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Regulatory effect of interleukin-4 (IL-4) on the expression and function of lymphocyte adhesion receptors involved in IL-2-induced cell aggregation

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SUMMARY

Human recombinant interleukin-4 (rIL-4) was studied for its capacity to inhibit rIL-2-induced lymphoid cell aggregation. In contrast to rIL-2, rIL-4 was unable to induce cluster formation by itself. However, when added simultaneously with rIL-2 to cultures of freshly isolated peripheral blood lymphocytes (PBL), rIL-4 inhibited cell aggregation in a dose-dependent way. In contrast, PBL, preactivated by a 4-day culture in the presence of 500 U/ml rIL-2, were not inhibited in their adhesive capacity by rIL-4. Inhibition of cell aggregation was most prominent at 24 hr and virtually lost after 72 hr of culture. Phenotypical analysis revealed that rIL-4, with similar kinetics, decreased the rIL-2-mediated up-regulation of the CD2, CD54 and CD49e adhesion molecules. In addition, it was observed that up-regulation of the activation epitope on CD11a recognized by the mAb NKI-L16, was prevented. During 24 hr of culture rIL-4 itself did not alter the expression of these antigens. Blocking experiments with mAb directed against adhesion structures did not reveal a direct role for CD49e, but obviously demonstrated involvement of CD11a/CD18–CD54 and CD2–CD58 interactions in the rIL-2-induced adhesion. Therefore, rIL-4 appears to inhibit the early phase of rIL-2-induced aggregation by preventing the up-regulation of CD54 and CD2 antigens and by inhibiting the generation of the activated state of the CD11a/CD18 receptor.

INTRODUCTION

During recent years the importance of cell–cell contacts in the activation of an immune response has become increasingly clear (for review see ref. 1). Adhesion receptors form initial contacts between effector and target cells before cognate events occur.[2,3] Subsequently, upon antigen recognition a phase of 'adhesion strengthening' occurs, during which conformational changes in adhesion structures are thought to play a role.[3-7] On lymphocytes activated states have been reported for the CD11a/CD18 β2 integrin receptor, the altered conformation of which is recognized by the mAb NKI-L16,[4,8] and for the CD49d, CD49e and CD49f molecules belonging to the VLA β1 subfamily of integrins.[7] Upon stimulation of lymphoid cells with interleukin-2 (IL-2), a strong cluster formation is induced. However, only limited knowledge is available on the effect of IL-2 on the expression of adhesion associated structures. Preferentially on natural killer (NK) cells, rather than on T lymphocytes, IL-2 was found to increase the expression of the receptor/ligand pairs CD11a/CD18–CD54 and CD2–CD58.[9] In another study IL-2-mediated increase in CD54 expression was detected on T cells as well, particularly on those of the memory cell type.[10] In preliminary experiments using human PBL, a diminished cluster formation was observed in cultures stimulated by recombinant (r)IL-2 in the presence of rIL-4. This observation bears resemblance to the reported inhibition by IL-4 of the IL-2-mediated proliferation and the generation of lymphokine-activated killer activity in cultures originating from resting T cells or NK cells.[11-14] In those studies the IL-4 inhibitory effect was not observed when IL-4 was added at least 24 hr after the start of the cultures. In addition, IL-4 itself did not have any functional effect on resting T cells or NK cells, which is in line with the minimal expression of IL-4R on these cells.[15] These data suggested that IL-4 inhibits an early IL-2-induced process. These observations prompted a detailed study on the effects of rIL-2 and rIL-4 on the expression of adhesion-associated structures during the process of cell aggregation, which precedes the induction of cellular proliferation. The results indicated that rIL-4 inhibits, early in culture, the expression of those adhesion molecules that are up-regulated by rIL-2, thereby delaying cell–cell contact. This may have direct consequences for the proliferation capacity of the cells.

Abbreviations: HE, hydroethidine; LL, large lymphocytes; MFI, mean fluorescence intensity; PFA, paraformaldehyde; SFDA, sulphofluorescein diacetate.

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MATERIALS AND METHODS

Monoclonal antibodies (mAb) and cytokines

The following mAb were used: SPV-T3b (anti-CD3, IgG2a),[16] CLB-FcRgran1 (anti-CD16, IgG2a),[17] CLB-IL2R/1 (anti-CD25, IgG2a),[18] CLB-T11.1/1 (anti-CD2, IgG1),[19] CLB-LFA-1/1 (anti-CD18, IgG1),[20] the latter four mAb kindly provided by Drs T. W. J. Huizinga, R. A. W. van Lier and F. Miedema from the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service (Amsterdam, The Netherlands); p358 (anti-CD54, IgG2a),[21] kindly provided by Dr J. P. Johnson (Institute for Immunology, Munich, Germany); F10.3 (anti-CD54, IgG1),[22] kindly provided by Dr A. Bloem (Academic Hospital, Utrecht, The Netherlands); NKI-L7 (anti-CD11a, IgG1),[23] NKI-L16 (anti-CD11a activation epitope, IgG2a),[8] NKI-P1 (anti-CD44, IgG1),[24] TS2/9 (anti-CD58, IgG1)[25] obtained through Dr T. A. Springer (Harvard Medical School, Boston, MA); HP1/3 (anti-CD49d, IgG1)[26] kindly provided by Dr F. Sanchez-Madrid (Hospital de la Princesa, Madrid, Spain); SAM-1 (anti-CD49e, IgG2b)[27] and 4F2 (IgG2a) directed against the activation antigen 4F2.[28]

The cytokines used were *Escherichia coli* derived rIL-2 (specific activity 3×10^6 Cetus U/mg; Eurocetus, Amsterdam, The Netherlands) and rIL-4, obtained as purified protein from supernatants of L cells transfected with the cDNA clone encoding IL-4 [kindly provided through Dr J. E. de Vries (specific activity $1{\cdot}2 \times 10^7$ U/mg; DNAX, Palo Alto, CA) by Dr S. Nagabhushan, Schering-Plough Corporation, Bloomfield, NY].

Cells and culture conditions

Peripheral blood lymphocytes (PBL) from normal donors were isolated by Ficoll–Hypaque density centrifugation, washed in Iscove's medium (Flow Laboratories, Irvine, U.K.) supplemented with 5% pooled, inactivated human serum (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service), penicillin 100 IU/ml and kanamycin 100 μg/ml (designated later as medium) and used either directly or after cryopreservation in 10% DMSO. As preactivated lymphocytes, PBL were used that were cultured for 4 days in the presence of rIL-2 500 U/ml. Small lymphocytes containing over 90% T cells and large lymphocytes (LL) enriched for up to 30% NK cells were isolated from the peripheral blood from normal donors by centrifugal elutriation as described previously.[29] Cell viability of all populations used was greater than 90% as detected by trypan blue dye exclusion. Cells at a concentration of 1×10^6/ml in a final volume of 1 ml were cultured at 37° in flat-bottomed 2-ml plates (Costar, Cambridge, MA) either with or without different concentrations of rIL-2 and/or rIL-4 for the periods indicated.

