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Role of LFA-1/ICAM-1 in interleukin-2-stimulated lymphocyte proliferation

Major adhesion routes between lymphoid cells involve the receptor/ligand pairs LFA-1/ICAM-1 and CD2/LFA-3, in addition to VLA or CD44 molecules. In this study we evaluated the role of these adhesion receptors in the proliferative response of lymphoid cells to interleukin-2 (IL-2). Blocking studies were performed with a panel of monoclonal antibodies (mAb) directed against these adhesion molecules. Selective inhibition of recombinant (r)IL-2-induced cell proliferation was observed with mAb directed against the α or β subunit of LFA-1 or to its ligand ICAM-1. Interestingly, rIL-2-induced proliferation was also inhibited by NKI-L16, an anti-1 α antibody known to enhance cell-cell interaction. Resting lymphocytes were preferentially susceptible to the inhibition, particularly in an early phase of culture and when stimulated with a relatively low dose of rIL-2. By using mAb that specifically could block distinct rIL-2 activation pathways, LFA-1/ICAM-1 interaction was found to be required for p55 IL-2 receptor (IL-2R)-mediated interaction of rIL-2 with its high-affinity receptor, but not for p75 IL-2R-mediated responses. Furthermore, it was shown that the rIL-2 response of T lymphocytes, but not of natural killer cells, was dependent on LFA-1/ICAM-1 interaction. This suggests that LFA-1/ICAM-1 interaction is required for an optimal rIL-2 response of cells capable of IL-2 secretion. Our data provide evidence for the hypothesis that adhesion receptor-directed release of IL-2 may result in a locally high concentration of IL-2 that triggers high-affinity IL-2R signaling and up-regulates p55 IL-2R to enhance cytokine responsiveness.

1 Introduction

Interleukin-2 (IL-2) is a T cell-derived cytokine involved in the proliferation and differentiation of T lymphocytes, B lymphocytes, NK cells as well as monocytes [1-4]. Upon antigen recognition by T lymphocytes, the induction of both IL-2 and IL-2R gene expression allows subsequent proliferation of activated clones, either in an autocrine or paracrine fashion [1, 5]. The IL-2R is composed of a complex of at least two different subunits, the α chain (p55 IL-2R, CD25) [6, 7] which has low affinity (K_d 10^{-8} M) and the β chain (p75 IL-2R) [8] which has intermediate affinity (K_d 10^{-9} M) for IL-2. Both subunits, together with a recently identified p64 γ chain [9], when complexed with IL-2, form a high affinity IL-2R (K_d 10^{-11} M [1, 8, 9]). The α chain is thought to function as a helper molecule, required to concentrate IL-2 on the surface of the cell and to enhance the affinity of the β chain for IL-2 upon complexation [10-13]. The β chain, which has a long intracellular domain, is responsible for internalization of the complex and is supposed to transduce the IL-2 signal into the cell [8, 14].

Upon activation by IL-2, the up-regulation of the p55 IL-2R by far exceeds that of the p75 IL-2R [1, 15, 16]. Peripheral blood NK cells and CD8⁺ T lymphocytes, both of which express p75 IL-2R [17], can be directly stimulated by IL-2 [2, 18-20].

Cellular immune functions are not only dependent on antigen/receptor and cytokine/receptor interactions, but are also determined by appropriate cell-cell contact [21, 22]. The importance of the interaction between the adhesion receptor LFA-1 (CD11a/CD18) and its ligand ICAM-1 (CD54) in TCR-mediated immune responses has been established and this adhesion pathway was found to contribute to both signaling and adhesion [23-34]. Anti-CD3-stimulated T cell proliferation was found to be regulated, either by LFA-1 mAb [30] or using purified ICAM-1 as a ligand [31], at the level of IL-2 production and p55 IL-2R expression. However, it remains to be established whether LFA-1/ICAM-1-mediated cell-cell contact, apart from its role in TCR-dependent signal transduction [30-34], directly participates in the interaction of IL-2 with its receptor(s). ICAM-1 (CD54), usually weakly expressed on hemopoietic cells, is strongly induced after stimulation of the cells with mediators of inflammation, *e.g.* IL-1 or IFN- γ [35], and also IL-2 [36-38]. As described previously, activation of LFA-1 by TCR triggering, is a prerequisite for interaction with ICAM-1 [39, 40] and can also be induced by stimulation with IL-2 as the primary stimulus [38, 41, 42]. Activation of lymphocytes by IL-2 results in the expression of the L16 epitope on LFA-1 which is thought to be associated with a conformational change of the molecule [43]. Recently, we showed that IL-2 as primary signal was capable of inducing cell clustering and up-regulation of the expression of both ICAM-1 and L16, as well as of CD2 and VLA-5 antigen, on peripheral lymphocytes [38]. Simulta-

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neous presence of IL-4 reduced both the IL-2-induced cell clustering and the surface expression of these adhesion molecules [38]. The well-known inhibitory effect of IL-4 on IL-2-induced cell proliferation [38, 44, 45], prompted us to investigate more directly the role of adhesion structures in the lymphocyte proliferation response to IL-2.

