CD30 Signals Integrate Expression of Cytotoxic Effector Molecules, Lymphocyte Trafficking Signals, and Signals for Proliferation and Apoptosis

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CD30 Signals Integrate Expression of Cytotoxic Effector Molecules, Lymphocyte Trafficking Signals, and Signs for Proliferation and Apoptosis

Hiromi Muta, Lawrence H. Boise, Lei Fang, and Eckhard R. Podack

Although CD30 has long been recognized as an important marker on many lymphomas of diverse origin and as activation molecule on B cells and T cells, its primary function has remained obscure. We now report that CD30 signals may serve to inhibit effector cell activity by integrating gene expression changes of several pathways important for cytotoxic NK and T cell effector function. In the large granular lymphoma line YT, CD30 signals down-regulate the expression of cytotoxic effector molecules, Fas ligand, perforin, granzyme B, and abrogate cytotoxicity. c-myc, a regulator of proliferation and an upstream regulator of Fas ligand expression, is completely suppressed by CD30. Furthermore, CD30 signals strongly induce CCR7, suggesting a role for CD30 signals in the homing of lymphocytes to lymph nodes. The up-regulation of Fas, death receptor 3, and TNF-related apoptosis-inducing ligand by CD30 indicates an increase in susceptibility to apoptotic signals whereas up-regulation of TNFR-associated factor 1 and cellular inhibitor of apoptosis 2 protect cells from certain types of apoptosis. Using gene microarrays, 750 gene products were induced and 90 gene products were suppressed 2-fold by CD30 signals. Signals emanating from CD30 use both TNFR-associated factor 2-dependent and -independent pathways. The integration of CD30 signals in a lymphoma line suggests that CD30 can down-modulate lymphocyte effector function and proliferation while directing the cells to lymph nodes and increasing their susceptibility to certain apoptotic signals. These studies may provide a molecular mechanism for the recently observed CD30-mediated suppression of CTL activity in vivo in a diabetes model. The Journal of Immunology, 2000, 165: 5105-5111.

The primary biological function of CD30 has been difficult to establish due to the pleiotropic nature of CD30 signals and the transience of CD30 expression in normal lymphocytes. Mice lacking a functional CD30 gene product have a defect in negative selection of thymocytes (21), whereas CD30-transgenic mice expressing CD30 in the thymus have enhanced activity for thymic negative selection (22). In accordance with this function in negative selection in the thymus, CD30L is expressed in the Hassal bodies of the thymus (23). Recently, a function of CD30 in modulating cytotoxic responses was reported by using TCR-transgenic, autoreactive, CD30-deficient CD8 T cells in a diabetes model. CD30-deficient T cells were 6000 times more active in causing autoimmune diabetes than their CD30-sufficient counterparts (24, 25). These data suggested that CD30 is an important negative regulator for cytotoxic lymphocytes in vivo. We have reported previously that signals emanating from CD30 down-regulate the cytotoxic activity of the large granular lymphocyte (LGL) lymphoma line, YT, for B7-expressing target cells (26). CD30 signals down-regulated CD28, which is required for recognition and triggering of cytotoxicity by YT (27).

Even though the signaling pathways of CD30 and the transcription factors activated by CD30 signals are well characterized, little is known about the genes targeted by CD30 for induction or repression. In part this is due to the difficulty of obtaining sufficient numbers of homogeneous lymphocyte populations expressing CD30 that would be suitable for analysis. The YT lymphoma, expressing high levels of CD30 constitutively and endowed with cytotoxic activity, therefore, is an ideal model to further define genes regulated by CD30 signals. As reported below, we find that CD30 down-regulates the expression of major effector molecules for cytotoxicity and represses genes required for proliferation. In
addition, CD30 up-regulates molecules critical for lymphocyte homing and increases the expression of proapoptotic and antiapoptotic molecules. The regulation of the expression of multiple gene products by CD30 can be interpreted as the synergistic suppression of cytotoxic cells by terminating cytotoxicity, diminishing proliferation, redirecting lymphocytes toward lymph nodes rather than inflammatory sites, and rendering them more susceptible to certain proapoptotic signals.

