RAPID COMMUNICATION

Pleiotropic Effects of the CD30 Ligand on CD30-Expressing Cells and Lymphoma Cell Lines

By Hans-Jürgen Gruss, Norman Boiani, Douglas E. Williams, Richard J. Armitage, Craig A. Smith, and Raymond G. Goodwin

CD30 is a member of the tumor necrosis factor receptor superfamily. CD30 was originally described as a cell surface antigen on primary and cultured Hodgkin’s and Reed-Sternberg cells. In this study, recombinant human CD30 ligand was expressed on the surface of CV-1/EBNA cells and tested for biologic activities on a variety of different CD30+ human lymphoma cell lines. CD30 ligand enhanced Ig secretion of Epstein-Barr virus (EBV)-immortalized, CD30+ lymphoblastoid B-cell lines, but not Burkitt lymphoma lines. Recombinant CD30 ligand enhanced proliferation of “T-cell-like” Hodgkin’s disease-derived cell lines and an adult T-cell leukemia cell line, but not “B-cell-like” Hodgkin’s disease-derived cell lines. CD30+, EBV-immortalized lymphoblastoid B-cell lines, or CD30+ and EBV+ tumor B-cell non-Hodgkin’s lymphoma cell lines. In addition, CD30 ligand mediated reduction of proliferation and viability, by induction of cytolysis cell death, of CD30+, large-cell anaplastic lymphoma cell lines. Two new antibodies, M44 and M67, against the CD30 antigen demonstrated similar biologic activities to the CD30 ligand.

In this report, we demonstrate that the recombinant CD30L and two new agonistic anti-CD30 MoAbs induce pleiotropic biologic effects on a variety of different CD30+ lymphoma cell lines representing models for various lymphoid malignancies. Signaling through the CD30 cell surface receptor enhances Ig secretion of EBV-immortalized lymphoblastoid B-cell lines, but not Burkitt lymphoma (BL) lines and proliferation of the “T-cell-like” HD-derived cell lines (HDLM-2 and L-540) and adult T-ALL cell line (KE-37), but not the “B-cell-like” HD-derived cell lines (KM-H2 and L-428). In addition, CD30 mediates reduction of proliferation and viability, by induction of cytolysis cell death, of CD30+ LCAL cell lines (Karpas 299, TS, DHL-1, HSC-M1, FL-LCAL, Michel, and HAKI). Thus, CD30L has pleiotropic biologic activities on several CD30+ lymphoma types.

MATERIALS AND METHODS

Cells and culture conditions. PBT cells were isolated from normal healthy donors by centrifugation over Histopaque (Sigma Chemical Co, St Louis, MO) and rosetting with 2-aminoethylisothiouronium bromide (AET)-treated sheep erythrocytes as described.

The recombinant human CD30 ligand (CD30L) enhanced the proliferation of an HD-derived lymphoma cell line and anti-CD3 activated PBT cells, but induced cell death of a CD30+ LCAL cell line.

In this report, we demonstrate that the recombinant CD30L and two new agonistic anti-CD30 MoAbs induce pleiotropic biologic effects on a variety of different CD30+ lymphoma cell lines representing models for various lymphoid malignancies. Signaling through the CD30 cell surface receptor enhances Ig secretion of EBV-immortalized lymphoblastoid B-cell lines, but not Burkitt lymphoma (BL) lines and proliferation of the “T-cell-like” HD-derived cell lines (HDLM-2 and L-540) and adult T-ALL cell line (KE-37), but not the “B-cell-like” HD-derived cell lines (KM-H2 and L-428). In addition, CD30 mediates reduction of proliferation and viability, by induction of cytolysis cell death, of CD30+ LCAL cell lines (Karpas 299, TS, DHL-1, HSC-M1, FL-LCAL, Michel, and HAKI). Thus, CD30L has pleiotropic biologic activities on several CD30+ lymphoma types.

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Contaminating monocytes were removed by plastic adherence for 1 hour at 37°C. The resulting T-cell preparations were always greater than 98% CD3, as determined by flow cytometric analysis. T cells were cultured in 1640 RPMI (GIBCO, Gaithersburg, MD) supplemented with 10% fetal calf serum (PCS; Interge Corp., Purchase, NY; lot no. J93410) and 100 μg/mL penicillin, 100 μg/mL streptomycin, and 2 mM L-glutamine (Sigma) (standard culture medium [SCM]) at 37°C in a humidified, 7% CO2 atmosphere.

