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Expert Commentary

CD40 LIGAND-BASED CANCER THERAPY

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ABSTRACT

Immunotherapy of cancer has intrigued the scientific community ever since it became clear that the immune system could eradicate tumor cells. To gain full capacity of immunotherapy it is important to activate tumor-specific cytotoxic T lymphocytes (CTLs) and defeat the suppressive actions of regulatory T (Treg) cells present in the tumor microenvironment. In experimental models of cancer, CD40 ligand (CD40L)-based therapy has reached these two goals. In addition to its immunostimulatory properties, CD40L can trigger apoptosis of tumor cells by mechanisms that are now beginning to emerge. Because of its dual properties as an immune activator and apoptosis inducer, CD40L is an attractive candidate for cancer therapy. It is currently under evaluation for its effect on human cancer.

CD40 LIGAND

The CD40 ligand (CD40L) is a member of the tumor necrosis factor (TNF) gene family and is expressed by a variety of cells including lymphoid, endothelial, and smooth muscle cells. However, it is the expression of CD40L on activated T helper (Th) cells of both Th1 and Th2 type that has been at major focus. CD40L binds to its receptor, CD40. Similar to CD40L, CD40 is expressed on a variety of lymphoid, endothelial, and smooth muscle cells but can also be found on fibroblasts, neuronal cells, keratinocytes and carcinomas [1]. CD40L submit signals as a single molecule but it is most potent as a trimer, binding to trimerized CD40. The action of CD40L-signaling depends on the interacting cell types and

includes diverse events such as apoptosis, proliferation, differentiation, cytokine production, and Ig class switch [2]. Mutations in the CD40L gene cause hyper IgM conditions and reduced capacity to defeat infections. On the other hand, overexpression of CD40L has been associated with several autoimmune diseases [1]. If CD40L expression causes autoimmunity or is merely a symptom of disease remains unknown. Indeed, in disease models of cancer, CD40L therapy does not appear to induce autoimmunity [2].

INDUCTION OF APOPTOSIS IN TUMOR CELLS VIA CD40 STIMULATION

The ability of CD40L to trigger apoptosis is intriguing given the absence of 'death domain' sequences in the cytoplasmic tail of CD40. However, apoptosis may occur indirectly via induction of death ligands such as TNFα, Fas ligand (FasL) and TNF-related apoptosis-inducing ligand (TRAIL) in stimulated cells. These death ligands may then promote apoptosis in an autocrine/paracrine manner [3-5]. Importantly, it is malignant cells (mainly CD40-positive tumor cells of epithelial origin) and not normal cells that undergo CD40L-dependent apoptosis. It has been demonstrated that CD40-stimulation of normal B lymphocytes or urothelial cells induces the rapid downregulation of the CD40-interacting proteins TRAF2 and TRAF3 thereby 'dampening' signals which promote apoptosis [6-8]. In contrast, CD40-stimulation of the malignant counterparts does not downregulate TRAF expression but utilizes TRAF3 to transduce signals leading to inhibition of proliferation [9] and induction of apoptosis [6]. The aforementioned in vitro studies led to the evaluation of CD40L as an apoptosis-induction therapy for human tumors. In SCID mouse models xenotransplanted with human ovarian carcinoma, breast cancer, or multiple myeloma cells CD40L administration induced significant inhibition of tumor growth [10-12]. Similarly, SGN-40, a humanized α-CD40 agonistic antibody, has demonstrated potent anti-proliferative and pro-apoptotic effects on B cell lymphomas and is currently under evaluation in a phase I clinical trial [13,14]. CD40L therapy, however, largely depends on functional immune system for effective tumor eradication. In a syngeneic mouse bladder cancer model, CD40L therapy has been thoroughly investigated. CD40L cured aggressively growing tumors in these mice but in an immunodeficient model, the tumor growth was only delayed [15,16]. These data clearly demonstrate the importance of the engagement of the immune system in CD40L-mediated therapy. Apoptosis induction may also contribute to this effect through antigen presentation to tumor-specific T cells. Several strategies are under development to enhance apoptosis upon CD40-stimulation. It has been demonstrated, for example, that CD40 ligation results in the engagement of the anti-apoptotic PI3 kinase signaling pathway and that PI3 kinase inhibition sensitizes tumor cells to CD40L-induced apoptosis. PI3 kinase pathway inhibitors could therefore be interesting drugs to use in combination with CD40-directed tumor therapies. Moreover, strong synergy between CD40L administration and chemotherapeutic agents such as 5FU and cis-platin has been observed in vitro and in mouse tumor models [9,10,17].

