |                        | +           | 27                                       |
|          |                             |                  |                        | Spleen      | 19                                       |
|          |                             |                  |                        | +           | 30                                       |
|          |                             |                  |                        | Spleen      | 19                                       |
|          |                             |                  |                        | +           | 21                                       |
|          |                             |                  |                        | Lymph node  | 26                                       |
|          |                             |                  |                        | +           | 29                                       |
|          |                             |                  |                        | Spleen      | 28                                       |
|          |                             |                  |                        | +           | 24                                       |
|          |                             |                  |                        | Spleen      | 45                                       |
|          |                             |                  |                        | +           | 40                                       |
|          |                             |                  |                        | Spleen      | 45                                       |
|          |                             |                  |                        | +           | 42                                       |
| 2        | TH-2                        | TH-2             | -                      | 5           | 3                                        |
|          |                             |                  |                        | +           | 41                                       |
|          |                             |                  |                        | Spleen      | 45                                       |
|          |                             |                  |                        | +           | 43                                       |
|          |                             |                  |                        | Spleen      | 42                                       |

* CTL were detected in cultures containing $10 \times 10^6$ B10.BR responder cells, $2.5 \times 10^6$ mitomycin-blocked B10 stimulator cells and $1 \times 10^6$ mitomycin-blocked B10.BR spleen cells as the target helper cell source. Cultures were incubated at $37°C$ in a CO₂ atmosphere for 5 days.

$+$ indicates that mitomycin-blocked B10.BR spleen helper cells were present; $-$ indicates no helper cells present.

§ Target cells for specific cytotoxicity assay were $^{51}$Cr-labeled B10 spleen cells; control target cells were $^{51}$Cr-B10.BR spleen cells.

Evidence was presented that helper effect requirements for TH-2α/TH-2β MLR could be supplied by a peripheral B cell, or solubilized B-cell membranes, and that it was trypsin sensitive. Further, identical helper activity was found in preparations of IgG, especially IgG2A, and the Fc fragment. Helper requirements were similarly explored as necessary to generation of CTL. Table V shows an illustrative experiment which established that a B-cell subpopulation also was responsible for help in generating CTL. Polyvalent IgG, explored using strains which shared K- or D-end alloantigens. TH-2α/TH-2β with syngeneic helper mediated maximal killing against B10.A which shares K-end alloantigens. Similarly, low level cytotoxicity was detected when B10.D2-stimulated responders were tested on B10.A targets. TH-2α/TH-2β stimulator cell which shares K-I-region alloantigens with TH-2β responders, did not generate any CTL.
TABLE II
Specificity of CTL Generated in TH-2\textsubscript{B10}/TH-2\textsubscript{B10} Cultures with Syngeneic (B10) Spleen Helper Cells

| Sources of cells cultured in sensitization phase* | % Specific \textsuperscript{51}Cr release from target spleen cells in cytotoxicity assay |
|-------------------------------------------------|--------------------------------------------------|
| B10.BR responder [B10 stimulator] [B10.BR spleen helper] | B10 | B10.BR | B10.D2 |
| TH-2 [TH-2] | – | 2 | 2 | 1 |
| | + | 31 | 3 | 2 |
| Spleen [–] | 33 | 2 | 3 |
| | + | 31 | 1 | 2 |
| Lymph node [TH-2] | – | 36 | 3 | 5 |
| | + | 35 | 5 | 5 |
| Spleen [–] | 33 | 2 | 6 |
| | + | 34 | 5 | 4 |

* 10 × 10\textsuperscript{6} B10.BR responder cells, 2.5 × 10\textsuperscript{6} mitomycin-blocked B10 stimulator cells, and 1 × 10\textsuperscript{6} mitomycin-blocked B10.BR spleen helper cells were cultured for 5 days; responder cells harvested from these cultures were assayed against the indicated \textsuperscript{51}Cr-labeled target spleen cells.

TABLE III
Specificity Requirements for Helper Cells Compared to Stimulator Cells In Generating TH-2\textsubscript{B10.BR} Responder Cytotoxicity Against Various Target Cells

| Experiment no. | Strain of origin of cells in culture generating B10.BR TH-2 responder cells* | Strain of origin spleen target cells (% specific \textsuperscript{51}Cr release) |
|----------------|--------------------------------------------------------------------------------|--------------------------------------------------|
|                 | [Stimulator TH-2 cells] [Spleen helper] | B10 | B10.D2 | B10.A |
| 1               | B10 | B10.BR | 51 | 3 | 2 |
|                 |     | B10 | 20 | 3 | 1 |
|                 |     | B10.A | 26 | 2 | 2 |
|                 |     | B10.D2 | 4 | 5 | 1 |
|                 |     | None | 3 | 1 | 3 |
| 2               | B10.D2 | B10.BR | 2 | 46 | 3 |
|                 |     | B10 | 2 | 7 | 1 |
|                 |     | B10.A | 3 | 14 | 6 |
|                 |     | B10.D2 | 2 | 11 | 2 |
|                 |     | None | 3 | 4 | 2 |
| 3               | B10.A | B10.BR | 3 | 2 | 16 |
|                 |     | B10 | 2 | 0 | 7 |
|                 |     | B10.A | 1 | 1 | 7 |
|                 |     | B10.D2 | 0 | 3 | 6 |
|                 |     | None | 0 | 1 | 2 |

