Proliferation of CD30+ T-Helper 2 Lymphoma Cells Can Be Inhibited by CD30 Receptor Cross-Linking with Recombinant CD30 Ligand


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ABSTRACT

Purpose: Cutaneous anaplastic large cell lymphomas (ALCLs) are characterized by the expression of CD30, spontaneous regression of skin lesions, and increased concentration of CD30 ligand (CD30L). We hypothesize that CD30-CD30L interactions explain the unusual clinical behavior of cutaneous ALCLs.

Experimental Design: Eight lymphoma cell lines established from four different patients were analyzed for T-cell clonality using PCR and subsequently denaturating gradient gel electrophoresis. The expression levels of CD30 were estimated using western blot analysis and cell cycle analysis revealed that growth regulation was dependent on cell culture conditions. Comparison of effects of ligation with CD30L and anti-CD30 agonistic antibody Hefi-1 revealed higher efficacy for CD30L in these ALCL lines.

Results: The results showed different levels of CD30 expression and a predominant T-helper 2 profile. In a cell kinetic analysis we found that ALCL cell growth is effectively inhibited by CD30L but only in those cell lines expressing CD30 molecules in sufficient amounts on the cell surface. Cell cycle analysis revealed that growth regulation was dependent on cell cycle status and signal strength. The binding of CD30 by its ligand provides new opportunities for controlling cell growth and treatment of CD30+ ALCL.

Conclusions: CD30+ ALCL cells can be growth inhibited by receptor ligation. Observed pleiotropic effects of CD30 signaling are most likely dependent on cell cycle status and signal strength. The binding of CD30 by its ligand provides new opportunities for controlling cell growth and treatment of CD30+ ALCL.

INTRODUCTION

Malignant lymphomas can originate from lymphocytes at any level of development and differentiation between pre-B/T cells and peripheral differentiated B or T lymphocytes. There is growing evidence that a clonal disease may have different clinical and histological features depending not only on the time point in the disease process but also probably on the corresponding state of lymphocyte activation (1). The precise mechanisms of lymphomagenesis are still obscure. However, it appears that various factors are involved including environmental, especially infectious agents, chromosomal translocations, insertions, or point mutations resulting from genetic instability of tumor cells (2, 3). The microenvironment in the skin contributes to the peculiar behavior of these neoplasms by providing various signals from adhesion molecules and cytokines. The CD30 antigen, a member of the tumor necrosis factor receptor superfamily, serves among others (4) as a prognostic marker in cutaneous T-cell lymphomas (5–9).

The biological function of tumor necrosis factor receptor family members is regulation of growth and elimination of peripheral T cells during their expansion in response to antigenic stimuli. CD30 is normally expressed on immunoblasts located in the perifollicular region around germinal centers. In addition, CD30 is expressed by Reed-Sternberg cells in virtually all cases of classical Hodgkin’s lymphoma and ALCL (10). The cognate CD30L is expressed on neutrophils, histiocytes, eosinophils, mast cells (11, 12), and a small subset of activated T cells (13). Furthermore, CD30L has been detected in Reed-Sternberg-like cells and smaller cells in lesions of patients with lymphomatoïd papulosis (14). The human CD30L is a M, 40,000, 234 aa residue transmembrane glycoprotein with 72% aa sequence identity to its ms counterpart.

Cutaneous CD30+ ALCL often are accompanied by spontaneous tumor regression (14–16). Although the underlying mechanisms are still unknown, we hypothesize that a selective increase in CD30L expression measured in clinical specimens of...
cutaneous ALCL may play an important role (14). CD30 cross-linking with Abs may result in proteolytic degradation of the protein and a release of soluble CD30 products into the extracellular compartment (17). However, not all of the anti-CD30 Abs can cause the described pleiotropic effects, and many of them are unable to produce any detectable biological changes (18). To additionally elucidate the function of CD30 and its potential impact on lymphoproliferative disorders, several murine models have been established. CD30 transgenic mice have shown enhanced cell death by CD30 receptor cross-linking (19), and CD30−/− mice showed an impaired negative selection in the thymus (20). When CD30+ ALCL cells were transplanted into mice, tumor growth could be inhibited by administration of the anti-CD30 mAb HeFi-1 (21, 22).

