

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
29 March 2007 (29.03.2007)

PCT

(10) International Publication Number
WO 2007/034465 A2

(51) International Patent Classification: **Not classified**

(21) International Application Number:
PCT/IE2006/000103

(22) International Filing Date:
21 September 2006 (21.09.2006)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
2005/0627 21 September 2005 (21.09.2005) IE

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declaration under Rule 4.17:

— of inventorship (Rule 4.17(iv))

Published:

— without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: COMPOSITIONS AND METHODS RELATING TO THE MODULATION OF AN IMMUNE-MEDIATED RESPONSE

(57) Abstract: Described is the novel finding that IL-1 F5 (IL-1 delta) and polypeptides derived therefrom bind to the receptor SIGIRR, with this binding interaction serving to modulate the immune response by stimulation the production of the cytokine IL-4. This induces an anti-inflammatory immune response. It has been further shown that PPARgamma is a key mediator in downstream signalling from SIGIRR following activation by the IL-1 F5 ligand. Modulation of the immune response occurs following binding of SIGIRR by IL-1 F5 in neuronal tissue and according methods for the treatment of neurodegenerative diseases are described.



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“Compositions and methods relating to the modulation of an immune-mediated response”

Field of the Invention

5 The present invention relates to novel methods for modulating an immune-mediated condition, in particular a neurodegenerative disease or inflammatory condition of the brain. The present invention further extends to the use of the cytokine IL-1F5 and modulation of the SIGIRR receptor in the induction of an anti-inflammatory immune response.

10

Background to the Invention

Cytokine Expression in the Brain

Originally discovered in the immune system, cytokines are secreted proteins now known to be expressed by both neurons and glia. Cytokines
15 are active during neural development as well as in the adult nervous system during both normal function and pathological conditions. Different cytokines can exert similar effects on a single cell type - a phenomenon that allows one factor to compensate for another. Additionally most cytokines induce multiple effects in multiple cell types – making it difficult
20 to determine the role of specific cytokines in the regulation of normal physiology.

Interaction of cytokines with specific cell surface membrane receptors is required for induction of biological activity. The precise mechanisms
25 invoked by cytokine binding at receptor sites is not clearly understood, however most cytokine binding induces a conformational change which allows the intracellular domain of the receptor to interact with accessory molecules. Receptor binding results in the activation of downstream effectors and activation of protein kinases which phosphorylate protein
30 substrates.

There are two major pathways of signal transduction involved in cytokine activation – the first pathway, used by mitogenic cytokines utilizes tyrosine kinases as the signal transducers, either directly or indirectly linked to the intracellular domain of the receptor. The second pathway involves activation of phospholipases that produce small mediators that activate serine-threonine kinases. Cytokines have been divided into “proinflammatory” and “anti-inflammatory” depending on their effects. As indicated by their name pro-inflammatory cytokines promote inflammation and anti-inflammatory cytokines suppress the activity of pro-inflammatory cytokines; they suppress genes for pro-inflammatory cytokines such as IL-1 and interferon- γ (IFN gamma). While IFN-gamma possesses anti-viral activity its ability to augment pro-inflammatory cytokine activity and induce nitric oxide have lead to its classification as a pro-inflammatory cytokine. The most thoroughly investigated of the proinflammatory cytokines are IL-1 and tumour necrosis factor-alpha (TNF- α) while the most studied of the anti-inflammatory cytokines are IL-4 and IL-10.

Anatomy of the hippocampal formation

The hippocampal formation, located on the medial aspect of each hemisphere beneath the cortical structures, comprises the dentate gyrus, the hippocampus proper and the subiculum, and is part of the limbic system. All 3 are composed of temporal lobe allocortex, tucked into an S-shaped scroll along the floor of the lateral ventricle. The largest afferent connection of the hippocampal formation is the perforant path, which projects from layers II and III of the entorhinal cortex in the temporal lobe. There are three major pathways in the hippocampus; the perforant fibre pathway from entorhinal cortex forms excitatory connections with the granule cells of dentate gyrus. The granule cells give rise to axons that form the mossy fibre pathway, which synapses with the pyramidal cells in

area CA3. The pyramidal cells of area CA3 project to the pyramidal cells in CA1 by means of the Schaffer collateral pathway. This is known as the trisynaptic circuit.

- 5 It is now widely accepted that the hippocampus plays a central role in the storage of memory. Studies involving lesions of the medial temporal lobe of monkeys demonstrated that these animals exhibited severe memory impairment. More recently, evidence for a role for the hippocampus in memory was demonstrated using magnetic resonance imaging and
- 10 positron emission topography. These techniques assessed blood flow and oxygen consumption in the hippocampus and identified that these parameters fluctuated during learning tasks.

Long term potentiation

- 15 In 1973 Bliss and Lomo reported that trains of high frequency stimulation applied to any of the perforant path in the anaesthetized rabbit increased the amplitude of the excitatory postsynaptic potentials (EPSPs) in the target hippocampal neurons. This sustained increase in synaptic efficacy was termed long-term potentiation (LTP). Hippocampal neurons have both
- 20 non NMDA (AMPA and kainate) and NMDA receptors. Non NMDA receptors participate in the early phase of the EPSP and gate ion channels with relatively low conductance that are permeable to both sodium and potassium but usually not to calcium. NMDA receptor-associated channels generate the late phase of the EPSP and have a higher
- 25 conductance being permeable to both sodium and potassium but particularly to calcium. Functioning of NMDA receptors depends on glycine being present in the extracellular fluid in sufficient quantities. The NMDA receptor-associated differs further in that its channel is dual regulated being not only ligand-gated but also voltage-gated. The
- 30 presence of extracellular magnesium in the pore of the channel means

that membrane depolarization must occur in order to allow ion influx through the channel. Membrane depolarization causes electrostatic repulsion of magnesium from its binding site in NMDA receptor-associated channel. Following production of a single action potential, the late phase
5 of EPSP is usually quite short, as magnesium returns to the pore. However as neurons fire repeatedly, the late phase becomes longer as magnesium takes longer to be replaced into the pore. These events help to explain some of the main features of LTP.

10 The Classical IL-1 family - IL-1 α and IL-1 β

Interleukin 1 is a cytokine that is produced by, and acts on, many different cell types. IL-1 has the ability to upregulate the expression of many genes important in the initiation and regulation of inflammatory conditions. As
such IL-1 is critical in the management of the host immune defence.

15 However, the potency of this cytokine means that any imbalance in IL-1 levels can rapidly lead to the development of many diseases, including rheumatoid arthritis, Alzheimer's disease and Parkinson's disease. The classical members of the IL-1 family consist of IL-1 α (IL-1 alpha), IL-1 β (IL-1 beta), IL-1 receptor antagonist (IL-1ra) and IL-18.

20 IL-1 α and IL-1 β were first isolated in the 1980s. Both IL-1 α and IL-1 β have similar properties in that they can activate the same signals and they both bind to the type 1 IL-1 receptor (IL-1R1). However differences in receptor affinities, cellular localization and sites of regulation and expression allow
25 for a divergence of IL-1 α and IL-1 β functions in vivo. Studies on knock out mice have shown that IL-1 β but not IL-1 α is required for T cell dependent antibody production and IL-1 β , but not IL-1 α , is required for fever development.

Both IL-1 α and IL-1 β are synthesized as precursor models of 31kDa. Pro-IL-1 α is biologically active but is retained within the cytoplasm, with little release in the circulation. In comparison IL-1 β is readily and rapidly exported from the cell. The method of release is thought to involve the packaging of IL-1 β into vesicles and subsequent ATP-driven exocytosis. Pro IL-1 β is cleaved by caspase-1 to form mature, biologically active IL-1 β .

IL-1 in the Brain

IL-1 β is constitutively expressed at low levels in healthy adult brain by a variety of cell types. In response to local brain injury or insult, IL-1 is over-expressed by the CNS macrophage equivalent cell, the microglia. Microglia are cells that exist in resting and active states and are activated in response to injury. They are thought to be one of the principal producers of IL-1 β . IL-1 β overexpression by microglia is also seen in response to acute head trauma, a recognized risk factor for the later development of Alzheimer's disease. IL-1 β has also been shown to be involved in the inflammatory pathology in multiple sclerosis (MS) and IL-1 receptor antagonist (IL-1ra) can ameliorate the symptoms of experimental autoimmune encephalitis (EAE), a murine model for MS.

IL-1F5

A biological role for IL-1F5 (IL-1 delta) has not yet been identified. IL-1F5 expression has been observed in a number of tissue types, including the brain, and also in keratinocytes, monocytes and dendritic cells. Expression of IL-1F5 can be upregulated following stimulation with LPS and a combination of IL-1beta and TNF alpha.

Studies carried out in T cells, fibroblasts and endothelial cells have demonstrated that IL-1F5 neither mimics nor antagonizes actions of IL-1 such as induction of IL-6.

SIGIRR

The single immunoglobulin related receptor (SIGIRR) is expressed primarily in mouse and human epithelial tissues such as kidney, lung and gut. To date, no ligand has been isolated for SIGIRR however recent evidence has suggested that it is a negative regulator of IL-1 and TLR signaling. It has been reported that SIGIRR-deficient mice exhibit increased inflammatory responses to LPS injection, suggesting an anti-inflammatory role for SIGIRR.

SIGIRR-deficient dendritic cells showed increased cytokine production in response to TLR agonists (lipopolysaccharide, CpG oligodeoxynucleotides). SIGIRR-deficient mice had normal susceptibility to systemic lipopolysaccharide toxicity and to intra-peritoneal (i.p). or sub-cutaneous (s.c), inflammation. However, SIGIRR-deficient mice were more susceptible to intestinal inflammation. Similarly it has been found that inflammation in response to LPS is enhanced in SIGIRR deficient mice. However the mechanism by which SIGIRR negatively modulates the immune response remains unclear.

US Patent Application No 2005/0058625 discloses a method of treating an inflammatory or autoimmune disease through the administration of the IL-1 delta polypeptide. However, no specific experimental evidence is provided to define the method of action or signalling pathway induced by IL-1 delta.

It is predicted therein that IL-1 delta mediates an anti-inflammatory response based on observed sequence homology between the IL-1 delta amino acid sequence and the amino acid sequence of IL-1ra. On the basis of this purported homology, it is predicted that IL-1 delta has the same function as IL-1ra and accordingly acts as an antagonist of IL-1 family members by means of antagonising the other members of the IL-1

family by competitively blocking IL-1 from binding to its receptor which mediate an inflammatory immune response.

5 The present inventors have now surprisingly found that IL-1F5 (IL-1 delta) mediates an anti-inflammatory effect, not by being an antagonist of IL-1 activity, but rather through binding to the IL-1 superfamily receptor SIGIRR (single immunoglobulin related receptor). IL-1F5 acts as an agonist to this receptor. Binding of SIGIRR by IL-1R results in the downstream
10 production of the anti-inflammatory cytokine IL-4 which serves to modulate an anti-inflammatory response.

The present inventors have further determined that in addition to upregulation of the cytokine IL-4, IL-1F5 mediates an upregulation of PPARgamma this modulation of activity also resulting in the induction of
15 an anti-inflammatory response.

Summary of the Invention

The nucleotide sequence of the human IL-1F5 protein (also known as IL-1 delta) that encodes amino acid SEQ ID NO:2 is provided as SEQ ID NO:1.
20

The amino sequence of the human IL-1F5 protein (also known as IL-1 delta) has been previously defined and this is described herein as SEQ ID NO:2 as follows:

25 mvlsgalcfr mkdsalkvly lhnnqllagg lhagkvikge eisvvpnrwl daslspvilg
vqggsqclsc gvgqeptltl epvnimelyl gakesksftf yrrdmgltsf fesaaypgwf
lctvpeadqp vrltqlpeng gwnapitdfy fqqcd

The nucleotide sequence of the murine IL-1F5 protein (also known as IL-1 delta) that encodes amino acid SEQ ID NO:4 is provided as SEQ ID NO:3.
30

The amino sequence of the murine IL-1F5 protein (also known as IL-1 delta) has been previously defined and this is described herein as SEQ ID NO:4 as follows:

5

mmvlsgalcf rmkdsalkvl ylhnnqllag glhaekvikg eeisvvpnra ldsalspvil
gvqggsqcls cgtekgpilk lepvnimely lgakesksft fyrrdmglts sfesaaypgw
flctspeakd pvrllqiped pawdapitdf yfqgcd

10 Accordingly in a first aspect of the present invention there is provided a method of suppressing a pro-inflammatory immune response, the method comprising the step of administering an effective amount of a polynucleotide or a fragment thereof which encodes a protein having SEQ ID NO:2 or SEQ ID NO:4 or a fragment thereof to a individual in need of
15 such treatment.

In one embodiment the individual is a mammal. In a further embodiment the mammal is a human. Where the individual is a human, the patient is preferably administered IL-1F5 having the amino acid as defined in SEQ
20 ID NO:2 or SEQ ID NO:4 or a fragment thereof.

A second aspect of the present invention provides the use of a polynucleotide or a fragment thereof which encodes a protein having SEQ ID NO:2 or SEQ ID NO:4 or a fragment thereof for the administration to an
25 individual for the suppression of an immune response, said immune response being characterised in that it is mediated through the activation of SIGIRR (single immunoglobulin related receptor).

In one embodiment the individual is a mammal. In a further embodiment
30 the mammal is a human. Where the individual is a human, the patient is

preferably administered IL-1F5 having the amino acid as defined in SEQ ID NO:2 or a fragment thereof.

5 A third aspect of the present invention provides the use of a polynucleotide or a fragment thereof which encodes a protein having SEQ ID NO:2 or SEQ ID NO:4 in the preparation of a medicament for the downregulation of an immune response.

10 A yet further aspect of the present invention provides a pharmaceutical composition comprising a therapeutically effective amount of a polynucleotide or a fragment thereof which encodes a protein having the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4 along with a pharmaceutically acceptable diluent, excipient or carrier.

15 According to a further aspect of the present invention there is provided a method for the prophylaxis and/or treatment of an immune-mediated disorder, the method comprising the step of administering a therapeutically effective amount of a peptide comprising the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4 or an analogue, derivative, fragment, variant or
20 peptidomimetic thereof to an individual in need of such treatment.

In one embodiment the individual is a mammal. In a further embodiment the mammal is a human. Where the individual is a human, the patient is preferably administered IL-1F5 having the amino acid as defined in SEQ
25 ID NO:2 or a fragment thereof.

Without wishing to be bound by theory, the inventors predict that the administration of the peptide having the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4 causes suppression of the pro-inflammatory
30 immune response by binding to and activating the receptor SIGIRR, which

in turn mediates downstream signalling which causes an upregulation in the production of the anti-inflammatory cytokine IL-4. IL-1F5 therefore exerts an anti-inflammatory function by binding to SIGIRR.

5 IL-1F5 peptide administration results in a decrease in the expression of the pro-inflammatory cytokine IL-1 and in particular IL-1 beta. This effect is seen in the IL-1 producing cells which are present in the brain, central nervous system (CNS) and other neuronal cells.

10 In one embodiment the invention extends to amino acid sequences which are at least 80% homologous with the sequence of SEQ ID NO:2 or SEQ ID NO:4. In further embodiments such sequences may be at least 85%, 90%, 95%, 96%, 97%, 98%, 99% or 99.5% homologous with the amino acid sequence of SEQ ID NO:2.

15 A further aspect of the present invention provides for the use of a peptide comprising the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4 or an analogue, derivative, fragment, variant or peptidomimetic thereof for the prophylaxis and/or treatment of an immune-mediated disorder.

20 In one embodiment the invention extends to amino acid sequences which are at least 80% homologous with the sequence of SEQ ID NO:2 or SEQ ID NO:4. In further embodiments such sequences may be at least 85%, 90%, 95%, 96%, 97%, 98%, 99% or 99.5% homologous with the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4.

25 In preferred embodiments of this aspect of the invention, the anti-inflammatory properties of IL-1F5 are exerted in the brain, CNS or other neuronal tissue, by virtue of the binding of SIGIRR by IL-1F5 and the resultant expression of the anti-inflammatory cytokine IL-4 which serves to

30

modulate immune responses. Such neuronal specific activity of IL-1F5 confers benefits in that it may be administered to a patient in need of therapy in order to selectively inhibit inflammation in the brain, while not affecting (through immune modulation or downregulation) beneficial
5 immune responses in the periphery.

A yet further aspect of the present invention provides the use of a peptide comprising the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4 or an analogue, derivative, fragment, variant or peptidomimetic thereof in the
10 preparation of a medicament for the suppression of an immune response, said immune response being characterised in that it is mediated through the activation of SIGIRR.

In one embodiment the immune response is suppressed in a mammal. In
15 a further embodiment the immune response is suppressed in a human. Where the individual is a human, preferably the peptide which is used comprises the amino acid as defined in SEQ ID NO:2 or a fragment thereof.

20 In one embodiment the invention extends to amino acid sequences which are at least 80% homologous with the sequence of SEQ ID NO:2 or SEQ ID NO:4. In further embodiments such sequences may be at least 85%, 90%, 95%, 96%, 97%, 98%, 99% or 99.5% homologous with the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4.

25

The present invention therefore has particular utility in the prevention and/or treatment of neurodegenerative and/or inflammatory conditions and diseases in and of the brain, CNS and neuronal tissue.

Accordingly, in a further aspect of the present invention, there is provided a method for the treatment and/or prophylaxis of a neurodegenerative disease or inflammatory condition of the brain, the method comprising the step of administering a therapeutically effective amount of a peptide
5 comprising the amino acid sequence of SEQ ID NO:2 or an analogue, derivative, fragment, variant or peptidomimetic thereof to an individual in need of therapy.

10 In one embodiment the individual is a mammal. In a further embodiment the mammal is a human. Where the individual is a human, the patient is preferably administered IL-1F5 having the amino acid as defined in SEQ ID NO:2 or a fragment thereof.