2 Materials and methods

2.1 mAb

The following mAb were used: NKI-L15 (anti-LFA-1 α , CD11a, IgG2a), NKI-L7 (anti-LFA-1 α , CD11a, IgG1; [46]), NKI-L16 (anti-LFA-1 α , CD11a activation epitope, IgG2a; [43]), NKI-P1 (anti-CD44, IgG1; [47]), SAM-1 (anti-VLA-5, CD49e, IgG2b, [48]), CLB-CD19 (anti-CD19, IgG2a; [49]), 1H4 (anti-CD20, IgG1), SPV-T3b (anti-CD3, IgG2a, [50]), CLB-T3/3 (anti-CD3, IgG2a; [30]), from R. van Lier, Amsterdam, CLB-LFA-1/1 (anti-LFA-1 β , CD18, IgG1; [51]), from F. Miedema, Amsterdam, CLB-FcR-gran1 (anti-Fc γ RIII, CD16, IgG2a; [52]), from P. Tetteroo, Amsterdam, GoH3 (anti-VLA-6, CD49f, rat IgG2a; [53]), from A. Sonnenberg, Amsterdam, 60.3 (anti-CD18, IgG2a; [25]), from M. Harlan, Seattle, Ts2/9 (anti-LFA-3, CD58, IgG1, [54]), from T. Springer, Boston, MA, HP1/3 (anti-VLA-4 α , CD49d, IgG1; [55]), from F. Sanchez-Madrid, Madrid, 4F2 (anti-4F2 activation antigen, IgG2a; [56]), F10.2 (anti-ICAM-1, CD54, IgG1; [57]), F10.3 (anti-ICAM-1, CD54, IgG1; [57]), both from A. Bloem, Utrecht, B-B10 (anti-p55 IL-2R, CD25, IgG1; [58]), from J. Wijdenes, Besancon, France, TU27 (anti-p75 IL-2R, IgG1; [59]), from K. Sugamura, Sendai, Japan, leu 19 (anti-CD56, IgG1, Becton Dickinson, San Jose, CA), leu M3 (anti-CD14, IgG2b, Becton Dickinson), DAKO-CD25-FITC (anti-CD25, Dakopatts, Glostrup, Denmark), anti-p75 IL-2R-FITC (Endogen, Boston, MA), anti-HLA-DR-FITC (Becton Dickinson), phycoerythrine (PE) - labeled leu 54 (anti-CD54, Becton Dickinson).

2.2 Cells and medium

PBL from normal donors were isolated by Ficoll Hypaque density centrifugation and washed in Iscove's medium (Flow Laboratories, Irvine, Scotland) supplemented with 5% pooled, inactivated human serum (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands), penicillin 100 IE/ml and kanamycin 100 μ g/ml (designated from here on as medium). As preactivated lymphocytes, PBL were used that were cultured for 4 days in the presence of rIL-2 500 (Cetus) U/ml (kindly provided by EuroCetus, Amsterdam, The Netherlands). Small lymphocytes, containing over 90% T cells, and large lymphocytes (LL), containing 20-30% NK cells and 70-80% T cells, were isolated from the peripheral blood of normal donors by centrifugal elutriation as described previously [60]. Cell viability of all populations was greater than 95% as detected by trypan blue dye exclusion.

2.3 Enrichment for NK cells

Resting NK cells were enriched from peripheral blood buffy coat by 1-h adherence to plastic at 37°C and depletion

of B cells, T cells and activated cells using mAb directed to CD19, CD3 and CD25, respectively and magnetic beads (Dynabeads, Dynal, Oslo, Norway) followed by selecting the negative cells on a FACStar cell sorter (Becton-Dickinson, Mountain View, CA). The resulting population contained at least 90% NK cells as determined by reactivity with CD56 and CD16 mAb and less than 1% T cells as tested with CD3 mAb.

2.4 Proliferation assay

To determine the proliferative capacity of the cells, 5×10^4 cells were incubated in round-bottom microtiter plates in a final volume of 200 μ l/well, in the presence of rIL-2, in the doses indicated, either with or without mAb directed to adhesion receptors or IL-2R mAb were used as $1/10^3$ diluted ascites or, were indicated, as purified mAb. For mAb NKI-L16, NKI-L15, CLB-LFA-1/1 and Ts2/9, $1/10^3$ diluted ascites corresponded with 1 μ g/ml, 10 μ g/ml, 1 μ g/ml and 8 μ g/ml IgG, respectively. The ascites dilution used for the mAb in functional assays was pre-titrated to be optimal in the binding assays. Unless indicated otherwise, mAb and rIL-2 were added at the start of the culture. In some experiments, wells of the microtiter plate were coated with CD3 mAb (CLB-T3/3, $1/10^6$ diluted ascites), followed by washing the wells five times with medium before the lymphocytes, either with or without rIL-2, were added. After culture for 4 days (or as stated otherwise in kinetic experiments), cells were pulsed with 14.8 kBq per well of [3 H]dThd (6.7 Ci/mmol, New England Nuclear, Boston, MA) for 4 h before being harvested as described previously [61]. Data are expressed as mean cpm \pm SD from triplicate cultures. In general, cells cultured in medium only generated less than 200 cpm. Statistical significance was calculated by comparison of experimental (antibody) values with controls in the same experiment using the paired *t*-test after logarithmic transformation of the data.

2.5 Aggregation assay

The formation of cell aggregates was determined by microscopic examination as described previously [38]. Briefly, cell clusters observed at the time of harvest of the culture were scored as the percentage of cells within clusters per total number of cells, $\times 100\%$. Scores ranged from: - = < 10%, + = 10-50% to ++ = > 50%.

2.6 Immunofluorescence assay

Surface marker analysis of cells stained with mAb and FITC-labeled goat anti-mouse Ig (GAM-FITC) was determined by FACScan analysis (Becton Dickinson), as described previously [38].

2.7 Assay for soluble p55 IL-2R

Soluble p55 IL-2R released in day 6 supernatants was assayed in duplicate using the soluble IL-2R ELISA kit of Eurogenetics (Tessenderlo, Belgium) according to the manufacturer's instructions.

3 Results

3.1 Involvement of LFA-1 in the rIL-2 response of small and large lymphocytes

In a previous study on the effect of IL-4 on IL-2-induced lymphocyte activation, it was found that cell aggregation, cell proliferation as well as expression of a number of adhesion receptors were co-regulated by both cytokines [38]. This prompted us to investigate whether adhesion receptors were directly involved in IL-2-mediated cell proliferation. Therefore small lymphocytes, representing a population enriched for T lymphocytes and depleted for monocytes and the bulk of NK cells, were incubated in the presence of rIL-2 either with or without mAb directed to a number of adhesion molecules known to be expressed on these cells. As shown in Table 1, only antibodies directed against the α subunit (NKI-L16, NKI-L15) or the β subunit (CLB-LFA-1/1) of LFA-1 inhibited the rIL-2-induced response. Surprisingly, also the LFA-1-activating mAb NKI-L16 inhibited the proliferation, comparably to the effect of the adhesion blocking mAb NKI-L15. Inhibitory effects did not correlate to effects of the mAb on cell aggregation measured in parallel by microscopic examination (Table 1). Differences in the effects of mAb were not due to differences in specific antibody concentrations, since all mAb were used at doses that were optimal for binding, and as shown in Table 1, effects on cell aggregation were apparent with most mAb. Lymphocytes that were pre-activated *in vitro* by culture for 4 days in the presence of 500 U/ml rIL-2, could no longer be blocked by NKI-L15 or CLB-LFA-1/1, but remained susceptible to inhibition by NKI-L16; although the latter inhibition was relatively weak, it was significant ($p < 0.01$) (Table 1). Table 2 indicates that the response of *in vivo* activated blasts (the fraction of large lymphocytes) to low dose rIL-2 (1 U/ml) was comparable to that of small, resting lymphocytes, but that their response to high dose rIL-2 (100 U/ml) was comparable to that of *in vitro* activated cells. The response of the *in vivo* activated blasts to high dose rIL-2 could not be blocked significantly