Materials and Methods

Cells

YT cells were cultured in Iscove’s modified Dulbecco’s MEM containing 10% heat-inactivated FBS and were treated with 5 μg/ml of the agonistic anti-human CD30 mAb C10 (26). Treated cells were harvested at different time points and analyzed.

To block TRAF2 signal transmission, YT cells were stably transfected with an expression vector containing truncated, dominant negative, murine dominant negative (DN)-TRAF2 using the Gene Pulser (Bio-Rad, Hercules, CA).

Multiprobe RNase protection assay

Total RNA was extracted from YT cells using the RNeasy Midi kit (Qiagen, Chatsworth, CA). Multiprobe RNase protection assay (PharMingen, San Diego, CA) was performed with 10 μg of total RNA according to the manufacturer’s instruction. Briefly, isolated RNA was hybridized for 17 h at 56°C with 32P-labeled multiprobe template sets and then treated with RNase. Protected RNA fragments were resolved on polyacrylamide gels and radioactive signals were analyzed by a phosphor imager (Bio-Rad). Digitized signals were normalized using as reference the signals of the housekeeping genes L32 or GAPDH. The relative expression levels of the gene products under analysis are presented as percentage to their expression in untreated cells, which is set to 100%.

RT-PCRs

One microgram of total RNA was used to synthesize cDNA using the SuperScript Preamplification System (Life Technologies, Rockville, MD). For PCR, 1/200 of the volume of the first-strand cDNA sample was used for amplification of c-myc, CCR7, or β-actin. The primers used for PCR were as follows: CCR7 (sense) 5'-TGGTGACTCTCTCTTGTATT-3', CCR7 (antisense) 5'-GGTGTAGCACAAGAGTGAAG-3'; c-myc (sense) 5'-ACTGGAGGAGGAAGAAC-3', c-myc (antisense) 5'-AAAGC CGCTCCACATACTGCT-3'; β-actin (sense) 5'-TCTGGCACCACAC TCTTAC-3', β-actin (antisense) 5'-GAAGAGAAGCTGGAAGAGTG-3'. The PCR products were resolved on agarose gels, visualized by ethidium bromide staining and quantitated using the Molecular Analyst software (Bio-Rad). β-actin was used as internal standard for normalization and relative expression levels of c-myc and CCR7 were expressed as percentage of untreated cells set to 100%.

Western blotting

YT cells were lysed in 150 mM NaCl, 1.0% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris (pH 8.0), with 1 μg/ml of each inhibitor, aprotinin, leupeptin, and pepstatin, and 1 mM PMSF. A total of 50 μg of the whole-cell lysate protein was analyzed on 10% SDS-polyacrylamide gels and blotted with anti-human TRAF1, TRAF2, and TRAF3 antisera, respectively (Santa Cruz Biotechnology, Santa Cruz, CA). Abs were detected by chemiluminescence (enhanced chemiluminescence; Amersham Pharmacia Biotech, Piscataway, NJ).

Flow cytometry

YT cells were cultured with or without C10 for 24 h, and 1 × 10^6 cells of each sample were stained with FITC conjugated anti-human Fas Ab or anti-human CD28 (PharMingen) and analyzed on a FACSscan flow cytometer (Becton Dickinson, Franklin Lakes, NJ).

Chromium release (cytotoxicity) assay

P815 or P815-Fas cells were labeled with 51Cr for 2 h and washed three times with PBS. Cytotoxicity of YT cells against P815 or P815-Fas cells was examined in 6-h assays in triplicate in 96-well microtiter plates at 1:1, 3:1, 10:1, and 30:1 E:T ratios. Results are expressed as percent specific lysis relative to HCl-lysed controls by subtracting the values of spontaneous Cr release, ranging between 5 and 12%.

LYMPHOCYTE EFFECTOR DOWN-REGULATION BY CD30

Gene microarray analysis

Human UniGem Microarrays (Genome Systems, St. Louis, MO) containing cDNAs of 4000 named genes and an equal number of expressed sequence tags (ESTs) were used to analyze the induction or suppression of mRNAs by CD30 signals. mRNA was extracted from untreated and C10-treated (17 h) YT cells according to the manufacturer’s instructions. After conversion to cDNA and differential fluorescent labeling, the fluorescent probes of C10-treated and untreated YT cells were mixed and hybridized to the gene microarrays. Differential binding of the two probes to the arrays is analyzed by the manufacturer and normalized according to known reference markers on the gene chip. A ratio of binding of one of the two probes indicates no change in gene expression due to CD30 signals. The data are given as percent induction or suppression of gene products by CD30 signals relative to untreated YT cells.

