The T-Cell CD2 Determinant Mediates Inhibition of Erythropoiesis by the Lymphokine Cascade

By St. Burdach, M. Shatsky, B. Wagenhorst, and L. Levitt

We examined the role of the T-cell antigen CD2 in the regulation of erythropoiesis by the lymphokine cascade. T-cell interleukin-2 (IL-2) receptors (p55) were induced via triggering of the antigen receptor–associated CD3 epitope. Before CD3 triggering, T cells were preincubated with a CD2-blocking (Leu-Bb) or isotype control antibody. T-cell pellets were employed during incubation to facilitate interaction between T-cell LFA-3 and CD2. CD2 blockade caused a 68% to 79% inhibition of p55 expression after three to six days of culture with IL-2. Next, we assessed the effect of CD2 blockade on IL-2–induced inhibition of BFU-E in autologous cocultures containing CD3-triggered T cells. IL-2 caused a dose-dependent inhibition (52% to 92%) of BFU-E in the presence but not in the absence of CD3-triggered T cells. T-cell CD2 blockade prior to CD3 triggering caused a 65% to 87% abrogation of IL-2–induced inhibition of BFU-E at 10 to 10² U/mL IL-2. Preincubation of CD3-triggered T cells with isotype control antibody had no effect on IL-2–induced erythroid inhibition. Day 3 supernatants from CD3-triggered T cells or CD2-blocked, CD3-triggered T cells established in the presence of IL-2 were next assessed for modulation of BFU-E. CD3-triggered T-cell supernatants caused a 77% ± 9% inhibition of BFU-E. Blockade of CD3 caused a 95% abrogation of T-cell–mediated BFU-E inhibition. In addition, CD2 blockade reduced interferon-γ (IFN-γ) release (84 to 128 U/mL) from CD3-triggered T cells by 81% at day 3 of culture. In control experiments, the addition of IFN-γ-neutralizing monoclonal antibody to CD3-triggered T-cell supernatant established in the presence of IL-2 caused 78% abrogation of IL-2 inhibition of BFU-E. We conclude that blockade of the CD2 T-cell determinant induces down modulation of (a) T-cell p55 IL-2 receptor expression, (b) IL-2–induced inhibition of BFU-E, and (c) IL-2–induced narrow T-cell IFN-γ release. These data suggest that the T-cell CD2 determinant can exert a regulatory effect on the control of erythropoiesis by the lymphokine cascade.

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MATERIALS AND METHODS

T-cell purification. Adult BM and peripheral blood (PB) Ficoll-Hypaque mononuclear cells were first depleted of monocytes to blunt endogenous IL-1 and subsequent IL-2 production as previously described.7,11 T cells were purified from the nonadherent layer by utilizing CD5 (Leu-1) pan T-cell monoclonal antibody.7,11 T cells were recovered from the bound fraction by differential elution and were 95% ± 3% pure. T-cell purity was assessed by an indirect three-step immunofluorescence procedure on separate cell aliquots employing CD5 (Leu-1) and CD3 (Leu-4) murine antihuman monoclonal antibodies.7,11