15 Typically the neurodegenerative disease or condition is selected from the group consisting of: Alzheimer's disease (AD), mild cognitive impairment (MCI), multiple sclerosis (MS), Parkinson's disease, Amyotrophic lateral sclerosis (ALS), Huntington's disease, prion diseases such as CJD, AIDS-related dementia, encephalitis, stroke and head trauma.

20 The neurodegenerative condition may also include acute inflammation conditions of the brain which result following bacterial and viral infections.

A further aspect of the present invention provides for the use of a peptide comprising the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4, a
25 sequence which is at least 80% homologous thereto, or an analogue, derivative, fragment, variant or peptidomimetic thereof for the prophylaxis and/or treatment of a neurodegenerative disease.

30 In one embodiment the neurodegenerative disease is treated in a mammal. In a further embodiment the neurodegenerative disease is

treated in a human. Where the individual is a human, preferably the peptide which is used comprises the amino acid as defined in SEQ ID NO:2 or a fragment thereof.

5 In one embodiment the invention extends to amino acid sequences which are at least 85% homologous with the sequence of SEQ ID NO:2 or SEQ ID NO:4. In further embodiments such sequences may be at least 90%, 95%, 96%, 97%, 98%, 99% or 99.5% homologous with the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4.

10

A yet further aspect of the present invention provides the use of a peptide comprising the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4, a sequence which is at least 80% homologous thereto, or an analogue, derivative, fragment, variant or peptidomimetic thereof in the preparation
15 of a medicament for the suppression of a neurodegenerative disease.

20

In specific embodiments, the neurodegenerative disease may be Alzheimer's disease. Alzheimer's disease exhibits a sustained, chronic over-expression of IL-1, with IL-1 over-expression being implicated in both the initiation and progression of the characteristic neuropathological changes, observed with Alzheimer's disease progression.

25

Accordingly, the invention extends to a method for the treatment and/or prophylaxis of Alzheimer's disease, the method comprising the steps of:
- administering a therapeutically effective amount of a peptide comprising the amino acid sequence of SEQ ID NO:2, or SEQ ID NO:4 or a sequence which is at least 80% homologous thereto, or an analogue, derivative, fragment, variant or peptidomimetic thereof to an individual in need of such treatment.

30

In one embodiment the individual is a mammal. In a further embodiment the mammal is a human. In one embodiment wherein the individual is a mammal the peptide which is administered comprises a peptide having the amino acid sequence of SEQ ID NO:2 or a fragment thereof or a
5 sequence with at least 80% homology thereto.

A further aspect of the invention extends to the use of an agent comprising a peptide comprising the amino acid sequence of SEQ ID NO:2, or SEQ ID NO:4 a sequence which is at least 80% homologous thereto, or an
10 analogue, derivative, fragment, variant or peptidomimetic thereof in the treatment of Alzheimer's disease.

A further still aspect relates to the use of a peptide comprising the amino acid sequence of SEQ ID NO:2, or SEQ ID NO:4 a sequence which is at least 80% homologous thereto, or an analogue, derivative, fragment,
15 variant or peptidomimetic thereof in the preparation of a medicament for the treatment of Alzheimer's disease.

A yet further aspect of the present invention provides a method of treating
20 Alzheimer's disease through direct administration of a therapeutically effective amount of a peptide comprising the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4, a sequence which is at least 80% homologous thereto, or an analogue, derivative, fragment, variant or peptidomimetic thereof to the brain, neuronal tissue or the CNS of an individual in need of
25 such treatment.

In one embodiment the individual is a mammal. In a further embodiment the mammal is a human. Where the individual is a human, the patient is preferably administered a peptide having the amino acid sequence of SEQ

ID NO:2 or a fragment thereof or a sequence with at least 80% homology thereto.

5 In specific embodiments of the present invention, the IL-1F5 polypeptide or fragment thereof may be delivered to the brain by means of an intracerebroventricular injection (icv / ICV) or through delivery into the CNS using minipumps.

10 Alternatively a viral vector may be used to target delivery of the IL-1F5 to the brain or neural tissue. Such a vector will include a construct which contains a gene encoding for IL-1F5 or a fragment, derivative, mimetic or analogue thereof. The construct may further contain a promoter which is provided adjacent to the gene and which controls expression of the gene. Viral vectors which may be suitable for such delivery and targeting may
15 be; (i) nonreplicative herpes simplex type 1 viruses (Poliani et al. Hum Gene Ther. 2001 May 20; 12(8):905-20.); (ii) Semliki Forest virus, (Jerusalmi et al. Mol. Ther. 2003 Dec;8(6):886-94.) and (iii) adenovirus, (for example see Braciack et al. J. Immunol. 2003 Jan 15;170(2):765-74.).

20 In further embodiments, naked plasmid DNA encoding for IL-1F5 or fragments, derivative, mimetics or analogues thereof may be directly administered.

25 A further still aspect relates to the use of a peptide comprising the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4, a sequence which is at least 80% homologous thereto, or an analogue, derivative, fragment, variant or peptidomimetic thereof in the preparation of a medicament for the treatment of Alzheimer's wherein the medicament is administered directly to the brain, neuronal tissue or the CNS of an individual in need of
30 such treatment.

In one embodiment the individual is a mammal. In a further embodiment the mammal is a human. Where the individual is a human, the patient is preferably administered a peptide having the amino acid sequence of SEQ
5 ID NO:2 or a fragment thereof or a sequence with at least 80% homology thereto.

According to a further aspect of the invention, the present invention extends to a method of treating an individual afflicted with Multiple
10 Sclerosis, comprising administering to the individual a peptide comprising the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4, a sequence which is at least 80% homologous thereto, or an analogue, derivative, fragment, variant or peptidomimetic thereof.

15 A further aspect of the invention extends to the use of an agent comprising a peptide comprising the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4, a sequence which is at least 80% homologous thereto, or an analogue, derivative, fragment, variant or peptidomimetic thereof in the treatment of Multiple Sclerosis.

20 A further still aspect relates to the use of a peptide comprising the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4, a sequence which is at least 80% homologous thereto, or an analogue, derivative, fragment, variant or peptidomimetic thereof in the preparation of a medicament for
25 the treatment of Multiple Sclerosis.

A yet further aspect of the present invention provides a method of treating Multiple Sclerosis through direct administration of a peptide comprising the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4, a sequence which

is at least 80% homologous thereto, or an analogue, derivative, fragment, variant or peptidomimetic thereof to the brain or CNS.

5 A further still aspect relates to the use of a peptide comprising the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4, a sequence which is at least 80% homologous thereto, or an analogue, derivative, fragment, variant or peptidomimetic thereof in the preparation of a medicament for the treatment of Multiple Sclerosis wherein the medicament is administered directly to the brain, neuronal tissue or the CNS.

10 Alternatively a viral vector may be used to target delivery of a peptide comprising the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4, a sequence which is at least 80% homologous thereto, or an analogue, derivative, fragment, variant or peptidomimetic thereof to the brain, neuronal tissue or the CNS.

15

The IL-1F5 polypeptide or fragment thereof may delivered to the brain by means of a intracerebroventricular injection (icv / ICV), through delivery by a minipump, by using a viral vector targeted to the brain or neural tissue, or by directly administering naked plasmid DNA encoding for IL-1F5 or fragments, derivative, mimetics or analogues thereof.

20

A further neural specific indication against which the present invention has utility is cognitive dysfunction.

25

Inflammatory changes in the brain exert a negative impact on cognitive function. In animal studies, such changes have been associated with impairment in hippocampal dependent learning paradigms and also in long term potentiation (LTP), which is a putative biological substrate for learning and/or memory.

30

It is well documented that LTP is impaired in the hippocampus of the aged animal. Among the changes which contribute to this impairment is an increased hippocampal concentration of the pro-inflammatory cytokine interleukin-1 beta (IL-1beta), and increased IL-1beta induced signalling.

5

According to a further aspect of the present invention there is provided a method for the prophylaxis and/or treatment of cognitive dysfunction, the method comprising the step of administering a therapeutically effective amount of an agent comprising a peptide comprising the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4, a sequence which is at least 80% homologous thereto, or an analogue, derivative, fragment, variant or peptidomimetic thereof to an individual in need of such therapy.

10

15

In one embodiment the individual is a mammal. In a further embodiment the mammal is a human. Where the individual is a human, the patient is preferably administered a peptide having the amino acid sequence of SEQ ID NO:2 or a fragment thereof or a sequence with at least 80% homology thereto.

20

A further aspect of the invention extends to the use of an agent comprising a peptide comprising the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4, a sequence which is at least 80% homologous thereto, or an analogue, derivative, fragment, variant or peptidomimetic thereof in the treatment of cognitive dysfunction.

25

A further still aspect relates to the use of a peptide comprising the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4, a sequence which is at least 80% homologous thereto, or an analogue, derivative, fragment, variant or peptidomimetic thereof in the preparation of a medicament for the treatment of cognitive dysfunction.

30

In a preferred embodiment, the administration of the a peptide comprising the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4, a sequence which is at least 80% homologous thereto, or an analogue, derivative, fragment, variant or peptidomimetic thereof results in the up-regulation of anti-inflammatory cytokines, for example IL-4 and the down-regulation of pro-inflammatory cytokines such as IL-1. Preferably this upregulation will be in the hippocampus, most preferably this upregulation of the anti-inflammatory cytokine profile will be present in the microglial cells. It is preferred that IL-1F5 be administered to the brain or directly to another area of the central nervous system (CNS).

Typically the anti-inflammatory cytokines which are upregulated are IL-4 and IL-10. The pro-inflammatory cytokines which are down-regulated are typically IL-1beta, and TNF-alpha. Preferably the modulation of pro-inflammatory and anti-inflammatory cytokine levels is in the hippocampus, most preferably this modulation of the cytokine profile will be seen in the microglial cells.

In preferred embodiments the fragment, derivative, analogue or mimetic of the IL-1F5 polypeptide confers IL-1F5 activity, that is that the peptide or peptide fragment can maintain long term potentiation, up-regulate the production of anti-inflammatory cytokines and down-regulate pro-inflammatory cytokines.

In preferred embodiments, the IL-1F5 polypeptide or fragment thereof may be administered directly to the brain or other suitable site of the central nervous system (CNS) in order to deliver the IL-1F5 polypeptide or fragment thereof to the neural tissue. In particular, the compositions will

be administered in such a way that they are directed to the hippocampus, and in particular can be directed to the microglial cells.

5 Accordingly, a yet further aspect of the present invention provides a method of treating cognitive dysfunction through direct administration of a peptide comprising the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4, a sequence which is at least 80% homologous thereto, or an analogue, derivative, fragment, variant or peptidomimetic thereof to the brain, neuronal tissue or the CNS.

10

A further still aspect relates to the use of IL-1F5 a fragment or derivative thereof in the preparation of a medicament for the treatment of cognitive dysfunction wherein the medicament is administered directly to the brain or CNS.

15

The IL-1F5 polypeptide or fragment thereof may delivered to the brain by means of a intracerebroventricular injection (icv / ICV), through delivery by a minipump, by using a viral vector targeted to the brain or neural tissue, or by directly administering naked plasmid DNA encoding for IL-1F5 or
20 fragments, derivative, mimetics or analogues thereof.

The present inventors have further identified that IL-1F5 binds to the IL-1 superfamily orphan receptor SIGIRR (single Ig IL-1R-related molecule). The identification of this binding association provides a further point at
25 which the immune response may be modulated. The binding of IL-1F5 to SIGIRR activates a signalling pathway which results in the upregulation of IL-4 production.

Accordingly, a further aspect of the present invention provides a method
30 for modulating the activity of the SIGIRR receptor through selectively

binding it with a peptide comprising the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4, a sequence which is at least 80% homologous thereto, or an analogue, derivative, fragment, variant or peptidomimetic thereof.

5

Binding of SIGIRR with a peptide comprising the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4, a sequence which is at least 80% homologous thereto, or an analogue, derivative, fragment, variant or peptidomimetic thereof serves to modulate the immune response through the downstream induction of an anti-inflammatory response, while competitive binding or functional blocking of the SIGIRR binding by a peptide comprising the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4, a sequence which is at least 80% homologous thereto, or an analogue, derivative, fragment, variant or peptidomimetic thereof results in the stimulation of an inflammatory response.

10
15

Accordingly, in addition to a peptide comprising the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4, a sequence which is at least 80% homologous thereto, or an analogue, derivative, fragment, variant or peptidomimetic thereof, further molecules may be employed to selectively influence the activity of the SIGIRR receptor, most specifically by influencing whether the receptor is in a bound or unbound state, the result of which will be an associated downstream modulation of the immune response.

20

25

As such the present invention further extends to methods for identifying molecules which may have a role in influencing the binding relationship which has been identified between IL-1F5 and SIGIRR.

Accordingly, a yet further aspect of the present invention provides a method for identification of modulator(s) of the binding of IL-1F5 and the SIGIRR receptor, said method comprising the steps of:

- 5 - providing first and second cellular samples containing the SIGIRR receptor,
- contacting said first sample with IL-1F5,
- contacting said first and second samples with a candidate molecule under conditions permissive of binding of said ligand, and monitoring the binding status of SIGIRR, and comparing the level of
- 10 downstream activation between said first and second samples, wherein a difference in downstream activation between said first and second samples identifies the candidate molecule as a modulator of IL-1F5 binding to SIGIRR.

15 In one embodiment the IL-1F5 is mammalian IL-1F5. In one embodiment, the IL-1F5 is human IL-1F5 or a fragment thereof, with human IL-1F5 being defined herein as comprising the amino acid sequence of SEQ ID NO:2.

20 The downstream activation of SIGIRR may be monitored by quantifying the upregulation of specific cytokines known the associated with an anti-inflammatory response, such as the cytokine IL-4.

25 Alternatively, or in addition, the activation of SIGIRR can be monitored by quantifying differences in PPAR activation, and in particular PPARgamma activation, which may result from downstream activation of SIGIRR.

30 As discussed briefly above, the present inventors have identified that IL-1F5 and fragments thereof mediate an anti-inflammatory response, not by IL-1F5 acting as an antagonist to IL-1 beta, but due to IL-1F5 acting as an

agonist to the SIGIRR receptor, wherein binding of IL-1F5 to SIGIRR results in activation of the receptor and downstream signalling. This signalling results in the activation of IL-4.

- 5 The inventors have recognised the utility of providing compounds which antagonise SIGIRR, with IL-1F5 having particular utility in this regard.

Accordingly, in a yet further aspect of the present invention there is provided a method of agonizing the SIGIRR receptor in a patient, the
10 method comprising the step of:

- administering to the patient a medicament comprising a peptide comprising the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4, a sequence which is at least 80% homologous thereto, or an analogue, derivative, fragment, variant or peptidomimetic
15 thereof,

in an amount effective to bind the SIGIRR receptor as an agonist.

In one embodiment the patient is a mammal. In a further embodiment, the patient is a human. Where the patient is a human, the patient is preferably
20 administered IL-1F5 having the amino acid as defined in SEQ ID NO:2 or a fragment thereof.

In one embodiment, the method can be used for the suppression of an aberrant immune response. The immune response may be causative of a
25 neurodegenerative disease, for example Alzheimer's disease, multiple sclerosis or mild cognitive impairment, Parkinson's disease, Huntington's disease, Amyotrophic lateral sclerosis, prion diseases, encephalitis and head trauma.

In a yet further aspect of the present invention there is provided the use of a peptide comprising the amino acid sequence of SEQ ID NO:2, a sequence which is at least 80% homologous thereto, or an analogue, derivative, fragment, variant or peptidomimetic thereof as an agonist in the treatment of a neurodegenerative disease.

In one embodiment the peptide comprising the amino acid sequence of SEQ ID NO:2, a sequence which is at least 80% homologous thereto, or an analogue, derivative, fragment, variant or peptidomimetic thereof is an agonist to SIGIRR.

In a still further aspect of the present invention there is provided the use of a peptide comprising the amino acid sequence of SEQ ID NO:2, a sequence which is at least 80% homologous thereto, or an analogue, derivative, fragment, variant or peptidomimetic thereof as an agonist of SIGIRR in the preparation of a medicament for the treatment of neurodegenerative diseases.

The present inventors have surprisingly found that PPAR activation, and in particular PPARgamma activation is effected following binding of SIGIRR by IL-1F5 or a fragment thereof.

Accordingly a yet further aspect of the present invention provides for the use of PPARgamma in the modulation of an immune response.

In one embodiment the immune response is an anti-inflammatory response mediated by IL-1F5.

The modulator(s) identified according to the above assay method may be a peptide or non-peptide molecule such as a chemical entity or

pharmaceutical substance. Where the modulator is a peptide it may be an antibody, or an antibody fragment. Further, where the modulator is an antibody, preferably it is a monoclonal antibody.

5 Novel compounds identified using the assays of the invention form a further independent aspect of the invention. Such compounds or modulators may be provided in pharmaceutical compositions. Such pharmaceutical compositions may be used in the treatment of conditions as hereinbefore described.

10

The assay of the present invention and compounds of biological significance to the SIGIRR signalling pathway which are realised by means of the use of the assays of the invention may have specific utility in the treatment in a number of medical conditions, particularly
15 neurodegenerative diseases, auto-immune conditions or immune mediated conditions.

15

According to a further aspect of the present invention there is provided an assay method for the detection of a binding ligand which causes
20 activation of SIGIRR, the assay comprising the steps of:

20

- providing a cellular sample comprising cells expressing SIGIRR, bringing said cells into contact with the ligand, and
- detecting the activation of PPAR,

wherein activation of PPAR is indicative of the binding of a ligand to
25 SIGIRR.

25

In one embodiment, the isotype of PPAR is PPARgamma.

30

In one embodiment the level of activation of SIGIRR can be compared to a control sample, such as the same type of cells which are not exposed to

the ligand. Alternatively, the test sample can be controlled to a known, pre-determined reference value.

