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Generation of an Inhibitory Circuit Involving CD8⁺ T Cells, IL-2, and NK Cell-Derived TGF-β: Contrastong Effects of Anti-CD2 and Anti-CD3

J. Dixon Gray, Makoto Hirokawa, Kazuo Ohtsuka, and David A. Horwitz

Although the phenomenon of immunosuppression is well established, the mechanisms involved in the generation of lymphocytes with down-regulatory activity are poorly understood. Unlike anti-CD3 antibodies, mitogenic combinations of anti-CD2 antibodies do not stimulate human PBL to produce IgM or IgG. In determining the reason for this difference, we have found that anti-CD2 triggers an inhibitory circuit facilitated by TGF-β provided by NK cells. Stimulation of PBL with anti-CD2, but not anti-CD3, generated substantial amounts of active TGF-β. NK cells were found to be a significant source of TGF-β and were the only lymphocyte population that constitutively produced this cytokine. Anti-CD2 enhanced the production of active TGF-β by purified NK cells. TGF-β. After the removal of NK cells or the addition of anti-TGF-β, anti-CD2 could stimulate Ig production. Anti-TGF-β had to be added within the first 24 h for a maximal effect. Moreover, a short, overnight exposure of CD8⁺ T cells to TGF-β could prime them for suppressor activity provided that IL-2 was also present. Thus, the presence of active TGF-β coincident with CD8⁺ T cell activation can condition these cells to mediate down-regulatory activity, and NK cells can serve as the source of this cytokine. The Journal of Immunology, 1998, 160: 2248–2254.

In humans, the 50-kDa CD2 molecule is expressed on T cells and NK cells (1–4). Although T cell activation via CD2 was first described as an “alternate pathway” (1), it has become evident that signaling through CD2 can enhance or inhibit T cell-B cell interactions. CD2 seems to play an obligatory role in T cell-mediated activation of resting B lymphocytes by two separate mechanisms. First, the binding of CD2 to specific ligands on the B cell surface increases the avidity of cognate recognition of Ag presented by the B cell (4). Second, CD2 initiates intracellular signals that synergize with those initiated by the CD3/TCR complex (2, 5). On the other hand, anti-CD2 mAbs can markedly inhibit anti-CD3-induced T cell proliferation (6) and Ab production in vitro (7). Anti-CD2 administered in vivo can prolong graft survival and ameliorate experimental autoimmune encephalomyelitis (8, 9). Mechanisms to account for these effects include the production of anergy (10), the generation of suppressor cells (11), or alteration of the traffic pattern of effector T cells (9). This report deals with anti-CD2-mediated suppressor cell generation.

Although it is well established that anti-CD3 mAbs induce T cell-dependent B cell differentiation (12, 13), mitogenic combinations of anti-CD2 mAbs generally lack this activity when added to PBMC. We report that this difference is caused when anti-CD2 triggers a novel inhibitory circuit that we have recently described (14). In this circuit, TGF-β generated by NK cells appears to serve as a critical costimulatory signal to induce T suppressor cells.

TGF-β is a multifunctional family of cytokines important in tissue repair, inflammation, and immunoregulation (15). TGF-β can inhibit T and B cell proliferation, NK cell cytotoxic activity, and the generation of T cell cytotoxicity (16). By contrast, TGF-β has been reported to promote the growth of murine CD4⁺ cells and CD8⁺ cells (17, 18). Besides its effect on T suppressor effector generation (14), TGF-β has costimulatory activity for human CD4⁺CD45RA⁺ cells (19). Although almost all cells have the capacity to produce TGF-β on stimulation, the cytokine is secreted as a latent complex and must be converted to its active form for functional activity (20).

We considered whether the different properties of anti-CD3 and anti-CD2 mAbs could be explained by effects of the latter on NK cells. At least 50% of NK cells display CD2 molecules (21), and anti-CD2 can stimulate these cells to produce IFN-γ (22) and can enhance killer cell function (23, 24). In this report, we show that anti-CD2 stimulates the production of TGF-β and that NK cells are the only lymphocyte population that constitutively secretes large amounts of this cytokine. We also report that the contribution of CD4⁺ T cells to the generation of CD8⁺ regulatory cells is the production of IL-2 and have demonstrated that the presence of both IL-2 and TGF-β at the time CD8⁺ T cells are activated conditions them to markedly down-regulate IgM and IgG production.

