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Effects of Toll-like receptor signals in T-cell neoplasms

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Abstract

T-cell neoplasms have poor prognosis and few effective therapeutic options. Therefore, identification of factors in T-cell leukemia/lymphoma that are associated with cancer progression may represent novel therapeutic targets. Recent studies have highlighted a previously unappreciated role for the expression of Toll-like receptors (TLRs) on T cells and their effects on cell survival and proliferation. TLRs can bind exogenous molecules derived from pathogens as well as endogenous self-ligands released from damaged cells. Recent reports demonstrate that TLR engagement on primary mouse or human T cells enhances proliferation and/or cell survival. The mechanisms by which TLR stimulation on T cells influences these parameters and the different T-cell subsets that are affected by TLR stimulation are currently under investigation. Furthermore, neither the biological importance of stimulating TLRs on neoplastic T cells nor the prevalence of TLR expression in T-cell malignancies have yet to be characterized. Based on published reports and compelling preliminary data, we propose that the activation of the TLR-MyD88 signaling pathway in neoplastic T cells contributes to disease progression by reducing cell death and enhancing cell division. In this article, we present both theoretical arguments and experimental data in support of this hypothesis.

Keywords

MyD88; T-cell apoptosis; T-cell leukemia; T-cell survival; Toll-like receptor

Toll-like receptors

Toll-like receptors (TLRs) belong to a family of host defense pattern recognition receptors that alert the immune system to infection by recognizing pathogen-associated molecular patterns derived from various microorganisms [1,2]. TLRs can also recognize intracellular danger-associated molecular patterns released from dying or stressed cells [3]. Table 1 summarizes various aspects of the different TLRs including their cellular localization, adapter molecules and agonists. A total of 13 TLRs in humans and mice have been identified to date; however, not all TLR ligands are known. The recent TLR ligands include protozoan profilin-like protein from *Toxoplasma gondii* (TLR11) [4] and a non-secreted, heat-stable molecule from *Salmonella* enteric serovar *typhimurium* (TLR15) [5]. Each TLR can recognize distinct molecules and can form homo- or hetero-dimers (such as TLR1 or

TLR2). The formation of TLR heterodimers aids in the detection of a broader array of microbial products.

Toll-like receptors are expressed primarily on cells of the innate immune system, such as dendritic cells (DCs), macrophages and B cells [1,2]. The engagement of TLRs by exogenous ligands functions as a 'danger signal' for the immune system, initiating a cascade of signaling events that trigger host defenses. Depending on which TLRs are stimulated and the cell type on which they are activated, TLR stimulation enhances immune responses by inducing cytokine production, augmenting the expression of costimulatory molecules and increasing cell numbers. The increase in cell numbers can occur by enhancing cell division, increasing cell survival or by promoting the generation of hematopoietic precursors.

The use of TLR agonists for the development of potent antitumor T-cell responses has been a subject of major focus in recent times. The role of TLR agonists is principally believed to occur by stimulating TLRs on professional antigen-presenting cells, which activate T-cell-mediated immunity to cancer cells [6,7]. Some of the most distinguishing features of TLR agonists are their aptitude to induce the expression of various costimulatory molecules including CD80 and CD86, to enhance the expression of MHC I and II, and to induce the production of the inflammatory cytokines (e.g., IL-12 and IFN- γ) and chemokines [6,8] necessary for the expansion of T cells. Although the activation of TLR signals in antigen-presenting cells is clearly important for the generation of vast numbers of T cells, emerging data from numerous groups, including ours, indicate that TLR engagement directly on T cells promotes their expansion.

Multiple effects of TLR engagement on primary T cells

The TLR expression profile and the effects of different TLR ligands on T cells vary between human and mouse as well as between diverse T-cell subsets [9–16]. The effects of TLR agonists on T cells are summarized in Table 1. In general, however, the TLR expression on T cells is heavily influenced by the activation status (i.e., naive, activated or memory) [11,17–24]. For example, whereas TLR proteins are barely detected or absent in naive T cells, TLR expression and function are detected within hours after T-cell activation. The sources of T-cell activation capable of conferring T cells with TLR function include stimulation with cognate MHC antigen, anti-CD3 antibodies or concavalin A [25–27]. The precise interplay between T-cell activation and induction of TLR expression is not known. However, T-cell activation may induce *de novo* synthesis of TLRs or induce the expression of other TLR signaling-related molecules such as adaptor proteins [25]. We recently showed that antigen-mediated T-cell activation induced *TLR2* gene transcription and protein expression, but only moderately increased MyD88 expression levels [25]. Furthermore, it is plausible that T-cell activation-dependent signals promote the translocation of TLRs from intracellular compartments to the cell surface where they can bind TLR ligands [9]. Memory T cells appear to maintain TLR expression levels above those of naive T cells but lower than levels expressed on blasting T cells [11,28]. Furthermore, unlike naive T cells, which require concomitant T cell receptor (TCR) stimulation for the transduction of TLR signals, human memory CD45RO⁺ CD4 T cells have been shown to respond to TLR5, TLR7/8 and TLR2 agonists, even in the absence of the TCR signal [19]; however, simultaneous TCR stimulation significantly enhances the costimulatory effects of TLR stimulation alone. Mansson *et al.* also reported that human CD8 T cells freshly isolated from the tonsil expressed elevated TLR2, TLR3 and TLR5 mRNA transcripts following infection with β -hemolytic streptococci [12]. CD8 T cells also expressed higher protein levels of TLR3 and TLR4 than CD4 T cells. However, the expression of TLR1 and TLR9 remained similar in CD4 and CD8 T cells.