by either NKI-L15, CLB-LFA-1/1 or NKI-L16 (in all cases $p > 0.1$). This indicated that the susceptibility to the inhibitory effect of LFA-1 mAb on the rIL-2 response was related to both the activation state of the cells and the strength of the rIL-2 stimulus.

Additional experiments using LL were set up to analyze the specificity of the inhibition of the LFA-1 mAb. As shown in Table 3, the proliferative response was also inhibited, although weakly (20–46%), by the anti-ICAM-1 mAb F10.2, indicating that LFA-1/ICAM-1 interaction was important for optimal responsiveness to IL-2. Comparable results were obtained using the ICAM-1 mAb F10.3 (data not shown). As shown in Fig. 1, a dose-dependent decrease in responsiveness to rIL-2 was obtained with purified LFA-1 mAb, ruling out the possibility that nonspecific ascites components were responsible for the inhibitory effects. Furthermore, it was excluded that the inhibition

Table 2. Effect of LFA-1 directed mAb on the rIL-2 response of large lymphocytes^{a)}

mAb	% of control response			
	rIL-2 100 U/ml	n	rIL-2 1 U/ml	n
–	100	5	100	4
NKI-L16	61 ± 17	3	68 ± 4	2
NKI-L15	83 ± 15	3	41 ± 9	2
CLB-LFA1/1	82 ± 22	5	23 ± 11	4
TS2/9	97 ± 7	5	100 ± 19	4

a) LL were obtained by centrifugal elutriation and were incubated at 5×10^4 cells/well in the presence of 100 U/ml rIL-2 or 1 U/ml rIL-2, without (control) or with mAb. After 4 days [³H]dThd incorporation was measured and expressed as % of control response. (Mean responses to 100 U/ml rIL-2 from all experiments: $9.2 \text{ cpm} \times 10^{-3}$ (range 3.6–16.8), to 1 U/ml rIL-2: $4.3 \text{ cpm} \times 10^{-3}$ (range 1.4–9.7)).

Table 1. Effect of adhesion-associated mAb on the rIL-2 response of resting and pre-activated lymphocytes

mAb	Antigen	CD	Resting cells			Preactivated cells	
			proliferation ^{a)} (% of control)	aggregation	n =	proliferation (% of control)	n =
–			100	+	6	100	6
NKI-L16	LFA-1 α	11a	52 ± 5	++	6	70 ± 9	6
NKI-L15	LFA-1 α	11a	48 ± 17	–	5	112 ± 8	5
CLB-LFA1/1	LFA-1 β	18	43 ± 5	–	3	112 ± 9	3
TS2/9	LFA-3	58	77 ± 5	±	2	95 ± 7	3
NKI-P1	HR	44	91 ± 3	++	3	100 ± 7	3
HP1/3	VLA-4	49d	93 ± 8	++	2	98 ± 11	3
SAM-1	VLA-5	49e	85 ± 5	+	2	94 ± 8	2
GOH3	VLA-6	49f	118 ± 0.5	+	2	102 ± 9	2
4F2	4F2		84 ± 8	+	6	91 ± 8	6

a) Resting cells (small lymphocytes obtained by centrifugal elutriation) or pre-activated lymphocytes (PBL pre-cultured for 4 days in the presence of 500 U/ml rIL-2) were incubated at 5×10^4 cells/well in the presence of 100 U/ml rIL-2, without (control) or with mAb. After 4 days [³H]dThd incorporation was measured and data are expressed as % of control response (mean response to rIL-2 from all experiments with resting lymphocytes was $4.7 \text{ cpm} \times 10^{-3}$ (range 1.6–9.4); mean response to rIL-2 from all experiments with pre-activated lymphocytes was $11.4 \text{ cpm} \times 10^{-3}$ (range 2.2–24.6). Background responses (medium or mAb only) were less than 5% of the rIL-2 response).

