

Apoptosis – an Ubiquitous T cell Immunomodulator

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Abstract

Apoptosis is a natural process where cells that are no longer required can be eliminated in a highly regulated, controlled manner. Apoptosis is important in maintaining the mammalian immune system and plays a significant role in immune response, positive and negative T cell selection, and cytotoxic death of target cells. When the apoptotic pathways are impaired or are not tightly regulated, autoimmune diseases, inflammatory diseases, viral and bacterial infections and cancers ensue. An imbalance in the anti-apoptotic and pro-apoptotic factors has been implicated in these diseases. Moreover, current therapies directed towards these diseases focus on the modulation of the apoptotic death pathways to regulate the immune response. In this review, we will focus on the process of T cell activation and apoptosis in autoimmune reactions, in response to tumor progression as well as in response to bacterial and viral infections.

Keywords: T cell; Apoptosis; Infection; Autoimmunity; Tumor

Introduction

In order to maintain tissue homeostasis, cells undergo a process called programmed cell death or apoptosis. All cells have the ability to undergo apoptosis, including normal cells, either because they are no longer needed, are diseased, have become senescent or the cells are harmful to the organism. The process of apoptosis is seen in multicellular organisms during development, homeostasis, defense mechanisms, metamorphosis, terminal differentiation and immune responses. In this review, we will focus on the process of apoptosis of T cells in autoimmunity, in response to bacterial and viral infections, and in response to tumor progression.

Apoptosis is a mechanism by which cells are destined to die as a result of either intrinsic signals or extrinsic signals [1]. Intrinsic pathway, also known as mitochondrial pathway, involves the interaction of pro-apoptotic proteins and anti-apoptotic proteins. Bcl-2 is an anti-apoptotic protein that is normally expressed on the outer membrane of the mitochondria. Internal damage to the cell signals the pro-apoptotic molecule, Bax to migrate to the mitochondrial outer membrane, acts as an antagonist to the Bcl-2 protein and leads to cytochrome *c* release by making pores in the outer mitochondrial membrane. The released cytochrome-*c* binds to the protein Apaf-1 (apoptotic protease activating factor-1) forming the apoptosome complex. The apoptosome complex can now bind and activate a protease called caspase-9. As a result of caspase-9 activation, downstream caspase-3 and -7 are cleaved and activated. The end result of this cascade of signals is the digestion of structural proteins in the cytoplasm and degradation of chromosomal DNA and phagocytosis of the cell. While the intrinsic pathway is in response to internal signals, the extrinsic pathway or the death receptor mediated pathway involves interaction of death receptors and its specific ligand, leading to the activation of caspases downstream. As a result of the receptor-ligand interaction and caspase activation, the cells are triggered to undergo programmed cell death. An example of the extrinsic apoptosis pathway is the Fas-FasL-dependent signaling where FasL binds to Fas, recruiting the Fas-associated death domain (FADD) and procaspase-8 to the C-terminus of the death receptor, namely Fas. Accumulation of several procaspase-8 molecules triggers the autocatalysis of caspase-8, followed by the activation of caspase-3 and caspase-7, ultimately resulting in the apoptosis of the cell. In addition to T cell apoptosis, chronic stimulation of the T cells could lead to terminal differentiation of the T cells resulting in an exhausted

T cell phenotype. Whereas an oxidative environment can result in T cell dysfunction and even T cell death, thereby affecting the immune response.

T Cell Tolerance vs. Immunity

Immune cells undergo apoptosis as part of the well known phenomenon of positive and negative selection of T cells in the thymus [2]. As a result of this selection process, self-reactive T cells are removed from the T-cell repertoire. T cells that have been exposed to antigen and expanded to mount an immune response, called effector T cells, can undergo programmed contraction (termed programmed cell death - PCD) or a rapid activation-induced cell death (AICD) on TCR restimulation to maintain homeostasis and contribute to generation of memory T cell. Generally, apoptosis under these conditions do not elicit an immune response. However, T cell death in the periphery can induce tolerance [3]. Immune cells are exposed to dead cells during normal processes such as cell turnover as well as cell injury and infection. The ability to elicit an immune response or induce tolerance depends on the context in which the T cells see the antigen. That is, the mammalian immune system reacts differentially to necrotic stimuli and apoptotic stimuli. While inflammation and adaptive immunity is seen in response to necrosis, apoptosis leads to immune tolerance and is anti-inflammatory [4]. Moreover, by blocking caspase activation, signals that would normally elicit a tolerogenic response are now converted to immunogenic signals. The immunostimulatory molecule HMGB1 (High-Mobility Group Box-1) has been shown to be important in distinguishing tolerogenic vs. immunogenic signals [4]. Gurung et al. [3] have shown that naïve apoptotic cells induce tolerance, whereas apoptotic cells previously activated by antigen can induce immunity. They suggest that the expression of CD154 and its

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interaction with CD40 on dendritic cells is important in generating an immunogenic response by apoptotic cells instead of a tolerogenic response. Interestingly, clinical trials using monoclonal antibodies specific to the CD3 T Cell Receptor (TCR) to treat Type 1 diabetes resulted in the induction of regulatory CD8⁺ CD25⁺ T cells [5]. They further show that immune tolerance in these patients can be attributed to the CD8⁺ Foxp3⁺ T cells detected in the peripheral blood.

The immune response is also subject to oxidative stress-mediated regulation where high concentration of reactive oxygen species (ROS) can lead to T cell apoptosis or necrosis. It has been shown that T cell subsets are differentially susceptible to oxidative stress-induced apoptosis [6]. Differences in the expression of oxidative-stress induced genes and ROS metabolism in the T cell subsets have been implicated in regulating their susceptibility to oxidative stress. Recent evidence have suggested that myeloid derived suppressor cells (MDSCs) induce production of ROS that inhibit ζ -chain expression on T cells, resulting in T cell dysfunction [7]. Persistent HCV infection is also associated with the accumulation of CD33⁺ MDSC cells, which in turn induce ROS production resulting in the suppression of T cell immune response to the HCV that has not been completely delineated. However, in addition to ROS-mediated suppression, arginase-1, COX-2 and iNOS pathways have been reported as possible candidates in MDSC-mediated suppression of T cell immunity [9]. Therefore, MDSC-induced production of ROS results in the suppression of T-cell mediated immune responses either by inducing T cell dysfunction or sensitizing T cells to accelerated death.

T Cell Exhaustion

Initial antigen presentation to naïve T cells with costimulatory molecules trigger the differentiation of naïve T cells to effector T cells. Subsequent to antigen clearance and post-infection, cytolytic effector T cells differentiate further into memory T cells that can survive long-term without antigen stimulation. Persistent viral infection has been shown to initiate T cell exhaustion, a form of T cell dysfunction, where T cells have impaired effector function, express inhibitory receptors on the cell surface, and unique transcriptional pathways different from effector and memory T cells [10]. Persistent viral-antigen stimulation leads to the decreased expression of IL-7 receptor α -chain, decreased expression of the anti-apoptotic Bcl-2 molecule and induces memory T cell exhaustion followed by recurrence of viral infection [11]. The prolonged infection could lead to the loss of viral-specific T cell response. Recent evidence has suggested that inhibitory receptor, programmed death 1 (PD-1) regulate T cell activation and is over expressed in T cells with exhaustive phenotype in response to chronic viral infections [10,12,13]. PD-1 expression is associated with increased sensitivity to spontaneous and Fas-FasL mediated apoptosis of HIV-specific CD8⁺ T cells [14] as well as with tumor evasion by inducing apoptosis of anti-tumor CD8⁺ T cells [15]. Previous reports have demonstrated that PD-1 ligand (PD-L) on tumor cells induces apoptosis of activated, tumor antigen-specific T cells (which express PD-1), resulting in the elimination of tumor-specific T cells at the tumor site thereby promoting host immune evasion [16]. Moreover, the expression of PD-1 and PD-L2 ligand, in a tissue-specific manner, can also influence the function of exhausted CD8⁺ T cells [17]. Based on these findings, PD-1 negatively regulates the function of exhausted CD8⁺ T cells and that blockade of PD-1 pathway is effective in restoring function of exhausted CD8⁺ T cells in a tissue-specific manner. Youngblood et al. have shown that in response to chronic infections, PD-1 expression is under epigenetic regulation, where exhausted