Proliferative & prosurvival effects of TLR stimulation on T cells

Among the various signals that TLR engagement induces on T cells, of critical importance for T-cell neoplasms are those that enhance cell survival and proliferation. For example, the engagement of TLR1/2, TLR5, TLR7/8 and TLR9 on helper CD4 T cells augments IL-2 production and consequently increases proliferation (Table 1). In addition, TLR stimulation has been shown to enhance the duration of IL-2 receptor (CD25/IL-2R α chain) expression [26]. Bendigs *et al.* demonstrated that TLR-ligated CD4 T cells circumvented the requirement for CD28 costimulation to induce IL-2 production and proliferation, stressing the effectiveness of TLR signals in T cells [29]. Recent studies by Cottalorda *et al.* and by our group demonstrate that the stimulation of TLR2 on CD8 T cells promoted T-cell expansion in part by decreasing the antigen threshold required to induce cell division/survival *in vitro* [25,26]. It is also worth noting that TLR signaling in several cell types including T cells can regulate proteins involved in cell cycle kinetics, including regulation of D-type cyclins (cyclins D1, D2 and D3) and cyclin-dependent kinases [11,25–27,30–32].

The addition of the TLR4 ligand lipopolysaccharide (LPS) to purified CD25⁺CD4⁺ T regulatory cells (Tregs) has also been shown to augment their proliferation and induce the upregulation of several activation markers [33]. Interestingly, although TCR activation and IL-2 augmented the effects of LPS, Tregs responded to LPS stimulation even in the absence of these signals, albeit to a lower extent. Thus, unlike CD4 helper T cells or cytotoxic CD8 T cells, Tregs do not appear to require TCR stimulation for functional TLR signaling. TLR5 stimulation of human Tregs has also been shown to augment their suppressive activity [10]. TLR8 (poly-G oligodeoxy nucleotides) ligation on Tregs abrogated their suppressive function [34]. Some reports indicate that TLR1/2 agonists (e.g., Pam₃CysK₄) can promote the proliferation of mouse CD25⁺CD4⁺ Tregs [11,35], while other studies found that TLR2 ligands reduce FoxP3 expression and lower their suppressive activity [33–35].

Engagement of TLRs on T cells can also increase cell numbers by modulating the expression of apoptosis-related molecules. For instance, Gelman and colleagues reported that TLR3 or TLR9 ligation on CD4 T cells augmented Bcl-x_L expression levels and enhanced cell survival [36,37]. Similarly, we reported that TLR9-stimulated CD4 T cells expressed higher levels of Bcl-2 and Bcl-x_L and maintained elevated levels of these molecules despite exposure to genotoxic stress (γ -radiation) [38]. In CD8 T cells, engagement of TLR2 increased the expression of bcl-x_L and another antiapoptotic molecule, A1 [26]. In addition, previous work from our group demonstrated that CD8 and CD4 T cells obtained from TLR9 ligand-injected mice expressed elevated levels of Bcl-2 and Bcl-x_L when compared with T cells obtained from control mice [39]. Activated T cells can also avoid death by expressing high levels of the antiapoptotic molecule Fas-associated death domain-like IL-1 β -converting enzyme-inhibitory protein (FLIP), which interferes with formation of the death-inducing signaling complex. We documented that T cells derived from TLR9 ligand-treated mice were more resistant against serum deprivation- and activation-induced cell death *ex vivo* [39]. Furthermore, enhanced T-cell survival was associated with increased expression levels of FLIP in both CD4 and CD8 T cells. Altogether, these studies emphasize the proliferative and prosurvival effects that different TLRs have on distinct T-cell subjects.

