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*J Immunol* 2008; 181:6051-6060; doi: 10.4049/jimmunol.181.9.6051
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IL-24 Induces Apoptosis of Chronic Lymphocytic Leukemia B Cells Engaged into the Cell Cycle through Dephosphorylation of STAT3 and Stabilization of p53 Expression

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Chronic lymphocytic leukemia (CLL) is characterized by the accumulation of long-lived monoclonal B cells mostly arrested at the G0/G1 phase of the cell cycle. CLL cells strongly express intracellular melanoma differentiation-associated gene-7 (MDA7)/IL-24. However, adenovirus-delivered MDA7 was reported to be cytotoxic in several tumor cell lines. We report herein that rIL-24 alone had no effect; however, sequential incubation with rIL-2 and rIL-24 reduced thymidine incorporation by 50% and induced apoptosis of CLL cells in S and G2/M phases of the cell cycle, but not of normal adult blood or tonsil B cells. IL-24 stimulated STAT3 phosphorylation in IL-24R1-transfected cells but not in normal or CLL B cells. In contrast, IL-24 reversed the IL-2-induced phosphorylation of STAT3 in CLL, and this effect was neutralized by anti-IL-24 Ab. Phospho-(P)STAT3 inhibition of p53, and therefore IL-24 induction of p53 secondary to PSTAT3 dephosphorylation may be sensed as a stress signal and promote apoptosis in cycling cells. This model explains why IL-24 can protect some resting/differentiated cells and be deleterious to proliferating cells. The Journal of Immunology, 2008, 181: 6051–6060.

IL-24 belongs to the IL-10 family of cytokines, which also includes IL-19, IL-20, IL-22, and IL-26 (1, 2). The expression of the corresponding gene was first described as being induced by in vitro treatment of melanoma cells with IFN-β plus mezerein (3). This resulted in growth arrest, loss of tumorigenic potential, and terminal differentiation of melanocytes, hence the name melanoma differentiation-associated gene-7 (MDA7) (4). Later it was found that the MDA7 gene encoded a secreted protein, which was named IL-24. Two sets of observations suggested that IL-24 had potential as an anticancer treatment: 1) its expression inversely correlated with progression toward malignancy (4, 5); 2) chronic lymphocytic leukemia (CLL) B cells, reminiscent of their mature differentiated/memory status (14). We have confirmed that MDA7/IL-24 was scarce or absent in normal adult B cells (8). This strong expression in CLL argued against a proapoptotic role for endogenous IL-24 protein. Indeed, IL-24 mRNA silencing promoted CLL cell apoptosis (14), indicating that IL-24 is a survival factor in CLL. Curiously, we failed to detect IL-24 in sera from 28 of 28 CLL patients, whereas concentrations of 100–500 pg/ml are commonly detected in healthy controls. This suggests that CLL cells do not secrete IL-24, or alternatively that they inhibit IL-24-producing cells or secrete a neutralizing anti-IL-24 activity.

Among cells of the immune system, IL-24 is expressed mostly by T cells and monocytes but not by B and NK cells (8). Forced expression of CD5 (9–11) in B cells increases the abundance of IL-10 mRNA and protein (12). DNA chip analysis revealed that other transcripts of the IL-10 family were also induced by CD5 (13). Importantly, IL-24/MDA7 mRNA and protein were both found to be abnormally abundant in 30 of 30 samples from CD5⁺ chronic lymphocytic leukemia (CLL) B cells, reminiscent of their mature differentiated/memory status (14). We have confirmed that MDA7/IL-24 was scarce or absent in normal adult B cells (8). This strong expression in CLL argued against a proapoptotic role for endogenous IL-24 protein. Indeed, IL-24 mRNA silencing promoted CLL cell apoptosis (14), indicating that IL-24 is a survival factor in CLL. Curiously, we failed to detect IL-24 in sera from 28 of 28 CLL patients, whereas concentrations of 100–500 pg/ml are commonly detected in healthy controls. This suggests that CLL cells do not secrete IL-24, or alternatively that they inhibit IL-24-producing cells or secrete a neutralizing anti-IL-24 activity.

