The Role of FasL/Fas Signaling Network in Infections and Tumorigenesis

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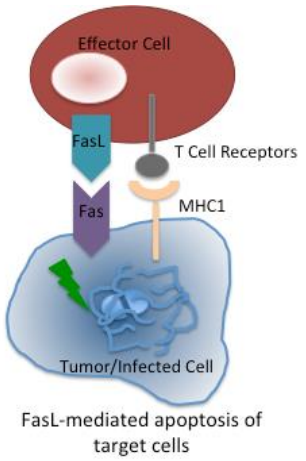
T Cell-Mediated Cytotoxicity:

All multicellular organisms are susceptible to tumor formation as well as infections from pathogenic viruses and bacteria, which serve as threats to the host organism. The host in turn deploys an immune response to ward off infections and eliminate malignant cells. T cell-mediated cytotoxicity is a complex and coordinated process that is critical for efficient immunological control of pathogenic infections and tumors (Kagi et al., 1996). This host defense mechanism is mediated by cytotoxic lymphocytes, a class of cells that includes $CD8^+$ cytotoxic T lymphocytes (CTLs). As important effectors of the adaptive immune system, CTLs scan tissues for cellular abnormalities and infections and have the ability to directly destroy malignant/infected cells by inducing apoptosis. Briefly, CTLs screen potential target cells for expression of foreign peptide fragments from cellular proteins and then destroy identified cells. The specificity of this process is mediated through cell surface T cell receptors on CTLs, which interact with antigen-derived peptides presented by the class I molecules of the major histocompatibility complex (MHC1) on the surface of target cells. Once the CTL identifies its target cell, it delivers the "lethal hit" by one of two distinct mechanisms described below (Takayama et al., 1995).

The initially characterized pathway is a calcium-dependent granule exocytosis pathway. In this pathway, effector cells secrete cytolytic proteins such as perforin and granzyme that penetrate target cells, initiate cytosolic and nuclear changes which culminate in apoptosis, thereby eliminating potentially harmful cells. The existence of an alternate pathway was proposed based on the observations that effector T cells from perforin knockout mice retained cytotoxic activity and were capable of inducing apoptosis in target cells. The calcium-independent pathway was identified as the FasL-Fas pathway, which was shown to have a crucial role in the homeostatic maintenance of immune cells in addition to an important role in T cell-mediated cytotoxicity (Henkart and Sitkovsky, 1994; Kagi et al., 1994) (Figure 1).

Figure 1



T Cell-Mediated Cytotoxicity by the FasL-Fas Pathway

Pathophysiological Roles of FasL-Mediated Apoptosis:

FasL-Fas Signaling Pathway:

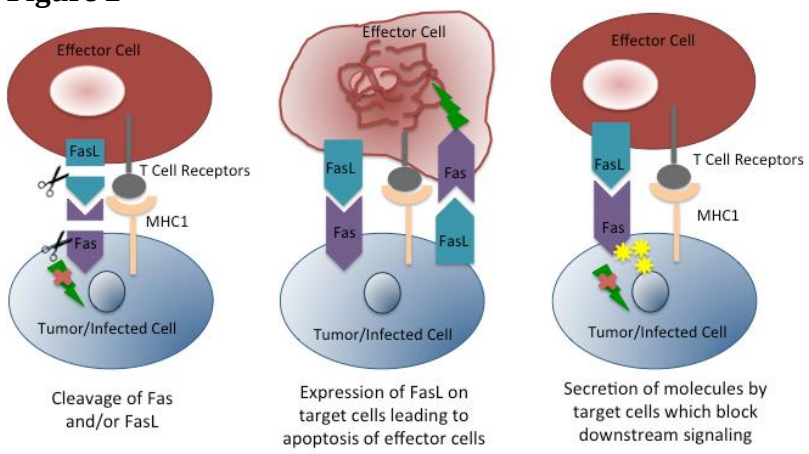
The Fas ligand (FasL), also known as CD95L, is a homotrimeric type II transmembrane protein belonging to the tumor necrosis factor (TNF) superfamily (Kavurma and Khachigian, 2003). FasL plays diverse roles in the immune system, including induction of apoptotic cell death in target cells. FasL binds to its receptor Fas (CD95R), a member of the TNFR subgroup of receptors termed the Death Receptors (DR). A hallmark of all DRs is the presence of the Death Domain (DD), which is responsible for transmitting the apoptotic signal. Binding of homotrimeric FasL to Fas triggers clustering of Fas and further recruitment of Fas-associated death domain (FADD) to the intracellular DDs of Fas. Capase-8 is bound to FADD and the oligomerization leads to self-cleavage and activation of caspase-8. Activated caspase-8 mediates apoptosis by direct activation of effector caspases like caspase-3 or through indirect activation of other downstream caspases through mitochondrial release of cytochrome c (Villa-Morales and Fernandez-Piqueras, 2012; Wajant et al., 2005).

