CD30 Is Involved in Inhibition of T-Cell Proliferation by Hodgkin’s Reed-Sternberg Cells

Che-Chun Su, Hsin-Hui Chiu, Chia-Che Chang, Jui-Chieh Chen, and Su-Ming Hsu

1Graduate Institute of Immunology and 2Graduate Institute of Biochemistry and Molecular Biology, National Taiwan University College of Medicine, Taipei, Taiwan; 3Department of Pediatrics, China Medical College Hospital, Taichung, Taiwan; and Institute of Biomedical Sciences, National Chung Hsing University, Taichung, Taiwan

ABSTRACT

CD30 is expressed on Hodgkin’s Reed-Sternberg (H-RS) cells, the tumor cells in Hodgkin’s disease. Increased levels of serum CD30 are observed in Hodgkin’s disease patients and are a good marker for predicting a poor prognosis and a poor response to therapy. In this study, we addressed the effect of CD30 on T cells. We showed that CD30, either as a membranous protein on H-RS cells and Chinese hamster ovary cells or as a plate-bound chimeric protein, inhibited T-cell proliferation. Anti-CD3-stimulated T cells in the presence of CD30 failed to increase tritium uptake and failed to express CD25 and CD26 and to produce interleukin 2. The inhibition of T-cell proliferation was, however, reversed with addition of exogenous interleukin 2 or pretreatment of H-RS cells with anti-CD30. Inability of T cells to express CD25 and CD26 in cocultures with H-RS cells or a plate-bound CD30 chimeric protein is in accordance with the results of immunohistochemistry on disease-involved tissues. We conclude that H-RS cells are able to inhibit the proliferation and activation of T cells through CD30-related interaction. The outcome of CD30-related interaction is an ineffective antitumor immunity, which is clearly in favor of the growth and survival of the tumor cells.

INTRODUCTION

Hodgkin’s disease (HD) is characterized by numerous infiltrating immune cells surrounding the malignant cells, which make up less than 1% of the total cell population in the tumor. How these tumor cells can survive in such a seemingly hostile environment has puzzled researchers (1). If left untreated, patients with HD will have a fatal course. This outcome manifests the failure of the reactive immune cells to eradicate the tumor cells and at the same time underscores the success of the tumor cells in evading attacks by these immune cells.

The neoplastic cells (i.e., Hodgkin’s Reed-Sternberg (H-RS) cells) in HD are characterized by abundant expression of a type I membrane protein, CD30, a member of the tumor necrosis factor receptor superfamily (2, 3). CD30 is rarely expressed by tumor cells from most T- or B-cell lymphomas and by most normal tissues (4). The restricted expression profile of CD30 explains why it is being used as a disease marker for B-cell lymphomas and by most normal tissues (4). The restricted expression profile of CD30 explains why it is being used as a disease marker for B-cell lymphomas and by most normal tissues (4).

CD30 is expressed on Hodgkin’s Reed-Sternberg (H-RS) cells, the tumor cells in Hodgkin’s disease. Increased levels of serum CD30 are observed in Hodgkin’s disease patients and are a good marker for predicting a poor prognosis and a poor response to therapy. In this study, we addressed the effect of CD30 on T cells. We showed that CD30, either as a membranous protein on H-RS cells and Chinese hamster ovary cells or as a plate-bound chimeric protein, inhibited T-cell proliferation. Anti-CD3-stimulated T cells in the presence of CD30 failed to increase tritium uptake and failed to express CD25 and CD26 and to produce interleukin 2. The inhibition of T-cell proliferation was, however, reversed with addition of exogenous interleukin 2 or pretreatment of H-RS cells with anti-CD30. Inability of T cells to express CD25 and CD26 in cocultures with H-RS cells or a plate-bound CD30 chimeric protein is in accordance with the results of immunohistochemistry on disease-involved tissues. We conclude that H-RS cells are able to inhibit the proliferation and activation of T cells through CD30-related interaction. The outcome of CD30-related interaction is an ineffective antitumor immunity, which is clearly in favor of the growth and survival of the tumor cells.

