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Review Article

ROLE OF TOLL LIKE RECEPTOR AND PATHWAYS INVOLVED IN MORPHINE WITHDRAWAL SYNDROME

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ABSTRACT

Opioids are powerful pain relievers, use of opioids for the treatment of pain has been aasociated with the potential disadvantages including development of tolerance, dependence. Identification of glial-mediated mechanism inducing opioid side effects include cytokine receptor, κ-opioid receptor, NMDA receptor and the recently elucidated Toll like receptor (TLR). TLRs are transmembrane proteins expressed by cells of the innate immune system, which recognize invading microbes and activate signaling pathways that launch immune and inflammatory responses to destroy the invaders. Toll like receptor is the generation of inflammatory cytokines such as TNF-α, IL-6 which are excellent targets for inflammatory diseases and also cause opioid withdrawal syndrome. Glial activation through TLR leading to the release of proinflammatory cytokines acting on neurons wich is important in the complex syndrome of opioid dependence and withdrawal. Moreover TLR stimulating drugs like imiquimod, CpGODN and antibodies which inhibit TLR are acrolein ,IRS954 used as therapeutic targets. Hence, suppression of glial cells proinlammatory cytokines through the toll like receptor can significantly reduce morphine withdrawal syndrome.

KEYWORDS: TLRS, opioid receptors, Second messengers, applications of TLR

INTRODUCTION:

Opiate addiction; a significant and social problem is complicated by the phenomena of tolerance and dependence[1]. It is kind of chronic relapsing brain disease characterized by the loss of control over intake[2,3,4]. Opioid drugs like morphine facilitate dopamine (DA) transmission. Various receptors are involved on opioid withdrawal like mu, kappa, delta, glutamate and toll like receptors. Toll-like receptors (TLRs) are well known as recognition of pathogens in the innate immune system aimed as defending the survival of the host[5,6]. TLRs and their functions have been established in immune cells. There are various types of TLR in which some are play important role in morphine withdrawal.Recent studies reveal that TLRs, including TLR2 and TLR4, are a key link between the innate immune system and the central nervous system (CNS)[7,8]. Microglial cells represent the resident

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immune host defense and are considered the major immune inflammatory cells of the CNS. TLR2 mediated signaling contributes to the impact on the CNS autoimmune diseases and inflammation[9]. TLR2 is required for morphine-induced microglia activation and inflammatory responses.

Opioid induced proinflammatory glial activation has been inferred from : (a) morphine inducesd upregulation of micogilal [10,11] and astrocytes [11,12].(b) morphine induced upregulation and/ or released proinflammatory cytokines [11,13,14, 15, 16, 17,18]. (c) enhanced morphine analgesia by co-administering the microglial attenuators minocycline [10,13,14,15] or AV411[11] and the astrocytes inhibitor fluorocitrate[12] (d) enhanced morphine analgesia by blocking proinflammatory cytokine action [13,14,19]. (e) opioid induced selective activation of microglial p38 MAPK and associated enhanced morphine analgesia [10]. As such, opioid induced proinlammatory glial activaiton through TLR is characterized by a cellular phenotype of enhanced reactivity and propensity to proinflammation in response to exposure of glial to opioids[13,14,15].

There are so many drugs which stimulate the toll like receptor like IPH-3201, TLR4/8 modulator to treat cancer, autoimmune and infectious diseases[20].

Monophosphoryl lipid A, Pam 3CSK4 and MALP, used as a adjuvant[21]. Imiquimod , topically active TLR7 agonist used for cancer treatment[22]. Antibodies which inhibit toll like receptor are acrolein used in sepsis , diabetes, rheumatoid arthritis and CVS diseases[23]. Eisai's eritoran tetrasodium – TLR4 antagonist used in sepsis and septic shock. It also inhibit TNF-α products .

RECEPTORS INVOLVED IN OPIOID WITHDRAWAL SYNDROME:

Opioid receptors are a group of G proteins coupled receptors with opioids as ligands. Opiate receptors are distributed widely in the brain and are found in spinal cord and digestive tract[24.25.26].

The four major subgroups of opiate receptors are delta(δ), kappa(κ), mu(μ) and nociception and each is involved in controlling different function in the brain[27]. For example: opiates and endorphins block pain signal by binding to the μ receptor site. The δ receptor in the brain is involved in pain relief, antidepressant effects and physical dependence. The κ receptor in the brain and spinal cord are linked with sedation , spinal analgesia and pupil constriction. The function of the μ receptor in the brain and spinal cord are physical dependence, respiratory depression, euphoria, pupil constriction and supraspinal analgesia. Nociception receptors in the brain and spinal cord are involved with appetite, depression, anxiety and the development of tolerance to μ agonists.

