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# Role of LFA-1 molecule in conjugate formation and recycling of human natural killer cells

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The leukocyte function-associated (LFA-1) glucoprotein is a heterodimer molecule with specific 177KD alpha(CD11a) and a common 95KD (CD18) beta chain that are non-covalently bound. The molecule has a specific function in various cell adhesions, cell-to cell interactions and is widely distributed on lekocytes; lymphocytes, NK-cells, thymocytes, granulocytes and monocytes [4]. Recently, as specific ligand for LFA-1 molecule the intercellular adhesion-1 molecule (ICAM-1) has been suggested and the molecule included into the integrin superfamily [3].

Blocking of the LFA-1 molecules on cytotoxic effector T-lymphocytes(CTL) by monoclonal antibodies (mAb) the cytotoxic function was found to be inhibited; the early adhesion (and recognition) step was blocked and similar mechanism was suggested for for NK-cytotoxicity that could also be inhibited by varios anti-LFA-1 mAbs [5].

In our recent experiments the inhibitory effects of mAbs directed to various epitopes of alpha and beta chain of LFA-1 molecule was analysed by in vitro assays suitable for detection of killing cycle of human NK cells.

## Materials and Methods

1. effector cells: Peripheral lymphocytes were separated on Ficoll-Hypaque that followed by adherence on plastic surface and nylon column separation. Nylon column passed (NCP), T-cell enriched effector cells were used in part of our experiments. Also large granular lymphocytes (LGL) separated on Percoll gradient were used as effector cells [6]. For single cell assay contaminant T-lymphocytes and monocytes were eliminated by mAb and complement (anti-CD5 and Mo-2). The purity of LGL was over 95% by morphology.

2. *in vitro* cytotoxicity assay: Our method has been published elsewhere [1]. Four stepwise dilution of effector cells were measured on K-562 target cells with 3 parallels.

3. monoclonal antibodies: MAbs to LFA-1 antigen of the IIIrd Monoclonal Workshop (Oxford) were used. Effector cells were four times washed after incubation.

4. single cell conjugate assay: A modified technique of Grinm and Bonavida [2] was used. Conjugates were carefully resuspended in aliquot of 0.07% agarose, kept at 42 °C and 0.25 umol propidium iodine was added and pipetted into a coverslip chamber. After 3h incubation the per cent of dead and living target-effector cell conjugates were counted in 200 conjugates.

## Results

1. Inhibitory effect of various anti--LFA-1 mAbs on NK-cytotoxicity: After 45 min preincubation of NCP effector cells by anti-LFA-1 mAbs it revealed that only the antibodies to the common beta chain exerted significant (30−77%) inhibitory effect, that may have biological relevance (Table I). The TS.1.18.1.1. mAb, specific to the beta subunit was further studied in various exeriments.

2. Time-course study of anti-LFA-1 mAb on NK cytotoxicity: In a time course preincubation of effector lymphocytes (30, 90 and 120 min) we found 30−57% inhibition, relative to control myeloma supernatant (X63Ag8) at each time point, though the magnitude of inhibition slightly decreased by shorter preincubation (data not shown). Also preincubation of labelled K-562 target cells with LFA-1 mAb made no inhibition of cytotoxicity.

By adding anti-LFA-1 mAb (TS 18.1.1.) to cytotoxicity assay at various times after the onset of the reaction, at the onset or 15, 40 and 80 minutes afterwards we observed no appreciable difference in the extent of inhibition which suggested the inhibition at later phase of target cell killing (Table II).

3. Single cell assay with highly purified large granular lymphocytes: In the soft agarose assay that may give information about the first cycle killing of LGL, we observed no inhibition of anti-LFA mAb. However the parallel in vitro cytotoxicity exerted well detectable effect on LGLs (48−73%) (Table III).

## Discussion

Earlier investigations have indicated that human and mouse long term T-cell lines with NK-like activity could be inhibited by mAb with LFA-1 specificity [4]. In CTL mediated kil-

Table I

Effect of LFA 1 pretreatment of lymphocytes by 3rd workshop sera

| WS | Antibody | Lymphocytes[1] | | | | Specificity |
|---|---|---|---|---|---|---|
| | Controls (5) | 38 | 24 | 28[2] | | |
| 706 | 60.3 Beatty | 18 | 8 | 12 | +++ | anti-beta |
| 707 | 3.9 Hogg | 40 | 26 | 38 | | anti-alpha |
| 708 | MHM23 | 30 | 11 | 12 | ++ | anti-beta |
| 709 | MHM24 | 31 | 22 | 25 | | anti-alpha |
| 710 | CLB. 54 Midema | 35 | 19 | 22 | | anti-alpha |
| 711 | CIMT | 39 | 21 | 33 | | anti-alpha |
| 713 | M232 Bernard | 27 | 10 | 16 | ++ | anti-beta |
| 724 | CLB-LFA 1/2 | 26 | 13 | 20 | ++ | anti-beta |
| 725 | IB4 Wright | 27 | 5 | 8 | +++ | anti-beta |
| 726 | 3.9 | 31 | 15 | 31 | | anti-alpha |
| 727 | L29 | 42 | 22 | 34 | | anti-alpha |
| 728 | 3KB43 | 41 | 23 | 29 | | anti-alpha |

[1] nylon column enriched lymphocytes preincubated (45 min, 37 C) and washed 4×
[2] NK-cytotoxicity to K-562, 18 : 1 E : T ratio, 4h incubation

TABLE II

Treatment of effector lymphocytes

| By anti-LFA 1 antibodies | | | | |
|---|---|---|---|---|
| Time | onset | 15 | 40 min. | 80 |
| Control | 60 | 54 | 55 | 53 |
| anti-LFA 1 | 21 | 20 | 16 | 15 |

treatment: see Table I, specific Cr-51 release, 70 : 1 E : T ratio

TABLE III

Single cell assay with highly purified LGL cells

| Conjugates cytotoxicity | | | | |
|---|---|---|---|---|
| | lytic | non lytic | 30% L.U. | % inhibition |
| Control | 82 | 118 | 500 | — |
| LFA-1 | 88 | 112 | 135 | 73 |
| Control | 123 | 77 | 3800 | — |
| LFA 1 | 125 | 75 | 2000 | 48 |

treatment of effector cells: see Table I
single cell assay of 230 min.
to K-562 cells of 210 min.

ling, LFA-1 antigen participates in the early phase of cytotoxic events. Recently anti beta-LFA-1 mAb-(CD18) have found to be inhibitory both to conjugate-formation and cytotoxicity of NK cells in fluid phase [5]. In our experiments with NK cytotoxicity, the early steps seem to be completely independent of LFA-1 structure: giving optimum dose at onset or later to the assay, it has resulted inhibition cytotoxicity which indicates that probably the later phase of it is sensitive to anti-LFA-1 mAb.

Our single cell assay in agarose and parallel conventional cytotoxicity with highly purified LGLs suggest that anti LFA-1 mAb has an exclusive inhibitory effect on the recycling pool of human LGLs. We want to stress that effect on killing cycle could only be analysed by immobilised assays. Our results indicate that probably the beta subunit of LFA-1 molecule may serve as a collaborating structure exclusively in second and multiple cycle killing. However, we have some evidence that anti alpha chain mAbs could also have effective inhibition on NK cells. Also functional synergistic effect was described suggesting independent mechanism of epitopes in inhibition of NK cells [5]. Recycling of effector cells may have altered sensitivity; recently Timonen

and others [6] have found the recycling pool alpha IFN sensitive. However, we found no increase of expression of LFA-1 molecule on NCP lymphocytes after IFN treatment (Kotlán, unpublished).

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