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“Role of Tumor Necrosis Factor Alpha (TNF- α) in Opioid Withdrawal Syndrome”.

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Abstract:

Opioids are powerful pain relievers; use of opioids for the treatment of pain has been associated with the potential disadvantages including development of tolerance, dependence. Drug addiction represents one of the major medical, social and economic burdens of human behavior. Various pathways and cytokines are involved in opioid withdrawal. The cytokine, tumour necrosis factor-alpha (TNF- α) plays a key role in the pathogenesis of many chronic inflammatory diseases and its increased level also caused withdrawal syndrome. Glial cells also play important role in opioid withdrawal. Identification of glial-mediated mechanism inducing opioid side effects includes cytokine receptor, κ -opioid receptor, NMDA receptor and toll like receptor (TLR). Glial activation through TLR leading to the release of proinflammatory cytokines (TNF- α) acting on neurons which is important in the complex syndrome of opioid dependence and withdrawal. Moreover, agents targeting the TNF- α such as AV411, thalidomide and rdp58, all show varied but promising files for providing significant relief from opioid side effects.

Keyword: Proinflammatory cytokines, opioid receptors, TNF- α , glial cell.

Introduction:

Opioid addiction is a complex syndrome which is characterised by tolerance, drug-seeking and physical dependence with withdrawal behaviors. It is kind of chronic relapsing brain disease characterised by the loss of control over intake^[1,2,3]. There are seven stages which are involved in withdrawal syndrome. Various pathways are involved in opiate withdrawal like DA dependent mechanism of opiate reinforcement, inhibition of adenylyl cyclase, activation of potassium conductance etc. Proinflammatory cytokines includes many

cytokines characterized as being inducible and belonging to different families, including IL-1, IL-6, TNF- α ^[4,5,6]. Other critical proinflammatory cytokines are IFN γ , IL-2 both products chiefly of TH1 cells as well as IFN α/β ^[7,8,9]. Newly synthesized pro TNF- α is expressed on plasma membrane^[10]. The biological response to TNF- α are mediated through 2 structurally distinct receptors: type 1 (TNFR1) and type 2 (TNFR 2)^[11,12]. Receptors which are involved in opioid withdrawal are mu, delta and toll like receptor (TLR). There are various TLRs in which some play important role in opioid

withdrawal. Recent studies reveal that TLRs, including TLR2 and TLR4, a key link between the innate immune system and CNS^[13,14]. Glial cells are also important for structural and metabolic maintenance of the nervous system, there are numerous reports demonstrating the ability of glial to respond to and send signals to neurons and synapses in the central and peripheral nervous system (CNS and PNS)^[15].

Opioid induced proinflammatory glial activation has been inferred from :

(a) morphine induced upregulation of microglial^[16,17] and astrocyte^[16,18].

(b) morphine induced upregulation and/ or released proinflammatory cytokines [16,19,20,21,22,23,24].

(c) enhanced morphine analgesia by co-administering the microglial attenuators minocycline^[16,19-24s] or AV411^[17] and the astrocytes inhibitor fluorocitrate^[18]

(d) enhanced morphine analgesia by blocking proinflammatory cytokine action^[19,20,25].

(e) opioid induced selective activation of microglial p38 MAPK and associated enhanced morphine analgesia^[16]. As such, opioid induced proinflammatory glial activation through TLR is characterized by a cellular phenotype of enhanced reactivity and propensity to proinflammation in response to exposure of glial to opioids^[19,20,21].

There are some drugs which are used as anti TNF- α therapy such as AVR11 (ibudilast) which is non-specific phosphodiesterase inhibitor that acts centrally by way of attenuation of glial cell activation and reduction of proinflammatory activating factors such as cytokines (TNF- α , IL-1 β) nitric oxide, and protein-1 and fractalkines^[26,27]. Thalidomide, it is used to treat moderate to severe leprosy and clinical trials are ongoing in cancer. Thalidomide has multiple effects that could account for its activity in myeloma. These include direct inhibition of tumour growth by altering the synthesis of cytokines that are involved in the growth and survival of myeloma cells, including TNF- α , IL-1 β , IL-6, IL-10^[28-30]. RDP58 is a small molecule that inhibits TNF- α synthesis by preventing the translation of the TNF- α mRNA.^[31]

Types of withdrawal syndrome

Two experimental paradigms of opiate withdrawal are used to study opiate dependence: spontaneous and precipitated withdrawal.