CD30 is originally thought to be a receptor that carried out its function through engagement with its respective ligand, CD30L. However, recent studies have shown a bidirectional mechanism leading to the growth and survival of H-RS cells. This outcome manifests the failure of the reactive immune cells to eradicate the tumor cells and at the same time underscores the success of the tumor cells in evading attacks by these immune cells.

The neoplastic cells [i.e., Hodgkin’s Reed-Sternberg (H-RS) cells] in HD are characterized by abundant expression of a type I membrane protein, CD30, a member of the tumor necrosis factor receptor superfamily (2, 3). CD30 is rarely expressed by tumor cells from most T- or B-cell lymphomas and by most normal tissues (4). The restricted expression profile of CD30 explains why it is being used as a disease marker for HD, and it suggests the possible involvement of CD30 in a yet undefined mechanism leading to the growth and survival of H-RS cells.

CD30 was originally thought to be a receptor that carried out its function through engagement with its respective ligand, CD30L, a member of the tumor necrosis factor receptor superfamily (2, 3). CD30 is rarely expressed by tumor cells from most T- or B-cell lymphomas and by most normal tissues (4). The restricted expression profile of CD30 explains why it is being used as a disease marker for HD, and it suggests the possible involvement of CD30 in a yet undefined mechanism leading to the growth and survival of H-RS cells.

CD30 is expressed on Hodgkin’s Reed-Sternberg (H-RS) cells, the tumor cells in Hodgkin’s disease. Increased levels of serum CD30 are observed in Hodgkin’s disease patients and are a good marker for predicting a poor prognosis and a poor response to therapy. In this study, we addressed the effect of CD30 on T cells. We showed that CD30, either as a membranous protein on H-RS cells and Chinese hamster ovary cells or as a plate-bound chimeric protein, inhibited T-cell proliferation. Anti-CD3-stimulated T cells in the presence of CD30 failed to increase tritium uptake and failed to express CD25 and CD26 and to produce interleukin 2. The inhibition of T-cell proliferation was, however, reversed with addition of exogenous interleukin 2 or pretreatment of H-RS cells with anti-CD30. Inability of T cells to express CD25 and CD26 in cocultures with H-RS cells or a plate-bound CD30 chimeric protein is in accordance with the results of immunohistochemistry on disease-involved tissues. We conclude that H-RS cells are able to inhibit the proliferation and activation of T cells through CD30-related interaction. The outcome of CD30-related interaction is an ineffective antitumor immunity, which is clearly in favor of the growth and survival of the tumor cells.

MATERIALS AND METHODS

Cell Lines and Cell Culture. H-RS cell lines (KM-H2, HDLM, and L428; Ref. 19) and U937 cells were maintained in complete medium composed of RPMI 1640 (Life Science Technologies, Gaithersburg, MD) supplemented with 10% fetal bovine serum, 2 mm t-glutamine, 1% sodium pyruvate, and 100 units/ml penicillin and streptomycin. Chinese hamster ovary (CHO) cells were purchased from the American Type Culture Collection (Manassas, VA) and maintained in complete medium composed of DMEM (Life Science Technologies) with the same supplement as described above.

Construction of CD30 Expression Plasmids. CD30 cDNA was generated by PCR from a cDNA pool of L428, a Hodgkin’s lymphoma cell line, by use of the sense oligonucleotides 5′-TTAAAGCCTGATGCCGGTTC-TCCCTCG and the antisense oligonucleotides 5′-TTGGAAATCCCTACCTTCCAGGAGCAGC. The resulting product was cloned between the HindIII and BamHI sites of the plasmid pcDNA3 (Invitrogen, San Diego, CA) to create the plasmid pcDNA3-CD30. Enhanced green fluorescent protein (EGFP) fusion constructs were created by use of the pEGFP-N1 vector (Clontech Laboratories, Palo Alto, CA) and the CD30 gene to create pEGFP-CD30. The cDNAs generated to fuse with EGFP lacked a STOP codon and were cloned between the HindIII and BamHI sites of the plasmid, upstream of and in frame with the EGFP coding region.

