INTERLEUKIN 2 IS NOT SUFFICIENT AS HELPER COMPONENT FOR THE ACTIVATION OF CYTOTOXIC T LYMPHOCYTES BUT SYNERGIZES WITH A LATE HELPER EFFECT THAT IS PROVIDED BY IRRADIATED I-REGION-INCOMPATIBLE STIMULATOR CELLS

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Interleukin 2-containing supernatants from concanavalin A-activated spleen cells (CSCS) were found to provide strong helper activity for cytotoxic T lymphocyte (CTL) responses against allogeneic stimulator cells in microculture systems, but provided usually insufficient help for CTL responses against I-region compatible allogeneic or TNP-haptenated syngeneic stimulator cells. The interleukin 2-containing supernatant from HGG-activated AODH 7.1 hybridoma cells also mediated only relatively weak CTL responses against TNP-haptenated syngeneic cells in microcultures. Both types of supernatants, however, supported substantial responses against TNP-haptenated syngeneic stimulator cells if irradiated allogeneically activated syngeneic T cells or irradiated allogeneic spleen cells were added to the cultures. The allogeneic cells and the activated syngeneic T cells provided little helper activity if they were added in the absence of the interleukin 2-containing supernatants, thus demonstrating a synergistic effect between these 2 helper components. An I-region difference was sufficient for the helper effect of the allogeneic cells and control experiments showed that the presence of foreign I-region determinants could not be substituted for the TNP-haptenated stimulator cells.

The AODH 7.1 supernatant was shown to provide a helper effect only if it was added at day 0, and had no effect at day 3 of the culture; but its helper effect was enhanced by irradiated allogeneic cells irrespective of whether these cells were added at day 0 or day 3. This indicated that the allogeneic cells provided a helper effect that was required during the late phase of the CTL response and therefore was qualitatively different from the early helper effect of interleukin 2. The late addition of allogeneic cells could not be substituted by the AODH 7.1 supernatant.

Some of our CSCS batches and concentrated CSCS preparations were found to provide the late helper effect and to support CTL responses equally well in the presence or absence of I-region-incompatible cells. This indicated that the late helper effect was also mediated by a soluble mediator.

Finally, our experiments showed that the helper cells that responded to the I-region differences and synergized with CSCS were provided by the irradiated allogeneic spleen cell populations and also by the responder cells from lymph nodes, but were less frequent in thymic responder cells. Alloreactive helper cells were not detected in the irradiated trinitrophenylated stimulator cell population, indicating that these helper cells were sensitive to the trinitrophenylation procedure but were relatively resistant to irradiation.

It is well established that the activation of cytotoxic T lymphocyte (CTL) precursor cells requires not only antigen, but also helper T cells and non-T accessory cells (1-15). Previous reports have shown 1) that accessory cells plus helper T cells of the phenotype Ly-1-2- are both required for the production of costimulator factor (interleukin 2) (11, 16, 2) that the accessory cells can be substituted by lymphocyte-activating factor (LAF, interleukin 1) from a macrophage-like cell line (11, 16), and 3) that the costimulator (interleukin 2) is required as a second signal for the activation of CTL precursor cells (11, 17-20). The CTL precursor cells recognize antigen mainly in association with cell surface structures encoded by the K- or D-region of the murine major histocompatibility complex (MHC) whereas helper T cell precursors (i.e., Ly-1-2- cells), recognize antigen mainly (3, 21), but not exclusively (18, 19), in association with I-region-encoded structures.

Our present studies on the activation of CTL against MHC-compatible target cells reveal that interleukin 2 is insufficient to support the activation of CTL against MHC-compatible stimulator cells, but synergizes with a helper effect from allogeneic (I-region) stimulated helper T cells. This latter type of helper effect in allogeneic responses is usually provided by the irradiated allogeneic stimulator cells, and conventional concanavalin A-activated spleen cell supernatant (conventional CSCS)3 preparations have therefore been successfully employed in the limiting dilution analysis of alloreactive CTL precursor frequencies (22). But this helper effect becomes limiting in CTL precursor frequency studies for MHC-compatible target cells.