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Receptors	Subtypes	Location[28,29]	Function[28,29]
Delta (δ) DOP OP1	Δ1 δ_2	Brain Pontine nuclei Amygdale Olfactory bulbs Deep cortex Peripheral sensory neurons	Analgesia Antidepressant effects Physical dependence
Kappa (κ) KOP OP2	κ1 κ2 κ3	Brain Hypothalamus Periqueductal gray Claustrum Substantia gelatinosa Peripheral sensory neurons	Analgesia Sedation Miosis Inhibition of ADH release Dysphoria
Mu (μ) MOP OP3	μ1 μ2 μ3	brain cortex thalamus striosomes periqueductal gray intestinal tract	μ1:- analgesia physical dependence μ2:- respiratory depression miosis euphoria reduced GI motility physical dependence μ3:- possible vasodilation

INVOVEMENT OF GLUTAMATE RECEPTORS IN OPIOID WITHDRAWAL SYNDROME:-

Types of glutamate receptors: Glutamate is one of the most abundant excitatory neurotransmitters in the central nervous system. Once released into the synaptic cleft, glutamate can bind to its receptors and exert its effect. According to pharmacological and molecular biological classification, glutamate receptors can be divided into two categories, ionotropic glutamate receptors (iGluRs) and metabotropic glutamate receptors (mGluRs).

The role of glutamate and its receptor in opioid addiction

It is known that certain glutamatergic projection could be impacted by addictive drugs. Data indicate that the activation of glutamatergic efferent fibers from the amygdala and prefrontal

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cortex is critical in the expression of addictive behaviors [30,31,32,33,34,35]. The alteration of glutamate-mediated transmission, especially the increase of glutamatergic transmission in the NAc may promote the seeking and relapse from abused drugs [36,37,38,39]. Microinjection of glutamate into the VTA can increase exploratory motor behaviors and the release of DA in NAc and mPFC. These results suggest that glutamate plays an important role in opioids dependence.

The role of iGluRs in opioid addiction[40]. NMDA receptors in rat brain following long-term treatment with morphine. Then it was shown that the expression of NMDA receptors was upregulated in morphine-dependent rat brains[41]. Recently, more studies showed that glutamatergic signal transduction can regulate drug effects, resulting in drug tolerance and dependence, including the tolerance and dependence of opioids. Glutamatergic afferents play a key role in regulating the firing of the VTA neurons. Activation of glutamatergic afferents and the VTA infusion of glutamate receptor agonists increase the firing rates of dopaminergic neurons and induce burst firing in vivo[42,43,44]. It was also demonstrated that MK801, an NMDA receptor antagonist, completely blocked the withdrawal symptoms induced by glutamate and naloxone[45,46]. MK-801 can also decrease morphine dependence, which may be related to the downregulation of NMDA receptors[40,41]. Western blot and microdialysis results showed that chronic intermittent use of morphine, cocaine and other addictive drugs can increase the level of glutamate in the VTA, upregulating the expression of AMPA receptor subtypes GluR1 and NMDAR1 in the VTA[47,48]. Co-application of opioids and NMDA receptor competitive or non-competitive antagonists can block the pain tolerance and physical dependence of opioid and the drug-seeking behaviors[49]. Glutamatergic signal transduction mediated by NMDA receptors was involved in the formation and maintenance of morphine dependence in human[50]. So that glutamate released in the central nervous system plays an important role in opioid withdrawal behaviors and that the iGluRs are involved in the process.

The role of mGluRs in opioid addiction

Metabotropic glutamate receptors, which mediate slow glutamate neurotransmission, are located throughout the limbic and cortical brain regions implicated in drug addiction. There is significant pharmacological and behavioral evidence that group I mGluRs are widely distributed in the projection neurons and intermediate neurons in the shell and core of NAc, providing the morphological evidence for their regulation and therapic effects in reward-related behaviors and drug addiction[51,52]. 3-[(2- methyl-1,3-thiazol-4-yl)ethynyl] pyridine (MTEP), an mGluR5 antagonist, dose-dependently inhibited morphine withdrawal symptoms induced by naloxone[53]. DA and glutamate play critical roles in the induction of LTP in the NAc through the activation of D1 dopamine receptors and group I mGluRs[54]. It was reported that the number and function of group II mGluRs upregulated the formation of opioids withdrawal. Group II mGluRs (mGluR2 and mGluR3) were involved in the negative regulation in the brain reward circuit and the formation of conditional offensive responses in drug dependence and withdrawal. However,

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the role of group III mGluRs in drug dependence is still not well investigated. Further investigation is required to fully understand the role of mGluRs in the pathological process of drug addiction.

PATHWAYS INVOLVED IN OPIOID DEPENDENCE:-

DA-Dependent mechanism of opiate reinforcement

Both the positive (rewarding) and negative (aversive) reinforcement of opiate μ and κ receptor agonist are mediated by the mesolimbic DA (system)[55,56,57]. Electrophysiological studies have demonstrated that systemic or iontrophoretic administration of morphine excites DA cells in the VTA and the substantia nigra[58,59].U50,488H(κ agonist) inhibits DA cells[60]. Microdialysis studies have demonstrated that intra cerebroventricular or VTA microinjection of μ agonists cause a significantly increase in extracellular NAcc DA[61,62,63,64,65,66] while κ agonist significantly decrease extracellular NAcc DA[62,63,65]. This push-pull or reciprocal modulation of the mesolimbic DA system by μ and κ receptor may, in part , underlie the neurochemical mechanism of opiate reinforcement.