Transfection and Selection of CD30-Expressing Stable Clones. CHO cells were grown in DMEM containing 10% fetal bovine serum and transfected with the sense oligonucleotides 5′-TTAAAGCCTGATGCCGGTTC-TCCCTCG and the antisense oligonucleotides 5′-TTGGAAATCCCTACCTTCCAGGAGCAGC. The resulting product was cloned between the HindIII and BamHI sites of the plasmid, upstream of and in frame with the EGFP coding region. The sequences of all DNA constructs were confirmed by sequencing in both directions by use of an ABI 377 automated sequencer and the ABI Prism Dye Terminator cycle sequencing system (Applied Biosystems, Foster City, CA) according to the manufacturer’s recommended procedure. Sequences were analyzed with Sequencer 3.1.1 software (Gene Codes, Ann Arbor, MI).

Received 5/19/03; revised 1/18/04; accepted 1/20/04.

Grant support: National Health Research Institute Grant NHRI-EX91-8704SL; National Science Council Grants NSC 892314B039009 (C.-C.Su), 892314B039026 (C.-C.Su), and NSC 912320B039030 (H.-H.Chiu); National Taiwan University Grant 89-B-FA01-1-4.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Su-Ming Hsu, Graduate Institute of Immunology, National Taiwan University College of Medicine, 1 Jen-Ai Road, Taipei, Taiwan. Phone: 886-2-23123456, extension 5781; Fax: 886-2-23947927; E-mail: smhsu@ha.mc.ntu.edu.tw.

2148

Downloaded from cancerres.aacrjournals.org on September 13, 2017. © 2004 American Association for Cancer Research.
with 0.6 μg DNA/10⁵ cells by use of the Effectene transfection reagent (Qiagen, Valencia, CA), according to the manufacturer’s instruction. CHO cells were selected with 1 mg/ml Genetin and analyzed after transient and stable transfection. Selected clones with strong green fluorescence were amplified first and stained with phycoerythrin-conjugated anti-CD30 to be selected further with fluorescence-activated cell sorting. To test whether EGFP induced artificial phenotypes, we conducted experiments with cells transfected with pEGFP only.

The stable clones were generally more than 99% EGFP positive and more than 90% CD30 positive, as assayed by confocal microscopy or FACSscan.

Construction and Purification of CD30-Fc Fusion Protein. The gene segment for the extracellular domain of CD30 was generated by PCR from pcDNA-CD30 by use of the sense oligonucleotides 5′-AAAACTTTCCTCCACAGGATCC-3′ and the antisense oligonucleotides 5′-TTGTCTACCTTCCCCGTTGAGGA-3′ and ligated to the gene for the Fc portion of human IgG1 (a gift from Dr. S.L. Hsieh, National Yang-Ming University, Taipei, Taiwan). The hinge region in the IgG gene was included in the construct. The gene segment for the CD30-Fc fusion protein was further transferred to the MIB vector (Invitrogen) for expression into the insect cells High Five (Invitrogen). High producers were selected with ELISA kits for the human MIB vector (Invitrogen) for transfection into the insect cells High Five (Invitrogen). High producers were selected with ELISA kits for human immunoglobulin Fc portion. Purification was done with protein A columns (Amersham Biosciences, Uppsala, Sweden). Bound antibodies were eluted with buffer containing 0.1 mol/liter glycine and 0.15 mol/liter NaCl (pH 2.4) and brought to neutral pH with 0.5 mol/liter sodium phosphate (pH 8.0). Purified proteins were dialyzed extensively with PBS and sterilized by filtration. The concentrations of proteins were determined by a bicinchoninic acid-based protein assay (Pierce, Rockford, IL).