MATERIALS AND METHODS

Animals. The A.TH, A.TL, and SJL mice were obtained from OLAC, Bicester, England, or in some cases from The Jackson Laboratory, Bar Harbor, ME. All other strains of mice were obtained from G1, Bomholtgaard, Ry, Denmark. The responder mice were usually 2 to 3 mo old.

Irradiation. Stimulator cells were irradiated with 1500 rad using a 60Co source if not indicated otherwise.

The preparation of CSCS. Two types of preparations were used: conventional CSCS (CSCS I) was prepared by culturing spleen cells from mice or

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1 Abbreviations used in this paper: CSCS, concanavalin A-activated spleen cell supernatant; CTL, cytotoxic T lymphocyte(s); CyP, cyclophosphamid; MHC, major histocompatibility gene complex; HEPES, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid; HGG, human γ-globulin.

2 The preparation of CSCS. Two types of preparations were used: conventional CSCS (CSCS I) was prepared by culturing spleen cells from mice or

3 Abbreviations used in this paper: CSCS, concanavalin A-activated spleen cell supernatant; CTL, cytotoxic T lymphocyte; CyP, cyclophosphamide; MHC, major histocompatibility gene complex; HEPES, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid; HGG, human γ-globulin.
rats at a density of 3 x 10^6 cells in 30 ml GIBCO medium 199 containing 0.15 mg Con A (GIBCO) and 3 x 10^{-3} M 2-mercaptoethanol for 24 hr. The supernatant was then isolated by centrifugation at 300 x G for 10 min and stored frozen. Individual batches were tested and titrated before the application in experiments. α-Methylmannoside at a final concentration of 1 mm (0.194 mg/ml plus HEPS (5 x 10^{-3} M) was added to the supernatant in order to neutralize the contaminating Con A. CSCS II was prepared according to the procedure of Warren et al. (23) with minor modifications. In brief, spleen cells from (C3H x DBA/2)F1 mice were cultured at a density of 10^7 cells/ml in 30-ml aliquots using RPMI 1640 GIBCO medium 199 containing glutamine, streptomycin-penicillin, HEPES, and 3 x 10^{-3} M 2-mercaptoethanol plus 0.005 mg/ml of Con A (GIBCO), but no fetal calf serum (FCS). The cultures were cultured in 75 cm² plastic tissue culture flasks (Falcon 3024) for 2 hr, the supernatants were discarded, and the cells were washed carefully 3 times with BSS and then cultured again in serum-free medium for 16 to 20 hr at 37°C. The supernatant was harvested, centrifuged to remove all cells, and concentrated 10X using an Amicon PM10 membrane. This factor was sterilized by filtration and stored at -20°C.

The preparation of an interleukin 2-containing supernatant from the hybridoma line AODH 7.1. The hybridoma line AODH 7.1 was originally produced by Kappler et al. (24) as an antigen-inducible interleukin 2-producing quadraple hybrid, and was kindly presented to us by Dr. C. G. Fathman. This line was maintained in RPMI 1640 containing 5 x 10^{-3} M 2-mercaptoethanol in tissue culture flasks at cell densities below 8 x 10^5/ml. For interleukin 2 production, 8 x 10^5 cells/ml were cultured in the presence of 10% FCS, 1 mg/ml human γ-globulin (HGG; SIGMA), and 8 x 10^5/ml 1500-rad irradiated B10.D2 spleen cells for 24 hr, and the supernatants were recovered by centrifugation and stored at -20°C.

Assay for interleukin 2 activity. The amount of interleukin 2 in the supernatants was measured by their ability to support the growth of the T cell clone W-2 (C57BL/6 spleen origin) (cloned by Michael Stock, Hanover, West Germany). The cells were grown in RPMI 1640 containing 30% Con A-stimulated rat spleen (Wistar) cell supernatant, and 5% FCS. The cells were then harvested and centrifuged to remove all cells, and concentrated 10X with an Amicon PM10 membrane. This factor was sterilized by filtration and stored at -20°C.

