Commentary

Regulation of CD30 Antigen Expression and Its Potential Significance for Human Disease

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CD30 antigen, a member of the tumor necrosis factor (TNF) receptor superfamily,^{1–3} was originally identified as a cell surface antigen on primary and cultured Hodgkin's and Reed-Sternberg cells by use of the monoclonal antibody Ki-1.^{4,5} CD30 antigen normally is expressed by a subset (15–20%) of CD45RO+ T cells after activation by a variety of stimuli.⁶ Its expression is stimulated by interleukin (IL)-4 during lineage commitment of human naïve T cells^{7,8} and is augmented by the presence of CD28 costimulatory signals.⁹ CD30 also is expressed at variable levels in different non-Hodgkin's lymphomas (NHL) as well as in several virally transformed T and B cell lines.^{5,10} In particular, CD30 is a specific marker of a subset of peripheral T cell NHLs known as anaplastic large cell lymphomas (ALCL).⁵ More recently, CD30 preferential expression has been detected on a subset of tissue and circulating CD4+ and CD8+ T cells producing mainly Th2 cytokines in immunoreactive conditions.^{11–14}

The biological significance of the CD30 molecule is related to the existence of a natural ligand, CD30L, a member of the TNF ligand superfamily recently identified in murine T cells and in human CD3-activated peripheral blood T cells, monocytes induced with lipopolysaccharide, and in eosinophils, at highest levels in pathological conditions such as Hodgkin's lymphoma (HL) and the hypereosinophilic syndrome.^{2,15} Cross-linking of CD30 results in signal transduction with increased levels of intracellular Ca²⁺.⁶ Engagement of CD30 by CD30L appears to up-regulate apoptosis of mature T lymphocytes in peripheral lymphoid organs of mice, rendering these lymphocytes susceptible to cell death after withdrawal of T cell receptor signaling.¹⁶

CD30 appears to have an important immunoregulatory role in normal T cell development. Within the thymus, CD30L is highly expressed on medullary thymic epithelial cells and on Hassall's corpuscles.¹⁷ In mice, CD30 overexpression helps to eliminate autoreactive T cells through negative selection of CD4+/CD8+ thymocytes by a caspase-dependent mechanism, a process that it totally

prevented by bcl-2.¹⁸ As expected, CD30–/– mice have elevated numbers of thymocytes and activation-induced death of thymocytes after CD3 cross-linking is impaired both *in vitro* and *in vivo*.¹⁹

The functional relevance of CD30/CD30L interaction in human lymphomas is beginning to be understood. Recombinant CD30L expressed on the surface of cultured cells was tested for biological activities on a variety of CD30+ human lymphoma cell lines.²⁰ It was demonstrated that CD30L+ cells enhance proliferation of HL cell lines with a T cell genotype/phenotype; in contrast they have no effect on HL cell lines with a B cell genotype/phenotype, and an antiproliferative effect on certain CD30+ T cell ALCL lines, including a cytolytic effect and a cytostatic component inducing cell cycle arrest.^{15,20} Therefore, CD30L is capable of transducing signals leading to either cell death or proliferation through its specific cognate molecule, CD30. Taken together, these data demonstrate pleiotropic biological activities of CD30L on different CD30+ lymphoma cell lines and indicate that CD30/CD30L interaction probably plays an important role in the pathophysiology of HL and ALCL. The molecular mechanism(s) underlying deregulated expression of CD30 in these lymphomas is unknown but appears to be independent of infection with Epstein-Barr virus (EBV) or human T-cell leukemia virus-1 (HTLV-1) since CD30 is strongly expressed on lymphoma cell lines which lack these viruses,²¹ and EBV is absent in approximately 40 to 50% of primary HL of immunocompetent patients in Western countries.²²

In this issue of *The American Journal of Pathology*, Croager and coworkers describe insightful experiments which begin to clarify one aspect of the regulation of CD30 expression.²³ They define three key regulatory re-

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gions important in the transcriptional control of CD30. In common with other members of the TNF receptor/TNF ligand superfamily, CD30 lacks a canonical TATA box commonly used by other genes as an anchor for basal transcriptional machinery. Croager et al found that transcription factor Sp1 acts as a surrogate for the TATA motif, recruiting TATA binding proteins which are normally required for initiation of transcription. A downstream promoter element was found to direct both the accuracy and level of CD30 transcription. Finally, an upstream microsatellite region that binds proteins which suppress transcriptional activity of the CD30 promoter was recognized. The essential feature of this microsatellite is a (CCAT)_n sequence which alone was capable of repressing transcription from the CD30 promoter. Croager et al have established that shortened versions of this microsatellite repressor have lower levels of repression *in vitro*, a finding that may have relevance in lymphomas and other diseases that exhibit increased CD30 expression.