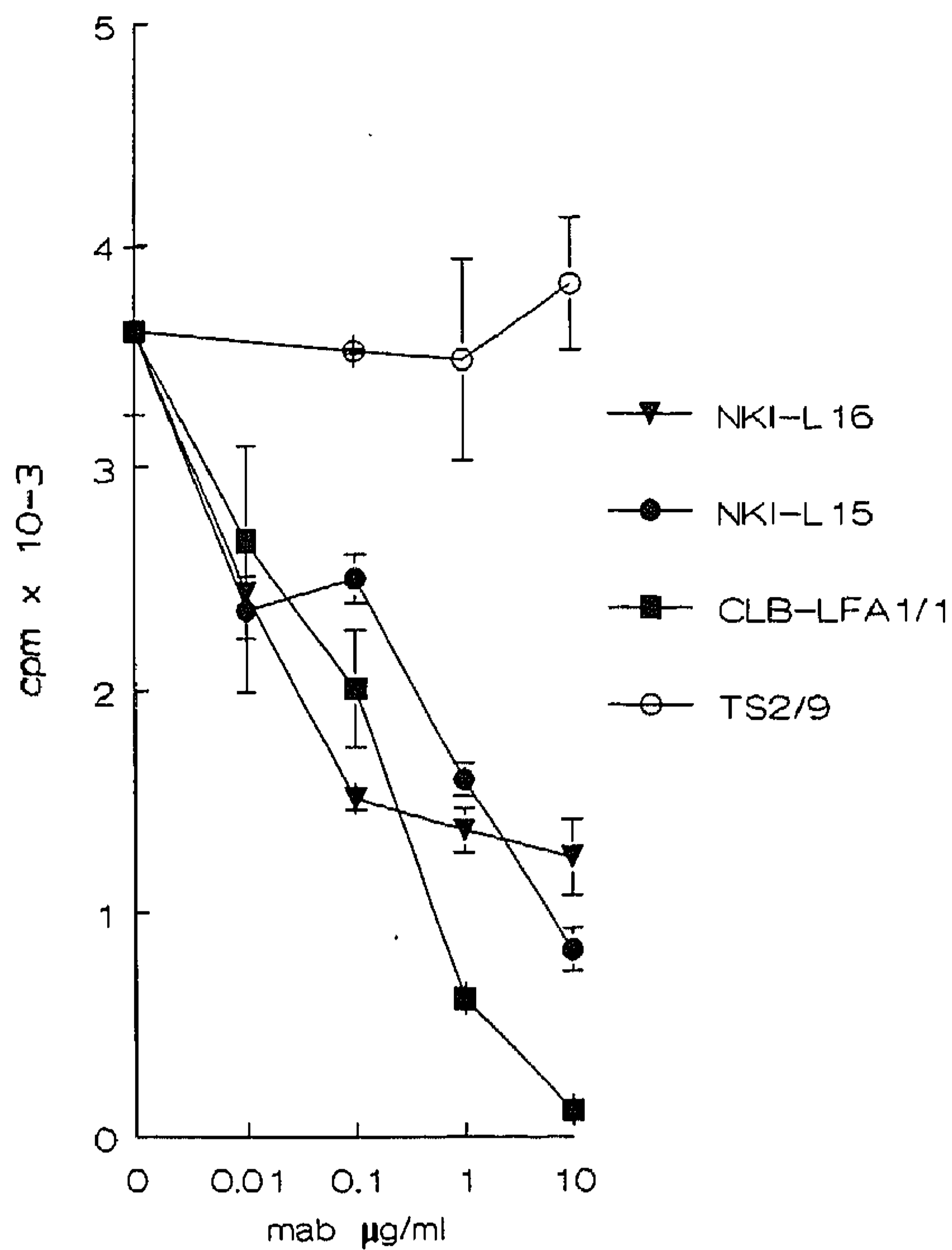


Figure 1. Dose-dependent inhibition of rIL-2 induced proliferation by LFA-1 directed mAb. LL were incubated at 5×10^4 cells/well in the presence of 1 U/ml rIL-2 and different doses of purified mAb NKI-L16, NKI-L15, CLB-LFA-1/1 and Ts2/9. [³H]dThd incorporation from triplicate cultures was measured at day 4. mAb in the absence of rIL-2 did not induce proliferation over background levels. One out of two experiments is shown.

was Fc receptor mediated or due to a shift in the kinetics of the response (data not shown).

3.2 LFA-1/ICAM-1 interaction during early phase of culture determines responsiveness to rIL-2

To assess in which phase of the culture cell-cell contact was important, the LL were incubated in the presence of rIL-2

(1 U/ml) and subsequently LFA-1 mAb were added at various time points. The data presented in Fig. 2 indicated that under the conditions used, LFA-1/ICAM-1 interaction during the first 24 h of culture determined optimal responsiveness to rIL-2 and that addition of blocking mAb NKI-L15 or CLB-LFA-1/1 after this initial phase did not influence the reaction. This interval was found to be dependent on the dose of rIL-2 used, being shorter with higher doses of rIL-2 (data not shown). Fig. 2 furthermore shows, that NKI-L16 inhibited the rIL-2-induced response, irrespective of the time of addition to the culture.