SECOND MESSENGERS / EFFECTORS

Activation of any of the three opioid receptor subtypes produces common cellular actions. Each receptor is coupled to pertussis toxin-sensitive G proteins, although some coupling to the pertussis toxin-insensitive G protein Gz has also been recognized[73]. The profile of coupling of the three opioid receptors to the spectrum of G proteins is similar[73].The most commonly reported actions include inhibition of adenylyl cyclase, activation of a potassium conductance, inhibition of calcium conductance, and an inhibition of transmitter release (Fig 1. given below).The actions of opioids to include the activation of protein kinase C (PKC), the release of calcium from extracellular stores, and the activation of the mitogen-activated protein kinase (MAPK) cascade, plays an important role in receptor function.

1. Inhibition of adenylyl cyclase

Until recently, nothing was known of the physiological consequences of the acute inhibition of adenylyl cyclase by opioids. Two effects have now been identified one is mediated by the modulation of a voltage-dependent current (*I*h), which is also termed the pacemaker current [74,75]. This cation nonselective current is activated at hyperpolarized potentials to cause an inward current that depolarizes the membrane potential toward threshold. The voltage dependence of this current is regulated by cAMP, being activated at less negative potentials when cAMP levels are elevated[74]. Opioids shift the voltage dependence to more negative potentials by decreasing intracellular cAMP. This inhibition was most easily observed after the activation of adenylyl cyclase with forskolin or PGE2[74] but has also been observed without prior activation[75]. The consequence of this action of opioids was to decrease the amplitude of the inward current that drives spontaneous activity and thus decreases excitability. This action of opioids could have been predicted based on work done on the pacemaker current in sino atrial nodal cells of the heart[69] where the activation of M2 muscarinic receptors shifted the voltage dependence of *I*h through an inhibition of adenylyl cyclase[76].

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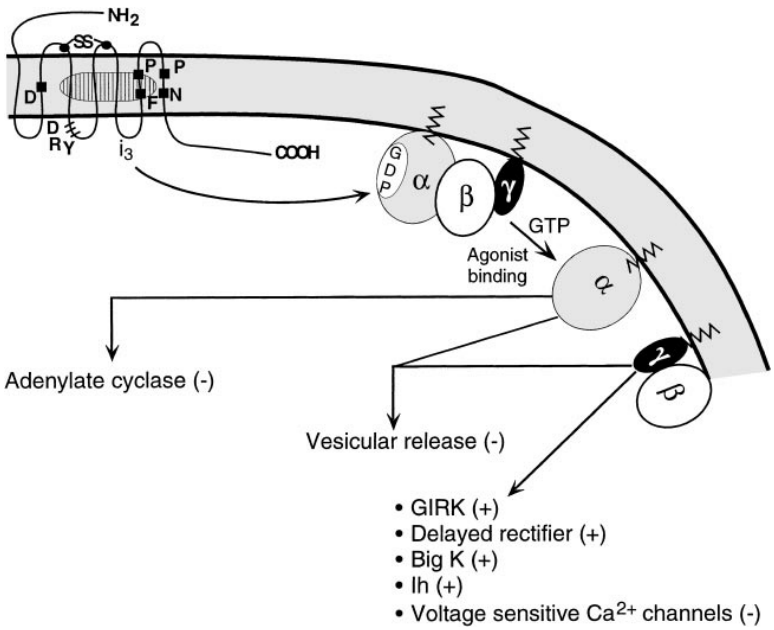

Figure 1. An illustration of the best-characterized pathway of effector activation of opioids. Three primary classes of effectors include the inhibition of adenylyl cyclase, inhibition of vesicular release, and interactions with a number of ion channels. These effectors are affected by both the GTP-bound form of the a-subunit as well as free b/g-subunits of pertussis toxin-sensitive G proteins. GIRK, G protein inwardly rectifying conductance[72].

2. Activation of potassium conductance

Opioids activate at least three separate potassium conductances. The most commonly observed is the G protein-activated inwardly rectifying conductance. All three opioid receptors have been shown to activate this conductance. The second messenger pathway is membrane delimited, mediated by a pertussis toxin-sensitive G protein[77], and it is presumed that the potassium conductance is activated by the b/g-subunits[78]. Rapid application and washout of opioids allowed the determination of the kinetics of opioid action using acutely dissociated cells[79]. The activation of the potassium conductance had a latency to onset of 50–100 ms and a time constant of activation of ;700 ms, which is similar to that observed for other receptors coupled to GIRK channels[79,80,81].The coupling of receptor to this potassium conductance was quite dependent on the experimental conditions. Opioids have been reported to activate the BK calcium sensitive potassium conductance[82]. This effect, coupled with recent reports of opioid-induced calcium release from internal stores[83,84,85,86,87], indicates the diversity of opioid action, which has been

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recognized to occur with other G protein-coupled receptors.

3. Inhibition of calcium conductance

There are many examples of the inhibition of calcium currents by activation of all opioid receptor subtypes. The inhibition of high-threshold calcium currents by opioids, in common with other receptors linked to pertussis toxin-sensitive G proteins, 1) is membrane delimited, 2) is mediated by the b/g-subunits of G proteins, 3) decreased the rate of current activation such that the inhibition was greater immediately after the voltage step, and 4) showed relief of inhibition following a depolarization to positive potentials [88].