Induction of T-cell p55 IL-2 receptors and preparation of T-cell supernatants. T-cell p55 (Tac) IL-2 receptors were induced via triggering of the T-cell antigen receptor–associated CD3 epitope.
Before antigen receptor triggering, T-cell pellets were preincubated (20 minutes, 0°C) with a CD2-blocking monoclonal antibody (NaCl-free Leu-5b, an IgG2α obtained from E. Engleman, Stanford University Blood Center, 10−7 to 10−6 g/106 cells) or an isotype control CD5 monoclonal antibody (NaCl-free Leu-1). T-cell pellets were employed to facilitate interaction between T-cell ILF-A and CD2. In control experiments, Leu-5b but not Leu-1 antibody completely abrogated T-cell rosetting with sheep RBCs. Following CD2 or CD5 preincubation, washed T-cell pellets were incubated with CD3 monoclonal antibody (OKT3, 1.25 × 10−6 g/106 cells), which recognizes a determinant associated with the T-cell receptor for antigen. Purified T cells when incubated with 1.25 × 10−6 g/106 CD3 antibody in the absence of exogenous IL-2 did not produce IL-2 as assessed by IL-2 concentration-dependent 3H-thymidine incorporation into a cloned IL-2-dependent murine cytotoxic T cell line.29 Both CD2-preincubated CD3-triggered T cells and control CD5-preincubated CD3-triggered T cells were cultured at 106 cells/mL. T cells were cultured in the presence of 102 U/mL recombinant DNA-derived IL-2 (Cetus Corp., Emeryville, CA) with or without their respective preincubation (10−4 g/mL) and triggering (1.25 × 10−6 g/mL) antibodies. At time 0 and after three and six days, cell aliquots were stained with OKT3-fluorescein isothiocyanate and p55 IL-2 receptor 2A3 (CD25)31 phycoerythrin antibodies. Lymphocyte-gated cells (106 cells per sample) were analyzed with a flow cytometer. T cells were negative for p55 at day 0. When compared with CD3-triggered cells cultured without preincubation, CD5 preincubation did not significantly affect p55 expression from CD3-triggered T cells. Supernatants were also harvested from these three- to six-day T-cell suspension cultures and subsequently assessed for IFNγ content and for effects on BM erythroid progenitor growth. IFNγ was measured by solid-phase radioimmunoassay as previously described.11

Clonal progenitor assay. In brief, heparinized BM aspirate was depleted of erythrocytes, granulocytes, and platelets by Ficoll-Hypaque density gradient fractionation. Interphase buoyant cells were subsequently depleted of monocytes by sequential polystyrene depletion and cultured with NAB-T cells at a ratio of 0.2:1.0 to NAB-T. Antibody incubations were performed with T-cell pellets to facilitate interaction between T-cell LFA-3 and CD2 antibody. Forty-two percent ± 9% of CD3-triggered T cells expressed p55 at day 3 and 53% ± 7% expressed p55 at day 6. CD2 blockade caused a 79% ± 9% inhibition of p55 expression at day 3, as compared with preincubation with isotype control CD5 antibody, and a 66% ± 11% inhibition of p55 expression at day 6 (Table 1). Preincubation with isotype control CD5 antibody did not affect CD3-mediated p55 expression (Table 1). When T cells were cultured in the absence of exogenous IL-2, CD2 blockade caused a 69% ± 8% abrogation of CD3-induced p55 expression. CD2 blockade had no effect on binding of either triggering antibody to CD3 or IL-2 receptor antibody to p55.

RESULTS

CD2 blockade inhibits p55 IL2 receptor expression. IL-2 receptors (p55) were induced on PB or BM T cells via triggering of the antigen receptor CD3 epitope. T cells were preincubated with CD2-blocking antibody before activation with CD3 antibody and subsequently cultured for three to six days in the presence of IL-2. Antibody incubations were performed with T-cell pellets to facilitate interaction between LFA-3 and CD2. Forty-two percent ± 9% of CD3-triggered T cells expressed p55 at day 3 and 53% ± 7% expressed p55 at day 6. CD2 blockade caused a 79% ± 9% inhibition of p55 expression at day 3, as compared with preincubation with isotype control CD5 antibody, and a 66% ± 11% inhibition of p55 expression at day 6 (Table 1).

Preincubation with isotype control CD5 antibody did not affect CD3-mediated p55 expression (Table 1). When T cells were cultured in the absence of exogenous IL-2, CD2 blockade caused a 69% ± 8% abrogation of CD3-induced p55 expression. CD2 blockade had no effect on binding of either triggering antibody to CD3 or IL-2 receptor antibody to p55.

CD2 blockade down regulates IL-2-induced inhibition of erythropoiesis. We first assessed the effect of preincubation with CD2 antibody v isotype control CD5 antibody on CD3-triggered BM T cell inhibition of BFU-E in autologous coculture. Progenitors were grown from nonadherent and T-cell-depleted BM mononuclear cells (NAB-T). In the presence but not in the absence of CD3-triggered T cells, IL-2 induced a dose-dependent inhibition of BFU-E (52% to 92%, Fig 1). CD2 blockade caused an 87% abrogation of IL-2-induced inhibition of BFU-E at 1 U/mL IL-2, a 65% abrogation of BFU-E inhibition at 10 U/mL IL-2, and an