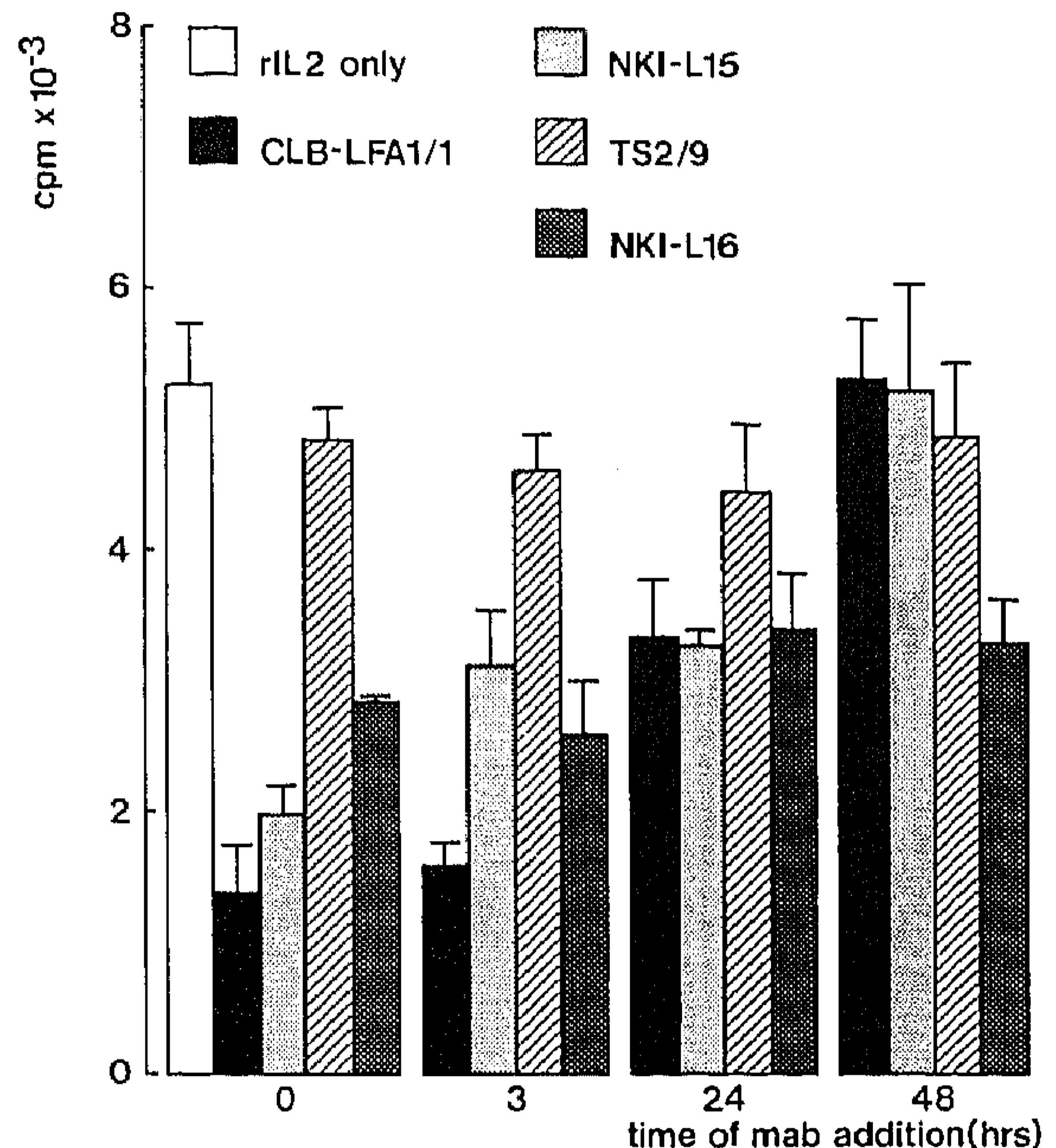


Figure 2. Kinetics of inhibition of rIL-2 response by LFA-1 directed mAb. LL were incubated at 5×10^4 cells/well in the presence of 1 U/ml rIL-2 and LFA-1 mAb were added at the time points indicated. [³H]dThd incorporation from triplicate cultures was measured at day 4. One experiment representative of three is shown.

Table 3. Inhibition of rIL-2-induced proliferation by ICAM-1 directed mAb^{a)}

Exp.	rIL-2	mAb	Antigen	[³ H]dThd incorporation (cpm × 10 ⁻³ ± SD)	% inhibition
Exp. 1	-	-		0.4 ± 0.1	
	+	-		4.4 ± 0.1	
	+	CLB-LFA1/1	CD18	1.8 ± 0.5	59
	+	F10.2	CD54	3.5 ± 0.5	20
	+	B-B10	CD25	0.1 ± 0.1	98
Exp. 2	-	-		2.3 ± 0.1	
	+	-		13.2 ± 1.6	
	+	CLB-LFA1/1	CD18	6.7 ± 1.4	49
	+	F10.2	CD54	7.1 ± 0.5	46
	+	B-B10	CD25	0.8 ± 0.1	94

a) LL were incubated at 5×10^4 cells/well in the presence or absence of rIL-2 (1 U/ml) and mAb F10.2. Data are expressed as $\text{cpm} \times 10^{-3} \pm \text{SD}$ from triplicate cultures harvested at day 4.

3.3 LFA-1 mAb selectively block p55 IL-2R-mediated responses to rIL-2

To analyze further whether a particular pathway of rIL-2 activation was affected by blockade of LFA-1/ICAM-1 interaction, LL were incubated in the presence of rIL-2 (100 U/ml) and CLB-LFA-1/1, either with the p55 IL-2R mAb B-B10, the p75 IL-2R TU27 or a combination of both mAb. As shown in Fig. 3, in the presence of B-B10, allowing interaction of rIL-2 with p75, CLB-LFA-1/1 hardly affected the rIL-2 response. However, in the presence of TU27, allowing interaction of rIL-2 with p55, CLB-LFA-1/1 profoundly reduced rIL-2 reactivity. Similar results were obtained with NKI-L15 or NKI-L16 (data not shown). This strongly suggested that either blocking or strongly enhancing the LFA-1/ICAM-1 interaction resulted in selective impairment of p55-mediated, high-affinity responses to rIL-2.