We recently demonstrated that TLR9 engagement imparts a radioprotective effect on activated CD4 T cells [38]. This radioprotective effect was manifested *in vitro* by decreased apoptosis. Intriguingly, we found that TLR9 engagement increased the rate of DNA double-strand break repair, and was associated with increased activation of the checkpoint kinases Chk1 and Chk2 and enhanced expression of the antiapoptotic molecules Bcl-2 and Bcl-x_L. *In vivo*, TLR9-stimulated wild-type T cells displayed greater radioresistance than TLR9-

stimulated MyD88^{-/-} T cells. In addition, Sohn *et al.* reported that TLR9 agonists protected primary mouse spleen cells, as well as human macrophage and B-cell lines, from death induced by γ -radiation [40]. Radioprotection was also accompanied by an increased expression of Bcl-x_L and Bcl-2. It is worth highlighting, however, that the radioprotective effects observed by Sohn *et al.* were without further differentiation of distinct immune cell subsets; therefore, whether T cells were amongst the protected cell subsets was not determined. Collectively, these findings indicate that TLR agonists can reduce γ -radiation-induced apoptosis. However, whether the activation of TLR signaling in T-cell neoplasms can contribute to radioresistance or resistance against other forms of anti-cancer therapy such as chemotherapy remain to be explored.

A role for TLR-MyD88 signaling in T cells *in vivo*

Emerging reports are beginning to emphasize an important role for TLR-MyD88 activation on T cells *in vivo*. For example, Bartholdy *et al.* [41] and Rahman *et al.* [42] demonstrated that MyD88 plays an indispensable role in the expansion of lymphocytic choriomeningitis virus (LCMV)-specific CD8 T cells. While MyD88 expression in CD8 T cells is not essential during early activation, it was indispensable for their sustained expansion. Similarly, Zhao *et al.* demonstrated that vaccinia virus-specific MyD88^{-/-} CD8 T cells are slow to expand *in vivo*, thus additionally stressing an important role for MyD88 signaling in T cells [43]. A deficiency in the expression of TRIF, TLR2, TLR4, TLR9 or IL-1R in T cells did not alter T-cell survival, emphasizing a critical and specific role for MyD88 signaling in T cells. Turka and colleagues also demonstrated a need for MyD88 expression in order for CD4 T cells to mount an effective response against *T. gondii* infection [44].

It has also been suggested that TLR9 agonists found in the synovium of rheumatoid arthritis patients can aggravate disease status by stimulating TLR9 on CD4 T cells [45]. Zanin-Zhorov *et al.* also reported that TLR signaling in human T cells altered their migration capacity, in part by changing the expression levels of chemokine receptors (e.g., CXCR4 and CCR7) [23]. Sobek and colleagues observed that activation of T cells with TLR2 agonists sustained pathogen-induced chronic inflammatory joint disease by inducing T-cell proliferation and IFN- γ secretion [27].

The physiological significance of TLR-MyD88 signals in T cells was further highlighted in recent studies by our group. We found that co-injection of CD8 T cells and TLR2 ligand into MyD88-deficient mice improved T-cell cytotoxicity [25] and increased T-cell numbers. Because cells from MyD88-deficient mice do not respond to TLR2 agonists, these results suggested that the increased number of CD8 T cells arises from direct stimulation of TLR2 on T cells. To demonstrate that the costimulatory effects of TLR2 ligand on T cells occurred in a MyD88- and TLR2-dependent fashion *in vivo*, we studied the expansion of MyD88-deficient and TLR2-deficient CD8 T cells *in vivo*. We found neither MyD88-deficient nor TLR2-deficient T cells responded to the TLR2 ligand *in vivo* [25,46].

In summary, accumulating evidence highlights the occurrence of TLR expression in T cells and the various effects that TLR stimulation has on diverse T-cell subsets. Whereas the engagement of certain TLRs enhances cytokine production, other TLR agonists can augment cell survival and proliferation or suppress T-cell responses. Mounting evidence also suggests that the activation of TLR signals on T cells may contribute directly to the pathophysiology of certain diseases, such as rheumatoid arthritis.

Do TLR signals in T-cell neoplasms contribute to disease progression?

The different effects that TLR agonists have on different T-cell subsets warrant further investigation of the role that these signals may play in the expansion and survival of T-cell

neoplasms. The following section provides an overview of published reports supporting the hypothesis that TLR–MyD88 signals in T-cell malignancies may contribute to disease progression.

T-cell neoplasms include several diseases that are markedly heterogeneous in their biology, presentation, natural history and therapy. T-cell leukemias include T-cell acute lymphoblastic leukemia (T-ALL), T-cell prolymphocytic leukemia (T-PLL), adult T-cell leukemia (ATLL) and T-cell large granular lymphocyte (T-LGL) leukemia. T-cell lymphomas include peripheral T-cell lymphoma, not otherwise specified (PTCL-NOS) and cutaneous T-cell lymphomas (CTLCs). Based on the diverse biology of these diseases, the importance of proliferative and antiapoptotic mechanisms is variably implicated.