CD8⁺ T cells have lost the ability to remethylate PD-1 DNA [18]. As a result, continual expression of the negative inhibitory receptor leads to impaired T cell function. Molecular studies of T cell exhaustion have shown that PD-1 expression is regulated by transcription factor Blimp1 in response to chronic antigen stimulation [19]. Moreover, Blimp1 is an important regulator of CD8⁺ T cell exhaustion and a repressor of memory CD8⁺ T cell differentiation. In addition, PD-1 induces the over expression of suppressor of cytokine signaling-1 (SOCS-1), which controls the downstream Jak/Stat signaling pathway effecting cytokine expression, in response to chronic HCV infection [20]. PD-1 and SOCS-1 are over expressed in patients with chronic HCV infection as compared to healthy controls, suggesting that crosstalk between these receptor molecules leads to T cell exhaustion by inhibiting the T-cell signaling pathway. Interestingly, based on recent evidence, the PD-1 and SOCS-1 negatively regulate the expression of another Jak/Stat pathway-regulated cytokine, IL-12 in monocytes, thereby affecting the host immunity to HCV infection during the initial phase of infection [21]. Decreased expression of IL-12 leads to impaired Th1 polarization and CTL response, resulting in the evasion of host immune response to HCV infection. Other inhibitory receptors include cytotoxic T lymphocyte-associated antigen-4 (CTLA-4), 2B4, CD160, GP49B, Tim3 and lymphocyte activation gene 3 (LAG-3) [10,22-26], all of which are important regulators of T cell dysfunction in chronic viral infections. Taken together, PD-1 is involved in regulating T cell exhaustion in cooperation with other negative inhibitory receptors, transcription factors and downstream signaling molecules. Therefore, persistent antigen stimulation results in the deletion of antigen-specific naïve or memory T cells and promotes chronic viral infections. In summary, the molecular mechanism involved in T cell exhaustion, T cell dysfunction and T cell apoptosis due to repetitive TCR stimulation modulates immune effector response and is an area of intensive research. In subsequent sections we discuss the various factors that affect apoptosis and in turn modulate infection and immunity.

Apoptosis in Autoimmunity

Apoptosis has been implicated in inducing autoimmune diseases including autoimmune thyroid diseases, systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) and multiple sclerosis (MS). In autoimmune diseases, auto-reactive T cells bypass the normal apoptotic signals resulting in the expansion of cells that attack self-antigens. Histone deacetylase proteins are important regulators of protein acetylation, chromatin remodeling and gene transcription. This type of transcriptional regulation is seen in tumor cells and it is now reported to occur in pro-apoptotic genes in auto-reactive T cells. Recent evidence has suggested that histone deacetylase 3 (HDAC3) is unregulated in PBMC of multiple sclerosis patients and as a result of this upregulation, self-reactive T lymphocytes are resistant to apoptosis [27]. Posttranslational modification is another method of triggering an immune response to self-antigens. For example, recent reports have suggested that isopartyl modification of cytochrome-c and small nuclear ribonucleoproteins (self-antigens), where the aspartic acid residues are non-enzymatically modified, can elicit an immune response to self-antigens in SLE patients [28]. Taken together, this would suggest that in autoimmunity, expression of self-reactive T cells is regulated at the transcriptional, post-transcriptional and post-translational levels.

Recent evidence suggests that defects in the anti-apoptotic protein BCL-2 leads to the development of autoimmune disorders [29,30]. Moreover, Fas-FasL (extrinsic) apoptosis pathway and the Bim (intrinsic) pathway have significant roles in regulating chronic immune

responses and thereby prevent autoimmunity [31,32]. Li et al. [33] have suggested that interleukin-7 is important in maintaining peripheral T-cell repertoire by inhibiting Bim activity at the posttranscriptional level. Based on studies using an animal model of spontaneous autoimmune disease, lymphocyte activation gene 3 (LAG-3) deficient mice, LAG-3 and PD-1, a negative regulator of activated T cells, work synergistically to prevent autoimmunity in mice [34]. Furthermore, an autoimmune susceptibility gene, FcRL3 has recently been identified in human T regulatory cells (Treg) [35]. While the function of this receptor is not known, and hence called an orphan receptor, the overexpression of this receptor leads to the dysfunction of Treg cells. The negative regulation of Treg cells results in autoimmune diseases. The FcRL3⁺ Treg cells resemble memory T cells with overexpression of PD-1 receptors where FcRL3 negatively regulates TCR signaling in Treg cells resulting in autoimmunity. Based on recent reports, SLE patients have increased apoptosis of circulating T cells and reduced clearance of apoptotic cells [36]. This increase in number of apoptotic cells is associated with increased production of reactive oxygen and nitrogen species as well as increased complement activation. This would suggest that these patients would benefit from therapies targeting the production of reactive oxygen and nitrogen intermediates, thereby reducing autoantigen presentation and subsequent autoimmune disease. However, the presence of auto-reactive T cells in SLE patients could be attributed to the defective regulation of PP2A B β , a regulator of IL-2 deprivation-induced T-cell apoptosis [37]. As a result of this defect in select SLE patients, auto-reactive T cells are resistant to apoptosis resulting in autoimmune disease. Contrary to the above methods of developing autoimmune diseases, current studies have also suggested that soluble Granzyme B, a serine protease expressed by cytotoxic T cells and natural killer cells, is responsible for generating auto-antigens in SLE [38] and in MS [39]. In brief, upon identifying the target cell, the membranes fuse so that the cytotoxic granules are released into the target cell to induce apoptosis. This is followed by the cooperation of another toxin, perforin. Perforin-granzyme B is now released by the cytotoxic T lymphocytes in the cytosol of the target cell leading to the death of virus-infected and tumor cells [40]. Thus, the difference in susceptibility of auto-reactive T cells to apoptosis is an important factor that governs their turnover and is responsible for autoimmune pathology.

Apoptosis in Viral Infection

One mechanism to counteract the pathogenesis of viral infections is to limit viral replication and spread by triggering the innate immune response. In response to viral infections, the host produces interferon-gamma (IFN- γ) to inhibit virus replication. Another common response to virus infection is to initiate suicide of the infected cell via premature apoptosis [41,42]. This study further suggested that the process of triggering premature apoptosis in the infected cell requires the interaction of interferon regulatory transcription factor-3 (IRF3) and pro-apoptotic protein, Bax. IRF3 and Bax are translocated to the mitochondria, followed by the activation of the intrinsic apoptotic pathway, killing the virus-infected cell. In an attempt to counteract the effects of interferon production by the host, virus-infected cells have the ability to interfere with IFN- γ induction and IFN JAK-STAT signaling [43]. Examples of this type of regulation are seen with NS1 protein of influenza-A virus [44], the NS3/4A of hepatitis C virus [45], and the V protein of paramyxovirus [46]. Moreover, current phase III clinical trials are testing the feasibility of NS3/4A protease inhibitors, telaprevir (VX-950) and boceprevir (SCH-503034), to treat hepatitis C infections. While it is known that interferon-stimulated gene 56

(ISG56) regulates virus-triggered signaling of IFN1 production, it has also been implicated in regulating cellular anti-viral responses [47]. Therefore, ISG56 inhibits viral replication and protein translation, and is a key regulator of cellular anti-viral responses.