* 10 × 10\textsuperscript{6} B10.BR TH-2 responder cells, 2.5 × 10\textsuperscript{6} mitomycin-blocked stimulator TH-2 cells, and 1 × 10\textsuperscript{6} mitomycin-blocked spleen helper cells of the indicated strains were cultured for 5 days and assayed against \textsuperscript{51}Cr-labeled target cells of the indicated strain.
**Table IV**

*H-2 Sublocus Target Cell Specificity of TH-2 B10 Responder Cells Generated in Culture with Various Stimulator Strains*

| Stimulator strain generating B10 TH-2 responder cells | Strain source of target spleen cells (% specific ⁵¹Cr release) |
|------------------------------------------------------|-------------------------------------------------------------|
| B10.BR                                               | B10.BR (kkkkkk) B10.A (khhddd) B10.D2 (ddddd) B10.A(5R) (bbddd) |
| B10.BR                                               | 48 31 4 4 |
| B10.A                                                | 29 56 9 9 |
| B10.D2                                               | 5 13 47 9 |
| B10.A(5R)                                            | 3 7 6 6 |

* 10 × 10⁶ B10 responder TH-2 cells, 1 × 10⁶ mitomycin-blocked B10 spleen helper cells, and 2.5 × 10⁶ of the indicated mitomycin-blocked TH-2 stimulator cells were cultured for 5 days, harvested for assay against the indicated ⁵¹Cr-labeled target cell strain. Subregion haplotypes of origin are indicated in parentheses.

**Table V**

*Characterization of the Spleen Helper Cell Subpopulation Supporting Generation of B10.BR CTL*

| Treatment of B10.BR spleen helper cells | % Specific ⁵¹Cr release from B10 target cells |
|----------------------------------------|---------------------------------------------|
| None                                   | 48                                          |
| Anti-Thy-1.2 plus C                    | 53                                          |
| Anti-Ig column-passed cells            | 6                                           |
| Nylon column-passed cells              | 5                                           |
| Nylon column-retarded cells            | 42                                          |

* 10 × 10⁶ B10.BR responder TH-2 cells, 2.5 × 10⁶ mitomycin-blocked B10 stimulator TH-2 cells, and 1 × 10⁶ mitomycin-blocked B10.BR helper spleen cells (pretreated as indicated) were cultured for 5 days and assayed for cytotoxicity against ⁵¹Cr-labeled B10 spleen cells.

IgG2A, and to a lesser extent, other Ig preparations could be substituted for intact helper B cells in TH-2ₙ(TH-2) cultures (Table VI). Not shown are experiments which indicate that, as in the MLR model, optimal concentration of Ig to produce cytotoxicity are narrowly restricted, inhibition occurring above 5–10 μg/culture. Intact B-helper cells always provided higher levels of cytotoxicity than any Ig preparations tested.

In the TH-2ₙ/TH-2ₙₙ MLR proliferation involved an initial rapid (1–2 h) interaction between stimulator and helper cell. Similar experiments revealed that generation of CTL requires interaction between helper and stimulator cells within the first 6 h of culture. Responder cells could be added much later in the culture period and still generate optimal levels of CTL (data not shown).

In order to further explore the mechanism of helper cell activity, B-helper cells were eliminated selectively at intervals from cultures containing all three cellular elements, by treatment with anti-Ig plus complement (C). The results are illustrated by the experiment shown in Table VII. The data are interpreted to indicate that a B-helper cell requirement continues through the first 72 h of culture. After this time, elimination of B-helper cells reduces, but does not eliminate the generation of CTL.
TABLE VI  
Helper Role of Various Immunoglobulin in Preparations in Generating Anti-B10.BR CTL*  

| Ig preparation used in cultures generating responder cells | % Specific ¹¹Cr release from target cells |
|------------------------------------------------------------|------------------------------------------|
| Ig (polyvalent)                                            | 27                                       |
| IgM                                                        | 18                                       |
| IgG1                                                       | 12                                       |
| IgG2a                                                      | 26                                       |
| IgG2b                                                      | 18                                       |
| None                                                       | 3                                        |
| B10 Spleen Cells                                           | 53                                       |

* 10 × 10⁶ B10 responder TH-2 cells, 2.5 × 10⁶ mitomycin-blocked B10.BR stimulator cells, and either 10 μg of the indicated B10 mouse immunoglobulin or 1 × 10⁶ mitomycin-blocked B10 spleen cells were cultured for 5 days, harvested, and assayed on ¹¹Cr-labeled B10.BR spleen cells.

