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An activating combination of CD2 antibodies stimulates tyrosine phosphorylation in a T lymphocyte cell line

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The activating combination of CD2 antibodies Leu-5b plus 9.1 stimulates tyrosine phosphorylation in the human T cell line Jurkat. The tyrosine phosphorylation has the same molecular weight pattern as that seen when cells are stimulated on the CD3 receptor with OKT3 antibodies. These data provide evidence that signal transduction by the CD2 receptor is coupled to an increase in tyrosine phosphorylation that is similar to that coupled to the CD3 receptor.

T cell; Tyrosine protein kinase; CD2 receptor; CD3 receptor

1. INTRODUCTION

Monoclonal antibodies against several different proteins present on the external surface of T lymphocytes have the ability to activate T cells. Among the proteins identified by these monoclonal antibodies are the CD2 and CD3 receptors. The CD3 receptor is composed of several polypeptides that form a complex. The CD3 complex interacts with the clonotypic polypeptides of the antigen receptor and there is considerable evidence that signal transduction through the antigen receptor is mediated by the CD3 complex [1]. Several studies suggest that signal transduction by CD2 also occurs, at least in part, via CD3. Signal transduction by CD2 requires the coexpression of CD3 [2–5] and stimulation of CD2 has been shown to activate the same second messengers systems as are activated by the stimulation of CD3. Thus both receptors are linked to the activation of the phosphatidyl inositol pathway with subsequent increases in cell calcium and diacylglycerol and activation of protein kinase C [6–10].

Several T cell surface receptors have been found to be coupled to the regulation of tyrosine protein kinase activity, suggesting that tyrosine phosphorylation has an important role in T cell activation and proliferation. Activation of T cells through the antigen/CD3 receptor complex and the Thy1 receptor results in activation of a tyrosine protein kinase [9,11]. Signal transduction by the CD4 and CD8 receptors as well as the receptor for interleukin-2 (IL-2) also involves the activation of tyrosine protein kinases [12–15]. The CD45 surface molecule has been identified as phosphotyrosine phosphatase [16,17]. Since signal transduction by several T cell surface receptors involves the regulation of tyrosine phosphorylation it seemed likely that stimulation of the CD2 receptor would also result in changes in tyrosine phosphorylation. We have tested for the ability of monoclonal antibodies to CD2 to regulate tyrosine phosphorylation in the Jurkat cell line, a line frequently used for studies of T cell activation.

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2. EXPERIMENTAL

The Jurkat 77 6.8 clone was obtained from Dr Kendall Smith, Dartmouth Medical College. CTLL-2 cells (ATCC) were used to assay for the production of IL-2. The CD3 antibody OKT3 was purified from the medium of the OKT3 hybridoma (ATCC) using protein A Sepharose. The CD2 antibody 9.1 [18,19] was generously provided as an ascites fluid by Dr Soo Young Yang, Sloan Kettering Cancer Center, New York, NY. The CD2 antibody Leu5b was obtained from Becton Dickerson. Rabbit antibodies to phosphotyrosine were produced as previously described [15]. Experiments examining levels of protein tyrosine phosphorylation were initiated by adding Jurkat cells (4×10^6 in 0.5 ml) to tubes that contained either CD3 or CD2 antibodies. The concentrations of antibodies that were used were those that gave a maximal response as determined from preliminary experiments. Following incubation for the indicated times the cells were pelleted with a 30 s centrifugation. The pellets were immediately dissolved in SDS-sample buffer and placed in a boiling water bath for 5 min. Samples were subjected to SDS-gel electrophoresis following blotting onto nitrocellulose. Proteins containing phosphotyrosine were detected by probing the blots with antibodies against phosphotyrosine followed by [^{125}I]protein A to detect the immune complexes [15,20]. CD3 was down modulated by incubating Jurkat cells for 16 h in the presence of 1 μg/ml OKT3 antibodies.

