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Bystander T Cells: A Balancing Act of Friends and Foes

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Abstract

T cell responses are essential for appropriate protection against pathogens. T cell immunity is achieved through the ability to discriminate between foreign and self-molecules, which relies heavily on stringent T cell receptor (TCR) specificity. Recently, bystander activated T lymphocytes, specific for unrelated epitopes during an antigen-specific response, have been implicated in various diseases. Numerous infection models have challenged the classic dogma of T cell activation as solely dependent on TCR and major histocompatibility complex (MHC) interactions, indicating an unappreciated role for pathogen-associated receptors on T cells. Here, we discuss specific roles of bystander activated T cells in pathogenesis, shedding light on the ability of these cells to modulate disease severity independently from TCR recognition.

Keywords

Bystander; T cell activation; signaling; pathogenesis; autoimmunity; infection; cancer

Conventional and Unconventional T Cell Activation

T cell responses are crucial to forming long-lived memory against foreign antigens and pathogens. MHC presentation of peptide to the TCR remains one of the most fundamental aspects of adaptive immunity and allows us to discriminate between self and foreign “non-self” antigen through the process of thymic selection. TCR signaling has been investigated thoroughly, and described as a system of intricate checks and balances consisting of both costimulatory and co-inhibitory receptor signaling [1]. Traditionally, T cell responses are considered successful when antigen-specific activation leads to the creation of clonal expansion and ultimately, memory. Disruptions to finely tuned T cell signaling have the potential for detrimental outcomes to the host [2, 3]. Although innate receptors on antigen-specific T cells have been described [4], activation of T cells in the absence of antigen and the downstream functional consequences have not been well characterized. Here, we discuss innate-like pathogen-recognition receptors that contribute to unconventional T cell signaling and examine mechanisms of TCR-independent activation and its potential significance in

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pathogenesis. We examine the implications of bystander T cell activation during microbial infections, autoimmunity, and during cancer immunotherapy. This topic is highly relevant as a better understanding of bystander T cell activation and the mechanisms by which these function in pathogenesis may provide insight into new and more directed putative therapeutic treatments for a variety of ailments.

Bystander T Cell Activation: Antigen-Independence

Bystander T cell activation reveals a role for T cell function independent of TCR signaling. This type of antigen-independent activation has been observed in inflammatory environments; including autoimmunity, cancer, and most commonly during infection [5-9]. Bystander T cells were first recognized in lymphocytic choriomeningitis virus (LCMV), vaccinia virus, and vesicular stomatitis virus murine models of viral infection, resulting in the proliferation and expansion of a diverse number (polyclonal) T cells [10]. Bacterial products such as LPS have also been linked to non-antigen-specific T cell stimulation, as demonstrated by direct injection of LPS into mice [11]. TCR-independent activation during pathogenesis has primarily been observed in CD8⁺ T cells, while less is known about CD4⁺ bystander T cell activation [10, 12-14]. Localized inflammatory-inducing conditions present during various diseases, can influence the threshold required to induce T cell mediated immune responses [15, 16], which may render T cells more receptive to secondary signals such as costimulatory receptors. In fact, microbial sensors such as **Toll-like receptors (TLRs)** and cytokines have all been implicated in initiating bystander T cell responses [7, 17, 18]. Other pathways of non-classical T cell activation can involve **superantigens**, **molecular mimicry** (Box 1), and dual T cell receptor signaling (Box 2). Superantigens greatly alter typical adaptive immune responses by activating polyclonal T cells and promoting constitutive B7-2/CD28 coreceptor engagement [19], which results in the sudden release of cytokines that can cause the acute condition known as cytotoxic shock syndrome or “cytokine storm” [20]. Antigen-independent activation often results in the secretion of pro-inflammatory molecules such as IFN γ and granzyme B, which act in conjunction to perpetuate inflammation as demonstrated in both mice [8, 21] and humans [22, 23]. Recent work has focused on the innate-like natural killer (NK) cell receptor NKG2D, which when engaged in human and murine CD8⁺ memory T cells, can lead to direct-killing of target cells expressing NKG2D-ligands [8, 24-27]. Murine CD8⁺ T cells are able to recognize and lyse *Listeria monocytogenes*-infected cells in an NKG2D-dependent manner, suggesting that bystander activated T cells might be beneficial to host defense [7]. Evidence of several innate NK cell receptors expressed by T cells is reviewed elsewhere [4], but it is now appreciated that T cells contain a collection of innate-like receptors which allow these cells to rapidly respond to microbial invasion. In addition, **virtual memory T cells (T_{VM})** in mice are also capable of bystander T cell function (Box 3), although these cells undergo antigen-specific signaling that is dependent on TCR recognition, thus rendering this T cell subset as an intermediary of classical and bystander groups.

As discussed below, T cell activation in the absence of TCR signaling is illuminating novel features and functions of T cell immunity. These TCR-independent pathways may have links to autoimmunity, usually defined by a loss of immunological tolerance, ultimately leading to the activation of self-reactive T and B cells. However, future research is clearly needed to

comprehensively define the molecular signatures of bystander T cell activation, and the mechanisms that contribute to either guiding protection against infectious agents or leading to increased disease susceptibility.