This study included tumor cell lines derived from patients with NHL of Burkitt’s and non-Burkitt’s type (n = 11). HD-derived cell lines (n = 6), LCAL (n = 8), and normal EBV-immortalized lymphoblastoid B-cell lines (n = 6). The African and American BL cell lines BL-APB, Raji, Daudi, and Namalwa (kindly provided by Dr D. Benjamin, Ohio State University, Columbus, OH) and Jijoye (purchased from American Type Culture Collection [ATCC], Rockville, MD; ATCC-CCL87) are EBV+ permanent BL cell lines and all cell lines have been established from a malignant pleural effusion (n = 6). The HD-derived permanent cell lines HDLM-2 and L-540 (kindly provided by Dr V. Diehl, University of Cologne, Germany) were established from a malignant pleural effusion in 1978, and the KM-H2 cell line from a malignant pleural effusion in 1982 under different primary culture stimulations, the L-428 cell line (DSM-ACC32; kindly provided by Dr H.G. Drexler, German Cancer Research Center, Heidelberg) and Ramos (ATCC-CRL1596), DOHH-2 (DSM-ACC47), and Karpas 422 (DSM-ACC32; kindly provided by Dr H.G. Drexler, German Collection of Microorganisms and Cell Cultures/DSM, Braunschweig, Germany) are EBV+ tumor B-cell lines with expression of B-cell surface antigens only. The EBV+ tumor B-cell line MC116 (ATCC-CRL 1649) has been derived from a patient with an undifferentiated lymphoma. The HD-derived permanent cell lines HDLM-1, -2, and -3; L-428; KM-H2 (kindly provided by Dr H.-G. Drexler); and L-540 (kindly provided by Dr V. Diehl, University of Cologne, Cologne, Germany) are EBV+ tumor cell lines from HD patients and have been described in detail previously.26-29 Briefly, the HDML cell lines have been established from a malignant pleural effusion of a 74-year-old man with endstage IVB NHL nodular sclerosis (NS) HD in 1982 under different primary culture stimulations, the L-428 cell line from a malignant pleural effusion of a 37-year-old woman with endstage NS HD in 1978, the KM-H2 cell line from a malignant pleural effusion of a 37-year-old man with stage IVB NS HD in 1974, and the L-540 cell line from the bone marrow of a 20-year-old woman with stage IVB NS HD.28-29 We screened the T-immunoblastic lymphoma line Sup-T1 (DSM-ACC140) and the adult T-ALL cell line KE-37 (DSM-ACC46) for CD30 expression (kindly provided by Dr H.-G. Drexler). The LCAL cell line Karpas 299 (DSM-ACC31) was established from peripheral blood blast cells of a 25-year-old white man with the diagnosis of CD30+ high-grade LCAL.28 The peripheral blast cells with pleiomorphic nuclei resembled primitive histiocytes, which bear the surface marker CD4, CD5, epithelial membrane antigen (EMA), HLA-DR, and CD30. The Karpas 299 cell line possesses the same cytochemical, immunologic, morphologic, and chromosomal profile with a 2;5 translocation, T-cell receptor β-chain gene rearrangement, typical anaplastic morphology, and CD4+, CD5+, EMA+, HLA-DR+, and CD30+ immunophenotype, as the original peripheral blood blast cells of the patient (kindly provided by Dr H.G. Drexler).29 Similarly, the permanent LCAL cell lines TS, DHL-1, HSC-M1 (kindly provided by Dr A. Lorenzana, Hospital for Sick Children, Toronto, Canada), JB-6 (kindly provided by Dr M.E. Kadin, Beth Israel Hospital, Boston, MA), FL-LCAL, Michel, and HAKI (kindly provided by Dr A.C. Feller, University of Lubeck, Lubeck, Germany) were established from primary CD30+ LCAL tumors. All six normal lymphoblastoid (nontumor origin) B-cell lines are EBV+ and were established from peripheral lymphocytes of patients with high-grade mononuclear cells (B-MNE, B-MNB, and B-MNK cell lines) or EBV-transformed cord blood lymphocytes (CB23 and CB33 cell lines). CB23, CB33, B-MNE, B-MNB, and B-MNK were kindly provided by Dr D. Benjamin. The MP-1 cell line is a spontaneously derived, EBV-transformed, B-lymphoblastoid cell line grown from peripheral mononuclear cells from a normal donor and has been described in detail previously.21 The cell lines were maintained in suspension culture in SCM at 37°C, in a humidified, 7% CO2 atmosphere, and were subcultured every 3 to 4 days. Only cultures in the log phase of growth were investigated. The cell lines have been previously studied for rearrangements, chromosomal alterations, cytochemical staining, cytokine secretion, and receptor expression.

Reagents and antibodies. In some experiments, cells were treated for the culture time, as indicated, with 12-0-tetradecanoylphorbol-13-acetate (TPA; Sigma) at a concentration of 24 nmol/L. CV-1/EBNA cells29 were transfected using the diethyl aminoethyl (DDEAE)/Dextran method with either vector alone (CV-1/HAV) or the huCD30L cDNA containing expression vector (CV-1/CD30L), and then fixed at 2 days posttransfection with 1% paraformaldehyde for 5 minutes at 25°C.22 Expression levels were measured by flow cytometry. The construction of the soluble CD30 fused to the Fc region of human IgG1 and purification of the CD30Fc fusion protein has been described previously.23 IL-2, IL-4, and TNF-α expressed in yeast were purified as described previously.23 IL-10 was purchased from Pepro Tech Inc (Rocky Hill, NJ).

To generate antibodies against the human CD30 antigen, CB6FI mice (provided by Jackson Laboratories, Bar Harbor, ME) were boosted twice intradermally with 10 μg CD30Fc in Ribi adjuvant (Ribi Immunocore Research, Hamilton, MT). One week after the second boost, peroxidase dot blot assays using CD30Fc showed a significant (>1/100) titer of anti-CD30 antibody in the serum. One week later, animals were boosted intravenously (IV) with 3 μg CD30Fc into the tail vein. Three days later, spleen was removed and spleen cells were fused to the X63-Ag8.653 mouse myeloma cell line by standard methods using a 50% polyethylene glycol/dimethyl sulfoxide (PEG/DMSO) solution (Sigma). Hybridoma cultures were established in 96-well plates (Costar, Cambridge, MA). Ten days later, culture supernatants were screened by an antigen capture assay using 121I-CD30Fc. Ninety-six-well plates were coated overnight with goat-antimouse serum (Zymed, San Francisco, CA) and blocked with 3% bovine serum albumin (BSA; Sigma); 50 μL of culture supernatant was incubated for 1 hour at room temperature. After three washes with phosphate-buffered saline (PBS), plates were incubated with 121I-CD30Fc for 1 hour and then washed with PBS again before being placed on film for overnight exposure. Positive wells were checked for reactivity with huLG by performing an ABC assay. Hybridoma cell lines reactive with huLG/HRP-CD30 were cloned. Positive supernatants were also tested by flow cytometry using CD30-expressing cells or CD30-transfected CV-1/EBNA cells. Large quantities of the human anti-CD30 M44 and M67 (mouse IgG1 isotype) MoAbs were purified from spent bulk culture supernatants from hybridoma cells grown in roller bottles. Antibodies were purified on a protein A affinity matrix using an automated purification system (BioRad MAPS system, Hercules, CA). Antibody concentration was determined by absorbance at 280 nm and purity assessed by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis and silver staining. Antibody concentrations were adjusted to 1 mg/mL and aliquots of purified antibody were stored frozen at −20°C in 0.05 μmol/L citrate buffer (pH 7.0). The monoclonal anti-CD30 antibodies Ki-1 and Ber-H2 have been described elsewhere and were purchased commercially (Dako Corp, Santa Barbara, CA).14