In the present study, we compare eight lymphoma cell lines with respect to surface marker expression, cytokine profile, and clonal TCR gene rearrangement. We correlate these findings with functional assays in which we inhibit cell growth by receptor cross-linking. In kinetic analyses we can clearly show the efficacy of recombinant CD30L to inhibit lymphoma cell growth when the CD30 receptor is expressed on the cell surface in sufficient amounts. Additional analysis revealed that cell death observed in our cultures is only partly because of apoptosis. Moreover, it is evident that cell culture conditions very much influence the lymphoma cell susceptibility to CD30 cross-linking. We believe our data help to explain controversial results presented in the literature (1, 25, 28–30). The human T-lymphoblastic lymphoma cell line Jurkat was obtained from the American Type Culture Collection (Manassas, VA). All of the cell lines were grown in RPMI 1640, supplemented with 10% heat-inactivated fetal bovine serum (Seromed, Berlin, Germany), 1% of an antibiotic mixture containing 10,000 units penicillin/ml and 10,000 µg streptomycin/ml, 5 mM glutamine, and 5 mM sodium pyruvate (all Life Technologies, Inc., Paisley, United Kingdom).

**TABLE 1** Phenotypic cell line description

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<th>CD15</th>
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* ALK, anaplastic lymphocyte kinase; n.d., not done; CLA, cutaneous lymphocyte antigen.

**MATERIALS AND METHODS**

**Cell Lines.** The cutaneous lymphoma cell lines Mac1, Mac2A, and Mac2B were derived from different clinical specimens of one patient showing progression from lymphomatoid papulosis to systemic ALCL (1, 15, 25, 26). In particular, the Mac1 cell line was derived from circulating tumor cells in the blood of the patient during an indolent course of his disease, whereas the Mac2A and Mac2B cell lines were derived from separate rapidly growing skin tumors 3 years later in the progression of the disease. The cell lines JK-A, JK-B, and JK-X represent different subclones, with a splicing variant of CD30 that lacks the extracellular and transmembrane domains of CD30, derived in vitro from a biopsy of a patient with a CD30+ cutaneous ALCL (26). The cell lines Karpas 299 and JB6 were derived from lymphoma cells of different patients with nodal/systemic CD30+ ALCL (27). Table 1 summarizes known characteristics of the cell lines from the literature (1, 25, 28–30). The human T-lymphoblastic lymphoma cell line Jurkat was obtained from the American Type Culture Collection (Manassas, VA). All of the cell lines were grown in RPMI 1640, supplemented with 10% heat-inactivated fetal bovine serum (Seromed, Berlin, Germany), 1% of an antibiotic mixture containing 10,000 units penicillin/ml and 10,000 µg streptomycin/ml, 5 mM glutamine, and 5 mM sodium pyruvate (all Life Technologies, Inc., Paisley, United Kingdom).

**PCR of TCR-γ Genes.** The method was performed according to Meyer et al. (31). Briefly, for PCR amplification 1 µg of total DNA was suspended in 50 µl PCR solution containing 40 pM of each primer, 200 µM of deoxynucleoside triphosphate, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 2.5 mM MgCl2 0.01% gelatin, and 2.5 units Taq DNA polymerase in a total volume of 50 µl. In a first step a PCR was performed with a primer set described elsewhere (32, 33). Initially, 25 cycles using the TCR-γ variable region (Vγ1–8) and the TCR-γ joining region Jγ1/2 primers were performed, then 10 µl of the first-round PCR product was added to 100 µl of fresh PCR buffer containing the nested set of Vγ1–8 and Jγ1/2 primers, and another 20 cycles were performed. Cycle parameters in both rounds were: initial denaturation step at 94°C for 2 min, denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 70°C for 40 s, followed by a terminal extension step at 72°C for 10 min. To amplify rearrangements involving Vγ9, the single Vγ9 primer replaced the two Vγ1–8 primers (31–33). This protocol has been performed on all of the cases as a standard. Cases that demonstrated no clonality, when amplified with primers Vγ1–8, Vγ9, and Jγ1/2, were reamplified with consensus primers for variable regions Vγ10/11 (34), joining region J1/2 (35) and J1/2 (36). With these primers a single-round PCR was performed with the following thermocycling conditions: initial denaturation step at 94°C for 4 min, 35 cycles of denaturation at 94°C for 1 min, annealing at 58°C for 1 min, and extension at 72°C for 1 min, followed by a terminal extension step at 72°C for 5 min. The reaction was performed in a Thermal Cycler 9600 (Perkin-Elmer, Kuesnacht, Switzerland). Primers as shown in Table 2 were obtained from Microsynth (Balgach, Switzerland).

