MEMBRANE GLYCOPROTEIN p150,95 OF HUMAN CYTOTOXIC T CELL CLONES IS INVOLVED IN CONJUGATE FORMATION WITH TARGET CELLS

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The p150,95 heterodimer, one of three members of the leukocyte function associated antigen (LFA) family, is expressed by monocytes, granulocytes, NK cells, and a small percentage of lymphocytes. We now report that the p150,95 glycoprotein is expressed by some cytotoxic T cell clones and that it is involved in cell-mediated cytotoxicity by these clones. Two CTL clones, clone JS-93 (CD3+ CD4+ CD8- ) and clone JS-102 (CD3+ CD4- CD8- ) expressed high levels of p150,95 and were shown to be specifically directed against HLA-DR and HLA-A2, respectively. Immunoprécipitations followed by two-dimensional gel electrophoresis demonstrated no heterogeneity in the p150,95 molecule isolated from both clones. Furthermore, we demonstrated that monoclonal antibodies (moab) directed against p150,95 could inhibit the cytotoxic activity of both clone JS-93 and clone JS-102 (50% and 47%, respectively). Single cell assays revealed the inhibition to occur at the level of conjugate formation rather than at the level of the lethal hit. Similar results were obtained with moab directed against LFA-1 (p170,95). The capacity of the moab directed against LFA-1 and p150,95 to inhibit CTL activity and conjugate formation were additive, resulting in a similar percentage of inhibition as found with moab directed against the common $\beta$-chain of these molecules. It is concluded that at least some CTL clones express the p150,95 antigen at their cell surface, and that this molecule, like LFA-1, acts at the level of conjugate formation between effector and target cells.

Cytolytic T lymphocytes (CTL) are crucial in the defense against intracellular parasites, viruses, and immunogenic neoplasms (1–3). CTL-mediated killing is a multistep process that takes place within 10 to 15 min at 37°C (4). The initial step is conjugate formation followed by the strengthening of the CTL-target cell interaction (4–7). The second step involves the delivery of the lethal hit, followed by lysis of the target cell independently of the presence of the CTL (4).

Two main phenotypes of CTL clones have been described, namely CD3+ CD4+ CD8- and CD3+ CD4- CD8+ subsets (8–10). CD3+ CD4+ CD8- CTL clones were primarily found to be directed against class II major histocompatibility complex (MHC) antigens, whereas CD3+ CD4- CD8+ CTL clones are mainly reactive with class I MHC antigens (9–12). Monoclonal antibodies (moab) directed against CD4 and CD8 are able to prevent conjugate formation (13–15). However, there is recent evidence that the CD8 antigen may be involved in the triggering of the lethal hit and has a regulatory role (16, 17). Furthermore, several investigators noted that moab directed against the CD3-T cell receptor complex (18) inhibited CTL-mediated lysis (13, 19–21), probably by preventing triggering of the lethal hit (13–15).

Another molecule involved in CTL-mediated lysis is the leukocyte function associated antigen-1 (LFA-1) (22–24). Monoclonal antibodies directed against this antigen have been shown to prevent conjugate formation (13, 25, 26), but are probably not involved in the delivery of the lethal hit (13). The role of LFA-1 is not restricted to CTL-target cell binding, since moab directed against this structure inhibit also various other cell-cell interactions, for instance B-B (27, 28), B-T (29–31), T endothelial cell (32), and T cell-macrophage interactions (29–31).

The LFA-1 antigen consists of an $\alpha$ subunit of 170 kildodalton (kD) and a $\beta$ subunit of 95 kD and is related to complement receptor 3 (CR3) (30, 33, 34). LFA-1 is expressed by all leukocytes in contrast to CR3 (35) and p150,95 by monocytes, granulocytes, and NK cells (36). Monoclonal antibodies directed against CR3 and p150,95 were unable to inhibit the mixed lymphocyte reaction and NK cell-mediated killing (36).

We now report that the p150,95 antigen can be expressed on both CD3+ CD4+ CD8- and CD3- CD4- CD8+ CTL clones. Furthermore, we provide evidence that moab reactive with this antigen can inhibit CTL-mediated killing at the level of conjugate formation.

MATERIALS AND METHODS

Monoclonal antibodies. The moab SPV-L7 (IgG1), S-HC1 3 (IgG2b), and Bear-1 (IgG1) are directed against the $\alpha$-chains of human CD3 (22–24). The moab SPV-L7 (IgG1), S-HC1 3 (IgG2b), and Bear-1 (IgG1) are directed against the $\alpha$-chains of human CD3 (22–24).