Flow cytometric analysis. Cells (1 × 107 cells/mL) of all cell lines were harvested and preincubated with 100 μg/mL human IgG (Sigma) in PBS plus 0.02% NaN3 and 10% goat serum (GIBCO) for 30 minutes at 4°C to prevent nonspecific binding of staining reagents. Surface expression of CD30 was assessed by staining with 1 μg/mL M44, M67, and Ber-H2 anti-CD30 MoAbs or IgG, isotype-matched controls for 30 minutes at 4°C. Cells were washed twice with PBS plus 0.02% NaN3 and incubated with goat-antimouse IgG-FITC or IgG, isotype-matched controls for 30 minutes at 4°C. Cells were analyzed on a FACScan (Beckton Dickinson, Mountain View, CA) and the data were acquired with Consort software (Tracor Northern, Enfield, CT).
NaNO₃ for 30 minutes at 4°C. CD30Fc, human IgG₁, and human IL-4RFc were biotinylated as described previously. For detection of CD30L, surface expression, cells were incubated with 5 μg/mL biotinylated CD30Fc, IgG₁, or IL-4RFc for 30 minutes at 4°C. After washing twice in PBS plus 0.02% NaNO₃, streptavidin-phycocerythrin (Becton Dickinson, Mountain View, CA; diluted 1:5 in PBS plus NaNO₃) was added to the cells for 30 minutes at 4°C. Cells were washed twice in PBS plus NaNO₃. Staining intensity was then analyzed using a FACSort (Becton Dickinson Corp, Sunnyvale, CA). A minimum of 5,000 cells were analyzed for each sample. Cells stained with isotype-matched control MoAb and biotinylated human IgG₁ or IL-4RFc were included to determine background fluorescence.

**Proliferation assay.** For activation of T cells, 96-well plates were coated with 10 μg/mL OKT3 (anti-CD3; ATCC-CRL8001) in 50 mmol/L Tris buffer (pH 8.5) and washed twice with PBS. Purified T cells (1 × 10⁶ cells/well) were cultured in the presence of immobilized OKT3 MoAb and a titration of fixed CV-1/EBNA cells transfected with huCD30L or vector alone. T cells were also cultured in costimulation with immobilized OKT3 MoAb plus a titration of immobilized M44 or M67 anti-CD30 MoAbs for 72 hours. The cultures with Hodgkin's, non-Hodgkin's, and B-cell lines were conducted in 96-well flat-bottom microtiter plates (Costar) in the presence of a twofold or 10-fold titration of immobilized M44, M67, Ber-H₂, or Ki-1 MoAbs, as indicated, or isotype-matched control MoAbs, for the culture times indicated. Cell lines were also cultured with either a titration of fixed CV-1/EBNA cells transfected with vector alone (CV-1/HAV) or the vector expressing human CD30L (CV-1/CD30L) for different culture times, as indicated. Cultures were pulsed with 1 μCi/well ³H-thymidine (³H.Tdr; 25 Ci/mmol: Amersham, Arlington Heights, IL) for the final 12 hours of culture. Cells were harvested and incorporated cpm determined by tritium-sensitive avalanche gas ionization detection on a Matrix 96 Beta Counter (Packard, Meriden, CT). Results are expressed as the mean cpm ± SD of ³H.Tdr incorporation of triplicate cultures.

**Determination of secreted Ig.** Levels of secreted IgM, IgG₁, IgA, and IgE were measured by enzyme-linked immunosorbent assay (ELISA) as described previously. Briefly, round-bottomed 96-well microtiter plates were coated with a 1/5,000 dilution of mouse ascites (Zymed, San Francisco, CA) containing MoAb specific for human IgE, IgA, and IgM or with 5 μg/mL purified MoAb for IgG₁ (Cappel, Malvern, PA). The plates were incubated with 20% goat serum for 30 minutes at 4°C to block nonspecific binding. After incubation of sample supernatants or Ig standards, the relevant conjugated, second-step antibody was added. All ELISA assays were developed by the addition of TMB substrate (Kirkegaard and Perry, Gaithersburg, MD) and the OD was measured at 520 nm. The level of sensitivity of ELISA assays was 300 pg/mL for IgG₁, IgM, and IgA and 900 pg/mL for IgE.

**RNA extraction and Northern blot analysis.** Medium- or TPA-treated HDLM-1, -2, or -3; L-428; KARPAS-299; KE-37; DOHH-2, or KARPAS-422 were CD30-. Three of five Burkitt NHL cell lines expressed CD30. BL-APB, Raji, and Jijoye were 40% to 80% positive, with an sMFI between 7 and 24 channels. The remaining two BL lines, Daudi and Namalwa, were CD30-. The cell line MC116, derived from an untransformed lymphoma, was also CD30-. The EBV- LCL cell lines were 100% positive for CD30 and had an sMFI of 133 ± SD of the six replicate cultures.

**RESULTS**

**CD30 expression of B-cell lines and lymphoma cell lines.** EBV-transformed normal B cells, lymphoma cells of the Burkitt type III NHL, LCL, and H-RS cells of HD have been shown to express CD30. For identification of biologic targets for the CD30L, we studied the constitutive expression of the CD30 surface antigen on a panel of EBV-immortalized B-cell lines, EBV⁺ or EBV⁻ NHL cell lines, and HD-derived cell lines. As shown in Table 1, all six EBV-immortalized normal lymphoblastoid B-cell lines studied expressed CD30 with 40% to 80% positive cells and a specific mean fluorescence intensity (sMFI) between 12 and 41 channels. The EBV⁻ NHL B-cell tumor lines WLS-L, Ramos, DOHH-2, or Karps 422 were CD30-. Three of five Burkitt NHL cell lines expressed CD30. BL-APB, Raji, and Jijoye were 40% to 80% positive, with an sMFI between 7 and 24 channels. The remaining two BL lines, Daudi and Namalwa, were CD30-. The cell line MC116, derived from an untransformed lymphoma, was also CD30-. The EBV⁺ LCL cell line Karpas 299 expressed high levels of the CD30 antigen on all cells with an sMFI of 255 channels (Table 1). The LCL cell lines TS, DHL-1, HSC-M1, FL-LCL, Michel, and HAKI were also strongly CD30⁺, with 80% to 100% positive cells (data not shown). All six EBV⁺ HD-derived cell lines expressed CD30 (Table 1). HDLM-2 and L-540 cell lines showed the highest expression levels. The populations were 100% positive for CD30, with an sMFI of 147 and 334, respectively. The EBV⁻ adult T-ALL cell line KE-37 was 92% positive for CD30 and had an sMFI of 133 channels. The four EBV⁻ cell lines Karps 299, KE-37, L-540, and HDLM-2 expressed the highest surface levels of the CD30 antigen.