Results

CD30 signals down-regulate Fas ligand (Fas-L) and up-regulate Fas and TNF-related apoptosis-inducing ligand (TRAIL)

CD30 signals are transmitted through adaptor proteins of the TRAF family, resulting in the activation of transcription factors including NF-kB and Jun N-terminal kinase. The target genes addressed by CD30 signals through these and other transcription factors remain largely undefined. Because few lymphocytes express CD30 under physiological conditions and CD30 expression is usually transient, it is difficult to obtain information about genes regulated by CD30. Using the YT cell line (28), a LGL lymphoma with cytotoxic activity constitutively overexpressing CD30, we generated CD30 signals using an agonistic anti-CD30 Ab, C10 (26), and studied the regulation of several groups of genes by CD30 signals.

We have published previously that CD30 signals induced the loss of YT cytotoxicity toward B7-expressing targets (26). Loss of cytotoxicity correlated with the down-regulation of CD28, which is known to be required for YT killing of B7-expressing target cells (27). In the current study, we investigated whether cytotoxic effector molecules and other gene products expressed by YT were regulated by CD30. YT cells contain cytolytic granules containing perforin and granzyme B and express functional Fas-L. To obtain reliable data on several groups of genes, the analyses combined the results of RNA expression with protein expression and with functional data. RNA expression data were obtained by several methods: RNase protection assays, RT-PCR assays, or gene microarray assays. Protein products were assayed by Western blot, flow cytometry, or by functional assays. In all cases, CD30 signals were examined by incubating YT cells with 5 μg/ml of the agonistic C10 Ab for various periods of time followed by the analysis of RNA levels, protein levels, or function.

To study regulation of Fas-L and a group of other gene products involved in regulating apoptosis by CD30, RNA protection assays were used (Figs. 1 and 2). The images of radiolabeled, hybridized, protected RNA fragments were quantitated and normalized by reference to housekeeping genes L32 or GAPDH using the phosphor imager. Agonistic anti-CD30 treatment of YT completely down-regulated Fas-L expression within 6 h. In contrast, TRAIL, Fas, and death receptor 3 (DR3) were up-regulated from 2- to 4-fold by CD30 signals; DR3 is only weakly expressed by YT whereas TRAIL showed a somewhat higher level of expression. The expression of other gene products such as caspase 8, Fas-associated factor, Fas-associated death domain protein, TNFR1, TNFR-associated death domain protein, and receptor-interacting protein, involved in signaling for apoptosis, was not significantly affected by CD30 signals (Figs. 1 and 2). It is evident from these regulatory effects that CD30 signals are highly specific for selected gene products rather than globally affecting transcriptional activity of proapoptotic and antiapoptotic genes.

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TRAF2 transmits signals for Fas and Fas-L regulation by CD30

To determine whether the TRAF2-dependent pathway was used by CD30 signals for the regulation of death receptor and ligand genes, YT cells were transfected with the DN mutant of TRAF2 (Fig. 2). DN-TRAF2 protein, after selection in G418, was expressed at a high level in bulk-transfected, uncloned YT, as seen in Western blots (Fig. 2A). The presence of DN-TRAF2 blocked CD30-mediated down-regulation of Fas-L by >50%. Similarly, DN-TRAF2 inhibited the up-regulation of Fas to the same degree (Figs. 2 and 3C). CD30 signals regulating the expression of death receptors and ligands, therefore, use primarily the TRAF2 pathway. These analyses were repeated in three independent experiments with essentially identical results. The effects of CD30 signals were also detected at the protein level or by functional assays.

Fas-L expression on untreated YT cells predicted that YT should induce apoptosis of Fas-expressing target cells. Fas-transfected P815 (P815-Fas), but not wild-type P815 (Fig. 3A) are indeed lysed by YT in 6-h assays, proving the use of Fas-L for the lytic activity. Treatment of YT with anti-CD30 Ab (C10) for 16 h completely abolished lysis of P815-Fas by YT, indicating that Fas-L protein in addition to its mRNA is down-regulated by CD30 signals. In DN-TRAF2-transfected YT in contrast, P815-Fas lysis was not inhibited by C10 treatment of YT cells (Fig. 3B).