Proliferation assay

The proliferation of the cells was determined by [^{3}H]thymidine ([^{3}H]TdR) incorporation as described previously.[30]

Aggregation assay by microscopic examination

The formation of cell aggregates was determined by at least two investigators using a light microscope as described previously.[4] Percentage aggregation was defined according to the formula:

$$\frac{\text{number of cells within clusters}}{\text{total number of cells}} \times 100\%.$$

Scores ranged from $<10\%$ indicating almost no aggregates, to $>90\%$, indicating cluster formation in large compact aggregates. Standard deviation of scores was generally less than 10%.

To analyse reaggregation, the cells were washed in medium, vigorously resuspended and seeded in flat-bottomed 96-well microtitre plates (Costar) at 2×10^5/well in the presence or absence of rIL-2 100 U/ml or NKI-L16 mAb (ascites dilution 1:1000). Over a time period of 5 hr aggregation was scored as described above.

For blocking experiments small lymphocytes were cultured in the presence of rIL-2 (100 U/ml) and mAb (1/1000 ascites dilution) and scored for cell aggregation after 48–96 hr as described above. Due to limited availability of the CD54 mAb p358, in blocking experiments F10.3 mAb was used.

Aggregation assay by FACS analysis

To analyse the early phase of aggregation, a FACS cluster assay was used,[31] as described previously.[4] Briefly, cells (1×10^6/ml) were stained with the red dye hydroethidine (HE; Polyscience Inc., Warrington, PA; 80 mg/ml in N,N-dimethylacetonide) at a concentration of 3 ng/ml or in another aliquot with the green dye sulphofluorescein diacetate (SFDA; Molecular Probes, Junction City, OR) at a concentration of 5 μg/ml. After 1 hr incubation at 37°, both cell aliquots were washed twice with medium and incubated for 30 min at 4°. Then 10^5 HE-labelled cells together with 10^5 SFDA-labelled cells were seeded in round-bottomed microtitre wells. NKI-L16 (ascites dilution 1:1000) was added to the wells and plates were directly spun for 2 min at 250 *g* [Hettich (Rotanta/RP) centrifuge]. Thereafter, cells were either fixed immediately with 0·5% paraformaldehyde (PFA) or upon subsequent culture at 37° for 5 min. The number of formed conjugates was determined using the FACScan (Becton Dickinson, Mountain View, CA). The percentage aggregation is quantitated by the percentage double fluorescent cells in quadrant 2, multiplied by a factor 2 to represent the percentage aggregation of a single fluorescent population.

Immunofluorescence assay

For surface marker analysis cells in PBS–BSA (0·5%; Sigma Chemical Co., St Louis, MO) containing 0·01% w/v sodium azide, were incubated in the appropriate concentration of mAb for 30 min at 4°, followed by washing twice in PBS–BSA–azide and subsequent incubation in fluorescein isothiocyanate (FITC)-labelled goat (Fab')$_2$ anti-mouse IgG antibody (Nordic, Tilburg, The Netherlands) for 30 min at 4°. After washing in PBS–BSA–azide the percentage positive cells and the mean fluorescence intensity (MFI) were determined by FACScan analysis. Antigen density was expressed as the relative MFI of cytokine-cultured cells compared to that of medium-cultured cells, the latter defined as 100%.

RESULTS

rIL-4 inhibits the rIL-2-induced cell aggregation of resting lymphocytes

To investigate the effect of rIL-2 and rIL-4 on cell aggregation, resting PBL were incubated overnight in medium alone, in the presence of rIL-2 or rIL-4, or with a combination of both lymphokines. After 24 hr aggregation of lymphocytes was scored microscopically. As shown in Table 1, rIL-2 strongly

Table 1. Inhibition of rIL-2-induced cell aggregation by rIL-4

Cell population*	Culture in the presence of			
	Medium	rIL-4†	rIL-2†	rIL-2+rIL-4
PBL ($n=3$)	8±8‡	9±3	53±11	33±5
Small lymphocytes ($n=3$)	6±9	7±9	41±13	21±8
Large lymphocytes ($n=4$)	20±7	20±7	62±4	22±8
Preactivated PBL ($n=3$)	83±6	85±5	85±5	87±3

* PBL were depleted from monocytes by 1 hr 37° adherence to plastic; small and large lymphocytes were obtained by centrifugal elutriation; preactivated lymphocytes were obtained by culturing PBL for 4 days in the presence of rIL-2 500 U/ml prior to use. n = number of experiments.

† rIL-2 used at 100 U/ml, rIL-4 at 100 U/ml.

‡ % aggregation determined microscopically at 24 hr; data are expressed as mean±SE.

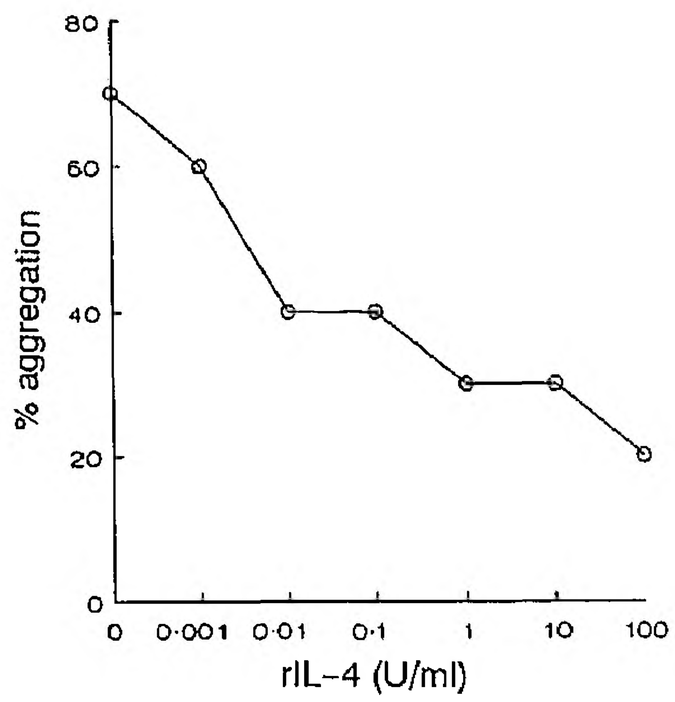

Figure 1. Dose-dependent inhibition by rIL-4 of rIL-2-induced cell aggregation. LL were incubated in 1000 U/ml rIL-2 in the presence of different doses of rIL-4 as indicated and scored for aggregation after 24 hr. In the absence of rIL-2, with or without rIL-4, the level of aggregation did not exceed the 20%.