T Cell Innate-like Receptors as Costimulatory Molecules

T cells express several stimulatory and inhibitory receptors which dictate T cell function and fate. In mice and humans, classic costimulatory molecules such as CD28 and CD40 ligand (CD40L) transduce positive signals during TCR engagement with antigen, needed to establish appropriate T cell responses. In the absence of costimulatory signals, T cells that encounter antigen often fail to respond and die, or enter a state of inactivation known as **anergy**. The repertoire of co-signaling receptors is highly versatile and adjusts to changes in the tissue environment. Signals sensed from surrounding cells and the microenvironment can determine which co-receptors are expressed on the T cell surface. Several co-signaling receptors that have been well characterized on **professional antigen presenting cells (APCs)** during infection have now been identified on T cells (Table 1). These molecules can drive a number of TCR-independent activation pathways and have been shown to influence disease outcomes [7, 9, 28].

NKG2D T Cell Signaling

One T cell innate-like receptor is NKG2D, a type II transmembrane protein originally identified on human NK cells [29]; it is an activating receptor capable of binding a number of diverse non-classical MHC I-like ligands including human MHC class I polypeptide-related sequence A (MICA), MICB, UL16 binding proteins (ULBPs), murine ribonucleic acid export 1 (Rae1), histocompatibility 60 (H60), and UL16-binding proteinlike Transcript (MULT) proteins [29, 30]. These binding partners possess elements of heat shock promoters and are generally absent in uninfected hosts but can become upregulated during cell stress, as in the case of bacterial and viral infections [31, 32]. T cell intrinsic NKG2D signaling utilizes adaptor proteins DAP10 and DAP12 (solely DAP10 in humans) resulting in the phosphorylation of ZAP70 and Syk, similar to TCR signaling [33]. Downstream events then lead to the activation of proinflammatory pathways including mTOR, Akt, MAPK Erk, Jnk, and NF- κ B [4, 24, 34]. Because there is functional heterogeneity of NKG2D signaling within T cells [30], the activation of NKG2D⁺ T cell subsets may have broad implications for disease pathogenesis; however, extensive and robust experiments are needed to determine if, how and when, protective or pathogenic responses are generated.

NKG2D in CD8⁺ T cells

NKG2D protein expression can be increased on both naïve and memory CD8⁺ T cells in the absence of TCR signaling through STAT-dependent cytokines IL-2 [35], IL-7 [36], and IL-12 [37], IL-15 [24, 38] in mice and humans. NKG2D expression on CD8⁺ T cells has also been linked to increased memory CD8⁺ T cell function independent of CD4⁺ T cell help. NKG2D signaling on CD8⁺ T cells in the presence of IL-15 confers high survival to mice lacking CD4⁺ T cells in a CD4-dependent influenza virus infection model [39]. Dysregulation of IL-15 has also been linked to altered NKG2D signaling and tissue damage

in patients with celiac disease [24, 40]. Thus, in other autoimmune diseases, the IL-15 and NKG2D signaling axis deserves further investigation.

Indeed, T cell intrinsic expression of NKG2D has been observed during several microbial infections (Table 1), with both beneficial and detrimental outcomes for the host. During intravenous injection of *L. monocytogenes* infection in mice, bystander-activated CD8⁺ T cells provide protection by limiting bacteria replication in an NKG2D-dependent manner and independent of TCR signaling, as evidenced from the degree of TCR signaling using transgenic GFP-Nur77 reporter mice [7]. Conversely, bystander NKG2D⁺CD8⁺ memory CD8⁺ T cells can exacerbate disease following infection with *Leishmania major*, as evidenced by large cutaneous lesions containing increased numbers of monocytes, neutrophils and CD8⁺ T cells relative to mice receiving an NKG2D blocking antibody [8]. Furthermore, *Leishmania* coinfection with acute (Armstrong) LCMV in mice can result in cutaneous tissue damage and inflammation as demonstrated from substantial tissue destruction and the presence of cellular infiltrates in leishmanial skin lesions; by contrast, treatment with a blocking NKG2D antibody could ameliorate this effect [21]. Moreover, viral infections such as influenza [41, 42], HIV-1 [43], and LCMV [10] have also been found to induce human and murine CD8⁺ T cell bystander activation in vivo and ex vivo. And, during hepatitis A virus infection, NKG2D⁺CD8⁺ T cells which were response-specific to unrelated viruses, were activated and trafficked to the liver in humans, subsequently targeting and lysing NKG2D ligand-overexpressing hepatocytes [44].