The protective effect of endogenous IL-24 on CLL cells seemed paradoxical, given the above-mentioned results from other groups (6, 7). One possible explanation is that IL-24 protects resting/terminally differentiated cells, whereas it is detrimental to proliferating cells. It is worth mentioning here that CLL is characterized by in vivo accumulation of long-lived and slow-dividing monoclonal B cells arrested at the G0/G1 phase with a genetic profile similar to that of memory B cells (15, 16). We therefore studied the effects of rIL-24 on malignant B cells and found that it exerted an inhibitory effect on thymidine incorporation by IL-2-stimulated CLL B cells. We explored its mechanisms of action further and found that, following stimulation with IL-2, rIL-24 induced apoptosis of cells engaged into the cell cycle. The mechanism probably involves dephosphorylation of phospho...
(P)STAT3 and enhancement/stabilization of p53 expression, thereby mediating cell cycle arrest and apoptosis of cells in the G1/M phase of the cell cycle. This mechanism was unexpected as it is opposite to that generally attributed to members of the class II family of cytokines from studies with epithelial cell lines. Additionally, and at variance with the previously reported JAK/STAT-independent action of adenovirus MDA7 (17), we demonstrate a specific induction of apoptosis by rIL-24.

Materials and Methods

Patients

The patients enrolled in the study were diagnosed according to cytologic and immunologic analyses and followed up at the Percy Military Hospital (Clamart, France) and Antoine Béclère Hospital (Clamart, France) between 2004 and 2007. Treatment-free period (if any) was at least 3 mo. Approval for these studies was obtained from the institutional review board of the University Hospital Antoine Béclère (Paris XI). All patients gave informed consent. B lymphocytes from leukemic donors were purified and maintained in culture as previously described (14). For B cell purification, a negative B cell selection was applied using a B cell enrichment RosetteSep kit (StemCell Technologies). The mean percentage of B cells was 92.5% (89.5–98%) in patients.

Reagents

The p53 transactivation activity inhibitor pifithrin-α and the caspase inhibitor zVAD-fmk (Calbiochem) and Troglitazone (Sigma-Aldrich) were used. Na-pervandate was prepared by mixing equal concentrations of Na2VO3 with H2O2 (100 mM) (both from Sigma-Aldrich) for 15 min at room temperature, and serial dilutions were added to cells.

Cell culture, proliferation, cell cycle, and apoptosis

BW5147 cell lines transfected with empty vector or with IL-20Rα plus IL-20Rβ (IL-24R1 heterodimer, given by Dr. J.-C. Renauld) were expanded in IMDM with 10% FCS, stimulated, and processed in the same way as CLL and control B cells. Tonsil and donor blood B cells were expanded in IMDM with 10% FCS, stimulated, and processed in the same way as CLL and control B cells. Tonsil and donor blood B cells were expanded in IMDM with 10% FCS, stimulated, and processed in the same way as CLL and control B cells.

Real-time PCR

Cells (2 × 106/ml) were incubated in complete medium for 24 h under various conditions, and RNA was purified using the RNeasy Mini kit (Qiagen). Reverse transcription was done with random hexamers using SuperScript II reverse transcriptase (Invitrogen) according to the manufacturer's instructions. First-strand cDNA was synthesized by using the supplied random hexamers. Real-time PCR (RT-PCR) was performed with a StepOnePlus System (Applied Biosystems). Reactions were performed in 96-well plates and consisted of 1× QuantiTect SYBR Green Master mix (Qiagen) and 100 nM of each of the primers. The Cq value was calculated and normalized to the house-keeping gene (GAPDH was used). Results were expressed as 2–ΔΔCq.

Immunoprecipitation and Western blot

Immunoprecipitation and Western blot analysis was performed as previously described. Briefly, B cells were harvested in a Wallac Printed Filtermat A with a Harvester 96 (Tomtec) and fixed with 100 mM Na3VO4 with H2O2 (100 mM) (both from Sigma-Aldrich) for 15 min at room temperature. The percentage of dou-bledts was estimated by forward scatter (FSC) linear vs FSC area analysis. Reagents

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Table I. rIL-24 inhibits IL-2-induced thymidine incorporation in CLL B cellsa