**DGGE.** The DGGE gel was performed with the D Gene System (Bio-Rad, Hercules, CA) according to the manufacturer’s instructions and as described elsewhere (37). The PCR products were precipitated and resuspended in 10 µl of deionized water, heat-denatured at 95°C, and then reannealed for 30 min at 30°C. The samples were loaded on a 6.5% polyacrylamide gel containing gradients of 30–60% urea/formamide and electrophoresed at 60°C for 6 h at 150V. The ethidium bromide-stained gel was photographed under UV light.

**Cell Kinetic Analysis.** Two 96-well flat-bottomed microtiter plates were coated with 0.5 µg of a murine recombinant CD30L (R&D Systems, Minneapolis, MN), mAb HeFi-1 (a kind gift of Dr. Longo, National Cancer Institute, Frederick, MD), and mIgG2a (Ancell, Bayport, MN) in 100 µl PBS/well. The murine CD30L cross-reacts with human CD30 (13). The recombinant CD30L was derived from a DNA sequence encoding aa residues Gln 68-Asp 239 of murine CD30L, and fused to the signal peptide of human CD30 and a polyhistidine-containing linker. After overnight incubation of the plates at 4°C, the coating solution was removed, and 200 µl RPMI 1640 containing
10,000 cells plus 0.5 μg of CD30L, HeFi-1, or ms IgG2a was added to each well. Cell cultures have been in logarithmic growth conditions at the time of transfer. The plates were incubated at 37°C in a 5% CO₂ atmosphere. After 1, 4, 6, and 8 days, aliquots of 20 μl were removed and stained with 100 μl PBS/trypan blue (Biochrom KG, Berlin, Germany). Living (nonstained) and dead (stained) cells were counted separately with a microscope (one visual field at ×200 magnification). At day 4, one volume of fresh RPMI 1640 was added to all of the wells, and all of the subsequent counts were multiplied by two.

**DNA Staining.** Micropipette plates were coated with 1 μg CD30L, HeFi-1, or ms IgG2a in 100 μl PBS/well overnight at 4°C. Then, the supernatant was removed and ~100,000 cells in 100 μl (a) serum-free medium or (b) complete medium were added. Cells in serum-free medium were kept under starved culture conditions for 12 h before use. After adding 100 μl of fresh serum-free medium, the cells were incubated for 24 h at 37°C in a 5% CO₂ atmosphere. The next day, BrdUrd (Sigma, St. Louis, MO) was added to the cells. The thymidine analogue BrdUrd was added to the medium at a concentration of 2 μg/ml. The plates were kept light protected and incubated for an additional 10 h. Then, the cells were harvested and stained with a FITC-conjugated anti-BrdUrd mAb (B44; BD Bioscience, Heidelberg, Germany). Briefly, the single cell suspensions were centrifuged for 10 min at 200 × g. The pellets were resuspended in 200 μl PBS and injected into 5 ml of 70% methanol. The cells were fixed for 20 min at room temperature and then centrifuged for 10 min at 200 × g. After removing the supernatant, the cells were resuspended in 2 ml 1 N HCl containing 0.5% Tween 20 (Sigma) and incubated for 15 min at 37°C. After another centrifugation step, the acid was neutralized with 200 μl 0.1 M sodiumtetraborate. Cells were washed in 1% BSA/PBS and then stained with an anti-BrdUrd-FITC Ab. For detection of incorporated BrdUrd, we used a FACScalibur (Becton Dickinson) and the CellQuestPro software. After measuring proliferation, the remaining cells in the tube were stained with 40 μg/ml propidium iodide (Sigma) for cell cycle analysis and measured again in the FACScalibur. Acquisition and analysis were performed in the “doublet discrimination mode.”