5 A yet further aspect of the invention provides a kit for the determination of the activation status of the SIGIRR receptor, the kit comprising a reference sample, means for determining the activation status of SIGIRR and instructions for the performance of any of the assays of the invention using the methods described in said aspects.

10 As discussed briefly above, the present inventors have made the further observation that PPAR activation results following IL-1F5 binding to SIGIRR. PPARgamma has been shown to have a role in IL-1F5 inhibition of LPS induced IL-1 expression in glial cells. As shown in the examples section, the use of a PPARgamma antagonist abolishes the IL-1F5
15 inhibition of LPS induced IL-1 expression indicating that PPARgamma has a role in IL-1F5 mediated activation of an anti-inflammatory response, and in particular the suppression of an IL-1 beta mediated pro-inflammatory response.

20 Peroxisome proliferators activated receptors (PPARs) are nuclear proteins that modulate gene expression. PPARs act as ligand activated transcription factors that increase transcription of target genes by binding a specific nucleotide sequence in the gene's promoter.

25 There are 3 isotypes of PPAR – alpha, beta and gamma. The present inventors have shown that IL-1F5 increases PPARgamma expression in mixed glial cells. PPARgamma (475/505 amino acids is located on chromosome 3p25.1). IL-1F5 PPARgamma activation is necessary for IL-1F5 induced inhibition of IL-1 beta.

Accordingly a further still aspect of the present invention provides a method for the prophylaxis and/or treatment of an immune-mediated disorder comprising the step of activating PPAR.

- 5 In one embodiment, the PPAR isotype which is activated is PPARgamma. This activation of PPARgamma modulates the increased expression of IL-4.

- 10 Accordingly, in a still further aspect of the present invention there is provided the use of PPARgamma to modulate the activity of IL-1F5.

- A further still aspect of the present invention provides a method of treating an immune-mediated condition in a subject comprising administering to said subject a therapeutically effective amount of a molecule which
15 induces activation and expression of PPARgamma.

In one embodiment the individual is a mammal. In a further embodiment the mammal is a human.

- 20 A yet further aspect of the present invention provides a pharmaceutical composition comprising a peptide comprising the amino acid sequence of SEQ ID NO:2, a sequence which is at least 80% homologous thereto, or an analogue, derivative, fragment, variant or peptidomimetic thereof.

- 25 A yet further aspect of the present invention provides an immunomodulator comprising a peptide comprising the amino acid sequence of SEQ ID NO:2, a sequence which is at least 80% homologous thereto, or an analogue, derivative, fragment, variant or peptidomimetic thereof.

A yet further aspect of the present invention provides a vaccine composition comprising a peptide comprising the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4, a sequence which is at least 80% homologous thereto, or an analogue, derivative, fragment, variant or
5 peptidomimetic thereof.

A yet further aspect of the present invention provides an antibody or binding fragment with specificity to a peptide comprising the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4, a sequence which is at least
10 80% homologous thereto, or an analogue, derivative, fragment, variant or peptidomimetic thereof.

A yet further aspect of the present invention provides an antibody or binding fragment with specificity to SIGIRR or a derivative or mutant or
15 fragment or variant or peptide thereof.

A still further aspect of the present invention provides an antibody or binding fragment with specificity to PPARgamma or a derivative or mutant or fragment or variant or peptide thereof.
20

Assays

The invention extends to assay systems and screening methods for determining modulators of SIGIRR activation and further to methods for monitoring SIGIRR activation by means of monitoring PPAR activation. As
25 used herein, an "assay system" encompasses all the components required for performing and analysing results of an assay that detects and/or measures a particular event or events.

A variety of assays are available to detect the activation status of PPAR,
30 these will be known to the person skilled in the art.

It is preferred, though not essential that the screening assays employed in the present invention are high throughput or ultra high throughput and thus provide an automated, cost-effective means of screening.

5

Analogues and derivatives

The present invention extends to peptides which are derivatives or homologues of IL-1F5, such peptides may have a sequence which has at least about 30%, or 40%, or 50%, or 60%, or 70%, or 75%, or 80%, or
10 85%, or 90%, 95%, 98% or 99% homology to the amino acid sequence of IL-1F5 as defined in SEQ ID NO:2 or SEQ ID NO:4. Thus, a peptide derivative of the IL-1F5 peptide of the invention may include a number of amino acid alterations, for example 1, 2, 3, 4, 5 or greater than 5 amino acid alterations.

15

Moreover, or in addition, the peptide may consist of a truncated version of IL-1F5 which has been truncated by 1, 2, 3, 4 or 5 amino acids.

20

The percentage identity of two amino acid sequences or of two nucleic acid sequences may be determined by aligning the sequences for optimal comparison purposes (e.g. gaps can be introduced in the first sequence for best alignment with the sequence) and comparing the amino acid residues or nucleotides at corresponding positions. The "best alignment" is an alignment of two sequences which results in the highest percent
25 identity. The percent identity is determined by the number of identical amino acid residues or nucleotides in the sequences being compared (i.e., % identity = number of identical positions/total number of positions x 100).

30

The determination of percent identity between two sequences can be accomplished using a mathematical algorithm known to those skilled in the

art. The NBLAST and XBLAST programs are examples of computer programs which perform such algorithms. BLAST nucleotide searches can be performed with the NBLAST program to obtain nucleotide sequences homologous to nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program to obtain amino acid sequences homologous to protein molecules of the invention.

To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilised. Alternatively, PSI-Blast can be used to perform an iterated search which detects distant relationships between molecules (Idem.). When utilising BLAST, Gapped BLAST, and PSI-Blast programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. See URL <http://www.ncbi.nlm.nih.gov>.

A derivative of a peptide for which the specific sequence is disclosed herein may be in certain embodiments the same length or shorter than the specific peptide. In other embodiments, the peptide sequence or a variant thereof may include a larger peptide.

As is well understood, homology at the amino acid level is generally in terms of amino acid similarity or identity. Similarity allows for 'conservative variation', such as substitution of one hydrophobic residue such as isoleucine, valine, leucine or methionine for another, or the substitution of one polar residue for another, such as lysine, glutamic acid for aspartic acid, or glutamine for asparagine.

Analogues of, and for use in, the invention as defined herein means a peptide modified by varying the amino acid sequence e.g. by manipulation of the nucleic acid encoding the protein or by altering the protein itself.

Such derivatives of the amino acid sequence may involve insertion, addition, deletion and/or substitution of one or more amino acids.

Mimetics of IL-1F5

5 In addition to fragments or derivatives of IL-1F5, non-peptide mimetics of the IL-1F5 polypeptide can be used in the performance of the present invention. Such mimetics may be prepared, either wholly or partly, by chemical synthesis. Generation of the peptides in this way can be performed by methods which are well known to the person skilled in the art.
10

Treatment / Therapy

The term 'treatment' is used herein to refer to any regime that can benefit a human or non-human animal. The treatment may be in respect of an existing condition or may be prophylactic (preventative treatment).
15 Treatment may include curative, alleviation or prophylactic effects.

More specifically, reference herein to "therapeutic" and "prophylactic" treatment is to be considered in its broadest context. The term
20 "therapeutic" does not necessarily imply that a subject is treated until total recovery. Similarly, "prophylactic" does not necessarily mean that the subject will not eventually contract a disease condition.

Accordingly, therapeutic and prophylactic treatment includes amelioration
25 of the symptoms of a particular condition or preventing or otherwise reducing the risk of developing a particular condition. The term "prophylactic" may be considered as reducing the severity or the onset of a particular condition. "Therapeutic" may also reduce the severity of an existing condition.

Administration

IL-1F5, or a variant, derivative, analogue or fragment thereof for use in the present invention may be administered alone but will preferably be administered as a pharmaceutical composition, which will generally
5 comprise a suitable pharmaceutical excipient, diluent or carrier selected depending on the intended route of administration.

IL-1F5, or a variant, analogue or fragment thereof for use in the present invention may be administered to a patient in need of treatment via any
10 suitable route. The precise dose will depend upon a number of factors, including the precise nature of the form of IL-1F5 to be administered.

Routes of administration may include; parenterally (including subcutaneous, intramuscular, intravenous, by means of, for example a
15 drip patch), some further suitable routes of administration include (but are not limited to) oral, rectal, nasal, topical (including buccal and sublingual), infusion, vaginal, intradermal, intraperitoneally, intracranially, intrathecal and epidural administration or administration via oral or nasal inhalation, by means of, for example a nebuliser or inhaler, or by an implant.

20 The composition may also be administered via microspheres, liposomes, other microparticulate delivery systems or sustained release formulations placed in certain tissues including blood. Suitable examples of sustained release carriers include semipermeable polymer matrices in the form of
25 shared articles, e.g. suppositories or microcapsules.

Examples of the techniques and protocols mentioned above and other techniques and protocols which may be used in accordance with the invention can be found in Remington's Pharmaceutical Sciences, 18th
30 edition, Gennaro, A.R., Lippincott Williams & Wilkins; 20th edition

(December 15, 2000) ISBN 0-912734-04-3 and Pharmaceutical Dosage Forms and Drug Delivery Systems; Ansel, H.C. et al. 7th Edition ISBN 0-683305-72-7 the entire disclosures of which is herein incorporated by reference.

5

Administration of IL-1F5 to the brain, neuronal tissue or CNS

In preferred embodiments, the IL-1F5 polypeptide or fragment may be administered directly to the brain or other suitable site of the central nervous system (CNS) in order to deliver the IL-1F5 to the neural tissue.

10 In particular, the compositions will be administered in such a way that they are directed to the hippocampus, and in particular can be directed to the microglial cells.

15 In specific embodiments of the present invention, the IL-1F5 polypeptide or fragment may delivered to the brain by means of intracerebroventricular injection (ICV) or through delivery into the CNS using minipumps.

20 Alternatively a viral vector may be used to target delivery of the the IL-1F5 polypeptide or fragment to the brain, CNS or neural tissue. Such a vector will include a construct which contains a gene encoding for IL-1F5 or a fragment or analogue thereof. The construct may further contain a promoter which is provided adjacent to the gene and which controls expression of the gene. Viral vector which may be suitable for such delivery and targeting may be nonreplicative herpes simplex type 1 viruses (see for example Poliani et al. (Delivery to the central nervous system of a nonreplicative herpes simplex type 1 vector engineered with the interleukin 4 gene protects rhesus monkeys from hyperacute autoimmune encephalomyelitis. Hum Gene Ther. 2001 May 20;12(8):905-20.); Semliki Forest virus, see Jerusalmi et al. (Effect of intranasal administration of Semliki Forest virus recombinant particles expressing reporter and

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cytokine genes on the progression of experimental autoimmune encephalomyelitis. Mol. Ther. 2003 Dec; 8(6):886-94.) and adenovirus, for example see Braciack et al. (Protection against experimental autoimmune encephalomyelitis generated by a recombinant adenovirus vector expressing the V beta 8.2 TCR is disrupted by co-administration with vectors expressing either IL-4 or -10. J Immunol. 2003 Jan 15; 170(2):765-74.)

In further embodiments, naked plasmid DNA encoding for IL-1F5 or fragments and analogues thereof may be directly administered.

Sequence homology

A particularly preferred nucleotide sequences of the invention is the human IL-1F5 sequence set forth in SEQ ID NO:1. The sequences of the amino acid encoded by the DNA of SEQ ID NO:1 is shown in SEQ ID NO:2.

Due to the known degeneracy of the genetic code, wherein more than one codon can encode the same amino acid, a DNA sequence can vary from that shown in SEQ ID NO:1 or SEQ ID no:3 and still encode a polypeptide having the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4 respectively. Such variant DNA sequences can result from silent mutations (e.g., occurring during PCR amplification), or can be the product of deliberate mutagenesis of a native sequence.

The invention thus provides isolated DNA sequences encoding polypeptides of the invention, selected from: (a) DNA comprising the nucleotide sequence of SEQ ID NO:1 or SEQ ID NO:3 (b) DNA encoding the polypeptide of SEQ ID NO:2 or SEQ ID NO:4 (c) DNA capable of hybridization to a DNA of (a) or (b) under conditions of moderate

stringency and which encodes polypeptides of the invention; (d) DNA capable of hybridization to a DNA of (a) or (b) under conditions of high stringency and which encodes polypeptides of the invention, and (e) DNA which is degenerate as a result of the genetic code to a DNA defined in (a), (b), (c), or (d) and which encode polypeptides of the invention. Of course, polypeptides encoded by such DNA sequences are encompassed by the invention.

"Stringency" of hybridization reactions is readily determinable by one of ordinary skill in the art, and generally is an empirical calculation dependent upon probe length, washing temperature, and salt concentration. In general, longer probes require higher temperatures for proper annealing, while shorter probes need lower temperatures. Hybridization generally depends on the ability of denatured DNA to reanneal when complementary strands are present in an environment below their melting temperature. The higher the degree of desired homology between the probe and hybridizable sequence, the higher the relative temperature which can be used. As a result, it follows that higher relative temperatures would tend to make the reaction conditions more stringent, while lower temperatures less so. For additional details and explanation of stringency of hybridization reactions, see Ausubel et al., Current Protocols in Molecular Biology, Wiley Interscience Publishers, (1995).

As herein defined, "stringent conditions" or "highly stringency conditions", may be identified by those that: (1) employ low ionic strength and high temperature for washing, for example 0.015 M sodium chloride/0.0015 M sodium citrate/0.1% sodium dodecyl sulfate at 50°C.; (2) employ during hybridization a denaturing agent, such as formamide, for example, 50% (v/v) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750

mM sodium chloride, 75 mM sodium citrate at 42°C.; or (3) employ 50% formamide, 5*SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5* Denhardt's solution, sonicated salmon sperm DNA (50 mug/ml), 0.1% SDS, and 10% dextran sulfate at 42°C., with washes at 42°C. in 0.2*SSC (sodium chloride/sodium citrate) and 50% formamide at 55°C, followed by a high-stringency wash consisting of 0.1*SSC containing EDTA at 55°C.

"Moderately stringent conditions" may be identified as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, New York: Cold Spring Harbor Press, 1989, and include the use of washing solution and hybridization conditions (e.g., temperature, ionic strength and %SDS) less stringent than those described above. An example of moderately stringent conditions is overnight incubation at 37°C. in a solution comprising: 20% formamide, 5*SSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5 x Denhardt's solution, 10% dextran sulfate, and 20 mg/ml denatured sheared salmon sperm DNA, followed by washing the filters in 1*SSC at about 37-50°C. The skilled artisan will recognize how to adjust the temperature, ionic strength, etc. as necessary to accommodate factors such as probe length and the like.

The invention thus provides equivalent isolated DNA sequences encoding biologically active forms of the IL-1F5 polypeptide selected from: (a) DNA derived from the coding region of IL-1F5; (b) DNA of SEQ ID NO:1, (c) DNA capable of hybridization to a DNA of (a) or (b) under conditions of moderate stringency and which encodes biologically active IL-1F5 polypeptides; and (d) DNA that is degenerate as a result of the genetic code to a DNA defined in (a), (b) or (c), and which encodes biologically active IL-1F5 polypeptides, such as those defined in SEQ ID NO:2.

As used herein, conditions of moderate stringency can be readily determined as defined above. The basic conditions are set forth by Sambrook et al. Molecular Cloning: A Laboratory Manual, 2 ed. Vol. 1, pp. 1.101-104, Cold Spring Harbor Laboratory Press, (1989). Conditions of
5 high stringency can also be readily determined as described above.

Also included as an embodiment of the invention is DNA encoding polypeptide fragments and polypeptides comprising inactivated N-glycosylation site(s), inactivated protease processing site(s), or
10 conservative amino acid substitution(s).

Embodiments of the invention extend to nucleic acid molecules that are at least 80% identical to the IL-1F5 sequence as provided in SEQ ID NO:1 or SEQ ID NO:3. Also contemplated are embodiments in which a nucleic
15 acid molecule comprises a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 99.5% identical to the native IL-1F5 sequence.

The percent identity may be determined by visual inspection and
20 mathematical calculation. Alternatively, the percent identity of two nucleic acid sequences can be determined by comparing sequence information using a computer programme. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways using publicly available computer software such as BLAST or ALIGN.
25 Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. In one embodiment, the GAP computer program, version 6.0 described by Devereux et al. (Nucl. Acids Res. 12:387, 1984) and available from the
30 University of Wisconsin Genetics Computer Group (UWGCG) is used.

The preferred default parameters for the GAP program include: (1) a unary comparison matrix (containing a value of 1 for identities and 0 for non-identities) for nucleotides, and the weighted comparison matrix of Gribskov and Burgess, Nucl. Acids Res. 14:6745, 1986, as described by Schwartz and Dayhoff, eds., Atlas of Protein Sequence and Structure, National Biomedical Research Foundation, pp. 353-358, 1979; (2) a penalty of 3.0 for each gap and an additional 0.10 penalty for each symbol in each gap; and (3) no penalty for end gaps. Other programs used by one skilled in the art of sequence comparison may also be used.

The invention also provides isolated nucleic acids useful in the production of polypeptides. Such polypeptides may be prepared by any of a number of conventional techniques. A DNA sequence encoding the IL-1F5 polypeptide, or desired fragment thereof, may be subcloned into an expression vector for production of the polypeptide or fragment. The DNA sequence advantageously is fused to a sequence encoding a suitable leader or signal peptide. Alternatively, the desired fragment may be chemically synthesized using known techniques. DNA fragments also may be produced by restriction endonuclease digestion of a full length cloned DNA sequence, and isolated by electrophoresis on agarose gels. If necessary, oligonucleotides that reconstruct the 5' or 3' terminus to a desired point may be ligated to a DNA fragment generated by restriction enzyme digestion. Such oligonucleotides may additionally contain a restriction endonuclease cleavage site upstream of the desired coding sequence, and position an initiation codon (ATG) at the N-terminus of the coding sequence.