NKG2D in CD4⁺ T cells

Although NKG2D is largely absent from CD4⁺ T cells in healthy individuals, the presence of NKG2D⁺CD4⁺ T cells in the periphery has also been linked to autoimmune diseases in mice and humans [45]. Specifically, NKG2D⁺CD4⁺ T cells from peripheral blood were suggested to exhibit regulatory functions, including the production of IL-10 and TGF- β in human patients with systemic lupus erythematosus (SLE) [46-48]. Moreover, cytotoxic activity and expansion of NKG2D⁺CD4⁺ T cells was found to inversely correlate with SLE disease activity, and increased frequencies of these cells were noted in juvenile-onset SLE patients undergoing spontaneous flares and remissions, relative to control donors [46-48]. High numbers of CD4⁺NKG2D⁺ T cells have also been documented in the synovial fluid from patients with rheumatoid arthritis, and are thought to contribute to joint disruption and disease, although the mechanisms for this are unknown [45]. Thus, the exact functions of NKG2D⁺CD4⁺ and their potential role in autoimmune pathogenesis remain unclear and warrant further investigation.

NKG2D⁺ T cells and disease

As discussed, inappropriate expression of NKG2D stimuli has been proposed to potentially lead to autoimmunity. In addition to rheumatoid arthritis [46], elevated NKG2D protein expression has been associated with inflammatory bowel diseases such as Crohn's disease and celiac disease [24, 49, 50], type I diabetes [23, 51], as well as multiple sclerosis [52] in mice and humans. Thus, further examination of bystander T cell activation during various human autoimmune diseases and other microbial infections should be explored. Accordingly, several studies have demonstrated that relative to controls, anti-NKG2D

antibody treatment could result in decreased inflammation by impairing the expansion and function of CD8⁺ T cells in mouse models of *Leishmania* infection, graft versus host disease (GVHD) or type I diabetes [8, 22, 23, 53]. Thus, we posit that augmenting NKG2D signaling, presumably in combination with antiviral or antibiotic therapies might potentially lead to faster and more complete resolution of disease, a strategy that may merit further attention. Moreover, a deeper understanding of the NKG2D-dependent T cell activation pathway could provide insight into novel putative treatments against infectious agents that might either be complementary, or replace current treatments that harbor toxic side effects or yield acquired antibiotic resistance.

Induction of bystander T cells through TLR signaling

Many infections result in bystander activation that can result in localized inflammation or systemic disease (Figure 1). Innate-like receptors directly recognize microbial molecules through **pathogen associated molecular patterns (PAMPs)** (see glossary). Through the activation of host **pattern recognition receptors (PRRs)**, antimicrobial molecules are released in efforts to control pathogen load and recruit adaptive immune responders. The ten PRRs of the TLRs family in humans and the 12 TLRs in mice are fundamental sensors of bacteria and viruses.

TLRs are expressed by both $\gamma\delta$ T cells and mucosal associated invariant T (MAIT) cells, and by conventional $\alpha\beta$ CD4⁺ and CD8⁺ T cells in mice and humans [54]. TLR engagement on T cells directly influences differentiation and effector function of CD4⁺ T cells. Early studies have demonstrated TLR involvement in **regulatory T cell (T_{reg})** signaling in humans and mice [55, 56]. In these studies, T cell intrinsic signaling of TLR4 when stimulated with lipopolysaccharide (LPS) enhanced T_{reg} survival proliferation and effector function. Further studies revealed that T cells could express a variety of TLRs [54], many of which are important regulators of disease during infection (Table 1).

T Cell Intrinsic TLR2 signaling

TLR ligands represent an important costimulatory signal modulating T cell immunity. Their recognition by TLR3, TLR4, TLR7, and TLR9 on T cells has been linked to worsened disease outcomes in models of infection, cancer, and autoimmunity [18, 68-71]. Thus, TLR signaling seems to represent a fundamental bridge between innate and adaptive immunity, working to recruit multiple players through the production of pro-inflammatory cytokines. It is surmised that tonic stimulation or dysregulation of TLR signaling can contribute to T cell-dependent increased inflammation and pathogenesis in certain instances.

TLR2 is a critical receptor responsible for the recognition of bacterial lipoproteins, peptidoglycans, lipoteichoic acid, and LTA. Multiple groups have demonstrated a functional role for TLR2 on both CD4⁺ and CD8⁺ T cells. TLR2 engagement on T cells lowers the activation threshold of T cells, while further enhancing cell survival and effector functions [57, 58]. Mouse models have demonstrated that CD4⁺TLR2⁺ T cells are phenotypically similar to memory Th1 cells, and are capable of producing IFN γ independent of TCR activation [59, 60]. Studies of the mucosal immune system in mice have also revealed that the commensal microbe *Bacteroides fragilis* can activate TLR2 on CD4⁺ T cells to establish