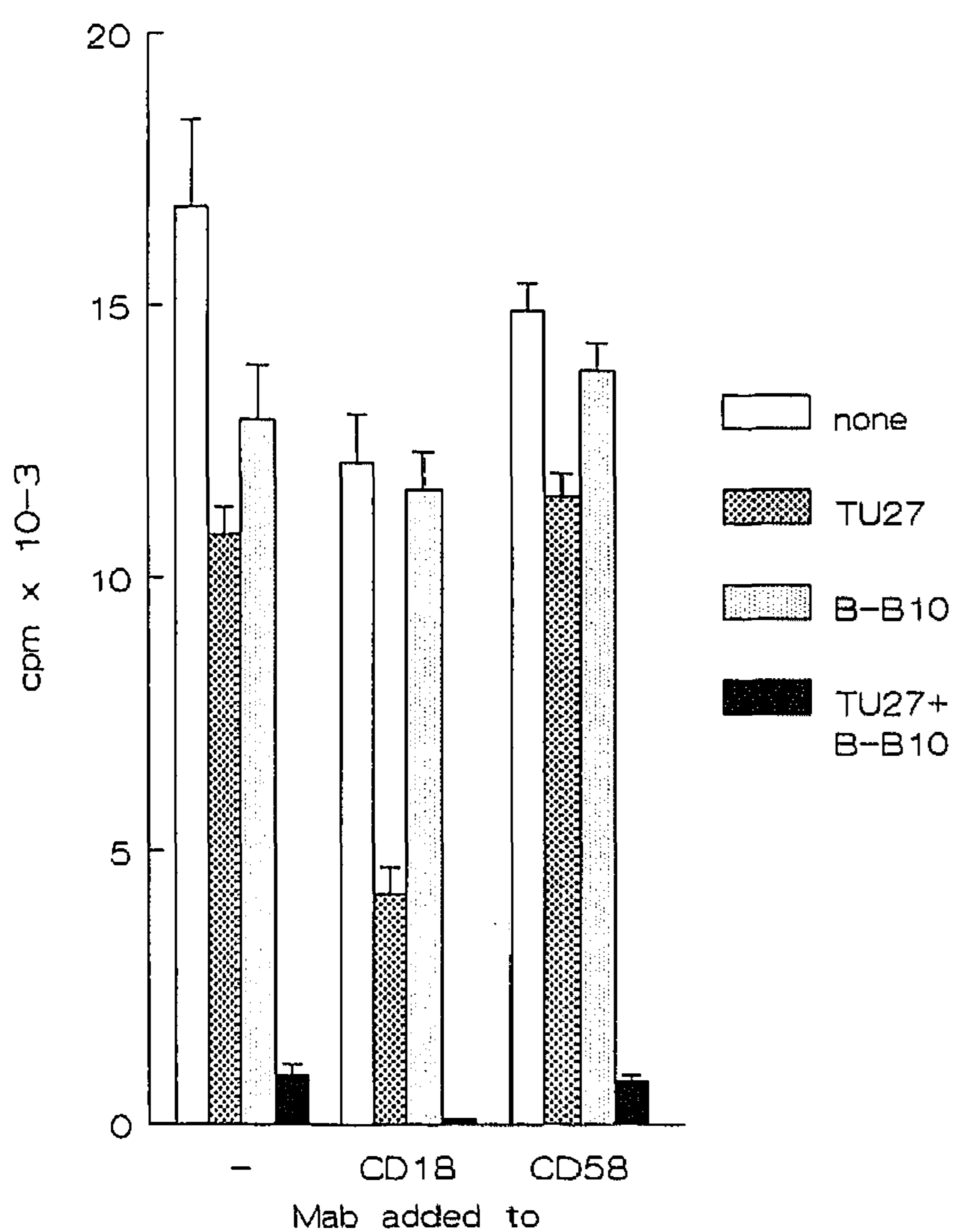


Figure 3. Inhibitory effect of CLB-LFA-1/1 on the p55 mediated rIL-2 response. LL were incubated in the presence of 100 U/ml rIL-2 either with or without mAb to CD18 (CLB-LFA-1/1) or CD58 (Ts2/9), in the presence or absence of p55 IL-2R directed mAb B-B10 and/or p75 IL-2R directed mAb TU27. [³H]dThd incorporation from triplicate cultures was measured at day 4. One experiment representative of five is shown.

3.4 Differential effects of LFA-1 mAb on NK cell- and T cell-enriched populations

To determine whether NK cells and T cells would be equally susceptible to the inhibitory effect of LFA-1 mAb, cell depletion experiments were performed as described in Sect. 2.3. NK cell-enriched populations (containing less than 1% T cells) were incubated in the presence of rIL-2 100 U/ml either with or without IL-2R-directed mAb, in the presence or absence of LFA-1 mAb NKI-L16 or CLB-LFA-1/1, or as control, LFA-3 mAb Ts2/9. As shown in Fig. 4, CLB-LFA-1/1 did not inhibit the rIL-2 response of

NK cells. However, NKI-L16 was capable of reducing NK cell rIL-2 reactivity, but only of p55-mediated, and not of p75-mediated responses.

Alternatively, T cells were investigated for their susceptibility to be inhibited by LFA-1 mAb in their responsiveness to rIL-2. Small lymphocytes, composed of over 90% T cells and less than 5% NK cells, were stimulated with a suboptimal dose of CD3 mAb (1/10⁶ diluted ascites, which gives approximately 1% of the proliferation of an optimal anti-CD3 response) and rIL-2, either with or without LFA-1 mAb NKI-L16, NKI-L15 or CLB-LFA-1/1, or as control, LFA-3 mAb Ts2/9. The data, shown in Table 4, indicate that NKI-L15, NKI-L16 and CLB-LFA-1/1 were inhibiting responsiveness to rIL-2 whereas Ts2/9 had almost no effect. Additional experiments, using T cell clones generated to EBV-transformed allogeneic B cells, indicated that responses of activated T lymphocytes to rIL-2 could not be blocked by CLB-LFA-1/1 or Ts2/9 and only to some extent by NKI-L16 (mean response to rIL-2 ± SD in the presence of mAb, as percentage of the control without mAb, from 10 separate experiments was 102 ± 12, 99 ± 13, 72 ± 12, respectively).

These data indicate that resting NK cells were inhibited in their responsiveness to rIL-2 only by LFA-1 mAb NKI-L16, but that resting T lymphocytes could be inhibited by both NKI-L16 and CLB-LFA-1/1.

