Cell Membrane Expression and Functional Role of the p75 Subunit of Interleukin-2 Receptor in Lymphoproliferative Disease of Granular Lymphocytes

By Renato Zambello, Livio Trentin, Giovanni Pizzolo, Pietro Bulian, Maria Masiarrelli, Cristina Feruglio, Carlo Agostini, Roberto Raimondi, Teodoro Chisesi, and Gianpietro Semenzato

The cell membrane expression and functional role of the interleukin-2 receptor (IL-2R) was analyzed in nine patients with lymphoproliferative disease of granular lymphocytes (LDGL) using monoclonal antibodies (MoAbs) specific for the p75 (TU27) and the p55 (anti-Tac) subunits of IL-2R. Four patients were characterized by the proliferation of CD3+CD8+ granular lymphocytes (GL) expressing the α/β T-cell receptor (Tac/α) and one case by the proliferation of CD3+CD4−CD8− GL expressing the γ/δ T-cell receptor (γ6δ); in four additional cases proliferating cells were CD3−GL. Consistent with data observed on normal GL, phenotypic analysis demonstrated that patients' GL lack the expression of the p55 IL-2R, whereas the p75 subunit is constitutionally expressed by expanding GL of both T-cell (either Tac/α and γ6δ) and natural killer (NK) origin in variable proportions (11% to 94% of cells). The analysis of the cytotoxic and proliferative activity demonstrated that the anti-p55 MoAb failed to inhibit IL-2-mediated activation, whereas a marked inhibition of both cytotoxicity and proliferation were obtained using the anti-p75 chain specific MoAb. These data indicate that the p75 chain of IL-2R is responsible for IL-2 signal transduction in both CD3+ and CD3−LDGL patients' GL.

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Table 1. Hematologic and Immunologic Findings in LDGL Studied Patients

<table>
<thead>
<tr>
<th>Patients</th>
<th>Age/Sex</th>
<th>WBC (x 10^3/L)</th>
<th>GL (mm^3)</th>
<th>CD3 (%)</th>
<th>CD4 (%)</th>
<th>CD8 (%)</th>
<th>CD16 (%)</th>
<th>CD25 (%)</th>
<th>TU27 (%)</th>
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<td>35/M</td>
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<td>2,900</td>
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<td>11</td>
<td>53</td>
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<td>23</td>
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<td>38</td>
<td>49*</td>
<td>20</td>
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</table>

Controls (n = 10) 6.5 ± 2 225 ± 85 75 ± 5 72 ± 4 4 ± 3 44 ± 7 28 ± 6 13 ± 5 <2 10 ± 3

TCR: T cell receptor. The WT31 and TCRα1 MoAbs were used to identify lymphocytes bearing the TCR αβ and TCR γδ, respectively.

*The majority of cells (48%) reacted with the ΔTCS1 MoAb, which recognizes a Cy2 associated δ chain determinant, while lacking the expression of the Cy1 associated δ chain determinant recognized by BB3 MoAb.

Results

Phenotypic analysis. Phenotypic evaluation of GL from our patients is summarized in Table 1. Patients no. 1 to 4 showed a proliferation of CD16+ GL in these cases, two-color analysis demonstrated that CD16+ GL expressed the CD3 marker, thus pointing to the NK lineage origin of these cells. In patient no. 2, CD16+ GL expressed the CD8 antigen at low density (CD8 "dim"). In the remaining five cases (no. 5 to 9), proliferating GL expressed the CD3 antigen. In four cases CD3+ GL coexpressed the T-cell receptor α/β (WT31 MoAb) and the CD8 antigen. In case no. 9, nearly 50% of cells were CD3+ CD4– CD8+ – WT31– and expressed the T-cell receptor γ/δ (TCRδ1 MoAb). Interestingly, TCRδ1+ GL expressed the unusual Cy2 gene product, recognized by the ΔTCS1 MoAb, but lacked the more common Cy1 gene product, recognized by the BB3 MoAb.