The coxsackievirus B (CVB3) uses a mechanism to suppress host-initiated antiviral signaling by cleaving mitochondrial antiviral signaling protein (MAVS) and Toll/IL-I receptor domain-containing adaptor inducing interferon-beta (TRIF) in order to evade host immune system [48]. These molecules are cleaved by the CVB3-derived 3C^{pro} cysteine protease. As a result, 3C^{pro} cysteine protease can suppress type I IFN and apoptotic signals whose purpose is to clear the CVB3 infection. In persistent herpes virus infections, BID and BIM pro-apoptotic proteins are involved in the regulation of activated CD8⁺ T cell death in vivo [49]. BID has been shown to act as a link between the extrinsic and intrinsic apoptotic pathways so that T cell immune responses are shutdown in conditions of chronic, persistent antigen exposure. Recent studies have also highlighted the role of small non-coding RNAs in translational regulation of viral transcripts. These small non-coding RNAs, known as microRNAs (miRNA), are approximately ~22 nucleotide in length, bind to messenger RNA and repress translation of the messenger transcript in viral pathogenesis. Interestingly, molecular techniques have identified 21 miRNAs and 400 mRNA that were differentially expressed in response to the HIV1 infection [50]. Based on these results, miRNAs are important in regulating the host cellular genes during the HIV-1 infection thereby changing the host's response to the infection. Moreover, HIV-1-derived TAR miRNA has been shown to protect the HIV-1 infected cell from apoptosis by down-regulating two apoptosis related genes, namely ERCC1 (Excision repair cross complementing-group1) and IER3 (Immediate Early Response 3) [51]. ERCC1, the excision repair enzyme, and IER3 protects cells from extrinsic type of apoptotic pathways. IER3 plays an important role in the induction of apoptosis in response to serum starvation and DNA damage. Similarly, KSHV-derived miRNA have been shown to induce IL-6 and IL-10 cytokines by macrophages and monocytes, thereby promoting tumor progression and suppressing anti-tumoral immune responses [52]. KSHV-derived miR-K12-3 and miR-K12-7 have been identified as key regulators of IL-6 and IL-10 production by targeted suppression of C/EBP β p20 (LIP) expression. LIP is a dominant-negative transcriptional repressor of other isoforms of C/EBP β . Therefore, KSHV-derived miR-K12-3 and miR-K12-7 up-regulate IL-6 and IL-10 production, which results in impaired dendritic cell maturation, inhibits Th1-, NK- and macrophage-derived cytokine production, influences T cell activation. Moreover, IL-6 has been shown to enhance angiogenesis in a VEGF- and FGF-mediated manner. Qin et al. [53] have also demonstrated that KSHV-derived miR-K12-11 up-regulates expression of xCT, enhances reactive nitrogen species (RNS) secretion by macrophages and protect these cells from RNS-induced cell death. Therefore, KSHV-derived miR-K12-11 promotes survival of KSHV-infected cells even under oxidative stress-promoting conditions. Taken together, virus-infected cells evade the host's immune response by inhibiting apoptosis of the virus-infected cells by regulating either host derived cytokines, reactive oxygen and nitrogen species, apoptotic pathways and excision repair enzymes. Based on the previous examples, it is quite clear that viral infection can not only affect T cell function, but also evade apoptosis by regulating the host lytic molecules that are involved in viral clearance.

Apoptosis in Bacterial Infections

The ability to evade apoptosis upon establishing an infection in the host is a requirement for bacterial survival. There are several bacterial mechanisms to accomplish this once cells are infected with bacteria. For example, bacteria can target macrophages and neutrophils and initiate apoptosis of these immune cells so that the bacterially infected cells survive and proliferate. That is, the macrophages and neutrophils can no longer attack the bacteria-infected cells. In contrast, there is current evidence that bacteria can also prevent apoptosis during infection. For example, *Chlamydia* have the ability to prevent apoptosis by preventing host-derived pro-apoptotic proteins, Bax and Bak from entering the mitochondria [54]. As a result of this mechanism, cytochrome-*c* is not released and therefore downstream caspase signaling is inhibited in the infected cells. *Chlamydia* can also target pro-apoptotic proteins, such as Bik, Puma and Bim, for degradation by a protease known as chlamydial proteasome-like activity factor (CPAF). It is also possible for *Chlamydia* to regulate extrinsic pathway of apoptosis by upregulating inhibitor of apoptosis proteins (IAPs). By preventing apoptosis of the *Chlamydia*-infected epithelial cell, the infected cells will continue to divide, undergo DNA synthesis and mitosis post-infection. Thereby ensuring that *Chlamydia* infected host cells continue to undergo cell division and prevent apoptosis of the infected cell. In contrast, *Chlamydia*-infected macrophages can induce apoptosis of activated T cells by secreting tumor necrosis factor- α (TNF- α) [55]. As a result of this apoptosis induction in host T cells, persistent Chlamydial infection is promoted. While it is known that CD8⁺ T cells are the primary cells that are activated in response to viral, bacterial infections, parasitic infections and tumor cells, current research has identified, B-cell lymphoma/leukemia 11b or Bcl11b as a critical transcription factor for CD8⁺ T cell immune response [56]. The results of this study further suggest that this zinc finger protein is required for antigen-dependent clonal expansion and cytolytic activity. Moreover, Bcl11b may regulate expression of perforin and granzyme B in response to bacterial infection. Other mechanisms can also regulate T cell activation and cell-mediated immunity. For example, Xie et al. [57] have suggested that T cell-mediated immunity and TCR/CD28 signaling in response to *Listeria monocytogenes* infection, is impaired in the absence of TNFR-associated factor 3 (TRAF3). There are examples of obligate intracellular bacteria that inhibit apoptosis by promoting cell survival pathways. While *Ehrlichia chaffeensis* inhibit apoptosis by up-regulating NF- κ B, as well as anti-apoptotic genes, namely bcl-2 [58], *Rickettsia rickettsii* inhibit apoptosis by upregulating NF- κ B, up-regulation of anti-apoptotic proteins, inhibition of cytochrome-*c* release and caspase activation [59]. Therefore, bacterial infections can take advantage of the host cell survival pathways and thereby allowing bacteria to replicate and evade the host immune response.

Apoptosis in Tumor Response

Tumor cells have developed mechanisms to evade the host immune system including downregulation of MHC class I molecules, secretion of immunosuppressive factors, and downregulation of co-stimulatory molecules. Recent reports have confirmed that tumor cells express B7-H1 co-stimulatory molecule, but normal tissues do not express B7-H1 [16]. Interestingly, B7-H1 has been described to regulate cellular and humoral immune responses through the PD-1 receptor on activated T and B cells. The end result would be to increase apoptosis of tumor reactive T cells. Moreover, Sakuishi et al. [60] have identified CD8⁺ tumor-infiltrating cells (TILs) that express PD-1 and T cell immunoglobulin mucin 3 (Tim-3), referred to as exhausted T

cells, which exhibit another form of immunosuppression. These T cells fail to proliferate, and cannot function as effector cells, that is, they fail to elicit a cytotoxic response to antigen stimulation, which results in unhindered tumor growth. By eliminating the exhausted T cell phenotype, via targeting of the Tim3 and PD-1 pathways, it is possible to effectively restore cytotoxicity function of these T cells in the tumor environment. Tumor cells can evade immune surveillance by modifying the surface antigen expression and promote immunosuppression. Tumor cells have also been shown to express galectin-1, which promotes tumor growth, angiogenesis and T-cell apoptosis [61]. Interestingly, the study establishes the importance of tumor-derived galectin-1 rather than host galectin-1 in tumor progression by regulating intratumoral immunomodulation via T-cell apoptosis. Another example of an immune escape mechanism is seen in colorectal cancers, where death receptor signaling is executed from tumor to T cell [62]. While tumor cells express TRAIL, the tumor-infiltrating immune effector cells, either IFN- γ -producing CD8⁺ effector T cells or CD56⁺ NK cells, express TRAIL-R1. The TRAIL and TRAIL-R1 interaction leads to the apoptosis of the CD8⁺ effector T cells, thereby evading the host immune system. Reports also suggest that CD8⁺ effector T cells infiltrating the tumors over-express Fas and cross-linking this molecule with its ligand, Fas ligand (FasL), on tumor cells leads to the apoptosis of the CD8⁺ effector cells, again resulting in the evasion of immunosurveillance and allowing tumor progression [63]. Based on in vitro studies, colon cancer cells over-expressing FasL can trigger apoptosis in T lymphocytes expressing Fas [64]. This would further support the process of inducing immune tolerance by initiating apoptosis of Fas-sensitive anti-tumor immune effector T lymphocytes. Immune evasion can also occur by tumor-induced signal transducer and activator of transcription 3 (STAT3) constitutive signaling in tumor cells and the tumor-associated immune cells [65,66]. STAT3 signaling is activated in the nonmalignant cells in the tumor microenvironment as a result of tumor-associated factors including IL-10, IL-6, β 2-microglobulin and VEGF. The antigen-presenting cells in the tumor, dendritic cells (DC), are negatively regulated by the STAT3 signaling resulting in the inhibition of DC maturation, decreased MHC II expression, decreased CD80/86 expression and decreased secretion of IL-12. As a result of this negative regulation by STAT3, there is a reduction in T cell cytotoxic activity and IFN γ production, an increase in the number of tumor-associated Treg cells. Together, they regulate anti-tumor response by the immune cells. While it is known that constitutive activation of STAT3 promotes angiogenesis and metastasis, other downstream targets of STAT3 signaling have been identified which includes CD46, a complement-regulatory protein [67]. CD46 expression is up-regulated in normal and tumor cells to prevent complement-mediated cytotoxicity. Therefore, STAT3 is a key mediator of tumor immune evasion at multiple levels. A significant number of CD4⁺CD25⁺ regulatory T cells were found in peripheral blood of patients with breast cancer as compared to the number these cells in the peripheral blood of patients with benign breast cancer and healthy volunteers [68]. Moreover, the increase in the number of Tregs, increased apoptosis of competent T cells, as well as the tumor size was associated with the expression of indoleamine 2,3-dioxygenase (IDO), which is required for the metabolism of tryptophan in the kynurenine pathway. This particular study has suggested that IDO is important in inducing tumor tolerance by up-regulating Treg cells and the subsequent apoptosis of competent cytotoxic T cells, thereby allowing the tumor to grow without triggering an immune response in the patient. In conclusion, molecular mechanism of immune surveillance is an important area of research that could shed some light into how