4. Inhibition of transmitter release

The opioid inhibition of acetylcholine release in the guinea pig ileum and ATP release in the vas deferens has been used as pharmacological assays for many decades[89,90,91]. In various peripheral preparations from different species, the activation of all three receptor subtypes has been found to cause inhibition of transmitter release[92,93,94]. The activation of potassium conductance and/or the inhibition of calcium conductance and not the inhibition of adenylyl cyclase have been argued to account for this action[95,96,97]

5. Activation of protein kinase C

A long-term, selective augmentation of *N*-methyl-Daspartate (NMDA)-mediated glutamate currents by activation of m-opioid receptors was observed in brain slices of trigeminal nucleus (80). This augmentation was mimicked by phorbol esters and blocked by the peptide inhibitor of protein kinase C (PKC). It was concluded that opioids activated PKC, which then increased the conductance activated by NMDA receptor agonists. This was the first and remains the strongest evidence that opioids augment postsynaptic glutamate currents by a mechanism involving the activation of PKC. There have, however, been more recent studies showing augmented NMDA receptor-mediated excitatory postsynaptic currents (EPSCs) by m-opioid agonists in both the nucleus accumbens and hippocampus[98,99,100,101]. This augmentation was not observed in the locus coeruleus (LC)[102], suggesting that it may be dependent on the makeup of NMDA receptor subunits and/or the isoforms of PKC present on any given cell type. The activation of PKC by opioids appears to result from the activation of phospholipase C and/or phospholipase A2, which is thought to result from an interaction of b/g-subunits of pertussis toxin-sensitive G protein and may require coactivation with the a-subunits of pertussis toxin-insensitive G proteins[103,104,105,106].

6. Release of calcium from internal stores

Initial studies demonstrating a transient increase in intracellular calcium in NG108–15 cells were unexpected (84). Most of the rise in calcium found in both NG108–15 and ND8–47 cells was blocked with dihydropyridine calcium channel blockers or removal of extracellular calcium, suggesting that calcium entry across the plasma membrane was the primary source[84,86].

TOLL LOKE RECEPTORS:

TLRs, a family of evolutionarily conserved pathogen recognition receptors, play a pivotal role in innate immunity. TLR family consists of 13 mammalian members. The cytoplasmic portions of TLRs show high similarity to that of the interleukin-1 receptor (IL-1R) family and are now called the Toll/IL-1

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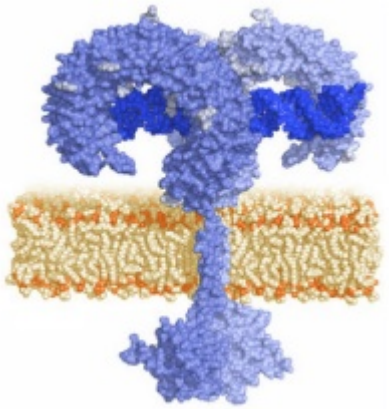
receptor (TIR) domain. A TIR domain is required to initiate intracellular signaling. The extracellular regions of TLRs and IL-1R are markedly different. Whereas IL-1R possesses an Ig-like domain, TLRs contain leucine-rich repeats in their extracellular domains. TLRs are pattern recognition receptors that sense a wide range of microorganisms, such as bacteria, fungi, protozoa, and viruses. Each TLR has its own intrinsic signaling pathway and induces specific biological responses against microorganisms such as dendritic cell maturation, cytokine production, and the development of adaptive immunity[107].

STRUCTURE OF TOLL LIKE RECEPTOR

TLRs are type I transmembrane proteins characterized by an extracellular domain containing leucine-rich repeats (LRRs) and a cytoplasmic tail that contains a conserved region called the



Toll/IL-1 receptor (TIR) domain. The structure of the extracellular domain of TLR3 was revealed by crystallography studies as a large horseshoe-shape[108]. TLRs are predominantly expressed in tissues involved in immune function, such as spleen and peripheral blood leukocytes, as well as those exposed to the external environment such as lung and GIT. Their expression profiles vary among tissues and cell types. TLRs are located on the plasma membrane with the exception of TLR3, TLR7, TLR9 which are localized in the endosomal compartment [109].Ten human and twelve murine TLRs have been characterized, TLR1 to TLR10 in humans,
and TLR1 toTLR9, TLR11, TLR12 and TLR13 in mice, the homolog of TLR10 being a pseudogene. TLR2 is essential for the recognition of a variety of PAMPs from Gram-positive bacteria, including bacterial lipoproteins, lipomannans and lipoteichoic acids. TLR3 is implicated in virus derived double stranded RNA. TLR4 is predominately activated by lipopolysaccharide. TLR5 detects bacterial flagellin and TLR9 is required for response to unmethylated CpG DNA. Finally, TLR7 andTLR8 recognize small synthetic antiviral molecules, and single-stranded RNA was reported to be their natural ligand[110]. TLR11[111] has been reported to recognize uropathogenic *E.coli*[112] and a profilin-like protein from *Toxoplasma gondii*[113]. The repertoire of specificities of the TLRs is apparently extended by the ability of TLRs to heterodimerize with one another. For example, dimers of TLR2 and TLR6 are required for responses to diacylated lipoproteins while TLR2 and TLR1 interact to recognize triacylated lipoproteins[114]. Specificities of the TLRs are also influenced by various adapter and accessory molecules, such as MD-2 and CD14 that form a complex with TLR4 in response to LP[115].