**The CD30L or anti-CD30 MoAbs induced biologic activities on CD30⁺ lymphoblastoid B-cell lines and NHL cell lines.** Recombinant human CD30L or anti-CD30 MoAbs were added to cultured CD30⁺ B cells and NHL cell lines, and the effect on proliferation was examined. The CD30⁺
### Table 1. Summary of CD30 Surface Expression

<table>
<thead>
<tr>
<th>Sample</th>
<th>Cell Line</th>
<th>Lineage*</th>
<th>EBV†</th>
<th>%§</th>
<th>sMFI‖</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Lymphoblastoid normal B-cell lines (a) Cord blood</td>
<td>CB23</td>
<td>B</td>
<td>+</td>
<td>++</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>CB33</td>
<td>B</td>
<td>+</td>
<td>+++</td>
<td>39</td>
</tr>
<tr>
<td>(b) Mononucleosis</td>
<td>B-MNE</td>
<td>B</td>
<td>+</td>
<td>+</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>B-MNB</td>
<td>B</td>
<td>+</td>
<td>+++</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>B-MNK</td>
<td>B</td>
<td>+</td>
<td>+++</td>
<td>32</td>
</tr>
<tr>
<td>(c) Peripheral blood</td>
<td>MP-1</td>
<td>B</td>
<td>+</td>
<td>+</td>
<td>12</td>
</tr>
<tr>
<td>2. HD-derived cell lines (a) Pleural effusion of NS HD</td>
<td>HDLM-1</td>
<td>&quot;T-cell-like&quot;</td>
<td>-</td>
<td>+++</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>HDLM-2</td>
<td>&quot;T-cell-like&quot;</td>
<td>-</td>
<td>+++</td>
<td>147</td>
</tr>
<tr>
<td></td>
<td>HDLM-3</td>
<td>&quot;T-cell-like&quot;</td>
<td>-</td>
<td>+++</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>L-428</td>
<td>&quot;B-cell-like&quot;</td>
<td>-</td>
<td>+++</td>
<td>35</td>
</tr>
<tr>
<td>(b) Bone marrow of NS HD</td>
<td>L-540</td>
<td>&quot;T-cell-like&quot;</td>
<td>-</td>
<td>+++</td>
<td>334</td>
</tr>
<tr>
<td>(c) Pleural effusion of MC HD</td>
<td>KM-H2</td>
<td>&quot;B-cell-like&quot;</td>
<td>-</td>
<td>+++</td>
<td>37</td>
</tr>
<tr>
<td>3. NHL cell line (a) Burkitt</td>
<td>BL-APB</td>
<td>B</td>
<td>+</td>
<td>++</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Raji</td>
<td>B</td>
<td>+</td>
<td>+++</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>Jijoye</td>
<td>B</td>
<td>+</td>
<td>+</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>Daudi</td>
<td>B</td>
<td>+</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Namalwa</td>
<td>B</td>
<td>+</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Ramos</td>
<td>B</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>(b) LCAL</td>
<td>Karpas 299</td>
<td>T</td>
<td>-</td>
<td>+++</td>
<td>255</td>
</tr>
<tr>
<td>(c) Immunoblastic</td>
<td>Sup-T1</td>
<td>T</td>
<td>-</td>
<td>-</td>
<td>2</td>
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<tr>
<td>(d) Undifferentiated</td>
<td>MC116</td>
<td>B</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>WILS-B</td>
<td>B</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>(e) Follicular cb/cc</td>
<td>DOHH-2</td>
<td>B</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>(f) Diffuse-large cell</td>
<td>Karpas 422</td>
<td>B</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>4. T-ALL</td>
<td>KE-37</td>
<td>T</td>
<td>-</td>
<td>+++</td>
<td>133</td>
</tr>
<tr>
<td>5. Myeloid cell lines (a) Promyelocytic</td>
<td>HL-60</td>
<td>M</td>
<td>-</td>
<td>+</td>
<td>4</td>
</tr>
<tr>
<td>(b) Histiocytic</td>
<td>U-937</td>
<td>M</td>
<td>-</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>(c) CML blast phase</td>
<td>K-562</td>
<td>M</td>
<td>-</td>
<td>+</td>
<td>7</td>
</tr>
</tbody>
</table>

* Phenotype and genotype association with T-cell (T), B-cell (B), or myeloid-monocytic (M) lineage. HD-derived cell lines present with some non-functional T cell ("T-cell-like") or B cell ("B-cell-like") lineage markers.
† Detection of EBV DNA and/or proteins.
§ Percentage of CD30 surface expression with -, negative; +, 20% to 40%; ++, 40% to 60%; ++++, 60% to 80%; and +++++, 80% to 100% positive cells.
‖ sMFI was calculated as mean channel number by subtracting control fluorescence values.

Normal lymphoblastoid B-cell lines and CD30⁺ Burkitt B-cell tumor cell lines did not respond with an increased ³H-thymidine uptake or cell number regardless of the culture conditions up to 96-hour culture period (data not shown). The addition of CD30L expressing CV-1/EBNA cells or the anti-CD30 MoAbs M44, M67, or Ki-1 did not alter the proliferation of the eight CD30⁺ nontumor or tumor B-cell lines investigated. Different culture times, conditions, or ligand and antibody concentration also had no effect on proliferation of the CD30⁺ nontumor and tumor B-cell lines (data not shown). Furthermore, cytokine secretion (eg, IL-1, IL-6, and TNF) from these cell lines was not affected by the addition of CD30L or anti-CD30 MoAbs (data not shown).