apoptosis of competent cytotoxic T cells, in the presence of Tregs, leads to tumor progression.

microRNA in T cell function

As discussed in previous sections, microRNA's (miRNA) can play an important role in modulating the host microenvironment, where tumor cells as well as virus-and bacteria-infected cells evade apoptotic signals regulated by innate effector molecules. However, T cell function could also be modulated by miRNA and could result in modulating the disease outcome. Recent studies have demonstrated the importance of miRNA's in the posttranscriptional regulation of gene expression, including genes associated with T cell activation. Curtale et al. [69] have shown that miRNA-146a plays a significant role in the activation of T-cell mediated immune response. They further suggest that miRNA-146a is important in regulating AICD, it acts as an antiapoptotic factor, targets the Fas-associated death domain (FADD), impairs both activator protein 1 (AP-1) activity and Interleukin-2 (IL-2) production upon TCR activation. Based on these results, it is evident that miR-146a is involved in the modulation of adaptive immunity. The importance of miRNA in regulating the differentiation of helper T cells has also been reported [70]. Based on these findings, miR-29 is also important in regulating IFN- γ production by T helper cells as well as the proliferation of helper T cells. Other miRNAs have been implicated in the negative regulation of T cell immunity, such as miRNA-155 [71]. MiR-155 is an oncogenic miRNA that is induced in dendritic cells upon maturation and has the ability to repress T cell

activation. MiRNA-155 may negatively regulate the expression of molecules required for lymph node migration, antigen presentation and T cell activation. Interestingly, Grigoryev et al. [72] have identified 71 differentially expressed miRNAs that can potentially regulate T cell activation. In addition to identifying two miRNAs, miR-155 and miR-221, as having anti-proliferative roles during T cell activation, they report several target genes for miR-155 and miR-221 including PIK3R1 (Phosphatidylinositol 3-kinase regulatory subunit alpha), IRS2 (Insulin receptor substrate 2, an adaptor of tyrosine kinase), IKBKE (Inhibitor of nuclear factor kappa-B kinase subunit epsilon) and FOS transcription factor. Taken together, these studies suggest significant role of miRNAs in regulating transcription of genes associated with T cell activation.

Pharmacological & Molecular Approaches to Modulate Apoptosis in Disease State

The ability to modulate apoptotic pathways in several diseases has been the focus of current pharmacological and molecular therapies. Table 1 illustrates various signaling molecules discussed above that are involved in regulating immune function and could be potentially targeted to improve various disease states. Patients with chronic hepatitis C infection develop fibrosis of the liver as a result of activated T cells, activated stellate cells and ultimately, apoptosis of the infected hepatocytes [73]. Moreover, HCV infection leads to apoptosis of activated CD4⁺ and CD8⁺ T cells via Fas-FasL apoptotic pathway, thereby promoting chronic HCV infection by evading the host

| Signaling Molecule | Involved in Regulating | Reference # |
|---------------------------|--------------------------------------|--------------|
| miR-146a | AICD | [69] |
| miR-29 | Helper T cell differentiation | [69] |
| miR-155, miR-221 | T cell activation | [71,72] |
| miR-K12-3, miR-K12-7 | IL-6 and IL-10 production | [52] |
| PD-1 | T cell exhaustion | [10, 12, 13] |
| Blimp-1 | T cell exhaustion | [19] |
| CTLA-4, 2B4, CD160, GP49B | T cell exhaustion | [10, 22-26] |
| LAG-3 | T cell exhaustion | [10, 22-26] |
| IRF-3 and Bax | T cell apoptosis | [41, 42] |
| NS1 protein | host immune system | [44] |
| NS3/4 A | host immune system | [45] |
| V protein | T cell apoptosis | [46] |
| ISG56 | IFN1 production | [47] |
| 3Cpro cysteine protease | IFN1 production | [48] |
| TAR miRNA | host ERCC1 and IER3 | [51] |
| CPAF | Bik, Puma and Bim degradation | [54] |
| TRAF3 | T cell activation | [57] |
| NF- κ B, bcl-2 | T cell apoptosis | [58] |
| NF- κ B, | cytochrome c T cell apoptosis | [59] |
| B7-H1 | apoptosis of tumor-reactive T cells | [16] |
| Tim-3 | immunosuppression, T cell exhaustion | [60, 10, 25] |
| Galectin-1 | T cell apoptosis | [61] |
| TRAIL-R1 | host immune system | [62] |
| Fas | host immune system | [63] |
| STAT-3 | immunosurveillance | [65, 66, 67] |
| IDO | immunosurveillance | [68] |
| Fas-FasL | T cell apoptosis | [74] |
| XIAP | apoptosis | [78, 79, 82] |
| Bcl-2, Bcl-XL, Bcl-W | apoptosis | [83] |

Table 1: Signaling Molecules and their role in T cell Regulation.

immune response [74]. Therapies directed to treat chronic HCV by limiting activated T cell apoptosis include caspase-inhibitor GS-9450 [75], and oral IDN-6556, another caspase inhibitor used to prevent apoptosis of hepatocytes in HCV infected patients [76]. However, the effect of IDN-6556 on apoptosis of activated T cells was not addressed in this study. Therefore, it is possible for IDN-6556 therapy to prevent apoptosis of hepatocytes and promote immune response by inhibiting apoptosis of activated T cells in HCV infected patients. The type of treatment administered is dependent on the type of viral infection. For example, HIV infected cells require a different approach to control viral replication and enhance immune response to HIV infection. By administering protease inhibitors to AIDS patients, neutrophils, polymorphonuclear leukocytes, and HIV-infected and uninfected CD4⁺ T cells are resistant to apoptosis signals by direct inhibition of the Calpain-Bax-associated apoptosis pathway [77]. This would increase the number of reactive T cells and thereby allow activation of host immune system.