RESULTS

Characterization of the various batches of CSCS and AODH 7.1 supernatant that were used in the subsequent experiments. The present experiments were essentially done with 2 preparations of AODH 7.1 supernatant (batches No. 1 and No. 27) and 3 conventional CSCS preparations. The experiments in the subsequent tables and figures were performed mostly with batch No. 9 MR if not indicated otherwise. This preparation was obtained from DBA/2 spleen cells. The CSCS batch No. 2 was obtained from spleen cells of Sprague Dawley rats. The CSCS batch No. 10 finally was obtained from (C3H x DBA/2)F1 spleen cells. All these interleukin 2 preparations revealed only moderate differences with respect to the interleukin 2 titers (Fig. 1), but marked differences with respect to their helper effect in cytotoxic responses (see Figs. 2 and 6). The interleukin 2 titers were relatively reproducible in different experiments, but the height of the 3H-TdR incorporation varied considerably. The exceptionally high cpm value for batch No. 1 in Figure 1 is therefore not meaningful.

The synergistic effect of irradiated I-region-incompatible cells and CSCS as helper components for CTL responses in microcultures. CSCS were previously shown to provide a convenient source for interleukin 2 and to support the long-term growth of T cell lines (28) as well as the primary activation of CTL in a number of systems (28–30). CSCS has also been successfully employed in studies on alloreactive CTL precursor frequencies (22), where it compensates for the insufficient numbers of helper T cells in the limiting-dilution conditions. In accordance with these reports, we found that small numbers of thymocytes or peripheral lymphocytes generated cytotoxic activity against allogeneic cells in microcultures in the presence, but not in the absence, of conventional CSCS (Fig. 2 and Table I). The 2 conventional CSCS preparations, No. 2 and No. 10, generated strong CTL activity in CBA lymph node cells against allogeneic SJL stimulator cells, but only batch No. 10 mediated a substantial CTL response against the TNP-haptenated syngeneic stimulator cells (Fig. 2). Experiments with a variety of CSCS batches revealed that most of our conventional CSCS preparations from rat or mouse spleen cells behaved like batch No. 2, whereas only a few preparations showed the
exceptional property of batch No. 10. The basis for this exceptional behaviour is presently under study.

Our experiments with responder cells from A.TH and A.TL mice (Table I) and A/J mice (Table II) revealed, moreover, that the conventional CSCS preparation (in this case, batch No. 9 MR) was not able to support the CTL responses against isolated H-2K or H-2D differences, but mediated strong responses against differences in the entire H-2 complex. This demonstrated a synergistic effect between the conventional CSCS and an I-region disparity in the system.

A similar synergy was also observed in CTL responses against TNP-haptenated syngeneic cells (Fig. 3). $4 \times 10^4$ (C3H x DBA/2)F1 lymph node responder cells plus $3 \times 10^5$ TNP-haptenated and 1500-rad irradiated syngeneic stimulator cells generated strong CTL responses against TNP-haptenated target cells if cultured in the presence of $3 \times 10^5$ irradiated SJL spleen cells and 0.03 ml conventional CSCS (batch No. 9 MR); but even the 2- to 3-fold higher concentration of the same CSCS preparation in the absence of the irradiated allogeneic cells mediated only weak responses and allogeneic cells without CSCS also mediated no substantial responses (Fig. 3). This indicated that CSCS and irradiated allogeneic cells acted synergistically and contributed helper effects of different quality.