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3. RESULTS

Initial experiments were performed to demonstrate the ability of CD2 antibodies to stimulate a physiological response such as IL-2 production in this Jurkat clone. Stimulation of the CD2 receptor by antibodies generally requires two different monoclonal antibodies that bind to distinct epitopes. An antibody such as Leu5b binds to an exposed epitope on CD2 but does not activate. However, Leu5b causes a conformation change that exposes a second, otherwise cryptic epitope, that binds an activating monoclonal antibody such as 9.1 [18,19]. In concordance with this mechanism table 1 shows that Leu5b and 9.1 together stimulate IL-2 production while neither antibody alone is effective. Table 1 also shows that the CD3 antibody OKT3 induces IL-2 production in this Jurkat clone. Activation through either CD2 or CD3 has the similar requirement for costimulation with a phorbol ester. Thus this Jurkat clone expresses significant levels of CD2 and CD3 receptors that can generate signals that result in a common physiological response. The results in table 1 also confirm the ability of Leu5b to bind to CD2 in such a manner as to expose the cryptic epitope for 9.1 allowing for this activating antibody to bind and activate the CD2 receptor [18,19].

Fig.1 shows how CD2 antibodies affect the level of tyrosine phosphorylation in Jurkat cells. In the absence of any stimulus Jurkat cells have basal tyrosine phosphorylation in several bands, the most prominent having molecular weights of 56000, 62000, and 115000. Treatment of these cells with the activating CD2 antibody 9.1 alone resulted in small but reproducible increases in tyrosine phosphorylation of several of the bands present in the basal state. The nonactivating antibody Leu5b had little or no effect on the level of tyrosine phosphorylation. When Leu5b and 9.1 were added together large increases in tyrosine phosphorylation were observed in bands with molecular weights of 115000, 96000, 78000, 65000 and 42000. Smaller increases in tyrosine phosphorylation were observed in the bands at 56000 and 62000. Thus while having no effect by itself, Leu5b greatly potentiated the effect of 9.1 on tyrosine phosphorylation. The ability of 9.1 alone to cause a small increase in tyrosine phosphorylation presumably reflects a low level of exposure of the CD2 epitope for this antibody on unstimulated Jurkat cells. However this low level of activation by 9.1 alone is not sufficient to stimulate IL-2 production (table 1).

Table 1

Effect of CD2 and CD3 antibodies on IL-2 production by clone 77 6.8 Jurkat cells

Condition	IL-2 production (U/ml)
Control	0
9.1	0
Leu5b	0
9.1 + Leu5b	46
OKT3	45
OKT3 + 9.1 + Leu5b	51
A23187	37

Jurkat cells were incubated with the various agents in the presence of 100 nM phorbol myristic acetate. After 24 h the supernatants were collected and assayed for the presence of IL-2. None of the agents stimulated significant IL-2 production in the absence of phorbol myristic acetate.

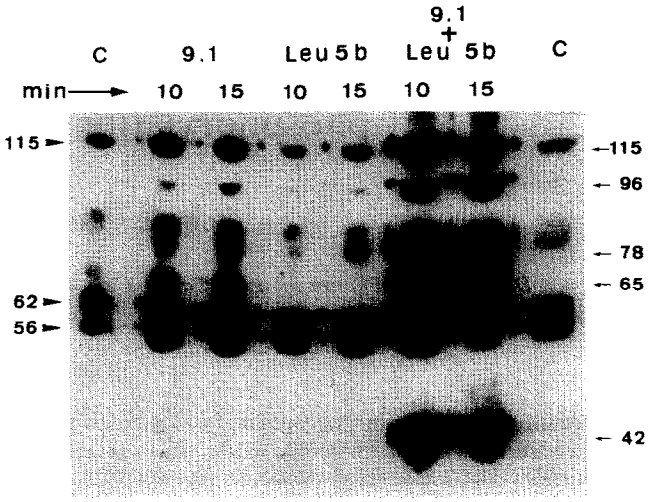

Fig.1. Effect of CD2 antibodies 9.1 and Leu5b on tyrosine phosphorylation in Jurkat cells. Cells were untreated (C) or treated with either 9.1, Leu5b or 9.1 + Leu5b for the indicated times in minutes. At the end of each incubation period the cells were pelleted and processed for an immunoblot with antibodies to phosphotyrosine. The figure shows the autoradiogram of the blot. The arrowheads indicate the position of the major phosphotyrosine-containing proteins present in the absence of any stimulus. The arrows indicate the molecular masses in kDa of the proteins that undergo large increases in phosphotyrosine.

The phosphotyrosine antibodies used for the blots are specific for phosphotyrosine. The signal in the immunoblots in fig.1 was unaffected by the inclusion of 1 mM phosphoserine or 1 mM phosphothreonine during the incubation with the phosphotyrosine antibodies while the presence of 10 μM phosphotyrosine caused a complete loss of the signal (data not shown).