Materials and Methods

Cell isolation

PBMC were prepared from heparinized venous blood of healthy adult volunteers by Ficoll-Hypaque (Pharmacia, Piscataway, NJ) density gradient centrifugation. Separation of PBMC into monocyte and lymphocyte fractions was performed by centrifugation on a continuous Percoll (Pharmacia, Piscataway, NJ) density gradient (25). Depletion of lymphocyte subpopulations was performed by staining with anti-CD4 (OKT4, American Type Culture Collection (ATCC), Rockville, MD), anti-CD8 (OKT8, ATCC), or anti-CD16 (3G8 kindly provided by Jay Unkeless, New York, NY). Reacting cells were then depleted using immunomagnetic beads (Dynal, Great Neck, NY).
To obtain NK cells, PBMC were added to a nylon wool column, and the eluted, nonadherent cells were immediately resorted with 2-aminoethylisothiouronium bromide-treated SRBC (26). The nonrosetting fraction was then stained with anti-CD3 and anti-CD74 (anti-HLA-DR) Abs and depleted of reacting cells using immunomagnetic beads (Dynal). This resultant population usually contained >98% CD56<sup>+</sup>, <0.5% CD3<sup>+</sup>, and <0.5% CD20<sup>−</sup> lymphocytes. In some experiments, the NK cells were isolated by cell sorting using a FACStar (Becton Dickinson, San Jose, CA). PBL were stained with anti-CD3 and anti-CD20, and the nonstaining cells were collected.

CD4<sup>+</sup> and CD8<sup>+</sup> cells were prepared from nylon-nonadherent lymphocytes by negative selection using immunomagnetic beads. For CD4<sup>+</sup> cells, the nylon-nonadherent cells were stained with Abs to CD8, CD16, CD11b, and CD74. The same Abs were used to obtain CD8<sup>+</sup> cells except that CD4<sup>+</sup> was substituted for CD8. Purity of CD4<sup>+</sup> and CD8<sup>+</sup> cells was 85 to 90%.

To obtain B cells, nylon wool-adherent cells were immediately resorted with SRBC to remove any T cells and treated with 5 mM L-leucine methyl ester for complete removal of monocytes and functional NK cells. The resulting population was >92% CD20<sup>+</sup> and <0.5% CD3<sup>+</sup>.

Reagents

Antibodies used were anti-CD2 (OKT11, ATCC); GT2 (27) made available by A. Bernard, Nice, France; T11, (1), kindly provided by S. Schlossman, Boston, MA; anti-CD3 (454 (28), a gift from W. Stohl, University of Southern California, Los Angeles, CA); anti-CD4 (OKT4, ATCC); anti-CD8 (OKT8, ATCC; CD8, Dako, Carpenteria, CA); anti-CD11b (OKM1, ATCC); anti-CD16 (3G8 (29), kindly provided by J. Unkeless, New York, NY); anti-CD20 (Becton Dickinson, San Jose, CA); anti-CD74 (L243, ATCC); anti-CD2 (CD8, Dako, Carpenteria, CA); anti-TGF-β (1D11.16), a murine IgG1, was kindly provided by J. Carlino (Celltrix Pharmaceuticals, Santa Clara, CA) and Bruce Pratt (Genzyme Corporation, Farmington, MA).