FasL-Fas-mediated apoptosis plays fundamental roles in several immune functions. The pathway is involved in functions including regulation of T cell homeostasis and maintenance of immune privilege, apart from its well-established role in T cell-mediated cytotoxicity. Cytotoxicity via the Fas pathway is critical for prevention of pathogenic infections as well as control of tumor cell growth. In response to infections and tumors, the host triggers FasL-mediated cell death, which in turn activates proinflmammatory cytokines and chemokines, all of which cumulatively contribute to immune-mediated elimination of infected cells as well as tumor cells. Fas-mediated cytotoxicity is tightly regulated during normal physiological processes, and deregulation of the Fas-FasL pathway leads to immune evasion by pathogens as well as tumor cells (Dockrell, 2003; Peter et al., 2015a).

FasL-Fas pathway in pathogenesis of infectious diseases:

Several pathogens have devised strategies to manipulate the host-orchestrated cell death programs to enhance their virulence (Edwards et al., 1999) (Figure 2). One such strategy involves inhibition of the Fas-FasL signaling pathway. In the case of pneuomonic plague by *Y. pestis*, the bacteria produces a protease Pla that directly cleaves FasL on effector cells, thereby circumventing apoptotic cell death. Enteropathogenic *E. coli* employ a different strategy in which they secrete an effector molecule into the cytoplasm of target cells, blocking downstream signaling following

Figure 2



Evasion of FasL-Fas-Mediated Cytotoxicity by Target Cells

Fas-FasL engagement (Caulfield and Lathem, 2014). Given that pathogens promote virulence by disabling the Fas-FasL signaling cascade, researchers have proposed administration of pro-apoptotic compounds such as exogenous FasL to overcome immune system manipulation. Promising results were obtained in a preliminary study conducted using TRAIL, a related pro-apoptotic ligand. Administration of exogenous TRAIL in mice with pneumococcal pneumonia led to restoration of apoptosis, and subsequent reduction in colonization by the pathogen *Streptococcus pneumonia* (Steinwede et al., 2012). This study provides evidence for the potential success of other pro-apoptotic modulating agents in augment the host immunity during pathogenic infections.

FasL-Fas pathway in tumorigenesis:

Just like pathogens, reports suggest that tumor cells have also conceived ways to evade T cell-mediated cytotoxicity (Figure 2). While in normal cells FasL expression is largely restricted to CTLs, many tumor types, including melanomas, colorectal carcinomas, hepatomas, and gliomas express high levels of FasL (Kim et al., 2004). Hence, FasL-expressing tumor cells can launch a powerful "counterattack" by inducing apoptosis in immune effector cells, such as Fas-expressing CTLs. Thus, tumor cells are capable of destroying tumor-infiltrating CTLs, thereby suppressing anti-tumor responses (Peter et al., 2015c). Studies in several tumor types report that apoptosis in tumor infiltrating lymphocytes is observed more frequently in FasL-positive tumor cells than FasL-negative tumors. However, other studies performed are inconsistent with the counterattack model of FasL-expressing tumors. Gene transfer of FasL in renal carcinoma cells enhanced apoptotic activity and tumor regression *in* vivo (Arai et al., 1997). The role of FasL in the counterattack by tumors is further confounded by another study in which mouse tumors overexpressing FasL were rapidly rejected via massive neutrophil infiltration, an effect attributed to apoptosis triggered by the host cells. In light of these experiments, the validity of the counterattack model has come into question (O'Connell, 2002). Other mechanisms devised by tumors to override Fas-mediated apoptosis include inducing cleavage of either FasL or Fas, epigenetic silencing of Fas resulting in impaired surface expression, and interfering with downstream signaling activity, all of which result in resistance to apoptosis and tumor propagation (Villa-Morales and Fernandez-Piqueras, 2012).