**Cell Surface Staining.** For surface receptor detection, 10⁶ cells were stained with anti-CD30 mAb (HeFi-1) at a concentration of 5 μg/ml for 2 h on ice in a total volume of 20 μl followed by a second staining with anti-ms IgG2a-FITC (Ancell). Aliquots of 10⁶ cells were incubated twice in a total volume of 20 μl with the appropriate labeled mAb. Then, cells were washed, resuspended, and subjected to FACScalibur analysis.

### Table 2 Primer sequences

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<th>Name</th>
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<td>Vγ9</td>
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<td>Jγ1/2</td>
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<td>JP1/2</td>
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**Fig. 1** Clonal TCR-Vγ chain rearrangement in lymphoma cell lines. PCR-DGGE analysis was performed for eight lymphoma cell lines with various primer combinations. Only positive results (Lanes 1, 3, 5, 7, and 9) and a corresponding negative control (Lanes 2, 4, 6, 8, and 10) are shown. Lane 1 represent a Vγ10/11:J1/2 rearrangement; Lanes 3, 5, and 7 show Vγ1–8:Jp1/2 rearrangements; and finally Lane 9 revealed clonality with Vγ1–8:Jγ1/2 primers. Lymphoma cell lines printed in bold (JK-X, Mac1, Mac2B, Karpas, and JB6) are shown. Lymphoma cell lines printed in *italics* below (JK-A, JK-B, and Mac2A) revealed similar/identical rearrangement pattern to the lymphoma cell lines above (data not shown). M is a molecular size marker (Marker VI; Roche Applied Science, Basel, Switzerland). Arrows indicate bands of clonality.

**Cytokine Profiling.** One million cells per ml RPMI 1640 were cultured for 3 days, then the supernatants were harvested and analyzed in ELISA for IFN-γ, IL-2, IL-4, IL-6, IL-8, and IL-10 in the Department of Clinical Immunology (University Hospital Zurich, Zurich, Switzerland). The amount of soluble cytokines was measured by Hbt Human IFN-γ ELISA test kit (HyCult Biotechnology, Uden, Netherlands), and IL-2, -4, -6, -8, and -10 ELISA test kit (R&D Systems Inc.), according to the manufacturer’s instructions. Appropriate reference material for all of the ELISA was included (Quantikine Controls, QC Controls Group 1; R&D Systems Inc.).
RESULTS

Clonal TCR Vγ Rearrangements in Lymphoma Cell Lines. All of the established lymphoma cell lines were subjected to clonal analysis with various combinations of TCR-γ chain-specific primers. As shown in Fig. 1, the JK-family carries a Vγ10/11:J1/2 rearrangement (very faint band). The Mac1,2A,2B-family showed a Vγ1–8:JP1/2 rearrangement. It is noteworthy that Mac2B, derived from the same patient but from a different lesion as Mac1 and Mac2A showed a different band pattern, thus emphasizing intraindividual heterogeneity of the tumor cells. Karpas 299 showed a Vγ1–8:JP1/2 rearrangement as well, and additionally Vγ1–8:J/H92531/2 rearrangements (data not shown). The lymphoma cell line JB6 gave positive results with the same primer pair Vγ1–8:J/H92531/2.

