Hematopoietic Growth Control by the T-Cell CD2 Determinant is Exerted at a Pretranslational Level*

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A. Introduction

Cellular growth control takes a delicate balance between stimulatory and inhibitory signals. Much previous research has focused on the identification of genes which encode for stimulatory signals. Genes that encode for inhibitory signals may be more difficult to analyze [1]. T-cell gene products are capable of inhibition of hematopoiesis in vitro and possibly in vivo [2–6]. The mechanisms regulating the inhibitory hematopoietic T-cell program are not well understood.

We have previously shown that triggering the T-cell antigen receptor associated epitope CD3 induces the p55 chain of the interleukin-2 (IL2) receptor on bone marrow T cells and renders marrow T cells responsive to low concentrations of IL2 [7]. In addition, we have demonstrated that IL2 inhibits the growth of marrow early erythroid progenitor cells (BFU-E) in the presence of IL2 receptor-positive T cells, and that interferon-γ (IF-γ) is an obligatory mediator of IL2-induced inhibition of BFU-E [4, 7]. We have also described a receptor-specific inhibition of myelopoiesis by IL2 which is mediated only in part by IF-γ [8]. Taken together, we have demonstrated a model for molecular regulation of hematopoiesis governed by an array of humoral and cellular signals, termed the lymphokine cascade. We have now examined the role of the early T-cell antigen CD2 in control of hematopoiesis by this lymphokine cascade.

CD2 has been identified for a long time as the receptor mediating sheep erythrocyte binding to T cells [9]. Later studies revealed that CD2 can serve as a receptor for a non-antigen-restricted pathway of T cell activation [10]. Lymphocyte function antigen 3 (LFA-3) has been identified as a natural ligand for CD2 [11, 12] and may induce T-cell activation in conjunction with additional activation signals [13]. LFA-3 is present on various cell types, including T-cells and mature red blood cells [14]. CD2-blocking monoclonal antibodies have been shown to inhibit binding of purified LFA-3 to CD2 [10–16]. Recent data suggest that interactions between CD2 and the antigen receptor may be essential for T-cell activation [17–19]. We utilized the CD2 antibody Leu 5b, which blocks a binding site for LFA-3 to examine the role of CD2 in IL2-induced inhibition of hematopoietic progenitors.

B. Induction of IL2 Receptors by CD2

IL2 receptors (p55) were induced on peripheral blood or marrow T cells via triggering of the antigen receptor associated CD3 epitope, as previously described [4, 8]. In brief, T cells were preincubated with CD2-blocking antibody before activation with CD3 antibody and subsequently cultured for 3–6 days in the presence of IL2. Antibody incubations were performed with T-cell pellets to facilitate interaction between LFA-3 and CD2.

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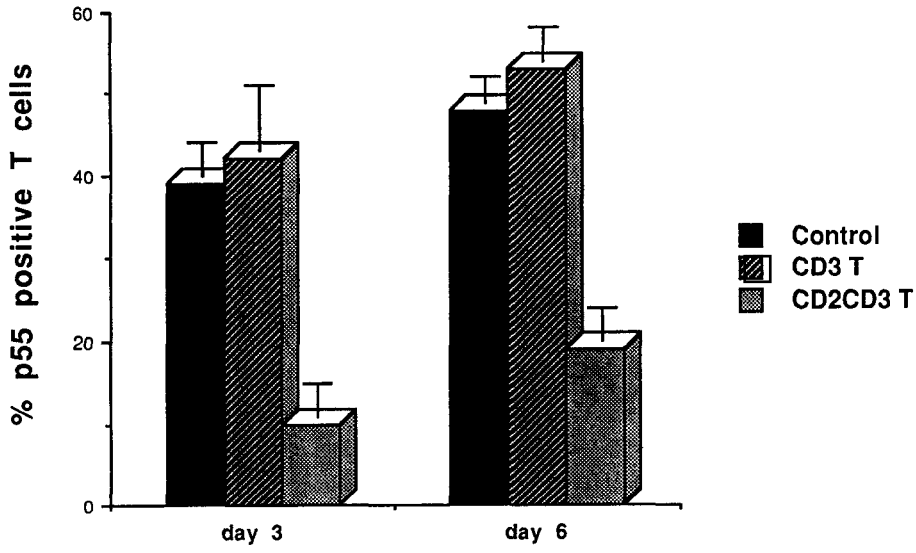

Fig. 1. CD2 blockade inhibits p55 IL2 receptor expression. *CD3 T,* CD3-triggered T cells; *CD2CD3 T,* CD2 blockade

42% of the CD3-triggered T cells expressed p55 at day 3, and 53% expressed p55 at day 6 (Fig. 1). CD2 blockade caused a 75% inhibition of p55 expression at day 3 as compared to preincubation with isotype control CD5 antibody and a 65% inhibition of p55 expression at day 6. Preincubation with isotype control CD5 antibody did not affect CD3-mediated p55 expression. CD2 blockade had no effect on binding of either triggering antibody to CD3 or IL2 receptor antibody to p55.