Western Blotting. To confirm the expression of CD30-EGFP fusion proteins by the stable clones, we isolated the whole-cell lysates for immunoblotting. The cell pellets of CD30-CHO cells and CD30-CHO cells were suspended in lysis buffer containing 50 mm Tris-HCl (pH 8.0), 150 mm NaCl, 0.6 mm phenylmethylsulfonyl fluoride, 0.1% SDS, and 1% Triton X-100. The samples were incubated at 4°C for 30 min and then centrifuged at 14,000 × g for 30 min. The supernatant was collected, and total protein was quantified. Thirty micrograms of lysate or 100 ng of fusion protein were separated by 7% SDS-PAGE and transferred to a polyvinylidene difluoride membrane blocked with 5% nonfat dry milk. Immunostaining was performed with goat polyclonal primary antibodies specific for human CD30 (R&D, Minneapolis, MN), followed by incubation with rabbit polyclonal antigoat IgG antibody conjugated to horseradish peroxidase (Santa Cruz Biotechnology, Santa Cruz, CA). Immunoreactive bands were visualized with enhanced chemiluminescence reagents (New England Nuclear Life Science, Boston, MA).

Purification of T Cells and Preparation of Accessory Cells. Total peripheral blood mononuclear cells were separated on Ficoll-Hypaque (Amer sham Biosciences). CD3 T cells in peripheral blood mononuclear cells were affinity-purified by use of the Pan-T MACS isolation kit (Miltenyi Biotech, Bergisch Gladbach, Germany) with a negative selection method. The resulting purified cells were more than 95% positive for CD3. The cells retained in the column were eluted outside the magnetic field, treated with 2% paraformaldehyde (J.T. Baker, Phillipsburg, NJ) for 2 h, and washed extensively with PBS. These cells were used as accessory cells in the T-cell proliferation assay.

Effect of H-RS Cells on T-Cell Proliferation. H-RS cells (KM-H2, HDLM, and L428) and U937 cells were first treated with mitomycin C (Kyowa, Tokyo, Japan) at 50 μg/ml for 2 h and washed extensively with PBS. A standard coculture system consisted of T cells and accessory cells (each 1 × 10⁶ cells/well) and H-RS cells or U937 cells at different ratios to T cells in triplicate in U-bottomed 96-well plates. Anti-CD3 (HT13a; BD Pharmingen, San Diego, CA) at 0.5 μg/ml was added, and the plates were incubated at 37°C/5% CO₂ for 3 days. During the last 6 h of incubation, cultures were pulsed with 0.5 μCi/well [³H]thymidine. Cells were then harvested with a Packard Cell Harvester onto unfilter plates. Cell-associated radioactivity was measured by scintillation counting (Topcount scintillation counter).

To rule out the effect of cytokines secreted from viable H-RS cells, we treated H-RS cells with 2% paraformaldehyde overnight at 4°C and washed them extensively with PBS. U937 cells received the same treatment. All of the fixed cells were positively stained by trypan blue. The coculture system and the procedures used were the same as above. An additional control, H-RS cells were treated with anti-CD30 (BerH2; DAKO, Glostrup, Denmark) or anti-CD40 (BD Pharmingen) at 50 μg/ml for 2 h and washed with PBS twice before use.

Effect of CD30-CHO Cells on T-Cell Proliferation. We also used CD30-CHO cells instead of H-RS cells for studying the effect of CD30 on T-cell proliferation. CHO cells or HDLM cells were cultured in triplicate in U-bottomed 96-well plates containing CHO cells, which expressed either CD30-EGFP or EGFP. Cells were pulsed, and the cell-associated radioactivity was measured with a scintillation counter, as described above.

