CD30-ligand and CD40-ligand expression in lymph nodes involved with Hodgkin's disease


Summary
Background: Reed-Sternberg cells of Hodgkin's disease express CD30 and CD40 receptors. The ligands for these receptors have been reported to have pleiotropic biologic activities in vitro, including induction of cell death and enhancing cell survival. Co-expression of the ligands for these receptors in lymph nodes involved with Hodgkin's disease is not known.

Purpose: The purpose of this study was to examine CD30 ligand (L) and CD40L expression in lymph nodes of patients with Hodgkin's disease, and to study CD30L expression on nodal lymphocyte subsets.

Materials and methods: CD30L expression on subsets of lymphocytes of five lymph nodes involved with Hodgkin's disease was determined by two-color FACScan. Messenger RNA expression of CD30L and CD40L was determined by the reverse-transcriptase polymerase chain reaction (RT-PCR) method performed on seven specimens involved with Hodgkin's disease (five lymph nodes and two spleens).

Results: Four of seven specimens (57%) contained cells that expressed CD30L mRNA and three specimens (43%) contained CD40L-expressing cells. The mean percentage of nodal lymphocytes expressing CD30L surface protein was ≤ 20%.

Conclusion: Hodgkin's disease lymph nodes and spleens frequently lack CD30L- and CD40L-expressing cells, and when CD30L is expressed, it is usually detected on few numbers of lymphocytes. The balance in the level of expression of these ligands in Hodgkin's disease lymph nodes may be related to the disease's clinical behavior.

Key words: CD30L, CD40L, FasL, Hodgkin's disease

Introduction
CD30 and CD40 are members of the tumor necrosis factor (TNF)-receptor family of cellular transmembrane proteins [1, 2]. In healthy individuals, CD30 is expressed on a subset of T cells while CD40 is expressed on mature B cells in addition to epithelial cells of several tissues. Predictably, CD30 and CD40 are expressed on the malignant counterparts of these benign cells. Thus, CD40 is expressed on B-cell malignancies and on several carcinomas, whereas CD30 is expressed on the malignant cells of anaplastic large-cell lymphoma. Both receptors, CD30 and CD40, are expressed on the malignant Reed-Sternberg (RS) cells of Hodgkin's disease [3–5].

CD30 ligand (L) and CD40L are members of the TNF-ligand family that are also transmembrane proteins predominantly expressed on the hematopoietic cells, especially lymphocytes and monocytes [1, 6]. Both CD30L and CD40L have pleiotropic biologic activities which range from growth stimulation to induction of cell death. CD30L (or anti-CD30) can induce apoptosis of CD30+ anaplastic large-cell lymphoma in vitro and in vivo [7, 8]. The biologic activities of CD30L in Hodgkin's disease remain unclear, mainly because there is no in vitro or animal model that is truly representative of the human disease. Although CD30L was reported to induce cell proliferation of several Hodgkin's- and RS-derived cell lines in vitro [7], SCID mice transplanted with the Hodgkin's disease-derived cell line L540CY were cured of their tumors when they were treated with anti-CD3-CD30 and anti-CD28-CD30 bispecific monoclonal antibodies followed by human T cells, a condition that is close to the human disease [9]. Thus, it is likely that CD30L can also mediate cell death of RS cells in vivo.

The role of CD40L in the growth regulation of RS cells is also unclear. Gruss et al. reported that CD40L shares biologic activities with CD30L such as induction of cytokine secretion by Hodgkin's-derived cell lines [10]. Carbone et al. reported that CD40L enhanced colony cell survival of several Hodgkin's disease-derived cell lines [4]. A similar protective role of CD40L has been previously observed in follicular center-cell lymphocytes [11].

In this study, we examined CD30L and CD40L expression in lymph nodes involved with Hodgkin's disease. We found that these ligands are not always expressed in Hodgkin's disease nodal cells, and when expressed, CD30L was detected on ≤ 20% of the nodal lymphocytes.
Materials and methods

Peripheral blood and tissue specimens were obtained from healthy donors and patients with Hodgkin's disease according to our institutional guidelines. Mononuclear cell separation and cell sorting by FACS were performed as previously described [12]. The sorted samples were > 99% pure for the target cells. Detection of CD30L on lymphocyte subsets by FACScan was performed as we have previously described using anti-CD30L monoclonal antibody (M80) from Immunex (Seattle, WA) [12]. RNA isolation and RT-PCR were performed as previously described using the following primers: for CD30L (5'CTCCTGGAGACACAGCCATGC, and 3'TGCTTGTATCTATG-TACTGGA); and for CD40L (5'ACTGGACTGCCCATCAGCATG, and 3'ACTGCTGGCCTCACTTATGAC) [12, 13].