host-microbial symbiosis [56]. The symbiosis factor Polysaccharide A (PSA) from *B. fragilis* activated TLR2 signaling on Foxp3⁺ regulatory T cells resulting in enhanced T_{reg} function and gene expression of cytokines *Il10* and *Tgfb*. The suppressive capacity was lost in Foxp3⁺ cells that were deficient in TLR2, demonstrating a unique role of TLR2 signaling on T cells that contribute to promoting mucosal tolerance and commensal homeostasis in the absence of antigen presenting cells [56]. Further studies have supported a role for T cell intrinsic expression of TLR2 during disease. For example, in concordance with the synovial inflammatory environment in Lyme-disease arthritis in humans, mouse models of *Borrelia burgdorferi* infection and Lyme disease-induced arthritis have documented increased numbers of CD4⁺TLR2⁺ and CD8⁺TLR2⁺ T cells in joints relative to controls, exacerbating disease symptoms, in a manner that was deemed independent of TCR signaling [9]. The outer surface lipoproteins (Osps) of *B. burgdorferi* have been well characterized, and are potent inducers of TLR2 [61, 62], resulting in the secretion of pro-inflammatory cytokines such as IL-1 β , IL-6, IL-12, CXCL9 and CXCL10 (Figure 2). Transfer of wild type (WT) T cells into *B. burgdorferi*-infected mice increased ankle swelling and histopathology, whereas transfer of TLR2-deficient T cells did not amplify arthritis [9]. Relying on experiments using TCR transgenic mice, TLR2 protein expression on T cells was found to be independent of TCR signaling. Moreover, expression of TLR2 on T cells enhanced production of T cell chemoattractants CXCL9 and CXCL10 as well as arthritis-promoting IFN γ , revealing a role for bystander activated T cells in a model of Lyme arthritis [9] (Figure 2).

In a murine model of multiple sclerosis (experimental autoimmune encephalomyelitis (EAE)), CD4⁺ **Th17 cells** expressing TLR2 led to enhanced Th17 proliferation and cytokine production, and clinically worsened disease outcome relative to mice deficient in CD4⁺ T cell-specific TLR2 expression, which in contrast, exhibited ameliorated immunopathology [28]. In addition, TLR2 signaling on CD8⁺ T cells may be beneficial to the host, based on evidence from a mouse model of hepatitis B virus (HBV) infection; HBV-specific CD8⁺ T cell responses were diminished in *Tlr2*^{-/-} mice relative to wild type mice, as evidenced from reduced numbers of IFN γ -producing HBV CD8⁺ T cells in peripheral organs [63]. Clinical evidence has also shown impaired TLR2/4 and TLR3 signaling in patients with chronic HBV-infection [64], although the role of TLR2 signaling in these patients remains to be investigated. TLR2 has been further characterized in rheumatoid arthritis (RA) patients, who have exhibited increased TLR2 expression in the perivascular regions of the joint, cartilage, and bone relative to healthy individuals [65]. The TLR2 ligand bacterial peptidoglycan has also been detected in the synovial fluid of patients with RA [66]. Relative to controls, in an RA synovial explant model, TLR2 expression in the joint was associated with increased cell migration and invasion, as well as with destructive enzymatic production of matrix metalloproteinase (MMP), thus linking TLR2 to exacerbation of pathology, given that TLR2 blockade significantly inhibited these features of disease [67]. Hence, T cell intrinsic expression of TLR2 requires further investigation as it might be implicated in disease pathogenesis [63, 65, 67].

Of note, endogenous molecules have also been hypothesized to act as TLR ligands, providing a potential mechanism for autoimmune development, usually defined by a loss of

immunological tolerance, ultimately leading to the activation of self-reactive T and B cells. For example, extracellular matrix proteins including fibronectin, fibrinogen and heat shock proteins are released during tissue injury and cell stress, and have been linked to innate cell activation through TLR2 and TLR4 in mice and humans [72-74]. Other damage-associated molecule patterns (DAMPs) such as chromatin complexes containing nucleic acid have been reported to associate with TLR3, TLR7, and TLR9 in mice [75]. The majority of TLRs signal through the adaptor protein MyD88 resulting in NF- κ B-dependent activation and pro-inflammatory responses [76]. Thus, overactivation of TLRs has can lead to disruptions in immune homeostasis, implicating TLR signaling in autoimmune syndromes including SLE, rheumatoid arthritis, asthma, gastrointestinal inflammation, and also cancer, which altogether constitutes a major field of investigation [77, 78]. Accordingly, inhibitors of TLR signaling are currently being developed as novel potential therapeutics for a variety of conditions and many are presently being tested in clinical trials [79].

The Environment Matters: Lowering the Sensitivity for T Cell Activation

Bystander activation occurs only in specific subsets of T cells in selective circumstances. Proliferation of bystander T cells predominantly occurs in memory T cells, which express high levels of the adhesion molecule CD44 [7, 10, 80], and less frequently in naïve T cells [81]. Memory T cells are more sensitive to cytokine-mediated signals than antigen-inexperienced T cells, and can be triggered to release their cytotoxic contents when exposed to far lower cytokine concentrations than naïve T cells [82, 83]. Paradoxically in one report, memory T cells were deemed less responsive to antigen and to display weaker TCR signaling than naïve T cells at the same concentration of antigen [84]. However, antigen-experienced memory T cells do not require MHC interactions and can undergo **homeostatic proliferation** in the presence of cytokines such as IL-7 and IL-15 [85, 86]. Thus, the induction of bystander T cell activation is heavily dependent on the cytokine milieu, for instance during infection, and is often confined to the site of pathogen localization [38, 43, 87-90]. Indeed, IL-12, IL-15, and IL-18 appear to be important regulators of bystander T cell responses, and are secreted by mononuclear cells in response to viral and bacterial infections in mice and humans [38, 43, 91, 92].