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Medium</th>
<th>IL-2</th>
<th>IL-2 Indexb</th>
<th>IL-2 + IL-24c</th>
<th>% Inhibitiond</th>
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<tr>
<td>1</td>
<td>98 ± 12.2</td>
<td>1,403 ± 20</td>
<td>17.3</td>
<td>859 ± 40</td>
<td>38.7</td>
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<td>2</td>
<td>67 ± 14.8</td>
<td>1,288 ± 14.8</td>
<td>19.2</td>
<td>1,088 ± 87</td>
<td>15.5</td>
</tr>
<tr>
<td>3</td>
<td>38 ± 22</td>
<td>591 ± 93.6</td>
<td>15.5</td>
<td>456 ± 196.8</td>
<td>22.8</td>
</tr>
<tr>
<td>4</td>
<td>79 ± 22.6</td>
<td>1,042 ± 55.6</td>
<td>13.1</td>
<td>1,112 ± 28.5</td>
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<tr>
<td>5</td>
<td>91 ± 35</td>
<td>812 ± 12</td>
<td>8.9</td>
<td>553 ± 137</td>
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<td>11</td>
<td>357 ± 58</td>
<td>11,050 ± 207</td>
<td>30.9</td>
<td>9,755 ± 415</td>
<td>11.7</td>
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<tr>
<td>12</td>
<td>98 ± 31</td>
<td>2,917 ± 274</td>
<td>30.3</td>
<td>1,915 ± 232</td>
<td>34.3</td>
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<td>13</td>
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<td>48.8</td>
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<tr>
<td>14</td>
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<td>34.2</td>
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<tr>
<td>16</td>
<td>91 ± 11</td>
<td>17,154 ± 313</td>
<td>151</td>
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<tr>
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<td>2,225 ± 154</td>
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<td>205</td>
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<td>1,639 ± 266</td>
<td>10.7</td>
<td>1,576 ± 111</td>
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<tr>
<td>33b</td>
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<td>32,487 ± 1,297</td>
<td>34.0</td>
<td>13,338 ± 996</td>
<td>59.0</td>
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<td>Tonsil B</td>
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<td>36,221 ± 1,289</td>
<td>42,175 ± 2,373</td>
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<td></td>
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<tr>
<td>Blood B</td>
<td>110 ± 30</td>
<td>294 ± 47</td>
<td></td>
<td>306 ± 194</td>
<td></td>
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</table>

a Thymidine incorporation in cpm/10⁶ cells after 3 days of culture of B cells from CLL patients, blood donors (n = 3) or pediatric tonsils (n = 3).
b Ratio of IL-2/medium counts.
c Cells were cultured overnight with IL-2, then with IL-24 for 3 more days.
d Percentage inhibition was calculated as cpm from IL-2 plus IL-24 cultures divided by cpm for IL-2 cultures.

Results
rIL-24 inhibits thymidine incorporation by activated CLL B lymphocytes
The effect of rIL-24 on CLL cell proliferation was investigated. Peripheral blood samples were collected from 33 patients with CLL with >20,000 lymphocytes/mm³ and B cells were cultured for 3 days in medium or medium plus IL-2, with or without IL-24; then, [³H]thymidine incorporation was tested. Spontaneous apoptosis was negligible in all samples cultured for 3 days (<2%) and therefore did not affect our results. As expected, most cell samples (22/33) proliferated in response to 50 ng/ml of IL-2 alone (18, 19), albeit to various degrees. The range of the IL-2 thymidine incorporation index was from 3 to >200 (Table I shows the results for B cells from 17 patients: proliferation as assessed from thymidine incorporation was 8.9–205 times that of cells cultured in medium alone). In contrast to IL-2, rIL-24 alone had no significant effect on cell proliferation, even when used at a dose of 300 ng/ml (data not shown). However, 20–100 ng/ml of IL-24 partially reversed the IL-2-induced proliferation in 14 of 17 samples: the inhibition was between 11.7% (patient 11) and 77.3% (patient 29) (³H]thymidine incorporation (cpm): IL-2 = 12,770; IL-2 + IL-24 = 8,868; p = 0.0004). Interestingly, cells from patient 33 underwent a Richter transformation, evolving from a CLL (33a) toward a large cell lymphoma (33b). After transformation, the cells retained strong CD5 expression and their protein phosphorylation profile on tyrosine was much more extensive than that in cells from the CLL stage (data not shown). Cells from both CLL and lymphoma stages were thawed and cultured in parallel in the same experiment. Lymphoma cells proliferated much faster than CLL cells both in medium alone and in the presence of IL-2. IL-2 inhibited both the spontaneous (data not shown) and IL-2-induced cell growth of lymphoma cells by 59% but had no effect on CLL cells (Table I). We tested combinations of IL-2 and IL-24 on six samples of B cells from tonsils or normal adult peripheral blood in parallel, and IL-24 did not exert any inhibitory activity on normal B cells.

