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Review Article

Toll-like receptors (TLRs) Review

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استعراض TLRs

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الملخص

مستقبلات شبيهة تول(TLR) هي مستقبلات يرتبط بها انماط جزيئية ذات علاقة بمسبب المرض(PAMPs) ولها دور مهم في المناعة الحامية من الإصابة بميكروب أو التهاب . وهي مكونات بدائية في جهاز المناعة الطبيعية والمكتسبة وهي فعالة كمتداخلات مركزية لنوعيات واسعة من الاستجابات , استجابة لتعكس تأثيرات لإفرازات الميكروب . تحفيز TLR بواسطة إفرازات الميكروب يؤدي إلى طرق استجابة لتنشيط ليس فقط المناعة الطبيعية ولكن المناعة المكتسبة أيضاً . روابط هذه المستقبلات TLR لها دور مهم غير مباشر في تحفيز استجابات مدعومة بخلايا تي خلال تأثيرها على خلايا المناعة الطبيعية مثل تنظيم ظهور الجسم المستضد على الخلايا واظهار الجزيء المحفز المساعد وانتاج محفز الخلايا عند الالتهاب . ليكن أكثر وضوحاً كون روابط مستقبلات TCR قادرة على التأثير المباشر على خلايا تي , ربما كجزيئات محفزة مساعدة . الدراسات على مستقبلات TLR تبدي بوضوح أهميتها في حدوث العديد من الأمراض , لتأسيس الميكانيكية المطلوبة بالضبط تحتاج تمحيص أكثر . بالعموم TLR تدعم استجابات خلايا تي الفعالة مثل انتاج محفز الخلايا والتكاثر والبقاء لخلايا تي الفعالة , في حين زيادة عدد خلايا تي المنظمة +CD4+CD25 مع فقدان مؤقت لوظيفة تثبيط المناعة . الميكانيكية الجزيئية لوظائف مدعومة بواسطة مستقبلات TLR في خلايا تي والتأثير المباشر لهذه المستقبلات على خلايا تي .

الكلمات الدالة : مستقبلات شبيهة تول – عمل الخلايا اللمفية تي - Th1/Th2

ABSTRACT

Toll-like receptors (TLRs) are pathogen-associated molecular patterns (PAMPs) recognition receptors that play important role in protective immunity against infection and inflammation. They are an essential component of the innate and adaptive immune systemans act as central integrators of a wide variety of signals, responding to diverse agonists of microbial products. Stimulation of Toll-like receptors by microbial products leads to signaling pathways that activate not only innate, but also adaptive immunity. TLR ligands indirectly play an important role in promoting T cell-mediated responses via their effects on innate immune cells including up-regulating antigen presentation, co-stimulatory molecule expression, and inflammatory cytokine production. It has also become increasingly evident that TLR ligands can also act directly on T cells, possibly as co-stimulatory molecules to modulate T cell response. Studies on TLRs clearly show their importance in induction of several diseases but establishing the exact underlying mechanism require further investigation. In general, TLRs can function as costimulatory receptor to enhance effector T responses including cytokine production, proliferation, and survival while expanding the CD4+ CD25+ Treg cell population with a transient loss of immunosuppressive function. The molecular mechanisms for the TLR mediated function in T cells and the direct effect of TLRs on T cell polarization need to be addressed.

Keywords: TLR, T Lymphocytes Function, Th1/Th2

INTRODUCTION

Protective immunity in mammal includes innate and adaptive immunity. The innate immune response is immediate, and it is the first line against pathogenic infection, but their function is limited and nonspecific. Adaptive immunity start later and is more specific and include cell mediated response by T cells and humoral response by B cells.