In accord with the RNase protection assay, CD30 signals up-regulated Fas protein expression by YT about 3-fold as measured in Western blots, whereas CD28 expression, as reported previously (26), decreased 3-fold. Both effects were blocked by the presence of DN-TRAF2 in YT (Fig. 3, C–F).

CD30 signals down-regulate perforin and granzyme B expression

We next determined whether the effector molecules perforin and granzyme B, expressed by YT cells, were regulated by CD30 similar to Fas-L. YT cells do not express granzyme A. The analysis was conducted by RNase protection assay and by hybridization to gene microarrays of fluorescently labeled cDNAs obtained from untreated YT and from cells treated for 17 h with C10, respectively. The microarray analysis gives information about the absolute level of gene expression (Table I, right column) and relative changes following CD30 signaling. For validation, the results of the microarray analysis were confirmed for the relevant genes by independent assays for RNA expression (Table I). Quantitative analysis of normalized expression of perforin and granzyme B mRNA after CD30 signaling revealed a 2-fold suppression from 100 to about 50% of the RNA levels for both gene products in both microchip assays and by RNase protection assay (Table I). Cathepsin C mRNA, coding for a lysosomal protease related to granzyme B, was also suppressed. Signals from CD30 thus down-regulate the two major cytotoxic pathways of lymphocytes; the apoptotic pathway important for lymphocyte homeostasis by down-regulating Fas-L and the cytolytic pathway important for immune defense by suppressing perforin and granzyme B expression. Since granzymes and perforin are stored in cytoplasmic granules, the effect of decreased mRNA levels will have only a delayed effect on the corresponding protein levels.
Fas-L expression in activated T cells via TGF-β up-regulate CCR7. CD30 signals completely down-regulated c-myc expression in YT within 24 h. Suppression of c-myc by CD30 therefore appears to be responsible for the suppression of downstream Fas-L. β-actin used as control in the RT-PCR analysis was not affected by CD30 signals.

Gene microarray analysis gives simultaneous information about the regulation of many gene products. We found a good correlation between gene microarray assays and assays for individual gene expression by other techniques (Table I). The microarray allows one to obtain a global overview of gene regulation in addition to the assessment at the individual gene level. In a global analysis of the microarray assay, CD30 signals up-regulated many more gene products than were down-regulated by CD30. We used as threshold for significant regulation a 2-fold or greater change of gene expression induced by CD30 (Table II). Of the ~8000 gene products analyzed by microarray analysis, 750 were up-regulated >2-fold by CD30, including ESTs, whereas only 90 gene products, including ESTs, were down-regulated to a similar extent (Table II). The remaining gene products were either not expressed by YT or not significantly regulated by CD30 signals. Twenty-four of the most highly induced or suppressed gene products by CD30 signals are given in Tables III and IV.

One of the up-regulated gene products that was discovered by microarray analysis is the chemokine receptor CCR7. Together with other receptors, CCR7 controls lymphocyte homing (30–32). CD30 signals increased the expression of CCR7 mRNA 5.8-fold by gene chip analysis. The microarray analysis was confirmed by RT-PCR of the same samples that were also used for RT-PCR of c-myc and quantitated relative to actin (Fig. 5). CCR7 mRNA was induced by CD30 signaling almost 3-fold within 24 h above the level of unstimulated YT as measured by PCR. Expression of CCR7 during the next 72 h slowly decreased (Fig. 5). According to microarray analysis, CCR5 and CCR6 are also expressed by YT cells but not affected by CD30 signals, whereas CCR1 and CCR2 and CXCR1 are not expressed.

CD30 signals suppress expression of c-myc and up-regulate CCR7

Expression correlates with cell proliferation and is known to regulate Fas-L expression in activated T cells via TGF-β (29).