Results

CD30L mRNA was expressed on FACS-sorted CD4+, CD8+, CD3+, CD14+, CD56+, and CD19+ peripheral blood cells obtained from healthy donors (Figure 1). Two normal tonsils and two peripheral blood specimens of patients with chronic lymphocytic leukemia also expressed CD30L, while three breast carcinoma cell lines and normal bronchoepithelial cells did not. Resting CD4+ and CD8+ cells expressed CD30L mRNA but had no detectable levels of surface CD30L protein by FACScan, while activated T cells (PHA stimulated or anti-CD3 stimulated) expressed CD30L mRNA and surface protein. In contrast, resting B cells expressed both mRNA and surface CD30L protein.

Five lymph nodes and two spleen specimens involved with Hodgkin's disease were studied for CD30L and CD40L expression by RT-PCR. Four specimens contained cells that expressed CD30L (57%) and three contained cells that expressed CD40L (43%). Both CD30L and CD40L were co-expressed in three specimens. We subsequently studied CD30L expression on lymphocyte subsets from five fresh lymph nodes involved with Hodgkin's disease. CD30L was detected on ≤20% of CD4+, CD8+, and CD19+ cells as determined by two-color FACScan (Figure 2).

Discussion and future directions

In this study, CD30L mRNA was found to be constitutively expressed in B and T lymphocytes (CD4+ and CD8+), monocytes, and natural killer cells of healthy donors. Prior reports also found its expression in granulocytes and eosinophils. Since the CD30 receptor is expressed only in a very small population of CD4+ cells, the reason for this global expression of CD30L on the mature hematopoietic cells and the physiologic function of CD30L in healthy individuals remain undetermined. It is possible that CD30L is involved in B-cell/T-cell communication and/or in the immune response against tumor cells and virally infected cells that express CD30 receptors.

The malignant RS cells of Hodgkin's disease are surrounded by abundant numbers of benign lymphocytes, monocytes, and eosinophils. RS cells express B7.1 and CD30L.
B7.2 costimulatory molecules, in addition to CD30, CD40, and Fas receptors [3, 4, 14, 15]. Thus, although RS cells are immunogenic and have receptors that can transduce cell death signals, they continue to thrive. The mechanism through which RS cells evade the immune cells, however, remains puzzling.

Although four of seven Hodgkin's disease nodal lymphocytes expressed CD30L mRNA, fewer than 20% of B and T lymphocytes had detectable levels of CD30L surface protein. It is not clear if the decreased detectable levels of CD30L surface protein described in this study is due to secreted cytokines by RS cells, such as IL-4, which favors the switch of Th0 to Th2 type of T-cells (which weakly express CD30L and FasL), or to soluble CD30L, which is known to be shed (Figure 3). Soluble CD30 may bind to surface CD30L protein and blocks its detection by anti-CD30L antibodies used in the detection method. The blockade of CD30L/CD30 interaction may be involved in the ability of RS cells to continue to thrive. Although CD30L has been reported to enhance cell proliferation in some RS-derived cell lines, its in vivo role has not been determined. Because patients with Hodgkin's disease and anaplastic large-cell lymphoma who have high soluble CD30 levels are reported to have a poor prognosis [16, 17], it is possible that soluble CD30 blocks CD30L-induced cell-death signals, therefore giving the CD30+ tumor cell survival advantage. Similar protective effect has been previously observed with soluble Fas [18, 19]. However, only studies on fresh Hodgkin's disease specimens will answer this question.

Finally, we have shown that not all Hodgkin's disease lymph node cells express CD40L by the RT-PCR method. This is in contrast to what has previously been reported using immunohistochemistry methods [20]. This discrepancy could be due to post-translational modification. It is hoped that studying larger numbers of patients will provide information about the clinical behavior of Hodgkin’s disease cases lacking CD40L expression.

Future areas of investigation should focus on studying the level of FasL expression in Hodgkin’s disease nodal lymphocytes, and to determine whether Fas and CD40 have opposing biologic activities on RS cells (Figure 3). Soluble CD40 and Fas in Hodgkin’s disease patients also need to be examined to determine if they have prognostic value. Finally, the potential therapeutic role of agents that can manipulate the function and expression of these receptors and ligands should be explored.

References

5. Smith C, Gruss HJ, Davis T et al. CD30 antigen, a marker for Hodgkin’s lymphoma, is a receptor whose ligand defines an emerging family of cytokines with homology to TNF. Cell 1993; 73: 1349-60.