- **Spontaneous opiate withdrawal** occurs after the sudden discontinuation or the rapid tapering of chronically administered opiates.
- **Precipitated opiate withdrawal** occurs after the use of an opioid receptor antagonist, which displaces the opioid receptor agonist. Both spontaneous and precipitated withdrawal

syndromes are accompanied by symptoms of abstinence; however, the onset is faster with precipitated opiate withdrawal. Naloxone and naltrexone are the most common competitive μ opioid receptor antagonists used to precipitate opiate withdrawal. The major difference between these two compounds is that naltrexone, which is orally active, has a much longer duration of action than naloxone^[32,33].

Pathways involved in opiate withdrawal syndrome

DA-Dependent mechanism of opiate reinforcement

Both the positive (rewarding) and negative (aversive) reinforcement of opiate μ and κ receptor agonists are mediated by the mesolimbic DA (system)^[34,35,36]. Electrophysiological studies have demonstrated that systemic or iontophoretic administration of morphine excites DA cells in the VTA and the substantia nigra^[37,38]. U50,488H (κ agonist) inhibits DA cells^[39]. Microdialysis studies have demonstrated that intra cerebroventricular or VTA microinjection of μ agonists cause a significant increase in extracellular NAcc DA^[40-45] while κ agonist significantly decrease extracellular NAcc DA^[41,42,44]. 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 G_z has also been recognized. The profile of coupling of the three opioid receptors to the spectrum of G proteins is similar^[46]. 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. 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, play 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^[47,48]. 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. 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^[47] but has also been observed without prior activation^[48]. 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^[49] where the activation of M₂ muscarinic receptors shifted the voltage dependence of *I_h* through an inhibition of adenylyl cyclase^[50].

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^[52], and it is presumed that the potassium conductance is activated by the b/g-subunits^[53]. Rapid application and washout of opioids allowed the determination of the kinetics of opioid action using acutely dissociated cells^[54]. 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^[54,55,56]. 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^[57]. This effect, coupled with recent reports of opioid-induced calcium release from internal stores^[58-62], indicates the diversity of opioid action, which has been 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^[63].

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^{[64-}

^{66]}. In various peripheral preparations from different species, the activation of all three receptor subtypes has been found to cause inhibition of transmitter release^[67-69]. 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.^[70-72]

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. 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^[73-76]. This augmentation was not observed in the locus coeruleus (LC)^[77], 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^[78-81].

6. Release of calcium from internal stores

Initial studies demonstrating a transient increase in intracellular calcium in NG108–15 cells were unexpected^[59]. 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^[59,61].

Stages involved in opioid withdrawal

- Stage I: Six to fourteen hours after last dose: Drug craving, anxiety, irritability, perspiration, and mild to moderate dysphoria.
- Stage II: Fourteen to eighteen hours after last dose: Yawning, heavy perspiration, mild depression lacrimation, crying, running nose, dysphoria, also intensification of the above symptoms. "yen sleep" (a waking trance-like state)
- Stage III: Sixteen to twenty-four hours after last dose: Rhinorrhea (runny nose) and increase in other of the above, dilated

pupils, piloerection (gooseflesh), muscle twitches, hot flashes, cold flashes, aching bones and muscles, loss of appetite and the beginning of intestinal cramping.