Cancer cells can evade apoptotic signals by over-expressing an anti-apoptotic protein called XIAP, X-linked inhibitor of apoptosis. Current pre-clinical and clinical trials have used anti-sense oligonucleotides directed toward XIAP to treat non-small cell lung cancer, pancreatic cancer, breast cancer, thereby making the cancer cells sensitive to chemotherapy and radiation therapy [78,79]. Under some circumstances, for example in response to chemotherapy, namely anthracyclins and oxaliplatin, cancer cells undergo immunogenic apoptosis where they trigger a protective immune response [29,80,81]. Dendritic cells engulf the apoptotic cancer cells and tumor cell-derived antigens are presented to tumor specific CD8⁺ T cells, which are now instrumental in preventing tumor progression. Cells that undergo immunogenic apoptosis express calreticulin on the surface of the cell, which is normally expressed in the endoplasmic reticulum. In contrast, cancer cells that are defective in presenting calreticulin on the surface are resistant to anticancer therapies like anthracyclins and oxaliplatin. For example the human neuroblastoma cell line, SH-SY5Y, fail to present calreticulin on the surface in response to anthracyclin treatment. Therefore, the ability of cancer cells to present calreticulin on the surface will determine its sensitivity to anthracyclin treatment. Current apoptosis-based therapies to treat cancer include caspase inhibitors, monoclonal antibodies directed towards TRAIL-receptors, CD95/Fas, TNF- α and anti-sense constructs against TNF- α , XIAP anti-sense oligo-nucleotides, XIAP RNAi and anti-survivin constructs, all of which are at either preclinical or phase I clinical trials [82]. Recently, BH3-mimetic therapy has been used to target pro-survival proteins, such as Bcl-2, thereby overcoming the resistance to apoptosis in hematological malignancies [83]. While Navitoclax and ABT-737 are examples of BH3-mimetics with affinity to Bcl-2, Bcl-XL and Bcl-w pro-survival proteins, Obatoclax and AT-101 BH3-mimetics induce cytotoxicity in tumor cells expressing Bcl-2 as well as Bcl-XL, Mcl-1, Bax and Bak. Interestingly, these BH3-mimetics have been used as single agents as well as in combination with conventional chemotherapeutic drugs to treat chronic lymphocytic leukemia (CLL), small lymphocytic lymphoma, NK/T cell lymphoma and follicular lymphoma (FL), acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), systemic mastocytosis and Hodgkin's lymphoma. Taken together, BH3-mimetics are used to overcome tumor-associated resistance to apoptosis and thereby limit tumor progression.

While kinase inhibitors and monoclonal antibodies have been used in clinical trials to treat tumors, new approaches to treat cancer include dendritic cell (DC) tumor vaccines and immunomodulatory agents that

enhance the host's tumor-specific T cell immune response. DC cancer vaccines are used to induce effector T cell differentiation and cytokine production specifically targeting the tumor [84]. However, DC, at the tumor site, is subjected to tumor induced immunosuppression by abnormal macrophages, myeloid-derived suppressor cells and Treg cells. Therefore, it is important for DC vaccines to circumvent the immunosuppressive tumor microenvironment in order to elicit a tumor-specific T cell-based immune response. For example, a phase III sipuleucel-T immunotherapy clinical trial to treat patients with castration resistant prostate cancer has some promise in eliciting a DC-directed anti-tumor response [85]. Sipuleucel-T is a cellular vaccine where autologous peripheral blood mononuclear cells are activated with a recombinant fusion protein PA2024. PA2024 fusion protein consists of human granulocyte/macrophage-colony stimulating factor (GM-CSF) and human prostatic acid phosphatase (PAP), and prostate antigen. Sipuleucel treatment targets cells that express GM-CSF-receptor, resulting in the activation and maturation of DC precursors. While overall patient survival was improved, the time to disease progression did not change with sipuleucel treatment. Other DC vaccine based therapies to induce tumor-antigen directed immune response include MHC class I and II-restricted peptide-loaded DC vaccines [86,87], DC co-transfected with mRNA encoding melanoma specific antigens and soluble human glucocorticoid-induced TNFR-related protein-ligand (GITR-L) and anti-CTLA-4 mAb [88] and DC transfected with the hTERT subunit of telomerase to treat pancreatic cancer [89].

Pharmacological agents that expand tumor-reactive T cells, NKT, NK cells and IFN- γ -producing killer DC, such as bryostatins, have shown to be effective as anti-tumor agents in animal models and in clinical trials. Adoptive cellular therapy was used to treat breast cancer in transgenic murine models where tumor-reactive T cells were expanded, ex-vivo, with bryostatin 1/ionomycin and cytokines IL-7/IL-15 + IL-2 [90]. Bryostatin is a protein kinase C modulator that mimics the CD3/TCR complex signaling resulting in the activation and proliferation of tumor-reactive T cells. Interestingly, these expanded T cells, which include memory and effector T cells, are resistant to MDSC-induced immunosuppression. Furthermore, Bryostatin 1 treatment has been used in combination with other cytotoxic chemotherapeutic agents, such as vincristine, to effectively treat select patients with non-Hodgkin lymphoma (NHL) who relapsed after autologous stem cell transplant [91]. In vitro studies using human B-cell lymphoma cell line, WSU-DLCL2, has suggested that administration of bryostatin/vincristine combination leads to the suppression of the anti-apoptotic protein Bcl-2 expression and the upregulation of the tumor suppressor protein, p53 [92]. Suggesting that this combination is effective in eliciting an anti tumor response. However, in this phase II clinical trial where patients with NHL were subjected to bryostatin1/vincristine treatment, the researchers could not unequivocally correlate clinical response with an increase in apoptotic frequency of T cells. Based on these results, further research in correlating clinical outcomes with apoptotic frequency of peripheral blood T cells is indicated. Nonetheless, current clinical trials have suggested that bryostatin can be used in combination with gemcitabine [93], fludarabine [94] and cisplatin [95] to effectively treat non-hematologic tumors, chronic lymphocytic leukemia and indolent NHL and refractory non-hematological tumors, including melanoma, sarcoma and head and neck, ovarian, cervical, esophageal pancreatic, renal and lung tumors. Taken together, bryostatin has the potential to effectively regulate T cell anti-tumor response in combination with other cytotoxic agents.

Ex-vivo expansion of human FOXP3-expressing Treg cells,

using pharmacological agents, is another area of research that has recently gained considerable interest, largely due to the plasticity of human Tregs cells. The immunosuppressive human Treg cells are capable of differentiating into FOXP3-negative, IL-17- and IFN- γ -secreting inflammatory/effector T cells depending on the cytokine microenvironment, thereby making it difficult to expand human FOXP3-positive Treg cells, ex-vivo [96]. Golovina et al. [97] have demonstrated that All-transretinoic acid (ATRA) in combination with rapamycin can expand human peripheral bloodpurified Tregs and that rapamycin plays a significant role in T cell homeostasis and T cell function. While rapamycin inhibits the expansion of effector T cells by inhibiting the mammalian target of rapamycin (mTOR) pathway, FOXP3⁺ Tregs have the ability to overcome this rapamycin-mediated inhibition and therefore, have the ability to expand, ex-vivo in response to rapamycin and ATRA. Taken together, FOXP3⁺ Treg are resistant to rapamycin since FOXP3 expression induces yet another signaling molecule, Pim2, a serine/threonine kinase that promotes resistance to rapamycin-mediated apoptosis [98]. Moreover, ex-vivo expanded Tregs retained the suppressive activity in the presence of ATRA and rapamycin and these Tregs can be used to effectively regulate autoimmune reactions in vivo. For example, rapamycin has been shown to reduce the severity of experimental osteoarthritis in an autophagy-activation manner [99]. Impaired autophagy results in the overproduction of ROS, abnormal gene expression and cell death. Interestingly, mTOR plays a significant role in inhibiting autophagy. This would then suggest that rapamycin has the ability to regulate ROS production and cell death by blocking mTOR signaling while promoting autophagy activation. Therefore, rapamycin treatment reduces the severity of osteoarthritis in mouse models by activating autophagy and inducing the expansion of immunosuppressive Treg, in vivo. Rapamycin therapy has also been used to restore insulin production by pancreatic β cells in patients with long-term type 1 diabetes by regulating the autoimmunity in these patients [100] as well as to induce immune tolerance in murine transplantation models, where rapamycin prevents or delays allograft rejection [101]. Taken together, the immunosuppressive drug, rapamycin can restore the function of Treg cells, maintain T cell homeostasis and regulate autoimmune reactions.

Conclusion

Apoptosis is a key component in health and disease. Normal development of the nervous system and immune system requires apoptosis of unwanted neuronal cells and immune cells, respectively. Understanding the molecular mechanisms of apoptosis will provide insight into the associated disease processes and may help in designing therapeutic regimens. While some conditions are attributed to upregulation of apoptosis, other conditions are due to decreased apoptosis of immune cells. Furthermore, apoptosis signaling pathway has several checkpoints and by elucidating the molecular basis for dysregulation of apoptosis in the disease states one can design more effective therapies to treat the diseases. Taken together, translational outcome of basic research on the molecular mechanisms of apoptosis in immunoregulation is warranted.