Cell cultures

Procedures for cell cultures have been described previously (30). In brief, the various lymphoid populations were added to the wells of a flat-bottom microtiter plate (Falcon, Lincoln Park, NJ) usually at 1 × 10<sup>5</sup>/well in medium. To measure Ig production, cultures were conducted in RPMI 1640 medium (Irvine Scientific, Santa Ana, CA) supplemented with 10% heat-inactivated FCS (Gemini Bioproducts Inc., Calabasas, CA), 10 g/ml gentamicin (Irvine Scientific), 2 mM l-glutamine (Flow Laboratories, McLean, VA), and 10 mM HEPES (U.S. Biochemical Corporation, Cleveland, OH). Since TGF-β can bind to serum components such as α2-macroglobulin or IgG (31, 32), supernatants to be assayed for TGF-β content were generated in Aim V serum-free medium (Life Technologies, Grand Island, NY). The variation between triplicate cultures was usually <10%.

Results

Neutralization of TGF-β enables anti-CD2 to induce T cell-dependent Ab production

As reported by others, PBMC were induced to produce IgM and IgG on stimulation with anti-CD3 (12, 13). With GT2 and OKT11, a mitogenic combination of anti-CD2 mAbs, there was generally

4 Abbreviation used in this paper: MLEC, mink lung epithelial cells.
little or no Ab production (Fig. 2) although both mitogens induced comparable levels of proliferative activity (25, 893 ± 5, 625 and 20, 619 ± 3, 605 cpm, respectively). A similar result was obtained with another mitogenic combination of anti-CD2 Abs, T112, and T113 (data not shown).

Consistent with our previous observations (14), we found that following depletion of CD8+ cells or NK cells, anti-CD2 was able to induce the production of considerable amounts of Ab. In the four experiments shown in Table I, depletion of monocytes had no effect on Ab production. Depletion of CD8+ cells resulted in the production of both IgM and IgG production in all experiments, and depletion of CD16+ cells had a similar effect in three of the four experiments.

Previously, we reported that both CD8+ cells and NK cells were important in the regulation of Ab production and that TGF-β was an important cytokine in mediating this activity (14). To determine whether the inability of anti-CD2 to stimulate Ab production was related to the production of TGF-β, we added anti-TGF-β to the cultures. As shown in Table II, in the presence of anti-TGF-β substantial amounts of IgM and IgG were induced by anti-CD2. Anti-TGF-β, however, had no effect on the lymphocyte-proliferative response to anti-CD2. As expected, the addition of anti-CD3 stimulated IgG production in the two donors shown in Table III. In contrast to the effect on anti-CD2, the addition of anti-TGF-β had no effect on anti-CD3-induced IgG production.

Since anti-CD2 could stimulate Ig production when anti-TGF-β Abs were included, we compared the ability of anti-CD2 and anti-CD3 to induce the production of active TGF-β. Figure 3 shows that anti-CD2, but not anti-CD3, stimulates the production of active TGF-β. Thus, the difference between anti-CD2 and anti-CD3 to induce Ig production is inversely related to their ability to stimulate active TGF-β production.

**NK cells are a principal source of TGF-β**

To determine the lymphocyte source of TGF-β, PBL were depleted of various lymphocyte populations and assayed for both latent and active TGF-β production either constitutively or after stimulation with anti-CD2 (Table IV). Production of total TGF-β, both constitutive and stimulated, was markedly reduced by removal of CD16+ cells but not by depletion of CD4+ or CD8+ cells. Similarly, depletion of CD16+ cells significantly reduced the production of active TGF-β. While the depletion of CD4+ cells had no effect, depleting CD8+ cells had variable effects ranging from none to a significant reduction. This variability probably reflects variation in the numbers of CD8+ CD16+ cells depleted in these experiments.

These studies suggested that NK cells were a primary source of TGF-β. To address this issue, we isolated various mononuclear cell populations and measured constitutive active and latent TGF-β production (Table V). Unlike T cells or B cells, purified NK cells secreted nanogram per milliliter amounts of latent TGF-β and significant amounts of active TGF-β. This level of TGF-β activity by NK cells was somewhat greater than that of monocytes, the more conventional hemopoietic source of TGF-β (34).

Although NK cells were the source of TGF-β in anti-CD2-stimulated PBL, it was not clear whether this was a direct effect of anti-CD2 on the NK cells or an indirect one from T cell activation. To address this, purified NK cells were negatively selected by cell sorting and cultured with or without anti-CD2. That NK cells could be directly stimulated by anti-CD2 to secrete active TGF-β is shown in Figure 4. By contrast, purified T cells produced negligible amounts of TGF-β after 48 h of culture. A similar finding was observed in five other experiments.