The invention encompasses polypeptides and fragments thereof in various forms, including those that are naturally occurring or produced through various techniques such as procedures involving recombinant DNA

technology. For example, DNAs encoding IL-1F5 polypeptides can be derived from SEQ ID NO:1 or SEQ ID NO:3 by *in vitro* mutagenesis, which includes site-directed mutagenesis, random mutagenesis, and *in vitro* nucleic acid synthesis. Such forms include, but are not limited to, derivatives, variants, and oligomers, as well as fusion proteins or fragments thereof.

The polypeptides of the invention include full length proteins encoded by the nucleic acid sequence of SEQ ID NO:1 or SEQ ID NO:3. A particularly preferred polypeptide comprises the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4. Also provided herein are polypeptide fragments of varying lengths. Naturally occurring variants as well as derived variants of the polypeptides and fragments are also provided herein.

An "IL-1F5 variant" as referred to herein means a polypeptide substantially homologous to IL-1F5, but which has an amino acid sequence different from that of the native IL-1F5 polypeptide because of one or more deletions, insertions, or substitutions. The variant has an amino acid sequence that preferably is at least 80% identical to an IL-1F5 polypeptide amino acid sequence, most preferably at least 90% identical. The percent identity may be determined, for example, by comparing sequence information using the GAP computer program, version 6.0 described by Devereux et al. (Nucl. Acids Res. 12:387, 1984) and available from the University of Wisconsin Genetics Computer Group (UWGCG).

Variants also include embodiments in which a polypeptide or fragment comprises an amino acid sequence that is at least 90% identical, at least 95% identical, at least 98% identical, at least 99% identical, or at least 99.9% identical to the preferred polypeptide or fragment thereof.

1 Variants include polypeptides that are substantially homologous to the native form, but which have an amino acid sequence different from that of the native form because of one or more deletions, insertions or substitutions. Particular embodiments include, but are not limited to, polypeptides that comprise from one to ten deletions, insertions or substitutions of amino acid residues, when compared to a native sequence.

10 A given amino acid may be replaced, for example, by a residue having similar physiochemical characteristics. Examples of such conservative substitutions include substitution of one aliphatic residue for another, such as Ile, Val, Leu, or Ala for one another; substitutions of one polar residue for another, such as between Lys and Arg, Glu and Asp, or Gln and Asn; or substitutions of one aromatic residue for another, such as Phe, Trp, or Tyr for one another. Other conservative substitutions, e.g., involving substitutions of entire regions having similar hydrophobicity characteristics, are well known.

Combinatorial Library

20 Combinatorial library technology (Schultz, JS (1996) Biotechnol. Prog. 12:729 – 743) provides an efficient way of testing a potentially vast number of different substances for ability to modulate activity of a polypeptide. Prior to or as well as being screened for modulation of activity, test substances may be screened for ability to interact with the polypeptide, e.g. in a yeast two-hybrid system (which requires that both the polypeptide and the test substance can be expressed in yeast from encoding nucleic acid). This may be used as a coarse screen prior to testing a substance for actual ability to modulate activity of the polypeptide.

The amount of test substance or compound which may be added to an assay of the invention will normally be determined by trial and error depending upon the type of compound used. Typically, from about 0.01 to 100 nM concentrations of putative inhibitor compound may be used, for example from 0.1 to 10 nM. Greater concentrations may be used when a peptide is the test substance.

Identification of Inhibitors

Compounds which may be used may be natural or synthetic chemical compounds used in drug screening programmes. Extracts of plants which contain several characterised or uncharacterised components may also be used. A further class of putative SIGRR inhibitor compounds can be derived from the IL-1F5 polypeptide and/or a ligand which binds the same. Peptide fragments of from 5 to 40 amino acids, for example from 6 to 10 amino acids from the region of the relevant polypeptide responsible for interaction, may be tested for their ability to disrupt such interaction.

Other candidate inhibitor compounds may be based on modelling the 3-dimensional structure of a polypeptide or peptide fragment and using rational drug design to provide potential inhibitor compounds with particular molecular shape, size and charge characteristics.

Following identification of a substance which modulates or affects IL-1F5 or SIGIRR binding activity, the substance may be investigated further. Furthermore, it may be manufactured and/or used in preparation, i.e. manufacture or formulation, of a composition such as a medicament, pharmaceutical composition or drug. These may be administered to individuals.

Mimetics

A substance identified as a modulator of SIGIRR binding and activation may be peptide or non-peptide in nature. Non-peptide "small molecules" are often preferred for many *in vivo* pharmaceutical uses. Accordingly, a
5 mimetic or mimic of the substance (particularly is a peptide) may be designed for pharmaceutical uses. The designing of mimetics to a known pharmaceutically active compound is a known approach to the development of pharmaceuticals based on a "lead" compound. This might be desirable where the active compound is difficult or expensive to
10 synthesise of where it is unsuitable for a particular method of administration, e.g. peptides are not well suited as active agents for oral compositions as they tend to quickly degraded by proteases in the alimentary canal. Mimetic design, synthesis and testing may be used to avoid randomly screening large number of molecules for a target property.

15 There are several steps commonly taken in the design of a mimetic from a compound having a given target property. Firstly, the particular parts of the compound that are critical and/or important in determining the target property are determined. In the case of a peptide, this can be done by
20 systematically varying the amino acid residues in the peptide, e.g. by substituting each residue in turn. These parts or residues constituting the active region of the compound are known as its "pharmacophore".

Once the pharmacophore has been found, its structure is modelled to
25 according its physical properties, e.g. stereochemistry, bonding, size and/or charge, using data from a range of sources, e.g. spectroscopic techniques, X-ray diffraction data and NMR. Computational analysis, similarity mapping (which models the charge and/or volume of a pharmacophore, rather than the bonding between atoms) and other
30 techniques can be used I this modelling process.

In a variant of this approach, the three-dimensional structure of the ligand and its binding partner are modelled. This can be especially useful where the ligand and/or binding partner change conformation on binding,
5 allowing the model to take account of this the design of the mimetic.

A template molecule is then selected onto which chemical groups which mimic the pharmacophore can be grafted. The template molecule and the chemical groups grafted on to it can conveniently be selected so that the
10 mimetic is easy to synthesise, is likely to be pharmacologically acceptable, and does not degrade *in vivo*, while retaining the biological activity of the led compound. The mimetic or mimetics found by this approach can then be screened to see whether they have the target property, or to what extent they exhibit it. Further optimisation or modification can then be
15 carried out to arrive at one or more final mimetics for *in vivo* or clinical testing.

Mimetics of substances identified as having ability to modulate the SIGIRR activation are included within the scope of the present invention. A
20 polypeptide, peptide or substance which can modulate the activity of a polypeptide according to the present invention may be provided in a kit, e.g. sealed in a suitable container which protects its contents from the external environment. Such a kit may include instructions for use.

25 Peptidomimetics

Whilst numerous strategies to improve the pharmaceutical properties of peptides found to exert biological effects are known in the art including, for example, amide bond replacements, incorporation of non-peptide
moieties, peptide small molecule conjugates or backbone cyclisation, the
30 optimisation of pharmacological properties for particular peptides still

presents those involved in the optimisation of such pharmaceutical agents with considerable challenges.

5 Peptides of and for use in the present invention may be modified such that they comprise amide bond replacement, incorporation of non peptide moieties, or backbone cyclisation. Suitably if cysteine is present the thiol of this residue is capped to prevent damage of the free sulphate group. Suitably a peptide of and for use in the present invention may be modified from the natural sequence to protect the peptides from protease attack.

10

Suitably a peptide of and for use in the present invention may be further modified using at least one of C and / or N-terminal capping, and / or cysteine residue capping. Suitably a peptide of and for use in the present invention may be capped at the N terminal residue with an acetyl group.

15

Suitably a peptide of and for use in the present invention may be capped at the C terminal with an amide group. Suitably the thiol groups of cysteines are capped with acetamido methyl groups.

Pharmaceutical Compositions

20

As described above, the present invention extends to a pharmaceutical composition for the treatment of immune-mediated, autoimmune and neurodegenerative conditions, wherein the composition comprises IL-1F5 or a fragment, derivative, mimetic or analogue thereof. Pharmaceutical compositions for use in accordance with the present invention may

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comprise, in addition to active ingredient (i.e. IL-1F5), a pharmaceutically acceptable excipient, carrier, buffer stabiliser or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material will depend on the route of administration,

which may be, for example, oral, intravenous, intranasal or via oral or nasal inhalation.

Dose

- 5 The IL-1F5 is preferably administered to an individual in a “therapeutically effective amount”, this being sufficient to show benefit to the individual.

The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of the condition
10 being treated.

Prescription of treatment, e.g. decisions on dosage etc, is ultimately within the responsibility and at the discretion of general practitioners, physicians or other medical doctors, and typically takes account of the disorder to be
15 treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners.

The optimal dose can be determined by physicians based on a number of parameters including, for example, age, sex, weight, severity of the
20 condition being treated, the active ingredient being administered and the route of administration.

Preferred features and embodiments of each aspect of the invention are as for each of the other aspects mutatis mutandis unless the context
25 demands otherwise.

Unless otherwise defined, all technical and scientific terms used herein have the meaning commonly understood by a person who is skilled in the art in the field of the present invention.
30

Throughout the specification, unless the context demands otherwise, the terms 'comprise' or 'include', or variations such as 'comprises' or 'comprising', 'includes' or 'including' will be understood to imply the inclusion of a stated integer or group of integers, but not the exclusion of any other integer or group of integers.

Detailed Description of the Invention

The present invention will now be described with reference to the following examples which are provided for the purpose of illustration and are not intended to be construed as being limiting on the present invention, and further, with reference to the figures.

Brief description of the drawings

Figure 1 LTP is impaired by LPS administration but maintained by treatment with IL-1F5. LTP in dentate gyrus was similar in saline treated rats (Δ) and IL-1F5 treated ($\blacksquare$). LTP is significantly impaired in LPS-treated rats (Δ) but is maintained in rats treated with LPS and IL-1F5 ($\square$). The mean slope of the population EPSP evoked by test stimuli delivered at 30 sec intervals before and after tetanic stimulation is shown. Population EPSP slope is expressed as a percentage of the slope recorded in the 5 min immediately prior to tetanic stimulation and values are expressed as means $\pm$ standard error of the man of 6 observations. SEMS are included for every 5th response,

Figure 2 shows LPS administration affects percentage change in EPSP slope; reversal by treatment with IL-1F5. A - The mean percentage in EPSP slope in the first 2 min post tetanic stimulation (compared with the 5 min period immediately proceeding tetanus) was decreased in LPS treated rats compared with saline treated

rats (n=6). LPS + IL-1F5 treated rats had significantly greater EPSP slopes compared to LPS treated rats (*p<0.05; ANOVA; n=6 in all cases). Values are expressed as the percentage change in EPSP slope and are means $\pm$ SEM of the means. B - The mean percentage change in EPSP slope in the last 5 min of recording (compared with the 5 min period immediately preceding tetanus) was significantly decreased in LPS treated rats (*p<0.05; ANOVA; n=6 in all groups). Values are expressed as percentage change in EPSP slope and are means $\pm$ SEM,

Figure 3 shows LPS administration is associated with an increase in the concentration of interleukin-1 β in hippocampus. Interleukin-1 β (IL-1 β) concentration was significantly increased in hippocampal tissue prepared from LPS-treated rats (n=6) compared with tissue prepared from saline treated rats (n=6; *p<0.05; ANOVA). There was no significant difference in IL-1 β concentration in tissue prepared from saline-treated rats and tissue prepared from IL-1F5 treated rats (n=6). IL-1 β concentration was significantly decreased in hippocampal tissue prepared from rats treated with LPS in combination with IL-1F5 treated rats compared with LPS treated rats (n=6; +p<0.05; ANOVA),

Figure 4 LPS administration is associated with enhanced immunopositive staining for Ox-6. Immunohistochemical images show enhanced Ox-6 positive staining in the hippocampus of LPS treated rats compared with control treated rats. There was no observable difference in staining in sections prepared from control treated rats compared with sections from IL-1F5 treated rats. There was no observable difference in staining between saline-treated rats and rats treated with LPS in combination with IL-1F5 (n=4 in all

cases). Magnification is at x40 and scale bar is 10µm. The blue colour present in the samples represents the counter stain which was used. Figure 4B represents a typical microglial cell in activated state,

5

Figure 5 shows IL-4 concentration is increased in hippocampus following IL-1F5 treatment. Hippocampal IL-4 concentration was significantly increased in hippocampal tissue prepared from rats treated with IL-1F5 compared with saline-treated controls (*p<0.05; ANOVA; n=6). IL-4 concentration was similar to control treated in the hippocampus of rats treated with LPS alone or LPS + IL-1F5. Values are expressed as pg IL-4/mg tissue corrected for protein and are means ± SEM,

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Figure 6 shows IL-1F5 treatment is associated with an increase in IL-4 protein in cultured cortical glial cells. Interleukin-4 (IL-4) concentration was significantly increased in IL-1F5 treated cells compared with control-treated cells (*p<0.05; students unpaired t test; n=6). Values are expressed as pg/mg supernatant and are means ± SEM,

20

Figure 7 IL-1F5 treatment is associated with an increase in IL-4 mRNA in cultured cortical glial cells. IL-4 mRNA was increased in IL-1F5 treated cells compared with control treated cells. One sample blot shows that IL-4 mRNA expression was increased in cells treated with IL-1F5 compared with control treated cells. Mean data was obtained from densitometric analysis and results are expressed as mean ± SEM,

25

Figure 8 shows pre-incubation with an antibody for the novel IL-1 receptor SIGIRR is associated with inhibition of IL-1F5 induced increase in IL-4 in cultured cortical glia. IL-4 concentration was significantly increased in cortical glial cells treated with IL-1F5 (n=6) compared with control-treated cells (n=6; *p<0.05; ANOVA). There was no significant difference between control-treated cells and cells treated with IL-1F5 following 4 hour pre-incubation with Anti-SIGIRR, or cells treated with Anti-SIGIRR alone (N=6 in all groups),

Figure 9 shows IL-1F5 treatment is associated with an increased surface expression of the novel IL-1 receptor SIGIRR in cultural cortical glial cells. Surface expression of SIGIRR was upregulated in cells treated with IL-1F5 compared with control-treated cells (n=4). There was no observable difference in SIGIRR expression in cells that were control-treated compared with cells treated with IL-1 β or IL-1F5 in combination with IL-1F5. Scale bar is 10 μ m,

Figure 10 shows IL-1 β induced inhibition of LTP is abrogated by IL-1F5. LTP in the dentate gyrus is similar in saline treated rats ($\bullet$), rats treated with IL-1F5 ($\square$) and rats treated with IL-1F5 and IL-1beta ($\diamond$). LTP in the dentate gyrus is significantly impaired in IL-1beta treated rats (Δ) but is maintained in rats co treated with IL-1beta and IL 1F5 ($\diamond$). The mean slope of the population EPSP evoked by test stimuli delivered at 30 second intervals before and after tetanic stimulation is shown. Population EPSP slope is expressed as a percentage of the slope recorded in the 5 min immediately prior to tetanic stimulation and values are expressed as means $\pm$ standard error of the mean of 6 observations. Standard errors are included for every 5th response,

Figure 11 shows IL-1beta administration affects percentage change in EPSP slope; reversal by treatment with IL-1F5. The mean percentage change in EPSP slope in the first 2 min post tetanic stimulation (compared with the 5 min period immediately proceeding tetanus) was decreased in IL-1beta treated with IL-1beta and IL-1F5 were significantly increased compared to LPS treated rats (*p<0.05; ANOVA; n=6 in all cases). Values are expressed as percentage change in EPSP slope and are means $\pm$ SEM. A - The mean percentage change in EPSP slope in the last 5 minutes of recording post tetanic stimulation (compared with the 5 minute period immediately preceding tetanus) was significantly decreased in IL-1beta treated rats (*p<0.005; ANOVA: n=6 in all groups). Values are expressed as percentage change in EPSP slope and are means $\pm$ SEM,

Figure 12 shows that IL-1beta administration is associated with an increase in JNK phosphorylation in the hippocampus. One sample immunoblot shows that phosphorylation of JNK (46kDa) was increased in hippocampal tissue prepared from IL-1beta treated rats (lane 2) compared with tissue prepared from saline-treated rats (lane 1), tissue prepared from IL-1F5 treated rats (lane 3) and tissue prepared from rats co treated with IL-1beta and IL-1F5 in combination (lane 4). A - Densitometric analysis revealed that mean JNK phosphorylation, expressed as a ratio with actin was increased in hippocampal tissue prepared from IL-1beta treated rats compared with tissue prepared from saline treated rats (*p<0.05; ANOVA; n=6 in both groups). There was no significant difference between control-treated, IL-1F5 treated and rats co-treated with IL-1beta and IL-1F5 (n=6 in all cases). The mean value was significantly reduced in tissue prepared from rats treated

with both IL-1 β and IL-1F5, compared with tissue prepared from IL-1 β treated rats (* p <0.05; ANOVA; n =6). Values are expressed as arbitrary units and are means $\pm$ SEM. Beta-actin expression was used to check for equal protein loading and remained unchanged in all samples,

Figure 13 shows that IL-4 concentration is increased in hippocampus following IL-1F5 treatment. Hippocampal interleukin-4 (IL-4) concentration was significantly increased in rats treated with IL-1F5 compared with saline-treated rats (* p <0.05; ANOVA; n =6). Mean IL-4 concentration was similar in tissue prepared from IL-1 β treated rats and rats treated with both IL-1 β and IL-1F5 treated rats. Values are expressed as pg IL-4/mg tissue corrected for protein and are means $\pm$ SEM,

Figure 14 shows that IL-1F5 treatment is associated with an increase in JAK1 phosphorylation in hippocampus. Densitometric analysis revealed that mean JAK1 phosphorylation was increased in hippocampal tissue prepared from IL-1F5 treated rats compared with tissue prepared from saline treated rats (* p <0.05; students unpaired t test; n =6 in both groups). Values are expressed as arbitrary units means $\pm$ SEM.