Effect of CD30-Fc Fusion Protein on Proliferation of Anti-CD3-Treated T Cells. To study the effect of the CD30-Fc fusion protein on T-cell proliferation, we used the plate-bound CD30-Fc fusion protein. CD30-Fc chimera protein or human IgG (Cappel, Aurora, OH) was coated at 1, 5, 10, or 50 μg/ml (50 μg/well) in carbonate-bicarbonate buffer (0.05 M (pH 9.6)) in 96-well U-bottomed tissue culture plates (Falcon; BD Biosciences, Mountain View, CA) and stored at 4°C overnight. The plates were washed with PBS before use for removal of unbound protein. Anti-CD3-treated T cells and accessory cells were added, and the plates were incubated at 37°C/5% CO₂ for 3 days. Next, cells were subjected to thymidine uptake assay, as described above.

We also used soluble CD153 to compete the interaction between plate-bound CD30 and CD153 on T cells. The experimental design was the same as above, except that for different concentrations of either human (h) CD153-mouse (m) CD8 or hCD154-mCD8 chimeric protein (ID Labs Inc., Ontario, Canada) was added.

IL-2 Measurement. T cells, accessory cells, and HM-H2 cells in different ratios were cocultured as described above. As an additional control, HM-H2 cells pretreated with anti-CD30 50 μg/ml for 2 h and washed with PBS twice before use. Plates were incubated at 37°C/5% CO₂ for 3 days. The supernatant was collected, and levels of IL-2 production were measured with an IL-2 ELISA kit (Diacalone, Besancon, France) according to the manufacturer’s protocol. In other studies, different concentrations of the plate-bound CD30-Fc chimeric protein were substituted for HM-H2 cells.

Effect of IL-2 on Proliferation of T Cells Cocultured with H-RS Cells or CD30-Fc Chimeric Protein. To study the effect of IL-2 on CD30-mediated T-cell inhibition, we added recombinant human IL-2 (United States Biological, Swampscott, MA) at 1 ng/ml to the coculture system described above. T cells were cultured, pulsed, and harvested as above.

Flow-Cytometric Analysis. The expression of CD30 and CD153 in T cells or H-RS cells was analyzed with fluorescence-activated cell sorting. T cells in the cocultures with H-RS cells or CD30-Fc chimeric protein were also analyzed for the expression of CD25 or CD26. The following antibodies were used: phycoerythrin-labeled anti-CD30 or anti-human CD153 and FITC-labeled anti-CD3, -CD25, or -CD26. For negative controls, FITC- and phycoerythrin-labeled mouse IgGs were substituted. All antibodies were purchased from BD Pharmingen. The histograms were gated on CD3-positive cells.

RESULTS

H-RS Cells Inhibit Anti-CD3-Induced T-Cell Proliferation. We first addressed the expression of CD30 and CD153 on T cells, accessory cells, and H-RS cells. As shown in Fig. 1A, T cells in our system expressed no or little CD30 and expressed CD153 after activation. Accessory cells expressed no or little CD30. All three lines of H-RS cells expressed CD30 and little or no CD153 (Fig. 1B).

We then addressed the effect of H-RS cells on the proliferation of T cells. Soluble anti-CD3 alone caused minimal T-cell proliferation (not shown here). Instead, the addition of both anti-CD3 and accessory cells was required for optimal T-cell proliferation. The tritium uptake was markedly decreased when mitomycin-treated KM-H2 cells were added to anti-CD3-treated T-cell/accessory-cell cocultures (Fig. 1C). This inhibition was also present when HDLM or L428 cells were substituted for KM-H2 cells, implying that H-RS cells exerted a potent suppressive effect on the proliferation of anti-CD3-treated T cells. In contrast, U937 cells exerted no inhibitory effect on T-cell proliferation.