We also tested the effect of IL-24, added to the cultures at various times before or after addition of IL-2 (Fig. 1a). The inhibitory

FIGURE 1. rIL-24 inhibits thymidine incorporation in activated CLL cells. a, IL-24 was added to cells cultured with IL-2 1 day before (D-1), at the same time (D0), or one day after (D1) IL-2. Counts of cells cultured with IL-2 were compared with those with IL-2 plus IL-24. b, IL-24 was added to CLL cells cultured for 24 h with medium alone, IL-2, IL-2 plus CD40 ligand, or anti-IgM plus CD40 ligand. For each condition, counts of cells cultured with IL-2 were compared with those with IL-2 plus IL-24.
IL-24 suppresses IL-2-induced STAT3 phosphorylation on tyrosine through a phosphatase-dependent mechanism

We studied the effects of IL-24 on IL-2 signaling in CLL cells. The IL-2 signaling pathway starts with its interaction with IL-2R, which is then phosphorylated; this triggers the phosphorylation of JAK1, which associates with the IL-2Rβ chain, and JAK3, which associates with the common γ chain; this results in activation of STAT5 and STAT3 (20–22). Protein extracts from CLL cells incubated with IL-2 were immunoprecipitated with anti-phosphotyrosine 4G10 Ab, and Western blots were performed with mAbs specific for proteins of the JAK/STAT pathway (Fig. 2a).

IL-2 induced a rapid phosphorylation (within 2 min, peaking at 10 min) of tyrosine residues in JAK1, which associates with the IL-2Rβ chain, and STAT5 proteins (Fig. 2a). Thus, IL-2 signaling stimulated the phosphorylation of JAK/STAT proteins in CLL cells in the same way as described in normal lymphocytes and lymphoid cell lines.

We cultured CLL B cells overnight with or without IL-2 and stimulated them or not with IL-24 (sequential incubation with IL-2 and IL-24 inhibits thymidine incorporation, see above) and assessed STAT3 phosphorylation on tyrosine with anti-PY705-STAT3. STAT3 was extensively phosphorylated in cells incubated overnight with IL-2 (evidence of sustained activity) but not in cells stimulated with IL-24 alone (Fig. 2b). In contrast, the presence of IL-24 inhibited IL-2-induced phosphorylation of STAT3. The total amount of STAT3 was similar under all conditions (Fig. 2b). The above results are shown on cells from five different patients. However, IL-24 has been reported to induce the phosphorylation of STAT3 in non-B cells (27), and this is inconsistent with our observations. We therefore investigated its action on the BW5147 thymoma cell line transfected with genes encoding an IL-24R1 heterodimer consisting of both IL-20R1 and IL-20R2 chains. We confirmed IL-24R expression in this cell line by immunofluorescence (data not shown). IL-24R-transfected cells displayed a strong STAT3 phosphorylation in the presence of IL-24 but did not respond to IL-2, whereas the untransfected control cell line did not respond to either IL-2 or IL-24 (Fig. 2c, left). Control experiments with purified tonsil B cells were performed in parallel and failed to show any effect of IL-24 on STAT3 (Fig. 2c, right). These experiments demonstrated the specific effect of IL-24 on CLL cells and validated the activities of recombinant cytokines used.