<table>
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<tr>
<th>Table 1. CD2 Blocking Monoclonal Antibody Inhibits T-Cell p55 IL-2 Receptor Expression</th>
<th>% T Cells Expressing p55 IL2 Receptor</th>
<th>Control T Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CD2-Blocked, CD3-Triggered T Cells</td>
</tr>
<tr>
<td>Presence of IL-2</td>
<td>3</td>
<td>9 ± 4</td>
</tr>
<tr>
<td>6</td>
<td>18 ± 6</td>
<td>53 ± 7</td>
</tr>
<tr>
<td>Absence of IL-2</td>
<td>3</td>
<td>8 ± 2</td>
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</table>

P55 was induced on purified T cells by triggering of the CD3 epitope as described in Methods. Before triggering CD3, T cells were preincubated with CD2-blocking antibody (CD2CD3) or isotype control antibody (CD5). The control represents CD3-induced p55 expression in the absence of preincubation antibody. T-cell suspensions were harvested after three and six days of culture in the presence or absence of 102 U/mL IL-2 and stained with IL-2 receptor phycoerythrin-conjugated monoclonal antibody. The data represent means ± SEM of four separate experiments.
85% abrogation at 10^2 U/mL IL-2, respectively (P < .01, Fig 1). CD2 blockade had no independent effect on BFU-E growth in the absence of IL-2: BFU-E growth was 123 ± 35 (with CD2 blockade) vs 126 ± 40 (control) per 1.5 x 10^5 NAB-T (mean ± SE of three separate experiments with samples from different individuals). IL-2-induced, CD3-triggered, T-cell-mediated inhibition of BFU-E was not affected by either preincubation of CD3-triggered T cells with control CD5 antibody or preincubation of non-CD3-triggered T cells with CD2-blocking antibody (data not shown).

Next we assessed whether CD2 blockade modulates IL-2–induced release of erythropoietic inhibitors from CD3-triggered T cells. Day 3 supernatants from BM CD3-triggered T cells with a free CD2 determinant or CD2-blocked, CD3-triggered T cells were assessed against BM NAB-T (Table 2). All supernatants were established in the presence of IL-2. CD3-blocked BM T-cell supernatants caused a 77% ± 9% inhibition of BFU-E. Blockade of CD2 caused a 95% abrogation of CD3-triggered, T-cell-mediated BFU-E inhibition. Preincubation with isotype control antibody had no effect on CD3-triggered T-cell supernatant inhibition of BFU-E (data not shown). Next, day 3 and day 6 supernatants from PB CD3-triggered T cells with free CD2 determinants or CD2-blocked, CD3-triggered T cells were assessed against BM NAB-T. Day 3 CD3-triggered PB T-cell supernatants caused a 69% ± 9% inhibition of BFU-E and day 6, CD3-triggered, PB T-cell supernatants caused a 69% ± 17% inhibition of BFU-E. Blockade of CD2 caused a 87% abrogation of CD3-triggered, T-cell-mediated release of humoral inhibitors of BFU-E at day 3 and a 60%

85% abrogation of BFU-E when supernatants were harvested at day 6.

CD2 blockade induces down regulation of BM and PB T-cell IFγ release. CD2 blockade reduced IFγ release (110 to 128 U/mL) from CD3-triggered BM T cells by 81% at day 3 and by 72% at day 6 of culture (Fig 2A). CD2 blockade reduced PB CD3-triggered T-cell IFγ release (84 to 87 U/mL) by 46% at day 3 and by 72% at day 6 (Fig 2B). Abrogation of IFγ release by CD2 blockade was not due to an alteration in the kinetics of IFγ release as assessed by repetitive IFγ immunoassay of T-cell supernatant established for three to 14 days. In control experiments, the addition of IFγ-neutralizing antibody to CD3-triggered T-cell supernatant established in the presence of IL-2 caused 75% abrogation of IL-2 inhibition of BFU-E (data not shown).