The effects of CD30L or anti-CD30 MoAbs on Ig isotype secretion of normal lymphoblastoid B-cell lines and the BL B-cell lines were then examined. The lymphoblastoid B-cell lines CB33 and B-MNK constitutively secreted IgM and IgA, respectively. The other lines (CB23, B-MNE, and B-MNB) did not spontaneously secrete any Ig subtypes. The addition of CD30L or anti-CD30 MoAbs to CB23, B-MNE, and B-MNB did not induce Ig secretion. Also, costimulation with IL-2, IL-4, and IL-10 did not induce Ig secretion. As shown in Fig 1, the addition of CD30L or the anti-CD30 MoAbs M44 and M67 enhanced IgM and IgA secretion by CB33 and B-MNK B cells, respectively. Costimulation with IL-2, IL-4, or IL-10 did not further enhance CD30L-induced Ig secretion (data not shown). The BL cell lines BL-APB and Raji constitutively secreted IgM, but there was no enhancement by addition of the CD30L either in the absence or presence of IL-2, IL-4, or IL-10. The CD30⁺ BL line...
CD30L INVOLVEMENT IN CD30+'LYMPHOMAS

Fig 1. Enhancement of Ig secretion by recombinant human CD30L. (A and B) Cells (1 x 10⁵/well) of the EBV-immortalized lymphoblastoid B-cell lines B-MNK (A) and CB33 (B) were cultured for 5 days with 2 x 10⁵/well CV-1/EBNA cells transfected with the empty vector (CV-1/HAV) or huCD30L containing vector (CV-1/CD30L) or with 10 μg/mL immobilized isotype control MoAb or M44 and M67 anti-CD30 (CV-1/HAV) or huCD30 containing vector (CV-1/CD30L) or with 10 ng/mL 1L2 and TNF-α for 48 hours as determined by ELISA. Results are expressed as mean values ± SEM from triplicate cultures and are representative of three experiments performed.

Jijoye did not secrete Ig isotypes constitutively or after stimulation with CD30L in the presence or absence of IL-2, IL-4, or IL-10 (data not shown). On the other hand, the CD30 BL cell line Ramos secreted 1,547 ± 120 ng/mL IgM in a 5-day culture. CD30L and IL-2 did not enhance IgM secretion, but IL-4 and IL-10 enhanced IgM secretion to 2,530 ± 94 and 3,136 ± 117 ng/mL, respectively. In summary, the Ig secretion of some normal lymphoblastoid CD30⁺ B-cell lines could be enhanced by the addition of CD30L and both anti-CD30 MoAbs M44 and M67, but was not dependent on costimulation with IL-2, IL-4, or IL-10. The CD30⁺ BL B-cell lines did not alter Ig secretion after addition of CD30L or anti-CD30 MoAbs with or without IL-2, IL-4, or IL-10 present.

CD30L and anti-CD30 MoAb effects on HD-derived cell lines and an adult T-ALL cell line. CD30L expressing CV-1/EBNA cells and anti-CD30 MoAbs (M44, M67, and Ki-1) were tested for their ability to enhance proliferation of the HD-derived cell lines HDLM-2, L-540 (both “T-cell-like”), KM-H2, and L-428 (both “B-cell-like”). As shown in Fig 2, neither of the “B-cell-like” HD-derived cell lines responded to either CD30L or anti-CD30 MoAbs (M44, M67, and Ki-1) up to 72 hours of culture (Fig 2C and D). In contrast, proliferation of the “T-cell-like” HD-derived cell lines was enhanced after addition of CD30L or anti-CD30 MoAbs M44 and M67 (Fig 2A and B). Both murine and human CD30L, expressed on CV-1 EBNA cells, induced a twofold to fivefold enhancement of [H]-thymidine uptake by HDLM-2 and L-540 H-RS cells compared with cells cultured with medium or CV-1/EBNA cells containing only vector (Fig 2A and B). Also, immobilized anti-CD30 MoAbs M44 and M67 enhanced proliferation of HDLM-2 and L-540 cells threefold to eightfold (Fig 2A and B). In contrast, the anti-CD30 MoAb Ki-1 did not induce proliferation of HDLM-2 and L-540 cells above that induced by isotype-matched control antibody (Fig 2A and B).

The murine and human CD30L enhanced proliferation of the “T-cell-like” cultured H-RS cells, HDLM-2, and L-540; a similar response could be observed for activated PB T cells (Fig 2E). Both anti-CD30 MoAbs M44 and M67, directed against the extracellular portion of the CD30 antigen, mimicked the CD30L-induced biologic activities. In contrast, the anti-CD30 MoAb Ki-1 and also Ber-H2 (data not shown) had no biologic effects. The enhanced proliferation of HDLM-2 and L-540 cells with CD30L or M44/M67 anti-CD30 MoAbs was time- and dose-dependent (Fig 3). CD30L induced maximal proliferation after 72 hours of culture (Fig 3A and D). The MoAbs M44 and M67 had maximal effects at concentrations of 10 μg/mL for a culture period of 48 to 72 hours (Fig 3B and E). The enhanced proliferative effect of the CD30L expressed on CV-1/EBNA cells or the both agonistic MoAbs appeared to be specific because it could be blocked by the addition of 50-fold excess of soluble CD30Fc protein (Fig 3C and data not shown).

To confirm these findings, other T-cell lymphoma/leukemia cell lines were screened for CD30 expression. The adult T-ALL cell line KE37 showed a strong constitutive surface expression of the CD30 antigen, but was negative for the B-cell antigen CD240 (Fig 4A). The addition of CV-1/EBNA cells expressing the CD30L or the immobilized anti-CD30 MoAbs M44 and M67 induced a threefold to sixfold enhancement of proliferation for KE37 cells (Fig 4B). The Ki-1 antibody did not alter proliferation (Fig 4B).

Expression of CD30L mRNA by T cells, monocytes, and HD-derived cell lines. The HD-derived cell lines HDLM-2, KM-H2, and L-428 did not express CD30L mRNA constitutively, or after stimulation with TPA for 24 to 72 hours or with 100 ng/mL IL-2 and TNF-α for 48 hours as determined by Northern blot analysis (Fig 5). Purified human T cells stimulated with TPA/PHA for 12 hours and peripheral blood monocytes activated for 12 hours with LPS showed induced expression of the CD30L mRNA (Fig 5). A control hybridization with GAPDH showed a similar total RNA loading for all lanes (data not shown). To confirm these results, flow cytometric studies and Scatchard analysis with the CD30Fc...
fusion protein were performed. The membrane-bound CD30L could not be detected constitutively or after different mitogen (eg, TPA/simocycin, PHA, and convalin A) or cytokine (eg, IL-1, IL-2, IL-4, IL-6, IL-9, and TNF) stimulation on the surface of all six HD-derived cell lines (data not shown).