**TGF-β and IL-2 are needed for the generation of CD8+ T cells that down-regulate Ab production**

Having shown that TGF-β contributes to the inability of anti-CD2 to stimulate Ab production, we considered the possibility that this cytokine has an important role in the generation of CD8+ suppressor activity. To determine when TGF-β was needed, anti-TGF-β was added at different times to PBL stimulated with anti-CD2. Figure 5 shows that, as before, the addition of anti-TGF-β at the start of the culture resulted in robust IgG and IgM production. A delay in the addition of this Ab for only 24 h reduced IgG production by 50% and IgM production by 80%. If anti-TGF-β was added at 48 h or later, the response was essentially abolished.

To show formally that TGF-β is involved in the generation of suppressor activity, CD8+ cells were cultured overnight in the presence or absence of TGF-β. We also included IL-2 because of

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**Table I. Depletion of either CD8+ or NK cells abolishes the inhibitory effects of anti-CD2**

<table>
<thead>
<tr>
<th>Expt.</th>
<th>PBMC (μg/ml)</th>
<th>PBL</th>
<th>CD8 dep</th>
<th>NK dep</th>
<th>PBMC (μg/ml)</th>
<th>PBL</th>
<th>CD8 dep</th>
<th>NK dep</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.30</td>
<td>0.30</td>
<td>6.10</td>
<td>35.10</td>
<td>1.20</td>
<td>0.50</td>
<td>14.00</td>
<td>28.00</td>
</tr>
<tr>
<td>2</td>
<td>0.10</td>
<td>0.22</td>
<td>4.70</td>
<td>3.90</td>
<td>0.20</td>
<td>0.50</td>
<td>2.60</td>
<td>1.90</td>
</tr>
<tr>
<td>3</td>
<td>0.85</td>
<td>0.87</td>
<td>3.75</td>
<td>0.75</td>
<td>0.30</td>
<td>0.30</td>
<td>2.28</td>
<td>0.15</td>
</tr>
<tr>
<td>4</td>
<td>0.63</td>
<td>0.48</td>
<td>6.50</td>
<td>16.25</td>
<td>0.28</td>
<td>0.10</td>
<td>9.75</td>
<td>15.50</td>
</tr>
</tbody>
</table>

*The cell populations indicated above were added to microtiter wells at 1 × 10^6/well. CD8+ or CD16+ cells were depleted (dep) by immunomagnetic beads. After culture in the presence of anti-CD2 (GT2 + T11), supernatants were assayed for Ig content by an ELISA. In Experiment 1 supernatants were collected at day 12, and in Experiments 2 to 4 supernatants were from 7-day cultures.

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**Table II. Anti-CD2 induces Ig synthesis following antagonism of TGF-β**

<table>
<thead>
<tr>
<th></th>
<th>IgM (ng/ml)</th>
<th></th>
<th>IgG (ng/ml)</th>
<th></th>
<th>[3H]TdR (cpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Medium</td>
<td>75</td>
<td>430</td>
<td>200</td>
<td>275</td>
<td>250</td>
</tr>
<tr>
<td>Anti-TGF-β</td>
<td>1,650</td>
<td>2,500</td>
<td>825</td>
<td>1,250</td>
<td>6,800</td>
</tr>
<tr>
<td>Mouse IgG1</td>
<td>175</td>
<td>270</td>
<td>175</td>
<td>550</td>
<td>310</td>
</tr>
</tbody>
</table>

*PBMC (1 × 10^6) were cultured with anti-CD2 (GT2 + T11) plus medium, anti-TGF-β (10 μg/ml), or control mouse IgG1 (10 μg/ml). Three separate experiments are shown. A, B, and C are donors.*
While monocytes are generally regarded as a significant source of TGF-β, and detectable amounts of the active form of this cytokine. Neither IL-2 nor TGF-β1 anti-CD2 stimulated CD4+ T cells, constitutively secrete substantial amounts of latent TGF-β. NK cells, but not resting B cells, CD4+ T cells and B cells. Figure 6 shows that neither IL-2 nor TGF-β alone were sufficient for the generation of suppressor activity. However, when CD8+ T cells were exposed to both cytokines, there was an 80% suppression of Ig production in the four experiments summarized.