Since Fas-L mRNA was completely down-regulated by CD30 signals, we investigated whether c-myc expression was also suppressed (Table I and Fig. 5). Using gene microarray analysis and RT-PCR, c-myc was found to be significantly suppressed by CD30 signals, despite the finding that proliferation was not significantly affected at that time (data not shown). In timed RT-PCR analysis, CD30 signals completely down-regulated c-myc expression in YT within 24 h. Suppression of c-myc by CD30 therefore appears to be responsible for the suppression of downstream Fas-L. β-actin used as control in the RT-PCR analysis was not affected by CD30 signals.

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Table I. mRNA expression levels in YT in the presence of CD30 signals

<table>
<thead>
<tr>
<th>Gene Product</th>
<th>RNAse protection (% of control)</th>
<th>RT-PCR (% of control)</th>
<th>Microarray (% of control)</th>
<th>Microarray, untreated control (arbitrary units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perforin</td>
<td>44</td>
<td>ND</td>
<td>50</td>
<td>2460</td>
</tr>
<tr>
<td>Granzyme B</td>
<td>58</td>
<td>ND</td>
<td>50</td>
<td>4237</td>
</tr>
<tr>
<td>Cathepsin C</td>
<td>ND</td>
<td>ND</td>
<td>43</td>
<td>1692</td>
</tr>
<tr>
<td>c-myc</td>
<td>ND</td>
<td>0</td>
<td>52</td>
<td>2043</td>
</tr>
<tr>
<td>CCR7</td>
<td>300</td>
<td>580</td>
<td>316</td>
<td></td>
</tr>
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</table>

*Analysis of expression of selected gene products by microarray analysis, RT-PCR, and RNase protection assay. YT cells were untreated or treated for 17 h with the agonistic anti-CD30 Ab C10 and used for RNA isolation. RNA was analyzed directly by RNase protection assay or converted to cDNA for analysis by RT-PCR or microarray analysis. RT-PCR data are normalized with reference to β-actin and expressed as percentage of the control (untreated YT). Microarray analysis was done by differentially labeling the cDNAs from untreated and C10-treated YT and hybridization to 8000 cDNAs printed on gene chips. Results are expressed as percentage of untreated control YT cells. The arbitrary units in the untreated control column correlate with absolute mRNA expression levels of untreated YT; the values represent the relative levels of untreated YT cDNA.
signals. CD30 signals likewise strongly induced TRAF1 mRNA (Fig. 6, A and B) and protein (Fig. 6C) within 6 h to about 12-fold above the level of unstimulated cells as measured by RNase protection assay. TRAF1 levels subsequently slowly decreased over 72 h to 6-fold above that in control cells. TRAF2 and TRAF4 levels did not change upon CD30 signaling; TRAF3 was not expressed by YT. The apoptosis inhibitor cIAP2, which can block activation of caspase 3, 7, and 8, was induced to a similar extent and with similar kinetics as TRAF1. In Western blots, TRAF1 protein was also induced in accordance with the results of RNase protection assay, whereas TRAF2 protein remained unchanged during the entire time course of CD30 signaling (Fig. 6C). TRAF3 was not detected in Western blots (data not shown). The presence of DN-TRAF2 in YT had little effect on TRAF1 and cIAP2 induction by CD30 in YT, in contrast to its effect on Fas-L, suggesting that these genes are induced independent of the participation of TRAF2 (Fig. 6D).

Discussion
The genes targeted by CD30 for induction or for down-regulation as revealed in this study can be grouped into distinct functional categories: cytotoxic and proapoptotic activity, proliferation, lymphocyte traffic, and susceptibility to apoptosis. The concerted modulation of gene expression by CD30 signals as described here has the potential to down-regulate cytolytic lymphocyte effector function by several synergistic pathways. Although the data were obtained with a cytotoxic lymphoma line expressing CD30, the pathways and gene products regulated by CD30 may also be affected in CTL and may provide a molecular mechanism for the previously reported effect of CD30 suppressing the cytotoxic activity of autoreactive, TCR-transgenic CD8 cells in a diabetes model in mice (24). Indeed, ongoing studies in our laboratory using CD30L-deficient mice confirm the suppressive effect of CD30L-CD30 interaction on CTL in vivo (H. Muta, G. Caceres, R. Levy, and E. R. Podack, unpublished observations).