induced the cells to form aggregates, whereas rIL-4 did not. However, when cells were cultured in the presence of both rIL-2 and rIL-4, the percentage of cell aggregation was much lower compared to the effect of rIL-2 alone. The inhibitory effect of rIL-4 on cell aggregation was not observed with PBL that were preactivated (Table 1). Inhibitory effects of rIL-4 were obtained with cultures of both small and large lymphocytes, although the latter showed somewhat higher reactivity to rIL-2 (Table 1). Therefore, in most experiments described below LL were used. The IL-4 inhibitory effect was observed with different rIL-2 concentrations (100 U/ml and 1000 U/ml) and depended on the dose of rIL-4 used (Fig. 1). The reported inhibitory effect of rIL-4 on rIL-2 induced cellular proliferation[12–14] was confirmed in this study and was found to correlate with the reduced cell aggregation (data not shown). These data indicate that resting lymphocytes can be induced to aggregate by culture in the presence of rIL-2, whereas rIL-4 inhibits this process.

rIL-4 does not affect the function of adhesion receptors

To investigate whether the inhibitory effect of rIL-4 was due to impairment of functional activity of adhesion receptors or to interference of IL-4 with the IL-2 signalling pathway leading to enhanced adhesion, reaggregation was studied using the mAb NKI-L16. Triggering of the L16 epitope of CD11a by this mAb has been found to be independent of intracellular signalling and is thought to induce a conformational change in the CD11a molecule resulting in the induction of firm aggregates within a few hours.[4,5,8] If rIL-4 interferes with IL-2 signalling and not with the activity of the adhesion receptors, cell aggregation would be expected to take place in the presence of NKI-L16 whether or not cells have been incubated in the presence of rIL-4. If rIL-4 affects the adhesion capacity of the receptors, cluster formation would be inhibited. LL were cultured for 24 hr in the presence or absence of rIL-2 or rIL-4, thoroughly washed and resuspended in 3 aliquots containing either medium alone, or rIL-2 or the mAb NKI-L16. During a period of 5 hr reaggregation was scored microscopically and the results are presented in Fig. 2. Cells cultured overnight in the presence of rIL-2 showed a rapid reaggregation. However, cells cultured overnight in medium alone did not aggregate unless NKI-L16 was added during the reaggregation period. Cells cultured overnight in the presence of rIL-4 behaved similarly to cells cultured overnight in medium alone. Although the kinetics of NKI-L16-induced reaggregation of rIL-4-precultured cells was somewhat slower than that of rIL-2-precultured cells, a comparable level of aggregation was obtained. This indicated that overnight culture in rIL-4 did not abolish the functional capacity of adhesion molecules but probably interfered with the signals induced by rIL-2 that result in the activation of adhesion receptors.

Kinetics of rIL-4-mediated inhibition of IL-2-induced cell aggregation

To investigate the effect of rIL-4 on the kinetics of cluster formation, LL were incubated for a period of 72 hr in the presence of rIL-2, with or without rIL-4. At 24, 48 and 72 hr aliquots of cells were washed and resuspended in rIL-2 or NKI-L16 and reaggregation determined. The data depicted in Fig. 3 demonstrated that the difference in reaggregation capacity between cells cultured in rIL-2 versus rIL-2 plus rIL-4 is most prominent at 24 hr (reaggregation in rIL-2 of 80 and 40%, respectively), declines at 48 hr and is lost at 72 hr. NKI-L16 induced reaggregation is presented as a control to show that rIL-2 plus rIL-4-precultured cells are capable of reaggregating comparably to rIL-2-precultured cells. However, careful investigation of clustering using FACS analysis instead of microscopic examination, indicated that the early kinetics of NKI-L16-induced reaggregation was more rapid for cells cultured overnight in the presence of rIL-2 than for cells cultured overnight in the presence of rIL-2 plus rIL-4 (Fig. 4). In addition, cell aggregation in the culture wells themselves, also showed the same prominent reduction at 24 hr and loss of inhibition at 72 hr (data not shown). Thus the data indicate that rIL-4 interferes early during culture with rIL-2 signalling leading to cell–cell aggregation.

Effect of rIL-4 on the expression of adhesion molecules

To test whether the delay in optimal rIL-2-mediated cell aggregation produced by rIL-4 would correspond to altered