The concentrated CSCS II preparation that was prepared according to the procedure of Warren et al. (23) (Fig. 3) and the conventional CSCS preparation No. 10 (for example, see Fig. 6) were found to mediate equally strong responses in the presence or absence of allogeneic cells, indicating that the helper effect of the allogeneic stimulus could also be substituted by a soluble factor.
Effect of additional irradiated allogeneic or MLR activated cells on the CTL response against TNP-haptenated syngeneic cells

Table III

<table>
<thead>
<tr>
<th>Gr</th>
<th>Responder Cells</th>
<th>Allogeneic Cells Added</th>
<th>CSCS Added</th>
<th>Responder Cells from Thymus</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A.TL (K<em>D</em>D)</td>
<td>None</td>
<td>+</td>
<td>11.7 (125 ± 13)</td>
</tr>
<tr>
<td>2</td>
<td>A.TL (K<em>D</em>D)</td>
<td>A.TH</td>
<td>-</td>
<td>1.5 (122 ± 17)</td>
</tr>
<tr>
<td>3</td>
<td>A.TL (K<em>D</em>D)</td>
<td>A.TH</td>
<td>+</td>
<td>38.1 (479 ± 43)</td>
</tr>
<tr>
<td>4</td>
<td>A.TL (K<em>D</em>D)</td>
<td>C57BL/6</td>
<td>+</td>
<td>49.6 (686 ± 35)</td>
</tr>
<tr>
<td>5</td>
<td>A.TH (K<em>D</em>D)</td>
<td>None</td>
<td>-</td>
<td>4.1 (42 ± 11)</td>
</tr>
<tr>
<td>6</td>
<td>A.TH (K<em>D</em>D)</td>
<td>A.TH</td>
<td>-</td>
<td>18.5 (204 ± 32)</td>
</tr>
<tr>
<td>7</td>
<td>A.TH (K<em>D</em>D)</td>
<td>C57BL/6</td>
<td>+</td>
<td>22.0 (248 ± 27)</td>
</tr>
</tbody>
</table>

Eight x 10^3 lymph node cells or 4 x 10^3 thymus cells were cultured as responder cells together with 3 x 10^5 TNP-haptenated syngeneic 1500 rad irradiated TNP-haptenated syngeneic cells in the presence or absence of 0.04 ml CSCS and/or additional 3 x 10^5 1500 rad irradiated allogeneic cells as indicated and were tested on haptenated syngeneic target cells 5 days later (for further details see legend to Table I).

The alloreactive helper T cells are located in the responder and in the irradiated allogeneic cell population. Combinations of homozygous responder cells and irradiated semi-allogeneic F_1 cells were used to locate the alloreactive helper T cell precursor in the system. It was found that thymic responder cells from DBA/2 mice responded against TNP-haptenated syngeneic stimulator cells only in the presence of conventional CSCS (batch No. 9 MR, 0.04 ml) plus irradiated allogeneic C3H, but not semi-allogeneic (C3H x DBA/2)_F1, spleen cells (Fig. 5). This experiment showed that the thymocytes contained substantial numbers of TNP-specific CTL precursor cells, but that even the large number of 10^5 thymocytes did not contain sufficient alloreactive helper T cell precursors to recognize the H-2^D_1 determinants on the semi-allogeneic cells. It was therefore concluded that the substantial response in the presence of the allogeneic C3H cells was mediated by alloactivated helper T cell precursors within this irradiated allogeneic cell population. DBA/2 lymph node cells, on the other hand, responded equally well in the presence of allogeneic and semi-allogeneic irradiated cells, indicating that even the small number of 12.5 x 10^3 peripheral lymphocytes contained sufficient numbers of alloreactive helper T cell precursors to recognize the foreign determinants on the semi-allogeneic cells (Fig. 5). CSCS in the absence of allogeneic cells may therefore help to identify and to assay helper cell precursors in the responder population. Control experiments also showed in this case that no cell combinations produced any detectable responses in the absence of CSCS (data not shown). This demonstrated again the synergistic effect. The experiment in Figure 5 implied also that the haptenated I-region determinants do not substitute for the TNP-haptenated helper T cell precursor in the system.