**Discussion**

This report confirms and extends our recent discovery of a negative regulatory circuit dependent on the production of active TGF-β generated by an apparent interaction between activated CD8+ T cells and NK cells (14). Here we demonstrate that a mitogenic combination of anti-CD2 mAbs is generally unable to induce T cell-dependent Ab production because of the induction of active TGF-β production in parallel with lymphocyte activation. This was suggested by the findings that IgM and IgG synthesis was observed following depletion of either CD8+ T cells or NK cells and that the addition of a neutralizing anti-TGF-β mAb concomitantly with anti-CD2 resulted in a vigorous Ab response. More definitive evidence was the ability of anti-CD2, but not anti-CD3, to stimulate the production of active TGF-β.

Our second observation was that NK cells are a major source of TGF-β. NK cells, but not resting B cells, CD4+ cells, or CD8+ cells, constitutively secrete substantial amounts of latent TGF-β, as well as detectable amounts of the active form of this cytokine. While monocytes are generally regarded as a significant source of TGF-β, unstimulated NK cells can secrete comparable amounts. Previously, we documented up-regulation of TGF-β mRNA in NK cells following interaction with CD8+ T cells in PWM-stimulated cultures, and subsequently these NK cells produced active TGF-β (14). In the present experiments, we found that NK cells could be directly stimulated with anti-CD2 to secrete increased amounts of active TGF-β. Since a significant percentage of NK cells express CD2 but not CD3, this would account for the differential effects of Abs to these molecules on TGF-β production and Ig production.

As is the case with other cellular sources of TGF-β (37), the cytokine is released predominantly as a latent complex and is converted to its active form extracellularly. Early studies indicated that cell-cell interaction was required for conversion. For example, with bovine cells, active TGF-β was produced when aortic endothelial cells and pericytes were in contact but not when separated (38, 39). More recently, however, Nunes et al. (40) found that activated macrophages could produce active TGF-β. Similarly, we have found that stimulated NK cells can also produce active TGF-β without the need for other interacting cell populations. Rifkin and colleagues (20) have demonstrated that an important step in active TGF-β production was the conversion of plasminogen to plasmin by the action of plasminogen activator. Unlike T cells, NK cells secrete the urokinase-type plasminogen activator (41), and ~50% express receptors for this activator (42). Therefore, NK cells have the potential to convert TGF-β by such a mechanism. Of note, NK cells stimulated by anti-CD2 increased active but not total TGF-β production, indicating that increased conversion was stimulated. However, whether this is by plasmin activity must be determined.

Active TGF-β has a variety of immunoregulatory activities (43, 44). We have previously shown that CD8+ cells stimulated in the absence of CD3 express TGF-β in vivo (35, 36), providing a possible explanation for the suppression observed in the present study. Thus, the results of the present study extend our previous in vivo observations and confirm that TGF-β can also be produced in vitro by CD8+ T cells. It should be noted, however, that our in vivo experiments were performed in the presence of a mitogenic stimulus (anti-CD3). Without this, the extent of TGF-β production by CD8+ T cells is unknown.

**Table III.** Lack of effect of anti-TGF-β on anti-CD3-stimulated IgG production

<table>
<thead>
<tr>
<th>Condition</th>
<th>Anti-CD2</th>
<th>Anti-CD3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Anti-TGF-β</td>
<td>5000</td>
<td>1300</td>
</tr>
<tr>
<td>Mouse IgG1</td>
<td>630</td>
<td>460</td>
</tr>
</tbody>
</table>

* PBMC (1 × 10^6) were cultured with anti-CD2 (GT2 + T11) or anti-CD3 in the presence or absence of anti-TGF-β (10 μg/ml) or control mouse IgG1 (10 μg/ml). Two separate experiments are shown. A and B are donors.