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References

1. Fadeel B, Orrenius S (2005) Apoptosis: a basic biological phenomenon with wide-ranging implications in human disease. *J intern med* 258: 479-517.
2. Owen JJ, Jenkinson EJ (1992) Apoptosis and T-cell repertoire selection in the thymus. *Ann N Y Acad Sci* 663: 305-310.
3. Kucaba TA, Ferguson TA, Griffith TS (2009) Activation-induced CD154 expression abrogates tolerance induced by apoptotic cells. *J Immunol* 183: 6114-6123.
4. Kazama H, Ricci JE, Herndon JM, Hoppe G, Green DR, et al. (2008) Induction of immunological tolerance by apoptotic cells requires caspase-dependent oxidation of high-mobility group box-1 protein. *Immunity* 29: 21-32.
5. Bisikirska B, Colgan J, Luban J, Bluestone JA, Herold KC (2005) TCR stimulation with modified anti-CD3 mAb expands CD8⁺ T cell population and induces CD8⁺CD25⁺ Tregs. *J Clin Invest* 115: 2904-2913.
6. Mehrotra S, Mougiakakos D, Johansson CC, Voelkel-Johnson C, Kiessling R (2009) Oxidative stress and lymphocyte persistence: implications in immunotherapy. *Adv Cancer Res* 102: 197-227.
7. Nagaraj S, Gabrilovich DI (2008) Tumor escape mechanism governed by myeloid-derived suppressor cells. *Cancer Res* 68: 2561-2563.
8. Tacke R, Lee HC, Goh C, Courtney J, Polyak SJ, Rosen HR, Hahn YS (2011) Myeloid suppressor cells induced by hepatitis C virus suppress T cell responses through the production of reactive oxygen species. *Hepatology*.
9. Greten TF, Manns MP, Korangy F (2011) Myeloid derived suppressor cells in human diseases. *Int Immunopharmacol* 11: 802-807.
10. Wherry EJ (2011) T cell exhaustion. *Nat Immunol* 12: 492-499.
11. Lang KS, Recher M, Navarini AA, Harris NL, Löhning M, et al. (2005) Inverse correlation between IL-7 receptor expression and CD8 T cell exhaustion during persistent antigen stimulation. *Eur J Immunol* 35: 738-745.
12. D'Souza M, Fontenot AP, Mack DG, Lozupone C, Dillon S, et al. (2007) Programmed death 1 expression on HIV-specific CD4⁺ T cells is driven by viral replication and associated with T cell dysfunction. *J Immunol* 179: 1979-1987.
13. Day CL, Kaufmann DE, Kiepiela P, Brown JA, Moodley ES, et al. (2006) PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression. *Nature* 443: 350-354.
14. Petrovas C, Casazza JP, Brenchley JM, Price DA, Gostick E, et al. (2006) PD-1 is a regulator of virus-specific CD8⁺ T cell survival in HIV infection. *J Exp Med* 203: 2281-2292.
15. Shi F, Shi M, Zeng Z, Qi RZ, Liu ZW, et al. (2011) PD-1 and PD-L1 upregulation promotes CD8⁺ T-cell apoptosis and postoperative recurrence in hepatocellular carcinoma patients. *International journal of cancer. Int J Cancer* 128: 887-896.
16. Dong H, Strome SE, Salomao DR, Tamura H, Hirano F, et al. (2002) Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. *Nat Med* 8: 793-800.
17. Blackburn SD, Crawford A, Shin H, Polley A, Freeman GJ, et al. (2010) Tissue-specific differences in PD-1 and PD-L1 expression during chronic viral infection: implications for CD8 T-cell exhaustion. *J Virol* 84: 2078-2089.
18. Youngblood B, Oestreich KJ, Ha SJ, Duraiswamy J, Akondy RS, et al. (2011) Chronic virus infection enforces demethylation of the locus that encodes PD-1 in antigen-specific CD8⁺ T cells. *Immunity* 35: 400-412.
19. Shin H, Blackburn SD, Intlekofer AM, Kao C, Angelosanto JM, et al. (2009) A role for the transcriptional repressor Blimp-1 in CD8⁺ T cell exhaustion during chronic viral infection. *Immunity* 31: 309-320.
20. Frazier AD, Zhang CL, Ni L, Ma CJ, Zhang Y, et al. (2010) Programmed death-1 affects suppressor of cytokine signaling-1 expression in T cells during hepatitis C infection. *Viral Immunol* 23: 487-495.
21. Zhang Y, Ma CJ, Ni L, Zhang CL, Wu XY, et al. (2011) Cross-talk between programmed death-1 and suppressor of cytokine signaling-1 in inhibition of IL-12 production by monocytes/macrophages in hepatitis C virus infection. *J Immunol* 186: 3093-3103.