Next we asked whether regulation of p55 IL2 receptor expression by CD2 is associated with regulation of p55 gene expression. RNA was extracted by phenol extraction in the presence of vanadyl ribonucleotides [21] or by a cesium chloride/guanidium isothiocyanate gradient [22] from immunopurified T cells or monocyte-depleted mononuclear cells (>90% T cells by three-stage indirect immunofluorescence with CD5 antibody). Following gel electrophoresis T-cell RNA was subjected to a modified Northern transfer employing Nylon membranes. Highly sensitive single-stranded probes were constructed utilizing a cDNA which recognizes the 5′ untranslated region and the first exon of p55 (obtained from G. Crabtree, Stanford, California, USA) [23]. Oligonucleotides were utilized as random primers in the presence of DNA polymerase, and the probe was subsequently hybridized to T-cell RNA [45]. The screen was exposed for 48 h.

Figure 2 depicts the results following 16 h of culture. The first lane from the left represents the negative control: RNA extracted from CD3-non-triggered T cells cultured in the absence of IL2. Only minute amounts of p55 3.5-kb mRNA

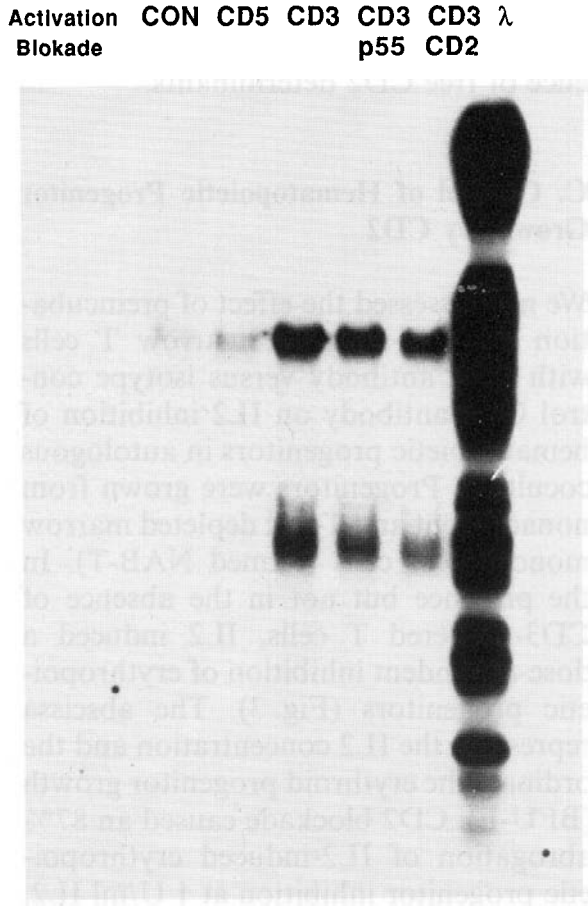

Fig. 2. CD2 blockade down-regulates p55 mRNA

are observed, and a 1.5-kb message is barely detectable. The second lane represents RNA from non-triggered T cells cultured in the presence of 10^2 U/ml: the presence of IL2 did not induce any substantial increase either of 3.5- or of 1.5-kb p55 mRNA. The third lane represents RNA extracted from CD3-triggered T-cells cultured in the presence of IL2: a strong p55 signal is detected. The fourth lane represents RNA from CD3-triggered T cells cultured in the presence of both IL2 (10^2 U/ml) and p55-blocking antibody. The fifth lane represents RNA from CD3-triggered T cells, which were preincubated with CD2-blocking antibody and cultured in the presence of 10^2 U/ml IL2. A definitive decrease of 1.5-kb p55 message is observed. All T cell samples except the CD2-blocked sample were preincubated with an isotype control antibody to rule out possible Fc-mediated effects. The last lane represents the size markers (λ DNA digested with Eco RI/Hind III). Thus, induction of p55 surface expression and accumulation of p55 mRNA are both dependent on the presence of free CD2 determinants.