The microsatellite was found to contain differences of as many as ten CCAT repeats in a preliminary study of DNA samples from seven random donors. A similar degree of microsatellite polymorphism was uncovered in a limited survey of human tumor cell lines, but none of these were derived from HL or CD30+ ALCL. With such a high degree of polymorphism in a small size sample, it may be anticipated that a more comprehensive study of patients with CD30+ lymphomas and autoimmune diseases (see below) may reveal recurrent polymorphisms that predispose them to overexpression of CD30 with corresponding disease phenotypes.

Supporting the hypothesis that polymorphisms resulting in variable microsatellite lengths can be associated with a predisposition for disease is the fragile X syndrome.²⁴ The fragile X syndrome results from variations in a (CGG)_n repeat found in the coding sequence of the *FMR-1* gene. Analysis of length variation in the region in normal individuals shows a range of allele sizes varying from a low of 6 to a high of 54 repeats. All alleles with more than 52 repeats are meiotically unstable with a mutation frequency of one, whereas meioses of alleles of 46 repeats and below have shown no mutation. The risk of expansion during oogenesis to the full mutation associated with mental retardation increases with the number of (CGG)_n repeats.

Microsatellite regions are also prone to insertion/deletion mutations, observed at highest frequency in individuals with defects in DNA mismatch repair enzymes hMHS2 and hMLH1.²⁵ Such individuals are at increased risk of developing colorectal cancers with inactivating mutations arising in microsatellites of the pro-apoptotic *BAX* gene²⁶ and the type II receptor for transforming growth factor- β , which normally has a growth inhibitory function.²⁷

The pleiotropic effects of CD30 signaling have been attributed to the nature of the target cell, its state of differentiation, transformation status and environmental co-factors.²⁰ These pleiotropic effects may also be explained by the divergent pathways of CD30 signal transduction through a family of adapter molecules called TNFR-associated factors (TRAFs) which has at least 6 family members.²⁸ Signals emanating from the TNFR

family diverge downstream of TRAF-2, leading to activation of two important transcription factors, NF- κ B and c-Jun N-terminal kinase.²⁹⁻³⁷

NF- κ B signaling is activated through recruitment and activation of a series of protein kinases which leads to the translocation of NF- κ B into the nucleus. The NF- κ B-inducing kinase (NIK) is a mitogen-activated protein (MAP) kinase kinase kinase (MAP3K).³⁸ A substrate of NIK is the I κ B complex (IKK), which consists of two subunits, IKK α and IKK β .^{39,40} The IKK complex is essential for the phosphorylation and inactivation of the NF- κ B inhibitor protein by I κ B.³⁹⁻⁴¹ I κ B is found in the cytosol associated with NF- κ B, keeping NF- κ B in an inactivated state. Upon phosphorylation and degradation of I κ B, NF- κ B translocates into the nucleus in an activated state.^{41,42} It can then stimulate transcription of a number of cellular genes, eg, IL-6,⁴³ that may account for the variable histopathology and clinical symptoms of patients with HL.⁴⁴

TRAF-2 also activates MEKK-1, another MAP3K, which selectively activates IKK- β , resulting in NF- κ B activation. MEKK-1 activation by TRAF-2 also results in activation of stress-activated kinase/c-Jun N-terminal kinase through activation of MEK-4, leading to various biological effects.^{37,45}