Downloaded from cancerres.aacrjournals.org on September 13, 2017. © 2004 American Association for Cancer Research.
H-RS Cells or CD30-Fc Chimeric Protein Inhibits IL-2 Production by Anti-CD3-Treated T Cells, and Addition of Recombinant IL-2 Reverses the Inhibition. We then tested whether the inhibitory effect of H-RS cells or CD30-Fc chimeric protein on T-cell proliferation is a result of defective IL-2 production from anti-CD3-treated T cells. IL-2 production from anti-CD3-treated T cells cocultured with KM-H2 cells was not detectable (Fig. 4A). However, IL-2 production was detected at a level similar to that by anti-CD3-treated T cells, if KM-H2 cells were pretreated with antagonistic anti-CD30. Similarly, we failed to detect IL-2 from anti-CD3-treated T cells when CD30-Fc chimeric protein (>5 μg/ml) was added (Fig. 4C). The inhibition of T-cell proliferation by KM-H2 cells or CD30-Fc fusion protein was reversed by addition of exogenous IL-2 (Fig. 4, B and D). These results indicate that H-RS cells or CD30-Fc chimeric protein abolished IL-2 production from anti-CD3-treated T cells; the CD30-related interaction may be responsible for this effect.

H-RS Cells or CD30-Fc Chimeric Protein Inhibits Expression of CD25 and CD26 by Anti-CD3-Treated T Cells. Tumor-infiltrating T cells in Hodgkin’s lymphoma generally lack expression of CD25 and CD26 (19). To test whether the inhibition of these activation markers is H-RS cell dependent or, more specifically, CD30 dependent, we assayed the expression of these molecules on T cells in our coculture system. As shown in Fig. 5A, the majority of anti-CD3-treated T cells cocultured with H-RS cells failed to express CD25 or CD26. However, if H-RS cells pretreated with anti-CD30 were used, the majority of the T cells in cocultures expressed both CD25 and CD26. Similar results were obtained with CD30-Fc chimeric protein. As shown in Fig. 5B, CD30-Fc protein or EGFP only were selected with fluorescence-activated cell sorting. The expression and cellular distribution of CD30 in these cells were demonstrated by confocal microscopy and Western blot analysis (Fig. 2, A and B). As shown in Fig. 2C, anti-CD3-induced T-cell proliferation was dramatically inhibited when the cells were cocultured with CD30+ CHO cells, either mitomycin C treated or paraformaldehyde fixed. However, the inhibition was reversed if CD30+ CHO cells were pretreated with blocking anti-CD30.

We produced CD30-Fc fusion proteins by ligating the extracellular domain of CD30 with the Fc portion of human IgG1. As shown in Fig. 3A, plate-bound CD30-Fc fusion protein had a dose-dependent inhibitory effect on the proliferation of anti-CD3-treated T cells. A dramatic inhibition was observed when the concentrations were equal to or greater than 10 μg/ml. As shown in Fig. 3B, soluble hCD153-mCD8, but not hCD154-mCD8 chimeric protein, was able to rescue T-cell proliferation inhibited by CD30-Fc fusion protein.

CD30 INHIBITS T-CELL PROLIFERATION
The neoplastic H-RS cells are characterized by abundant expression of CD30, which is rarely expressed by tumor cells from most T- or B-cell lymphomas. The restricted expression profile of CD30 suggests a close relationship between CD30 and HD. CD153→CD30 signaling drives nuclear factor κB activation and leads to constitutive cytokine expression, enhancing the cell proliferation and survival of H-RS cells (24). CD30 signals down-regulate the expression of CD28, Fas ligand, perforin, and granzyme B, and they abrogate cytotoxicity in a lymphoma cell line, suggesting that CD30 can down-modulate lymphocyte effector function and proliferation (25, 26). We showed here that CD30, either as a membranous protein on H-RS cells and CHO cells or as a plate-bound CD30 chimeric protein, inhibited T-cell proliferation via a CD30→CD153 signaling pathway. When cocultured with H-RS cells, anti-CD3-treated T cells failed to reveal increased tritium uptake, failed to enhance the expression of CD25 and CD26, and failed to produce IL-2. The inhibition of T-cell proliferation was, however, reversed with addition of exogenous IL-2 or pretreatment of H-RS cells with an antagonistic antibody against CD30. The inability of T cells to express CD25 and CD26 in cocultures with H-RS cells is in accordance with the results of immunohistochemistry on disease-involved tissue (19).