C. Control of Hematopoietic Progenitor Growth by CD2

We next assessed the effect of preincubation of CD3-triggered marrow T cells with CD2 antibody versus isotype control CD5 antibody on IL2 inhibition of hematopoietic progenitors in autologous coculture. Progenitors were grown from nonadherent and T-cell depleted marrow mononuclear cells (termed NAB-T). In the presence but not in the absence of CD3-triggered T cells, IL2 induced a dose-dependent inhibition of erythropoietic progenitors (Fig. 3). The abscissa represents the IL2 concentration and the ordinate the erythroid progenitor growth (BFU-E). CD2 blockade caused an 87% abrogation of IL2-induced erythropoietic progenitor inhibition at 1 U/ml IL2, a 65% abrogation at 10 U/ml IL2, and a 55% abrogation at 100 U/ml IL2. CD2 blockade had no independent effect on erythropoietic progenitor growth in the absence of IL2 or in the presence of non-CD3-triggered T cells. IL2-induced CD3-triggered T cell mediated inhibition of erythropoietic progenitors was not affected by preincubation of CD3-triggered T-cells with control CD5 antibody.

In contrast to the abrogation of erythropoietic inhibition, CD2 blockade did not abrogate IL2-induced inhibition of monocyte/macrophage progenitors (Fig. 4). Likewise, CD2 blockade did not affect IL2-induced inhibition of total myeloid progenitors (Fig. 4, insert).

Next we assessed whether CD2 blockade modulates IL2-induced release of hematopoietic inhibitors from CD3-triggered T cells. Day 3 supernatants from marrow CD2-non-blocked, CD3-triggered T cells or CD2-blocked, CD3-triggered T cells (Fig. 5) were assessed against nonadherent, T-depleted marrow target cells (NAB-T). All supernatants were established in the presence of IL2. CD3-triggered marrow T-cell supernatants caused a 79% inhibition of erythropoietic progenitors. Blockade of CD2 caused an almost complete abrogation of CD3-triggered T-cell mediated inhibition of erythropoietic progenitors. Preincubation with isotype control antibody had no effect. In contrast, CD2 blockade did not reconstitute growth of monocyte/macrophage progenitors inhibited by IL2.

D. Regulation of Lymphokine Production by CD2

CD2 blockade reduced IF-γ release from CD3-triggered marrow T cells by 81% at day 3 and by 72% at day 6 of culture (Fig. 6). Similar results were obtained with peripheral blood T cells (data not shown).

We then asked whether regulation of IF-γ release by CD2 is associated with regulation of IF-γ gene expression. Total RNA was extracted from immunopurified T cells or monocyte-depleted