In agreement with previous studies [9,10], stimulation of the CD3 receptor on Jurkat cells also causes increases in tyrosine phosphorylation. Fig.2 compares the time course and pattern of tyrosine phosphorylation induced by stimulating either the CD3 or the CD2 receptor. The pattern of tyrosine phosphorylation induced by CD3 antibodies is identical to that observed when cells are stimulated by antibodies to CD2. The tyrosine phosphorylation induced via the CD3 receptor was maximal by the earliest time point of 2.5 min, begins to decline by 10 min and has almost returned to basal levels by 40 min. In contrast the tyrosine phosphorylation stimulated by CD2 antibodies is considerably slower than that seen with the CD3 antibodies, requir-

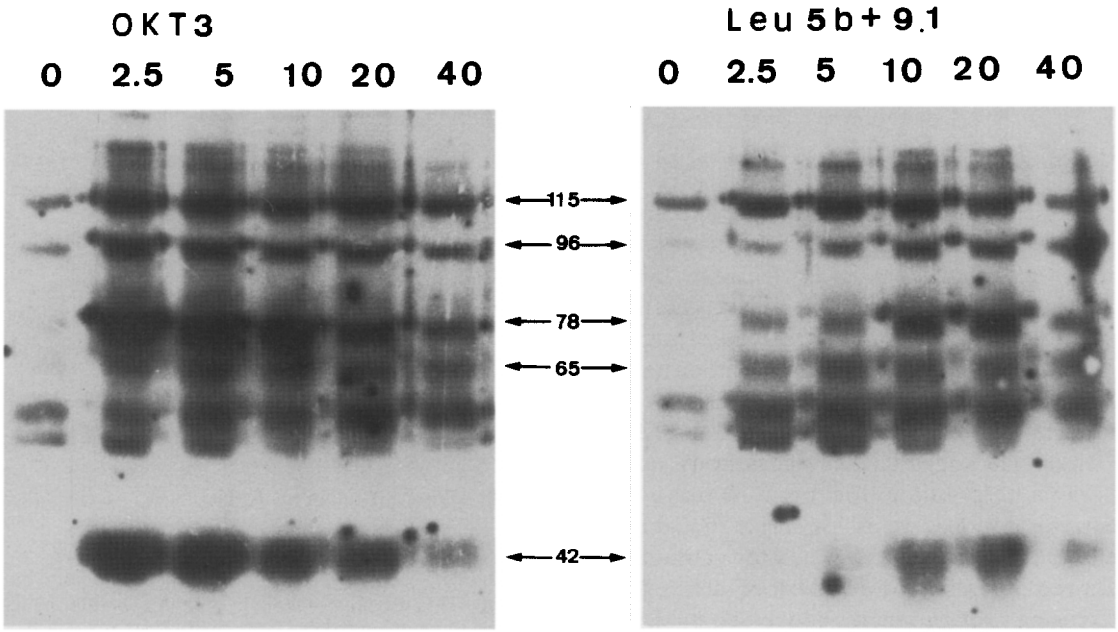

Fig.2. Comparison of the kinetics and patterns of tyrosine phosphorylation induced by either CD3 or CD2 antibodies. Jurkat cells were treated with saturating concentrations of either OKT3 or the combination of Leu5b and 9.1 antibodies. At the indicated times in minutes the cells were spun down and dissolved in SDS buffer. The samples were then analyzed by immunoblots with antibodies to phosphotyrosine. The arrows indicate the positions of the proteins that undergo large increases in tyrosine phosphorylation.

ing 5 to 10 min to reach a maximum. In addition the maximal levels of tyrosine phosphorylation seen with the CD2 antibodies is less than that observed with the CD3 antibodies. However, like activation through CD3 the tyrosine phosphorylation nearly returned to basal levels after 40 min of incubation with the CD2 antibodies.