The most direct effect of CD30 on cytotoxic activity is mediated via the complete down-regulation of Fas-L, eliminating the ability of YT to lyse P815-Fas. Regulation of Fas-L expression by CD30 is a novel finding and has important implications in the homeostatic regulation of lymphocytes. CD30 also down-regulates perforin and granzyme B. Since perforin and granzyme B are storage proteins, the effect of transcriptional regulation on protein levels depends on the half-life of the cytotoxic proteins and may take

![FIGURE 4](44x454) Suppression by C10 of perforin and granzyme B mRNA expression in YT and in DN-TRAF2-transfected YT upon CD30 signaling (5 µg C10, 24 h). Upper panel, Analysis by RNase protection assays. Lower panels, Quantitative analysis of perforin and granzyme B expression by phosphor imager analysis and of the RNA protection array and normalization with GAPDH.

![FIGURE 5](44x454) Suppression of c-myc and up-regulation of CCR7 by CD30 signals. RT-PCR analysis of c-myc, CCR7, and β-actin in YT following CD30 signaling after culture for the indicated time with 5 µg/ml C10 (lower panel). The ethidium bromide-stained bands are normalized by the use of β-actin and quantitated relative to untreated YT cells which are set to 100%.

Table II. Effect of CD30 signals on the expression of multiple genes microarrayed on gene chips for analysis

<table>
<thead>
<tr>
<th>Effect of CD30 Signals</th>
<th>No. of Gene Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Up-regulated &gt;2-fold⁴</td>
<td>750</td>
</tr>
<tr>
<td>Up-regulated 1.4–2-fold</td>
<td>1600</td>
</tr>
<tr>
<td>Not significantly regulated</td>
<td>2560</td>
</tr>
<tr>
<td>Down-regulated 1.4–2-fold</td>
<td>510</td>
</tr>
<tr>
<td>Down-regulated &gt;2-fold</td>
<td>90</td>
</tr>
<tr>
<td>Genes not expressed by YT</td>
<td>3000</td>
</tr>
</tbody>
</table>

⁴ Fold regulation refers to the difference to the uninduced state (without anti-CD30).

![Table III](44x479) Genes and ESTs up-regulated by CD30

<table>
<thead>
<tr>
<th>Name</th>
<th>GenBank ID</th>
<th>Fold induction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homo sapiens BCE-1</td>
<td>AI248988</td>
<td>8</td>
</tr>
<tr>
<td>Chemokine receptor 7</td>
<td>L08176</td>
<td>5.8</td>
</tr>
<tr>
<td>Liver carboxylesterase-2</td>
<td>U60553</td>
<td>5.2</td>
</tr>
<tr>
<td>Apoptosis inhibitor 2</td>
<td>AI581499</td>
<td>4</td>
</tr>
<tr>
<td>EST</td>
<td>AI081356</td>
<td>3.6</td>
</tr>
<tr>
<td>Human mRNA for KIAA0033 gene</td>
<td>D26067</td>
<td>3.2</td>
</tr>
<tr>
<td>Cadherin 14</td>
<td>D83542</td>
<td>3.2</td>
</tr>
<tr>
<td>EST</td>
<td>AI341451</td>
<td>3.1</td>
</tr>
<tr>
<td>rab 8-interacting protein</td>
<td>AF900145</td>
<td>3.1</td>
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<tr>
<td>Brain-heart-protocadherin</td>
<td>AB006757</td>
<td>3</td>
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<tr>
<td>EST</td>
<td>AI692207</td>
<td>3</td>
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<td>EST</td>
<td>Incyte 2598990</td>
<td>3</td>
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<tr>
<td>Pre-B cell leukemia</td>
<td></td>
<td></td>
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<tr>
<td>transcription factor 3</td>
<td>X59841</td>
<td>2.9</td>
</tr>
<tr>
<td>EST</td>
<td>N51730</td>
<td>2.9</td>
</tr>
<tr>
<td>KIAA0432</td>
<td>AA219672</td>
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</tr>
<tr>
<td>Vinculin</td>
<td>M33308</td>
<td>2.9</td>
</tr>
<tr>
<td>Aldehyde dehydrogenase</td>
<td>M63967</td>
<td>2.9</td>
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<tr>
<td>Caspase 1</td>
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<td>EST</td>
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<tr>
<td>Phosphofructokinase</td>
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<td>AI609112</td>
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<td>EST</td>
<td>AI298083</td>
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⁴ Twenty-four of the most highly up-regulated genes, as detected by gene microarray analysis, are listed.
several days to become detectable. CD30 thus suppresses the granule-dependent and granule-independent cytotoxic pathways of cytolytic lymphocytes. A similar effect of CD30 signals on cytotoxic lymphocytes infiltrating tissues in vivo, that may be mediated by local CD30L expression on monocyte/macrophages or activated T cells, is likely to substantially dampen cytotoxic activity in situ.