T-acute lymphoblastic leukemia

Acute lymphoblastic leukemia (ALL) is the most common cancer in children, accounting for 29% of all cancers (23.6% in ages 0–14 years; 5.8% in ages 15–19 years) [47,48], making ALL the most common cancer in this age group [49]. Nearly 5000 cases of ALL are diagnosed annually in the USA. A total of 15% of ALLs in children and 25% in adults are T-ALL, which has been historically linked to a poor prognosis. The use of conventional cancer therapies together with a strict use of prognostic factors has resulted in an overall complete remission rate of 85% of childhood T-ALL and only 40% of adult T-ALL. The poor outcome in adult patients with T-ALL has been associated with drug resistance and limited effective therapeutic options [50,51].

The most specific diagnostic marker of T-cell lineage is surface or cytoplasmic CD3 expression [47]. Other cell surface markers that may be expressed on T-ALL cells include CD2, CD5 and CD7, as well as T-cell subset markers, including CD1 (thymocytes), CD4 (T helper cells) and CD8 (cytotoxic T cells). Given the heterogeneity of T-cell antigen expression on leukemic T cells and considering that T-cell subsets express distinct TLR expression profiles, we postulate that the expression of TLRs or other TLR-related proteins might be utilized as a means to further characterize disease heterogeneity. In support of this assertion, preliminary data from our group indicate that the T-ALL cell line CCRF-CEM expresses TLR1 and TLR2 (Figure 1A). Furthermore, the engagement of the TLR1–TLR2 heterodimer with Pam₃Cysk₄ significantly augments the proliferation of the T-ALL lines (Figure 1B). By contrast, the T-lymphoma line HUT-78 expresses TLR2 but low levels of TLR1 (Figure 1A). The reduced levels of TLR protein correlated with decreased numbers of *TLR* gene transcripts (not shown) and a lack of response to the TLR1–TLR2 ligand (Figure 1B). We are currently probing the idea that the levels of TLR protein/mRNA transcripts in T-cell neoplasms or that the proliferative responses to TLR agonists by malignant T cells are associated with clinical data such as pretreatment characteristics, response to treatment and relapse.

Notch signaling also plays a key role in normal T-cell development, and aberrations in this pathway are seen in malignant transformation [52–55]. It is estimated that 50% of human T-ALLs have activating mutations in Notch-1. Notch signaling is initiated when the extracellular domain of the receptor binds to a membrane-bound ligand of a neighboring cell, including the δ (or δ -like) and Jagged/Serrate families of membrane-bound ligands. Engagement of Notch with its ligand leads to its proteolytic cleavage, which releases the Notch intracellular domain from the cell membrane. The Notch intracellular domain translocates to the nucleus, where it forms a complex with the DNA binding protein CSL, MAML1 and histone acetyltransferases (HATs), resulting in transcriptional activation of Notch target genes [54,55]. Intriguingly, activation by a combination of TLR ligands has been shown to augment δ -like ligand expression on DCs [56,57]. Furthermore, a lack of

MyD88 in DCs significantly reduces the expression levels of δ -like ligand [58,59]. Although a link between TLR signals and Notch signals in T-cell malignancies remain unknown, recent studies, showing that Notch signaling can be activated by TLR stimulation (in macrophages), merit further investigation of this topic [60].

γ -secretase inhibitors (GSIs) to block Notch signaling are in clinical trials in the treatment of T-ALL [61–63]. Specifically, GSIs have been shown to block proteolytic activation of Notch receptors. Interestingly, Elzinga reported the characterization of a conserved TRAF6 consensus binding site within the IL-1R, which rendered IL-1R susceptible to γ -secretase-mediated cleavage [64]. γ -secretases also target TRAF6, which is instrumental in TLR–MyD88 signaling [64]. These data suggest that an alternative mechanism by which GSIs inhibit T-ALL progression may be reducing TRAF6/MyD88/IL-1 signals; however, this concept has yet to be examined.

Approximately 85% of T-ALL patients display an increased activation of the PI3K–Akt–mTOR pathway resulting from Notch1 activation, cytokine signaling (i.e., IL-4) and/or mutations in PI3K, Akt or PTEN. An increased activation of the PI3K–Akt–mTOR pathway is associated with cell proliferation, survival and drug resistance. Recent findings highlight that inhibiting the PI3K–Akt–mTOR pathway reduces the proliferation and survival of T-ALL [65]. We recently demonstrated that the activation of TLR2–MyD88 in nonmalignant T cells resulted in part from the enhanced activation of the mTOR pathway [66]. Inhibiting mTOR or Akt in T cells abolished the costimulatory effects of the TLR2 agonist. These data suggest that the constitutive activation of PI3K–Akt–mTOR pathway may in part be a result of active TLR signaling in T-cell neoplasms; however, this concept has yet to be demonstrated.