Abbreviations used in this paper: LFA-1, leukocyte function associated antigen 1; moab, monoclonal antibody; TEA, triethanolamine; kD, kilodalton; E/T, effector to target cell.
LFA-1, p150,95, and CR3 (Mo1, Mac-1), respectively (30, 36). The moab CLB-54 (IgG1) reacts with the common β-chain of this antigen family and was kindly provided by Dr. F. Miedema (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands) (30, 39). SPV-7 and CLB-54 were obtained after fusion of SP2/0 myeloma cells with spleen cells of mice, immunized with T lymphocytes. The moabs were selected for their capacity to inhibit CTL activity. The moab Bear-1 and S-HC1 3, respectively. The following moabs were used as controls: SPV-T3A (anti-CD3, IgG2b), CEM (anti-KLH, IgG1), anti-Leu-14 (B cell specific, IgG2b).

Cell lines and T cell clones. Two different human cytotoxic T cell clones were mainly used in this study, a CD4+ CD8- (clone JY) (14, 39, 45) and a CD3+ CD4+ CD8+ (clone JS-93) (12, 43, 47) cell line. The clones were directed against HLA-DR and HLA-A2, respectively (40). The specificities of the CTL clones JS-104, -107, -122, -132, and -141, all directed against JS-93; the J-line cell clones were cultured in RPMI 1640 supplemented with 5% fetal calf serum (FCS).

Immunofluorescence. Cells were incubated (30 min, 0°C) in phosphate-buffered saline (PBS) containing 1% bovine serum albumin (BSA; Sigma Chemical Co., St. Louis, MO) and 0.01% sodium azide with appropriate dilutions of the different moab followed by incubation with fluorescein isothiocyanate (FITC)-labeled goat anti-mouse IgG antibody (Nordic, Tilburg, The Netherlands) for 30 min at 0°C. The relative fluorescence intensity was measured by FACS IV analysis, which was calculated as

\[
\text{Fluorescence intensity of a moab reactive with the cells} = \frac{\text{Fluorescence intensity of a moab nonreactive with the cells}}{\text{Fluorescence intensity of a moab nonreactive with the cells}} \times 100\%.
\]

CR-release assay. Cytotoxicity assays were carried out in microtiter plates with U-shaped wells as described (8). The capacity of moab to block the cytotoxic activity of CTL clones was determined by incubating 1 or 5 × 10^5 effector cells with or without moab for 15 min at room temperature. After this incubation period, 1 × 10^6Cr-labeled JY cells were added. Subsequently, the microtiter plates were centrifuged at 1 × 10^5 × G (Beckman Instruments, Palo Alto, CA) for 15 min at 4°C. Material insoluble in the lysis buffer was removed by centrifugation at 100,000 × G for 30 min in an air-driven centrifuge (Beckman Instruments, Palo Alto, CA). Lysates were precleared further by successive incubations with formalin-fixed Staphylococcus aureus bacteria (strain Cowan I) and mouse IgG coupled to protein A-Sepharose. Precleared lysates were incubated for 3 hr with 4 hr with a nonspecific moab coupled to protein A-Sepharose. The immunoprecipitates were removed from the lysates by centrifugation at 13,000 × G. Precipitates were resuspended in 0.2 ml TEA/NaCl buffer with 0.5% sodium deoxycholate and were centrifuged for 15 sec at 13,000 × G. Subsequently immunoprecipitates were washed extensively in 0.1 M TEA/HCl, pH 7.8, 0.2% Nonidet P-40.

Enzyme treatments. Neuraminidase treatment was carried out on immunoprecipitates for which protein A-Sepharose beads were resuspended in 50 ml of 0.05 M sodium acetate, pH 5.5, 0.9% NaCl, 0.1% Carb, 1 mM phenyl methyl sulfonil fluoride (PMSF) and 0.1 U per neuraminidase sample (Sigma N2967), and incubation took place for 3 hr at 37°C while shaking.