Differential CD30 Expression of Lymphoma Cell Lines. Nine lymphoma cell lines were tested for CD30 expression (Fig. 2). fluorescence-activated cell sorter analysis revealed three expression patterns. The cell lines JK-X, JK-A, and JK-B are negative; Jurkat T-cell lymphoblastic lymphoma and Mac2B are weakly positive, Mac1, Mac2A, and JB6 are strongly positive for CD30 expression and comparable with the known CD30 expression of Karpas 299 and JB6, although the isotype control of JB6 exhibits a small side peak. Peripheral blood leukocytes (PBL) of healthy donor served as a negative control.

Th2 Cytokines Are Dominantly Expressed in the Lymphoma Cell Lines. The cytokine expression profile for IL-2, IL-4, IL-6, IL-8, IL-10, and IFN-γ/H9253 was tested for seven lymphoma cell lines. As shown in Table 3, the lymphoma cell lines Mac1, Mac2A, and Mac2B show high expression of Th2-like cytokines. In contrast, the lymphoma cell lines JK-X, JK-A, and JK-B show low expression of Th2-like cytokines. The lymphoma cell line Karpas 299 shows intermediate expression of Th2-like cytokines. The lymphoma cell line JB6 shows high expression of Th1-like cytokines.

Table 3. Cytokine expression profiles

<table>
<thead>
<tr>
<th>Cell line</th>
<th>IFN-γ (units/ml)</th>
<th>IL-2 (pg/ml)</th>
<th>IL-4 (pg/ml)</th>
<th>IL-6 (pg/ml)</th>
<th>IL-8 (pg/ml)</th>
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* n.d., not done.  
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Fig. 2. Fluorescence-activated cell sorter staining of CD30 expression of lymphoma cell lines. The thin line represents the isotype control; the thick line represents the Hεβ-1 (anti-CD30) stainings. JK-X, JK-A, and JK-B are negative; Jurkat T-cell lymphoblastic lymphoma and Mac2B are weakly positive, Mac1, Mac2A, and JB6 are strongly positive for CD30 expression and comparable with the known CD30 expression of Karpas 299 and JB6, although the isotype control of JB6 exhibits a small side peak. Peripheral blood leukocytes (PBL) of healthy donor served as a negative control.

Fig. 3. Origin and characteristics of the cell lines including their phenotypes. + = strong expression; +/- = weak expression; – = no expression; n.d. = not done.

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<table>
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<tr>
<th>Cell line</th>
<th>IFN-γ (units/ml)</th>
<th>IL-2 (pg/ml)</th>
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"Normal serum levels."
Fig. 4 Kinetics of tumor cells. At indicated days (1, 4, 6, and 8) 20-μl aliquots of cells were removed from the culture, stained with 100 μl of PBS/trypan blue and counted under a microscope. One visual field at ×200 magnification has been counted. Living cells (A) and dead cells (B) were counted separately. The experiments were done in triplicates. The symbols represent the mean. Statistically significant differences between the pairs CD30L versus % (nothing) and HeFi-1 versus iso (isotype) are indicated; bars, ±SD.
B

Kinetic of dead cells

Fig. 4 Continued.
phoma cell lines under standardized conditions, whereas JK-A was tested only for IL-2, IL-4, and IL-10. Besides individual differences, the cell lines expressed more Th2 cytokines than Th1 cytokines (Table 3). In detail, we see high levels (>100 pg/ml) of IL-6 and IL-10, and low levels (<10 units or pg/ml) of IFN-γ, IL-2, IL-4, and IL-8 in Mac2A, JK-X, JK-B, and JK-A (note: JK-A was not tested for IFN-γ, IL-6, and IL-8). Mac2B was high only for IL-6, whereas Mac1 showed no remarkable cytokine expression. Karpas 299 and JB6 showed high levels (>100 pg/ml) only for IL-10.