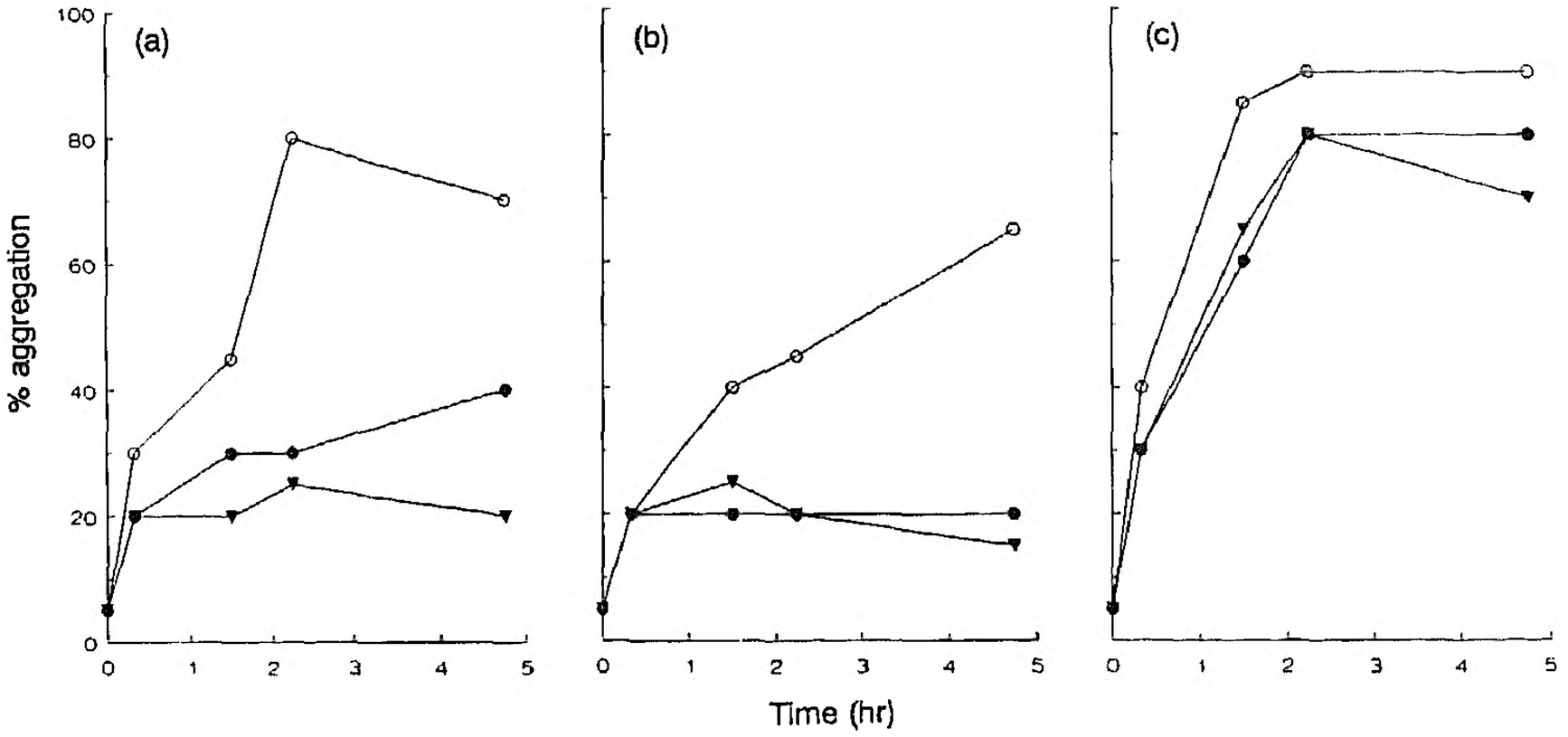

Figure 2. Effect of overnight incubation in cytokines on the subsequent reaggregation capacity of LL. Cells were cultured overnight in medium (a), rIL-4 100 U/ml (b) or rIL-2 100 U/ml (c) washed and resuspended in medium (▼), rIL-2 100 U/ml (●) or NKI-L16 (ascites dilution 1:1000) (○). Reaggregation was scored for the time period indicated.

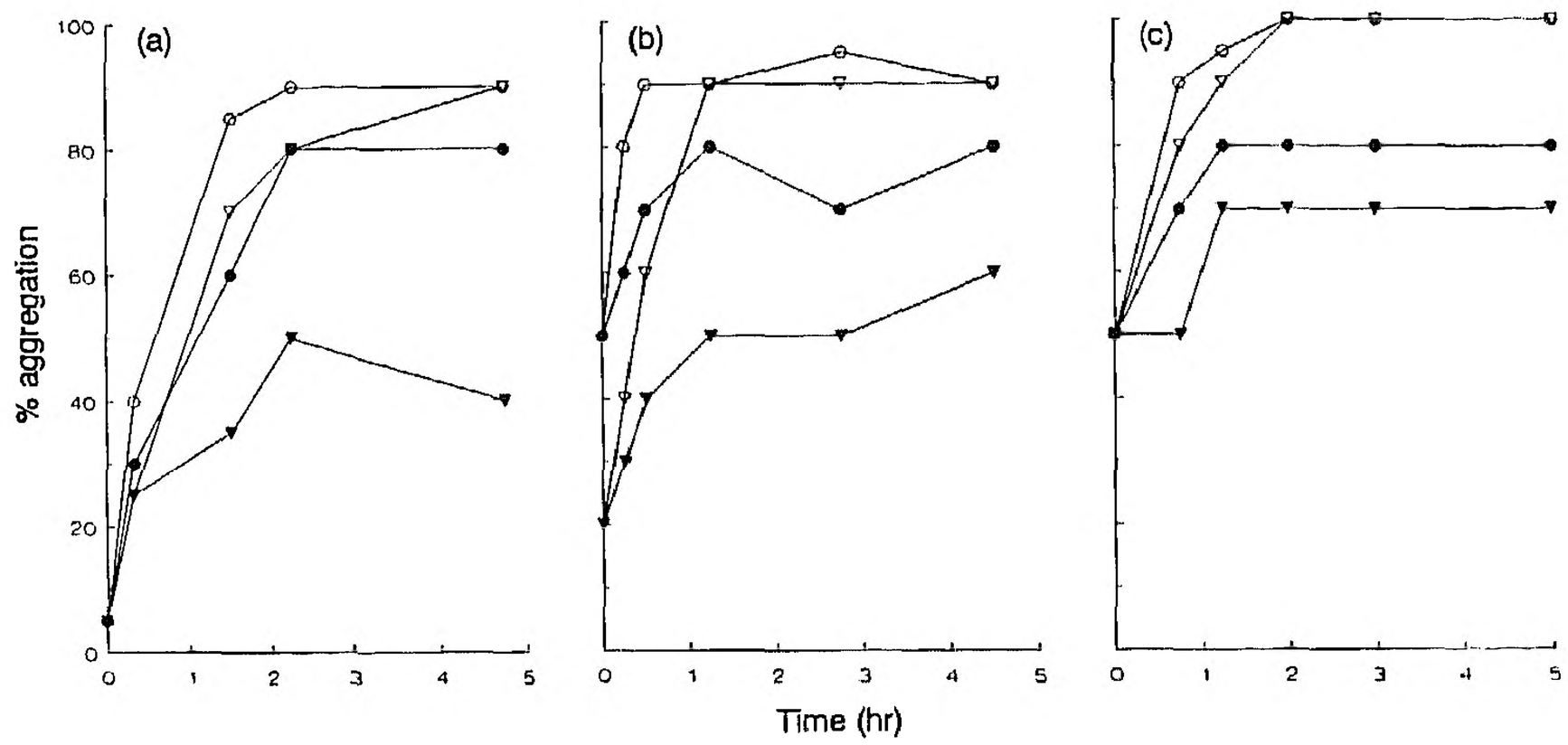

Figure 3. Effect of preincubation in the presence of rIL-4 on the subsequent reaggregation capacity of LL. Cells were cultured for 24 (a), 48 (b) or 72 hr (c) in the presence of rIL-2 100 U/ml in the absence (●, ○) or presence (▼, ▽) of rIL-4 100 U/ml. At each time-point cells were washed, resuspended in rIL-2 100 U/ml (●, ▼) or NKI-L16 (1:1000) (○, ▽) and scored for reaggregation capacity.