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TYPES OF TOLL LIKE RECEPTOR

TLR1, TLR2 and TLR6

TLR2 recognizes a variety of microbial components. These include lipoproteins/lipopeptides from various pathogens, peptidoglycan and lipoteichoic acid from Gram-positive bacteria, lipoarabinomannan from mycobacteria, glycosylphosphatidylinositol anchors from Trypanosoma cruzi, a phenol-soluble modulin from Staphylococcus epidermis, zymosan from fungi and glycolipids from Treponema maltophilum[116]. There are two aspects proposed for mechanisms that could explain why TLR2 recognizes a wide spectrum of microbial components. The first explanation is that TLR2 forms heterophilic dimers with other TLRs such as TLR1 and TLR6, both of which are structurally related to TLR2. Macrophages from TLR6-deficient mice did not show any production of inflammatory cytokines in response to mycoplasma-derived diacyl lipopeptides. However, these cells showed normal production of inflammatory cytokines in response to triacyl lipopeptides derived from Gram-negative bacteria[117]. In contrast, macrophages from TLR1-deficient mice showed a normal response to mycoplasma-derived diacyl lipopeptides, but an impaired response to triacyl lipopeptides[118]. Thus, TLR1 and TLR6 functionally associate with TLR2 and discriminate between diacyl or triacyl lipopeptides. Moreover, the involvement of TLR1 in the recognition of the outer surface lipoprotein of Borrelia burgdorferi has also been shown[119]. The second explanation involves recognition of fungal-derived components by TLR2[120]. In this model, TLR2 has been shown to functionally collaborate with distinct types of receptors such as dectin-1, a lectin family receptor for the fungal cell wall component b-glucan. Thus, TLR2 recognizes a wide range of microbial products through functional cooperation with several proteins that are either structurally related or unrelated.

TLR3

Expression of human TLR3 in the double-stranded RNA (dsRNA)-non-responsive cell line 293 confers enhanced activation of NF-κB in response to dsRNA. In addition, TLR3-deficient mice are impaired in their response to dsRNA[121]. dsRNA is produced by most viruses during their replication and induces the synthesis of type I interferons (IFN-a/b), which exert anti-viral and immunostimulatory activities. Thus, TLR3 is implicated in the recognition of dsRNA and viruses. However, TLR3-independent mechanisms of dsRNA recognition exist.

TLR4

TLR4 is an essential receptor for LPS recognition[122,123]. In addition, TLR4 is implicated in the recognition of taxol, a diterpene purified from the bark of the western yew (Taxus brevifolia)[124,125]. TLR4 involved in the recognition of endogenous ligands, such as heat shock proteins (HSP60 and HSP70), the extra domain A of fibronectins, oligosaccharides of hyaluronic acid, heparan sulfate and fibrinogen. However, all of these endogenous ligands require very high concentrations to activate TLR4. In addition, it has been shown that contamination of LPS in the HSP70 preparation confers ability to activate TLR4[126]. LPS is a very potent immuno-activator, and accordingly, TLR4 can be activated by a very small

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amount of LPS, contaminating these endogenous ligand preparations.

TLR5

Expression of human TLR5 in CHO cells confers response to flagellin, a monomeric constituent of bacterial flagella[127]. TLR5 has further been shown to recognize an evolutionarily conserved domain of flagellin through close physical interaction between TLR5 and flagellin[128]. TLR5 is expressed on the basolateral, but not the apical side of intestinal epithelial cells[129]. TLR5 expression is also observed in the intestinal endothelial cells of the subepithelial compartment[130]. In addition, flagellin activates lung epithelial cells to induce inflammatory cytokine production[131]. These findings indicate the important role of TLR5 in microbial recognition at the mucosal surface. A common stop codon polymorphism in the ligand-binding domain of TLR5 has been shown to be associated with susceptibility to pneumonia caused by the flagellated bacterium Legionella pneumophila[131].

TLR7 and TLR8

TLR7 and TLR8 are structurally highly conserved proteins, and recognize the same ligand in some cases. Analysis of TLR7- deficient mice revealed that murine TLR7 recognizes synthetic compounds, imidazoquinolines, which are clinically used for treatment of genital warts associated with viral infection[132]. Human TLR7 and TLR8, but not murine TLR8, recognizes imidazoquinoline compounds[133]. Murine TLR7 has also been shown to recognize another synthetic compound, loxoribine, which has anti-viral and anti-tumor activities[134,135]. Therefore, TLR7 and human TLR8 were predicted to recognize a nucleic acid-like structure of the virus. This prediction has recently been shown to be true from the finding that TLR7 and human TLR8 recognize guanosineor uridine-rich single-stranded RNA (ssRNA) from viruses such as human immunodeficiency virus, vesicular stomatitis virus and influenza virus[136,137,138]. ssRNA is abundant in the host, but usually the host-derived ssRNA is not detected by TLR7 or TLR8. This might be due to the fact that TLR7 and TLR8 are expressed in the endosome, and host-derived ssRNA is not delivered to the endosome.