In contrast to innate immunity, adaptive immunity is specific for each pathogen, the response last longer because of the memory cells induction. After antigens encounter the system, the naïve T cells become activated and differentiate into T helper type 1 (Th1), Th2, TH17 or regulatory T cells based on the structure of antigen, co-stimulation and cytokine milieu. Each subsets of T cells have different function. For example, Th1 acts against intracellular pathogen but may cause inflammatory diseases. Th2 cells control against extracellular pathogens, but are also responsible for allergic responses, and there enough evidences indicate the key role TH17 cells in pathogenesis of autoimmune diseases such as RA, Psoriaris, Psoriatic arthritis, ankylosing spondylitis Therefore, It is always fascinating to an immunologist how innate and adaptive immunity is regulated. The results from these studies help us understand the etiology of some of these diseases.

The immune system has evolved primarily to combat pathogens. However, irrational exuberant of the immune response can lead to a range of autoimmune diseases. Thus, the immune system serves the mammalian hosts

in three key aspects: a. To mount an immediate defence against infection, involving innate defence (rapid but non-specific), and primary immune response (delayed but specific). B) To form a rapid and effective recall mechanism in response to re-infection(specificTandBcell memory). C)Toavoidautoimmune pathology(toleranceand regulation/suppression).

The host defence response to pathogens depends on the immune system.Adaptive immunity is a highly sophisticated system that is mediated by antigen-specific T and B cells and is observed only in vertebrates. In contrast, innate immunity is conserved from invertebrates to vertebrates. Even invertebrates and plants harboring only partial innate immunity have an effective host defence system. Studies of the host defence system in fruit flies (Drosophila) provided the first clue as to the mechanism of innate immune recognition.

Toll was initially identified as an essential protein that plays a central role in the establishment of dorsoventral polarity in the embryo of Drosophila. In Drosophila, a family of Toll receptors plays an important role in combating the invasion of pathogens (1). Adult fruit flies which are mutated in Toll are susceptible to infection by fungi and bacteria, respectively (2-3). It indicates the importace of Toll in protection of Drosophila against infection.Subsequently, homologues of Drosophila Toll were identified in mammals and are termed Toll-like receptors (TLRs) (4). TLRs compose a large family with at least 11 distinct TLRs (PAMPs) have now been identified in humans and 13 in mice.

Among the 11 known mammalian TLR family members, TLR2, 4, 5, 6 and 9 have been implicated in the recognition of bacterial components. TLR2 is responsible for the recognition of peptidoglycan and lipoprotein, whereas TLR4 recognizes LPS. TLR3 is implicated in the recognition of dsRNA and viruses which is produced by most viruses during their replication and TLR9 is a receptor for CpG DNA. TLR5 has been shown to be a receptor for flagellin in bacteria.

Several ligands have been characterized as TLR7 and/or TLR8 ligands, classified in synthetic compounds and natural nucleoside structures. Most or all of the TLRs, like Toll are believed to be functional multimers. Some, like the TLR2 complexes with TLRs 1 or 6, are heteromeric. Some appear to be homomeric, and in some cases, non-TLR subunits are part of the signaling complex. For example, TLR4 seems not to detect LPS directly, but only as a complex with MD2 and CD14, a small tightly associated LPS binding subunit. (2, 5-11).

Some synthetic compounds were already produced and used as immune activators before they were characterized as TLR7/TLR8 ligands. TLR10, which exists in humans and is most closely related to TLRs 1, 2, and 6, has been lost from the mouse genome. Its ligand cannot be explored in the mouse and remains uncertain. TLRs 11, 12, and 13 have been lost from the human genome, and of the 3, only one ligand for TLR11 has been identified (11-12).
They detect a broad range of pathogen-associated molecular patterns (PAMPs) to recognize different microbial as a means to distinguish 'non-self' from 'self., and in some cases they also recognize endogenous ligands, which are considered damage-

associated molecular patterns (DAMPs) (10, 13). PAMPs are integral structural components of the pathogens and are thus essential to the survival of the infectious organisms. Therefore, PAMPs are expected to be conserved among a range of pathogens, including virus, bacteria and fungus (10).