expression of adhesion structures on the surface of the cells, FACS analysis was carried out in parallel with cell aggregation measurements. Cells from the experiment depicted in Fig. 3 were assayed at 24 and 72 hr for expression of adhesion molecules known to be involved in adhesion pathways between lymphoid cells, i.e. CD11a, CD18, CD54, CD2, CD58, CD49d, CD49e and CD44. As controls the CD25 IL-2R α-chain and the activation antigen 4F2 were measured, both known to be up-regulated by rIL-2. The results, representative for three experiments, indicate that only small variations occurred in the percentage $CD3^+$ or $CD16^+$ cells indicating that the composition of the cell population did not vary significantly between the different cytokine cultures (Table 2). Of all markers tested, only the percentage $CD25^+$ and $CD54^+$ cells varied among the different cytokine cultures and these percentages were still reduced in rIL-4-supplemented cultures after 72 hr. On the other hand, as shown in Table 3, antigen density of certain adhesion receptors more closely, and with similar kinetics, correlated with the adhesion capacity of the cells in the different cytokine cultures. Early enhancement by rIL-2 (24 hr) of the expression of the L16 epitope, CD54, CD2 and CD49e antigens was completely reduced to background levels in cultures supplemented with rIL-4. The rIL-2-enhanced expression of the activation markers CD25 and 4F2 was only partially reduced by additional rIL-4. Incubation of cells in rIL-4 alone for 24 hr did not affect the expression of adhesion-associated molecules compared to cells cultured in medium alone. The rIL-4-mediated reduction in the rIL-2 up-regulated expression of L16 epitope, CD54, CD2 and CD49e observed at 24 hr, was virtually lost at 72 hr. Thus the expression profile of these antigens closely paralleled the observed differences in aggregation pattern of cells cultured in rIL-2 versus rIL-2 plus rIL-4 (compare Fig. 3). At 24 hr neither rIL-2 nor rIL-4 affected the expression of the adhesion molecules CD11a, CD18 or CD49d (Table 3). The up-regulation of the expression of these antigens observed at 72 hr, preferentially in rIL-2 and less in rIL-2 plus rIL-4-cultured cells, did not result in differences in lymphocyte aggregation pattern between these cultures.

Adhesion routes involved in rIL-2-induced cell aggregation

These data indicated that rIL-4 inhibited the early phase of rIL-2-induced cell adhesion and that this was correlated with

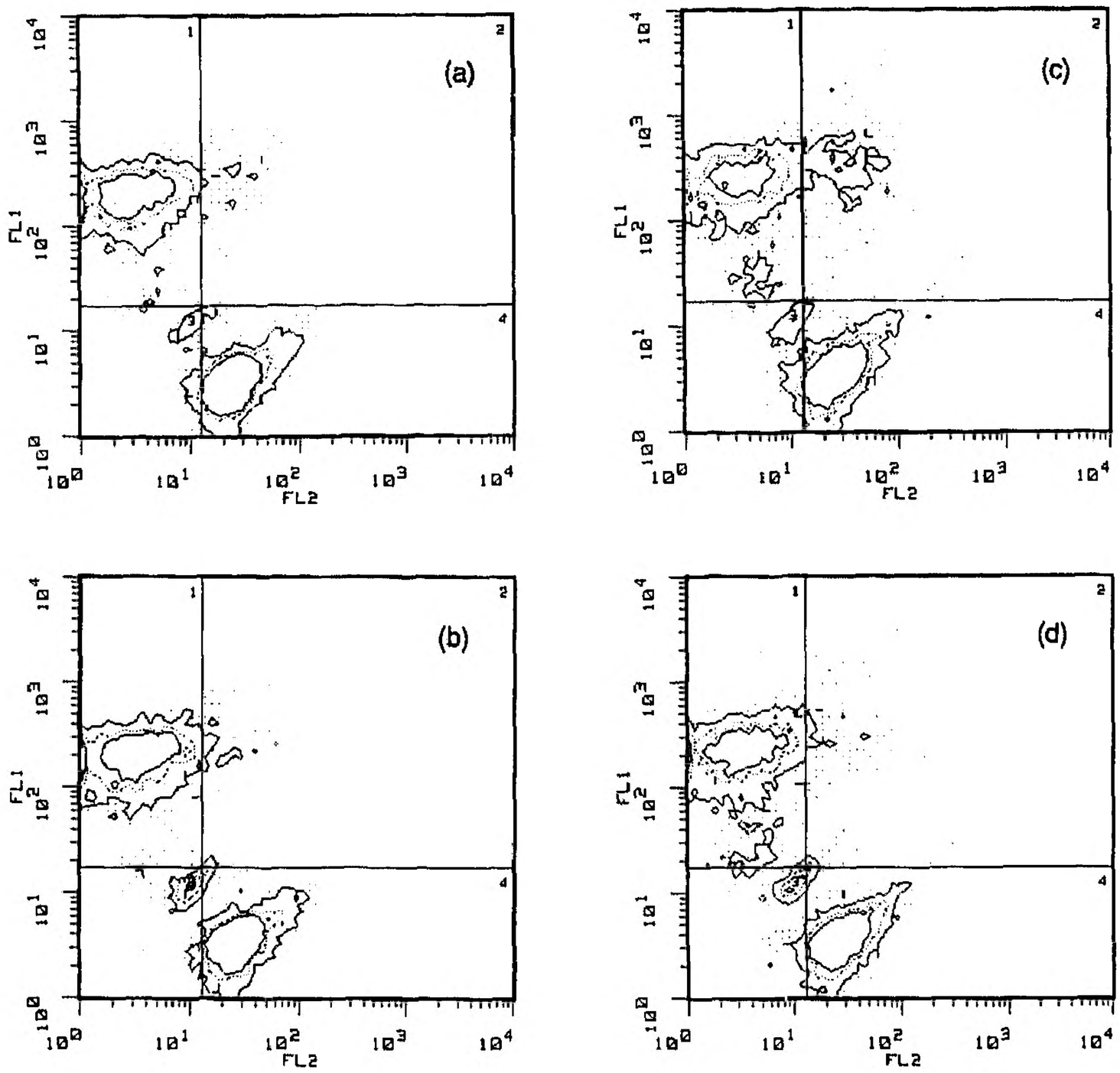

Figure 4. FACS analysis of cluster formation upon reaggregation in the presence of NKI-L16. LL cultured for 24 hr in medium (a), rIL-4 100 U/ml (b), rIL-2 100 U/ml (c) or rIL-2 + rIL-4 (d) were processed as described in Materials and Methods. Clustered cells are shown as double fluorescent cells in quadrant 2 and are quantitated as percentage of single fluorescent cells as follows: medium (a) 7·8%, rIL-4 (b) 8·1%, rIL-2 (c) 19·0% and rIL-2 + rIL-4 (d) 10·4%. Data are shown for cells fixed immediately after the start of reaggregation in the presence of NKI-L16. Upon incubation for an additional 5 min at 37° before fixation, cluster formation of cells cultured overnight in the presence of rIL-2 or rIL-2 + rIL-4 raised to 30·0% and 19·2%, respectively (not shown).

early reduction in the expression of the adhesion molecules L16 epitope, CD54, CD2 and CD49e. To test whether these structures were essential for rIL-2-induced cluster formation, blocking experiments with the respective mAb were performed. As shown in Table 4, mAb directed to CD11a, CD18, CD54 and CD58, were capable of blocking rIL-2-induced cell aggregation. This confirmed the direct involvement of these adhesion receptors in rIL-2-induced lymphocyte aggregation. No inhibiting effects were observed using mAb SAM-1 directed to CD49e. Additional experiments with two VLA β1 mAb (TS 2/16 and AIIB2) did not result in blocking of rIL-2-induced cell adhesion (data not shown). This suggested that the CD49e antigen does not play a primary role in rIL-2-induced cell aggregation.