IL-12, IL-15, and IL-18 can boost bystander T cell responses during microbial infection

The amount of IL-12 can directly influence the formation of short-lived effector cytotoxic lymphocytes [91]. CD8⁺ T cells stimulated with IL-12 release exosomes capable of directly activating other naïve CD8⁺ T cells in the absence of antigen, as shown in *ex vivo* models using OT-I transgenic T cells [93]. IL-15, in turn, has been reported by multiple groups to globally activate T cell responses. Specifically, IL-15 stimulation of memory CD8⁺ T cells can result in a similar response to TCR stimulation, as evidenced from elevated cellular proliferation, gene expression changes, and synthesis of effector molecules IFN γ , perforin, and granzyme B in peripheral blood from mice and humans [88, 94]. In addition, Untreated HIV-1 patients can exhibit increased numbers of bystander-activated CD8⁺ T cells in peripheral blood in the presence of IL-15 *in vitro*, as evidenced from the increased proliferation and production of granzyme B with TCR specificity for non-HIV-1 epitopes relative to untreated cells[43]. CD8⁺ T cells from patients infected with Hepatitis A virus

(HAV) can be activated in an IL-15-dependent and TCR-independent manner, contributing to liver injury, given that CD8⁺ T cells that were non-HAV-specific could be detected in patient liver samples and which could exert cytotoxicity [44]. In the case of IL-18, this cytokine can stimulate the production of IFN γ by memory CD8⁺ T cells during bacterial and parasitic infections [38, 92], and has been shown to synergize with IL-12 for optimal IFN γ production by CD4⁺ Th1 cells and CD8⁺ T cells during viral infections in humans and mice [95, 96]. This suggests a complex landscape of cytokine interactions that may modify T cell function during infections, and which based on a deeper understanding, may provide insight into potential cytokine-based treatments. Moreover, airway resident memory CD8⁺ T cells stimulated with IL-12 and IL-18 produce IFN γ independent of TCR stimulation during mouse models of allergic asthma [90]. Specifically, bystander-activated T cells could modulate the degree and frequency of allergic responses in the lung, which could help suppress the migration of allergy-promoting T cells into the airway [90]. Of note, IL-18 is readily induced during pathogenesis via TLRs, **nucleotide-binding oligomerization domain-like receptors (NLRs)**, or TNF α receptor signaling in response to PAMPs, resulting in the rapid recruitment and mobilization of T cells, and implicating this cytokine as an important mediator of host defense [90]. IL-18 has also been demonstrated to activate both memory [97] and bystander T cells [7, 38] in the absence of antigen, and this is relevant as it could potentially cause harm in a host.

Together, these inflammatory mediators can work in combination to promote antimicrobial function and control pathogen load. However, during chronic inflammation or infection, this mechanism may be destructive to surrounding host tissue. Indeed, the potential for T cells to become activated by cytokines in the absence of cognate antigen may provide an environment for inappropriate T cell responses during pathogenesis. Further work is needed to establish the contribution of antigen-independent signals to memory T cells and how this might influence memory T cell function and protection efficacy.

Bystander T cell Activation and Cancer Immunotherapy

Immunotherapy, a treatment that boosts the body's own immune system to identify and eradicate cancer, is proving to be an effective form of resorting T cell-mediated immune responses for certain cancers. Immunotherapy regimens can include inhibitory receptor checkpoint blockade such as by using anti-CTLA-4 and anti-PD-1 antibodies, which have led to sustained increased responses in selective patients and cancer types. Patients and tumors respond variably to immunotherapy, which is attributed in part, to the diversity of the immune repertoires, heterogeneity of the **tumor-infiltrating lymphocytes (TILs)** and the complexity of the tumor itself [5, 98]. Recent research has shown that TILs can recognize a wide range of epitopes unrelated to the tumor in cancer patients, many of which possess specificity to viral pathogens [5]. Of relevance to our discussion, these bystander-activated T cells correlate with multiple clinical parameters, such as the mutation status of lung tumor growth factors receptors [5]. This suggests a role for antigen-independent T cell activation in human tumor-infiltrates, which merits further investigation.

Recent reports indicate that bystander T cell activation can correlate with positive responses to immunotherapy, as evidenced by increased numbers of non-antigen specific CD8⁺ TILs in

patients with either lung or colorectal cancers [5]. These CD8⁺ TILs did not express CD39, a marker of chronic antigen stimulation. In addition, CD8⁺ T cell expression of NKG2D could increase antitumor effects during immunotherapy independent of CD4⁺ T cell help, as shown in melanoma mouse models and human tissue biopsies [99, 100]. Moreover, using an *in vitro* system of human antigen-specific CD8⁺ T cell differentiation, IL-12 was found to enable TCR-independent activation through the NK cell receptor DNAM1 (CD226), [101]. Specifically, the addition of IL-12 to CD8⁺ T cells dramatically increased TCR-independent tumor cell cytotoxicity through DNAM1. This prompted the suggestion that IL-12 might be considered in potential immunotherapeutic strategies to treat cancers via activation of non-tumor specific CD8⁺ T cells, although this warrants in-depth studies. With regard to IL-18, this cytokine has been reported to promote enhanced chimeric antigen receptor (CAR) T cell functions, including proliferation and secretion of IFN γ [102, 103], although its role in bystander activation of TILs clearly needs further investigation.