TLRs act as primary sensors of microbial products and activate signaling pathways that lead to the induction of immune and inflammatory genes (10). TLRs belong to a broader family of proteins, which include receptors for the pro-inflammatory cytokines IL-1, IL-18 and the orphan receptor T1/ST2 (14). All members of this superfamily signal inflammation in a very similar manner. This is due to the presence of a conserved protein sequence in the cytosolic domain called the Toll/IL1 receptor (TIR) domain, that activates common signaling pathways, most notably those that activate the transcription factor NFκB and stress-activated protein kinases (14).

It was initially thought that TLRs are primarily expressed by antigen-presenting cells (APCs), such as macrophages and dendritic cells, and that interactions between microbial ligands and TLRs in these cells will indirectly result in activation of cells of the adaptive immune system, especially T cells. Evidence is now accumulating that TLRs play an important role in the recognition and activation of components of pathogens not only in innate immunity but also in adaptive immunity. It has now become clear that TLRs are also expressed by various T cell subsets, such as conventional αβ T cells, regulatory T cells, CD8 T cells (15-17), and γδ T cells as well as natural killer T cells (18-19). Importantly, it appears that at least in some of these T cell subsets, TLRs are functionally active, because stimulation of these cells with TLR agonists in the absence of APCs results in exertion of effector or regulatory functions of T cells.

METHODS

Toll-like Receptors on T cells

Most investigations on TLRs have focused on cells of the innate system because TLRs are closely associated with innate response. However, there is no *a priori* reason why TLRs may not have a direct function in adaptive immunity. Expression of TLRs on innate and adaptive immune cells seems to be important in immune systems for elimination of pathogen. TLRs are expressed widely in many types of immune cells, including DCs, neutrophils, eosinophils, mast cells, macrophages, monocytes and epithelial cells (9-10, 20). Interestingly, we and others reported TLR express functionally on different subtype of T cells. TLR-3, -6, -7 and -9 have been reported to be expressed on CD4+ T cells (21).

TLR messages have been sporadically reported in T cells (15-16, 18, 22-25). We have for the first time demonstrated that TLR2, and TLR2 only**,** is functionally expressed on the surface of activated T cells and memory cells (16-22). Resting naïve human $CD4^+$ T cells (99.9% pure from human cord blood) express intracellular TLR messages but no detectable cell surface TLRs. However, following a few hours activation *in vitro* with plate-bound anti-CD3, and particularly in the presence of IFNα, these cells express clear cell surface TLR2 and TLR4 as shown by flow cytometry and immunofluorescence microscopy (15-17). Such cell surface expression was also

seen with Epstein-Barr virus (EBV)-specific human $CD8^+$ T cells following re-stimulation with EBV peptide *in vitro*. This finding suggests clinical relevance of TLR expression in T cells. Activated T cells responded to BLP (synthetic bacterial lipoprotein, $Pam_3Cys\text{-}SK_4$, a specific TLR2 ligand) to proliferate and produce markedly enhanced levels of cytokines, including IL-2, IFNγ, and TNFα. In contrast, activated T cells did not respond to LPS (a TLR4 ligand). This is most likely explained by the lack of CD14 (a co-receptor of TLR4) expression on T cells. The BLP-induced T cell proliferation can be specifically blocked by anti-TLR2 antibody, and is unlikely to be mediated by potential contamination of antigen presenting cells (APC), since anti-CD3 activated T cells from TLR2 knock out mice did not respond to BLP even in the presence of 5% APC from wild-type mice. We then went on to show that $CD4^+CD45RO^+$ memory T cells from adult peripheral blood constitutively expressed TLR2 and rapidly produced more IFNγ in response to BLP than naïve $CD4^+CD45RA^+$ T cells cultured with immobilised anti-CD3 antibody. Interestingly, BLP also significantly enhanced the proliferation and IFNγ production of memory $CD4^+CD45RO^+$ (but not naïve $CD4^+CD45RA^+$) T cells cultured with IL-2 or IL-15 alone, in a bystander manner.