T-cell prolymphocytic leukemia

T-cell prolymphocytic leukemia is a rare chronic leukemia, representing approximately 2% of adult chronic lymphocytic leukemia, and is characterized by proliferation of small-to-medium-sized prolymphocytes [65]. The immunophenotype of T-PLL cells is that of a mature post-thymic T cell: TdT, CD2⁺, CD3⁺, CD5⁺ and CD7⁺ [67].

T-cell prolymphocytic leukemia cells demonstrate structural abnormalities involving the rearrangement of the T-cell leukemia/lymphoma (*TCL*)1 oncogene and the TCR- α/δ locus, resulting in *TCL1* overexpression [68]. High-level *TCL1* expression in TCR-expressing T-PLL is associated with faster cell doubling, resulting, in part, from enhanced responses to TCR stimulation [69]. Interestingly, TLR costimulation on T cells has been shown to significantly lower the TCR activation threshold, resulting in increased proliferation. Whether TLR–MyD88 signals can amplify TCR signals or alter *TCL1* expression in T-PLL cells and whether MyD88 signals play an important role in T-PLL survival and/or proliferation merit investigation.

Adult T-cell lymphoma/leukemia

Adult T-cell lymphoma/leukemia is a mature peripheral T-cell malignancy caused by the human T-cell lymphotropic virus (HTLV) type-1 [70]. HTLV-1 is passed by sexual transmission, from mother to child (mainly from breastfeeding) and from contaminated blood products. The infected population is estimated to be 15–20 million worldwide, but only approximately 2.1% of females and 6.6% of males infected by HTLV-1 will develop ATLL [70–72].

NF- κ B is one of the signaling pathways that are constitutively activated in these cells, suggesting that it plays a critical role in proliferation and/or survival. Based on the fact that

the TLR signaling pathway activates NF- κ B, Mizobe and colleagues investigated whether MyD88-mediated signals contributed to NF- κ B activation in HTLV-I-transformed T cells [73]. The authors found a constitutive association of MyD88 with IRAK1 in several HTLV-I-transformed T-cell lines but not in Jurkat, HuT78 and MOLT4, which were not infected with HTLV-I. Furthermore, MT2 cells, which are transformed by HTLV-1, expressed TLR1, 6 and 10 mRNA. Constitutive activation of NF- κ B was inhibited by overexpressing dominant-negative forms of MyD88, resulting in reduced proliferation and enhanced apoptosis. These data highlight an indispensable role for MyD88 in the survival of HTLV-infected cells. Furthermore, HTLV-I tax, a viral protein involved in allowing random integration of HTLV-1 proviral DNA into the host genome, augmented TLR expression and could activate NF- κ B. These findings suggest that TLR–MyD88 signaling may contribute to constitutive activation of downstream signaling events, such as NF- κ B activation, in malignant T cells.

Not all TLR agonists promote T-cell expansion. Intriguingly, Moarbes *et al.* found that a EAPB0203, a molecule closely related to the TLR7 ligand imidazoquinoline, inhibited cell proliferation and induced apoptosis of fresh ATL cells as well as HTLV-I-transformed malignant T cells, while T cells from healthy individuals remained resistant to TLR7 ligand-mediated apoptosis [74]. EAPB0203 treatment was associated with the downmodulation of several antiapoptotic proteins, including c-IAP-1 and Bcl-x_L, and loss of mitochondrial membrane integrity. However, despite being a TLR7 agonist, EAPB0203 treatment did not appear to alter NF- κ B activation, suggesting that the TLR-mediated activation of NF- κ B may be primarily associated with cell survival.

Adult T-cell lymphoma/leukemia cells also express the T-cell antigens CD2, CD3 and CD5, but usually not CD7, and nearly all express CD52 and CD25. CD25 is the α -chain of the IL-2R, which in association with CD122 (b chain) and CD132 (γ -common chain), form the high affinity IL-2R. Leukemic cells from ATLL patients have been shown to proliferate in response to IL-2 [48,75,76]. The engagement of various TLRs on healthy T cells can enhance production of cytokines, which augment cell division (Table 1) [11,19,29,77]. Furthermore, the activation of TLR signals in T cells increases the expression levels of cytokine receptors (i.e., CD25 and CD132) on the T-cell surface and prolongs the duration of the receptor expression. Whether TLR signaling in T-cell neoplasms promote division by increasing cytokine production and/or receptor expression remains an intriguing question that has yet to be addressed.

T-cell large granular lymphocyte leukemia

T-cell large granular lymphocyte leukemia is a rare, indolent chronic lymphoproliferative disorder characterized by clonal expansion of T-LGLs, which typically express CD3, CD8 and CD57, with or without CD16 and/or CD56, and clonal *TCR* gene rearrangements.