![Figure 4](image-url) Effect of small numbers of allogeneic cells and of preactivated syngeneic helper T cells on the CTL response against TNP-haptenated syngeneic cells. The indicated numbers of responder cells from (C3H x DBA/2)_F1, lymph node cells were cultured together with 3 x 10^5 TNP-haptenated syngeneic cells in the presence or without 0.08 ml conventional CSCS (batch No. 9 MR) and the indicated numbers of 1500 rad irradiated SJL spleen cells or preactivated syngeneic helper T cells. These syngeneic helper T cells have been activated by co-culturing with irradiated SJL-stimulator cells for 2 days in macrocultures and have been enriched for T cells by a fractionation over nylon wool columns and subsequently irradiated with 1500 rad (q).
TABLE IV
Specificity of the CTL responses against TNP-coupled haptenated stimulator cells with an I-region difference*

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Gr Responder Cells</th>
<th>Stimulator Cells</th>
<th>Target Responder Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A.TH (K&quot;I&quot;D&quot;)</td>
<td>TNP-ATL (K&quot;I&quot;D&quot;)</td>
<td>TNP-ATH (K&quot;I&quot;D&quot;)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>51.0 (713 ± 80)</td>
<td>57.4 (1121 ± 124)</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>11.7 (125 ± 12)</td>
<td>14.5 (156 ± 24)</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>31.8 (352 ± 51)</td>
<td>64.9 (1048 ± 106)</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>35.0 (430 ± 26)</td>
<td>58.3 (880 ± 56)</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>39.7 (506 ± 104)</td>
<td>58.5 (880 ± 83)</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>12.3 (131 ± 24)</td>
<td>21.8 (224 ± 36)</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>28.0 (326 ± 93)</td>
<td>58.0 (666 ± 123)</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>15.6 (170 ± 30)</td>
<td>36.8 (458 ± 71)</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>7.9 (82 ± 16)</td>
<td>14.2 (153 ± 27)</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>19.0 (211 ± 62)</td>
<td>51.1 (716 ± 123)</td>
</tr>
<tr>
<td>11</td>
<td></td>
<td>9.7 (102 ± 25)</td>
<td>15.9 (173 ± 36)</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td>TNP-ATL (K&quot;I&quot;D&quot;)</td>
<td>TNP-SUL (K&quot;I&quot;D&quot;)</td>
</tr>
<tr>
<td>13</td>
<td></td>
<td>0.4 (4 ± 12)</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td></td>
<td>TNP-ATH (K&quot;I&quot;D&quot;)</td>
<td>TNP-DBA (K&quot;I&quot;D&quot;)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>19.9 (221 ± 46)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>A.TL (K&quot;I&quot;D&quot;)</td>
<td>29.6 (350 ± 37)</td>
</tr>
</tbody>
</table>

* One x 10^5 lymph node cells or 4 x 10^5 thymus cells from the indicated responder strains were cultured in the presence of 0.05 ml CSCS and 3 x 10^5 TNP-haptenated stimulator cells, and were finally tested on the indicated target cells. For other details see legend to Table I.

TABLE V
Specificity of the CTL response of A.TH responder cells against nonhaptenated stimulator cells with an I-region difference*

<table>
<thead>
<tr>
<th>Gr</th>
<th>Target Cells</th>
<th>Cytotoxic Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CBA (K&quot;I&quot;D&quot;)</td>
<td>11.7 (125 ± 10)</td>
</tr>
<tr>
<td>2</td>
<td>C57BL/6 (K&quot;I&quot;D&quot;)</td>
<td>0.0 (1 ± 10)</td>
</tr>
<tr>
<td>3</td>
<td>A.TH</td>
<td>0.0 (0 ± 10)</td>
</tr>
<tr>
<td>4</td>
<td>TNP-ATH</td>
<td>0.1 (1 ± 10)</td>
</tr>
</tbody>
</table>

* Three x 10^5 thymic responder cells from A.TH donors were cultured together with 0.05 ml CSCS and 3 x 10^5 nonhaptenated A.TL stimulator cells and tested on the indicated target cells. For other details see legend to Table I.