CD30L and anti-CD30 MoAbs induced cytolytic cell death of cultured LCAL cells. CD30+ LCALs are characterized by the presence of strong CD30 surface expression on the anaplastic lymphoma cells. Using the LCAL cell line Karpas 299 as a model for this NHL subgroup, we examined the biologic activity of CD30L on this cell line. Karpas 299 cells have the typical features of a CD30+ LCAL with 2;5 translocation; T-cell receptor P-chain rearrangement; surface expression of CD4, CD5, EMA, CD30, and HLA-DR; and a typical anaplastic morphology. As shown in Fig 6, Karpas 299 cells incubated with CD30L, M44, or M67 anti-CD30 MoAbs showed a threefold to sixfold reduced proliferation in comparison to cells cultured with CV-1/EBNA cells transfected with the vector alone, isotype-matched control MoAb, or medium (Fig 6A and B). A significant reduction of 3H-thymidine uptake of Karpas 299 cells is measurable after 24 hours in culture with CD30L, M44, or M67 anti-CD30 MoAbs (Fig 6C). The reduction of proliferation was time-dependent and was minimal 72 hours after initiation of the cultures (Fig 6C). To exclude nonspecific cytotoxic effects of the CD30L or the anti-CD30 MoAbs M44 and M67, specific 51Cr release was determined as a measurement of specific cell killing. As shown in Fig 6D, the huCD30L, muCD30L, M44, and M67 anti-CD30 MoAbs induced 15% to 23% specific 51Cr release from labeled Karpas 299 cells after 12 hours and approximately 30% specific 51Cr release after 24 hours. The reduction of proliferation in the presence of CD30L or anti-CD30 M44 and M67 MoAbs was dose-dependent (Fig 6A and B). This inhibition of proliferation could be almost completely reversed by the addition of 50-fold excess soluble CD30Fc (Fig 6A and B).

The effect of CD30L and anti-CD30 MoAbs M44 and M67 on the viability and total number of viable cells are shown in Fig 6E. Karpas 299 cells were incubated with CV-1/EBNA cells expressing the vector alone or the CD30L, with an isotype-matched control MoAb, or with M44/M67 anti-CD30 MoAbs. Every 12 to 24 hours, the absolute cell number and viability were determined by trypan-blue staining. Karpas 299 cells stimulated with control reagents showed exponential growth without significant reduction in viability (Fig 6E). In contrast, Karpas 299 cells incubated with CD30L or both M44/M67 anti-CD30 MoAbs showed a reduction of cell viability (30% to 40%) and arrested cell growth (Fig 6E). The cytolytic effect of the CD30L was dose-dependent and could be blocked by the addition of 50-fold excess CD30Fc (Fig 6F). Excess CV-1/EBNA cells per well elevated the background of nonspecific 51Cr release, which could not be blocked by CD30Fc. The CD30L-induced cytolytic effect on the CD30+ Karpas 299 cells was confirmed using multiparameter flow cytometric Hoechst staining and fluorescence confocal microscopy to distinguish between live and dead cells (data not shown). The mechanism for the cytolytic cell death of Karpas 299 cells caused by CD30L is presently unclear. Analysis of Karpas 299 cellular DNA showed a small amount of spontaneous DNA fragmentation, similar to that seen for apoptotic cells, which is not further enhanced by the CD30L (data not shown). Unstimulated, mitogen-activated, or cytokine-activated Karpas 299 cells were analyzed by flow cytometry using the CD30Fc protein to determine CD30L membrane expression. CD30L could not be detected for any preparation of Karpas 299 cells (data not shown).

As shown in Table 2, the CD30L and anti-CD30 MoAbs M44 and M67-induced cytolytic effect was found for seven
CD30L INvolvement in CD30+ Lymphomas

Fig 3. Proliferation of HD-derived cell lines HDLM-2 and L-540. Proliferation of HD-derived cell lines HDLM-2 (5 x 10^4 cells/well) (A, B, and C) and L-540 (2 x 10^4 cells/well) (D and E) cultured for varying times with (A and D) medium alone or fixed CV-1:EBNA cells (1 x 10^6 cells/well) transfected with huCD30L (CV-1/huCD30L), muCD30L (CV-1/muCD30L), or vector alone (CV-1/HAT); (B and E) immobilized anti-CD30 MoAbs M44, M67, and Ki-1 at 10 μg/mL or 1 μg/mL or an isotype-matched control MoAb at 10 μg/mL; (C) immobilized anti-CD30 MoAb M44, M67, and Ki-1 and isotype-matched control antibody at 10 μg/mL with and without the presence of 50-fold excess of CD30Fc for 72 hours. Tritiated thymidine incorporation was determined. Results are expressed as a percentage of medium control cultures ± SEM of triplicate cultures and are representative of three experiments performed.

Discussion

The expression of the lymphoid activation antigen CD30 on the presumed neoplastic H-RS cells of HD and a subset of malignant NHLs, like LCAI and BL, has received considerable attention as a useful clinical and pathologic marker for the identification of malignant lymphocytic cells in these types of lymphomas. CD30, which is a member of the TNF/NGF receptor superfamily, is also expressed by a small subset of normal activated T and B cells. We recently reported the cloning with molecular and biochemical characterization of a ligand for the CD30 receptor, as a new member of the TNF/NGF ligand superfamily. The CD30 ligand (CD30L) is, like TNF-α, a membrane-bound protein and a product of activated T cells and macrophages with classic pleiotropic cytokine activities. Whether CD30L exists in an alternative soluble secreted or cleaved form, like TNF-α, is presently unclear. The data presented in this report demonstrate that the recombinant human CD30L, expressed as a membrane protein on the surface of CV-1:EBNA cells, elicits a variety of pleiotropic biologic responses on different CD30+ lymphoma cells in the absence of additional cytokines.