- Stage IV: Twenty-four to thirty-six hours after last dose: Increase in all of the above including severe cramping and involuntary leg movements ("kicking the habit"), loose stool, insomnia, elevation of blood pressure, moderate elevation in body temperature, increase in frequency of breathing and tidal volume, tachycardia (elevated pulse), restlessness, nausea
- Stage V: Thirty-six to seventy-two hours after last dose: Increase in the above, fetal position, vomiting, free and frequent liquid diarrhea, which sometimes can accelerate the time of passage of food from mouth to out of system to an hour or less, involuntary ejaculation, which is often painful, saturation of bedding materials with bodily fluids, weight loss of two to five kilos per 24 hours, increased white cell count and other blood changes.
- Stage VI: After completion of above: Recovery of appetite ("the chucks"), and normal bowel function, beginning of transition to post-acute and chronic symptoms that are mainly psychological but that may also include increased sensitivity to pain, hypertension, colitis or

other gastrointestinal afflictions related to motility, and problems with weight control in either direction^[82]

Proinflammatory cytokines

Proinflammatory cytokines include inducers of the immune and inflammatory response, with many molecules involved in both processes. Macrophages are capable of producing a plethora of cytokines, including TNF- α , IL-1, IL-6, IL-12, IL-15, IL-18, chemokines such as IL-8, MIP-1 α , MIP-1 β , MCP-1, interferons, etc.

TNF superfamily of cytokines

There are a number of TNF-related cytokines which have been molecularly defined in recent years. The first were TNF- α and TNF- β , which both react with the TNF receptors (p55 type I and p75 type II). When these receptors were cloned it became apparent that they were related in structure and sequence to nerve growth factor receptor: apart from the latter the TNF/TNFR superfamily are primarily involved in the immune and inflammatory mechanisms^[83]. The TNF/TNFR superfamily molecules are involved in a variety of processes, many of which involve cell activation, growth, and regulation of apoptosis. Many of the TNFR family members can induce apoptosis (e.g. Fas, TNFR, death receptor 3). The former include the protein interaction domain termed the 'death domain', but other TNFR family members lacking it, e.g. TNFR p75 (type II) and CD30, can also cause apoptotic cell death.

Members of the TNF family, e.g. TNF- α , LT- α , CD40L, CD27L, 4-1BBL, induce cell activation and growth in the short term. There is a marked overlap in function of many of the proteins in the TNF/ TNFR family, which is explained in large part by sharing of receptors and intracellular signaling pathways. There has been considerable progress in the analysis of TNFR family signaling. There are shared structural aspects of the TNF/TNFR family, which has helped recognize functionally uncharacterized 'orphan' members. TNF family members are all type II transmembrane proteins (with the C-terminal on the outside of the cell), with a transmembrane region and a cytoplasmic region, which for TNF- α is very long. LT- α is an exception, it is a secreted protein with a typical leader sequence. While most of the remainder are active principally on the cell surface e.g. Fas, CD40, these proteins are shed by enzymes which belong to the ADAMS family (a disintegrin and metalloproteinase). The TNF α -converting enzyme (TACE), is now termed ADAMS 17. TNF- α is active in both surface and shed forms, the latter diffusing more widely. Cell surface TNF- α preferentially acts on p75 TNFR. The bioactive form of all the TNF family is believed to be the trimer.

IL-1R

There are two IL-1 receptors: type I and type II. They are related members of the immunoglobulin super- family. Type I IL-1R is the signaling receptor and is widely

expressed. Type II IL-1R is a related molecule but only has a short cytoplasmic domain (20 amino acids) and has not so far been shown to signal. It is expressed on myeloid cells and may act as a decoy receptor. It is shed upon cell activation and may function as an inhibitor.

IL-6

IL-6 has some undeniably proinflammatory features, for example it induces fever. However, it also has some anti-inflammatory features, such as the capacity to partly downregulate TNF α production. IL-6 also has major effects on the immune system, activating B cells to Simmunoglobulin, and T cells to upregulate receptors for IL-2.^[84]

Cytokine inducers of immunity

Macrophages, in common with other antigen-presenting cells (APCs), produce a range of cytokines which have potent effects on the immune system. Major proinflammatory cytokines such as IL-1, TNF α , and IL-6 also are proimmune, upregulate IL-2 receptors and activate B cells. There is also another series of molecules produced chiefly by macrophages and other APCs which are important in the activation of the immune response, such as IL-15, IL-12, and IL-18.