22. Crawford A, Wherry EJ (2009) The diversity of costimulatory and inhibitory receptor pathways and the regulation of antiviral T cell responses. *Curr Opin Immunol* 21: 179-186.
23. Grosso JF, Goldberg MV, Getnet D, Bruno TC, Yen HR, et al. (2009) Functionally distinct LAG-3 and PD-1 subsets on activated and chronically stimulated CD8 T cells. *J Immunol* 182: 6659-6669.
24. Nakamoto N, Cho H, Shaked A, Olthoff K, Valiga ME, et al. (2009) Synergistic reversal of intrahepatic HCV-specific CD8 T cell exhaustion by combined PD-1/CTLA-4 blockade. *PLoS Pathog* 5: e1000313.
25. Jones RB, Ndhlovu LC, Barbour JD, Sheth PM, Jha AR, et al. (2008) Tim-3 expression defines a novel population of dysfunctional T cells with highly elevated frequencies in progressive HIV-1 infection. *J Exp Med* 205: 2763-2779.
26. Kaufmann DE, Kavanagh DG, Pereyra F, Zaunders JJ, Mackey EW, et al. (2007) Upregulation of CTLA-4 by HIV-specific CD4+ T cells correlates with disease progression and defines a reversible immune dysfunction. *Nat Immunol* 8: 1246-1254.
27. Zhang F, Shi Y, Wang L, Sriram S (2011) Role of HDAC3 on p53 expression and apoptosis in T cells of patients with multiple sclerosis. *PLoS One* 6: e16795.
28. Yang ML, Doyle HA, Gee RJ, Lowenson JD, Clarke S, et al. (2006) Intracellular protein modification associated with altered T cell functions in autoimmunity. *J Immunol* 177: 4541-4549.
29. Tischner D, Woess C, Ottina E, Villunger A (2010) Bcl-2-regulated cell death signalling in the prevention of autoimmunity. *Cell Death Dis* 1: e48.
30. Hughes PD, Belz GT, Fortner KA, Budd RC, Strasser A, et al. (2008) Apoptosis regulators Fas and Bim cooperate in shutdown of chronic immune responses and prevention of autoimmunity. *Immunity* 28: 197-205.
31. Hutcheson J, Scatizzi JC, Siddiqui AM, Haines GK 3rd, Wu T, et al. (2008) Combined deficiency of proapoptotic regulators Bim and Fas results in the early onset of systemic autoimmunity. *Immunity* 28: 206-217.
32. Weant AE, Michalek RD, Khan IU, Holbrook BC, Willingham MC, et al. (2008) Apoptosis regulators Bim and Fas function concurrently to control autoimmunity and CD8+ T cell contraction. *Immunity* 28: 218-230.
33. Li WQ, Guszczynski T, Hixon JA, Durum SK (2010) Interleukin-7 regulates Bim proapoptotic activity in peripheral T-cell survival. *Mol Cell Biol* 30: 590-600.
34. Okazaki T, Okazaki IM, Wang J, Sugiura D, Nakaki F, et al. (2011) PD-1 and LAG-3 inhibitory co-receptors act synergistically to prevent autoimmunity in mice. *J Exp Med* 208: 395-407.
35. Swainson LA, Mold JE, Bajpai UD, McCune JM (2010) Expression of the autoimmune susceptibility gene FcRL3 on human regulatory T cells is associated with dysfunction and high levels of programmed cell death-1. *J Immunol* 184: 3639-3647.
36. Oates JC, Farrelly LW, Hofbauer AF, Wang W, Gilkeson GS (2007) Association of reactive oxygen and nitrogen intermediate and complement levels with apoptosis of peripheral blood mononuclear cells in lupus patients. *Arthritis Rheum* 56: 3738-3747.
37. Crispin JC, Apostolidis SA, Finnell MI, Tsokos GC (2011) Induction of PP2A Bbeta, a regulator of IL-2 deprivation-induced T cell apoptosis, is deficient in systemic lupus erythematosus. *Proc Natl Acad Sci U S A* 108: 12443-12448.
38. Shah D, Kiran R, Wanchu A, Bhatnagar A (2011) Soluble granzyme B and cytotoxic T lymphocyte activity in the pathogenesis of systemic lupus erythematosus. *Cell Immunol* 269: 16-21.
39. Niland B, Miklossy G, Banki K, Biddison WE, Casciola-Rosen L, et al. (2010) Cleavage of transaldolase by granzyme B causes the loss of enzymatic activity with retention of antigenicity for multiple sclerosis patients. *J Immunol* 184: 4025-4032.
40. Trapani JA, Sutton VR (2003) Granzyme B: pro-apoptotic, antiviral and antitumor functions. *Curr Opin Immunol* 15: 533-543.
41. Chattopadhyay S, Marques JT, Yamashita M, Peters KL, Smith K, et al. (2010) Viral apoptosis is induced by IRF-3-mediated activation of Bax. *EMBO J* 29: 1762-1773.
42. Chattopadhyay S, Sen GC (2010) IRF-3 and Bax: a deadly affair. *Cell cycle* 9: 2479-2780.
43. Zou X, Xiang X, Chen Y, Peng T, Luo X, et al. (2010) Understanding inhibition of viral proteins on type I IFN signaling pathways with modeling and optimization. *J Theor Biol* 265: 691-703.
44. Wang X, Li M, Zheng H, Muster T, Palese P, et al. (2000) Influenza A virus NS1 protein prevents activation of NF-kappaB and induction of alpha/beta interferon. *J Virol* 74: 11566-11573.
45. Naggie S, Patel K, McHutchison J (2010) Hepatitis C virus directly acting antivirals: current developments with NS3/4A HCV serine protease inhibitors. *J Antimicrob chemother* 65: 2063-2069.
46. Andrejeva J, Childs KS, Young DF, Carlos TS, Stock N, et al. (2004) The V proteins of paramyxoviruses bind the IFN-inducible RNA helicase, mda-5, and inhibit its activation of the IFN-beta promoter. *Proc Nat Acad Sci U S A* 101: 17264-17269.
47. Li Y, Li C, Xue P, Zhong B, Mao AP, et al. (2009) ISG56 is a negative-feedback regulator of virus-triggered signaling and cellular antiviral response. *Proc Nat Acad Sci U S A* 106: 7945-7950.
48. Mukherjee A, Morosky SA, Delorme-Axford E, Dybdahl-Sissoko N, Oberste MS, et al. (2011) The coxsackievirus B 3C protease cleaves MAVS and TRIF to attenuate host type I interferon and apoptotic signaling. *PLoS pathog* 7: e1001311.
49. Masson F, Kupresanin F, Mount A, Strasser A, Belz GT (2011) Bid and Bim collaborate during induction of T cell death in persistent infection. *J Immunol* 186: 4059-4066.
50. Gupta A, Nagilla P, Le HS, Bunney C, Zych C, et al. (2011) Comparative Expression Profile of miRNA and mRNA in Primary Peripheral Blood Mononuclear Cells Infected with Human Immunodeficiency Virus (HIV-1). *PLoS one* 6: e22730.
51. Klase Z, Winograd R, Davis J, Carpio L, Hildreth R, et al. HIV-1 TAR miRNA protects against apoptosis by altering cellular gene expression. *Retrovirology* 6: 18.
52. Qin Z, Kearney P, Plaisance K, Parsons CH (2010) Pivotal advance: Kaposi's sarcoma-associated herpesvirus (KSHV)-encoded microRNA specifically induce IL-6 and IL-10 secretion by macrophages and monocytes. *J leukoc Biol* 87: 25-34.
53. Qin Z, Freitas E, Sullivan R, Mohan S, Bacelieri R, et al. (2010) Upregulation of xCT by KSHV-encoded microRNAs facilitates KSHV dissemination and persistence in an environment of oxidative stress. *PLoS pathog* 6: e1000742.
54. Miyairi I, Byrne GI (2006) Chlamydia and programmed cell death. *Curr opin microbiol* 9: 102-8.
55. Jendro MC, Fingerle F, Deutsch T, Liese A, Köhler L, et al. Chlamydia trachomatis-infected macrophages induce apoptosis of activated T cells by secretion of tumor necrosis factor-alpha in vitro. *Med microbiol immunol* 193: 45-52.
56. Zhang S, Rozell M, Verma RK, Albu DI, Califano D, et al. Antigen-specific clonal expansion and cytolytic effector function of CD8+ T lymphocytes depend on the transcription factor Bcl11b. *J Exp Med* 207: 1687-1699.
57. Xie P, Kraus ZJ, Stunz LL, Liu Y, Bishop GA (2011) TNF receptor-associated factor 3 is required for T cell-mediated immunity and TCR/CD28 signaling. *J Immunol* 186: 143-155.
58. Zhang JZ, Sinha M, Luxon BA, Yu XJ (2004) Survival strategy of obligately intracellular *Ehrlichia chaffeensis*: novel modulation of immune response and host cell cycles. *Infect Immun* 72: 498-507.
59. Joshi SG, Francis CW, Silverman DJ, Sahni SK (2004) NF-kappaB activation suppresses host cell apoptosis during *Rickettsia rickettsii* infection via regulatory effects on intracellular localization or levels of apoptogenic and anti-apoptotic proteins. *FEMS microbiol lett* 234: 333-341.
60. Sakuishi K, Apetoh L, Sullivan JM, Blazar BR, Kuchroo VK et al. (2010) Targeting Tim-3 and PD-1 pathways to reverse T cell exhaustion and restore anti-tumor immunity. *J Exp Med* 207: 2187-2194.