TABLE VII  
Timing of Effect of Anti-Ig Antiserum Plus C on Generation of CTL in TH-2B10/TH-2(BR) Cultures*  

| Time of anti-Ig plus C treatment | % Specific ¹¹Cr release from B10.BR spleen target cells |
|----------------------------------|--------------------------------------------------------|
| Anti-Ig NRS$                      | Anti-Ig  NRSt                                           |
| h                                | 5  41                                                  |
| 12                               | 3  40                                                  |
| 24                               | 3  35                                                  |
| 48                               | 5  41                                                  |
| 72                               | 20 45                                                  |
| 96                               | 21 43                                                  |
| 120                              | 41 41                                                  |
| No treatment                     | 45 52                                                  |

* 10 × 10⁶ B10 responder TH-2 cells, 2.5 × 10⁶ mitomycin-blocked B10.BR stimulator TH-2 cells, and 1 × 10⁶ mitomycin-blocked B10 spleen helper cells were cultured for 5 days and cytotoxicity assayed against ¹¹Cr-labeled B10 spleen target cells. Rabbit anti-mouse immunoglobulin antiserum plus C (0.2 ml of 1:5 anti-Ig plus 0.5 ml of 1:4 guinea pig C) was added to the culture wells at the indicated interval after initiation of culture. After incubating 60 min, the cells were washed three times, fresh medium was added, and the cultures were allowed to incubate for the remainder of the 5-day period.

$ NRS, normal rabbit serum.

Discussion  
The presence of a critical proportion of B cells is required in order for proliferation to occur in 3-day MLR between allogeneic immunocompetent thymus cells (1). This requirement is now shown to apply to a generation of specifically CTL when such cultures are extended to 5–7 days (CML). As in the TH-2a/TH-2(B) MLR, IgG2A and, to a lesser extent other immunoglobulins, also appear able to substitute for the helper effects of intact B cells, although
somewhat less efficiently under conditions tested. The B-helper cell effect in CML appears to involve an initial interaction with the stimulator population within a few hours after establishing the culture. After 6 h in cultures of responder and stimulator cells alone, no CTL were generated by adding helper cells. Once "help" has been initiated, however, B-helper cell requirements continue almost to the end of the culture period. Treatment of cultures with anti-Ig and C as late as 96 h after initiation presumably eliminating B cells, and binding any immunoglobulin effectively prevented generation of CTL.

The TH-2a/TH-2e plus the helper cell model appears to have histocompatibility requirements similar to other CML. K-I-region differences are required in order to generate CTL within the limits of strains tested, and K- or D-end specificity of cytotoxicity to stimulator cells was demonstrated. As was shown for MLR, optimal helper activity was attained if the B-helper cell was syngeneic with responder. The reason for syngeneic preference in this context remains unknown. Curiously, syngeneic "help" is trypsin sensitive, while congenic help is not in the MLR model. 2

Several types of evidence have accumulated recently which suggest that the subset of T cells responsible for most proliferation in CML may not be identical with that ultimately providing the cytotoxic effect. For example, peak proliferation in TH-2a/TH-2e cultures yielding CTL occurs by 72 h and falls off sharply thereafter (1). 2 CTL generation begins at 3 days and peaks at 5-6 days. Similar data are reported for peripheral T cells which led to the conclusion that CTL generation may not require proliferation. Further, CTL generated in peripheral T-cell cultures are of a subclass of T cells carrying the Ly2,3 phenotype (11, 12). This phenotype is not necessarily involved in the earlier proliferative-phase characteristic of such cultures (13). The data reported here do not permit the conclusion that CTL generated in TH-2a/TH-2e cultures are of a subclass of thymus T cells distinct from those proliferating.

It seems clear that the helper cell requirement is imposed at the beginning, before cell proliferation, and continues well into the phase of CTL generation. "Help" in this context might represent a similar signal requirement, but of differing sensitivity on the part of different T-cell types. Favoring this is the observation that IgG2A is as efficient a helper in the proliferative response as intact B cells, whereas IgG2A was far less efficient than intact B cells in CTL generation. It is also conceivable that the B-helper cell produces two helper products, perhaps Ig and Ia in this system, each acting at different times to provide helper activity in CTL-generating cultures. The TH-2a/TH-2e plus helper model should permit more adequate analysis both of T-cell contributions and of the role of B-helper activity in cultures generating specifically cytotoxic T cells.

Summary

Immunocompetent mouse thymus cell subsets (TH-2) cultured with allogeneic TH-2 cells, require a peripheral lymphoid cell in order to generate specifically cytotoxic T cells (CTL) in vitro. The helper cell is identified as a B cell, and can be supplied either in the mixture of target or responding peripheral cells, or in optimal ratios of 10:1 to 20:1 as nonproliferating cells syngeneic to the responder,
when both responder and target are TH-2. Allogeneic helper cells are much less effective. Kinetic studies indicate that helper cell activity involves initial interaction with stimulator cell and is then required throughout the period of generation of cytotoxic lymphocytes. Addition of critical levels of certain IgG preparations, particularly IgG2A appears to support generation of CTL in TH-2/TH-2 cultures but is considerably less effective than intact B cells. Cytotoxicity generated under these conditions has the same genetic requirements for I-region histoincompatibility between stimulator and responder, and for K or D identity of stimulator and target cell, as has been reported for peripheral cell CTL.

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