IL-15

IL-15 is one of the major early activators of T cells and NK cells. This is because functionally IL-15 has a very similar spectrum of activity to IL-2, now understood because as the signal transduction /chains of

the IL-2R (the α and β chains) are also used by IL-15. However, as the IL-15 α chain (unlike the IL-2 β chain) is constitutively expressed, this permits IL-15 to be involved in the early activation of T and NK cells, prior to the CD25 component of the high-affinity upregulation of the IL-2 receptor. In view of its powerful effects on the immune system, IL-15 is produced in small amounts, and the presence of 10 potential AUG transcriptional start sites in the IL-15 promoter is probably of major importance in limiting the rate of protein production. Once produced, the properties of IL-15 would be similar to those of IL-2, namely growth and activation of T lymphocytes, B lymphocytes, macrophage activation, NK activation, etc.^[85]

IL-12

IL-12 is structurally unusual, as it is a heterodimeric cytokine. The p40 chain is produced relatively abundantly, but only when coupled to the p35 chain, which is very sparingly produced, is the bioactive form (p70) generated. IL-12 acts on T cells and NK cells to activate them. On T cells it activates naïve CD4⁺ 'TH0' cells towards the CD4⁺ 'TH1' cells which have a proinflammatory phenotype. These are T cells which produce IL-2 and IFN but not IL-4 and help downregulate the CD4⁺ TH2 'allergic' phenotype (cells which produce IL-4 and IL-5). IL-12 is a very potent inducer of IFN, but its capacity to generate TH1 cells is not solely dependent in this property. IL-12 has strong

adjuvant activity and is produced by APC ± most abundantly by dendritic cells, macrophages, and B lymphocytes.^[86]

IL-18

IL-18 was first described as 'interferon-inducing factor', but is now known to act as a cofactor with IL-12 in the generation of TH1 type cells which produced IFN. Structurally, it has been found to be conformationally related to IL-1, and its receptor uses a similar signal transduction pathway. TH1 cells have receptors for IL-18, but not for IL-1, and the TH2 cells have receptors for IL-1 but not for IL-18. The strong proinflammatory effects of IL-12 are much diminished in the absence of IL-18.

Structure, Synthesis and Receptors of

TNF- α : Human TNF- α is translated as a 26-kDa protein that lacks a classic signal peptide. Newly synthesized pro TNF- α is expressed on the plasma membrane, and is then cleaved in the extracellular domain through the action of matrix metalloproteinases to release a mature soluble 17-kDa protein. In both its cell-associated and secreted forms, trimerization is required for biological activity. Both the cell-associated 26-kDa and secreted 17-kDa forms are biologically active, and the cell associated form is often thought to be responsible for juxtacrine signalling secondary to cell-to-cell contact. The specific functions of cell-associated and secreted TNF- α remain

controversial, although it is clear that the two forms of TNF- α have both overlapping and distinct biological activities. For example, Kollias *et al.* used a novel transgenic mouse that expresses only cell-associated TNF- α to demonstrate that these animals can develop chronic inflammatory diseases such as rheumatoid arthritis^[87]. In fact, the development and progression of RA in rodent models seems to be dependent on both the cell-associated and secreted forms of TNF- α ^[88]. By contrast, mice expressing only a cell-associated form of TNF- α seem to be resistant to endotoxin-induced lethality, indicating that the shock-producing properties of TNF- α are due primarily to the production of the soluble 17-kDa form^[89]. The primary enzyme responsible for processing cell associated TNF- α to a secreted form is TNF- α -converting enzyme or (TACE, also known as ADAM-17)^[90]. TACE is an adamalysin, a member of a class of membrane associated enzymes that contain both disintegrin and matrix metalloproteinase domains. These enzymes are crucial for the processing of several membrane-associated proteins including TNF- α , Fas ligand, the TNF receptors and the epidermal growth factor receptor. TACE seems to have a number of crucial biological functions in addition to its processing of cell-associated TNF- α . Mice deficient in TACE are developmentally lethal^[91], whereas mice deficient in TNF- α or its two receptors survive gestation.