Figure 15 shows that IL-1F5 treatment is associated with an increase in STAT6 phosphorylation in hippocampus. Densitometric analysis revealed that mean STAT6 phosphorylation was increased in hippocampal tissue prepared from IL-1F5 treated rats compared with tissue prepared from saline treated rats (* p <0.05; students t test; n =6 in both groups) Values are expressed as arbitrary units of STAT6 phosphorylation and are means $\pm$ SEM.

Figure 16 shows that IL-1F5 treatment is associated with an increase in ERK1 and ERK2 phosphorylation in hippocampus. A - One sample immunoblot shows that both ERK 1 and ERK 2 phosphorylation was increased in hippocampal tissue prepared from IL-1F5 treated rats compared with tissue prepared from saline-treated rats. B - Densitometric analysis revealed that mean ERK 1 phosphorylation was significantly increased in hippocampal tissue prepared from IL-1F5 treated rats compared with tissue prepared from saline treated rats (* $p < 0.05$; student t test; $n = 6$ in both groups). Values are expressed as arbitrary units of ERK phosphorylation and are means $\pm$ SEM. C - A sample immunoblot shows no change in the expression of beta-actin in hippocampal tissue prepared from saline-treated rats and tissue prepared from IL-1F5 treated rats. Densitometric analysis revealed similar mean β -actin expression in hippocampal tissue prepared from both groups ($n = 6$ in both cases). Values are expressed as arbitrary units of beta-actin expression and are means $\pm$ SEM,

Figure 17 shows that IL-1F5 attenuates IL-1 β induced IL-6 protein and mRNA expression in mouse glia,

Figure 18 shows that intracerebroventricular injection of IL-1F5 attenuates LPS induced IL-6 in rat hippocampus,

Figure 19 shows that intracerebroventricular injection of IL-1F5 attenuates LPS-induced JNK phosphorylation in rat hippocampus,

Figure 20 shows that IL-1F5 suppresses LPS-induced IL-1 β production by mouse mixed glia cells but not by murine macrophages or dendritic cells,

5 Figure 21 shows that IL-1F5 suppresses LPS-induced IL-1 β protein production by mixed glia cells but not by macrophages,

10 Figure 22 shows that IL-1F5 induced IL-4 production from murine mixed glial cells but not from dendritic cells, spleen cells or macrophages,

Figure 23 shows that IL-1F5 induces IL-4 protein and mRNA expression in rat mixed glial cells,

15 Figure 24 shows that intracerebroventricular injection of rats with IL-1F5 significantly increased IL-4 expression in the hippocampus,

20 Figure 25 shows that IL-4 mediates the IL-1F5 attenuation of LPS-induced IL-1 β production from glial cells,

Figure 26 shows that the attenuation of LPS-induced IL-1 β production by IL-1F5 in the hippocampus is mediated through IL-4,

25 Figure 27 shows PPARgamma protein expression levels in primary cultured cortical glia following treatment with IL-1F5 (24 hour incubation),

Figure 28 shows a graph indicating a significant amount of PPARgamma expression induced by IL-1F5,

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Figure 29 shows a graph which indicates IL-1beta expression. As observed previously IL-1F5 inhibits LPS induced increases in IL-1beta expression. Co-treatment with a PPARgamma antagonist reduces this inhibitory effect of IL-1F5, indicating that PPARgamma plays a role in IL-1F5 functioning. Pre-incubation with a PPARgamma antagonist is associated with a reduction in IL-1F5 induced inhibition of IL-1beta in cultured cortical glia. IL-1beta concentration was significantly increased in cortical glial cells treated with LPS and this increase was inhibited following co-treatment with IL-1F5. Pre-treatment with a PPAR gamma antagonist abolished this inhibitory effect, indicating that IL-1F5 PPARgamma activation is necessary for IL-1F5 induced inhibition of IL-1beta,

Figure 30 shows the defined nucleotide sequence of human IL-1F5,

Figure 31 shows the defined amino acid sequence of human IL-1F5,

Figure 32 shows the defined nucleotide sequence of murine IL-1F5, and

Figure 33 shows the defined amino acid sequence of murine IL-1F5.

EXAMPLES

The effect of IL-1F5 administration on LPS induced effects in the brain was considered. The effect of IL-1F5 on IL-4 production was also examined as was the effect of IL-1F5 on the receptor SIGIRR.

Materials and Methods

Housing of Animals

An inbred strain of male Wistar rat was used for all experiments, aged between 2 and 4 months, supplied by the BioResources Unit, Trinity College Dublin. The animals weighed between 200 and 350g. Animals were housed in groups of 4, 5 or 6 and were maintained under a 24 hour light dark cycle in the BioResources Unit. Food, consisting of normal Laboratory chow and water was available ad libitum. Ambient temperature was controlled between 22 and 23°C.

Lipopolysaccharide (LPS) administration

In experimental treatment groups rats were anaesthetised by intraperitoneal (ip) injection of urethane (1.5g/kg; 33% w/v). Depth of anaesthesia was determined by the absence of the pedal reflex and if needed a top up dose of urethane was used (maximum 2.5g/kg). Rats were then injected ip with sterile 0.9% w/v saline or lipopolysaccharide (LPS; 100µg/kg in sterile 0.9% w/v saline) from Escherichia coli serotype 0111:B4 (Sigma, Dorset, UK). Three hours following administration of saline/LPS, rats were assessed for their ability to maintain long-term potentiation (LTP).

IL-1beta and IL-1F5 administration

In experimental treatment groups where rats were challenged with LPS, rats were given an intracerebroventricular (icv) injection. Fur on the scalp was clipped and the head was positioned in a head holder in a stereotaxic frame (ASI Instruments). A midline incision was made with a scalpel and the skin pulled back to reveal the skull. The peristeum was scraped clear and a Bregma and Lambda were identified. A dental drill was used to form a small hole in the skull 2.5mm posterior and 0.5mm lateral, to Bregma, through which agents could be injected. Rats were administered

IL-1F5 (3µg/ml; recombinant E.coli expressed) and 5 minutes later were administered saline or LPS by ip injection. Animals were assessed for their ability to sustain LTP 3 hours after injection. In some experiments rats were given IL-1beta (3.5ng/ml; R& D systems, UK) and IL-1F5 (3µg/ml) icv and in this case were assessed for their ability to maintain LTP after 30minutes.

Induction of LTP *in-vivo*

Preparation of animals

Following icv injection animals were assessed for their ability to sustain LTP. A window of the skull was removed using a dental drill, allowing correct placement of electrodes. The dura mater was pierced and pulled back to expose the brain. The recording chamber, consisting of the stereotaxic frame and amplifiers was surrounded by a Faraday cage to inhibit outside interference. All instruments within the cage were grounded to eliminate 50Hz cycle noise.

Electrode implantation

Bipolar stimulating electrodes and unipolar recording electrodes (Clark Electromedical, UK) were used in this study. The stimulating electrode was placed on the surface of the brain, 4.4mm lateral to lambda. The recording electrode was placed on the surface of the brain 2.5mm lateral and 3.9mm posterior to Bregma. The positions of the stimulating and recording electrodes were carefully monitored as they were lowered in increments through the cortical and hippocampal layers into the perforant path and granule cell layer of the dentate gyrus respectively, until the characteristic perforant path granule cell synapse response was observed. The depth of electrodes was finely adjusted so as to maximize the response. This was carried out by generating 4 V pulses of 0.1 msec duration through the stimulating electrode at a frequency of 0.1 Hz.

Evoked responses were picked up by the recording electrode and displayed on an Apple Macintosh computer (Performa 5200). The final depth of the recording electrode was between 2.5 and 3.5 mm and for the stimulating electrode was between 2.5 and 3 mm. Stimuli were then
5 delivered at 30 second intervals.

EPSP recordings

The population field post-synaptic potential (field EPSP) was used as a measure of excitatory synaptic transmission in the dentate gyrus. EPSPs
10 were recorded by passing a single square wave of current at low frequency (0.033Hz, 0.1sec, 2msec delay) which was generated by a constant isolation unit (IsoFlex, UK), to the bipolar stimulating electrode. The evoked response was transmitted via a pre-amplifier (DAM 50; Differential Amplifier; gain 75, World Precision Instruments, USA) to an
15 analogue digital converter (Maclab/2e, Analog Digital Instruments). This digitised system was then linked to an Apple Macintosh computer (Performa 5200) via a specifically written software package (Scope, Version 3.36).

20 The slope of EPSP was taken as an indicator of excitatory synaptic transmission. After a period of stabilization, test shocks at 1/30 sec were recorded for a 10min control period to establish baseline recordings. This was followed by a delivery of 3 trains of stimuli (250 Hz for 200 msec) at 30 second intervals. Recording at test shock frequency then resumed for
25 40 minutes.

Preparation of tissue

Dissection

Rats were killed by cervical dislocation and decapitation. The brains were
30 rapidly removed. One half of the brain was coated in OCT compound

(Sakura Tissue-Tek, Netherlands), immersed in liquid N₂ and stored at -80°C until sections were prepared. From the remaining half of the brain the hippocampus and cortex were quickly dissected free on ice.

5 Preparation of slices for freezing

Freshly dissected tissue was sliced bidirectionally to a thickness of 350µm using a McIlwain tissue chopper (Mickle Laboratory Engineering Co., Surrey, UK). Tissue was added in Eppendorf tubes containing 1ml Krebs solution (composition in mM: NaCl 136, KCl 2.54, KH₂PO₄ 1.18, 10 MgSO₄·7H₂O 1.18, NaHCO₃ 16, glucose 10) with added CaCl₂ (2mM final concentration). Tissue slices were vortexed and allowed to settle, before being washed twice more in this Krebs solution. The slices were rinsed with Krebs solution containing 2mM CaCl₂ containing 10% dimethyl sulphoxide and stored at -80°C until required for later analyses.

15

Protein Quantification

Protein quantification was assessed according to Bradford (1976). Standards were prepared from a 200µg/ml stock solution of bovine serum albumin (BSA; Sigma Dorset, UK). A range of standards was prepared from this solution from 200µg/ml to 3.125µg/ml. Standards were added in duplicate to the 96-well plate and Bio-Rad dye reagent concentrate (40µl; Bio-Rad, Hertfordshire, UK) was added to both standards and samples. Absorbance was measured at 630nm using a 96-well plate reader (Labsystems Multiskan RC, UK). A regression line was plotted (GraphPad Prism, US) and the concentration of protein was calculated and converted to mg protein/ml.

25

Analysis of Cytokines ex vivo

Preparation of samples

Hippocampal slices were thawed rapidly and washed 3 times in Krebs solution (composition in mM: NaCl 136, KCl 2.54, KH_2PO_4 1.18, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 1.18, NaHCO_3 16, glucose 10) containing 2mM CaCl_2 . The slices were homogenized (x60 strokes) in Krebs solution (400 μl) containing 2mM CaCl_2 using a 1ml glass homogeniser (Jencons, Bedfordshire, UK). Protein concentrations were assessed and equalized with Krebs solution containing 2mM CaCl_2 . Samples were stored at -80°C until required.

10 Analysis of interleukin-1 β concentration

IL-1 β concentration was assessed by Enzyme Linked ImmunoSorbent Assay (ELISA). 96-well plates (Nunc-Immuno plate with MaxiSorp surface) were coated with capture antibody (100 μl ; 1 $\mu\text{g}/\text{ml}$; goat anti-rat IL-1 β in PBS (137mM NaCl, 2.7mM KCl, 8.1mM Na_2HPO_4 and 1.5mM KH_2PO_4 pH7.3; R&D systems, Minneapolis, USA) and incubated overnight at room temperature (RT). A wash buffer of PBS containing 0.05% Tween-20, pH 7.4 was used to wash the plate 3 times and a blocking buffer (300 μl ; PBS containing 1% BSA, 5% sucrose and 0.05% NaN_3) was added to the wells and the plates were incubated at room temperature for 1 hour. Standards were prepared from rat recombinant IL-1 β (R&D systems Minneapolis, USA) diluted in PBS containing 1% BSA. Plates were washed 3 times in wash buffer and 100 μl of standards and samples were incubated for 2 hours at RT. Following this incubation, plates were washed 3 times with wash buffer and streptavidin-horseradish peroxidase conjugate (100 μl ; 1:200 dilution in PBS containing 1% BSA; R&D systems, Minneapolis, USA) was added and incubated at RT for 20 min. The plates were washed 3 times with wash buffer and substrate solution (100 μl ; 1:1 dilution Reagent A (H_2O_2) and Reagent B (tetramethylbenzidine); R&D systems, Minneapolis, USA) was added to the well. The plates were incubated in the dark for 40 min, creating a colour change to blue. A stop solution (1M

H₂SO₄; 50µl) was added to each well and plates were read at 450nm on a 96-well plate reader (Labsystems Multiskan RC). A standard curve was produced and results were expressed as pg IL-1beta/mg tissue corrected for protein or as pg/ml supernatant for in vitro experiments.

5

Analysis of interleukin-4 concentration

IL-4 concentration was assessed by Enzyme Linked ImmunoSorbent Assay (ELISA). 96-well plates (Nunc-Immuno plate with MaxiSorp surface) were coated with capture antibody (100µl; 2µg/ml; monoclonal mouse anti-rat IL-4 in PBS; R&D systems, Minneapolis, USA) and incubated overnight at RT. A wash buffer of PBS containing 0.05% Tween-20, pH 7.4 was used to wash the plate 3 times and a blocking buffer (300µl; PBS containing 1% BSA, 5% sucrose and 0.05% NaN₃) was added to the wells and the plates were incubated at RT for 1 hour. Standards were prepared from rat recombinant IL-4 (R&D systems Minneapolis, USA) diluted in PBS containing 1% BSA. Plates were washed 3 times in wash buffer and 100µl of standards and samples were incubated for 2 hours at RT. Following this incubation, plates were washed 3 times with wash buffer and detection antibody (100µl; 50ng/ml; biotinylated goat anti-rat IL-4 in PBS containing 1% BSA; R&D systems, Minneapolis, USA) was added and incubation continued for 2 hours at RT. The plates were washed 3 times in wash buffer and streptavidin horseradish peroxidase conjugate (100µl; 1:200 dilution in PBS containing 1% BSA; R&D systems, Minneapolis, USA) was added and incubation continued at RT for 1 hour. The plates were washed 3 times with wash buffer and substrate solution (100µl; 1:1 dilution Reagent A (H₂O₂) and Reagent B (tetramethylbenzidine); R&D systems, Minneapolis, USA) was added to the well. The plates were incubated in the dark for 40 minutes, creating a colour change to blue. A stop solution (1M H₂SO₄; 50µl) was added to each well. The plates were read at 450nm on a 96-well plate reader (Labsystems Multiskan RC). A

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standard curve was produced and results were expressed as pg IL-4/mg tissue corrected for protein (see section 2.5) or as pg/ml supernatant for in vitro experiments.

5 Experiments in-vitro

Preparation of cultured cells

Preparation of sterile coverslips

Glass coverslips (13mm diameter; Chance Propper, UK) were soaked in 70% ethanol for 1h followed by overnight exposure to ultraviolet light.

10 Coverslips were coated with poly-L-lysine (40µg/ml in sterile dH₂O; Sigma, UK) for 1 hour at 37°C so as to provide a suitable surface for cells to adhere. Coated coverslips were air-dried, placed in 24-well plates (Greiner, Austria) and stored at 4°C until required.

15 Primary culture of cortical glia

Glia were isolated from cerebral cortices of Wistar rats 1 day postpartum (BioResources Unit, Trinity College, Dublin). Rats were decapitated and cortices dissected as described above. Cortices were placed in 3 ml Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% foetal bovine serum (Gibco, UK), penicillin (100U/ml; Gibco, UK) and streptomycin (100U/ml; Gibco, UK). Tissue was triturated (x7), passed through a sterile nylon mesh filter and centrifuged (2500g for 3 min at 20°C). The pellet was resuspended in DMEM. Resuspended glia were placed on the centre of each coverslip (65µl per coverslip) and allowed to
25 adhere to the glass coverslip for 2 hours in a humidified incubator containing 5% CO₂: 95% air at 37°C before 400µl of pre-warmed DMEM was added to each well. Cells were grown for 10 days prior to treatment and media replaced every 3 days.

30 Cell Treatments

Treatment with IL-1F5

IL-1F5 was diluted to a stock concentration of 300µg/ml in PBS and stored at -80°C. For cell treatment IL-1F5 was diluted to a final concentration of 3µg/ml in media. Cells were treated with IL-1F5 for 24 hours.

5

Treatment with IL-1beta

Recombinant rat IL-1beta (R&D Systems, USA) was prepared as a stock solution of 1µg/ml in sterile PBS and used at a final concentration of 5ng/ml in media. Cells were treated with IL-1beta for 24 hours.

10

Treatment with anti-SIGIRR

An antibody to the SIGIRR receptor (anti-SIGIRR; R&D Systems, Minneapolis, USA) was diluted to a concentration of 100 µg/ml in PBS. Working concentrations of 20 µg/ml were prepared in media. Cells were incubated in anti-SIGIRR for a period of 4 hours.