From another angle, there are other cancer treatments that aim to boost the immune system and induce potent antitumor effects, such as TLR agonists, cytokines, or inhibitory antibodies [115, 119]. Inflammatory cytokines have been administered to increase efficacy of cancer therapeutics. For instance, several cytokines have been approved for the treatment of cancer therapy including IL-2 for the treatment of melanoma and metastatic renal cancer [104], and IFN α for stage 3 melanoma and hairy cell leukemia [105]. Other cytokines such as IL-12, IL-15, IL-21, and granulocyte macrophage colony-stimulating factor (GM-CSF) have been assessed in clinical trials, however these therapies have not reached their full potential due to their insufficient concentrations in tumors, in addition to toxic side effects [106]. Several TLR agonists are currently in clinical trials for the development of antitumor responses. Various TLRs have been reported in a diversity of cancers, including TLR2, TLR3, TLR4, TLR5, TLR7/8, and TLR9, but found to display both antitumor and protumor properties [78]. CD8⁺ T cells deficient in TLR2 displayed weak antitumor activity including the inability to survive and generate memory T cells compared to CD8⁺ T cells sufficient in TLR2 [107]. Furthermore, CD8⁺ T cells overexpressing TLR2 enhanced the antitumor activity of these cells. This might suggest that TLR bystander activated T cells might be useful in combating the tumor environment -- an unexplored area which requires further investigation. Another relatively successful therapeutic intervention includes the use of neutralizing antibodies targeting immune checkpoints CTLA-4 and PD-1 for certain tumors [108]. However, the effect of these therapies on the bystander activation of T cells will require in-depth examination to provide insight into developing improved anticancer treatments.

The contribution of bystander T cells during cancer immunotherapy or other regimens remains an open question, but nevertheless, one which will be critical also to addressing possible personalized therapeutics. Indeed, it is clear that extensive and rigorous work is needed to understand bystander T cell activation and how it can influence patient prognosis.

Concluding Remarks

Bystander activation of T cells can occur through different receptors and costimulatory signals. The threshold for T cell activation is often lowered due to a dysregulated

inflammatory environment which may occur during infection, autoimmunity, chronic inflammation, or cancer, providing an opportunity for T cell signaling to occur outside of the stringent and conventional TCR triggering pathway. Pathogen recognition by innate receptors on T cells can deliver unconventional costimulatory signals which subsequently influence disease outcomes, as in the case of microbial infections, autoimmune diseases, and even cancer. Numerous questions remain unanswered in this rapidly emerging field (see Outstanding Questions), but increasing evidence suggests that bystander T cell activation, a crucial component to inflammation, may represent a key defense mechanism (“a friendly fire”), but one which may also contribute to disease pathogenesis for a variety of conditions (“a foe”). Consequently, it will be important to fully dissect the molecular regulators that govern bystander T cell activation, and to critically evaluate the functional role of these T cell subsets in health, as well as in disease pathogenesis, and in response to treatment regimens. This may ultimately provide a platform for developing novel putative targets, and presumably, preventative and therapeutic treatments for certain human maladies.

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Glossary

Anergy

a state of T cell unresponsiveness, important in the maintenance of peripheral tolerance to antigen

Central memory T cells

T cells poised to respond to antigen and which access peripheral lymphoid tissues

Cross-presentation

For CD8⁺ T cells -- The presentation of extracellular antigens by MHC I on antigen-presenting cells to CD8⁺ T cells

Cross reactivity

The ability of the T cell receptor to recognize more than one peptide-MHC structure

Dual T cell receptor

Two T cell receptors on the same T cells with different antigen specificities or overlapping peptide repertoires

Epitope spreading

the development of immune responses to endogenous antigen secondary to the release of self-antigens during a chronic autoimmune or inflammatory response.

Homeostatic proliferation

long-term survival and gradual turnover of mature T cells in the periphery

Molecular mimicry

sequential or structural similarities between foreign and self-antigens

Negative selection

the process by which T lymphocytes expressing auto-reactive TCRs are deleted during T cell development in the thymus

Nucleotide-binding oligomerization domain-like receptors (NLRs)

intracellular sensors of pathogen associated molecular patterns or danger associated molecular patterns that enter host cells through phagocytosis

Pathogen associated molecular patterns (PAMPs)

conserved molecular motifs common among bacteria and or viruses

Pattern recognition receptor (PRR)

germline-encoded host receptors which recognize conserved molecular motifs essential for pathogen survival

Professional antigen presenting cells

cells (including macrophages, dendritic cells, B lymphocytes) that possess a specialized ability to present processed peptides via surface MHC to T lymphocytes

Regulatory T cells (Treg)

T cells capable of suppressing the activation of auto-reactive cells in the periphery and modulating inflammatory T cell responses

Superantigens

microbial products such as bacterial toxins that activate large numbers of T cells through binding of constant domains expressed by families of TCR beta chains

T helper 17 (Th17) cells

a subset of proinflammatory CD4⁺ T cells characterized by the production of IL-17

Toll-like receptor (TLR)

family of receptors recognizing structurally conserved molecules on bacteria and viruses

Tumor-infiltrating lymphocyte (TIL)

immune cell migrating from circulation into a tumor cell.