The pathogenesis of T-LGL leukemia includes the presence of chronic antigenic stimulation, resulting in persistence of a clone of antigen-driven terminal memory effector T-lymphocytes [78]. Exogenous signals, such as cytokines, can also influence apoptosis-related protein expression. A role for TLR signals in T-LGLs has yet to be determined. However, as previously mentioned, TLR activation on nonmalignant T cells have recently been shown to increase responses to antigenic stimulation, resulting in increased T-cell numbers. The precise mechanisms by which TLR signals augment T-cell numbers are not known but likely involve regulation of apoptosis-related molecules [26,36,38] and the induction of cytokines and cytokine receptors, which promote cell division. In support of this claim, TLR2 engagement on activated T cells increases the expression of Bcl-x_L and A1 while reducing the levels of the proapoptotic molecule Bim [26,46]. Ongoing studies by our

group also indicate that overexpressing MyD88 in CD8 T cells significantly augments TCR signals and enhances survival even in the absence of TLR ligand. Our preliminary data are in agreement with emerging studies revealing an indispensable role for MyD88 in T-cell survival [36–38,41–43].

Autoimmune disorders, such as rheumatoid arthritis, arise in approximately 35% of patients with T-LGL. Interestingly, studies by van der Heijden and colleagues found that TLR9 and TLR2 agonists in the synovium of rheumatoid arthritis costimulated T-cell responses [45]. Sobek *et al.* reported that TLR2 agonists sustained pathogen-induced chronic inflammatory joint disease by inducing T-cell infiltration, proliferation and cytokine production [27]. These observations lead us to question whether some of the endogenous TLR agonists that exacerbate autoimmune T-cell-mediated responses may also promote the expansion of malignant T cells by stimulating TLR–MyD88 signals directly in leukemia cells.

Constitutive activation of PI3K, Akt, NF- κ B, JAK-signal transducer and activator of transcription (STAT) and sphingolipid-mediated (sphingosine kinase [SPHK]1) signaling in T-LGL has also been demonstrated [79–82]. Recent studies by Quigley *et al.* [31] and by our group [66] demonstrate that the costimulatory effects of TLR2 signals in CD8 T cells are unequivocally dependent on the PI3K–Akt pathway [31]. Furthermore, cells from T-LGL patients are resistant to Fas-mediated apoptosis [79–82]. This is similar to T cells derived from TLR9-treated mice [39], which exhibit resistance to activation-induced cell death and an increased expression of FLIP. These data underscore some of the similarities in the signaling profiles of T-LGL cells and TLR-stimulated T cells.

The two available treatments with demonstrated efficacy in series of patients with T-LGL leukemia are the immunosuppressive agents cyclosporine A (CsA) and low-dose methotrexate [83–85]. Both produce high response rates and durable responses. CsA functions to inhibit calcineurin, which is responsible for activating the transcription factors NF-AT in T cells. Suppression of NF-AT results in reduced T-cell proliferation. Recent studies demonstrate that calcineurin can also negatively regulate TLR signaling by interacting with MyD88, TRIF, TLR2 and TLR4 (but not TLR9 nor TLR3) [86], suggesting that therapies such as CsA may also function to reduce T-LGL growth by inhibiting TLR signaling pathways.

Peripheral T-cell lymphoma & cutaneous T-cell lymphomas

T-cell lymphomas currently include various sub-types including PTCL-NOS and CTCLs [87]. PTCL is a subtype of non-Hodgkin's lymphoma (NHL) and is an aggressive cancer that generally has a poor prognosis and accounts for 10% of all non-Hodgkin's cases. CTCL is a rare lymphoma of low indolence, which accounts for approximately 5% of all non-Hodgkin's diagnoses. A potential role for TLRs in PTCL and CTCL has only recently begun to be examined. Smith *et al.* examined the expression of TLR1 to TLR9 mRNA levels and found TLR1, TLR2 and TLR4 to be highly expressed in PTCL cells [88]. Furthermore, immunostaining revealed an increase in the number of cells expressing both TLR2 and TLR4. Jarrousse *et al.* also examined the expression levels of several TLRs in the CTCLs – mycosis fungoides and Sézary syndrome – at different stages of the disease [89]. Using paraffin-embedded biopsies, they found cutaneous lesions to express higher levels of TLR2, TLR4 and TLR9 compared with normal skin. Mycosis fungoides showed increased TLR2, TLR4 and TLR9 expression. Biopsies from Sézary syndrome patients also demonstrated detectable levels of TLR2, TLR4 and TLR9. Although the expression levels of other TLRs or the effects of ligating TLRs on T lymphomas has yet to be characterized, Suchin *et al.* described a case report showing that administration of a topical cream containing 5% of the TLR7 agonist imiquimod successfully treated a stage IA CTCL [90]. These results are

especially encouraging considering that the patient did not respond to prior treatment with a number of topical steroids or topical nitrogen mustard and carmustine. This case study further emphasizes the diverse effects of TLR agonists, highlighting that while some TLR agonists may promote the expansion of T-cell neoplasms other TLR ligands may induce apoptosis.