These results, therefore, show that TLR2 serves as a co-stimulator receptor for antigen-driven T cell development, and may help maintain T cell memory. These finding suggests that pathogen, via their PAMPs, may contribute directly to the activation and perpetuation of T cell memory in antigen dependent and independent manner. It should also be noted that BLP alone did not activate naïve or memory T cells. It does so only in the presence of TCR activation or via a bystander effect of cytokines such as IL-2 or IL-15.

This dual-signalling mechanism should avoid excessive T cell proliferation by BLP alone (15-16, 22). We also have preliminary data showing that BLP could enhance the proliferation and survival of memory T cells *in vivo,* in an adoptive transfer of OVA/TCR transgenic (D0.11) mouse model (Komai-Koma M et al., unpublished data). By contrast, TCR stimulation down-modulates significantly surface TLR-5 expression on human CD4+ T cells (29). TLR expression on T cells may be regulated by TCR signalling, which needs further investigation in the future. These data thus offer the possibility that pathogens, via their PAMPs, may contribute directly to the perpetuation and activation of T cells.

At least some TLRs may function as a co-stimulatory receptor for antigen-specific T cell responses and participate in the maintenance of T cell memory (15, 30-31). It has been shown that ligands for TLR-2, -3, -4, -5 and -9 enhance the proliferation and/or biological functions of conventional effector T cells (15, 17, 30, 32). Co-stimulation of CD4+ T effector cells with anti-CD3 mAb and TLR-5 ligand flagellin results in enhanced proliferation and production of IL-2 at levels equivalent to those achieved by co-stimulation with CD28 (33-34).

CpG-containing oligodeoxynucleotides (CpG-ODN) can co-stimulate primary T cells in the absence of APCs (35). In the presence of the TCR signal, CpG-ODN induces IL-2 production, IL-2R expression and thus T cell proliferation. Furthermore, CpG-co-stimulated T cells differentiate into cytolytic T lymphocytes in vitro (35). Co-stimulation of antigen-activated murine CD8+ T cells

with the lipopeptide Pam3CysSK4 (Pam), a TLR-1/2 ligand, enhances the proliferation, survival and effector functions of these cells (17-33) TLR-2 engagement on CD8+ T cells reduces significantly their need for co-stimulatory signals delivered usually by mature APCs (17).

It was reported that activated neonatal naive $CD8^+$ T cells are functionally responsive to direct stimulation by TLR2 or TLR5 agonists. Flagellin and Pam_3Cys functioned directly to enhance cellular activation, clonal expansion, and cell effector function beyond that which was achieved by normal cellular activation. They suggest that the combined and sustained dual stimulation of this cell type may represent an attractive new avenue in adjuvant design for future neonatal vaccination strategies requiring a $CD8^+$ component (33). It is also reported that TLR3 agonists might also directly influence some $CD8^+$ T cell effector functions. The increased IFN-γ production provided by TLR3 signaling in $CD8^+$ effector T cells which could be beneficial in therapeutic vaccines, and may lead to better responses against tumors or chronic viral infections (32). Application of bacteria and their product such as LPS and Lipid A is not new concept in immunology and was practiced before. Traditionally, activation of TLRs in APCs would lead to the production of IFN-α, pro-inflammatory cytokines such as TNF-α, IL-1 and IL-6, and the cytokines IL-12 and IL-18 that instruct Th1 to differentiate, whereas an increased Th2 response was observed in MyD88 deficient mice with impaired TLR signaling (36-37).