Electrophoresis and autoradiography. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out on vertical slab gels according to the procedure of Laemmli (47). Two-dimensional gel electrophoresis was done according to the method of O’Farrell (48). For isoelectric focusing, amphotoles (LKB, Bromma, Sweden) of pl 3.5 to 10, 4 to 6, and 5 to 8 were used in a ratio of 1:1:4 sample buffer and gel. Samples were dissolved in 8.8 M urea, 0.4% SDS, 5% 2-mercaptoethanol, and 0.2% amphotoles. After 45 min, an equal volume of water was added, followed by 60°C for 1 hr at 800 V. The second dimension consisted of a 7.5% SDS-polyacrylamide gel. Kodak XAR-5 film was used in combination with intensifier screens (Cronex Lightning Plus, Dupont Chemical Co., Newton, CT) for autoradiography of 125I-labeled materials.

RESULTS

Expression of p150,95 by CTL clones. The p150,95 antigen has been shown to be expressed on only a minor population of peripheral blood lymphocytes (36). Furthermore, it has been reported that immunoprecipitates carried out with antibodies to the common β-chain of the LFA-1 family from CTL clones sometimes show the presence of the p150,95 antigen (33, 49). This prompted us to screen our CTL clones for reactivity with the S-HC1 3 moab (anti-p150,95α). The expression varied widely between the 16 CTL clones that were tested and was also variable in time within the individual clones (results not shown). Two clones were selected for detailed analysis, clone JS-93 (CD3+ CD4+ CD8-) and clone JS-102 (CD3+ CD4+ CD8+), which expressed high levels of the p150,95 antigen (relative fluorescence intensity >10) as shown by immunofluorescence (Fig. 1). Furthermore, these clones were found to be reactive with moab directed against LFA-1, but not with moab directed against CR3, the third member of the LFA-1 family (Fig. 1). Similar results were obtained with the other CTL clones (not shown).

Inhibition of the cytotoxic activity of the CTL clones. It is well established that moab directed against the LFA-1 α-and β-chain are able to inhibit the lytic activity of CTL clones. Because the α-chains of LFA-1 and p150,95 are associated with a common β-chain, and are therefore structurally related, we tested whether antibodies directed against p150,95α could block CTL activity. As depicted in Table 1, moab directed against the α- and β-chains of LFA-1 and p150,95 were able to inhibit the cytotoxicity of both clone JS-93 and clone JS-102. In addition, the moab SPV-T3b (anti-CD3) blocked the cytotoxicity of both clones. In contrast, the moab Bear-1, which reacts with CR3, and the moab CEM (anti-KLH) were ineffective. Similarly, other moab directed against CR3 (OKM-1) were unable to inhibit cytotoxicity. At low effector to target cell (E:T) ratios, the inhibition was more
pronounced than at high E:T ratios.

Combinations of the antibodies directed against the pl50,95 and LFA-1 α-chains resulted in additive blocking effects on the cytolytic action of both clones (Table II). Although anti-LFA-1α moab could inhibit CTL activity, a much higher inhibition was observed if a combination of anti-LFA-1α and anti-pl150,95α moab were used, indicating that anti-pl150,95 under these conditions can synergize with anti-LFA-1. Furthermore, we noted that inhibition of CTL activity by anti-β-chain moab was comparable to the inhibition found with mixtures of anti-LFA-1α or anti-pl150,95α moab (Table II). These results indicate that both LFA-1 and pl150,95 are involved in the process of cell-mediated cytolyis.

Inhibition of conjugate formation by moab. Several investigators have shown that moab directed against LFA-1 block cell mediated cytolyis at the level of conjugate formation (13, 25, 26). To determine whether the inhibition of CTL activity by moab directed against pl150,95 was also caused by the inhibition of conjugate formation, single cell assays were carried out. As shown in Table III, both moab directed against pl150,95α and the LFA-1α were able to inhibit conjugate formation of clone JS-93 and clone JS-102 with JY cells. Mixtures of these moab had additive blocking effects and were as effective in inhibiting conjugate formation as moab directed against the common β-chain. In addition, it is shown that SPV-T3b (anti-CD3) did not prevent conjugate formation but was able to inhibit CTL-mediated killing (Table III). Conjugates formed in the presence of moab directed against the LFA-1 family antigens showed the same ratio viable/nonviable target cells as control samples. These results indicate that the LFA-1 family antigens do not play a significant role in the delivery of the lethal hit.