Specific Cell Growth Inhibition of CD30+ Lymphoma Cell Lines by CD30L. After characterization of the cell lines, our main interest was to investigate the influence of CD30L and anti-CD30 mAbs on the proliferation of lymphoma cell cultures. As demonstrated in Fig. 4A, several effects could be observed. First, significant differences in tumor cell growth kinetics are seen in five cell lines (Karpas 299, Mac1, Mac2A, Mac2B, and JB6), all of them expressing CD30 on the surface. JB6 was the only cell line showing significant growth inhibition with both CD30L and HeFi-1. JK-X, JK-A, and JK-B, all CD30 negative, remained unaffected by CD30L or HeFi-1. Despite expressing small amounts of CD30, Jurkat cells remained unresponsive to both reagents when compared with untreated or isotype control cultures. All of the experiments were performed in parallel to assure comparable conditions. However, some lymphoma cell lines, such as Mac2B, showed only marginal overall cell proliferation in this setting, whereas others (JK-B) reached an early plateau and subsequently decreased in numbers. Secondly, Fig. 4A reflects different growth characteristics of the tested lymphoma cell lines. In all of the cases the amount of starting cells was the same. However, after transfer into microtiter plates the untreated populations developed differently. JK-X, JK-A, Karpas 299, Mac1, and Jurkat showed increasing cell number during the first days in culture until a plateau was reached. In contrast, JK-B, Mac2A, Mac2B, and JB6 became static or even decreased in cell number probably because of adaptation problems to the new culture conditions, because this phenomenon was seen in both the treated and the untreated/isotype control groups of the respective cell cultures.

In correspondence to the kinetics of living cells we looked at the development of the dead cell population. Significant differences were seen again for Karpas 299, Mac1, Mac2A, and to a certain extent for JB6 when treated with CD30L. However, treatment with HeFi-1 revealed no significant effect on the dead cell counts. In each case reduced amounts of dead cells were observed in the CD30L-treated cultures compared with the control and HeFi-1 groups. Fig. 4B shows increasing numbers of dead cells in all of the untreated or isotype-treated cultures over time except for Mac2B. In Mac2B cultures we see high numbers of dead cells already after 1 day reflecting most likely adaptation of the population to new culture conditions, which is analogous to what we have observed in the kinetics of living Mac2B cells. The overall increase of dead cell counts in the cultures (with the exception of Mac2B) took place after ~4 days, compared with the increase of living cells observed in Fig. 4A. Therefore, we believe that exponential cell growth in our cultures is limited by the experimental setting to ~4 days followed by an increased cell death that keeps the number of living cells at a relatively constant level. This hypothesis may apply to Jurkat, JK-X, JK-A, Karpas 299, and Mac1. However, JK-B, Mac2A, Mac2B, and JB6 behave differently as described above.

To visualize the effect of CD30L on cell-cell interactions, we observed the lymphoma cells in vitro. CD30L inhibits the formation of colonies in CD30-positive Mac1 cells but not in CD30-negative JK-X cells as shown in Fig. 5. Additionally, the CD30L-treated Mac1 culture shows a large amount of cell debris.

CD30L-mediated Cell-Cycle Alterations. To additionally investigate the observed growth inhibition of lymphoma cell lines by CD30L, we analyzed the DNA content of treated cells and observed BrdUrd incorporation for a 10-h time interval. Karpas 299 and JB6 represent cell lines with high CD30 expression levels, whereas Mac2B represents intermediate CD30 expression levels and JK-A represents cells with no detectable CD30 expression, and serves as a negative control. Fig. 6A demonstrates a reduced proliferation of Karpas 299, Mac2B, and JB6 cells when incubated with CD30L, whereas JK-A remained unaffected by CD30L. At the same time, Fig. 6B revealed differential results for apoptosis measurements. Here, under starving culture conditions, Karpas 299 showed significantly reduced apoptosis, whereas Mac2B and JB6 showed increased apoptosis. Under starving culture conditions, CD30 ligation reduces cell proliferation and simultaneously reduces apoptosis, but only in Karpas 299 (CD30 high) cells, whereas apoptosis in Mac2B (CD30 low) and JB6 (CD30 high) is increased. Under nonstarving culture conditions the proliferation of Karpas 299, Mac2B, and JB6 was inhibited by CD30L (Fig. 6C), similar to what was observed in Fig. 6A for starving cells. A difference was observed for apoptotic values (Fig. 6D). Karpas 299 and, as a negative control, JK-A appeared unaffected by CD30L, whereas Mac2B and JB6 exhibited a slightly increased apoptosis after CD30 ligation. Under nonstarving culture conditions, CD30 ligation inhibits proliferation of Karpas 299,
Mac2B, and JB6 cells, and simultaneously increases apoptosis slightly in Mac2B and JB6 cultures, compared with the negative control culture, JK-A. Taken together, we conclude that cell proliferation is reduced under both culture conditions by CD30L. Moreover, we found starving cells to be more susceptible to CD30L-mediated apoptosis than nonstarving cells with the exception of Karpas 299 (Fig. 6E). Fig. 6E shows clearly that Karpas 299 behaves differently than other cell lines. Here, under starving culture conditions Karpas 299 appears more resistant to apoptosis upon CD30 ligation. Whereas BrdUrd incorporation, shown in Fig. 6, A and C, depict development (proliferation) of a population over a certain time period, propidium iodide staining with cell cycle analysis, as shown in Fig. 6, B and D, represents a snapshot of the same population.