Several studies have also explored whether genetic differences in TLR genes influence other hematopoietic malignancies such as NHL and Hodgkin's lymphoma (HL) pathogenesis. Mollaki *et al.* investigated the association between *TLR9* and *MYD88* gene polymorphisms and the risk for HL [91]. The results to this study demonstrated an association between *TLR9/1237C* and *TLR9/2848A* gene polymorphisms and the risk for HL. Purdue *et al.* examined *TLR* gene variants in a pooled analysis of 1946 NHL cases and 1808 controls and found that two TLR10–TLR1–TLR6 variants in moderate linkage disequilibrium were significantly associated with NHL risk [92]. Moreover, the analysis provided evidence that TLR2 variants may influence susceptibility to marginal zone lymphoma. A study by Nieters *et al.* recognized that the TLR2 -16933T>A variant was linked to a 2.8-fold increased risk of follicular lymphoma but a reduced risk of chronic lymphocytic leukemia [93]. The TLR4 Asp299Gly variant was also associated with the risk of HL and mucosa-associated lymphoid tissue lymphoma. These studies suggest an effect of TLR/TLR-related polymorphisms and various lymphoma subtypes.

Concepts for overcoming TLR-MyD88 signaling in T-cell neoplasms

The last decade has witnessed important advances in the field of TLR research. Various compounds have been developed to target TLR signals for the treatment of severe sepsis and various diseases. These include TLR4 antagonists, such as LPS, from *Rhodobacter sphaeroides* and *Porphyromonas gingivalis*, in other words, Tak242 and eritoran [94], which are currently in Phase III clinical trials to treat patients with sepsis. The use of soluble TLRs as well as antibodies to neutralize TLRs or antibodies against TLR ligands [95] represents another potentially effective means to inhibit specific TLR signals. Recent insights indicate that several drugs already in clinical use to treat cancer, allergies and chronic infections can also attenuate TLR signals. For example, statins, which are generally used to lower cholesterol levels, have been shown to inhibit TLR-mediated inflammatory responses. Niessner *et al.* showed that treatment with simvastatin significantly reduced TLR2 and TLR4 protein expression on monocytes, and lowered MCP-1 levels in healthy volunteers [96]. Methe *et al.* also demonstrated that treatment with statins lowered TLR4 expression levels and was associated with decreased IRAK-1 activity on monocytes [97]. In addition, pretreatment of the mice with cerivastatin reduced serum levels of TNF- α and IL-1 β in response to the TLR4 agonist LPS [98].

Angiotensin-receptor blockers, such as candesartan cilexetil, have also been shown to have TLR antagonist activity [99]. Candesartan is widely used to treat hypertension [100]. Several groups have reported that candesartan also attenuates inflammation by reducing TLR expression levels. Sanchez-Lemus *et al.* demonstrated that treatment with candesartan prior to LPS administration decreased COX-2 and IL-6 gene expression and reduced cytokine production *in vivo* [101]. Dasu *et al.* demonstrated that pretreating monocytes with candesartan reduced TLR2 and TLR4 expression at the mRNA and protein levels, which consequently reduced NF- κ B activity and expression of IL-1 β , IL-6, TNF- α and MCP-1 [102]. Similarly, candesartan reduced TLR2 and TLR4 expression and curbed responses to TLR agonists *in vivo* [101].

The drug glycyrrhizin, the main active compound in liquorice, has also been found to antagonize TLR signaling. Glycyrrhizin has been used to treat patients with various viral-

induced diseases. In Japan, for example, glycyrrhizin has been used to treat patients with hepatitis C, influenza, HIV-I and the severe acute respiratory syndrome virus [104–107]. Although the precise mechanism of action remains undefined, glycyrrhizin has been shown to impede TLR4 and TLR9 signaling, resulting in a reduction of proinflammatory cytokines such as IL-8, IL-1 β , TNF- α and eotaxin-1 [108–111]. Glycyrrhizin has also been shown to inhibit signal transduction in response to the ligand HMGB1, which is recognized by TLR2 and TLR4 [112].

Conclusion

Recent advances underscore the biology of TLRs in normal T cells. New insights have provided us with a greater appreciation for the diverse effects that different TLR agonists have on the survival and proliferation of various T-cell subsets. Ongoing studies are just now beginning to reveal a potential role for TLR signaling in T cells and suggest that TLRs may play an important role in the pathogenesis of T-cell malignancies.