We screened EBV-transformed normal lymphoblastoid B-cell lines, different types of EBV+ and EBV- NHL cell lines, like Burkitt’s, centroblastic/centrocytic (cb/cc), undifferentiated, and LCAI, and also HD-derived cell lines of B- and T-cell phenotype for CD30 expression as potential targets for CD30L activities on CD30+ lymphoma cell lines. In agreement with published reports, we found that EBV-transformed lymphoblastoid B cells (eg, CB23, CB33, B-MNE, B-MNB, B-MNK, and MP-1) and some type III BL cells (eg, BL-APB, Raji, and Jijoye) were CD30+. Other B-cell NHL types (eg, undifferentiated, diffuse large cell, and cb/cc follicular lymphoma cell lines) were CD30-. Cell lines derived from HD (eg, HDLMS, KM-H2, L-428, and L-540) and primary T-cell NHL of the LCAI type (eg, Karpas 299,
Fig 4. CD30 expression and proliferative response of the T-ALL cell line KE-37 to CD30L. (A) KE-37 cells (1 × 10^6) were stained with 10 μg/mL M67 anti-CD30 MoAb (IgG1) or M2 anti-CD40 MoAb (IgG1) and phycoerythrin. Staining intensity was analyzed by flow cytometry. Control samples were stained with an isotype-matched (IgG1) control MoAb to determine background fluorescence. A minimum of 5,000 cells was analyzed for each sample. The fluorescent intensity is shown in log_{10} intervals and are representative of four experiments. (B) KE-37 cells (5 × 10^6) were cultured for 72 hours with 1 × 10^5 CV-1/EBNA cells transfected either with the empty vector (CV-1/HAV) or huCD30L (CV-1/CD30L) and 10 μg/mL immobilized isotype-matched control MoAb or anti-CD30 MoAbs M44, M67, or Ki-1. Tritiated thymidine incorporation was determined after 72 hours. Data are the percentage of medium control cultures ± SEM of triplicate cultures and are representative of three experiments performed.

Fig 5. Northern blot analysis of huCD30L mRNA expression of HD-derived cell lines. RNA was prepared from the HD-derived cell lines HDLM-2, KM-H2, and L-428 or from PBTs and blood monocytes, as indicated. HD-derived cell lines were stimulated before harvest with 24 mmol/L phorbol myristate acetate (PMA) or 100 ng/mL IL-2 and TNF-α-activated, PBTs were activated with 32 mmol/L PMA, 1 mg/mL PHA, and peripheral blood monocytes were activated with 0.5 μg/mL lipopolysaccharide (LPS) for different culture times. Fractionation was performed on a 1% formaldehyde gel and hybridized with a radiolabeled antisense riboprobe corresponding to the coding region of the huCD30L cDNA.

TS, DHL-1, HSC-M1, FL-LCAL, Michel, and HAKI) were strongly CD30+, whereas other T-cell NHLs were negative (e.g., immunoblastic lymphoma line Sup-T1). In contrast, the HTLV-1, HTLV-2, and HIV-1 negative adult T-ALL cell line KE-37 was strongly positive for the CD30 receptor. These data confirm that EBV-transformed B-cell lymphoma lines can constitutively express CD30, but higher levels are detected on EBV− HD-derived cell lines, and the highest levels are found on "T-cell–like" forms (e.g., HDLM-2 and L-540), CD30+ LCAL cells with T-cell phenotype (e.g., Karpass 299, TS, DHL-1, FL-LCAL, and Michel), and the adult T-ALL cells (e.g., KE-37). The molecular mechanisms underlying the deregulated CD30 expression for these lymphomas are unclear, but are EBV and HTLV independent. CD30 expression in primary HD and LCAL cases is a typical marker for these forms of lymphomas, and is found in EBV+ and EBV− cases. Only 50% of HD and LCAL cases are EBV−.34,35

In contrast to the biologic activities of the CD40L, which is capable of delivering a mitogenic stimulus to B cells in the absence of additional cytokines,19,20,30,34 the CD30L was not able to enhance the proliferation of CD30− lymphoblastoid B cells or BL cells in the presence or absence of additional cytokines (IL-2, IL-4, and IL-10), shown previously to costimulate with CD40L.34 CD40L has stimulatory effects on resting and activated B cells, inducing B-cell growth, Ig secretion, and expression of CD23.19,20,30,34 Similarly, the CD30L is capable of enhancing secretion of Ig isotypes from EBV-transformed lymphoblastoid B cells, but not BL cells. Other B-cell–stimulatory cytokines (e.g., IL-2, IL-4, and IL-
CD30L INVOLVEMENT IN CD30+ LYMPHOMAS

A.

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10

0.01 0.1 1 10

CV-1/EBNA Cell x 10^{-3}

CV-1/huCD30L

CV-1/muCD30L

CV-1/huCD30L + CD30Fc

B.

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20

10

0.01 0.1 1 10

CV-1/EBNA Cell x 10^{-3}

CV-1/huCD30L + CD30Fc

C.

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10

0.01 0.1 1 10

CV-1/EBNA Cell x 10^{-3}

CV-1/huCD30L + CD30Fc

Fig 6. CD30L-induced cytotoxicity to the LCAL cell line Karpas 299. (A, B, and C) Karpas 299 cells (5 × 10^6) were cultured for 72 hours with 1 × 10^4 CV-1/EBNA cells transfected with the huCD30L (CV-1/huCD30L), muCD30L (CV-1/muCD30L) containing vector, or the vector alone (CV-1/HAV) (A), or with 10 μg/mL immobilized anti-CD30 MoAbs M44 and M67 or isotype-matched control MoAb (B). Some experiments were performed in the presence of 50-fold excess of CD30Fc. (C) Karpas 299 cells were harvested at different culture periods. Results are expressed as the mean cpm ± SEM tritiated-thymidine incorporation of triplicate cultures and are representative of four separate experiments performed. (D and F) CD30L-induced cytotoxicity was measured by specific ^51Cr release for different culture periods. ^51Cr-labeled Karpas 299 cells (5 × 10^6 cells/well) were incubated with CV-1/EBNA cells (1 × 10^6 cells/well) transfected with vector alone (CV-1/HAV) or huCD30L (CV-1/huCD30L), 10 μg/mL immobilized anti-CD30 MoAbs M44 and M67, or isotype control MoAb. (D) Percent specific ^51Cr release was measured after 12 hours or (E) after 24 hours with titration of CV-1/EBNA cells with and without 50-fold excess of CD30Fc. Each stimulus was performed in six replicate cultures. Specific ^51Cr release was calculated as the mean mean of specific ^51Cr release ± SEM. (E) Karpas 299 cells (2 × 10^6/mL) were cultured in the presence of CV-1/EBNA cells (5 × 10^6/mL) expressing the vector alone (CV-1/HAV) or the huCD30L (CV-1/huCD30L), with 10 μg/mL isotype-matched control MoAb or with M44 and M67 anti-CD30 MoAbs. Every 12 to 24 hours the absolute cell number and viability was determined by trypan-blue staining. Results are representative of three experiments performed and are shown as absolute cell number/mL ± SEM of triplicate cultures.