**DISCUSSION**

In this study we analyzed eight anaplastic T-cell lymphoma and one lymphoblastic T-cell lymphoma lines in terms of genotypic, phenotypic, and functional characteristics. Analysis of TCR gene rearrangement is widely used for the detection of T-cell clonality in lymphoproliferative diseases (31). We found TCR-Vγ chain gene clonality in all eight of the anaplastic T-cell lymphoma lines tested, although the band for JK-A was weak. The detected clones often underwent uncommon rearrangements using pseudogene JP1/2 as reported for Sézary patients (37), thus confirming the T-cell lymphoma origin of the cell lines.

The marked increase in IL-6 secretion into the tissue culture medium by Mac2A cells corresponds to our published results on the comparative gene expression profile of the cell lines. We could show a 20-fold amplification of IL-6 gene expression by Mac2A cells over Mac1 cells (38). This finding corresponded to the onset of systemic B symptoms, which are best correlated with serum levels of IL-6 in large cell lymphoma and Hodgkin’s disease (39). In progression of disease, we also reported loss of response of Mac2A cells to growth inhibition by transforming growth factor β (25) because of an inactivating mutation of the receptor type II (40).

Furthermore, cytokine profiling of the cell lines provided new results relevant to the ongoing debate about the T-helper 1/2 dichotomy in the context of neoplastic T-cell disorders in the skin (41–45). In the murine model, two major subdivisions of the T-helper system have been defined. T-helper 1 clones secrete mainly IL-2 and IFN-γ, whereas Th2 clones produce IL-4, IL-5, IL-6, and IL-10 (46). This system of dichotomy is also applicable to humans if Th 1/2 functions are understood as predominant but not exclusive poles (41, 42, 47). Our cell lines preferentially express Th2-like cytokines (i.e., IL-4, IL-6, IL-8, and IL-10). This is in accordance with observations made by Yagi et al. (48). They detected IL-10 mRNA in biopsies from primary cutaneous large cell lymphoma and lymphomatoid papulosis. As a consequence, they could demonstrate beneficial effects of a treatment with IFN-γ that selectively inhibits the growth of Th2 cells (48). Th2 cytokine mRNA was detected in skin lesions at all stages of the disease (47). Indeed, IFN therapy had become an important treatment modality in lymphoproliferative disorders (49–52). On the basis of our genotypic and phenotypic analysis, we feel encouraged that the lymphoma cell lines we studied closely represent the original malignancy and, therefore, serve as a suitable in vitro model for the biology and treatment of human cutaneous lymphoproliferative disorders.