Virtual memory T cell (T_{VM})

In mice, Antigen-specific T cells that possess a memory phenotype without seeing cognate antigen

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Highlights

- T cell activation independent of T cell receptor signaling (bystander activation) can be beneficial but also detrimental to the host, as in certain microbial infections. It is driven by T cell intrinsic expression of pattern recognition receptors and other costimulatory signals.
- Bystander activation of T cells has been associated with the exacerbation of disease symptoms for several infections including *Borrelia burgdorferi* (Lyme disease), *Leishmania major* (Leishmaniasis), Human Immunodeficiency Virus, hepatitis A virus, Epstein Barr virus, and others.
- TCR-independent activation may be beneficial in the context of cancer immunotherapy, where bystander T cells might promote anti-tumor responses. Present studies are aiming to better elucidate these pathways.

Outstanding Questions

- What factors determine a protective versus a pathogenic bystander T cell response?
- What molecular signals define a bystander activated T cell? What markers or receptors are involved in bystander T cell activation and in different tissue contexts? Further characterization of possible molecular markers is needed.
- What signals influence bystander CD4⁺ versus CD8⁺ T cell activation?
- In which specific CD4⁺ and CD8⁺ T cell subsets does bystander T cell activation occur?
- Which particular microbial fragments and pattern recognition receptors (PRRs) are involved in bystander T cell activation?
- How is bystander activation controlled? Are Tregs able to suppress bystander T cell activation? Are Tregs themselves activated through bystander activation events?
- What therapeutic strategies can be developed to suppress or activate bystander T cells during transplantation, cancer immunotherapy, autoimmunity, immunosuppression, or infections?

Box 1: T cell recognition of pathogen epitopes mimicking host structures

Molecular mimicry describes the presence of common epitope structure or sequence between host and microbe, providing a situation in which infection could lead to autoimmunity. Pathogens possessing cross-reactive epitopes with host proteins that induce T cell activation include hepatitis B virus, group A *Streptococcus pyogenes*, and *B. burgdorferi* [109-111]. Additionally, several autoimmune diseases are hypothesized to result from self-destructive responses initiated by structural homology between microbes and self-antigens, including multiple sclerosis [112, 113], cardiomyopathy [114], celiac disease [115], type I diabetes [116], psoriasis [117], rheumatic fever [118], and herpes stromal keratitis [119]. In the case of molecular mimicry, it appears that failure to eliminate T cells with self-reactive potential in the thymus during negative selection or failure to regulate those that escape into the periphery by T_{regs} results in anti-pathogen responses that turn on the host.

Activation of self-reactive T cells is enhanced through linear sequence homology in amino acid motifs as well as environmental cues. Major histocompatibility complex (MHC) binding specificities to the TCR are highly dependent on chemical properties, with amino acid sequences possessing similar structural properties able to bind at the same location in the MHC binding groove [120]. TCRs are able to bind multiple peptides with similar structures, resulting in the binding of both host and pathogen epitopes. Although distinct from bystander activated T cells, which do not rely on activation from the TCR, T cells activated through molecular mimicry with a foreign pathogen could contribute to explaining the generation of some autoimmune diseases.

Box 2: Dual T Cell Receptors

Contrary to the classic T lymphocyte dogma that each T cell expresses a single antigen receptor that recognizes a single foreign antigen, there are T cells that express **dual TCRs** that recognize unique sequence and are capable of responding to distinct stimuli. Evidence also suggests that this second TCR reacts to a broad array of unrelated pMHC, furthering the ability to respond to a breadth of antigens [121]. The distinct antigen receptors generally result from a single beta chain that dimerizes with different alpha chains [122-124]. Up to 33% of human and 15% of murine $\alpha\beta$ T cells express two functional α chains on the cell surface [122, 124, 125]. Dual TCR's have several implications for host defense which may threaten the ability to discriminate the between host and foreign molecules, possibly leading to autoimmunity [125-127]. Indeed, dual TCRs have been identified in autoimmune models of multiple sclerosis and rheumatoid arthritis [128, 129] and have also been implicated in lung inflammation through the recognition of self and a member of the microbiota [130, 131]. Bearing two different TCRs significantly expands the repertoire for foreign antigen [126, 132], and could be advantageous in tumor suppression models [133-135]. In the context of alloreactive T cells, particularly graft-versus-host disease, the enrichment of dual TCR T cells in patients has correlated with poor prognosis [136, 137]. Lastly, in a recent study, NOD mice that lacked the ability to create dual TCR T cells due to hemizygous deletion of TCR alpha and beta were more resistant to type 1 diabetes than controls [138]. Although distinct from bystander activated T cells, the expression of multiple TCRs on a single cell also warrants further investigation in the context of infection and autoimmune generation.