Expression of p75 IL-2R on GL of LDGL patients. Although in all patients cells lacked the expression of the p55 IL-2R, both CD3+ and CD3– GL expressed the p75 IL-2R (range 11% to 94% of cells). The expression of TU27 MoAb showed different patterns of positivity (Fig 1), possibly related to the different densities of the p75 IL-2R on GL surface. Examination of the contour plots (Fig 2), demonstrated the coexpression on cell surface of the p75 IL-2R and CD16+ in CD3– LDGL patients and of p75 IL-2R and CD8+ on CD3+ Tα/β+ LDGL patients. In both groups, CD4+ cells were TU27 negative. In case no. 9, TCRα1+ CD4– CD8– GL were shown to express the p75 IL-2R, which was not detected on CD4+ and CD8+ cells.

Effect of anti-p75 IL-2R MoAb on activation of patients’ GL. As shown in Fig 3A, CD3+ LDGL patients demon-
strated low baseline cytotoxicity against the K-562 target cells, which could be boosted by rIL-2. While anti-Tac MoAb did not provide any effect on cytotoxic function, the addition of TU27 MoAb dramatically inhibited the IL-2 induced cytotoxicity in these cases. Since GL of CD3–LDGL patients expressed high basal values of cytotoxicity against the K562 target cells (275 LU, 711 LU, 147 LU and 73 LU for patients no. 1, 2, 3, and 4, respectively), the NK-resistant Raji targets were used to test the cytotoxicity induced by IL-2. At baseline conditions, three cases showed low levels of lysis against the Raji targets, whereas in case no. 3, GL were spontaneously cytotoxic for these targets (81 LU). The addition of TU27 MoAb to the culture markedly inhibited the IL-2 induced lytic activity also in these CD3− cases. Neither anti-p75 IL-2R nor anti-p55 IL-2R MoAb affected the cytotoxicity against target cells mediated by

![Fig 1](image1.png)

Fig 1. Expression of p75 IL-2R on GL in three representative LDGL patients. The histograms of TU27 stained cells were superimposed over the histograms of control IgG1 stained cells (histograms nearest the ordinate). Markers were set up to include greater than 95% of the control IgG stained cells.

![Fig 2](image2.png)

Fig 2. Peripheral blood leukocytes were stained with FITC conjugated IgG1 or IgG2a control MoAb and purified control IgG1 MoAb (A, D and G); FITC conjugated Leu-11a (IgG1) MoAb (B); OKT3 MoAb (IgG2a) (C); OKT8 MoAb (IgG2a) (E and I); OKT4 MoAb (IgG2a) (F and L); TCRβ1 (IgG1) MoAb (H); and purified TU27 (IgG1) MoAb (B, C, E, F, H, I and L). Biotinilated goat anti-mouse IgG1 and streptavidin PE were used to detect TU27 MoAb, as reported in detail in the Materials and Methods section. Markers limiting quadrants were set to include greater than 95% of the control IgG stained cells in the lower left quadrant. A, B, and C are referred to a representative CD3− LDGL case (no. 4); D, E, and F to a representative CD3−Tαβ+ LDGL patient (no. 6) and G, H, I, and J to CD3+Tγδ+ LDGL case no. 9.
Fig 3. Effects of anti-IL-2R MoAbs on GL cytotoxicity (A) and proliferation (B) in LDGL patients. Peripheral blood lymphocytes were cultured in medium alone or with IL-2 (100 U/mL) for 72 hr in the presence of isotype matched control Ig, anti-p55 IL-2R MoAb, anti-p75 IL-2R MoAb, or both anti-p55 and anti-p75 IL-2R MoAbs. Cytotoxic activity was tested against the Raji cells in the case of CD3- LDGL (n.: 4) or K562 cells in the case of CD3+ LDGL (Tαβ n.: 4; Tγδ n.: 1) and controls (n.: 4). Results indicate the mean ± SEM.

resting lymphocytes (data not shown). Proliferation of either CD3+ and CD3- GL sustained by IL-2 was slightly inhibited by anti-Tac MoAb alone (Fig 3B) and, to a greater extent, by TU27 MoAb. Although anti-p55 IL-2R MoAb alone did not significantly modify the IL-2 induced proliferative activity in both CD3+ and CD3- LDGL patients, in the majority of cases the combination of anti-p55 and anti-p75 IL-2R MoAbs provided a synergistic effect in inhibiting the [3H]-Thymidine incorporation, the phenomenon being more evident in CD3- cases.