15

Fluorescent Immunocytochemistry

SIGIRR Expression

Following treatment of cultured glia, coverslips were washed in TBS, and fixed with absolute alcohol. Cells were permeabilised with 0.1% Triton X-100 and non-reactive sites were blocked with goat serum (blocking buffer; 5% in PBS). To determine the surface distribution of SIGIRR, cells were incubated overnight with an anti-SIGIRR antibody (20 µl; 1:500 dilution in 10% blocking buffer; R&D systems, UK) purified from goat serum. This antibody was raised against a peptide corresponding to a short amino acid sequence of SIGIRR of mouse origin. Coverslips were washed 3 times in PBS and secondary antibody was added (100µl; 1:100 dilution; anti-goat IgG conjugated to FITC; Sigma, UK) for 1 hour at room temperature. Coverslips were washed several times in distilled H₂O (dH₂O), before being mounted onto microscope slides using a mounting medium for

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fluorescence (Vector, USA) and the perimeter of each coverslip was sealed using nail varnish. Mounted coverslips were viewed under x40 magnification by fluorescence microscopy (Leitz Orthoplan Microscope, Germany) using Improvision software (Improvision, UK). Cells were
5 observed at excitation, 490nm; emission, 520nm for FITC labelled antibodies.

Immunostaining

Preparation of cryostat sections

10 Half brains were maintained at -80°C until required for processing. Using a cryostat (Leica CM1900), 20µm sections were prepared. The sections were mounted on gelatine coated slides and air-dried for 30 minutes. Sections were stored at -20°C until required for immunohistochemical analysis.

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Staining sections for MHC class II

Frozen cryostats sections were thawed at room temperature. Sections were fixed using ice-cold absolute ethanol for 10 min, washed using TBS (containing in mM: 137mM NaCl, 2.7mM KCl, 8.1mM Na₂HPO₄ and
20 1.5mM KH₂PO₄ pH 7.3; R&D systems, Minneapolis, USA) and then blocked using as solution of 10% normal goat serum (NGS; Vector Laboratories, USA) containing 4% BSA in TBS . Sections were incubated overnight in a humidified chamber at 4°C in the presence of OX-6 antibody (10µl/ml; 1:100 in TBS; Serotec, UK). Sections were then washed in TBS
25 and incubated in the presence of a secondary antibody, anti-mouse IgG (1:200 in TBS; Sigma, Dorset, UK). Washing in PBS was repeated and sections were placed in avidin-biotin-horseradish peroxidase solution, diluted in TBS for 1 hour (Vectastain elite ABC kit, Vector Laboratories, USA) and then reacted with 3,3'-diaminobenzidine (DAKO Corporation,
30 USA) and H₂O₂ for colour development. The reaction was terminated by

washing the sections in double distilled H₂O, and positively stained cells were visualized under the light microscope at X4 and X40 magnification.

SDS-polyacrylamide gel electrophoresis

5 Preparation of samples

Hippocampal slices were thawed rapidly, washed 3 times in Krebs solution (composition in mM: NaCl 136, KCl 2.54, KH₂PO₄ 1.18, MgSO₄ 1.18, NaHCO₃ 16, glucose 10) containing 2mM CaCl₂ and homogenized using a 1ml glass homogeniser (Jencons, Bedfordshire, UK). Protein concentrations were assessed (see section 2.5) and equalized with Krebs containing 2mM CaCl₂. sample buffer (0.5M Tris-Hcl pH 6.8; 20% glycerol (w/v); 2% SDS (w/v); 5% mercaptoethanol (v/v); 0.05% bromophenol blue (w/v)) was added to generate a final concentration of 1ng/ml and samples were boiled for 5 minutes.

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Preparation of polyacrylamide gels

Polyacrylamide gels (1mm thick) were prepared with either a monomer concentration of 10% or 12% overlaid with 4% stacking gel, between 10cm wide glass plates, mounted on an electrophoresis unit (Sigma Techware, Dorset, UK; Laemmli, 1970). Electrode running buffer (25mM Tris base; 200mM glycine; 17mM SDS) was added to the upper and lower reservoirs of the unit. Samples were loaded into the wells using a Hamilton MicroLiter syringe (10µl per well). A pre-stained molecular weight marker (5µl; Sigma, Dorset, UK or Santa Cruz, California, USA) was also loaded. A 32mA current was passed through the unit in order to separate proteins, according to their molecular weight. The current was switched off when the blue dye band reached the bottom of the gel.

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Semi-dry electrophoretic blotting of proteins

The gel was removed from the gel apparatus, placed on top of a sheet of nitrocellulose membrane (0.45µm pore size; Sigma, Dorset, UK), moistened in transfer buffer (25mM Tris-base; 192mM glycine; 20% methanol (v/v); 0.05% SDS (w/v)) and cut to the size of the gel. A sandwich was made by placing filter paper (Standard Grade no.3, Whatman, Kent, UK) was placed on top and beneath the nitrocellulose/gel. The sandwich was soaked in transfer buffer and placed on a titanium electrode (anode) of a semi dry blotter (Sigma, Dorset, UK). Air bubbles were removed. A constant current of 225mA was applied for 75 minutes.

Western Immunoblotting

The nitrocellulose membrane was blocked for non-specific binding and then probed with an antibody raised against the appropriate protein. The membrane was then washed and incubated with horseradish peroxidase (HRP) conjugated secondary antibody. A chemiluminescent detection agent was added and the membrane was exposed to 5 by 7 inch photographic film (Hyperfilm ECL, Amersham, Buckinghamshire, UK) and developed using a Fuji X-ray processor.

JNK phosphorylation

Non-specific binding was blocked by incubating nitrocellulose membranes overnight at 4°C in Tris-buffered saline (TBS 10ml per membrane; TBS; 20mM Tris-HCL; 150mM NaCl; pH7.6) containing 5% BSA. Membranes were washed 3 times for 15 minutes in TBS-T. The primary antibody used was a mouse monoclonal IgG antibody raised against a peptide corresponding to a short sequence of JNK1 human origin, which identifies the phosphorylated form of JNK (10ml; 1:300 dilution in TBS containing 0.1% BSA; Santa Cruz, California, USA). Membranes were incubated in the presence of the primary antibody for 2 hours at RT, and then washed for 15 minutes 3 times in TBS-T. the secondary antibody (10ml; 1:600

dilution; goat anti-mouse HRP in TBS containing 0.1% BSA; Sigma, Dorset, UK) was added and incubation proceeded for 1 hour at room temperature. Membranes were washed 3 times for 15 minutes with TBS-T. Supersignal (Pierce, Illinois, USA) was added for 5 minutes after which
5 membranes were exposed to photographic film for 1 second in the dark, after which time the film was developed.

Phosphorylation of JAK1 and STAT6

Non-specific binding was blocked by incubating nitrocellulose membranes
10 overnight at 4°C in Tris-buffered saline (10ml; TBS; 20mM Tris-HCl; 150mM NaCl; pH7.6) containing 5% BSA. Membranes were washed for 15mins 3 times in TBS-T (10ml). The primary antibody used was a rat monoclonal IgG antibody raised against a peptide corresponding to a short sequence of JAK1 of mouse origin (10ml; 200µg/ml; 1:200 dilution in TBS
15 containing 0.1% BSA; Santa Cruz, California, USA), or a rat monoclonal antibody raised against a peptide corresponding to a short sequence of STAT6 of (10ml; 200µg/ml; 1: 100 dilution in TBS containing 0.1% BSA; Santa Cruz, California, USA). Membranes were incubated in the presence of the primary antibody for 2 hours at room temperature, and then washed
20 for 15 min 3 times in TBS-T. The secondary antibody (10ml; 1:600 dilution; rat anti-goat HRP in TBS containing 0.1% BSA; Sigma, Dorset, UK) was added and incubation proceeded for 1 hour at RT. Membranes were washed for 15min 3 times with TBS-T. Supersignal (Pierce, Illinois, USA) was added. Samples were incubated for 5 minutes after which time
25 membranes were exposed to photographic film for 1 sec in the dark, after which time the film was developed.

Phosphorylation of ERK

ERK phosphorylation was assessed in whole-cell lysate using a rat
30 monoclonal IgG1 antibody raised against a peptide corresponding to a

sequence of short sequence of ERK of mouse origin, which recognizes the phosphorylated form of ERK (10ml; 200µg/ml; 1:200 dilution in TBS-T containing 1% BSA; Santa Cruz, USA). Blotting proceeded as described above.

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Actin Expression

Following Western Immunoblotting to assess the phosphorylation of JNK, JAK1, STAT6 and ERK blots were stripped with an antibody stripping solution and reprobed for analysis of total actin expression to confirm equal loading of the protein. The primary antibody used was a mouse monoclonal IgG antibody corresponding to amino acid sequence mapping the terminus of actin (10ml; 1:200 dilution in TBS containing 0.1% BSA; Santa Cruz, California, USA). Membranes were incubated for 2 hours in the presence of the primary antibody and then washed 3 times for 15 min with TBS-T. The secondary antibody (10ml; 1:500 dilution; goat anti-mouse IgG HRP in TBS containing 0.1% BSA; Sigma, Dorset, UK) was added and incubation resumed for 1 hour at room temperature. Membranes were washed for 15 minutes, 3 times in TBS-T. Supersignal (Pierce, Illinois) was added and membranes were incubated for 5 minutes and the membranes were exposed to film and developed.

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Densitometry

In all cases quantification of protein bands was achieved by densitometric analysis using an Ultraviolet Transilluminator (GelDoc-It Imaging system, BioImaging Systems, UK). Values were expressed as arbitrary units.

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RT-PCR

RNA extraction from cultured cortical glia

Total RNA was extracted from cultured cortical glia using TRI reagent (Sigma, Dorset, UK). Cultured glia were rinsed with sterile PBS and lysed

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directly on 24 well culture plates by adding 50µl reagent per well and scraping the cells using the rubber end of a 1ml syringe piston (B.Braun Medical Ltd, Melsungen, Germany). Cells were incubated at room temperature for 5 minutes to allow dissociation of nucleoprotein complexes. Chloroform reagent (0.2ml; Sigma, Dorset, UK) was added and samples were incubated at room temperature for 5-10 min to allow sample to separate into phases. Samples were centrifuged at 12,000 x g for 15 min after which the aqueous phase was removed and transferred to a new tube. RNA was precipitated by adding isopropanol (0.5ml; Sigma, Dorset, UK). Samples were incubated at room temperature for 10 minutes and centrifuged at 12,000 x g for 20 min. RNA pellets were washed with 75% ethanol (Sigma, Dorset, UK), allowed to air dry and resuspended in DEPC treated water.

Analysis of RNA by gel Electrophoresis

Samples were run on a 1% (w/v) agarose gel to ensure RNA was intact and had not been degraded. Agarose gel was prepared in 1X Tris borate EDTA (TBE) buffer (100ml; 0.08 M Tris; 0.04M Boric acid; 1mM EDTA; pH 8.3). Ethidium Bromide (EtBr; Sigma, Dorset, UK) was added to give a final concentration of 0.5µg/ml. RNA samples were mixed with 3.5µl of H₂O and 1ml X6 gel loading buffer (60% (w/v) glycerol, 0.4% (w/v) bromophenol blue) in preparation for gel electrophoresis. Samples were loaded onto gels and RNA was separated by application of a 90 V voltage to the gel apparatus. Migration of the bromophenol blue was monitored and the voltage was switched off when the blue dye band reached the bottom of the gel. The gel was visualized under UV light.

DNase 1 treatment of RNA

Contaminating DNA was removed from RNA preparations. The RNA was treated with RNase-free DNase I (Invitrogen, USA) and 10X DNase 1

reaction buffer at 1 unit/ μ g of RNA for 15 min at RT. EDTA solution was then added to inactivate the DNase and the samples were incubated at 65°C for 10 minutes.

5 RT-PCR

Equal amounts of cDNA were used for PCR amplification using Superscript II RNase reverse transcriptase enzyme (Invitrogen, UK). 1 μ g of sample RNA was mixed with 1 μ l of Oligo dT Primer (Invitrogen, UK) and 1 μ l of dNTP mix (Promega, UK). The sample was incubated at 65°C for 5
10 min after which it was removed to ice. 5X reaction buffer (4 μ l), 0.1M dithiothreitol (DTT; 2 μ l) and RNase Inhibitor (1 μ l; Promega, USA) was added to the reaction mixture and the reaction was heated to 42°C then Superscript reverse transcription enzyme was added (1 μ l). The reaction was incubated at 42°C for 50 min for cDNA synthesis and then at 75°C for
15 10 minutes to inactivate the reverse transcription.

A mastermix PCR solution was prepared (25 μ l; 2.5 μ l 10X reaction, 2.5 μ l MgCl₂, 1 μ l dNTP mix (Promega, USA) 1 μ l of upstream and downstream primers, 13 μ l double distilled (ddH₂O), 0.5 μ l of Taq polymerase). 2.5 μ l of
20 sample was added to the mastermix and PCR was run with a total of 35 cycles. Primers were pre-tested through an increasing number of cycles in order to obtain RT-PCR products in the exponential range. The following sequences of primers were used to determine IL-4 Expression: upstream 5'-CCT TGC TGT CAC CCT GTT CTG C-3'; downstream 5'-GTT GTG
25 AGC GTG GAC TCA TTC ACG-3'; and for rat beta-actin mRNA expression: upstream 5'-GAA ATC GTG CGT GAC ATT-3'; downstream 5'-TCA GGA GGA GCA ATG ATC TTG A-3'. The cycling conditions were as follows: 95°C for 60 seconds followed by cycles of 94°C for 45 seconds, 60°C for 45 seconds and 72°C for 90 seconds. The reaction was

stopped by final extension for 10 minutes at 70°C. These primers generated IL-4 PCR products of 352bp and beta-actin PCR products of 360 bp. Equal volumes of PCR product from each sample was loaded onto 1.5% agarose gels, bands were separated by application of 90V, photographed and quantified using densitometry.

Densitometry

In all cases quantification of mRNA bands was achieved by densitometric analysis using an Ultraviolet Transilluminator (GelDoc-It Imaging system, BioImaging Systems, UK). Values were expressed as arbitrary units.

Statistical Analysis

Data are expressed as means $\pm$ standard error of the means. A Student's t-test for independent means or a one-way analysis of variance (ANOVA) was performed where appropriate to determine whether significant differences existed between conditions. When this analysis indicated significance (at the 0.05 level), post hoc student Newmann-Keuls test analysis was used to determine which conditions were significantly different from each other (GraphPadPrism).

Results

Example 1 - LPS administration is associated with impairment in LTP but abrogated with IL-1F5 treatment

LTP was similar in saline treated rats to those treated with IL-1F5 in perforant path granule cell synapses was markedly attenuated in rats injected with LPS (* $p < 0.001$; ANOVA; Figure 1). The data indicate that mean percentage change in EPSP slope in the 2 minutes immediately following tetanic stimulation, compared with the mean value in the 2 minutes prior to stimulation was significantly decreased in LPS-treated rats (137.32 $\pm$ 2.81; mean $\pm$ SEM; n=6) compared with controls (159.88 $\pm$ 3.25;

mean $\pm$ SEM; n=6; **p<0.001; Figure 2) Similarly there was a significant difference between the mean values in the last 5 minutes of the experiment in the LPS-treated group (97.35 ± 1.26 ; mean $\pm$ SEM; n=6), compared with the control treated group (133.00 ± 1.17 ; mean $\pm$ SEM; n=6; **p<0.001; ANOVA; Figure 2). Treatment with IL-1F5 did not significantly alter the response to the tetanus.

Example 2 - LPS administration is associated with an increase in the concentration of IL-1beta in the hippocampus

It has been shown that the LPS-induced inhibition of LTP is coupled with an increased IL-1beta concentration in the hippocampus and the data presented in Figure 3 support these findings. Thus mean IL-1beta concentration in the hippocampus of LPS-treated (80.58 ± 17.76 ; n=6) rats was significantly greater than that in control animals (36.22 ± 3.09 ; *p<0.05; ANOVA). This increase was not observed in LPS-treated rats which received IL-1F5; in this case, mean IL-1beta concentration was similar to that in tissue prepared from control rats (50.56 ± 15.51 ; n=6).

Example 3 - LPS administration is associated with an increase in OX-6 positive staining in the hippocampus

It has been shown that the cell source of IL-1beta is likely to be activated microglia (Tikka et al., 2001) and to assess whether IL-1F5 attenuates the LPS-induced increase in IL-1beta by downregulating microglial activation, cryostat sections were prepared from rats in each of the four treatment groups. Sections were stained for evidence of MHCII expression by investigating OX6 staining. Figure 4 shows that LPS markedly increased MHCII expression, and that while IL-1F5 alone did not appreciably modulate MHCII expression; it had the ability to abrogate the LPS-induced change (Figure 4).

Example 4 - IL-4 concentration is increased in the hippocampus following administration of IL-1F5

Previous findings have revealed that IL-4 can block the LPS-induced increase in IL-1beta mRNA and protein and therefore we investigated the effects of IL-1F5 administration on IL-4 concentrations. Figure 5 shows that IL-4 concentration was significantly increased in hippocampal tissue prepared from IL-1F5 treated rats (186.12 ± 8.36 ; $*p < 0.05$; Figure 5) and IL-4 concentration was similar in control-treated rats and rats treated with LPS alone or with IL-1F5.

Example 5 - IL-4 protein concentration and mRNA is increased in cultured cortical glial cells following treatment with IL-1F5

Past studies have indicated that IL-4 is produced by glia and to confirm this, glial cells were prepared and treated with and without IL-1F5.

Analysis of the supernatants, prepared from glial cells indicate that IL-1F5 significantly increased IL-4 concentration (22.79 ± 2.54 compared with 9.58 ± 1.319 ; $*p < 0.05$; students unpaired t test; Figure 6). Figure 7 shows that IL-1F5 has the ability to increase IL-4 mRNA. These data indicate an interaction of IL-1F5 with glial cells.

Example 6 - The IL-1F5 mediated increase in IL-4 is abrogated following treatment with anti-SIGIRR

The possibility that IL-1F5 may mediate its effects via an interaction with SIGIRR was considered. SIGIRR is known to be distributed in the brain.

The data here indicate that while incubation of cortical glia in the presence of IL-1F5 caused a significant increase in IL-4 concentration (22.33 ± 2.865 pg/ml compared with 13.12 ± 1.829 pg/ml; $*p < 0.05$; ANOVA; $n=10$; Figure 5), incubation in the presence of an anti-SIGIRR antibody blocks this effect.