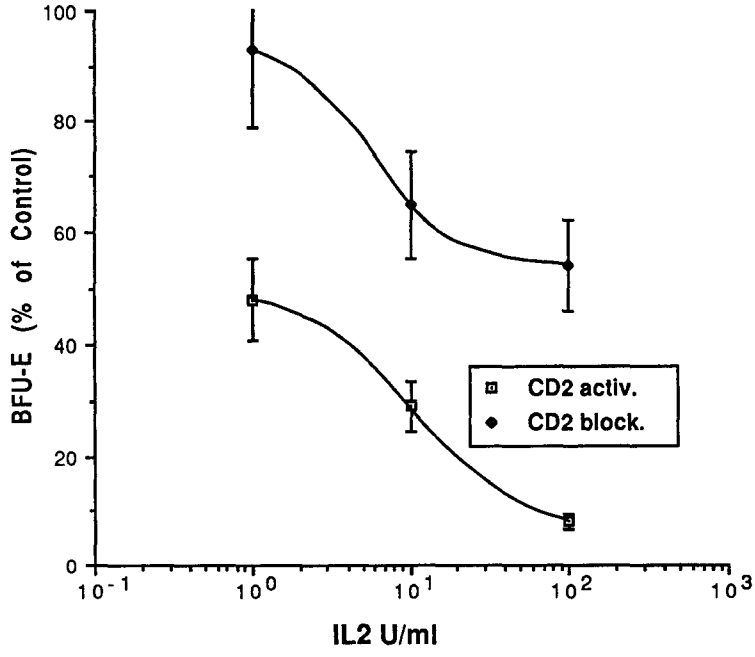

Fig. 3. CD2-mediated control of erythropoiesis

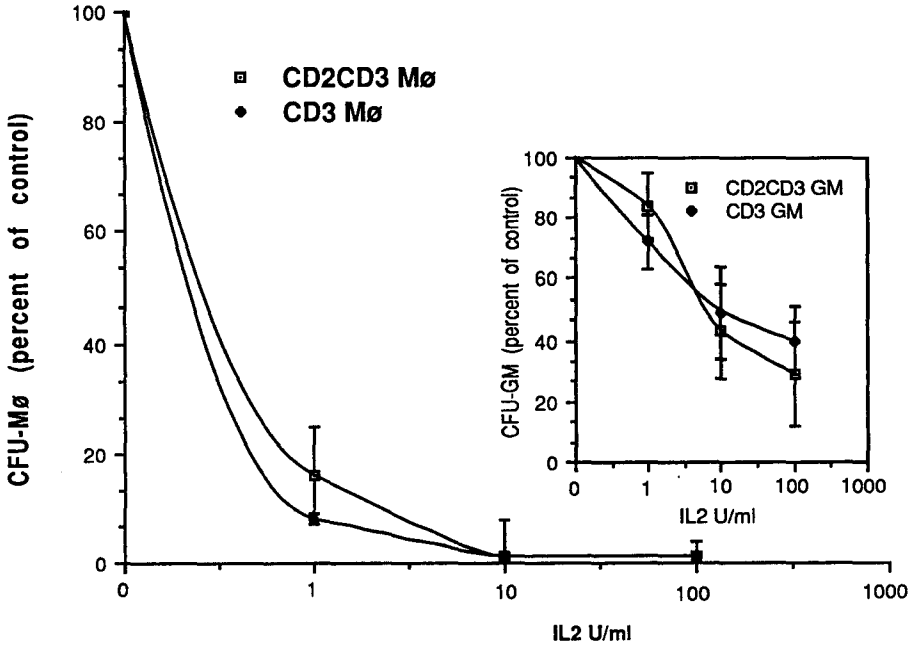

Fig. 4. CD2 blockade does not affect inhibition of colony-forming units – macrophages ($M\Phi$). *Insert,* CD2 blockade does not affect inhibition of colony-forming units – granulocyte-macrophages (*GM*) *CD2CD3,* CD2 blockade