T cells represent most of the tumor-infiltrating lymphocytes in HD. They consist predominantly of CD4+ TCRαβ+ cells and are CD45RO+/CD45RA−/CD45RBdim, indicating that they might be Th2-type memory T cells. They express the early activation markers CD38 and CD69, but little or no CD25 or CD26 (16, 19), suggesting that these T cells are blocked at a later stage after activation. Loss of CD25 and/or CD26 significantly impairs the production of IL-2 and the proliferative capacity of T cells after adequate stimulus (27). These CD26− cells have been shown to display a Th2-like cytokine pattern, secreting IL-4 and IL-5 upon appropriate stimulation. The secretion of IL-4 by CD26− T cells and H-RS cells (28) could lead to a preferential influx of Th2-type T cells (29) and suppress Th1-type immune responses.

H-RS cells are known to secrete a large number of other cytokines, including various types of ILs (e.g., IL-4 and -5) and tumor necrosis factors, colony stimulating factors, and transforming growth factors (3, 15, 17). Among these, transforming growth factor β is a potent immunosuppressant (17). However, expression of transforming growth factor β is widely expressed by many types of neoplasms, and its presence in H-RS cells may not be solely responsible for the anergic response seen in these patients. Thus, in addition to the involvement of cytokines, the CD30→CD153 interaction via cell-cell contact may contribute to a greater extent to facilitate a diversion to Th2-like and/or anergic immune responses.

**CD30 INHIBITS T-CELL PROLIFERATION**

chimeric protein was as effective as KM-H2 cells in inhibiting the expression of CD25 and CD26 in anti-CD3-treated T cells. At the same time, addition of IL-2 rescued the expression of CD25 on T cells inhibited by CD30-Fc chimeric protein. These results suggest that CD30 may account for the inhibitory effect.

**DISCUSSION**

HD is characterized by numerous infiltrating T cells, which are incompetent in terms of tumor eradication. The ineffective immune responses are not only limited to the tissue level, but also detected systemically (20). Patients with HD usually have a reduced number of T cells, especially CD4+ T cells, in the circulation (21). When untreated, a reduced proliferative response to anti-CD3 and IL-2 was observed in peripheral-blood lymphocytes. The production of IL-2 by lymphocytes was also reduced (22, 23).

The neoplastic H-RS cells are characterized by abundant expression of CD30, which is rarely expressed by tumor cells from most T- or B-cell lymphomas. The restricted expression profile of CD30 suggests a close relationship between CD30 and HD. CD153→CD30 signaling drives nuclear factor κB activation and leads to constitutive cytokine expression, enhancing the cell proliferation and survival of H-RS cells (24). CD30 signals down-regulate the expression of CD28, Fas ligand, perforin, and granzyme B, and they abrogate cytotoxicity in a lymphoma cell line, suggesting that CD30 can down-modulate lymphocyte effector function and proliferation (25, 26). We showed here that CD30, either as a membranous protein on H-RS cells and CHO cells or as a plate-bound CD30 chimeric protein, inhibited T-cell proliferation via a CD30→CD153 signaling pathway. When cocultured with H-RS cells, anti-CD3-treated T cells failed to reveal increased tritium uptake, failed to enhance the expression of CD25 and CD26, and failed to produce IL-2. The inhibition of T-cell proliferation was, however, reversed with addition of exogenous IL-2 or pretreatment of H-RS cells with an antagonistic antibody against CD30. The inability of T cells to express CD25 and CD26 in cocultures with H-RS cells is in accordance with the results of immunohistochemistry on disease-involved tissue (19).

T cells represent most of the tumor-infiltrating lymphocytes in HD. They consist predominantly of CD4+ TCRαβ+ cells and are CD45RO+/CD45RA−/CD45RBdim, indicating that they might be Th2-type memory T cells. They express the early activation markers CD38 and CD69, but little or no CD25 or CD26 (16, 19), suggesting that these T cells are blocked at a later stage after activation. Loss of CD25 and/or CD26 significantly impairs the production of IL-2 and the proliferative capacity of T cells after adequate stimulus (27). These CD26− cells have been shown to display a Th2-like cytokine pattern, secreting IL-4 and IL-5 upon appropriate stimulation. The secretion of IL-4 by CD26− T cells and H-RS cells (28) could lead to a preferential influx of Th2-type T cells (29) and suppress Th1-type immune responses.