10) are not required for this enhancement of Ig secretion. The role of CD30L on primary human B cells is presently unclear and under investigation, but primary murine B cells can be induced to proliferate and secrete polyclonal Ig by CD30L costimulation with IL-4 and IL-5 (K. Grabstein et al, manuscript in preparation). It seems possible that the CD30L, like the CD40L, may have a biologic role in B-cell proliferation, maturation, and/or differentiation. The relevance of CD30 expression on BL remains unclear and needs further investigation.

Primary HD cases are usually characterized by the presence of CD30+ and CD15+ H-RS cells in reactive normal tissue. Primary H-RS cells express, to a variable degree, some typical B- and T-cell surface markers and can have either nonfunctional T-cell receptor (TCR) or heavy/light chain Ig rearrangements. Primary HD cases can be subdivided into "B-cell-like" or "T-cell-like" subforms, with a predominance of each type in different histologic subtypes. The few existing HD-derived cell lines represent an in vitro model of the presumed malignant component of HD, the H-RS cells, and also express either "T-cell-like" (eg, HDLM-2 and L-540) or "B-cell-like" (eg, L-428 and KM-H2) phenotypes. Similar to PBT costimulated with anti-CD3 and CD30L, the "T-cell-like" HD-derived cell lines HDLM-2 and L-540 showed enhanced proliferation in the presence of the CD30L, which could not be further increased by costimulation with mitogens, such as PHA, ionomycin, or TPA. For the HDLM-2 and L-540 H-RS cells, both the...
murine and human CD30L are capable of enhancing proliferation and the two new anti-CD30 MoAbs M44 and M67 show agonistic biologic activities, in contrast to the established anti-CD30 MoAbs (eg, Ki-1 and Ber-H2). For the “B-cell-like” HD-derived cell lines KM-H2 and L-428, no enhancement of proliferation was observed with the CD30L. This might be explained by different biologic involvement and functional roles of the CD30-CD30L network for “T-cell-like” and “B-cell-like” primary HD cases. We cannot exclude the possibility that some of the HD-derived cell lines have, in vitro, lost the ability to respond to CD30L or other cytokines.

To exclude a CD30L-dependent autocrine growth loop for the HD-derived cell lines, cultured H-RS cells were analyzed for CD30L expression at the mRNA and protein level. None of the six HD-derived cell lines examined showed CD30L mRNA or protein expression constitutively or after stimulation with TPA or cytokines (eg, K-1, IL-2, IL-4, IL-6, IL-9, and TNF). The HD-derived cell lines appear not to use the CD30-CD30L interaction in an autocrine fashion. Recently, different groups have shown that primary and cultured H-RS cells produce and secrete a panel of different cytokines, which correlate with typical clinical and pathologic features of HD.\(^{39-43}\) Heterogeneous clinical presentation of HD cases might be caused by variable cytokine expression.\(^{39-43}\) These studies have identified HD as a tumor of cytokine-producing cells, which are responsible for the hyperpressive tissue involvement (eg, sclerosis, eosinophilia, T-cell accumulation and activation, and plasmacytosis). The CD30L is expressed on activated T cells and macrophages, but also constitutively on granulocytes.\(^{22}\) All three cell types are usually found in HD-involved tissue and might support the proliferation of primary H-RS, modulating cytokine expression and secretion, or cell-cell interactions. Like the “T-cell-like” HD-derived cell lines (HDLM-2 and L-540), the adult T-ALL cell line KE-37 strongly expressed CD30L, and exposure to CD30L enhances the proliferation of these cells. In summary, the CD30L delivers a mitogenic activity to activated PBTs and also to some “T-cell-like” lymphoma/leukemia cells, such as HD and adult T-ALL.

In contrast, the CD30+ LCAL cell line Karpas 299, which has a T-cell phenotype and a characteristic T:2:5 translocation, responded to CD30L with a reduction in proliferation. Similar data were obtained for six other CD30+ LCAL cell lines (eg, TS, DHL-1, HSC-M1, FL-LCAL, Michel, and HAKI). Further experiments with Karpas 299 cells showed a cytotoxic effect of the CD30L on these LCAL cells. All eight LCAL cell lines were negative for CD30L protein expression either constitutively or after different stimulations. Normal activated human PBT cells are able to express CD30 and CD30L, but the “T-cell-like” lymphoma cell lines investigated here, like HD and LCAL cell lines, are able only to express the CD30 activation antigen and not the CD30L. The molecular mechanism of the CD30L-mediated cytotoxicity on the LCAL cell lines is presently unclear and occurs rather late in comparison to the induction of programmed cell death by antibodies against the Fas/Apo-1 antigen.\(^{44-45}\) Investigation of the histologic distribution of the CD30L in primary LCAL and HD cases will further evaluate the involvement of the CD30-CD30L interaction in the pathobiology of these CD30+ lymphomas. HD and CD30+ LCAL share some characteristic common pathologic features, such as IL-9, CD30, and CD-lectin expression, and might originate from a similar lymphoid cell.\(^{3,46-49}\) It remains unclear if HD and CD30+ LCAL are closely related diseases; however, the typical T:2;5 translocation and CD45+, EMA−, CD15− phenotype of CD30+ LCAL cases is different from the presentation of typical HD cases.\(^{3,37,48,50}\) The analysis of the role of the CD30-CD30L interaction in the pathobiology of CD30+ lymphomas (eg, HD, LCAL, and BL) should increase our understanding of these lymphomas.

The data presented here suggest that the CD30-CD30L interactions may play a role in the pathophysiology of CD30+ lymphomas and function of immune system cells. The full spectrum of CD30-CD30L interaction and biologic activities for lymphomas and immune cells is currently under investigation.

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Pleiotropic effects of the CD30 ligand on CD30-expressing cells and lymphoma cell lines

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