Recently it has been described that CTL clones can form nonspecific conjugates with target cells that do not express the specific antigen to which the CTL is directed (13, 26). Although conjugates are formed, lysis of these target cells does not occur (13). This process was shown to be LFA-1 dependent. To investigate whether pl150,95 is also involved in this “antigen nonspecific” conjugate formation, clone JS-93 and clone JS-102 were conjugated to Daudi cells, which cannot be lysed by these clones, since they do not express class I or proper class II molecules (40). As shown in Table IV, formation of these nonspecific conjugates could be inhibited both by moab directed against pl150,95 and against LFA-1. From these data it is concluded that the inhibition of cell-mediated cytolyis by moab directed against pl150,95 and LFA-1 is primarily caused by inhibition of conjugate formation and not by blocking of the delivery of the lethal hit.

Biochemical analysis of pl150,95 on CTL clones. As mentioned above, clone JS-93 has the CD3+ CD4+ CD8+ phenotype, whereas clone JS-102 has the CD3+ CD4+ CD8+ phenotype. To investigate whether the different CTL clones expressed biochemically identical pl150,95, two-dimensional gel electrophoresis was performed from iodinated material. The pl150,95 α- and β-chains were

<table>
<thead>
<tr>
<th>moab</th>
<th>Antigen</th>
<th>Clone JS-93</th>
<th>Clone JS-102</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>% Inhibition of CTL Activity</td>
<td>E:T ratio</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5:1</td>
</tr>
<tr>
<td>Bear-1</td>
<td>CR3α</td>
<td>2 ± 3</td>
<td>3 ± 3</td>
</tr>
<tr>
<td>SPV-L7</td>
<td>LFA-1α</td>
<td>34 ± 5</td>
<td>55 ± 10</td>
</tr>
<tr>
<td>S-HCl 3</td>
<td>p150,95α</td>
<td>26 ± 6</td>
<td>50 ± 9</td>
</tr>
<tr>
<td>S-HCl 3 + SPV-L7</td>
<td>Common β</td>
<td>38 ± 8</td>
<td>65 ± 4</td>
</tr>
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<td>SPV-T3b</td>
<td>CD3*</td>
<td>40 ± 6</td>
<td>72 ± 8</td>
</tr>
<tr>
<td>CEM</td>
<td>KLH</td>
<td>78 ± 12</td>
<td>98 ± 4</td>
</tr>
</tbody>
</table>

* CTL clones were mixed with 51Cr-labeled JY cells and incubated for 4 hr in the presence of moab (10 μg/ml) as indicated. In the absence of moab the cytotoxicity always exceeded 30%. Mean percentages inhibition ± SD of four experiments are shown. The phenotypes of clones 93 and 102 are CD3+ CD4+ CD8+ and CD3+ CD4+ CD8+, respectively.
found to be slightly heterogeneous in charge, but no significant differences were observed between the clones (Fig. 2A). To reduce the charge heterogeneity, sialic acids were removed by neuraminidase treatment. The PI of the p150,95 a-chain shifted for both clones from 5.7-6.1 to a PI of 6.0-6.2 (Fig. 2B). Interestingly, the $\beta$-chain of p150,95 separated into four distinct spots, probably due to incomplete digestion or other posttranslational modifications. No significant differences were observed between the clones. Taken together, these results indicate that the p150,95 antigen is nonpolyorphic expressed by CTL clones with different phenotypes.

**TABLE II**

<table>
<thead>
<tr>
<th>moab</th>
<th>Antigen</th>
<th>JS-104</th>
<th>JS-107</th>
<th>JS-122</th>
<th>JS-123</th>
<th>JS-141</th>
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<tr>
<td>Bear-1</td>
<td>CR3a</td>
<td>4</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>SPV-L7</td>
<td>LFA-1a</td>
<td>25</td>
<td>25</td>
<td>48</td>
<td>27</td>
<td>30</td>
</tr>
<tr>
<td>S-HCI 3</td>
<td>p150,95a</td>
<td>14</td>
<td>10</td>
<td>3</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>CLB-54</td>
<td>Common $\beta$</td>
<td>52</td>
<td>41</td>
<td>62</td>
<td>54</td>
<td>38</td>
</tr>
<tr>
<td>S-HCI 3 + SPV-L7</td>
<td></td>
<td>48</td>
<td>37</td>
<td>64</td>
<td>44</td>
<td>42</td>
</tr>
<tr>
<td>SPV-T3b</td>
<td>CD3</td>
<td>72</td>
<td>66</td>
<td>83</td>
<td>68</td>
<td>92</td>
</tr>
<tr>
<td>CEM</td>
<td>KLH</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>% p150,95-positive cells</td>
<td></td>
<td>55</td>
<td>78</td>
<td>25</td>
<td>33</td>
<td>18</td>
</tr>
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</table>