H-RS cells are known to secrete a large number of other cytokines, including various types of ILs (e.g., IL-4 and -5) and tumor necrosis factors, colony stimulating factors, and transforming growth factors (3, 15, 17). Among these, transforming growth factor β is a potent immunosuppressant (17). However, expression of transforming growth factor β is widely expressed by many types of neoplasms, and its presence in H-RS cells may not be solely responsible for the anergic response seen in these patients. Thus, in addition to the involvement of cytokines, the CD30→CD153 interaction via cell-cell contact may contribute to a greater extent to facilitate a diversion to Th2-like and/or anergic immune responses.
responses. A recent paper (30) on a CD30 homologue encoded by *Ectromelia* virus also coincides with the function of the human CD30. This *M*_{12,000} secreted vCD30 protein abrogates T-cell proliferation and type I cytokine-mediated inflammation *in vivo* but has no effect on type II cytokine-mediated inflammation.

Increased serum levels of soluble CD30 are observed in HD patients and are a useful marker for predicting the prognosis and responses to therapy for the disease (31). Increased levels of soluble CD30 are also observed in various conditions, such as rheumatoid arthritis (32), colorectal cancer, and viral (HV, human T-cell lymphotropic virus, and EBV) infection (33), and seem also to be correlated with impaired Th1 immune responses and dominant Th2 immune responses. High serum levels of soluble CD30 have been reported better to predict the response to second-line therapy in rheumatoid arthritis patients (34). An increased level of soluble CD30 might be a marker for identifying a patient subset in which IL-2 treatment may help to restore the impaired immune system (35).

The role of CD30→CD153 interaction in physiological and most pathological conditions remains to be determined, however. Mice lacking a functional CD30 gene show defective negative thymocyte selection (36), whereas transgenic mice overexpressing CD30 in T cells have enhanced thymic negative selection (37), suggesting a role of CD30 in negative selection or apoptosis for thymocytes. CD30-deficient islet-specific T cells are much more autoaggressive than are wild-type cells in inducing destruction of pancreatic islets and the onset of diabetes (38), implying an effect of CD30 on limiting the proliferative potential of autoreactive T cells and protecting the body against autoimmune disorders. Furthermore, expression of CD30 may confer an immune privilege on a given tissue or organ. For example, CD30⁺ placental T cells as well as CD30⁺ decidual cells may contribute to inhibiting the antibody response to fetal allotransplants by maternal CD153⁺ B cells (39).

In conclusion, we established that CD30 is involved in the inhibition of T-cell proliferation, IL-2 production, and expression of CD25 and CD26 by T cells. These CD30⁻/CD69⁺/CD25⁻/CD26⁻ lymphocytes surrounding the H-RS cells are consistent with anergic and/or Th2-type T cells. Therefore, these T cells in HD do not support a cytotoxic antitumor response. The outcome of CD30-related interaction is an ineffective antitumor immunity, which is clearly in favor of the growth and survival of the tumor cells.

**ACKNOWLEDGMENTS**

We are grateful to Chih-Cheng Lu for assistance in preparing the illustrations. We also thank Dr. C.-L. Chien (National Taiwan University, Taipei, Taiwan) for guidance on confocal microscopy.

**REFERENCES**


CD30 Is Involved in Inhibition of T-Cell Proliferation by Hodgkin's Reed-Sternberg Cells

Che-Chun Su, Hsiu-Hui Chiu, Chia-Che Chang, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/64/6/2148

Cited articles
This article cites 36 articles, 11 of which you can access for free at:
http://cancerres.aacrjournals.org/content/64/6/2148.full#ref-list-1

Citing articles
This article has been cited by 5 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/64/6/2148.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.