Moreover, it has been demonstrated that the dose of antigen plays an important role in directing Th1/Th2 differentiation driving by DCs. A lower concentration of ovalbumin (OVA) peptide (1 and 10 ng/mL) induce Th2 commitment while higher concentrations (1 μg/mL and 100 ng/mL) failed to elicit Th2 development. Stimulation of $CD4^+$ T cells with DCs along with TLR2 or TLR9 agonists in the presence of the 10 ng/mL of OVA peptide, the optimal antigen concentration for Th2 development resulted in suppression of IL-4 production and Th2 development. This suggests that TLR-activated DCs can block Th2 lineage commitment independent of antigen dosage (39). A lower dose of LPS (0.1 μg), through TLR4 signaling, induced a Th2 response to inhaled antigens in a murine allergic sensitization model.

In contrast, high doses of LPS (100 μg) with antigen resulted in a Th1 response (40). However, repeated administration of TLR2 ligand Pam_3CSK_4 or TLR4 ligand LPS leads to tolerance of TLR2 or TLR4 (41-42) with reduced cytokine release and expression of IRAK-1 and IRAK-4 proteins (41). Additionally, activation of TLR4 resulted in a MyD88-dependent Th17 response in memory $CD4^+$ T cells in the absence of TRIF molecule (38).

RESULTS

TLR and $CD4^+CD25^+$ Treg cells

There is considerable interest in the functional role of regulatory T (Treg) cells, which subsume the role of the much-maligned suppressor T cells. There are currently at least three major types of Treg cells: Th3, Tr1 and $CD4^+CD25^+$ T cells with overlapping functions (43-45). $CD4^+CD25^+$ T cells are arguably the best characterized so far and have been implicated in the prevention of a range of inflammation in infectious and autoimmune diseases (46). $CD4^+CD25^+$ Treg cells are found in mice and

human and represent 5-10% of peripheral blood $CD4^+$ T cells and are regarded as memory T cells. These Treg cells originate from the thymus through intermediate-affinity selection and are hypo-responsive to allogenic or polyclonal activation *in vitro*. However, they suppress the proliferation of conventional $CD25^-$ T cells in co-culture in a cell-contact dependent and antigen nonspecific manner.

The exact mechanism by which $CD4^+CD25^+$ Treg cells exert their suppressive effect is unclear but may involve the inhibition of IL-2 transcription in the responder cell populations. The suppressive function is critically dependent on the presence of Foxp3 (47-48). $Foxp3^{-/-}$ mice failed to produce $CD4^+CD25^+$ Treg cells and developed spontaneous autoimmune diseases. We reported that $CD4^+CD25^+$ Treg cell suppress the differentiation and function of Th1 and Th2 cells, *Leishmania major* infection and colitis in mice (49). We also have found that $CD4^+CD25^+$ Treg cells can be directly activated by BLP (but not LPS) and that this may be correlated with the expression of Foxp3.

Furthermore, we found that BLP, together with anti-CD3 antibody, could activate Treg cells but reversibly abolish the suppressive activity of these cells. This series of study demonstrate that TLR2 provides a strong positive signal for the amplification of T cells response (16, 50).

On the other hand, engagement of TLR2 resulted in human $CD8^+CD25^+Foxp3^+$ Treg cells expansion that directly suppressed $CD4^+$ T-cells proliferation by cell-contact inhibition and triggered $CD4^+CD45RO^+$ memory T-cell apoptosis inhibiting allergen induced Th2 immune responses (51). Treg cells are able to regain their suppressive property in the presence of IL-2 once the TLR2 ligand is removed (16, 52). Although TLR2-stimulated Treg cells readily lost their ability to suppress proliferation of effector T cells, cytokine production by effector T cells was still repressed.

This suggests that the activity of Treg cells was cytokines independent (53). Treg and Th17 cells are considered divergent and mutually inhibitory. It has been reported that when naive $CD4^+$ T cells were stimulated with TLR2 agonists, Th17 differentiation in vitro and Th17 cytokine production occurred (54). Thus, the reduced suppressive function of Treg cells induced by TLR2 stimulation may be a result of imbalanced phenotype and function between Treg and Th17 (55).

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