* CTL clones were mixed with $^{35}$Cr-labeled JY cells (ratio 2:1) and incubated for 4 hr in the presence of moab (10 $\mu$g/ml), as indicated. In the absence of moab the cytotoxicity always exceeded 25%. The percentage of inhibition of cytotoxicity of representative experiments of five CTL clones are shown. The clones 104, 107, 122, and 141 expressed CD4 but not CD8, whereas clone 132 was shown to be CD4+ CD8+. The percentage of p150,95-positive cells was determined by FACS IV analysis. The relative fluorescence intensity did not exceed 3 (see Materials and Methods).

**TABLE III**

<table>
<thead>
<tr>
<th>moab</th>
<th>Antigen</th>
<th>Clone JS-93 $^a$</th>
<th>Clone JS-102 $^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bear-1</td>
<td>CR3a</td>
<td>0 ± 4</td>
<td>0 ± 3</td>
</tr>
<tr>
<td>SPV-L7</td>
<td>LFA-1a</td>
<td>38 ± 8</td>
<td>42 ± 12</td>
</tr>
<tr>
<td>S-HCI 3</td>
<td>p150,95a</td>
<td>27 ± 3</td>
<td>24 ± 6</td>
</tr>
<tr>
<td>CLB-54</td>
<td>Common $\beta$</td>
<td>65 ± 12</td>
<td>64 ± 5</td>
</tr>
<tr>
<td>S-HCI 3 + SPV-L7</td>
<td></td>
<td>55 ± 10</td>
<td>68 ± 7</td>
</tr>
<tr>
<td>SPV-T3b</td>
<td>CD3</td>
<td>2 ± 4</td>
<td>0 ± 2</td>
</tr>
<tr>
<td>CEM</td>
<td>KLH</td>
<td>2 ± 4</td>
<td>1 ± 3</td>
</tr>
</tbody>
</table>

$^a$ Single cell assays were carried out as described in Materials and Methods. In the absence of moab the percentage of conjugate formation and target cell lysis was 56 ± 10% and 45 ± 15%, respectively, for both clones. Mean percentages of inhibition ± SD of four experiments are shown. The moab were added at a concentration of 10 $\mu$g/ml. The phenotypes of clones 93 and 102 are CD3+ CD4+ CD8- and CD3+ CD4- CD8+, respectively.

$^b$ E:T ratio 1:1.

**TABLE IV**

<table>
<thead>
<tr>
<th>moab</th>
<th>Antigen</th>
<th>Clone JS-93 $^a$</th>
<th>Clone JS-102 $^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bear-1</td>
<td>CR3a</td>
<td>2 ± 4</td>
<td>1 ± 1</td>
</tr>
<tr>
<td>SPV-L7</td>
<td>LFA-1a</td>
<td>65 ± 7</td>
<td>65 ± 4</td>
</tr>
<tr>
<td>S-HCI 3</td>
<td>p150,95a</td>
<td>30 ± 8</td>
<td>35 ± 7</td>
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<tr>
<td>CLB-54</td>
<td>Common $\beta$</td>
<td>85 ± 5</td>
<td>75 ± 11</td>
</tr>
<tr>
<td>S-HCI 3 + SPV-L7</td>
<td></td>
<td>85 ± 9</td>
<td>86 ± 8</td>
</tr>
<tr>
<td>SPV-T3b</td>
<td>CD3</td>
<td>3 ± 2</td>
<td>1 ± 1</td>
</tr>
<tr>
<td>CEM</td>
<td>KLH</td>
<td>0 ± 2</td>
<td>1 ± 2</td>
</tr>
</tbody>
</table>

$^a$ Single cell assays were carried out as described in Materials and Methods. Both clones 93 and 102 were not able to lyse Daudi cells as measured by the chromium-release assay (40). In the absence of moab the percentage of conjugate formation was 65% ±15 for both clones. Mean percentages of conjugate formation and target cell lysis was 50 ± 10% and 48 ± 8%, respectively, for both clones. Mean percentages of inhibition ± SD of four experiments are shown. The moab were added at a concentration of 10 $\mu$g/ml. The phenotypes of clones 93 and 102 are CD3+ CD4+ CD8- and CD3+ CD4+ CD8+, respectively.

$^b$ E:T ratio 1:1.