Down-regulation of Fas-L by TGF-β is mediated by c-myc; the DN c-myc mutant has been shown to block TGF-β-mediated Fas-L down-regulation (32). CD30 signals, as shown here, completely down-regulate c-myc in addition to Fas-L, supporting the link between c-myc and Fas-L expression. The down-regulatory signal for c-myc and Fas-L is traveling via TRAF2, because DN-TRAF2, the DN mutant, blocks the effect of CD30 signaling on Fas-L down-regulation. The down-regulation of c-myc by CD30 may also be responsible for the diminished proliferative capacity of CD30-expressing Ag-specific CTL in tissues (24).

The strong up-regulation of CCR7 by CD30 was an unexpected finding resulting from gene microarray analysis. CCR7 expression allows cells to enter lymph nodes via secondary lymphoid tissue chemokine expressed on high endothelial venules (29–31). CCR7-expressing, central memory T cells have reduced effector function, including sharply diminished cytokine production (31). Our studies show that CD30 signals can strongly up-regulate CCR7, coincident with the loss of cytotoxic function. CD30L signals delivered to CD30 expresing tissue-infiltrating cytotoxic cells, therefore, have the potential to shut off cytotoxicity within the tissue and redirect CTL to the draining lymph node.

CD30 signals can up-regulate Fas and DR3, making cells potentially more susceptible to apoptotic signals by Fas-L or the DR3-L. It is possible that cells that are redirected to lymph nodes via CCR7 expression, reencounter APCs and undergo activation-induced cell death due to the increased level of Fas and DR3 expression. The concurrent up-regulation by CD30 of antiapoptotic genes like TRAF1 and cIAP2 on the other hand, which is associated with resistance to TNF-induced apoptosis and to caspase 3, 7, and 8 activation, may render the cells more resistant to specific apoptotic signals (33, 34). Among CD4 cells, CCR7 is expressed exclusively on TH1 cells (29). Reentry into lymph nodes of CCR7-positive memory cells triggered by CD30 and expression of antiapoptotic molecules, therefore, may serve to provide TH1 helper function for the generation of additional effector cells. Further studies will be needed to understand the role of CD30 regulating lymphocyte traffic and apoptosis in vivo.

The importance of TRAF2 in mediating CD30 signals is well documented (18, 20). As shown here, TRAF2 is used by CD30 for up- or down-regulating many genes on YT including Fas-L, CD28, and Fas. Interestingly, however, the up-regulation by CD30 of TRAF1 and cIAP2 is not dependent on TRAF2 since the DN mutant was unable to block the induction of these genes. It has been reported previously, and is confirmed here, that TRAF-independent signaling can emanate from CD30 (35). However, the molecular nature of that signaling pathway remains to be defined.

The analysis of gene products modulated by CD30 signals on an LGL lymphoma allows the formulation of the hypothesis that CD30 terminates cytotoxic lymphocyte responses by several synergistic pathways. The molecular mechanisms appear to comprise a synergistic pattern for suppression of cytotoxicity by decreasing the expression of cytotoxic effector molecules, down-regulating cosimulatory CD28, slowing down proliferation, potentially redirecting lymphocyte traffic, and increasing lymphocyte susceptibility to apoptosis. It remains to be established that these molecular processes can be induced by CD30 signals in vivo for the generation of additional effector cells. Further studies will be needed to understand the role of CD30 regulating lymphocyte traffic and apoptosis in vivo.

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mechanisms occur in T cells in vivo due to local CD30L expression. The reported finding that CD30 dampens cytotoxic activity of CTL in a diabetes model in mice (24) provides strong support for this hypothesis. Moreover, in our own initial studies, CD30L-deficient mice show increased in vivo CTL activity supporting the role of CD30 as a negative regulator of cytotoxic lymphocytes.

References