**Table IV.** Effect of depleting different lymphocyte subpopulations on the production of TGF-β

<table>
<thead>
<tr>
<th>Condition</th>
<th>Expt. 1</th>
<th>Expt. 2</th>
<th>Expt. 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium</td>
<td>Anti-CD2</td>
<td>Medium</td>
<td>Anti-CD2</td>
</tr>
<tr>
<td>PBL</td>
<td>400</td>
<td>2000</td>
<td>464</td>
</tr>
<tr>
<td>CD4+</td>
<td>ND</td>
<td>ND</td>
<td>560</td>
</tr>
<tr>
<td>CD8+</td>
<td>200</td>
<td>1040</td>
<td>344</td>
</tr>
<tr>
<td>CD16</td>
<td>128</td>
<td>376</td>
<td>48</td>
</tr>
</tbody>
</table>

**Table V.** Constitutive TGF-β production by blood mononuclear cell populations

<table>
<thead>
<tr>
<th>Condition</th>
<th>NK</th>
<th>CD4</th>
<th>CD8</th>
<th>B</th>
<th>Monocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>3585</td>
<td>± 798</td>
<td>&lt;5</td>
<td>167 ± 30</td>
<td>2058 ± 346</td>
</tr>
<tr>
<td>Active</td>
<td>84 ± 22</td>
<td>&lt;5</td>
<td>6 ± 1</td>
<td>43 ± 20</td>
<td></td>
</tr>
</tbody>
</table>

* The various populations were cultured in serum-free medium for 48 h, and the supernatants were tested in triplicate for TGF-β activity using genetically engineered mink cells. The supernatants were heated at 80°C for 2 min to measure of total TGF-β and expressed as picograms per milliliter.
presence of TGF-β generated suppressor activity for Ab production (14). However, TGF-β alone was insufficient, and CD4+ cells were also required to generate activity. This finding was highly consistent with previous reports describing a role for CD4+ suppressor-inducer cells. In the present study, we were able to replace CD4+ inducer cells with IL-2. A role for this cytokine in the generation of suppressor activity has been described in other studies (45–47). Consistent with our findings, IL-2 alone was not sufficient to generate this activity.

Our next significant observation was the critical timing of TGF-β in suppressor cell generation. The presence of active TGF-β was needed early, within the first 24 h. This was evident from studies where anti-TGF-β was added at various times after lymphocyte stimulation with anti-CD2. At least a half-maximal Ab response reappeared if the addition of anti-TGF-β was delayed for 24 h. Furthermore, conditioning of CD8+ cells with IL-2 and TGF-β during the first 24 h of culture was sufficient for them to become suppressor cells.

The mechanism of action of CD8+ suppressor cells has not been determined. However, it is known that TGF-β can up-regulate itself by paracrine and autocrine effects (48). Moreover, Kehrl et al. (17) reported that T cell-derived TGF-β was not detectable until after 72 h of culture. We also found that T cells had no endogenous TGF-β activity and speculate that NK cell-derived TGF-β can prime them to produce large amounts of this cytokine. TGF-β producing regulatory T cell clones have been described by others (49).

Although the administration of either anti-CD2 or anti-CD3 in vivo (9–11, 50, 51) has immunosuppressive effects, only the infusion of anti-CD3 results in severe systemic toxic side effects.

FIGURE 4. Purified NK cells stimulated with anti-CD2 produce active TGF-β. Unfractionated PBL or NK cells (1 × 10⁶) and T cells purified by cell sorting were cultured in the presence or absence of anti-CD2 (GT2 plus T11) in Aim V medium. After 48 h, supernatants were harvested, and the active TGF-β content was measured by the mink luciferase assay.

FIGURE 5. Anti-TGF-β must be added early to promote Ig production. PBL were cultured with anti-CD2. At the times indicated, anti-TGF-β (10 μg/ml) or control mouse IgG1 (10 μg/ml) was added to the wells. The medium value refers to baseline Ig production by cells cultured with anti-CD2 for 7 days. At day 7 of culture, supernatants were assayed for IgM and IgG production by an ELISA. This experiment has been repeated twice with similar results.