We studied the kinetics and dose response of STAT3 phosphorylation following incubation with IL-2 and IL-24. STAT3 phosphorylation was inhibited by IL-24 doses as low as 10 ng/ml of IL-24 (Fig. 3b), and inhibition was rapid (2 min) (Fig. 3a). In
IL-24 induces the apoptosis of B cells engaged into the cell cycle

We next determined whether the inhibition of thymidine incorporation into B cells reflected cell cycle block and/or apoptosis. CLL B cells were synchronized with cold thymidine for 24 h and cultured with or without IL-2, then washed and stimulated or not with IL-24 for an additional 24 h; they were then fixed and stained thereafter with PI and subjected to FACS analysis. The proportion of hypodiploid cells as assessed by the analysis of the cell cycle was very similar to that estimated by staining with annexin V (data not shown). Hypodiploid (apoptotic) cells, cells in G2/M phases of the cell cycle, and hyperdiploid cells were counted (M1 to M4 populations, respectively, in Fig. 5, a and b). The number of cycling (S + G2/M + hyperdiploid) cells increased 4-fold under IL-2, whereas IL-24 alone had no significant effect (see the one representative sample in Fig. 5a). In contrast, the number of (S + G2/M + hyperdiploid) cells was reduced by more than half under IL-2 plus IL-24. A parallel increase in hypodiploid cells was observed under this condition (Fig. 5a). In this figure, we show all ungated cells. The percentage of cell doublets was estimated in eight experiments to be 4.4 ± 1.5, and these doublets localized mostly in the hyperdiploid population.

The mean ± SD percentage of cells in each phase of the cell cycle was determined (Fig. 5b). Analysis of 24 samples indicated that the most significant inhibitory effect of IL-24 in IL-2-cultured cells vs IL-2 alone was on the G2/M population (7.34 ± 2.30% vs 4.01 ± 1.4%), whereas its effect was lower in hyperdiploid cells (5.42 ± 2.85% vs 2.71 ± 1.58%). IL-24 significantly increased the proportion of the cell population that was hypodiploid (4.0 ± 1.7% vs 6.79 ± 2.8%) but increased only slightly that in G0/G1 (83.4 ± 5.3 vs 87.2 ± 5.3%) (Fig. 5b).

These results suggested that IL-24 induced apoptosis at the expense of proliferating cells. To verify this, we incubated cells with CFSE, with IL-2 and with or without IL-24; the cells were then stained with Apo2.7 mAb after 2 days. Apoptosis was evidenced in 27.7% of cells that had been stimulated with IL-2 plus IL-24 and only in those that had undergone one division (CFSElow cells) but not in those that did not divide (CFSEhigh cells) (Fig. 5c). In contrast, the proportion of cells cultured with IL-2 alone and that had divided and underwent apoptosis was much lower (10.5%) (Fig. 5c). The percentage of annexin V-positive, PI-negative apoptotic cells was also higher under IL-2 plus IL-24 (20%) than under IL-2 (8%) (see the representative sample in Fig. 5c).

**IL-24 induces apoptosis through stabilization/enhancement of p53 expression**

Oncogenic pathways that signal through STAT3 inhibit p53 expression (24, 25). STAT3 is a latent transcription factor activated by several growth factors and cytokines (26), including cytokines of the γc family, which cause its phosphorylation on tyrosine and translocation into the nucleus. Work with nonlymphoid cells shows that several members of the class II family of cytokines, which includes IL-24 (2, 27, 28), activate STAT3; also, IL-24-mediated apoptosis was reported to be independent of STATs in several nonlymphoid cell lines (17). However, our results demonstrate that IL-24 abolishes IL-2 signaling in CLL.

