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 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. Subsequently, upon antigen recognition a phase of 'adhesion strengthening' occurs, during which conformational changes in adhesion structures are thought to play a role. 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, and for the CD49d, CD49e and CD49f molecules belonging to the VLA β1 subfamily of integrins. 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. 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. 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. 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. 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.
MATERIALS AND METHODS

Monoclonal antibodies (mAb) and cytokines

The following mAb were used: SPV-T3b (anti-CD3, IgG2a),
CLB-FcγRα1 (anti-CD16, IgG2a),
CLB-IL2R/1 (anti-CD25, IgG2a),
CLB-T11.1/1 (anti-CD2, IgG1),
CLB-LFA-1/1 (anti-CD18, IgG1),
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),
TS2/9 (anti-CD58, IgG1),
NKI-L16 (anti-CD11a activation epitope, IgG2a),
NKI-P1 (anti-CD44, IgG1),
TS2/9 (anti-CD58, IgG1)
obtained
through Dr T. A. Springer (Harvard Medical School, Boston,
MA); HP1/3 (anti-CD49d, IgG1) kindly provided by Dr F.
Sanchez-Madrid (Hospital de la Princesa, Madrid, Spain);
SAM-1 (anti-CD49e, IgG2b) and 4F2 (IgG2a) directed
against the activation antigen 4F2.

The cytokines used were Escherichia coli derived rIL-2
(specific activity 3 × 10^6 Catus 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
Sanchez-Madrid (Hospital de la Princesa, Madrid, Spain);
Iscove’s medium (Flow Laboratories, Irvine, U.K.) supple-
dimented 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 (desig-
nated 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°C 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 [3H]thymidine
([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 aggrega-
tes. 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-dimethylacetamide) 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°C, both cell aliquots were washed twice with
medium and incubated for 30 min at 4°C. 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°C for 5 min. The number
of formed conjugates was determined using the FACSScan
(Becton Dickinson, Mountain View, CA). The percentage
aggregation is quantitated by the percentage double fluorescence
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°C, 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°C. After washing in
PBS–BSA–azide the percentage positive cells and the mean
fluorescence intensity (MFI) were determined by FACSScan
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
lymophokines. 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

<table>
<thead>
<tr>
<th>Cell population*</th>
<th>Culture in the presence of rIL-4 (U/ml)</th>
<th>Medium</th>
<th>rIL-4†</th>
<th>rIL-2†</th>
<th>rIL-2 + rIL-4†</th>
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<tr>
<td>PBL (n = 3)</td>
<td></td>
<td>8 ± 8‡</td>
<td>9 ± 3</td>
<td>53 ± 11</td>
<td>33 ± 5</td>
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<td>Small lymphocytes (n = 3)</td>
<td></td>
<td>6 ± 9</td>
<td>7 ± 9</td>
<td>41 ± 13</td>
<td>21 ± 8</td>
</tr>
<tr>
<td>Large lymphocytes (n = 4)</td>
<td></td>
<td>20 ± 7</td>
<td>20 ± 7</td>
<td>62 ± 4</td>
<td>22 ± 8</td>
</tr>
<tr>
<td>Preactivated PBL (n = 3)</td>
<td></td>
<td>83 ± 6</td>
<td>85 ± 5</td>
<td>85 ± 5</td>
<td>87 ± 3</td>
</tr>
</tbody>
</table>

* PBL were depleted from monocytes by 1 hr 37°C 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 proliferation15-18 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 NK1-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.5,8,9 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 NK1-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 NK1-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 NK1-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 NK1-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 NK1-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. NK1-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 NK1-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
Regulatory effect of IL-4 on IL-2-induced cell aggregation

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 NK1-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 NK1-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
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 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, 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

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<th>Antigen</th>
<th>mAb</th>
<th>Time of culture (hr)</th>
<th>Culture in the presence of</th>
<th>Medium</th>
<th>rIL-4</th>
<th>rIL-2</th>
<th>rIL-2 + rIL-4</th>
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<tr>
<td>CD3</td>
<td>SPV-T3b</td>
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<td>74*</td>
<td>74</td>
<td>77</td>
<td>79</td>
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<tr>
<td>CD16</td>
<td>CLB-FeRgran1</td>
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<td>78</td>
<td>76</td>
<td>75</td>
<td>75</td>
<td>73</td>
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<tr>
<td>CD25</td>
<td>CLB-IL-2R/l</td>
<td>0</td>
<td>31</td>
<td>27</td>
<td>26</td>
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<td>29</td>
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<tr>
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<td>4F2</td>
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* % positive cells.

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

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<tr>
<th>Antigen</th>
<th>rIL-4</th>
<th>rIL-2</th>
<th>rIL-2 + rIL-4</th>
<th>rIL-4</th>
<th>rIL-2</th>
<th>rIL-2 + rIL-4</th>
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<td>CD11a-L16 epitope</td>
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<td>114</td>
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<td>112</td>
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</tr>
</tbody>
</table>

* % of MFI of medium-cultured cells.

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, 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

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

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 IL-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 not 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., 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. Upon subsequent T-cell triggering, adhesion structures become activated, and adhesion strengthening occurs and firm effector/target interaction results in optimal effector function. 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 artifically 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, 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

Regulatory effect of IL-4 on IL-2-induced cell aggregation