Example 7 - IL-1F5 treatment is associated with an increase in SIGIRR expression in cultured cortical glia

These data suggest that IL-1F5 may mediate its effect via interaction with the SIGIRR receptor. Figure 9 shows that SIGIRR is expressed in glia and that treatment of cultured cortical glia with IL-1F5 results in an increase in SIGIRR expression when compared to controls.

Example 8 - IL-1beta administration is associated with impairment in LTP but is restored by treatment with IL-1F5

These results suggest that the effect of IL-1F5 is mediated through its ability to initiate IL-4 production, therefore it must be predicted that IL-4 will mimic the effects of IL-1F5. Previous studies have indicated that many actions of IL-1beta are inhibited by IL-4 (Nelms et al., 1990) and the evidence suggests that the ability of IL-4 to downregulate IL-1R1 expression may be responsible for these effects (Nolan et al., 2004). If IL-4 mediates the effects of IL-1F5, then it can be hypothesized that IL-1F5 will mimic the effects of IL-4 in blocking IL-1beta induced inhibition of LTP. Figure 10 shows that while LTP was attenuated in IL-1beta treated rats, IL-1F5 had the ability to abrogate this effect so that the change in EPSP slope induced by tetanic stimulation in control-treated rats was similar to that in rats treated with IL-1beta in combination with IL-1F5. Analysis of changes in the 2 minute immediately following tetanic stimulation showed that the mean percentage change in slope of EPSP was 121.32 ± 1.49 in control-treated and 119.3 ± 2.462 in IL-1beta treated rats compared with 127.02 ± 1.47 and 126.34 ± 0.85 in rats treated with IL-1F5 and IL-1F5+IL-1beta respectively (Figure 11). The corresponding changes in the last 5 minutes of the experiment were 118.54 ± 0.65 , 104.73 ± 0.51 , 12.45 ± 0.77 and 115.50 ± 0.30 in control, IL-1beta-treated, IL-1F5-treated and IL-1beta+IL-1F5 treated rats respectively.

Statistical analysis revealed a significant difference between control-treated and IL-1beta treated rats (*p<0.05; ANOVA; Figure 11), but not between control-treated rats and rats in either of the other treatment groups.

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Example 9 - IL-1beta administration is associated with an increase in JNK in the hippocampus

Several studies have reported that the IL-1beta induced impairment in LTP is accompanied by an increase in activation of JNK and the data shown here provide further support for a correlation between these measures. To confirm the inhibitory effect of IL-1F5 on IL-1beta induced changes we assessed the interaction between these cytokines. Figure 12 shows that the significant IL-1beta-induced activation of JNK was attenuated in hippocampal tissue prepared from rats treated with IL-1F5 (2.05 ± 0.24 compared with 1.46 ± 0.11 ; *p<0.05; ANOVA; n=6). Importantly the data indicate that IL-1F5 alone exerted no effect.

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IL-4 concentration is increased in the hippocampus following IL-1F5 treatment

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Hippocampal tissue was prepared from these rats was assessed for IL-4 concentration by ELISA and the findings demonstrate that the mean cytokine concentration was significantly increased in hippocampal tissue prepared from IL-1F5-treated rats, compared with control-treated rats (p<0.05; ANOVA; Figure 13), while the concentration was similar in tissue prepared from rats treated with LPS and those treated with IL-1beta and IL-1F5 (Figure 13).

Example 10 - IL-1F5 administration is associated with an increase in JAK1 and STAT6 phosphorylation in the hippocampus

In parallel with these findings the effect of IL-1F5 on downstream modulators of IL-4 function – JAK1 and STAT6 was examined. The data represented in figures 15 and 16 indicate there was a significant increase in JAK1 phosphorylation in hippocampal tissue prepared from IL-1F5 treated rats (222.83 ± 61.51 arbitrary units), compared with control treated rats (106.45 ± 6.69 arbitrary units; $p < 0.05$; students unpaired t test; Figure 14). This finding was coupled with a significant increase in downstream STAT6 phosphorylation in hippocampal tissue prepared from IL-1F5 treated rats (163.14 ± 25.38), compared with control-treated rats (95.40 ± 4.01 ; $*p < 0.05$; students unpaired t test; Figure 15).

Example 11 - IL-1F5 treatment is associated with an increase in ERK phosphorylation in the hippocampus

Results indicate an increase in both ERK1 and ERK2 in hippocampal tissue prepared from IL-1F5 treated rats. Densitometric analysis revealed that ERK2 phosphorylation was significantly increased in hippocampal tissue prepared from IL-1F5 treated rats (9.48 ± 0.98 arbitrary units) compared with control treated rats (4.00 ± 1.24 ; $*p < 0.05$; students unpaired t test; $*p < 0.05$; students unpaired t test; Figure 16).

Example 12 - IL-1F5 attenuates IL-1 β induced IL-6 protein and mRNA expression in mouse glia

Mixed glial cell cultures were prepared from C57BL/6 mice and treated with IL-1 β (5 ng/ml) alone or in the presence of IL-1F5 (3 μ g /ml). A) Supernatants were removed after 24 hours and IL-6 concentrations were determined by ELISA. B) Cells were lysed and IL-6 mRNA expression was determined by RT-PCR. Results are expressed as arbitrary units relative to β -actin. $** p < 0.01$, $*** p < 0.001$ IL-1 β versus control; $+ p < 0.05$, $+++ p < 0.001$, IL-1 β versus IL-1 β + IL-1F5 by ANOVA; $n=6$.

Results

The results, as shown in Figure 17, show that IL-1 β significantly increased IL-6 mRNA ($p < 0.01$) and protein expression ($p < 0.001$) and these changes were significantly ($p < 0.05$ and $p < 0.001$) attenuated by co-treatment with IL-1F5.

Example 13 - Intracerebroventricular injection of IL-1F5 attenuates LPS induced IL-6 in rat hippocampus

Rats received an intracerebroventricular injection of IL-1F5 (30 ng/ml) or vehicle only followed immediately by an intraperitoneal injection of LPS (100 μ g/kg) or vehicle only. Rats were sacrificed after 3 hours, hippocampal tissue prepared and IL-6 concentrations determined by ELISA. * $p < 0.05$, LPS versus vehicle; + $p < 0.05$, LPS alone vs LPS+IL-1F5 by ANOVA

Results

The results, as shown in Figure 18, show that IL-6 concentration was significantly increased in hippocampal tissue prepared 3 hours following intraperitoneal injection of LPS ($p < 0.05$). Intracerebroventricular injection of IL-1F5 given immediately before LPS significantly attenuated the LPS-induced change ($p < 0.05$)

Example 14 - Intracerebroventricular injection of IL-1F5 attenuates LPS-induced JNK phosphorylation in rat hippocampus

Rats received an intracerebroventricular injection of IL-1F5 (30ng/ml) or vehicle only followed 15 minutes later by an intraperitoneal injection of LPS (100 μ g/kg) or vehicle only. Rats were sacrificed after 3 hours, hippocampal tissue prepared and phosphorylation of JNK was determined by western blotting with a specific antibody. * $p < 0.05$, LPS versus control by ANOVA; $n=6$

Results

The results, as shown in Figure 19 show that intraperitoneal injection of LPS significantly increases JNK phosphorylation in rat hippocampal tissue (p<0.05) and these changes were attenuated by intracerebroventricular injection of IL-1F5.

Example 15 - IL-1F5 suppresses LPS-induced IL-1 β production by mouse mixed glia cells but not by murine macrophages or dendritic cells

Mouse mixed glia cells, macrophages or dendritic cells were cultured with medium only or LPS (100 ng/ml) alone or in the presence of IL-1F5 (1 or 3 μ g/ml). After 24 hours cells were lysed and IL-1 mRNA expression was determined by RT-PCR. Results are expressed as arbitrary units relative to β -actin. ***p<0.001 LPS versus control, +++ p<0.001 LPS versus LPS + IL-1F5 by ANOVA; n=6.

Results

The results, as shown in Figure 20 show that LPS induces IL-1 β mRNA expression in mouse mixed glial cells, macrophage and dendritic cells (p<0.001) and that IL-1F5 significantly reduce LPS-induced IL-1 mRNA expression in glial cells but not in macrophages or dendritic cells.

Example 16 - IL-1F5 suppresses LPS-induced IL-1 β protein production by mixed glia cells but not by macrophages

Rat mixed glia cells or the murine macrophage cell line J774 cells were cultured with medium only or LPS (100 ng/ml) alone or in the presence of IL-1F5 (3 μ g/ml). After 24 hours supernatants were removed and IL-1 β concentrations determined by ELISA. ***p<0.001, LPS versus control; +++ p<0.001 LPS versus LPS + IL-1F5 by ANOVA.

Results

The results, as shown in Figure 21 show that LPS induces IL-1 β production in rat mixed glial cells ($p<0.001$) and J774 cells and that IL-1F5 significantly reduce LPS-induced IL-1 mRNA expression in glial cells ($p<0.001$) but not in the macrophages.

Example 17 - IL-1F5 induced IL-4 production from murine mixed glial cells but not from dendritic cells, spleen cells or macrophages

Mouse mixed glial cells (A), dendritic cells (B), spleen cells (C) or peritoneal macrophages (D) were stimulated with medium only (Con) or IL-1F5 (1-3 $\mu\text{g/ml}$). After 24 hours, supernatants were removed and IL-4 concentrations were determined by ELISA. ** $p<0.01$, LPS versus control by ANOVA; $n=6$.

Results

The results, as shown in Figure 22 show that treatment of murine mixed glia with IL-1F5 significantly increases IL-4 protein production ($p<0.01$), but that IL-1F5 exerted no significant effect on IL-4 production by dendritic cells, spleen cells or macrophages, although treatment with PMA and ionomycin significantly increased IL-4 in spleen cells and macrophages (data not shown).

Example 18 - IL-1F5 induces IL-4 protein and mRNA expression in rat mixed glial cells

Rat mixed glial cells were cultured for 24 hours with medium only (Con) or IL-1F5 (3 $\mu\text{g/ml}$). (A) supernatants were removed and IL-4 concentrations were determined by ELISA. (B) Cells were lysed and IL-4 mRNA expression was determined by RT-PCR. Results are expressed as arbitrary units relative to β -actin. *, $p<0.05$, ** $p<0.01$ IL-1F5 versus control by unpaired Students-t test; $n=6$

Results

The results, as shown in Figure 23 show that treatment of rat mixed glial cells with IL-1F5 significantly increases IL-4 protein and mRNA expression (p<0.01).

Example 19 - Intracerebroventricular injection of rats with IL-1F5 significantly increased IL-4 expression in the hippocampus

Rats received an intracerebroventricular injection of IL-1F5 (30 ng/ml) or vehicle only. Rats were sacrificed after 3 hours, hippocampal tissue homogenates were prepared and A) IL-4 mRNA was determined by RT-PCR relative to β -actin and B) IL-4 protein concentrations were determined by ELISA. *, p<0.05, **p<0.01 IL-1F5 versus control by ANOVA; n=6

Results

The results, as shown in Figure 24 show that intracerebroventricular injection of significantly enhances IL-1F5 IL-4 mRNA (p<0.01) and protein (p<0.05) expression in the hippocampus.

Example 20 - IL-4 mediates the IL-1F5 attenuation of LPS-induced IL-1 β production from glial cells

Mixed glial cells prepared from C57BL-6 and IL-4^{-/-} mice were cultured with medium only or LPS (100 ng/ml) alone or in the presence of IL-1F5 (1-3 μ g/ml). After 24 hour supernatants were removed and IL-1 β concentrations determined by ELISA. *p<0.05 LPS versus control; +p<0.05, LPS versus LPS + IL-1F5 by ANOVA; n=6.

Results

The results, as shown in Figure 25 show that treatment of cultured glia prepared from wild type or IL-4^{-/-} mice resulted in a significant increase in

IL-1 β concentration ($p < 0.05$) although the LPS-induced IL-4 was lower in cells prepared from IL-4 $^{-/-}$ mice. IL-1F5 significantly attenuated the LPS-induced change in glial cells from wildtype C57BL-6 ($p < 0.05$), but not the IL-4 $^{-/-}$ mice.

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Example 21 - The attenuation of LPS-induced IL-1 β production by IL-1F5 in the hippocampus is mediated through IL-4

C57BL/6 or IL-4 knockout (IL-4 $^{-/-}$) mice received an intracerebroventricular injection of IL-1F5 (30 ng/ml) or vehicle only followed 15 minutes later by an intraperitoneal injection of LPS (200 μ g/kg) or vehicle only. Mice were sacrificed after 3 hours, hippocampal tissue was prepared, homogenized and IL-1 β concentrations determined by ELISA. *** $p < 0.001$, LPS versus control, +++ $p < 0.001$, LPS versus LPS + IL-1F5, X $p < 0.05$, IL-4 $^{-/-}$ versus wildtype mice by ANOVA; $n=6$)

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Results

The results, as shown in Figure 27 show that i.p. injection of LPS significantly increases concentration of IL-1 β in hippocampus of wildtype mice ($p < 0.001$) and that IL-1F5 significantly attenuated the LPS-induced change in wildtype ($p < 0.001$), but not IL-4 $^{-/-}$, mice. Constitutive expression of IL-1 β was significantly higher in hippocampal tissue prepared from IL-4 $^{-/-}$ compared with wildtype mice ($p < 0.05$).

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Example 22 – Effect of PPAR γ expression on IL-1F5 mediated inhibition of LPS induced IL-1 β in glial cells

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Primary cultures of cortical glia cells were prepared as follows. Glia were isolated from cerebral cortices of Wistar rats 1 day postpartum (BioResources Unit, Trinity College, Dublin). Rats were decapitated and cortices dissected. Cortices were placed in 3ml Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% foetal bovine serum

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(Gibco, UK), penicillin (100U/ml; Gibco, UK) and streptomycin (100U/ml; Gibco, UK). Tissue was triturated (x7), passed through a sterile nylon mesh filter and centrifuged (2500g for 3 min at 20°C). The pellet was resuspended in DMEM. Resuspended glia were placed on the centre of each coverslip (65µl per coverslip) and allowed to adhere to the glass coverslip for 2 hours in a humidified incubator containing 5% CO₂: 95% air at 37°C before 400µl of pre-warmed DMEM was added to each well. Cells were grown for 10 days prior to treatment and media replaced every 3 days. Cells were treated with LPS (100ng/ml), IL-1F5 (3µg/ml), GW9962 (20µM) or combinations of these treatments. Twenty four hours later, supernatant was collected for analysis of IL-1beta and cells were harvested for analysis of PPARgamma expression by gel electrophoresis and immunoblotting.

IL-1beta was analysed by ELISA as previously described (Barry et al., J Neurochem. 93, 221-231 2005). PPARgamma was assessed by standard procedures (Barry et al., 2005) using anti-rabbit polyclonal PPARγ (10ml; 1:400 dilution in TBS containing 0.1% BSA; Calbiochem, USA) as the primary and anti-rabbit IgG linked antibody (1:1000 dilution in TBS containing 0.1% BSA; Santa Cruz, USA) as the secondary antibodies.

Results:

Figure 28 shows that PPARgamma protein expression was found in primary cultured cortical glia following treatment with IL-1F5 (24 hour incubation). Figure 29 shows that the level of expression of PPARgamma expression induced by IL-1F5 is significant, and Figure 30 shows that IL-1F5 inhibits LPS induced increase in IL-1beta expression. Further, co-treatment with a PPARgamma antagonist reduces this inhibitory effect of IL-1F5, indicating that PPARgamma may play a role in IL-1F5 functioning.

Discussion

A number of studies have identified the fact that the LPS-induced inhibition of LTP is coupled with, and probably at least partially dependent on an increase in hippocampal concentration of IL-1beta. It is hypothesized that this increase in IL-1beta concentration is due to an increase in the activity of the brain like macrophages, microglia. These cells are thought to be the principal producers of IL-1beta. Once produced, IL-1beta induces a sequence of signaling cascades, which results in alteration of transcription factors. One such transcription factor, which has been the subject of extensive research, is the c-Jun N terminal kinase (JNK). An increase in phosphorylation of JNK was associated with increased expression of the death inducer Fas ligand (FasL) in cultured cortical neurons. Here the inverse correlation between increased IL-1beta concentration and the inhibition of LTP is confirmed and, significantly, these LPS-induced changes are inhibited following IL-1F5 treatment. In parallel with an increase in IL-1beta concentration, the data indicate that LPS increases JNK activation, as previously described and this increase was also attenuated in tissue prepared from rats which received both IL-1F5 and LPS and IL-1F5 in combination.

In an effort to explore the underlying mechanism by which IL-1F5 acts to inhibit these changes, microglial activation was assessed, since the most likely source of the LPS-induced increase in IL-1beta concentration is activated microglia. It was demonstrated that the marker for microglia activation, the MHCII was clearly upregulated in hippocampal sections prepared from LPS treated rats and that IL-1F5 attenuated this effect.

IL-4 Expression

IL-4 has been the subject of extensive research, as it emerges that it plays a pivotal role in modulating the inflammatory process. IL-4 has been

shown to reverse impairment of LTP and lead to increase in the release of anti-inflammatory agents such as TGF- β . Activation of a number of signalling pathways leads to IL-4 influencing gene expression – phosphatidylinositol-3 kinase, phosphorylation of insulin receptor substrates by IL-4R α chain, activation of Ras/MAP kinases including ERK, and activation of the JAK/STAT pathway. Binding of IL-4 to its receptor results in the translocation of STAT6 to the nucleus. Once in the nucleus, STAT6 binds to STAT-binding elements to activate gene transcription. Genes induced by IL-4 include IL-4R α , IL-1 α , IL-4 and MHC II.