DISCUSSION

The results of the present study show that rIL-4 delayed the rIL-2-induced aggregation of lymphoid cells in a dose-dependent way. The effect was observed when rIL-4 was added simultaneously with rIL-2 in cultures containing freshly isolated lymphocytes and not when preactivated cells were used. Diminished cell aggregation was most prominent at 24 hr of culture whereas it was lost at 72 hr. With similar kinetics rIL-4 decreased the rIL-2-mediated enhancement of expression of the adhesion-associated surface markers L16 epitope of CD11a, CD2, CD54 and CD49e. Recombinant IL-4 itself had no effect on cell aggregation or adhesion marker expression early in culture. In addition, the reported inhibitory effect of rIL-4 on rIL-2-mediated proliferation[12–14] was confirmed in our study and found to correlate with the effect of rIL-4 on cell aggregation (data not shown). Despite the fact that the down-regulating effect of rIL-4 on the adhesive capacity of the cells was not observed at 72 hr, the level of activation of the cells at 72 hr was still markedly reduced compared with rIL-2-cultured cells. Notably the reduced expression of the p55 IL-2R (CD25), which together with the p75 IL-2R forms the high-affinity IL-2R,[32–34] may be responsible for the subsequent reduction in proliferative capacity of the cells.

Under conditions that rIL-4 inhibited rIL-2-induced cell aggregation, it also inhibited the rIL-2-induced cell proliferation. Notably, with both functional assays the effect was only observed with resting and not with preactivated lymphocytes. This suggested a causal relationship between both cellular functions. If this is the case, specific blocking of adhesion by mAb to adhesion receptors is expected to result in blocking of subsequent proliferation. Experiments are in progress which indicate that CD11a/CD18–CD54 interactions are indeed essential for rIL-2-induced proliferation (F. A. Vyth-Dreese, manuscript in preparation). Thus the rIL-4-mediated inhibition of lymphocyte aggregation early in culture may have direct consequences for the reduced proliferative capacity of IL-2-stimulated cells.

Table 2. Phenotypic analysis of LL cultured in the presence of rIL-2 and/or rIL-4

Antigen	mAb	Time of culture (hr)	Culture in the presence of: Medium	rIL-4	rIL-2	rIL-2+rIL-4
CD3	SPV-T3b	0	74*			
		24	78	77	79	77
		72	76	75	75	73
CD16	CLB-FcRgran1	0	31			
		24	27	26	30	29
		72	22	21	27	28
CD25	CLB-IL-2R/1	0	33			
		24	48	46	58	40
		72	36	37	63	39
CD54	p358	0	10			
		24	13	13	51	29
		72	15	15	73	52
CD11a L16 epitope	NKI-L16	0, 24, 72	>90	>90	>90	>90
CD11a	NKI-L7					
CD18	CLB-LFA-1/1					
CD2	CLB-T11.1/1					
CD58	TS2/9					
CD49d	HP 1/3					
CD49e	SAM-1					
CD44	NKI-P1					
4F2	4F2					

* % positive cells.

Table 3. Regulation of surface marker expression by rIL-2 and/or rIL-4

Antigen	Relative MFI: 24 hr rIL-4	24 hr rIL-2	24 hr rIL-2+rIL-4	72 hr rIL-4	72 hr rIL-2	72 hr rIL-2+rIL-4
CD11a-L16 epitope	99*	140	103	114	130	121
CD11a	110	106	112	109	157	138
CD18	93	95	93	94	161	105
CD54	100	890	110	89	833	733
CD2	93	132	108	187	389	335
CD58	96	120	105	79	124	94
CD49d	99	118	108	96	151	118
CD49e	101	148	110	66	93	66
CD44	107	91	85	130	78	76
CD25	110	335	180	128	648	367
4F2	102	173	129	81	213	190
CD3	98	111	101	80	101	96

* % of MFI of medium-cultured cells.

The ratio for MFI values of mAb versus background (negative control) for the above listed antigens varied from 7 to 200 in freshly obtained LL, from 9 to 230 for 24 hr and from 11 to 280 for 72 hr cultured cells. The exception is the anti-CD54 mAb that did not stain the fresh and medium-cultured cells above background level.

Whereas previous studies have shown that the IL-4 inhibitory effect on cell proliferation and the generation of lymphokine-activated killer activity is an early effect taking place in the first 24 hr of culture,[11–14] our data demonstrate that the rIL-4 effect on cell aggregation is transient, being visible only in the first 24–48 hr. In view of the shared homology of the extracellular domains of the p75 IL-2R and the IL-4R,[35] it is tempting to speculate that hindering by rIL-4 of the interaction between rIL-2 and the p75 IL-2R would play a role. The transient character of the inhibition by rIL-4 may be explained by the fact

Table 4. Adhesion structures involved in IL-2-induced cell aggregation

Culture in presence of rIL-2 supplemented with mAb	Antigen	Aggregation range (%)
—		30–60 (6/6)*
NKI-L16	CD11a L16 epitope	> 60 (6/6)
NKI-L15	CD11a	< 10–30 (5/6)
CLB-LFA-1/1	CD18	< 10 (5/6)
F10.3	CD54	10–30 (3/4)
TS2/9	CD58	10–30 (3/5)
HP1/3	CD49d	> 60 (5/5)
SAM-1	CD49e	30–60 (3/4)
4F2	4F2	30–60 (5/5)

Small lymphocytes were cultured in the presence of rIL-2 (100 U/ml) and adhesion-associated mAb (1/1000) and scored for cell aggregation after 48–96 hr of culture. In the absence of rIL-2, only NKI-L16 and HP1/3 induced cell aggregation above background level (which was < 10%).