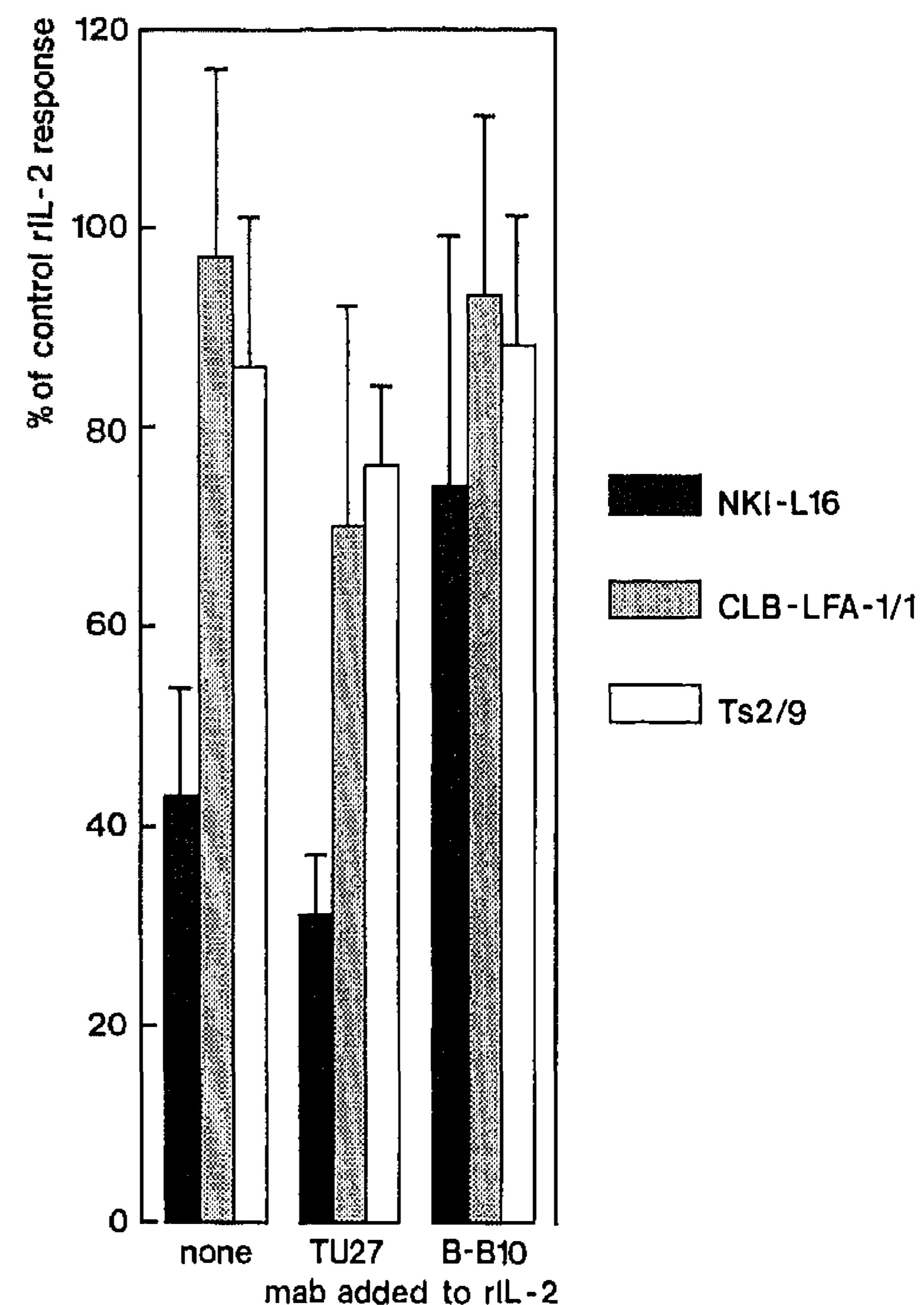


Figure 4. Differential effects of LFA-1 directed mAb on the response to rIL-2 of NK cell-enriched populations. NK cell-enriched cell populations were incubated at 5×10^4 cells/well in triplicate cultures in the presence or absence of rIL-2 (100 U/ml) and mAb and harvested for [³H]dThd incorporation at day 4. Data are presented as the mean % of control responses (to rIL-2 only) ± SD from three separate experiments (responses to rIL-2: 10.8, 15.1, and 19.0 cpm × 10³, respectively; absolute cpm × 10⁻³ to rIL-2 in the presence of TU27 and B-B10: 3.4, 18.5, 16.6, and 5.0, 12.0, 22.7, respectively).

Table 4. Inhibitory effect of LFA-1 mAb on T cell proliferation and surface antigen expression upon stimulation with suboptimal anti-CD3 plus rIL-2

Culture in presence of ^{a)}	rIL-2 present	cpm × 10 ⁻³ ± SD	Mean fluorescence intensity (% positive cells)			
			CD25	p75	HLA-DR	CD54
-	-	0.2 ± 0.1	8(11)	30(24)	192(17)	29(64)
-	+	41.7 ± 1.6	165(63)	22(19)	594(63)	61(74)
NKI-L15	+	22.6 ± 2.5	100(52)	20(20)	707(58)	91(84)
NKI-L16	+	11.6 ± 1.1	45(34)	29(23)	518(47)	66(80)
CLB-LFA-1/1	+	8.0 ± 0.1	81(28)	23(20)	247(32)	65(76)
Ts2/9	+	33.9 ± 4.0	160(62)	19(15)	537(65)	67(77)
B-B10	+	1.9 ± 0.1	N.D. ^{b)}	22(20)	151(18)	32(70)

a) T cells (small lymphocyte fraction) were incubated at 5×10^4 cells/well in suboptimal anti-CD3 (1:10⁶ diluted), either with or without rIL-2 (10 U/ml) and/or mAb for 6 days. Data are expressed as [³H]dThd incorporation from triplicate cultures, and as mean fluorescence intensity (% positive cells). Cells cultured in the presence of mAb, but without rIL-2, did not proliferate above background level (in the presence of suboptimal anti-CD3 only). Neither did mAb alone change the background surface antigen expression. One experiment representative of four to six is shown.

b) N.D., not determined.

Table 5. Effect of LFA-1 mAb on the level of soluble p55IL-2R in cultures stimulated with suboptimal anti-CD3 and rIL-2^{a)}

mAb	rIL-2 present	Soluble p55IL-2R (U/ml)
-	-	119 ± 9
-	+	117 ± 0.7
NKI-L15	+	186 ± 10
NKI-L16	+	179 ± 8
CLB-LFA1/1	+	139 ± 9
Ts2/9	+	180 ± 0.7

a) T cells (small lymphocyte fraction) were incubated at 5×10^4 cells/well in suboptimal anti-CD3 (1:10⁶ diluted), either with or without rIL-2 (10 U/ml) and/or mAb. Supernatants from triplicate cultures were pooled at day 6 and assayed in duplicate for soluble p55IL-2R. Data are expressed as the mean ± SD from two separate experiments.

3.5 Effect of LFA-1 mAb on the activation state of T lymphocytes

To establish effects of LFA-1 mAb on the activation state of rIL-2-stimulated cells, T lymphocytes were stimulated with a suboptimal concentration of CD3 mAb and rIL-2, in the presence of either LFA-1 mAb NKI-L15, NKI-L16, CLB-LFA-1/1 or the control Ts2/9. After 4 days of culture, the cells were stained with FITC-conjugated mAb directed to p55 IL-2R, p75 IL-2R, HLA-DR and ICAM-1. The data show that CLB-LFA-1/1 and NKI-L16, and to a lesser extent NKI-L15, prevented activation of the lymphocytes as shown by decreased percentages of cells expressing p55 and HLA-DR. Inhibition of surface antigen expression was observed concomitantly with inhibition of cell proliferation (Table 4). Expression of p75 or ICAM-1 was not decreased by LFA-1 or LFA-3 mAb (Table 4), whereas the antigen density of the latter was reduced by anti-p55 IL-2R mAb B-B10 (data not shown). In addition, CLB-LFA-1/1 reduced the release of soluble p55 IL-2R, whereas NKI-L15, NKI-L16 and Ts2/9 showed no effect or slightly increased the level of this receptor in the supernatant (Table 5).