Because CD30L was detected in regressing cutaneous lymphoid lesions and often exhibits coexpression with CD30 (14), we explored the possible influence of recombinant CD30L on established lymphoma cell lines. We hypothesized that CD30-CD30L interactions in vivo may have an impact on the mechanism of self-regression of cutaneous lymphoma lesions. In our cell kinetic studies the two endpoints we measured, living cells and dead cells, complemented each other to provide a dynamic picture of the tumor cell population. In general, we saw inhibition of proliferation when CD30-positive cell lines were incubated with CD30L. These findings are supported by data published recently, showing that soluble trimeric CD153 (CD30L) was effective in triggering cell death of target cells with membrane anchored CD30 (53). This is in contrast to some of the effects reported with HeFi-1. Franke et al. (18) have concluded that HeFi-1 does not directly induce CD30 signaling, which additionally stimulated our interest to directly test the effects of CD30L. The proliferative effect of cross-linking...
CD30 with HeFi-1 on cutaneous lymphoma cells, as described in the literature, can be explained by nuclear translocation and activation of NF-κB (15). The intracellular domain of CD30 can bind tumor necrosis factor receptor-associated factor, resulting in activation of NF-κB. According to Mir et al. (24), NF-κB plays the determining role in the sensitivity or resistance of lymphoma cells to CD30-induced apoptosis. The quality of the signal (binding site, affinity) provided by CD30L or HeFi-1 binding to CD30 may be different and, thus, may influence the resulting biological effect.

Pleiotropic effects of CD30 activation have already been described by Gruss et al. (54). They found induction of cell death of systemic CD30+ ALCL after CD30L exposure. In contrast, it has been shown that CD30L enhances proliferation of peripheral T cells and the Hodgkin’s cell line HDLM-2 associated with tyrosine kinase phosphorylation of a cytosolic protein but exhibit an opposite effect on ALCL cell lines without tyrosine kinase phosphorylation (55). Schneider and Huber (56) stated recently that CD30-mediated signaling can promote cell proliferation as well as cell death depending on cell type and costimulatory signals. In that light, we believe that different parameters influence the fate of lymphoma cells on CD30 activation. Firstly, the microenvironment or cell cycle state at time of CD30 activation is of importance, whether lymphoma cells are starving or in a logarithmic growth phase. This would be similar to the action of certain drugs, e.g., methotrexate, and is important for planning treatments involving CD30 activation. Secondly, the mode of receptor cross-linkage influences the transduced signal, and finally, the receptor density on the lymphoma cell contributes to signaling effects. It might be possible that under starving conditions the level of membrane-bound CD30 changes, increasing susceptibility to CD30L-mediated cell death, as we have observed for Mac2B and JB6, or vice versa. These questions will be addressed in additional experiments. Interestingly, Huber et al. (57) observed different effects of CD30 activation in Karpas 299 and JB6 lymphoma cell lines. Whereas Karpas 299 responded with growth inhibition, JB6 appeared unaffected by treatments with anti-CD30Abs. Consistent with our results (23), growth arrest of Karpas 299 cells correlated with up-regulation of the cell cycle inhibitor p21 rather than the induction of apoptosis (57).

Other experimental approaches indicate that CD30 can serve as a suitable target for tumor lysis (29, 58, 59). Hombach et al. (58) demonstrated that lymphoma cell cross-priming with recombinant anti-CD30-γ receptor-transfected T cells can effect specific lysis of lymphoma cells. We had already shown that an immunotoxin consisting of Saporin-6 conjugated to an anti-CD30 Ab, Ber-H2, has potent antitumor activity in vitro and in severe combined immunodeficiency disease mice engrafted with human CD30+ systemic ALCL (29). Huhn et al. (59) found that a newer CD30L-based fusion toxin (Ang-CD30L) using the human RNAase angiogenin, exhibits specific cytotoxicity against CD30-positive Hodgkin’s lymphoma cell lines. Our current study suggests that naked CD30L, without toxin, also has the potential for treatment of human CD30+ ALCL. We provide evidence that CD30 ligation can inhibit cell proliferation; additionally, we show a slightly increased rate of apoptosis that can be enhanced when cells were kept under starving culture conditions with the exception of Karpas 299 cells. Karpas 299 cells, in contrast, exhibit reduced apoptosis levels and proliferation under starving culture conditions indicating a cell cycle arrest.

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Proliferation of CD30+ T-Helper 2 Lymphoma Cells Can Be Inhibited by CD30 Receptor Cross-Linking with Recombinant CD30 Ligand

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