Chronic treatment of T cells with CD3 antibodies such as OKT3 causes the down modulation of the CD3 complex [21,22]. Thus if the CD2-induced tyrosine phosphorylation is dependent on the expression of the CD3 complex then down modulation of this complex should obliterate the effects of CD2 antibodies on tyrosine phosphorylation. Fig.3 shows how down modulation of the CD3 complex affects the ability of CD2 and CD3 antibodies to increase tyrosine phosphorylation in Jurkat cells. As expected, pretreatment of the cells with OKT3 antibodies causes a complete loss of the CD3-dependent tyrosine phosphorylation. Down modulation of the CD3 complex also significantly inhibited the ability of CD2 antibodies to increase tyrosine phosphorylation. An exception to this loss of CD2-stimulated tyrosine phosphorylation is a protein of molecular weight of 65000. This protein was consistently observed to undergo increases in tyrosine phosphorylation in response to CD2 antibodies even after down modulation of CD3 resulted in a complete loss of response to CD3 antibodies. These results suggest that while the major pathway for CD2-dependent tyrosine

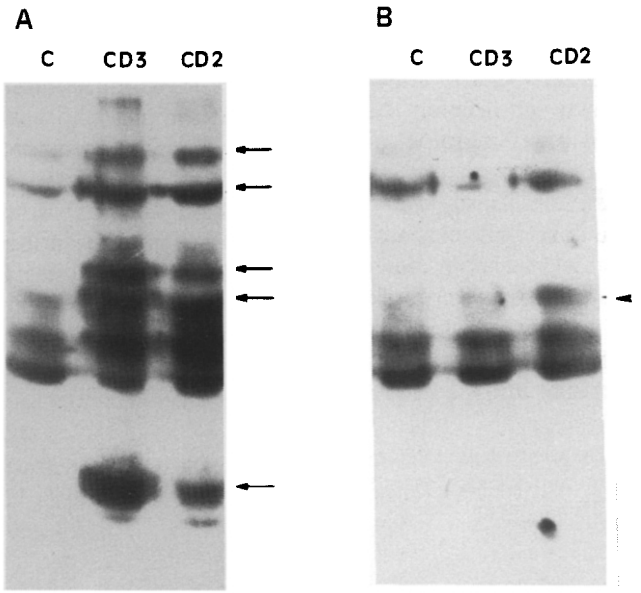

Fig.3. Effect of down modulation of the CD3 receptor on CD3- and CD2-induced tyrosine phosphorylation. Jurkat cells were preincubated in the absence (A) or presence (B) of OKT3 antibodies for 16 h. The cells were then washed and were either untreated (C), restimulated on the CD3 receptor with OKT3 antibodies (CD3) or on the CD2 receptor with9.1 + Leu5b (CD2). Cells were incubated for 5 min with OKT3 antibodies and 15 min with CD2 antibodies. Following these incubations the cells were pelleted and processed for immunoblots. The arrows in A indicate those proteins that undergo a large increase in phosphotyrosine upon stimulation of either CD3 or CD2 while the arrowhead in B indicates the 65 kDa protein whose tyrosine phosphorylation still increases in response to CD2 antibodies after down modulation of CD3.

phosphorylation is via CD3, CD2 can also stimulate tyrosine phosphorylation by a mechanism independent of CD3.

4. DISCUSSION

We have found that stimulation of the CD2 receptor on Jurkat cells results in a large increase in tyrosine phosphorylation of several distinct proteins. In agreement with earlier work [9,11] we have also found that stimulation of the CD3 on Jurkat cells causes an increase in tyrosine phosphorylation. The similarity in the pattern of proteins that undergo increases in tyrosine phosphorylation when either CD2 or CD3 receptor is stimulated suggests that the same tyrosine protein kinase is responsible for both increases in tyrosine phosphorylation. The observation that down modulation of the CD3 complex eliminates or reduces both of these responses provides further evidence that a common tyrosine protein kinase is involved in signal transduction by the CD2 and CD3 receptors. Our observation that CD2 antibodies stimulate tyrosine phosphorylation is somewhat at variance with the report of Weissman et al. [23]. These investigators were unable to detect an effect of CD2 antibodies on the level of tyrosine phosphorylation in the ζ chain of the antigen receptor in human peripheral leukocytes. Under these same conditions increases in tyrosine phosphorylation of the ζ subunit were detected when the cells were stimulated with OKT3 antibodies. However, these investigators studied the phosphorylation of a single possible substrate and examined this phosphorylation at only one time point. It is possible that a more general examination of tyrosine phosphorylation at several time points after stimulation would have revealed an increase in peripheral leukocytes in response to CD2 antibodies. It is clear from the data presented in this paper that in Jurkat cells stimulation of CD2 is coupled to increases in tyrosine phosphorylation.

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