TLR9

Analysis of TLR9-deficient mice revealed that TLR9 is a receptor for CpG DNA[139]. There are at least two types of CpG DNA, termed A/D-type CpG DNA and B/K-type CpG DNA. B/K-type CpG DNA is conventional, which was identified first, and is a potent inducer of inflammatory cytokines such as IL-12 and TNF-a. A/D-type. CpG DNA is structurally different from conventional CpG DNA and has a greater ability to induce IFN-a production from plasmacytoid dendritic cells (PDC), but less ability to induce IL-12[140,141]. TLR9 has been shown to be essential for the recognition of both types of CpG DNA[142]. TLR9 recognition of A/Dtype CpG DNA leads to induction of an anti-viral cytokine IFN-a in PDC indicates that TLR9 is involved in viral recognition. Indeed, in addition to bacterial CpG DNA. TLR9 appears to be involved in the pathogenesis of several autoimmune diseases through recognition of the chromatin structure. Chloroquine is clinically used for treatment of rheumatoid arthritis and SLE, but its mechanisms are unknown. Since chloroquine also blocks TLR9-dependent signaling through inhibition

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of the pH-dependent maturation of endosomes by acting as a basic substance to neutralize acidification in the vesicle[143], it may act as an anti-inflammatory agent by inhibiting TLR9-dependent immune responses.

TLR11

TLR11 expressed in bladder epithelial cells and mediate resistance to infection by uropathogenic bacteria in mouse[144]. TLR11- deficient mice are highly susceptible to uropathogenic bacterial infection. TLR11 mediates antiuropathogenic bacterial response[144].

TLR SIGNALLING MEDIATORS GLIAL CELLS ACTIVATION IN THE CNS : Innate immunity in the cns depends primarily on the function of glial cells , especially in microglia which are important for the activation of adaptive system[145].

TLR in microglia:- TLR mediated signalling promotes the production of a variety of inflammatory mediators [146,147]. Exogenous and Endogenous TLR ligands activate microglial cells. TLR may mediate different pathway in microglial leading to either neuroprotective or neurotoxic phenotype [148]. However , activated microglia with TLR ligands also produce neurotoxic molecules such as nitric oxide (NO), reactive oxygen species (ROS), peroxynitriate, proinflammatory cytokines (TNF-α, IL-1β) which leads to withdrawal syndrome[149].

TLR appear to activate very similar signaling pathways to IL-1 and some researchers now refer to this pathway as the TLR-IL signaling pathway[150]. That is, TLRs work through activation of an adaptor protein known as myeloid differentiation factor 88 (MyD88).This factor leads to activation of the IL-1 receptor- associated kinases (IRAKs) and TNF receptor – associated factor-6 (TRAF6), which finally culminates in activation of NF-Kb[151]. Other TLR- associated pathways include the JNK and interferon (IFN) pathways. Both TLR2 and TLR4 are important in recognizing endogenous pain-mediating signals. These studies have shown a highly interconnected web of pathway involving TLRs and other well–defined proinflammatory pathways which are associated with glial activation and opioid side effects[152,153].

ROLE OF TLR IN MORPHINE WITHDRAWAL :- Functional studies have implicated a role of TLR2 in the morphine withdrawal syndrome. TLR are well knows as recognition of pathogens in the innate immune system[153,154]. Microglial cells represent the resident host defence and are considered the major immune inflammatory cells of CNS. TLR2 mediated signaling contributes to the impact on the CNS autoimmune diseases and inflammation. In present study , it is revealed that TLR2 is required for morphine – induced microglia activation and inflammation responses.

TOLL LIKE RECEPTOR AND PROINFLAMMATORY CYTOKINES:- TLRs are group of pattern recognition receptors found on astrocytes and mainly micoglial that can be activated by exogenous (pathogenic proteins) and endogenous (IL-1β, TNF-α) molecules; when activated , they produce an immune response resulting in the release of of cytokines. TLR activation has been positively linked to the development of opioid tolerance and other side effects, decreased opioid efficacy and the development and maintenance of neuropathic pain[155].

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TOLL LIKE RECEPTOR AND OPIOID TOLERANCE AND DEPENDENCE:
Researchers have postulated that an independent mechanism is responsible for tolerance, physical dependence , reward and respiratory depression than those effects mediated by classical opioid receptors such as μ, κ, and δ receptors. In a recent study , the induction of opioid-induced hyperalgesia in triple receptor knockout mice suggests that opioids also act through different mechanism separate from the classic opioid receptors on neurons. It has been shown that opioids can bind to TLR-4 receptors on glial cells leading to their activation and synthesis of nociceptive cytokines which counteracting the opioid analgesic effects[156].