61. Banh A, Zhang J, Cao H, Bouley DM, Kwok S, et al. (2011) Tumor galectin-1 mediates tumor growth and metastasis through regulation of T-cell apoptosis. *Cancer Res* 71: 4423-4431.
62. Grimm M, Kim M, Rosenwald A, von Raden BH, Tsaur I, et al. (2010) Tumour-mediated TRAIL-Receptor expression indicates effective apoptotic depletion of infiltrating CD8⁺ immune cells in clinical colorectal cancer. *Eur J Cancer* 46: 2314-2323.
63. Whiteside TL (2009) Tricks tumors use to escape from immune control. *Oral Oncol* 45: 119-123.
64. Zhang W, Ding EX, Wang Q, Zhu DQ, He J, et al. (2005) Fas ligand expression in colon cancer: a possible mechanism of tumor immune privilege. *World J Gastroenterol* 11: 3632-3635.
65. Yu H, Kortylewski M, Pardoll D (2007) Crosstalk between cancer and immune cells: role of STAT3 in the tumour microenvironment. *Nat Rev Immunol* 7: 41-51.
66. Kortylewski M, Yu H (2007) Stat3 as a potential target for cancer immunotherapy. *J Immunother* 30: 131-139.
67. Buettner R, Huang M, Gritsko T, Karras J, Enkemann S, et al. (2007) Activated signal transducers and activators of transcription 3 signaling induces CD46 expression and protects human cancer cells from complement dependent cytotoxicity. *Mol Cancer Res* 5: 823-832.
68. Sun J, Yu J, Li H, Yang L, Wei F, et al. (2011) Upregulated expression of indoleamine 2,3-dioxygenase in CHO cells induces apoptosis of competent T cells and increases proportion of Treg cells. *J Exp Clin Cancer Res* 30: 82.
69. Curtale G, Citarella F, Carissimi C, Goldoni M, Carucci N, et al. (2010) An emerging player in the adaptive immune response: microRNA-146a is a modulator of IL-2 expression and activation-induced cell death in T lymphocytes. *Blood* 115: 265-273.
70. Steiner DF, Thomas MF, Hu JK, Yang Z, Babiarz JE, et al. (2011) MicroRNA-29 Regulates T-Box Transcription Factors and Interferon-gamma Production in Helper T Cells. *Immunity* 35: 169-181.
71. Mao CP, He L, Tsai YC, Peng S, Kang TH, et al. (2011) In vivo microRNA-155 expression influences antigen-specific T cell-mediated immune responses generated by DNA vaccination. *Cell Biosci* 1: 3.
72. Grigoryev YA, Kurian SM, Hart T, Nakorchevsky AA, Chen C, et al. (2011) MicroRNA Regulation of Molecular Networks Mapped by Global MicroRNA, mRNA, and Protein Expression in Activated T Lymphocytes. *J Immunol* 187: 2233-2243.
73. Rockey DC, Bissell DM (2006) Noninvasive measures of liver fibrosis. *Hepatology* 43: 113-120.
74. Iken, K, Huang L, Bekele H, Schmidt EV, Koziel MJ (2006) Apoptosis of activated CD4⁺ and CD8⁺ T cells is enhanced by co-culture with hepatocytes expressing hepatitis C virus (HCV) structural proteins through FasL induction. *Virology* 346: 363-372.
75. Arends JE, Hoepelman AI, Nanlohy NM, Höppener FJ, Hirsch KR, et al. (2011) Low doses of the novel caspase-inhibitor GS-9450 leads to lower caspase-3 and -8 expression on peripheral CD4⁺ and CD8⁺ T-cells. *Apoptosis* 16: 959-966.
76. Pockros PJ, Schiff ER, Shiffman ML, McHutchison JG, Gish RG, et al. (2007) Oral IDN-6556, an antiapoptotic caspase inhibitor, may lower aminotransferase activity in patients with chronic hepatitis C. *Hepatology* 46: 324-329.
77. Lichtner M, Mengoni F, Mastroianni CM, Sauzullo I, Rossi R, et al. (2006) HIV protease inhibitor therapy reverses neutrophil apoptosis in AIDS patients by direct calpain inhibition. *Apoptosis* 11: 781-787.
78. LaCasse EC, Cherton-Horvat GG, Hewitt KE, Jerome LJ, Morris SJ, et al. (2006) Preclinical characterization of AEG35156/GEM 640, a second generation antisense oligonucleotide targeting X-linked inhibitor of apoptosis. *Clin Cancer Res* 12: 5231-5241.
79. Hotchkiss RS, Strasser A, McDunn JE, Swanson PE (2009) Cell death. *N Engl J Med* 361: 1570-1583.
80. Zitvogel L, Kepp O, Senovilla L, Menger L, Chaput N, et al. (2010) Immunogenic tumor cell death for optimal anticancer therapy: the calreticulin exposure pathway. *Clin Cancer Res* 16: 3100-3104.
81. Kepp O, Tesniere A, Schlemmer F, Michaud M, Senovilla L, et al. (2009) Immunogenic cell death modalities and their impact on cancer treatment. *Apoptosis* 14: 364-375.
82. Fischer U, Schulze-Osthoff K (2005) New approaches and therapeutics targeting apoptosis in disease. *Pharmacol Rev* 57: 187-215.
83. Khaw M, Huang DCS, Roberts AW (2011) Overcoming blocks in apoptosis with BH3- mimetic therapy in haematological malignancies. *Pathology* 43: 525-535.
84. Schuler G (2010) Dendritic cells in cancer immunotherapy. *Eur J Immunol* 40: 2123-2130.
85. Kantoff PW, Higano CS, Shore ND, Roy Berger E, Small EJ, et al. (2010) Sipuleucel-T immunotherapy for castration-resistant prostate cancer. *The New England journal of medicine* 363: 411-422.
86. Schadendorf D, Ugurel S, Schuler-Thurner B, Nestle FO, et al. (2006) Dacarbazine (DTIC) versus vaccination with autologous peptide-pulsed dendritic cells (DC) in first-line treatment of patients with metastatic melanoma: a randomized phase III trial of the DC study group of the DeCOG. *Ann Oncol* 17: 563-570.
87. von Euw EM, Barrio MM, Furman D, Levy EM, Bianchini M, et al. (2008) A phase I clinical study of vaccination of melanoma patients with dendritic cells loaded with allogeneic apoptotic/necrotic melanoma cells. Analysis of toxicity and immune response to the vaccine and of IL-10 -1082 promoter genotype as predictor of disease progression. *J Transl Med* 6: 6.
88. Pruitt SK, Boczkowski D, de Rosa N, Haley NR, Morse MA, et al. (2011) Enhancement of anti-tumor immunity through local modulation of CTLA-4 and GITR by dendritic cells. *Eur J Immunol* 41: 3553-3563.
89. Suso EM, Dueland S, Rasmussen AM, Vetthrus T, Aamdal S, et al. (2011) hTERT mRNA dendritic cell vaccination: complete response in a pancreatic cancer patient associated with response against several hTERT epitopes. *Cancer Immunol Immunother* 60: 809-818.
90. Kmiecik M, Basu D, Payne KK, Toor A, Yacoub A, et al. (2011) Activated NKT cells and NK cells render T cells resistant to myeloid-derived suppressor cells and result in an effective adoptive cellular therapy against breast cancer in the FVB/N202 transgenic mouse. *J Immunol* 187: 708-717.
91. Barr PM, Lazarus HM, Cooper BW, Schluchter MD, Panneerselvam A, et al. (2009) Phase II study of bryostatins 1 and vincristine for aggressive non-Hodgkin lymphoma relapsing after an autologous stem cell transplant. *Am J Hematol* 84: 484-487.
92. Mohammad RM, Diwakaran H, Maki A, Emara MA, Pettit GR, et al. (1995) Bryostatins 1 induces apoptosis and augments inhibitory effects of vincristine in human diffuse large cell lymphoma. *Leuk Res* 19: 667-673.
93. El-Rayes BF, Gadgil S, Shields AF, Manza S, Lorusso P, et al. (2006) Phase I study of bryostatin 1 and gemcitabine. *Clin Cancer Res* 12: 7059-7062.
94. Roberts JD, Smith MR, Feldman EJ, Cragg L, Millenson MM, et al. (2006) Phase I study of bryostatin 1 and fludarabine in patients with chronic lymphocytic leukemia and indolent (non-Hodgkin's) lymphoma. *Clin Cancer Res* 12: 5809-5816.
95. Pavlick AC, Wu J, Roberts J, Rosenthal MA, Hamilton A, et al. (2009) Phase I study of bryostatin 1, a protein kinase C modulator, preceding cisplatin in patients with refractory non-hematologic tumors. *Cancer Chemother Pharmacol* 64: 803-810.
96. Komatsu N, Mariotti-Ferrandiz ME, Wang Y, Malissen B, Waldmann H, et al. (2009) Heterogeneity of natural Foxp3⁺ T cells: a committed regulatory T cell lineage and an uncommitted minor population retaining plasticity. *Proc Natl Acad Sci U S A* 106: 1903-1908.
97. Golovina TN, Mikheeva T, Brusko TM, Blazar BR, Bluestone JA, et al. (2011) Retinoic acid and rapamycin differentially affect and synergistically promote the ex vivo expansion of natural human T regulatory cells. *PloS one* 6: 15868.
98. Basu S, Golovina T, Mikheeva T, June CH, Riley JL (2008) Cutting edge: Foxp3-

- mediated induction of pim 2 allows human T regulatory cells to preferentially expand in rapamycin. J Immunol 180: 5794-5798.
99. Carames B, Hasegawa A, Taniguchi N, Miyaki S, Blanco FJ, et al. (2011) Autophagy activation by rapamycin reduces severity of experimental osteoarthritis. Ann Rheum Dis.
100. Piemonti L, Maffi P, Monti L, Lampasona V, Perseghin G, et al. (2011) Beta cell function during rapamycin monotherapy in long-term type 1 diabetes. Diabetologia 54: 433-449.
101. Charbonnier LM, Le Moine A (2012) Rapamycin as immunosuppressant in murine transplantation model. Methods Mol Biol 821: 435-445.

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