* No. of experiments in which aggregation was scored within the range shown/total no. of experiments. In the other experiments mAb did change aggregation.

that inhibition of CD25 expression at 24 hr is not complete (see Table 3). Even in the presence of rIL-4, IL-2 may continue to bind to p75 and p55 subunits, thereby up-regulating p55 further to a level sufficient to activate the cells to form clusters.

The inhibitory effect of rIL-4 on rIL-2-induced lymphocyte aggregation was found to correlate with a reduced expression of the adhesion molecules L16 epitope, CD54, CD2 and CD49e. The data from Table 4 confirm that CD11a/CD18–CD54 and CD2–CD58 interactions are directly involved in rIL-2 induced adhesion. Thus it can be concluded that rIL-4 inhibits rIL-2-induced cell aggregation by preventing the early up-regulation of the CD2 and CD54 receptors. In the case of the CD11a/CD18 antigen, rIL-4 appears to inhibit the generation of the activated state of the receptor as shown by the reduced expression of the L16 epitope. On the other hand, the mAb used to detect the CD49e antigen, SAM-1, did not block rIL-2-induced aggregation (Table 4), nor did two CD29 (VLA β1) mAb (data not shown). Therefore, it is unlikely that the CD49e antigen is directly involved in rIL-2-induced adhesion. In agreement with previous observations of Campanero *et al.*,[26] the CD49d mAb HP1/3 was found to stimulate aggregation (Table 4). This effect was related to the activation state of the cells since it was observed more profoundly upon prolonged incubation in the presence of rIL-2 (data not shown). Recombinant IL-2, in turn, was found to up-regulate CD49d expression, but only after several days of culture (see Table 3). Therefore, the CD49d molecule seemed to play a secondary role in facilitating cell–cell interaction after the onset of IL-2-induced adhesion had taken place. And even during the later stages of IL-2-induced cell aggregation, differences in CD49d expression seemed to have less impact on the adhesion capacity of the cells compared with that of L16 epitope, CD2 and CD54 adhesion receptors. Therefore it is concluded that the latter molecules are the main determinants of IL-2-induced cell adhesion.

In the cascade of events that take place when an antigen-specific T lymphocyte encounters its target cell, non-specific contact is thought to precede cognate events.[2] Upon subsequent T-cell triggering, adhesion structures become activated,[3–7] adhesion strengthening occurs and firm effector/target interaction results in optimal effector function.[3] This pathway may be mimicked by stimulation with IL-2 (ref. 5 and the present study). Thus the physiological role of IL-4 would be to prevent the phase of initial, non-specific cell–cell contact, and subsequent progression to the phase of adhesion strengthening in the absence of antigen. In the presence of antigen, or by perturbation of adhesion molecules artificially by mAb triggering, the activated state of the adhesion structures would be favoured and the effect of IL-4 would be counteracted. Indeed, incubation of lymphocytes in the presence of NKI-L16, known to induce the activation state of the CD11a molecule,[3–5,8] was found to abrogate the effect of rIL-4 in rIL-2-stimulated cultures supplemented with rIL-4 (see Fig. 3).

Thus, it is concluded that rIL-4 inhibits the early phase of rIL-2-mediated cell aggregation by reducing the expression of those molecules that are important for initial non-specific cell–cell contact. These structures determine the subsequent phase of adhesion strengthening in the presence of antigen. The latter process is mimicked by NKI-L16 induced reaggregation and under these conditions the negative effect of rIL-4 is overruled. These data confirm the importance of IL-2 and IL-4 as regulatory molecules for lymphoid cell activation and differentiation, and stress the essential role of cell–cell interactions in these processes.

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REFERENCES

1. SPRINGER T.A. (1990) Adhesion receptors of the immune system. *Nature*, **346**, 425.
2. SPITS H., VAN SCHOOTEN W., KEIZER H., VAN SEVENTER G., VAN DE RIJN M., TERHORST C. & DE VRIES J.E. (1986) Alloantigen recognition is preceded by nonspecific adhesion of cytotoxic T cells and target cells. *Science*, **232**, 403.
3. FIGDOR C.G., VAN KOOYK Y. & KEIZER G.D. (1990) On the mode of action of LFA-1. *Immunol. Today*, **11**, 277.
4. VAN KOOYK Y., VAN DE WIEL-VAN KEMENADE P., WEDER P., KUIPERS T.W. & FIGDOR C.G. (1989) Enhancement of LFA-1 mediated cell adhesion by triggering through CD2 or CD3 on T lymphocytes. *Nature*, **342**, 811.
5. VAN KOOYK Y., WEDER P., HOGERVORST F., VERHOEVEN A.J., VAN SEVENTER G., TE VELDE A.A., BORST J., KEIZER G.D. & FIGDOR C.G. (1991) Activation of LFA-1 through a Ca^{2+}-dependent epitope stimulates lymphocyte adhesion. *J. Cell Biol.* **112**, 345.
6. DUSTIN M.L. & SPRINGER T.A. (1989) T-cell receptor cross-linking transiently stimulates adhesiveness through LFA-1. *Nature*, **341**, 619.
7. SHIMIZU Y., VAN SEVENTER G.A., HORGAN K.J. & SHAW S. (1990) Regulated expression and binding of three VLA (β1) integrin receptors on T cells. *Nature*, **345**, 250.