CD40L-Stimulation of the Immune System

Platelets have been considered as cell fragments only important for blood clot formation during vessel damage. However, platelets release preformed CD40L molecules within seconds after stimulation at the site of injury [18]. Potential targets are antigen-engulfing tissue residing DCs that upon CD40-activation will differentiate into a mature stage and migrate to local lymph nodes for contact with lymphocytes. Hence, platelets are very important for the initiation of adaptive responses. Immature DCs have high capacity of engulfing antigens in their surroundings. They express low to medium levels of antigen presenting molecules e.g. the major histocompatibility complex (MHC) molecules I and II. They also express low amounts or lack expression of the costimulatory molecules CD80/86 and the cytokines IL1β, IL6, IL12, and IFNγ [19]. Upon CD40L/CD40 interaction, however, the DCs decrease their capacity for antigen uptake and up-regulate the antigen processing and presentation machinery. Thus, MHC I and II molecules as well as the costimulatory molecules CD80/CD86 increases on the cell surface. CCR7 is upregulated to ensure DC migration to lymph nodes [20]. The DCs will produce a variety of cytokines upon activation including IL1β, IL6, IL12 and IFNγ [19]. The T cells receive the so-called 'activation signal 1' via MHC interactions with the T cell receptor (TcR) and 'signal 2' via costimulation (B7/CD28) and CD40/CD40L interactions. The first signal provides activation of antigen-specific T cells. Without costimulation, the antigen-stimulated T cells would undergo anergy or even apoptosis [21]. Continuous CD40-stimulation of the DCs will prolong their IL12 producing phase and, hence, their capacity to drive Th1 type responses [2]. IL12 enhances activation of both T cells and NK cells which produce IFNγ. Release of IFNγ will in turn promote MHC-I upregulation on target cells and enhance the activation of macrophages that will participate as effector cells in the Th1 response. Although B lymphocytes (Th2 response) are dependent on CD40 for full differentiation, Ig-class switch and proliferation, they do not appear to significantly participate in the anti-tumor effects of CD40L [2].

The CD40L/Tumor Cycle

The role of CD40L in tumor therapy is multifaceted (Figure 1). First, engagement of CD40 can directly trigger apoptosis and inhibition of proliferation in CD40-positive tumor cells. Apoptosis induction may also enhance uptake of tumor material by DCs. CD40-activation of DCs dramatically enhances tumor antigen presentation and costimulation to CTLs. CD40L may also upregulate MHC-I, B7, and death receptors such as Fas and TNFR on the tumor cell surface [19,22], thus enhancing their recognition and killing by tumor-specific CTLs. CD40L-based therapies have shown potent effects in various murine models of cancer including neuroblastoma, mastocytoma, fibrosarcoma, colon,, ovarian, lung, prostate and bladder cancer, malignant melanoma, myeloma and pre-B lymphoma [2]. The main effector mechanism has shown to be dependent on a functional specific immune system as discussed above. Cell depletion studies have shown that CTLs are crucial for the effects of CD40L administration on tumor cell lysis and memory induction. In an orthotopic bladder cancer model, immunosuppressive regulatory T cells (Treg) were present in the tumors of

CD40L treated as well as control treated mice. However, the number of Treg in lymph nodes was decreased upon CD40L therapy. Further, T cells from these mice were not able to inhibit proliferation of T cells from naïve mice as opposed to T cells from control treated, tumor-bearing mice. CD40L therapy may, hence, both activate the immune system as well as play a key role in the inhibition of Treg cells [15].