**DISCUSSION**

Evidence is provided that the p150,95 antigen can be expressed by both class I and class II HLA-specific CTL clones. Monoclonal antibodies directed against p150,95 are demonstrated to inhibit the cytotoxic reaction at the level of conjugate formation. Furthermore, the mode of action of p150,95 is similar to that of LFA-1, and additive inhibitory effects were observed if mixtures of moab directed against both antigens were used. In addition, synergistic effects were observed in those situations where anti-p150,95 moab had no significant blocking effects. Finally, the p150,95 antigen was not found to be heterogeneous, since biochemical analysis of the p150,95 antigen derived from different CTL clones showed identical results.

Sanchez-Madrid et al. (33) described a variable presence of the p150,95 antigen in lysates of CTL clones, since they frequently observed the presence of a 150 kD band in immunoprecipitations from CTL clones with moab directed against the $\beta$-chain of the LFA-1 family. These researchers suggested that the variability of expression might be attributed to variations in the presence of feeder cells. Immunofluorescence experiments with moab directed against p150,95 revealed the expression of this antigen on CTL clones (Fig. 1). During the preparation of this paper, similar findings were reported by Miller et al. (50). The possibility that our results are due to the expression of the p150,95 antigen on monocytes present in the feeder cell suspensions, instead of on CTL clones, could be excluded for several reasons.
First, some CTL clones (clone JS-93 and JS-102) expressed the p150,95 molecule, with a high intensity on 100% of the cells (Fig. 1) in the absence of feeder cells. Second, some CTL clones were extensively washed and subsequently cultured for 3 wk or longer in the presence of IL-2 but without the addition of feeder cells and still expressed the p150,95 antigen (not shown). Third, the CTL clones 93 and 102 are shown to have a functionally active p150,95 antigen.

It is well documented that LFA-1, CR3, and p150,95 share a common β subunit (30, 33-38). Monoclonal antibodies directed against the LFA-1 α- or β-chain have been described to inhibit CTL function (13, 22-25, 35). In addition to the structural resemblance between LFA-1 and p150,95, our present data also show functional similarities, since mab directed against p150,95α were also able to inhibit CTL function (Table I). Monoclonal antibodies directed against CR3 were ineffective due to the absence of this antigen on CTL clones.

Furthermore, we (Table I) and other investigators observed that mab directed against the common β-chain of the human LFA-1 family antigens are generally more potent inhibitors of CTL function than mab to LFA-1 alone (34, 49-52). Because the presence of p150,95 on CTL clones has not been described in this respect, these results may be explained by the assumption that the β-chain plays a major role in CTL-target cell interactions (52). On the basis of our results, we do not reject such a possibility, but advocate that the potent inhibitory effects of anti-common β-chain antibodies can be attributed to the blocking of both p150,95 and LFA-1. This notion is supported by the finding that mixtures of mab directed against the α-chains of LFA-1 and p150,95 are at least as effective in inhibiting CTL function as mab directed against the common β-chain (Table I). In addition, results of preliminary experiments indicate that p150,95-negative CTL clones can be equally well blocked by anti-LFA-1α mab as by anti-β-chain mab.

The cytotoxic activity of CTL clones with a low expression of p150,95 could not be inhibited significantly by mab directed against p150,95 alone. These clones were blocked more effectively by anti-common β-chain antibodies than by mab directed against the α-chain of LFA-1. Interestingly, combinations of mab directed to p150,95 and LFA-1 α-chains are as potent in inhibiting the function of these CTL clones as anti-β-chain mab alone (Table II). The experiments suggest that under certain conditions LFA-1α and p150,95 can act synergistically.

Of interest are the findings that conjugate formation between monocytes and target cells can also be inhibited by mab directed against LFA-1 and p150,95 (53). In this combination, target cell lysis was not observed. These results are comparable to our data obtained with Daudi cells that were conjugated to CTL (Table IV) without being killed due to the absence of the antigens needed for specific recognition. Because alloantigen recognition is probably preceded by nonspecific adhesion of cytotoxic T cells and target cells (13, 26), we concluded that both LFA-1 and p150,95 play an important role in the initial
(specific) conjugation formation of effector cells and target cells.

Until now, the mechanism that regulates the variable expression of p150,95 on T cells is unknown and requires further study. Preliminary experiments demonstrated that IFN-γ, IL-1, and IL-2 cannot modulate the expression of p150,95. The effects of different feeder mixtures are currently under investigation.

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REFERENCES