EXPRESSION OF TLRs IN CNS GLIAL CELLS

Microglia are CNS tissue resident macrophages and act as immune sentinels of the brain. In accordance with this view, primary microglia in vitro constitutively express a wide complement of TLRs (TLRs1-9) at varying levels[157,158]. In comparison, primary astrocytes also express a wide variety of TLRs, but at lower levels. Murine astrocytes express TLRs1-9, with particularly high levels of TLR3[159,160], suggesting that astrocytes may be particularly important for anti-viral responses in the CNS. To date, human astrocytes have been reported to express TLRs1-5 and TLR9, also with particularly high expression of TLR3[157,161,162].The lack of TLR6-8 may be a difference between species or the result of varying isolation and culture conditions. There is also evidence that both oligodendrocytes and neurons can express TLRs, but their role in innate immune responses during CNS[157,163,164. Under resting conditions in vivo. Constitutive expression of TLRs is primarily in microglia and largely restricted to the circumventricular organs (CVOs) and meninges, areas with direct access to the circulation, although they may be expressed at lower levels in other regions as well[165,166,167]. The levels of TLRs in the CNS can be upregulated by viral and bacterial infection, treatment with TLR stimuli, or CNS autoimmunity[157,160,167,168], providing a mechanism for amplification of inflammatory responses to pathogens infecting the CNS. These stimuli upregulate multiple TLRs in a coordinated fashion, not only the TLR involved in recognition of a particular pathogen or class of pathogens. For example, treatment of astrocytes with the dsRNA synthetic mimic polyinosinic-polycytidylic acid (poly I:C), a viral stimulus, upregulates its own receptor TLR3, and also upregulates TLR2 and TLR4, which are normally used to recognize bacterial product[159]. Similarly, infection of mice with rabies broadly increases CNS expression of TLRs1-4 and 6–9[160].The pattern of TLR upregulation is not fixed and varies with the particular pathogen encountered, even with pathogens of a similar class. For example, in contrast to rabies virus, Semliki forest virus infection in the CNS fails to upregulate TLR4 and TLR6, but does increase expression of TLR13[160]. The upregulation of TLRs in the CNS is likely in part due to the infiltration of TLR-expressing inflammatory cells, and in part due to the upregulation of receptor expression on astrocytes and microglia, which occurs in response to a variety of inflammatory stimuli[158,159].

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THERAPEUTIC APPLICATIONS OF TOLL LIKE RECEPTOR:

Drugs stimulating toll like receptors

IPH-3201. There are currently approximately twenty drugs in pre-clinical development, with a further dozen or so in clinical trials[157]. IPH-3201, a series of TLR7/8 modulators to treat cancer, autoimmune and infectious diseases. Also IPH-3102, a double-stranded RNA and natural ligand of TLR3. Activation of the TLR3 pathway leads to the activation of NF-kB and the production of type I interferons to elicit antiviral defenses, and it is hoped that this may be an effective method of destroying cancerous cells. TLR3 detects virus invasion and initiates the antiviral immune response via TRIF/IKK signaling in the activation and maturation of dendritic cells (DCs) and monocytes, allowing for the regulated processing and presentation of antigens, the upregulation of major histocompatibility complex, and costimulatory molecules and secretion of pro-inflammatory chemokines and cytokines[158]. These events then mediate the activation of antigen-specific T and B-cell responses.

Monophosphoryl lipid A (MPL) derived from detoxifying Salmonella minnesota lipid A, produced its adjuvant effect through the stimulation of the TRAM/TRIF signal transduction pathway of TLR4 and deactivation of Mal/MyD88 signaling[159], thereby acting as a partial rather than full agonist at the receptor and has been licensed for use as a vaccine adjuvant[160]. It is also

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recognized that the major use for compounds that activate TLR2 are as adjuvant.

Pam3CSK4 and MALP: The synthetic compounds, such as Pam3CSK4 and mycoplasma-derived lipopeptide (MALP)-2, may be developed for adjuvant usage[161,162].TLR5 is the receptor for bacterial flagellin monomers and is the only TLR that recognizes a protein ligand[163].

CBLB502, CBLB502 an engineered flagellin derivative was found to have potent NFα B activation and reduced immunogenic characteristics. A single injection of CBLB502 before lethal total body irradiation protected mice and rhesus monkeys from both gastrointestinal (GI) and hematopoietic acute radiation symptoms and resulted in improved survival and yet, importantly, did not decrease tumor radio sensitivity[164]. These results imply that TLR5 agonists may be valuable as adjuvants for cancer radiotherapy. The activation of TLR5 has also been recently reported to be an efficient adjuvant for influenza A vaccine. A recombinant protein containing a consensus extracellular domain of M2 protein (M2e) sequence linked to the TLR5 ligand provides an effective approach to developing vaccines against wide-spread epidemic and pandemic influenza[165].