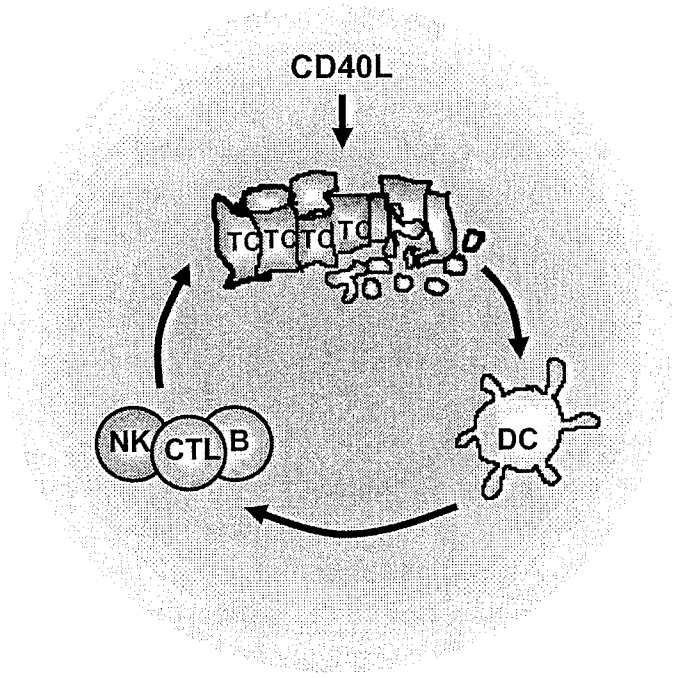

Figure 1. The CD40L/tumor cycle. CD40L therapy of tumors will induce apoptosis of CD40+ tumor cells (TC). This will enhance antigen uptake by DCs. CD40L will also stimulate the DCs to mature, migrate to lymph nodes and activate immune cells to mount responses against the tumor. Upon CD40L-stimulation, tumor cells may enhance their expression of MHC-I and death receptors making them better targets for CTLs.

CD40L in Clinical Cancer Trials

Soluble trimerized CD40L has been investigated in 32 patients with advanced solid tumors including renal cell carcinoma, head and neck cancer, cervical cancer, sarcoma, rectal carcinoma, esophageal carcinoma, breast carcinoma, peritoneal carcinomatosis, adenocarcinoma (unknown primary tumor) and non-Hodgkin's lymphoma. Patients received daily subcutaneous injections for 5 days/dose. Toxicity, maximum tolerated dose (MTD) and pharmacokinetics were evaluated. In one patient with breast carcinoma, grade 4 elevations of hepatic transaminases (AST, ALT) were seen. Otherwise, only a few cases of anemia (not dose dependent), neutropenia, lymphopenia and pain were observed. Dyspnea, asthenia, dyspepsia and pneumonia were seen in one patient each at the MTD level. Two patients had partial responses. Twelve patients had stable disease, however, only 6 of them remained stable during the final two treatment courses [23].

Two studies have been done on B cell chronic lymphocytic leukemia (B-CLL). In the first study, 11 patients were infused with their own ex vivo treated (AdCD40L gene transfer) B-CLL cells. No dose-limiting toxicity was seen in these patients. However, general flu-like

symptoms were seen such as fever, fatigue, arthralgia, myalgia, nausea and anorexia by 12 hours post infusion. Less common adverse reactions included headache, edema, dehydration and diarrhea. In a few patients increased, but transient, levels of transaminases were seen. All abnormalities resolved within a few days post treatment. As expected, IL12 and IFNγ were increased upon treatment demonstrating Th1 responses and their importance as biological markers of CD40L therapy. Most patients demonstrated decreased lymphocyte numbers (>97% were B-CLL cell prior therapy) and lymph node size post treatment. The effect could be sustained weeks to months after treatment [24]. In the second study on B-CLL, 9 patients received 3-8 subcutaneous vaccinations with cells activated by IL2 and CD40L gene therapy. Local pain, redness and swelling were observed at the vaccination site. In this study, no hepatic dysfunction was observed. A third of the patients had >50% decrease in lymph node size at 6-week evaluation. This effect was not sustained longer than 12 weeks [25].

Adenovirus-mediated CD40L therapy is currently under evaluation for bladder cancer in humans and malignant melanoma in dogs, both studies taking place at Uppsala University in Sweden.

CONCLUSIONS

The anti-tumor effects of CD40L are intriguing. The mechanism by which CD40 activation exerts these effects includes inhibition of tumor cell proliferation, induction of apoptosis, sensitization to other anti-cancer agents and stimulation of potent anti-tumor immune responses. Apoptosis induced by CD40L in CD40-positive carcinomas not only directly reduce tumor burden, but also increase antigen uptake and presentation by dendritic cells, thereby amplifying CTL and NK cell responses. Despite the theoretical risk of autoimmunity by this potent immune activator, no such adverse effect has been observed in experimental animal models or in phase-I/II clinical trials in humans. The absence of severe side-effects further supports the therapeutical potential of CD40L for the treatment of cancer. This potential is currently under evaluation in phase I clinical trials.

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