Box 3: Naïve T cells acting experienced-like: Virtual memory T cells

Memory T cells characteristically form following challenge with antigen, however recent studies have demonstrated that memory CD8⁺ T cells are found in all mice, even those unexposed to pathogens [89]. These ‘**virtual memory**’ T cells (**T_{VM}**) constitutively express high levels CD44 and CD122 and represent up to 15-25% of naïve murine CD8⁺ T cells, however, their prevalence in humans is not well defined [139, 140]. T_{VM} cells share common characteristics with T cells that can be activated in a bystander fashion including the ability to bypass TCR signaling, rapidly respond to general inflammation including IL-15, IL-12, IL-18 [94], and express the innate receptor NKG2D. Similar to common memory CD8⁺ T cells, T_{VM} secrete IFN γ in response to antigen-specific stimulation, and are capable of contributing to the clearance of pathogen and host defense [141].

T_{VM} cells also show a distinct tendency to differentiate into central memory T cells, and originate from cells with increased affinity for self-antigens [89]. This has direct implications for autoimmune development, and T_{VM} are thought to contribute to the severity of psoriatic lesions in humans [142]. T_{VM} expression of effector molecules NKG2D and granzyme B is dependent on IL-15, as it their ability to provide a robust initial response to a number of harmful pathogens that can eliminate the need for a complete adaptive immune response. As the host ages, T_{VM} cells have been shown to preferentially traffic and populate the liver [89]. Although T_{VM} are capable of bystander T cell function, they also undergo antigen-specific signaling dependent on TCR recognition, making them intermediaries of classical and bystander T cell groups.

Figure 1. Reported Human Anatomical Locations of Infections and Diseases Associated with Bystander T Cell Activation.

Bystander T cells have been implicated in both viral and bacterial infections as well as in various autoimmune and inflammatory diseases.

Figure 2. Bystander T cell activation during *Borrelia burgdorferi* infection in humans and mice results in exacerbation of Lyme disease.

Borrelia burgdorferi spirochetes disseminate to the synovial joint resulting in the infiltration of mononuclear cells, including dendritic cells and T cells into the synovial tissue. (1) *B. burgdorferi* stimulates TLR1/2 signaling through its outer surface lipoproteins (Osps), which results in the secretion of several proinflammatory cytokines including IL-1 β , IL-12, IL-6 and T cell chemoattractants CXCL9 and CXCL10. (2) Inflammatory T cell responses concentrated in the joint fluid may become bystander activated cells through TLR1/2 and cytokine receptor signaling (3). Together, cytokine signaling and TLR1/2 signaling within T cells results in the secretion of proinflammatory IFN γ . (4) T cell responses can alter fibroblast-like synoviocytes, leading to synovial pathology including the erosion of bone and cartilage.

Table 1.

Reported Bystander T cell activation markers, ligands, and disease associations

Receptor	Ligand(s)	T Cell	Disease or Infection	Expression Impacting Disease Outcome	Species	Refs
CD31/PECAM-1	CD31/PECAM-1	CD4 CD8	Juvenile idiopathic arthritis	Worsened	Human	[143]
NKG2D	MICA/B (human)	CD8	Leishmaniasis	Worsened	Mouse	[8, 21]
	ULBP1-6 (mouse) RAE-1 (mouse)	CD8	Listeriosis	Ameliorated	Mouse	[7]
		CD8	Hepatitis A	Worsened	Human	[44]
		CD8	Graft versus host disease	Worsened	Human and mouse	[22]
		CD4	Rheumatoid Arthritis	Worsened	Human	[45, 46]
		CD8	Type I Diabetes	Worsened	Mouse	[23, 53]
		CD8	Influenza	Worsened	Mouse	[41]
		CD8	Celiac	Worsened	Mouse	[24]
		CD4	Systemic lupus erythematosus	Ameliorated	Human	[46]
		CD8	Hepatitis A virus	Worsened	Human	[44]
NKp30	BAG-6 B7-H6	CD8	Toxoplasmosis	Worsened	Mouse	[13]
KLRG1	E-cadherin	CD4, CD8	Lyme Arthritis	Worsened	Mouse	[9],
TLR2	Lipoprotein	CD8	Epstein Barr Virus Infection	Ameliorated	Mouse	[58]
TLR3	dsRNA	CD8	Listeriosis	Worsened	Mouse	[144]
		CD4 CD8	Human immunodeficiency virus Infection	Worsened	Human	[145, 146]
TLR4	Lipopolysaccharide	CD8	Rheumatoid Arthritis	Worsened	Human	[69]
TLR7	ssRNA	CD4	Experimental autoimmune encephalitis (EAE)	Worsened	Mouse	[6, 147]
		CD4	Experimental Autoimmune encephalomyelitis	Worsened	Mouse	[18]
TLR9	CpG DNA	CD8	Type I Diabetes	Worsened	Mouse	[71]