To investigate the mechanisms of IL-24-mediated apoptosis, we incubated CLL cells with various combinations of IL-2, IL-24, zvad (a caspase inhibitor), and pifithrin (pft)-α, an inhibitor of p53 transactivation (29). Cell cycle analysis was performed using cells stained with PI. The number of cells in the G2/M phase of the cell cycle was, under IL-2 plus IL-24, half that...
FIGURE 5. rIL-24 induces apoptosis and decreases the population of CLL cells engaged into the S/G2/M phase of the cell cycle. a. CLL cells were synchronized with cold thymidine, cultured in medium only or medium supplemented with IL-24, IL-2, or IL-2 then IL-24, and then fixed and stained with PI. M1, hypodiploid cells (dead); M2, cells in G0/G1; M3, cells in S + G2/M; M4, hyperdiploid cells. The increase in the M3 population under IL-2, the decrease of this population, and the increase in the M1 population under IL-2 plus IL-24 are shown. b, Analysis of the cell cycle. Mean ± SD (n = 8) of the M1 to M4 populations calculated as in a from CLL cells cultured as indicated. In each population, IL-2 vs IL-2 plus IL-24 conditions were compared using Wilcoxon’s test. c, Top, Cells were incubated in CFSE, stimulated with IL-2 or IL-2 plus IL-24 and stained with Apo2.7 Ab. Bottom, Cells cultured for 3 days with IL-2 or IL-2 plus IL-24 were stained with annexin V-FITC and PI; the histogram shows the population gated on annexin V plus PI cells.
under IL-2 (3.36 ± 1.3% vs 7.1 ± 1.5%). Pft-α almost totally reversed the loss of cells from the S/G2/M phases induced by IL-24 (6.44 ± 2.2% in the presence of pft-α, zvad, or anti-IL-24 vs 3.36 ± 1.3% in its absence) (Fig. 6, a and b). The effect of IL-24 on the proportion of cells in the S/G2/M phase was also reduced albeit to a lesser extent by zvad (IL-2 + IL-24 + zvad: 4.8 ± 1.77%) (Fig. 6, a and b). These observations suggest that cells driven to proliferation by IL-2 undergo a p53-mediated block of the cell cycle followed by (at least partly) caspase-dependent apoptosis. IL-24 increased p53 expression in IL-2-stimulated CLL cells as assessed by Western blots and quantitative PCR (Fig. 6, c and d), consistent with this model. Note that, unlike p53, there were no detectable effects on Bcl-2 and p38 MAP kinase Abs. d, Relative gene expression in CLL cells cultured for 48 h with medium only and medium containing IL-24, IL-2, and IL-2 plus IL-24 (histograms in that order). RNA was extracted on day 3, reverse-transcribed, amplified, and analyzed by real-time PCR. Means ± SD samples for samples from four to six different patients are shown. Values for medium- vs IL-2 plus IL-24-treated samples were compared using Wilcoxon’s test.

FIGURE 6. The decrease in the S/G2/M population in IL-2 plus IL-24-cultured cells is dependent on IL-24-mediated induction of p53 expression. a and b, The pharmacological inhibitor of p53, pft-α, reverses the IL-24-mediated decrease of cells engaged into cell cycle. Cells were cultured overnight with medium or IL-2 and then supplemented with pft-α (40 μM), zvad-fmk (zvad) (10 μg/ml), IL-24, IL-24 plus pft-α, IL-24 plus zvad, or IL-24 plus anti-IL-24 for 24 h and stained with PI for cell cycle analysis. a, A representative experiment from one patient with CLL is shown. b, The percentage of CLL cells in the S/G2/M phases of the cell cycle (open histograms) or hypodiploid cells (filled histograms). Means ± SD for cells from eight different patients. For black-and-white histograms, comparisons were made between IL-2 plus IL-24 vs IL-2 and between IL-2 plus IL-24 and IL-2 plus IL-24 plus either pft-α, zvad, or anti-IL-24. Only significant results (p < 0.03) using Wilcoxon’s test are indicated (*). c, IL-24 augments p53 expression and phosphorylation in CLL. Cells were lysed after 3 days of culture as indicated and analyzed by Western blotting with anti-p53, anti-Pser15-p53, anti-Bcl-2, and anti-p38 MAP kinase Abs. d, Relative gene expression in CLL cells cultured for 48 h with medium only and medium containing IL-24, IL-2, and IL-2 plus IL-24 (histograms in that order). RNA was extracted on day 3, reverse-transcribed, amplified, and analyzed by real-time PCR. Means ± SD samples for samples from four to six different patients are shown. Values for medium- vs IL-2 plus IL-24-treated samples were compared using Wilcoxon’s test.

The phosphatase activator troglitazone induces p53 expression, STAT3 dephosphorylation, and apoptosis of CLL cells

We showed that IL-24-induced apoptosis in CLL is dependent on p53 expression (see above). As PSTAT3 is a repressor of p53 transcription, IL-24-induced apoptosis in CLL is presumably also dependent on PSTAT3 dephosphorylation. Therefore, a phosphatase activator may reproduce some of the effects of IL-24.