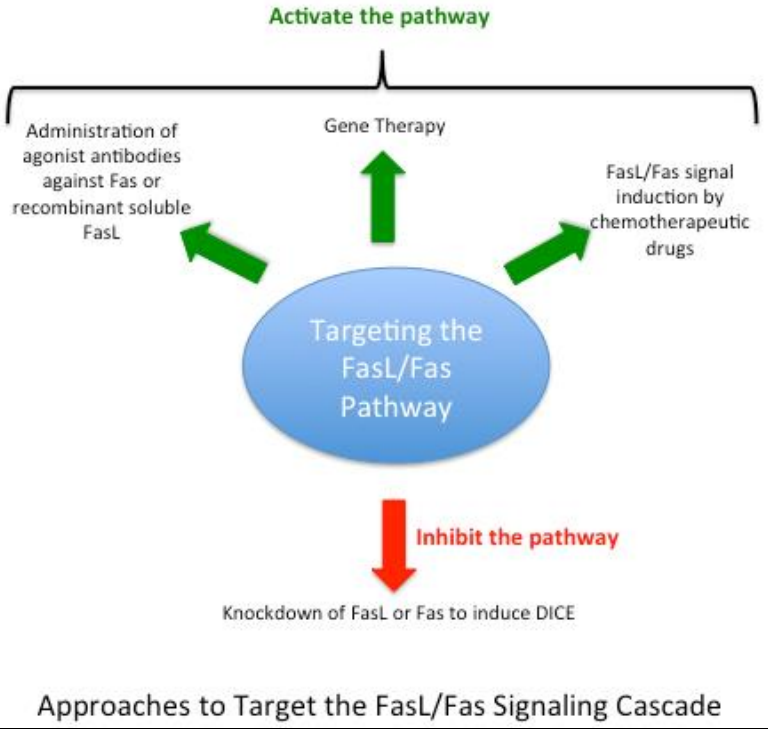
Therapeutic Benefits of Targeting FasL-Fas signaling pathway – To Activate or Inhibit?

Given the prominent role of FasL-Fas signaling network in tumors, research efforts are directed towards harnessing the apoptotic potential of the pathway in cancer therapy (Bremer, 2013; Bremer et al., 2009; Villa-Morales and Fernandez-Piqueras, 2012) (Figure 3). Initial experiments with Fas agonists and systemically delivered recombinant FasL led to severe hepatotoxicity and subsequent death in mice, thereby negating their systemic use in human cancer therapy (Bremer, 2013). However, intraperitoneal injections of soluble FasL resulted in efficient elimination of murine lymphoma cells with minimal toxicity in a mouse model (Rensing-Ehl et al., 1995). These data indicate that compartmentalized administration of Fas agonists is imperative for success of FasL-Fas therapy in cancer. In dogs with osteosarcoma, intratumoral FasL gene therapy delivered in an adenovirus vector (Ad-FasL) improved the rates of disease-free interval and overall survival in a subset of animals (Modiano et al., 2012). These data have spurred researchers to explore the potential of Ad-FasL therapy in human pediatric patients with osteosarcoma. The preclinical success of FasL gene therapy

in canines underscores the benefits of animal models that serve as biologically relevant tools to further develop novel and clinically translatable therapeutic approaches.

While amplifying the FasL-Fas pathway to achieve cell death for therapeutic gain has received considerable attention, there is mounting evidence for tumor-promoting effects of the FasL-Fas pathway since it can serve as a critical survival signal for cancer cells (Chen et al., 2010). Hence, rather than augmenting FasL activity in cancer therapy, some efforts are now focused on strategies to inhibit the FasL-Fas pathway for therapeutic gain. In a process termed Death induced by CD95R/L elimination (DICE), researchers reported that elimination or either FasL or Fas results in death of transformed cells (Hadji et al., 2014). The induced death is independent of caspase-8 and is characterized by an increase in cell size, production of mitochondrial reactive oxygen species, and DNA damage. DICE is a novel concept that is gaining traction particularly from the standpoint of an attractive therapeutic alternative for cancer patients. Inducing DICE as a stand-alone therapy or in combinatorial regimens is being explored as viable options for cancer therapy (Peter et al., 2015a).

Figure 3



Approaches to Target the FasL/Fas Signaling Cascade

While the field is still nascent, there is enough evidence to support a role for apoptotic modulators as important signaling hubs for clinical intervention. Continued investigation into mechanisms by which pathogens and tumor cells manipulate apoptotic pathways to alter host responses is crucial for development of successful and viable therapeutic options.

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