**DISCUSSION**

We have demonstrated that blockade of the T-cell determinant CD2 induces down modulation of (a) T-cell p55 IL-2 receptor expression, (b) IL-2–induced inhibition of BFU-E, and (c) IL-2–induced BM and PB T-cell IFγ release. Earlier studies indicated that CD2-blocking antibodies inhibit both IL-2 receptor expression and IL-2 production. It remained to be clarified whether abrogation of IL-2 receptor expression by CD2 blockade is due to abrogation of IL-2 release since IL-2 upregulates its own receptor on antigen receptor–triggered T cells. Thus, we assessed the effects of CD2 blockade both in the absence of IL-2 and in the presence of an excess of exogenous purified recombinant DNA–derived IL-2 (10^3 U/mL). We were able to demonstrate abrogation of IL-2 receptor expression in the presence as well as in the absence of exogenous IL-2 when employing experimental conditions under which endogenous IL-2 production is completely abrogated. These experimental conditions include utilization of an immunopurified T-cell population that is vigorously monocyte depleted as well as the use of nonmitogenic concentrations of CD3 antibody. Our results indicate that down regulation of IL-2 receptor expression by CD2 blockade is not solely due to abrogation of IL-2 release but is in part independent of that release. **Table 2. CD2 Blockade Down Modulates the IL-2–Induced Release of Humoral Inhibitors From CD3-Triggered T Cells**

<table>
<thead>
<tr>
<th>Source of CM</th>
<th>BFU-E Growth in Presence of</th>
<th>BM, day 3</th>
<th>PB, day 3</th>
<th>PB, day 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-Cell Supernatant</td>
<td>CD2-Blocked</td>
<td>CD2-Blocked</td>
<td>CD2-Blocked</td>
<td>CD2-Blocked</td>
</tr>
<tr>
<td>CD3-Triggered T-Cell CM</td>
<td>115 ± 27</td>
<td>28 ± 11</td>
<td>121 ± 19</td>
<td>110 ± 25</td>
</tr>
<tr>
<td>CD3-Triggered T-Cell CM</td>
<td>126 ± 40</td>
<td>35 ± 20</td>
<td>131 ± 23</td>
<td>121 ± 15</td>
</tr>
</tbody>
</table>

**Fig 1.** CD2-blocking monoclonal antibody abrogates IL-2–induced inhibition of BFU-E. BM mononuclear target cells depleted of adherent cells and T cells were cultured in the presence or absence of autologous CD2-blocked, CD3-triggered T cells (termed CD2CD3T) or T cells preincubated with an isotype control antibody prior to CD3 triggering (termed CD3T). Cultures were established in the presence of various concentrations of IL-2 as indicated on the abscissa. The ordinate represents the ratio of BFU-E grown in the presence of IL-2 plus the respective T-cell population to BFU-E grown in the absence of IL-2 but in the absence of the same T-cell population. BFU-E controls were 128 ± 40 (presence of CD2CD3 T cells and absence of IL-2), 121 ± 15 (presence of CD3 T cells and absence of IL-2), and 123 ± 34 (absence of T cells and absence of IL-2). The data represent means ± SEM of three separate experiments.
ERYTHROID REGULATION BY T CELL CD2 RECEPTOR

We next asked whether CD2 has a role in IL-2–induced inhibition of erythropoiesis. We demonstrated here that CD2 blockade of T cells, activated under conditions to facilitate interaction between LFA-3 and CD2, abrogates IL-2–induced inhibition of erythropoietic progenitors cocultured with autologous T cells. This observation suggests that CD2 may participate in T-cell–mediated hematopoietic immunoregulation. Subsequently, we asked whether the effect of CD2 blockade on erythropoiesis is mediated by a cellular or a humoral mechanism. Abrogation of IL-2–induced inhibition, demonstrated by coculture of erythropoietic progenitors with CD2-blocked T cells, was reproduced by culturing erythropoietic progenitors with supernatants established from CD2-blocked T cells. This finding indicates that CD2 blockade can modulate IL-2 regulation of erythropoiesis by interfering with the release of an IL-2–induced humoral inhibitor of erythropoiesis from activated T cells.