FIGURE 6. Conditioning of CD8+ T cells to down-regulate IgG production. Purified CD8+ cells were incubated overnight with IL-2 (10 U/ml) and/or TGF-β (0.1 ng/ml) as described in Materials and Methods. The washed CD8+ cells (5 × 10⁶/well) were added to CD4+ cells (5 × 10⁶/well) and B cells (5 × 10⁶/well) stimulated with anti-CD2, and the amount of IgG produced after 7 days was measured. The bars indicate the relative amount of IgG produced compared with cultures without added CD8+ cells. The mean ± SEM of four experiments is shown where IgG values ranged from 1.3 to 10.5 μg/ml. The relative effect of CD8+ T cells on IgG production was calculated by the formula: [1 – (CD4 + CD8 + B cells)/ (CD4 + B cells)] × 100.
(52–54). This is because of massive polyclonal T cell activation with up-regulation of many cytokines which include IL-2, IL-6, TNF-α, and IFN-γ. The reasons for these differences between anti-CD2 and anti-CD3 are poorly understood. CD2 molecules on the T cell surface are in close proximity to the CD3/TCR complex and signaling through the CD2 pathway in T cells is dependent on intact CD3 ζ-chains. Synergism between the CD2 and the CD3/TCR receptor pathways has been demonstrated (1, 2, 55). However, perturbation of certain epitopes on the large cytoplasmic domain of CD2 can have inhibitory effects. For example, the binding of an LFA-3 fusion protein to the CD58 binding site on CD2 inhibits T cell activation to a variety of stimuli and results inergy (56). The absence of severe toxic side effects following the administration of anti-CD2 could, therefore, be due to negative signaling.

Alternatively, these differential effects of anti-CD2 and anti-CD3 in vivo might possibly have a less complex explanation. Since NK cells display CD2 molecules (21), the action of anti-CD2 on both T cells and NK cells could promote the conversion of latent TGF-β and trigger the TGF-β-dependent inhibitory circuit. The resulting immunosuppressive effects could, therefore, abort the cytokine release associated with anti-CD3 therapy. While anti-CD2 administration in vivo results in the prolongation of graft survival and the amelioration of experimental autoimmune encephalomyelitis, neither of these effects is dependent on anergy (10, 11). Consistent with this suggestion, we found that following the depletion of NK cells, the effects of anti-CD2 mAbs on T cell-dependent B cell differentiation were equivalent to that of anti-CD3. Thus, once anti-CD2 up-regulation of TGF-β production was blocked, the effects of each of these mAbs were now indistinguishable.

In addition to facilitating the generation of CD8+ T suppressor cells, NK cells have also been reported to have a role in the induction of CTL activity (57). Similar to our observation on T suppressor cell induction, CD4+ T cells by themselves were unable to induce CD8+ T cells to develop potent cytotoxic activity against allogenic target cells. The addition of NK cells provided costimulatory signals for the development of CTL activity. A possible relationship between the costimulatory signals supplied by NK cells in facilitating each of these CD8+ T cell effectors activities remains to be investigated. Of interest, it has been reported that TGF-β can up-regulate CTL activity (58).

It is likely that NK cells are involved in suppressor cell generation in vivo. They are abundant adjacent to the mucosal membranes that line the intestines (59), the lungs (60), and female reproductivic organs (61). In each of these organ systems, the response to Ag stimulation is predominantly negative with an associated up-regulation of TGF-β (62–64). Related to this is the predominant IgA Ab response (65, 66) and generation of regulatory cells that mediate oral tolerance (67, 68). We propose that the exposure of CD8+ T cells (and perhaps other T cell subsets) to TGF-β coincident with activation may trigger the up-regulation of endogenous TGF-β and enable these cells to produce immunosuppressive amounts of this or other inhibitory cytokines. In these organ systems, therefore, the presence of NK cell-derived TGF-β may explain the predominantly negative immune response that occurs following Ag challenge to T cells.

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References