8. KEIZER G.D., VISSER W., VLIEM M. & FIGDOR C.G. (1988) A monoclonal antibody (NKI-L16) directed against a unique epitope on the α-chain of human leukocyte function-associated antigen 1 induces homotypic cell–cell interactions. *J. Immunol.* **140,** 1393.
9. ROBERTSON M.J., CALIGIURI M.A., MANLEY T.J., LEVINE H. & RITZ J. (1990) Human natural killer cell adhesion molecules. Differential expression after activation and participation in cytolysis. *J. Immunol.* **145,** 3194.
10. BUCKLE A.-M. & HOGG N. (1990) Human memory T cells express intercellular adhesion molecule-1 which can be increased by interleukin 2 and interferon-γ. *Eur. J. Immunol.* **20,** 337.
11. SPITS H., YSSEL H., PAILLARD X., KASTELEIN R., FIGDOR C.G. & DE VRIES J.E. (1988) IL-4 inhibits IL-2 mediated induction of human lymphokine-activated killer cells, but not the generation of antigen-specific cytotoxic T lymphocytes in mixed leukocyte cultures. *J. Immunol.* **141,** 29.
12. NAGLER N., LANIER L.L. & PHILLIPS J.H. (1988) The effects of IL-4 on human natural killer cells. A potent regulator of IL-2 activation and proliferation. *J. Immunol.* **141,** 2349.
13. KAWAKAMI Y., CUSTER M.C., ROSENBERG S.A. & LOTZE M.T. (1989) IL-4 regulates IL-2 induction of lymphokine-activated killer activity from human lymphocytes. *J. Immunol.* **142,** 3452.
14. MARTINEZ O.M., GIBBONS R.S., GAROVOY M.R. & ARONSON F.R. (1990) IL-4 inhibits IL-2 receptor expression and IL-2 dependent proliferation of human T cells. *J. Immunol.* **144,** 2211.
15. PARK L.S., FRIEND D., SASSENFELD H.M. & URDAL D.L. (1987) Characterization of the human B cell stimulatory factor 1 receptor. *J. exp. Med.* **166,** 476.
16. SPITS H., KEIZER G., BORST J., TERHORST C., HEKMAN A. & DE VRIES J.E. (1984) Characterization of monoclonal antibodies against cell surface molecules associated with cytotoxic activity of natural and activated killer cells and cloned CTL lines. *Hybridoma*, **2,** 423.
17. HUIZINGA T.W.J., VAN DER SCHOOT C.E., JOST C., KLAASSEN R. KLEIJER M., VON DEM BORNE A.E.G.KR., ROOS D. & TETTEROO P.A. (1988) The PI-linked receptor FcRIII is released on stimulation of neutrophils. *Nature*, **333,** 667.
18. VAN LIER R.A.W., BOOT J.H.A., VERHOEVEN A.J., DE GROOT E.R., BROUWER M. & AARDEN L.A. (1987) Functional studies with anti-heavy chain isotype switch-variant monoclonal antibodies. Accessory cell-independent induction of interleukin 2 responsiveness in T cells by ε-anti-CD3. *J. Immunol.* **139,** 2873.
19. VAN LIER R.A.W., BROUWER M. & AARDEN L.A. (1988) Signals involved in T cell activation. T cell proliferation induced through the synergistic action of anti-CD28 and anti-CD2 monoclonal antibodies. *Eur. J. Immunol.* **18,** 167.
20. MIEDEMA F., TETTEROO P.A.T., HESSELINK W.G., WERNER G., SPITS H. & MELIEF C.J.M. (1984) Both Fc receptors and lymphocyte-function-associated antigen on human Tγ lymphocytes are required for antibody-dependent cellular cytotoxicity (killer cell activity). *Eur. J. Immunol.* **14,** 518.
21. JOHNSON J.P., STADE B.G., HUPKE U., HOLZMANN B. & RIETHMÜLLER G. (1988) The melanoma progression-associated antigen P3.58 is identical to the intercellular adhesion molecule, ICAM-1. *Immunobiology*, **178,** 275.
22. BLOEMEN P., MOLDENHAUER G., VAN DIJK M., SCHUURMAN H.J., GMELIG MEYLING F.H.J. & BLOEM A.C. (1992) Multiple ICAM-1 (CD54) epitopes are involved in homotypic B cell adhesion. *Scand. J. Immunol.* **35,** 517.
23. KEIZER G.D., BORST J., FIGDOR C.G., SPITS H., MIEDEMA F., TERHORST C. & DE VRIES J.E. (1985) Biochemical and functional characteristics of the human leukocyte membrane antigen family LFA-1, Mo-1 and p150,95. *Eur. J. Immunol.* **15,** 1142.
24. PALS S.T., HOGERVORST F., KEIZER G.D., THEPEN T., HORST E. & FIGDOR C.G. (1989) Identification of a widely distributed 90-kDa glycoprotein that is homologous to the Hermes-1 human lymphocyte homing receptor. *J. Immunol.* **143,** 851.
25. SANCHEZ-MADRID F., KRENSKY A.M., WARE C.F., ROBBINS E., STROMINGER J.L., BURAKOFF S.J. & SPRINGER T.A. (1982) Three distinct antigens associated with human T lymphocyte-mediated cytolysis, LFA-1, LFA-2 and LFA-3. *Proc. natl. Acad. Sci. U.S.A.* **79,** 7489.
26. CAMPANERO M.R., PULIDO R., URSA M.A., RODRIGUEZ-MOYA M., DE LANDAZURI M.O. & SANCHEZ-MADRID F. (1990) An alternative leukocyte homotypic adhesion mechanism, LFA-1/ICAM-1-independent, triggered through the human VLA-4 integrin. *J. Cell. Biol.* **110,** 2157.
27. KEIZER G.D., TE VELDE A.A., SCHWARTING R., FIGDOR C.G. & DE VRIES J.E. (1987) Role of p150,95 in adhesion, migration, chemotaxis and phagocytosis of human monocytes. *Eur. J. Immunol.* **17,** 1317.
28. HAYNES B.F., HEMLER M.E., MANN D.L., EISENBARDT G.S., SHELHAMER J, MOSTOWSKI H.S., THOMAS C.A., STROMINGER J.L. & FAUCI A.S. (1981) Characterization of a monoclonal antibody (4F2) that binds to human monocytes and to a subset of activated lymphocytes. *J. Immunol.* **126,** 1409.
29. FIGDOR C.G., BONT W.S., DE VRIES J.E. & VAN ES W.L. (1981) Isolation of large numbers of highly purified lymphocytes and monocytes with a modified centifugal elutriation technique. *J. immunol. Meth.* **40,** 275.
30. VYTH-DREESE F.A., VAN DER REIJDEN H.J. & DE VRIES J.E. (1982) Phorbol-ester mediated induction and augmentation of mitogenesis and interleukin-2 production in human T-cell lymphoproliferative disease. *Blood*, **60,** 1437.
31. KUIJPERS T.W., KOENDERMAN L., WEENING R.S., VERHOEVEN A.J. & ROOS D. (1990) Continuous cell activation is necessary for stable interaction of complement receptor type-3 with its counterstructure in the aggregation response of human neutrophils. *Eur. J. Immunol.* **20,** 501.
32. TSUDO M., KOZAK R.W., C.K., GOLDMAN R.W. & WALDMANN T.A. (1986) Demonstration of a non-Tac peptide that binds interleukin 2: a potential participant in a multichain interleukin 2 receptor complex. *Proc. natl. Acad. Sci. U.S.A.* **83,** 9694.
33. TESHIGAWARA K., WANG H.-M., KATO K. & SMITH K.A. (1987) Interleukin 2 high affinity receptor expression requires two distinct binding proteins. *J. exp. Med.* **165,** 223.
34. HATEKEYAMA M., TSUDO M., MINAMOTO S., KONO T., DOI T., MIYATA T., MIYASAKA M. & TANIGUCHI T. (1989) Interleukin 2 receptor β chain gene: generation of three receptor forms by cloned human α and β chain cDNA's. *Science*, **244,** 551.
35. BAZAN J.F. (1990) Haemopoietic receptors and helical cytokines. *Immunol. Today*, **11,** 350.