Future perspective

T-cell leukemias are a heterogeneous group of disorders for which novel and improved therapies are needed. Further elucidation of the biology of TLR signals in T-cell neoplasms has the potential to lead to novel targeted therapies for these malignancies. Furthermore, evaluation of several key TLR-related molecules could help establish a molecular profile of distinct T-cell leukemias, disease progression and/or prognosis of therapy-resistant patients.

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Executive summary

Costimulatory effects of activating Toll-like receptor-MyD88 signals in nonmalignant & leukemic T cells

- Activating Toll-like receptor (TLR)–MyD88 signals in nonmalignant T cells augments cytokine production, increases proliferation, enhances cell survival and can confer radioresistance.

TLR-MyD88 signals in malignant T cells

- Constitutive activation of MyD88 signaling in adult T-cell lymphoma/leukemia cells promotes cell survival.
- Toll-like receptor engagement on leukemic T-cell lines augments cell division.
- The engagement of certain TLRs on T-cell neoplasms can augment proliferation/survival, other TLR agonists can induce cell death.
- Polymorphisms and haplotypes in TLR/TLR-related molecules are associated with the development of T-cell leukemia/lymphoma.

Conclusion

- Recent advances emphasizing the biology of TLRs in normal T cells suggest that TLRs may play an important role in the pathogenesis of T-cell malignancies.
- Current data indicate that malignant and nonmalignant T lymphocytes express functional TLRs.
- Depending on which TLR is activated, and on which cell type TLR stimulation occurs, the engagement of TLRs and/or MyD88 signals can augment cell numbers or induce apoptosis.
- Assessment of several key TLR/TLR-related molecules could help establish molecular profiles of distinct T-cell leukemias, disease progression and/or prognosis of therapy-resistant patients.

Figure 1. Proliferative effects of Toll-like receptor 1/2 agonists and Toll-like receptor expression on T-cell ALL and T-lymphoma cells

(A) The basal protein levels of TLR1, TLR2 and MyD88 in CCRF-CEM and Hut-78 cells were determined by western blot. Flotillin was used as a loading control for membrane proteins. (B) The T-ALL CCRF-CEM and T-lymphoma Hut-78 cell lines were cultured in the presence of varying concentrations of the synthetic TLR1/2 ligand, tripalmitoylated lipopeptide, Pam₃Cysk₄. A total of 72 h later, proliferation was determined by measuring tritiated thymidine incorporation. cpm: Counts per minute; Pam₃Cysk₄: Tripalmitoyl-*S*-glyceryl-cysteine; T-ALL: T-cell acute lymphocytic leukemia; TLR: Toll-like receptor.

Table 1

Pleiotropic effects of Toll-like receptor engagement on various T-cell subsets.

TLR	Adapter molecule	TLR ligand	Effects of TLR engagement on T cells	T-cell subset	Ref.
TLR1/2	MyD88	Triacylated lipopeptides Diacylated lipopeptides Lipoproteins Heat shock proteins HMGB1	Augments production of IFN- γ , perforin, granzyme B and IL-2 Increases proliferation Decreases antigen threshold Upregulates the expression of antiapoptotic molecules	$\delta\gamma$, CD4 and CD8 T cells Memory T cells	[11,13-16,21,23,25-27,31,33,35,46]
TLR3	TRIF	dsRNA PolyI:C	Augments production of IFN- γ	$\delta\gamma$, CD4 and CD8 T cells	[12-14,18,22]
TLR4	MyD88 TRIF	Lipopolysaccharides HMG-B1 Heat-shock proteins	Upregulates the expression of CD69 and CD25	CD4 and CD8 Treg	[28,45]
TLR5	MyD88	Flagellin	Augments production of IFN- γ and IL-2 Suppresses Treg function?	CD4 and CD8 Treg	[9,10,19,29]
TLR2/6	MyD88	Diacyl lipoproteins	Increases CD4 Treg proliferation	CD4 Treg	[32]
TLR7	MyD88	ssRNA	Augments production of IFN- γ and IL-4	CD4	[18,19,29]
TLR8	MyD88	ssRNA	Reverses Treg function Augments production of IFN- γ by CD4 helper T cells	Treg CD4 helper T cells	[19,28,34]
TLR9	MyD88	Unmethylated CpG DNA	Augments production of IL-2 Increases proliferation Radioprotection	CD4	[28,29,36,38]
TLR10	Unknown	Unknown	Unknown	-	-
TLR11	MyD88	<i>T. gondii</i> profilin	Unknown	-	-
TLR12	Unknown	Uropathogenic bacteria	Unknown	-	-
TLR15	Unknown	Unknown	Unknown	-	-

T. gondii: Toxoplasma gondii; *TLR*: Toll-like receptor; *Treg*: Regulatory T cell.