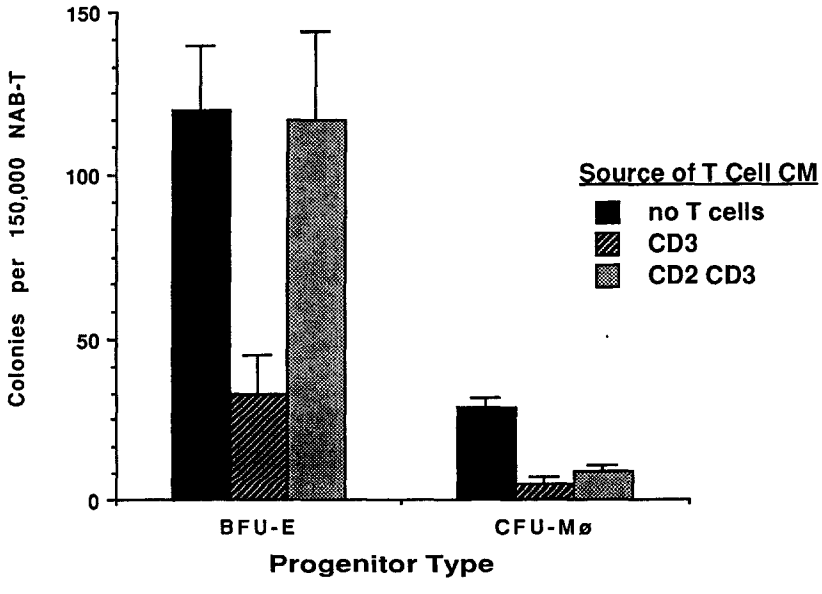

Fig. 5. Differential effect of CD2 blockade on humoral progenitor inhibition

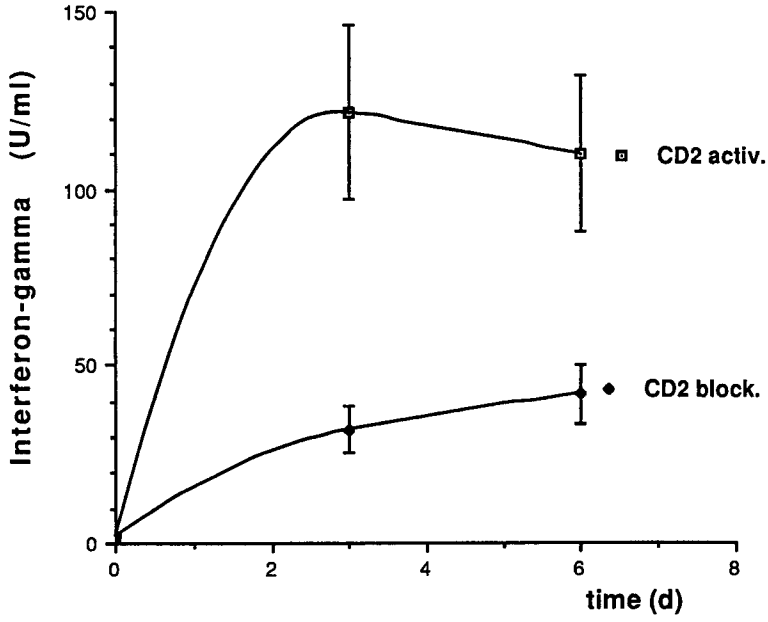

Fig. 6. Abrogation of IF-γ release by blockade of CD2

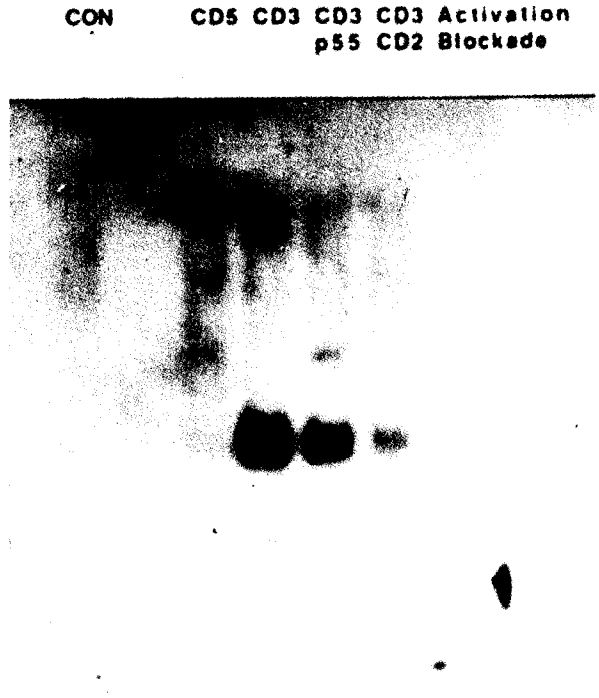

Fig. 7. CD2 blockade down-regulates IF-γ mRNA

mononuclear cells by the cesium chloride/guanidium isothiocyanate method [22]. Following gel electrophoresis of equal amounts, T-cell RNA was subjected to a modified Northern transfer utilizing Nylon membranes. Probes were constructed from a full-length cDNA for IF-γ [25] utilizing oligonucleotides as random primers in the presence of Klenow DNA polymerase. The IF-γ cDNA probes were hybridized to T-cell RNA.

Figure 7 depicts the results of a representative experiment. The first lane on the far left represents the negative control: RNA extracted from T cells before the begin of cultures. The second lane demonstrates that IL2 in the absence of high affinity IL2 receptors does not induce IF-γ mRNA. In contrast, IL2 in the presence of high-affinity IL2 receptors induces a strong signal for IF-γ mRNA (third lane). The fourth lane demonstrates that blocking of the p55 chain of IL2 receptor-positive T cells partially abrogates the IL2-induced increase in IF-γ mRNA. The suboptimal p55-blocking antibody concentration utilized in this study abrogates only about 50% of IL2-induced T-cell proliferation. Of interest, CD2 blockade prior to triggering of CD3 also abrogates the IL2-induced increase of IF-γ mRNA, as indicated in the fifth lane. Thus abrogation of IF-γ protein release by blockade of CD2 is preceded by an abrogation of IF-γ mRNA. This data suggests that CD2-mediated IF-γ production is regulated at a pretranslational level.

E. Conclusion

We conclude that blockade of the T-cell CD2 receptor induces down-modulation

of (a) T-cell p55 IL2 receptor mRNA accumulation and membrane receptor expression, (b) IL2-induced inhibition of erythroid but not myeloid progenitors, and (c) IL2-induced marrow and peripheral blood T-cell IF-γ protein release and IF-γ mRNA accumulation.

This 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. These results demonstrate that the alternate receptor not only serves to promote T-cell proliferation and amplification of the immune response against non-self but also participates in the activation of an immunoregulatory T-cell program. The data also indicate that blockade of CD2 down-regulates the whole sequence of inhibitory signals induced by IL2 and provided by the lymphokine cascade for the erythropoietic progenitor cell. On the other hand, failure of CD2 blockade to abrogate inhibition of myelopoiesis indicates that specific regulatory T-cell programs can be triggered via CD3 independently of CD2. CD2 thus participates in hematopoietic differential regulation by the lymphokine cascade.

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