LATE-ACTING HELPER EFFECT OF ALLOGENIC CELLS

The AODH 7.1 supernatant batch No. 1 (A) and the conventional CSCS preparation No. 10 (C) that was selected to mediate equally good CTL responses in the presence or absence of allogeneic cells were added at day 0 and/or day 3 as helper components, with or without irradiated SJL cells (S), to cultures of CBA lymph nodes or thymus cells and irradiated TNP-haptenated CBA stimulator cells (Fig. 6). One hundred microliters of the culture supernatants were removed after centrifugation at day 3 and were replaced by 100 μl of fresh culture medium containing the helper component indicated in Figure 4. The experiment revealed that the AODH 7.1 supernatant by itself supported a clear-cut CTL response only in the case of 5 x 10^5 lymph node responder cells, but not with 2 x 10^5 lymph node or 2 x 10^5 thymic responder cells (Fig. 6, lines 3 and 4).

The CSCS No. 10 preparation, in contrast, supported considerable responses also in the case of the thymic responder cells or the smaller numbers of lymph node cells (lines 8 and 11), indicating that sufficient numbers of CTL precursor cells were present in the thymus, and that endogenous helper cells in the lymph nodes were required to synergize with the AODH 7.1 supernatant. A more direct demonstration of a synergistic effect was revealed by lines 1 to 3: The CBA lymph node cells generated substantial responses when interleukin 2 (A) was given at day 0 and allogeneic cells (S) at day 3 (see line 2), whereas either component alone generated only weak or no substantial responses (lines 1 and 3). The AODH 7.1 supernatant provided a detectable helper effect only if added at day 0 and not at day 3 (compare lines 3, 4, and 5), and the same applied to its synergistic effect (compare lines 2 and 7). Moreover, the addition of the AODH 7.1 supernatant at day 3 did not substitute for the irradiated SJL cells (compare lines 2 and 4). The irradiated SJL cells, on the other hand, synergized equally well whether added at day 3 or day 0 (compare lines 1 and 6), indicating that their synergistic contribution was required only in the late phase of the response and was therefore qualitatively different from the early effect of the AODH 7.1 supernatant. The selected CSCS preparation No. 10, on the other hand, supported equally strong CTL responses in the absence and presence of the allogeneic cells (lines 8 to 11), but again only if added at day 0 and not at day 3 (lines 12 and 15).

The experiments in Figure 7 showed finally that CSCS batch No. 2 behaved like the AODH 7.1 supernatant (batch No. 2.7.) and provided no detectable helper effect in the late phase of the cytotoxic response, whereas CSCS batch No. 10 and the irradiated allogeneic SJL cells mediated a strong enhancement when added at day 3 to the microcultures (Fig. 7).

DISCUSSION

Our experiments revealed that the conventional CSCS preparations are sufficient to support cytotoxic responses against allogeneic cells but are usually insufficient as helper components to support CTL responses against MHC- or I-region-compatible stimulator cells in microcultures and the same applied to the interleukin 2-containing supernatant from HGG-activated AODH 7.1 hybridoma cells. However, both types of supernatants mediated substantial CTL responses in the presence of irradiated I-region-incompatible cells or allogeneically activated irradiated syngeneic T cells, which by themselves were not able to support substantial CTL responses in microcultures. This observation indicated that interleukin 2 synergized with a 2nd type of helper effect that was activated in the presence of an I-region difference. Only some exceptions of CSCS preparations (i.e., batch No. 10) or the concentrated

The CSCS preparation No. 10 behaved like the AODH 7.1 supernatant (batch No. 2.7.) and provided no detectable helper effect in the late phase of the cytotoxic response, whereas CSCS batch No. 10 and the irradiated allogeneic SJL cells mediated a strong enhancement when added at day 3 to the microcultures (Fig. 7).
and partially purified CSCS preparation according to the procedure of Warren et al. (23) were able to support equally good CTL responses in the presence or absence of I-region-incompatible cells (see Figs. 3 and 6), indicating that the allogeneically activated helper effect is also mediated by a soluble factor.