4 Discussion

The data obtained in the present study indicate that resting peripheral T lymphocytes require LFA-1/ICAM-1 interac-

tions for optimal responsiveness to IL-2. Previous studies have shown the involvement of these adhesion molecules in cell proliferation induced by TCR triggering by antigen, allo-antigen or mitogen [23-34]. Our findings extend these observations in several aspects: (1) the inhibitory effects of LFA-1 mAb are observed with cells stimulated with IL-2 as the primary signal; (2) the extent of inhibition of responsiveness to rIL-2 depends on the state of activation of the lymphocytes and on the levels of rIL-2 used; (3) prevention of LFA-1/ICAM-1 interactions selectively impairs p55 rIL-2R-mediated responses, but does not affect interaction of IL-2 with the intermediate p75 IL-2R; (4) the requirement for the LFA-1/ICAM-1 pathway in IL-2 reactivity is restricted to T lymphocytes, and is not observed for NK cells; (5) strengthening of LFA-1/ICAM-1 interactions can also lead to reduced responsiveness to rIL-2.

First, it was of interest to investigate whether blocking of the LFA-1/ICAM-1 and CD2/LFA-3 pathways of cell interaction would directly interfere with the proliferative response of the cells in response to IL-2. Despite the fact that both LFA-1 and LFA-3 mAb are known to profoundly inhibit cell conjugate formation [22, 36, 62], only the former inhibited the rIL-2 proliferation response, irrespective of their effect on cell aggregation (Table 1).

To discriminate which IL-2 activation route was blocked by LFA-1 mAb, either that initiated through interaction of IL-2 with p75 or through interaction of IL-2 with the high-affinity receptor, or both, experiments were performed with blocking mAb specifically directed to the IL-2R subunits p55 and p75. The results show that rIL-2 responses mediated through the p55 α chain are inhibited by LFA-1 mAb, whereas rIL-2 responses mediated through p75 only, are unaffected. This finding strongly argues in favor of a functional relationship between p55 and components of the LFA-1/ICAM-1 adhesion route, as proposed by the group of Waldmann [63, 64]. These investigators showed a partial association of p55 and ICAM-1 on cells of the HTLV-1-positive T cell line HUT-102 [63, 64]. Our attempts to reveal any association of p55 and ICAM-1 molecules on the surface of PBL, LL, rIL-2-activated PBL or T cell clones, either by co-modulation studies using the FACS or by microscopic examination of fluorochrome-labeled cells, were negative (data not shown). Therefore, although it remains to be established whether these molecules are associated on peripheral lymphocytes, such association might explain our present findings: inhibition of cell-cell contact would prevent a locally, high level of IL-2 to

bind to the low-affinity IL-2R p55 supposedly present at the site of cell-cell contact. Thus, subsequent rapid up-regulation of p55 would be prevented, resulting in reduced activation. Since T cells [5] in contrast to NK cells [65] are capable of producing IL-2 in response to the exogenous IL-2 signal, such a hypothesis may explain our finding that T lymphocytes, but not NK cells, are inhibited in their responsiveness to rIL-2 by the blocking LFA-1 mAb CLB-LFA-1/1.

Literature data show that LFA-1 may serve both as an adhesion receptor as well as a signaling molecule [30-34]. In our study, upon stimulation with CD3 mAb in the presence of suboptimal rIL-2 levels, NKI-L15 and NKI-L16 were found to potentiate responses (data not shown). Under optimal IL-2 stimulation of T cells, the main function of LFA-1/ICAM-1 interaction is to provide cell-cell contact, as shown by the finding that both LFA-1 α and LFA-1 β mAb block the response (Table 4).

T lymphocytes from patients, suffering from the leukocyte adhesion deficiency (LAD) syndrome, which fail to express LFA-1 on their cell surface, show impaired proliferative responsiveness to mitogenic and antigenic triggering [66]. However, repetitive stimulation ultimately results in a level of proliferation, comparable to that of normal lymphocytes, despite lack of LFA-1 expression of the expanded cell population [66]. This may be explained by assuming that LAD T lymphocytes can be induced by antigenic triggering to express minimal levels of IL-2R, but fail to up-regulate these receptors in the presence of exogenous IL-2. Only after repetitive TCR stimulation, p55 IL-2R may be expected to be up-regulated, via endogenous routes, to a level sufficient to bind exogenous IL-2.

In addition, our data indicate that not only blocking LFA-1 mAb, but also the adhesion-inducing mAb NKI-L16 inhibited proliferation in response to rIL-2. In comparison with the LFA-1 β mAb CLB-LFA-1/1 or the LFA-1 α mAb NKI-L15, inhibition by NKI-L16 was found to be less dependent on the state of activation of the cells, on the strength of the rIL-2 stimulus or on the kinetics of addition to the culture, suggesting that its action was not restricted to an early phase of the IL-2 activation process. Moreover, NKI-L16 reduced the activation of the T lymphocytes to a lesser extent than CLB-LFA-1/1 as determined by phenotype analysis and the release of soluble p55 IL-2R. NKI-L16 was shown to profoundly inhibit the p55-, but not the p75-mediated response of NK cells to IL-2, suggesting that its target too was the high-affinity IL-2R. In addition, this indicated that strong cluster formation itself was not responsible for the observed inhibition of proliferation. Presumably, the strong activation of the LFA-1 molecule induced by NKI-L16, which leads to prolonged increased interaction with ICAM-1 [38, 39, 41, 42], results in reduced mobility of the putatively associated p55, which may explain the inhibitory effect of NKI-L16. Further experiments should prove whether this hypothesis is correct. Although tested with different cells (HUT-102), lateral diffusion measurements by Edidin and coworkers [67] have indicated that immobilization of ICAM-1 can lead to reduced mobility of p55. The effect of the anti-LFA-1 mAb 24 that locks the high-affinity state of LFA-1 in antigen-stimulated cultures and concomitantly inhibits cell prolifer-

ation [68], bears resemblance to the effect of NKI-L16 and may be explained similarly.

In conclusion, our data indicate that cell-cell contact through LFA-1/ICAM-1 interactions provides the T lymphocyte with an important tool for rapidly up-regulating its high affinity IL-2R to effectively respond to IL-2.

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