Imiquimod: Imiquimod is the first approved topically active TLR7 agonist. It is prescribed for treatment of external virus induced skin lesions, such as the genital and perianal warts resulting from papillomavirus infections[166]. There is also a growing evidence to indicate therapeutic interest in TLR7/TLR8 agonists for cancer treatment. As such, imiquimod is now also used as a treatment for cancer and has shown itself to be efficacious against primary skin tumors and cutaneous metastases[167]. In fact, imiquimod has been approved for the treatment of external genital and perianal warts, but has also been found to be effective for a host of other virus-associated dermatologic lesions, including common and flat warts, molluscum contagiosum and herpes simplex. Oncological lesions showing improvement with the use of imiquimod include basal cell carcinoma, actinic keratosis, squamous cell carcinoma in situ, malignant melanoma, cutaneous T-cell lymphoma, and cutaneous extramammary Paget's disease[168]

Cytosine-phosphate-guanosine oligodinucleotide (CpGODN),Cytosine-phosphate-guanosine oligodinucleotide (CpGODN), the common TLR9 agonist has shown substantial potential as vaccine adjuvants, and as mono- or combination therapies for the treatment of cancer, infectious and allergic diseases[169]. Phase I and II clinical trials have indicated that CpG-ODNs have antitumor activity as single agents and enhance the development of antitumor T-cell responses when used as therapeutic vaccine adjuvants. CpG-ODNs have shown benefit in multiple rodent and primate models of asthma and other allergic diseases, with encouraging results in some early human clinical trials.

Antibodies inhibiting toll like receptors

Antagonists of lipid A have been under clinical development before the discovery of TLRs as treatments for Gram-negative sepsis and endotoxemia[170]. The following analogs or natural molecules E5564 (eritoran), curcumin, auranofin (an antirheumatic gold compound), cinnamaldehyde, and

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acrolein are just a few of the sample candidates currently under investigation.

Acrolein: Acrolein with an alpha, beta-unsaturated carbonyl group inhibits LPS-induced homodimerization of TLR4[171].

Small molecules that inhibit MyD88 binding to TLR4 are also emerging. Cell penetrating peptides fused with the BB loop (a highly conserved sequence in the TIR that is situated between the second -strand and the second helix) sequences of TLR2 and TLR4 also inhibit LPS-induced signaling, probably by interfering with either receptor dimerization or adapter recruitment[172].

Numerous diseases such as sepsis, diabetes, rheumatoid arthritis, and cardiovascular diseases, seem to be associated with both TLR2 and TLR4

Eisai's eritoran tetrasodium:- Another TLR4 antagonist, Eisai's eritoran tetrasodium, has reached Phase III trials for the treatment of sepsis and septic shock. In Phase I trials it proved its ability to dose-dependently inhibit TNF-α production.

IRS954:- TLR7/9 antagonist, IRS954, has shown early signs of efficacy; in a murine model of lupus, it reduced serum levels of nucleic acid-specific antibodies and decreased proteinurea, glomerularnephritis and end-organ damage.

RECENT RESEARCH ON TOLL LIKE RECEPTOR

Legionella lipoprotein activates toll like receptor-2 and induces cytokine production and expression of costimulatory molecules in peritoneal macrophages :- A report 'Legionella lipoprotein activates toll like receptor-2 and induces cytokine production and expression of costimulatory molecules in peritoneal macrophages', is newly published data in experimental and molecular medicine. "Legionella bacterium an intracellular pathogens of mononuclear phagocyte, causes acute fatal pneumonia, especially in patients with impaired cellular immune response. The researchers concluded that these results indicates that legionella PAL might activate macrophages via a TLR-2 dependent mechanism which thus induce cytokine production and expression of costimulatory and MHC molecules[67].

The influence of prolonged cycling on monocyte Toll like receptor 2 and 4 expression in healthy men:- It is reported that some immune cells function including monocyte Toll like receptor(TLR) expression and antigen presentation are temporarily impaired following acute bouts of strenuous exercise, which could represent an 'open window' to upper respiratory tract infection(URTI)[67].

Lipoteichoic acid-induced TNF-α and IL-6 gene expression and oxidative stress production in macrophages are supreessed bt ketamine through down regulating tool like receptor -2 mediated activation of ERK1/2 A NO NF kappa B :- Lipoteichoic acid-induced TNF-α and IL-6 gene expression and oxidative stress production in macrophages are supreessed bt ketamine through down regulating tool like receptor -2 mediated activation of ERK1/2 A NO NF kappa B, new data in gene therapy. According to recent research" lipoteichoic acid (LTA) , a gram positive bacterial outer membrane component , can cause septic shock. Previous studies showed that ketamine has anti-inflammatory and antioxidant effects on gram negative LPS-induced macrophages activation[67].

The researchers concluded that : "this study shows that one possible mechanism involved in

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ketamine –induced inhibition of LTA induced TNF-α and IL-6 gene expression and oxidative stress production is through down regulating TLR-2 mediated phosphorylation of ERK1/2 and the subsequent translocation and transactivation of NF kappa B[67].

CONCLUSION:- Addiction to opioids is a complex syndrome involving tolerance, drug –seeking and physical dependence with withdrawal avoidance behavior. There are number of exciting directions for the use of glial modifying agents as opioid adjuvants for the treatment of acute and chronic withdrawal syndrome. Important targets include cytokine receptors, TLRs, glutamate receptors and κ receptors. TLR play important role in withdrawal syndrome as it activates the glial cells and release the proinflammatory cytokines like IL-1, IL-6 and TNF-α. TLR 2 and TLR 4 are responsible for withdrawal syndrome and rest of the receptors are responsible for bacteria, fungi infections. There are so many drugs such as TLR stimulating and inhibiting drugs used in various diseases.

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