Signal transduction and effector mechanisms

The biological responses to TNF- α are mediated through two structurally distinct receptors: type 1 (TNFR1, also known as p60, p55, CD120a) and type II (TNFR2, also known as p80, p75, CD120b). Both receptors are transmembrane glycoproteins with multiple cysteine-rich repeats in the extracellular N-terminal domains. Although their extracellular domains share structural and functional homology, their intracellular domains are distinct, and transduce their signals through both overlapping and distinct pathways. The primary characteristic that distinguishes the intracellular domains of TNFR1 and TNFR2 is the presence of a death domain in TNFR1, which is not present in TNFR2. The death domain is a sequence of approximately 70 amino acids that is pivotal to the ability of TNF- α to trigger cellular apoptosis. In common with the death domain of Fas ligand, this intracellular domain provides a docking site for a number of accessory proteins, including Fas-associated death-domain-containing protein (FADD), TNFR1-associated death domain-containing protein (TRADD), and TNFR-associated factor-2 (TRAF-2). TRADD and TRAF2 provide the branching points for the proapoptotic and inflammatory signalling pathways that are characteristic of TNFR1. Under physiological conditions, signalling through TNFR1 seems to be

primarily responsible for the proinflammatory and shock-producing properties of TNF- α . Mice deficient in TNFR1 are resistant to endotoxin-induced lethality, whereas mice deficient in TNFR2 remain sensitive^[89,92]. By contrast, other biological responses to TNF- α seem to be dependent on signalling through both receptors. Hepatic injury to a T-cell mitogen is reduced in mice deficient in either receptor^[88], and the development of RA secondary to overexpression of cell-associated TNF- α is diminished by the absence of TNFR1 or TNFR2^[87]. All nucleated cells express TNF receptors, although their distribution varies with cell type. TNFR1 is expressed constitutively on most cell types, whereas expression of TNFR2 can be induced. In addition, TNFR2 is restricted to certain cell types and can discriminate TNF- α from different species. The receptors also differ significantly in their binding affinities for homotrimeric TNF- α . Although both receptors can be considered high-affinity, the on-off kinetics of the two differ dramatically. Binding of homotrimeric TNF- α to TNFR1 is thought to be essentially irreversible, whereas binding to TNFR2 is associated with both rapid on and off kinetics^[93]. This has fuelled speculation that TNFR2 might function as a 'ligand passer' in some cells^[94], transferring TNF- α to TNFR1. However, TNF- α signalling through TNFR2 seems to have a dual role in T cells. In the absence of TNFR1 signalling, TNF- α

promotes the proliferation of naive T-cells through the actions of TNFR2^[95]. Moreover, despite the absence of a death domain, TNFR2 initiates apoptosis in activated CD8+ T cells independent of TNFR1 signalling^[96,97]. These data indicate that TNFR2 is likely to function as more than just a ligand passer in T cells, and that these functions might vary depending on the cell type and the presence of key intracellular signalling molecules. Both TNF receptors can be cleaved from the cell surface by members of the matrix metalloproteinase family in response to inflammatory signals, such as TNF- α receptor binding. The shed extracellular domains of the receptors retain their ability to bind TNF- α and therefore probably function as either endogenous inhibitors or facilitators of the biological activity of TNF- α ^[98], depending on their concentrations and the concentrations of the ligand. Both receptors are excreted in the urine immunologically intact. In fact, the original TNF- α -binding proteins were fragments of these receptors^[99-101].

Toll like 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 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^[102].

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*^[103]. 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^[104]. In contrast, macrophages from TLR1-deficient mice showed a normal response to mycoplasma-derived diacyl lipopeptides, but an impaired response to triacyl lipopeptides^[105]. 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^[106]. The second explanation involves recognition of fungal-derived components by TLR2^[107]. 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 β -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- κ B in response to dsRNA. In addition, TLR3-deficient mice are impaired in their response

to dsRNA^[108]. 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^[109,110]. In addition, TLR4 is implicated in the recognition of taxol, a diterpene purified from the bark of the western yew (*Taxus brevifolia*)^[111,112]. 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^[113]. LPS is a very potent immuno-activator, and accordingly, TLR4 can be activated by a very small 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^[114]. TLR5 has further been shown to recognize an evolutionarily conserved domain of flagellin through close physical interaction between TLR5 and flagellin^[115]. TLR5 is expressed on the basolateral, but not the apical side of intestinal epithelial cells^[116]. TLR5 expression is also observed in the intestinal endothelial cells of the subepithelial compartment^[117]. In addition, flagellin activates lung epithelial cells to induce inflammatory cytokine production. 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*^[118].