To rule out the possibility that normal residual lymphocytes and not the abnormal GL population may account for the findings associated with IL-2 mediated activation, highly purified GL were studied in three representative patients (two CD3- and one CD3+ LDGL) in the same experimental conditions. As shown in Fig 4, the patterns observed for the cytotoxic and proliferative abilities were consistent with those shown using PBMC, with the exception of patient no. 8, in whom a relevant inhibition of proliferative activity of purified CD3+ GL was demonstrated by anti-p55 IL-2R MoAb.

DISCUSSION

In this study we demonstrated that in patients with LDGL proliferating GL constitutionally express the p75 IL-2R. Both CD3+ (either Tαβ or Tγδ) and CD3- GL are equipped with p75 IL-2R, whereas detectable levels of surface p55 IL-2R were not found on cell surface. Using MoAb to the p75 IL-2R, we provided direct evidence that the initial events leading to the IL-2 activation of GL in LDGL patients are mediated by the p75 chain of IL-2R.

Previous reports on LDGL patients had already suggested that the IL-2 activation was mediated through the p75 IL-2R. These results were based on an indirect demonstration that made use of radiolabeled IL-2. Together with the p75 IL-2R, however, other proteins could cross-link with [125I]-IL-2 on the membrane of cells, such as the not well defined 70 Kd protein or MHC class I antigens. The
Recent studies on the expression of the p75 IL-2R using MoAbs have demonstrated that the majority of normal CB16+ circulating lymphocytes (NK cells) and a variable percentage of CD3+ T lymphocytes (mostly CD8+ or CD4–CD8–γδ+ T cells) constitutionally expressed the p75 IL-2R, although this point is still controversial. Because these cell populations represent discrete subsets with low frequency in peripheral blood, further studies are needed to precisely characterize these cells for the presence and the kinetic of expression of membrane p75 IL-2R either in the blood or in other compartments. To explain the findings that CD8+ cells do not express the p75 IL-2R, an alternative hypothesis supports the fact that residual T cells in this disorder are to some extent abnormal. Taken together, our data indicate that the expression and the functional properties of the p75 IL-2R are maintained in the pathologic counterparts of cells with which we are dealing, pointing to a possible role for the p75 IL-2R on the abnormal proliferation of GL. In this regard, although LDGL encompasses a wide spectrum of clinical, immunologic and biologic conditions, the expression of the p75 IL-2R represents a consistent marker of all different types of proliferating GL in this disorder.

Interestingly enough, although anti-p55 IL-2R MoAb did not significantly inhibit the IL-2-induced cytotoxicity against the targets used and exerted only a minimal effect on the IL-2 mediated proliferative activity, the combination of anti-p75 and anti-p55 IL-2R MoAbs showed slightly higher levels of inhibition than those detected in experiments using anti-p75 IL-2R MoAb alone, particularly in terms of induction of the proliferative activity of CD3− cases (Fig 3). As to cytotoxic function, the pattern is more evident following enrichment of GL (Fig 4B). The differences in blocking experiments using anti-p75 IL-2R MoAb as compared with the combination are not statistically significant, perhaps because of the low number of cases studied. However, it is tempting to suggest that low levels of the p55 IL-2R subunit, possibly not detectable by flow cytometry, are expressed and functional on GL of patients, irrespective of their T-cell or NK-cell origin. According to this interpretation we have recently shown in LDGL patients that IL-2 is able to stimulate the synthesis of mRNA for p55 IL-2R in CD3+ GL even in the absence of a detectable CD25 antigen on cell surface of cells under study (manuscript in preparation).

In conclusion, the results obtained from the present study directly implicate the p75 IL-2R as the structure responsible for the functional properties of the p75 IL-2R observed in LDGL.
for IL-2-mediated activation of GL in patients with LDGL, irrespective of the lineage (T or NK) of proliferating cells.

ACKNOWLEDGMENT

The authors thank the Glaxo Institute for Molecular Biology S.A., Geneva, Switzerland, for providing IL-2; Drs T. Uchiyama, K. Sugamura, S. Taki and L. Moretta for kindly providing anti-Tac, TU27 and BB3 MoAbs; Adele Munari and Dr Mariella Sanzari for their expert technical assistance, and Martin Donach for his help in the preparation of the manuscript.

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