Several experimental observations indicated that the MHC disparity in the culture provided an antigenic stimulus mainly for helper T cell precursors rather than for the CTL precursor cells under test. First, it was established that an I-region disparity was required, sufficient for the synergistic effect to occur (Tables I–III), and it was previously shown (4–6) that helper T cell precursors preferentially recognize I-region determinants, whereas CTL precursor cells preferentially recognize K- or D-region determinants. The synergistic effect was observed regardless of whether the foreign I-region determinants were presented on the stimulator cells for the CTL precursor cells (i.e., on the TNP-haptenated cells) or on separate cells (unlinked recognition). This observation was at variance with the model proposed by Paetkau et al. (17), but was in line with the earlier experiments of Bach, Alter, et al. (4–6) and of others (18), and demonstrated that this helper function is nonspecific in its effect. Our experiments also showed that the allogeneic cells could not be substituted for the TNP-haptenated stimulator cells, indicating again that they did not stimulate the TNP-specific CTL precursor cells under test, but rather some helper cells (Table V). Previous experiments of Billings et al. (31) suggested the possibility that the allogeneic I-region determinants might have recruited additional CTL precursor cells with specificity for TNP-haptenated syngeneic I-region determinants. Our experiments on this point showed, however, that the cytotoxic activity on TNP-haptenated syngeneic I-region determinants was low and would not have contributed significantly to the strong responses against the TNP-haptenated syngeneic K- or D-region determinants (see Table IV). The experiments of Billings et al. (31) were performed in macrocultures and indeed showed substantial kill on TNP-haptenated syngeneic I-region determinants only at relatively high killer-to-target cell ratios (i.e., 100:1).

Two lines of evidence supported the assumption that interleukin 2 and the additional I-region disparity provided helper signals of different quality and not merely quantity. First, the various conventional CSCS preparations and the AODH 7.1 supernatant showed similar interleukin 2 titers (Fig. 1) but differed strikingly in the ability to support a CTL response in an MHC-compatible system (Fig. 2). Moreover, titration of the

The synergistic effect of allogeneic and semi-allogeneic cells with conventional CSCS (batch No. 9 MR) on the CTL responses of thymic and peripheral lymphocytes against TNP-haptenated syngeneic cells. Graded numbers of responder cells from DBA/2 lymph nodes or thymus were cultured together with 3 x 10^5 TNP-haptenated syngeneic stimulator cells and 0.04 ml CSCS No. 9 MR with or without 3 x 10^5 irradiated C3H or (C3H x DBA/2)F1 spleen cells. The cytotoxic activity was tested after 5 days on TNP-haptenated DBA/2 target cells. Control cultures without CSCS were included in the experiment for all experimental groups but generated in all cases not more than background cytotoxicity.
cells (Fig. 3) revealed that the combination of allogeneic cells and a low concentration (i.e., 30×10^6) together with a 2- or 3-fold higher concentration of CSCS supported a 2nd type of helper effect. Our experiments with I-region-compatible, K-/D-region-incompatible stimulator cells indicated, on the other hand, that allogeneic I-region determinants stimulate this synergistic helper effect more efficiently than K- or D-region determinants. This is in line with earlier observations on the specificity of helper T cells (4–6), but not in line with 2 more recent reports that helper T cells are also activated to some extent by K- or D-region determinants (18, 19).

Plate (34) reported recently that the activation of TNP-specific CTL in macrocultures requires an MHC-restricted helper factor if Ia+ T cells are removed from the responder cell population. These studies thus suggested that the activation of CTL requires different helper components but it was not clear from the experiments whether the MHC-restricted helper factor was needed in addition to interleukin 2, or whether it was a part of the same helper pathway and able to overcome the requirement for interleukin 2. Our observation that the CSCS batch No. 10 from (C3H × DBA/2)F1 spleen cells, in contrast to most other CSCS preparations, was able to support the response of the CBA responder cells in the absence of I-region-incompatible stimulator cells suggested the possibility that an MHC-restricted 2nd helper factor was indeed detected in these experiments. But the helper effect of C3H cells on DBA/2 responder cells (Fig. 5) was not consistent with this interpretation. Clearly, this point needs further investigation.

REFERENCES