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^[119]. Human TLR7 and TLR8, but not murine TLR8, recognizes imidazoquinoline compounds^[120]. Murine TLR7 has also been shown to recognize another synthetic compound, loxoribine, which has anti-viral and anti-tumor

activities^[121,122]. 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 guanosine or uridine-rich single-stranded RNA (ssRNA) from viruses such as human immunodeficiency virus, vesicular stomatitis virus and influenza virus^[123-125]. 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^[126]. 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- α . A/D-type CpG DNA is structurally different from conventional CpG DNA and has a greater ability to induce IFN- α production from plasmacytoid dendritic cells (PDC), but less ability to induce IL-12^[127,128]. TLR9 has been shown to be essential for the recognition of both types of CpG DNA^[129]. TLR9 recognition of A/Dtype CpG DNA leads to induction of an anti-viral cytokine IFN- α 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 of the pH-dependent maturation of endosomes by acting as a basic substance to neutralize acidification in the vesicle^[130], 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. TLR11-deficient mice are highly susceptible to uropathogenic bacterial infection. TLR11 mediates antiuropathogenic bacterial response^[131].

Toll like receptor and proinflammatory cytokines: TLRs are group of pattern recognition receptors found on astrocytes and mainly microglial that can be activated by exogenous (pathogenic proteins) and endogenous (IL-1 β , TNF- α) molecules; when activated, they produce an immune response resulting in the release 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].

Glial cells

Glial cells, also called neuroglia or simply glia are non-neuronal cells that maintain homeostasis, form myelin and provide support and protection for neurons in the brain. Glial cells comprise 90% of the human brain.^[132]

Types of glial cells

Glia are divided into two subtypes, the microglia which function largely as scavengers to engulf apoptotic cell debris and the macroglia comprised of oligodendrocytes that myelinate axons, and astrocytes.^[133]

- **Microglia:-** Microglia are specialized macrophages capable of phagocytosis that protect neurons of the central nervous system ^[134] They are derived from hematopoietic precursors rather than ectodermal tissue; they are commonly categorized as such because of their supportive role to neurons. These cells comprise approximately 15% of the total cells of the central nervous system. They are found in all regions of the brain and spinal cord. Microglial cells are small relative to macroglial cells, with changing shapes and oblong nuclei.

They are mobile within the brain and multiply when the brain is damaged. In the healthy central nervous system, microglia processes constantly sample all aspects of their environment (neurons, macroglia and blood vessels).

- **Macroglia**

Role of astrocytes in opioid withdrawal

Astrocytes are the most abundant glial cell type in the central nervous system^[135]. Like other glial cell types, astrocytes were once thought to play only a secondary, non-regulatory and permissive role in nervous function. The cell membranes of astrocytes bear receptors for most neurotransmitters and peptides: glutamate, dopamine, norepinephrine, serotonin, gammaaminobutyric acid, acetylcholine and opioid peptides^[136]. They also bear in their plasma membranes neurotransmitter^[137-139] and glucose^[140] transporters, and aquaporin-4 channels for water transport^[141,142]. Administration of cocaine, amphetamines and most psychostimulants induces activation of astrocytes^[143-145]. This activation is defined by an increase in the expression of glial fibrillary acidic protein (GFAP), a main component of the cytoskeleton of astrocytes. GFAP is known to be upregulated in response to brain injury and neurotoxicity^[146,147], although changes in GFAP expression are not limited to overt brain injury and many other plastic changes in the neuropil also result in

increased GFAP expression^[148-150]. For instance, treatment with ethamphetamine (METH, “speed”) results in loss of dopaminergic terminals without detectable loss of neurons^[151], but induces astrogliosis with increased GFAP in the striatum, hippocampus and frontal cortex^[152].