CD2 blockade abrogated T-cell IFγ release when T cells were cultured under conditions designed to facilitate interaction between LFA-3 and its CD2 receptor. This observation, coupled with previously described effects of recombinant IFγ on BFU-E\(^2,27,29\) and the ability of IFγ neutralizing antibody to markedly reduce CD3-triggered supernatant inhibition of BFU-E, suggests that the effects of CD2 receptor blockade on erythroid growth are mediated at least in part by diminished release of IFγ from antigen-activated T cells. CD2 blockade may well modulate the release of lymphokines other than IFγ from antigen-activated T cells.

Recent studies have demonstrated that CD2 functions as a receptor for a non–antigen-restricted pathway of T-cell activation.\(^{11,12}\) The CD2 receptor molecule contains three distinct epitopes (T11.1, T11.2, and T11.3) as defined by oligonucleotide mutation analysis and antibody-binding studies.\(^{13,16}\) One of the three epitopes represents the binding site for LFA-3 and is recognized by Leu-5b/T11.1-type antibodies. The physiological ligands for the other CD2 epitopes have to date eluded identification.\(^{14,31}\) LFA-3 is involved in T-cell activation via the alternate pathway, but LFA-3 alone is not sufficient to induce that activation. T-cell activation via CD2 requires either costimulation of various CD2 epitopes or stimulation of the LFA-3 receptor in conjunction with triggering of the T-cell antigen receptor–associated epitope CD3.\(^{14,30,31}\) A number of precedents exist that suggest that interactions between CD2 and the antigen receptor complex are essential in T-cell activation. The stimulation of T cells with a combination of a CD2 antibody and a weakly mitogenic CD3 antibody induces a full proliferative response, whereas each antibody alone provides only a minimal signal.\(^{30}\) Stimulation of T cells via the alternate receptor with various CD2 antibodies (T11.2 and T11.3) induces phosphorylation of CD3 chains, also suggesting linkage of the antigen receptor complex and CD2.\(^{32,33}\)

Our results suggest that the CD3 antigen receptor complex and the CD2 alternate pathway receptor are jointly involved in T-cell activation and erythropoietic immunoregulation. In the presence of T-cell LFA-3, T-cell activation via CD3 appears to provide the signal equivalent of T11.2 plus T11.3 antibodies to induce augmented IL-2 receptor expression and to render T cells permissive for IL-2–induced IFγ release and inhibition of erythropoiesis. We postulate that the cooperative interaction between the LFA-3 receptor and the antigen receptor constitutes a prerequisite for the expression of an immunoregulatory T-cell program.

Our study indicates that T-cell erythropoietic immunoregulation is not confined solely to antigen-restricted T-cell activation but also involves an antigen-independent pathway of T-cell activation. The results demonstrate that the LFA-3 receptor not only serves to promote T-cell proliferation and amplification of the immune response against nonself but can also participate in the activation of an immunoregulatory T-cell program. The data also indicate that the LFA-3 receptor modulates the immunoregulatory effects of IL-2. Moreover, blockade of CD2 down regulates the whole sequence of inhibitory signals that is induced by IL-2 and that may be provided by the lymphokine cascade for the erythropoietic progenitor cell. The LFA-3 receptor thus participates in the hematopoietic immunoregulatory network of the lymphokine cascade. Our model may serve as a
prototype to further assess hematopoietic immunoregulation by the alternate T-cell receptor.

We have previously demonstrated that hematopoietic control by regulatory T-cell lymphokines is dependent on the specific receptor and activation status of their respective regulatory target cells.\textsuperscript{7,10,11} Inhibition of erythropoiesis by IFN requires either the presence of both antigen-presenting monocytes and resting T cells or the presence of antigen receptor--triggered T cells.\textsuperscript{7,20} In addition, the hematopoietic regulatory effects of IL-2 require the presence of functional IL-2 receptors\textsuperscript{3,11} and, as demonstrated in this study, the participation of CD2. Delineation of model systems controlling for expression of cellular receptors mediating the effects of these T-cell lymphokines is a prerequisite for studying regulatory effects of T-cell lymphokines in normal hematopoiesis and for assessment of their role in immune-mediated suppression of erythropoiesis in humans.

REFERENCES

15. Peterson A, Seed B: Monoclonal antibody and ligand binding sites of the T cell erythrocyte receptor (CD2). Nature 329:842, 1987
26. Burdach St, Levitt L: The role of the T cell


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