Constitutive activation of NF- κ B-RelA prevents Hodgkin/Reed-Sternberg (H/RS) cells in HL from undergoing stress-induced apoptosis; HL cell lines depleted of constitutive NF- κ B are no longer able to produce tumors in immunodeficient mice.⁴⁶ It is possible that in HL tissues, NF- κ B is up-regulated by CD30/CD30L interactions. The source of CD30L in HL tissues appears to be from activated T cells and eosinophils.^{15,47} The density of eosinophils correlates with a poorer prognosis in nodular sclerosing HL, possibly as their expression of CD30L serves to promote the survival of H/RS cells.⁴⁸ A direct stimulating effect of CD30L expressed by eosinophils on proliferation of HL cell line HDLM-2 was reported by Pinto and coworkers.¹⁵ However, HDLM-2 has a T cell phenotype and genotype,⁴⁹ and therefore represents only a small fraction of HL cases.^{50,51} In contrast, Gruss and coworkers, who showed similar CD30L enhanced proliferation results for HDLM-2 and L-540, another HL line with T cell characteristics, found that HL cell lines of B cell phenotype and genotype were not further stimulated to proliferate by recombinant CD30L.²⁰ A possible explanation for these results is that the HL lines of B cell type have a high constitutive activation of NF- κ B and cannot be further stimulated by engagement with CD30L.⁴⁶

The Sp1 transcription factor, required for initiating transcription of the CD30 gene, is up-regulated by viral activation. Accordingly, Croager and colleagues suggest that in HL, EBV may up-regulate Sp1 leading to H/RS cell overexpression of CD30, and possibly CD40, another TATA-less member of the TNF family.²³ CD40 protects germinal center B cells from apoptosis,^{52,53} and therefore could also promote survival of H/RS cells.⁵⁴ Because EBV-latent membrane protein-1 (LMP-1) and CD30 use the same signal transduction pathway of TRAFs to transmit growth signals from the cell membrane to the nucleus,⁵⁵⁻⁵⁸ both direct and indirect effects of EBV on H/RS cell survival are plausible. In two of three cases of EBV-

negative HL, clonal deleterious mutations of I κ B, which normally maintains NF- κ B in an inactive state in the cytosol, were found in H/RS cells, suggesting an alternative pathway for activation of NF- κ B in EBV-negative cases.⁵⁹

CD30 lacks the death domain of the TNF receptor and Fas antigen, which is required for transduction of an apoptotic signal.^{2,3,60,61} A recent study indicates that cytotoxic effects induced by CD30 are mediated by endogenous production of TNF and autocrine or paracrine activation of TNF receptor-1.⁶² CD30/CD30L-associated cell death relies on interaction of the cytoplasmic domain of CD30 with TRAF-1, -2, -3, and -5.²⁹⁻³⁶ Deletion analysis shows that the COOH-terminal 66 amino acids of CD30, a region of CD30 that interacts with TRAF-1 and -2, is required to induce cell death.⁶³ Binding of TRAF-2 to the cytoplasmic domain for CD30 causes rapid proteolytic degradation of TRAF-2 and associated protein TRAF-1, limiting the ability of CD30 to transduce cell survival signals through TRAF-2, and increasing the sensitivity of cells to undergo apoptosis induced by the TNF receptor 1.³⁴

The cytostatic and cytolytic antiproliferative effect of CD30 activation on ALCL lines can be used to therapeutic advantage. We and others have shown that activation of the CD30L binding domain by an unconjugated agonist antibody (HeFi-1) causes lymphoma regression and prolongs survival of immunodeficient mice xenografted with human systemic CD30+ ALCL.^{64,65} Clinically, increased amounts of CD30L are associated with spontaneous regression of skin lesions in primary CD30+ cutaneous lymphomas,⁶⁶ a spectrum ranging from lymphomatoid papulosis, in which spontaneous regression is a consistent feature, to CD30+ lymphoma, in which regression occurs in 25% of lesions.^{67,68} With this tendency for spontaneous regression, it is not surprising that CD30 expression is associated with a significantly better survival for patients with primary cutaneous lymphomas.^{69,70} These experimental and clinical studies indicate that expression of CD30 antigen is not only an important prognostic marker but also could be used as the target of novel therapies.

A soluble 88-kd form of CD30 is released by CD30+ cells *in vitro* and *in vivo*, probably as a result of proteolytic cleavage of the membrane-bound CD30.⁷¹ Serum levels of soluble CD30 (sCD30) are elevated in most untreated patients with HL and correlate with tumor burden, response to therapy, and event-free survival.⁷² Serum sCD30 levels greater than 100 U/ml were predictive of a poor outcome for patients with HL in a multivariate analysis of risk factors.⁷² Elevations of serum sCD30, greater than those in stage-matched cases of HL, occurred in patients with CD30+ ALCL.⁷³ Serum sCD30 values returned to the normal range when patients achieved complete remission, and increased to abnormal values again at relapse, indicating that serum sCD30 can be used effectively to monitor disease activity in patients with CD30+ ALCL.