Chronic treatment with morphine also results in increased GFAP expression or enlarged astrocytes in VTA, NAcc, frontal cortex, locus coeruleus and nucleus of the solitary tract of the rat^[153-157]. The morphine-induced increases in GFAP expression and the astroglial activation are probably mediated by $\alpha 2$ -adrenoceptors since the antagonist yohimbine inhibits upregulation of $\alpha 2$ -adrenoceptors and prevents the increase in GFAP expression caused by chronic morphine treatment^[156,157]. The responsiveness of astrocytes of morphine administration^[158,159] that astrocytes might contribute to morphine tolerance, and more recent evidence supports that hypothesis^[160]. For instance, inactivation of astrocytes by the gliotoxin fluorocitrate attenuates both tolerance to morphine analgesia and morphine-induced increase in GFAP immunostaining^[155]. Tolerance to morphine has been also related to downregulation of glial glutamate transporters GLT-1 and GLAST in the spinal cord^[161] suggesting a link between structural and functional features of astrocytes involved in tolerance to morphine.

The neurotrophic activity of astrocytes may also be relevant to the effects of cocaine in VTA. Glial-derived neurotrophic factor (GDNF) is present in neurons astrocytes and microglial cells, although it seems to be mainly produced by astrocytes^[162,163]. GDNF supports the survival and differentiation of dopaminergic cells and protects those cells against METH-induced neurotoxicity as shown in wild-type mice^[164] and in heterozygous mice with a partial deletion of the GDNF gene^[165]. Glial-derived neurotrophic factor, when infused into the VTA reduces the increase in the formation of key proteins induced by cocaine exposure^[166]. Furthermore mice lacking expression of GDNF display increase behavioral sensitization to cocaine^[167] and treatment of mice with the dipeptide Leu-Ileu an inducer of GDNF (and TNF-alpha, also produced by astrocytes) expression blocked the acquisition of METH-induced place preference and sensitization^[168,169].

Tumor necrosis factor alpha, produced by astrocytes and microglial cells, has been shown recently to prevent METH neurotoxicity and dependence in mice possibly through the enhancement of dopamine uptake in the striatum and the prevention of METH-induced increases in extracellular dopamine^[170].

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^[171].

- TLR in microglia: TLR mediated signalling promotes the production of a variety of inflammatory mediators^[172,173]. Exogenous and Endogenous TLR ligands activate microglial cells. TLR may mediate different pathway in microglial leading to either neuroprotective or neurotoxic phenotype^[174]. However, activated microglia with TLR ligands also produce neurotoxic molecules such as nitric oxide (NO), reactive oxygen species (ROS), peroxynitrate, proinflammatory cytokines (TNF- α , IL-1 β) which leads to withdrawal syndrome^[175].

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^[176]. 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- κ B^[177]. 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^[178,179].

Relation between glial cells and proinflammatory cytokines

Glial cells of the CNS such as astrocytes, oligodendrocytes and microglia can respond to and also produce many of the proinflammatory cytokines initially attributed to lymphocytes and macrophages. These cytokines include IL-1, IL-2, IL-3, IL-4, IL-6, IL-8, IL-10, IFN- α , IFN- β , IFN- γ , TNF- α , TNF- β , TGF- β and colony stimulating factor (CSFs)^[180]. Opioid withdrawal induces glial activation and proinflammatory cytokines (TNF- α) expression in different sites of the brain.

Approaches to anti-tnf- α therapy

Ibudilast (AV411)

AV411 (Avigen, Inc., Alameda, CA) is a blood-brain permeable, nonspecific phosphodiesterase inhibitor that acts centrally by way of attenuation of glial cell activation and reduction of proinflammatory activating factors, such as cytokines (TNF- α , IL-1 β),

nitric oxide, and chemokines such as monocyte chemo-attractant protein-1 and fractalkines; it increases production of anti-inflammatory IL-10^[180,181]. It was initially used to treat bronchial asthma and poststroke dizziness, which has been attributed to its ability to reduce inflammation and cause vasodilatation^[182]. In a recent study, AV411 was shown to reduce mechanical allodynia caused by neuropathic pain as well as noxious neuropathy induced by chemotherapeutic agents (paclitaxel and vincristine); it was also shown to reduce morphine tolerance. When administered systemically, AV411 was shown to be distributed to the spinal cord and to attenuate morphine-induced glial cell activation in certain brain regions. AV411 has also been shown to inhibit peripheral inflammatory cells^[183,184], which has been suggested as a possible cause of reduced pain perception peripherally. Other recent published data demonstrate that AV411 reduced spontaneous opioid withdrawal, protected naloxone-induced morphine withdrawal when given during the period of development of morphine dependence, and simultaneously enhanced analgesic effects (three to five times increases in acute potency), even in situations in which opioid dependence had already been established^[185]. These effects were seen with both morphine and oxycodone, with no changes in plasma morphine levels. Another study shows that AV411 decreases a morphine-induced