Troglitazone (TG) is an activator of protein tyrosine phosphatase 1B (PTP1B); it reduces PY705-STAT3 and promotes apoptosis in human primary gliomas (34). We tested whether TG stimulated p53 expression and apoptosis in CLL. TG induced dose-dependent dephosphorylation of PSTAT3 in CLL cells cultured in the presence of IL-2. It also induced p53 production, but had no effect on total STAT3 abundance (Fig. 7a). Cell cycle...
Discussion

We report the first description of the conditions under which IL-24 exerts a proapoptotic effect on CLL as well as the associated molecular events. The effect is dose dependent and reversed at both cellular and molecular levels by anti-IL-24 Abs; it is therefore specific. CLL cells contain abundant IL-24 mRNA and protein (14), and this led us to study the effect of exogenous IL-24 on these cells. Our findings appeared not to be entirely coherent with the published (5–7) apoptotic effect of adenovirus-encoded IL-24. This effect was described as being independent of both IL-24R and the JAK/STAT pathway (17). However, we found that rIL-24 is proapoptotic, provided that CLL cells are stimulated to enter the cell cycle, and this reconciles the various diverse observations. IL-24 expression inversely correlates with the proliferation of malignant melanocytes (4, 5), which is consistent with IL-24 having protective and growth inhibition activities in terminally differentiated cells, which is the case in CLL cells (13–16). However, intracellular IL-24 did not apparently counteract the apoptotic effect of rIL-24 in IL-2-stimulated CLL cells. To explain this, we observed that IL-24 transcripts are rapidly down-regulated in activated B cells (unpublished data). We think therefore that once cells have received a proliferation signal (here by IL-2), intracellular IL-24 is down-regulated; this allows cells to enter the cell cycle. If cells are exposed shortly after a “stop signal” (here by rIL-24), they undergo apoptosis (this is also consistent with the role of p53, see below).

Note that the effects of IL-24 on B cells are similar to those of IL-4. IL-4 exerts an inhibitory effect after B cells receive an activation signal, from, for example, IL-2 (35, 36). However, IL-4 and IL-24 may inhibit B cell proliferation by different molecular mechanisms, as their respective expressions are inversely correlated. We indeed observed that IL-4 is a potent inhibitor of both CD5 and IL-24 expression in B cells (our unpublished results), which is also consistent with CD5 being an IL-24 inducer (14). CLL cell death was diminished by zvad-fmk, and thus caspases are involved to some extent and need to be studied more in detail. In contrast, cell death was abolished by a pharmacological inhibitor of p53, and therefore the mechanism involves p53 induction.

p53 is a major transcription factor that potently inhibits cell cycle progression at G1/S and G2/M checkpoints (37, 38). Its expression and function are impaired in many tumors (39, 40) due to down-regulation or inactivation by mutations. Restoring endogenous p53 induced regression of murine liver carcinomas in vivo. The mechanism did not involve apoptosis but instead involved induction of a senescence program associated with the production of M-CSF, CCL2, CXCL1, and IL-15 (41). Note that IL-24 induces the production by human PBMC of inflammatory cytokines, including TNF-α, GM-CSF, IL-6, IL-12, and IFN-γ (42). Restoration of p53 function in vivo can lead to tumor regression through induction of cellular senescence in sarcomas and apoptosis in lymphomas (43). These studies pave the way for the use of p53 activators to treat human cancers. Here, however, we could not reverse death completely with zvad-fmk, so both senescence and apoptosis may be at work in CLL. The patterns of regulation of downstream genes indicate that p53 accumulates in cells following stress signals that stop its degradation and/or enhance its transcription and its activity as a transcription factor (Fig. 6). IL-24 may act as a stress signal that turns off the IL-2-signaling cascade and induces p53 expression, probably by inactivating the p53 transcriptional repressor PSTAT3. Other mechanisms may also be operating through the stabilization of p53 and/or inhibition of its degradation through the phosphorylation of Ser15 in p53 by at least eight

![FIGURE 7. Effect of TG on dephosphorylation of PSTAT3, p53 induction, and cell death in CLL.](image-url)
different Ser/Thr kinases (30), including p38 MAPK, which is activated by IL-24 (14). However, the complexity of p53 regulation by posttranslational mechanisms is such that dedicated analysis is required to understand the effects of IL-24 more fully. Our experiments support the role of PSTAT3 dephosphorylation on p53 de-repression and downstream induction of cell death. TG, an antidiabetic drug and a PPAR-α agonist (34) that activates the tyrosine kinase 78: 745–752.