Increased CD30 expression appears to play a role in non-neoplastic diseases as well. CD30 expression is increased in autoimmune and cutaneous disorders, AIDS, and other diseases with increased levels of CD4 or CD8

lymphocytes expressing Th2 cytokines.⁷⁴⁻⁷⁶ CD30 expression is protective in a murine model of experimental autoimmune diabetes where signaling through CD30 limits the expansion of autoreactive CD8+ T cells.⁷⁷ In the absence of CD30 signaling, pancreatic islet specific CD8+ T cells gain the ability to proliferate extensively on secondary contact with pancreatic islet antigen. This model identifies a novel mechanism for control of autoimmunity involving CD30 that is distinct from Fas (CD95)-dependent deletion of autoreactive CD8+ T cells.⁷⁸

Patients with progressive systemic sclerosis have numerous cutaneous CD4+ T cells with a Th2 cytokine profile, increased expression of CD30 antigen, and elevated levels of soluble CD30 (sCD30) in their sera.⁷⁹ Elevated serum sCD30 is present also in patients with systemic lupus erythematosus and correlates with disease activity.⁸⁰ CD30+ T cells, both CD4+ and CD8+, are present in the synovial fluid and elevated serum levels of sCD30 are found in patients with rheumatoid arthritis.⁸¹ Serum sCD30 is elevated in patients with active Hashimoto's thyroiditis where it is associated with transient destructive thyroiditis, and in patients with Graves' disease, particularly in thyrotoxic patients, where it correlates with TSH receptor antibody activity.⁸²

Large numbers of lymphocytes expressing CD30 and producing Th2 type cytokines are found in the skin and elevated sCD30 in the serum of patients with acute atopic dermatitis but not in patients with allergic contact (nickel-induced) dermatitis.^{83,84} CD30+ cells decline with clinical improvement of atopic dermatitis following phototherapy with UV-A and UV-B.⁸⁵ CD30 expression also is increased in the skin and serum of patients with chronic graft-versus-host disease.⁸⁶

CD30 appears to be important in AIDS, where activation of CD30 enhances HIV replication in CD4+ T cells from HIV-infected individuals.⁸⁷ CD30 activation leads to enhanced HIV transcription via TRAF-2-mediated NF κ B activation,³⁶ possibly leading to the reactivation of HIV expression from latently infected CD4+ T cells.⁸⁸ HIV infection of CD4+ T cells from HIV-seronegative individuals leads to CD30 expression which precedes and is associated with the T cell death.⁸⁹ Expression of CD30 and elevated serum sCD30 persists in the late stages of AIDS, largely as a function of CD8+ T cells.¹²

CD30 expression by numerous tissue-infiltrating activated T lymphocytes and elevated serum sCD30 are characteristic of Omenn's syndrome, a severe immunodeficiency characterized by clinical and laboratory features of a Th2 type response, including erythroderma, hypereosinophilia, elevated IgE, and lymphadenopathy.⁹⁰

In summary, CD30 is an important molecule that regulates the growth and death of lymphocytes in malignant lymphomas, autoimmune and cutaneous disorders, and AIDS. Insights into the mechanism of CD30 expression such as those described by Croager and coworkers have potentially far-reaching effects for control of these diseases. Understanding the mechanism(s) of CD30 expression could help to identify individuals or families at highest risk to develop certain lymphoproliferative and autoimmune diseases. Novel therapies that inhibit CD30 expression, the TRAF signaling molecules, and/or activa-

tion of NF- κ B can hold promise for treatment of HL where expression of CD30 and TRAF-mediated activation of NF- κ B promote survival of H/RS cells and possibly for AIDS in which CD30 activation promotes HIV replication. Monoclonal antibodies or other pharmacological reagents that can mimic the antiproliferative effects resulting from CD30/CD30L interactions could be used to treat chemotherapy resistant and minimal residual disease of patients with CD30+ ALCL. Because not all CD30+ ALCL lines are growth inhibited by CD30L,²⁰ it would be advantageous to employ *in vitro* and murine xenograft assays to identify patients with CD30+ lymphomas who are likely to respond to therapies which activate CD30.^{64,65} Finally, measuring serum levels of sCD30 should be evaluated on a broader scale as a non-invasive method to determine prognosis and monitor disease activity in CD30+ lymphomas, autoimmune diseases, and cutaneous lymphoproliferative disorders.

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