increase of dopamine in the nucleus accumbens, a nucleus known to be associated with morphine-induced drug reward as well as withdrawal^[186,187], thus further illustrating the beneficial role of AV411 in regulation of drug reward and dependence.

Thalidomide.

Thalidomide has been approved by the US FDA to treat moderate to severe leprosy, and clinical trials are ongoing in cancer (multiple myeloma)^[188-193]. Thalidomide has multiple effects that could account for its activity in myeloma. These include direct inhibition of tumour growth by altering the synthesis of cytokines that are involved in the growth and survival of myeloma cells, including TNF- α , IL-1 β , IL-6 and IL-10. Thalidomide also seems to inhibit the production of two major growth factors involved in angiogenesis: vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF). Further understanding the mechanisms of action for this molecule is crucial because of the known drug-induced congenital malformations it can cause in humans. However, thalidomide has been a successful development drug to treat multiple myeloma. It is important to note that the history of thalidomide illustrates the limits of the current pharmacological models that are available to evaluate potential teratogenicity^[194]. Thalidomide is metabolized differently by various species and, in fact, by diverse strains

within species. Thalidomide does not induce malformations in pregnant rodents typically used to test for teratogenicity. Moreover, rabbits exhibit different malformations from those observed in humans. Primates such as the marmoset seem to have similar susceptibility to humans^[195-197].

Two pilot studies have been conducted with thalidomide in patients with active inflammatory Crohn's disease. One study evaluated thalidomide in low dosage (50–100 mg per day) for 12 days and led to a 67% patient response and 0–33% disease remission. A second pilot study used thalidomide at 200–300 mg per day for 12 days in patients with active inflammatory and fistulizing Crohn's disease. Results showed that 80% of the patients with FISTULIZING DISEASE had fistula closure and 50% of patients with active inflammatory disease had a clinical response. New distant relatives of thalidomide or selective cytokine inhibitory drugs (SelCIDs) are under development for the treatment of cancer^[198-202]. SelCIDs have been shown to inhibit phosphodiesterase type 4 enzyme (PDE4), which indirectly decreases TNF- α production. TNF- α synthesis can be blocked by antidepressant and has been shown to be selective for PDE4. Rolipram and other PDE4 inhibitors that cross the blood–brain barrier cause nausea, and clinical trials have been discontinued. In addition, a clinical trial with CellTech's CDP-840 demonstrated dose-limiting side effects.

However, CDP-840 did not induce nausea. Second-generation, orally active PDE4 inhibitors with decreased side effects have resulted in the generation of SB207499 (Ariflo) by SmithKline Beecham.

RDP58 (Rationally Designed Peptide)

RDP58 is a small molecule (SangStat Medical) that inhibits TNF- α synthesis by preventing the translation of the TNF- α mRNA. In addition to TNF- α , RDP58 inhibits the expression of IFN- γ and IL-12 and upregulates haeme oxygenase *in vivo*. RDP58 consists of nine D-amino acids and glycine. The use of D-amino acids can make molecules resistant to degradation by human proteases. RDP58 is not systemically available, indicating that it is locally active after oral administration.

CONCLUSION

Opioid addiction, a significant and social problem is complicated by the phenomena of tolerance and dependence. There are various receptors which are involved in opioid withdrawal like glutamate receptor, κ -opioid, toll like receptor (TLR). 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 . The signalling pathways that inhibit cytokine synthesis are complex and there are many approaches. Hence suppression of proinflammatory cytokines (TNF- α) through TLR can significantly reduce opioid withdrawal syndrome.

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