Furthermore, IL-4 has the ability to inhibit activation of genes associated with inflammation including IL-1 β , IL-12, TNF- α and iNOS which generates nitric oxide. Ultimately, IL-4 binding results in the suppression of macrophage activity and the differentiation of T-helper cells towards a Th2 cell phenotype further promoting anti-inflammatory activity. Once activated IL-4 initiates a series of signalling events leading to the activation of protective transcription factors. This sequencing events involves activation of the janus activates kinases (JAKs) and signal transducers and activation of transcription (STATs). Aged rats, like LPS treated rats exhibit a deficit in LTP and this is associated with an increased hippocampal concentration of IL-1 β coupled with a decrease in the concentration of IL-4. The data here confirms the importance of IL-4 in the maintenance of LTP by showing that IL-4 partially restores the age-related deficit and abrogates the LPS-induced and IL-1 β induced deficit in LTP (Nolan et al., 2004).

IL-1F5 exerts its anti-inflammatory effect by increasing IL-4 concentration in the hippocampus and the evidence presented supports this. In addition to demonstrating an increase in IL-4 concentration in the hippocampus of

rats treated with IL-1F5, it can be reported that IL-1F5 acts to increase IL-4 mRNA and IL-4 protein in glial cells.

SIGIRR

5 Hitherto, no known ligand for SIGIRR has been described. Analysis of differential expression of SIGIRR has indicated it is highly expressed in the kidney; moderately expressed in the colon, small intestine, lung, spleen and liver; and weakly expressed in the brain (Wald et al., 2003). Overexpression of SIGIRR in Jurkat and HepG2 cells led to a substantial
10 reduction in IL-1- and IL-18-mediated activation of NF κ B. These results indicate that SIGIRR may function as a negative regulator of IL-1 and IL-18 signalling. The mechanism by which SIGIRR negatively regulates IL-1 signalling remains unclear. As SIGIRR interacts with IL-1R1, IRAK and TRAF 6 after IL-1 treatment, SIGIRR may negatively regulate the IL-1
15 pathway through its interaction with the IL-1R complex.

Conclusions

It is shown herein for the first time that IL-1F5 acts on glial cells causing IL-4 release and that this effect relies on an interaction with SIGIRR, since
20 the IL-1F5-induced increase in IL-4 is blocked in the presence of the SIGIRR antibody. A specific anti-inflammatory action of IL-1F5 has been identified. IL-1F5 is shown to be a ligand for SIGIRR, and downstream signalling from SIGIRR activation results in an anti-inflammatory effect. Further, IL-1F5 upregulates IL-4 and IL-4 dependent signalling pathways
25 in the brain.

All documents referred to in this specification are herein incorporated by reference. Various modifications and variations to the described
embodiments of the inventions will be apparent to those skilled in the art
30 without departing from the scope of the invention. Although the invention

has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes of carrying out the invention which are obvious to those skilled in the art are intended to be covered by the present invention.

5 Reference to any prior art in this specification is not, and should not be taken as, an acknowledgment or any form of suggestion that this prior art forms part of the common general knowledge in any country.

Claims

1. A method of suppressing a pro-inflammatory immune response, the method comprising the step of administering a therapeutically effective
5 amount of a polynucleotide or a fragment thereof which encodes a protein having SEQ ID NO:2 or a fragment thereof to a individual in need of such treatment.
2. The use of a polynucleotide or a fragment thereof which encodes a
10 protein having SEQ ID NO:2 or a fragment thereof for the administration to an individual for the suppression of an immune response, said immune response being characterised in that it is mediated through the activation of SIGIRR (single immunoglobulin related receptor).
3. The use of a polynucleotide or a fragment thereof which encodes a
15 protein having SEQ ID NO:2 in the preparation of a medicament for the downregulation of an immune response.
4. A pharmaceutical composition comprising a therapeutically effective
20 amount of a polynucleotide or a fragment thereof which encodes a protein having the amino acid sequence of SEQ ID NO:2 along with a pharmaceutically acceptable diluent, excipient or carrier.
5. A method for the prophylaxis and/or treatment of an immune-
25 mediated disorder, the method comprising the step of administering a therapeutically effective amount of a peptide comprising the amino acid sequence of SEQ ID NO:2 or an analogue, derivative, fragment, variant or peptidomimetic thereof to an individual in need of such treatment.

6. The use of a peptide comprising the amino acid sequence of SEQ ID NO:2 or an analogue, derivative, fragment, variant or peptidomimetic thereof for the prophylaxis and/or treatment of an immune-mediated disorder.

5

7. The use of a peptide comprising the amino acid sequence of SEQ ID NO:2 or an analogue, derivative, fragment, variant or peptidomimetic thereof in the preparation of a medicament for the suppression of an immune response, said immune response being characterised in that it is mediated through the activation of SIGIRR.

10

8. A method for the treatment and/or prophylaxis of a neurodegenerative disease or inflammatory condition of neuronal tissue, the method comprising the step of:

15

- administering a therapeutically effective amount of a peptide comprising the amino acid sequence of SEQ ID NO:2 or an analogue, derivative, fragment, variant or peptidomimetic thereof to a patient in need of therapy.

20

9. Method as claimed in claim 8 wherein the neurodegenerative disease or condition is selected from the group consisting of: Alzheimer's disease (AD), mild cognitive impairment (MCI), multiple sclerosis (MS), Parkinson's disease, Amyotrophic lateral sclerosis (ALS), Huntington's disease, prion disease, CJD, AIDS-related dementia, encephalitis, stroke and head trauma.

25

10. A method as claimed in claim 8 or claim 9 wherein the polypeptide or fragment thereof may delivered to the brain by means of a intracerebroventricular injection (icv / ICV) or through delivery into the CNS using minipumps.

30

11. The use of a peptide comprising the amino acid sequence of SEQ ID NO:2, a sequence which is at least 80% homologous thereto, or an analogue, derivative, fragment, variant or peptidomimetic thereof in the preparation of a medicament for the suppression of a neurodegenerative disease.
12. Use as claimed in claim 11 wherein the neurodegenerative disease or condition is selected from the group consisting of: Alzheimer's disease (AD), mild cognitive impairment (MCI), multiple sclerosis (MS), Parkinson's disease, Amyotrophic lateral sclerosis (ALS), Huntington's disease, prion diseases such as CJD, AIDS-related dementia, encephalitis, stroke and head trauma.
13. The use of a peptide comprising the amino acid sequence of SEQ ID NO:2, a sequence which is at least 80% homologous thereto, or an analogue, derivative, fragment, variant or peptidomimetic thereof in the preparation of a medicament for the treatment of Alzheimer's wherein the medicament is administered directly to the brain, neuronal tissue or the CNS to an individual in need of such treatment.
14. A method of treating an individual afflicted with Multiple Sclerosis, comprising administering to the individual a therapeutically effective amount of a peptide comprising the amino acid sequence of SEQ ID NO:2, a sequence which is at least 80% homologous thereto, or an analogue, derivative, fragment, variant or peptidomimetic thereof.
15. A method for modulating the activity of the SIGIRR receptor through selectively binding it with a peptide comprising the amino acid sequence of

SEQ ID NO:2, a sequence which is at least 80% homologous thereto, or an analogue, derivative, fragment, variant or peptidomimetic thereof.

16. A method for identification of modulator(s) of the binding of IL-1F5 and the SIGIRR receptor, said method comprising the steps of:
- providing first and second cellular samples containing the SIGIRR receptor,
 - contacting said first sample with IL-1F5,
 - contacting said first and second samples with a candidate molecule under conditions permissive of binding of said ligand, and monitoring the binding status of SIGIRR, and comparing the level of downstream activation between said first and second samples, wherein a difference in downstream activation between said first and second samples identifies the candidate molecule as a modulator of IL-1F5 binding to SIGIRR.

17. A method of agonizing the SIGIRR receptor in a patient, the method comprising the step of:
- administering to the patient a medicament comprising a peptide comprising the amino acid sequence of SEQ ID NO:2, a sequence which is at least 80% homologous thereto, or an analogue, derivative, fragment, variant or peptidomimetic thereof,
- in an amount effective to bind the SIGIRR receptor as an agonist.

25

18. A method as claimed in claim 17 wherein the method can be used for the suppression of an aberrant immune response.

19. A method as claimed in claim 18 wherein the aberrant immune response is causative of a neurodegenerative disease.

30

20. A method as claimed in claim 19 wherein the neurodegenerative disease is selected from the group comprising; Alzheimer's disease (AD), mild cognitive impairment (MCI), multiple sclerosis (MS), Parkinson's disease, Amyotrophic lateral sclerosis (ALS), Huntington's disease, prion diseases such as CJD, AIDS-related dementia, encephalitis, stroke and head trauma.

21. The use of a peptide comprising the amino acid sequence of SEQ ID NO:2, a sequence which is at least 80% homologous thereto, or an analogue, derivative, fragment, variant or peptidomimetic thereof as an agonist in the treatment of a neurodegenerative disease.

22. The use of a peptide comprising the amino acid sequence of SEQ ID NO:2, a sequence which is at least 80% homologous thereto, or an analogue, derivative, fragment, variant or peptidomimetic thereof as an agonist of SIGIRR in the preparation of a medicament for the treatment of neurodegenerative disease.

23. An assay method for the detection of a binding ligand which causes activation of SIGIRR, the assay comprising the steps of:

- providing a cellular sample comprising cells expressing SIGIRR, bringing said cells into contact with the ligand, and
- detecting the activation of PPAR,

wherein activation of PPAR is indicative of the binding of a ligand to SIGIRR.

24. An assay as claimed in claim 23 wherein the isotype of PPAR is PPARgamma.

25. The use of PPARgamma in the modulation of an immune response, wherein said response is characterised in that it is mediated by SIGIRR activation.
- 5 26. The use of an antibody or binding molecule with specificity for PPARgamma for the modulation of an immune response.
26. An antibody or binding fragment with specificity for SIGIRR, wherein said antibody or binding fragment binds to SIGIRR at the same active site
- 10 as IL-1F5.

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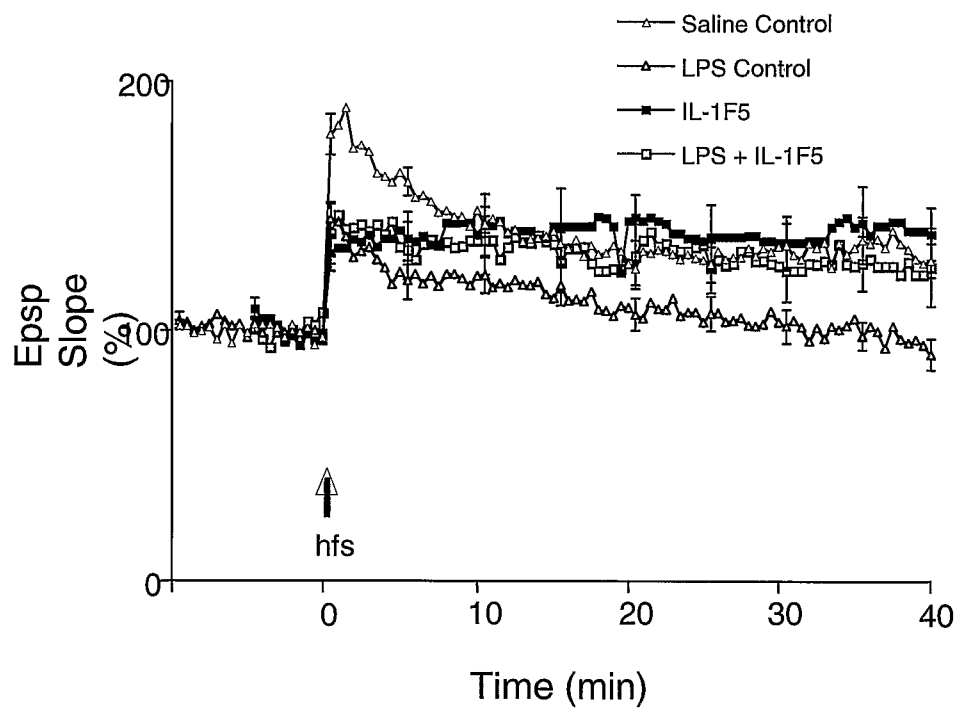


Figure 1 - LTP is impaired by LPS administration but maintained by treatment with IL-1F5

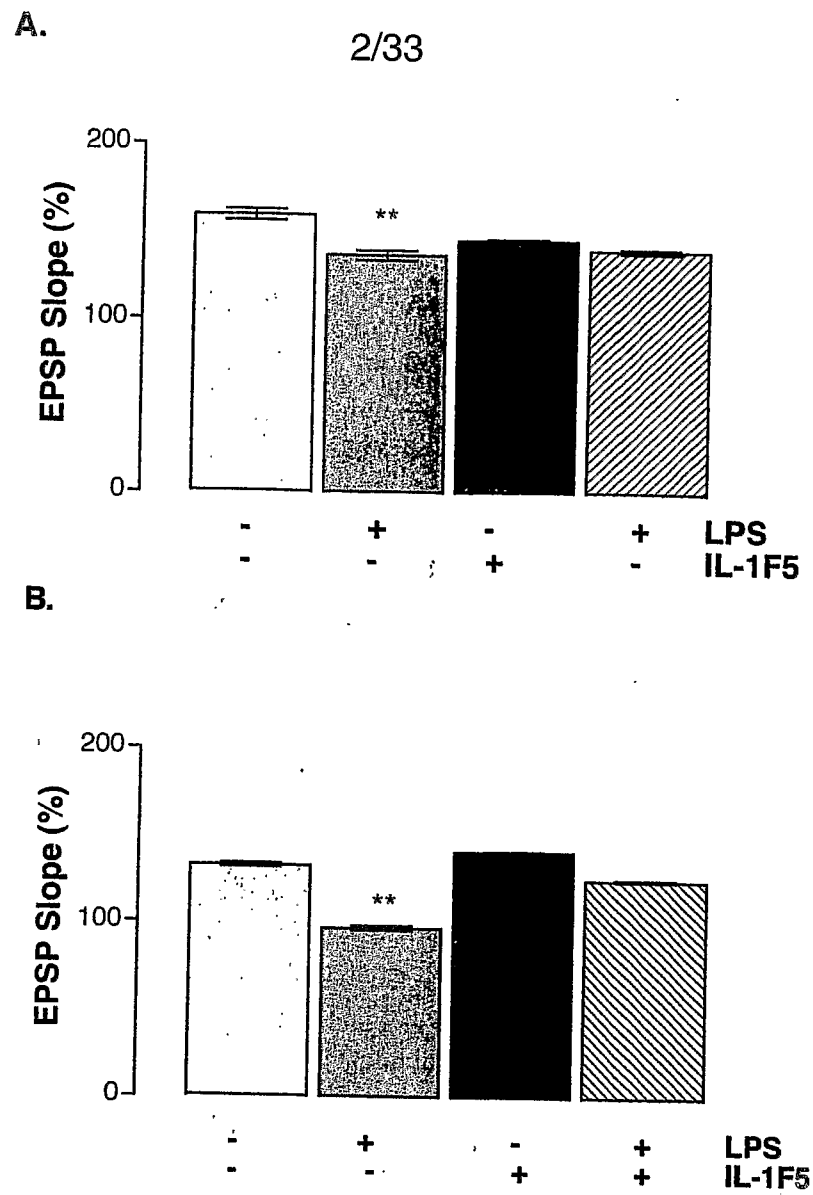


Figure 2 - LPS administration affects percentage change in EPSP slope; reversal by treatment with IL-1F5

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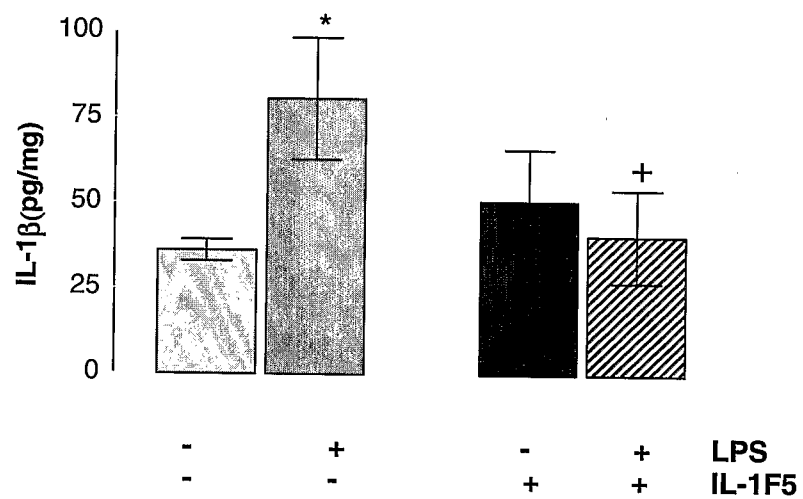
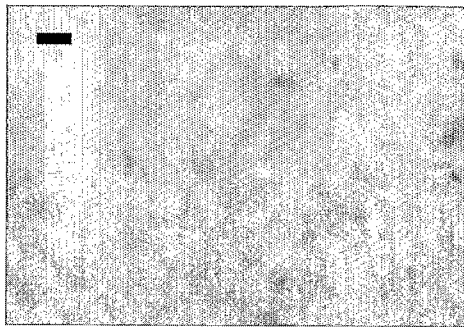


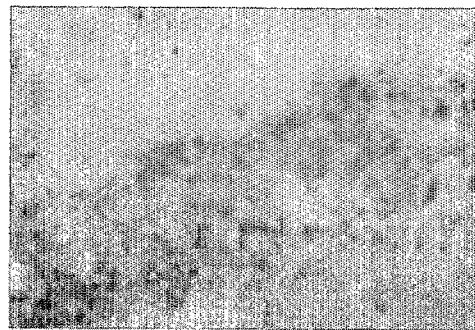
Figure 3 - LPS administration is associated with an increase in the concentration of interleukin-1 β in hippocampus

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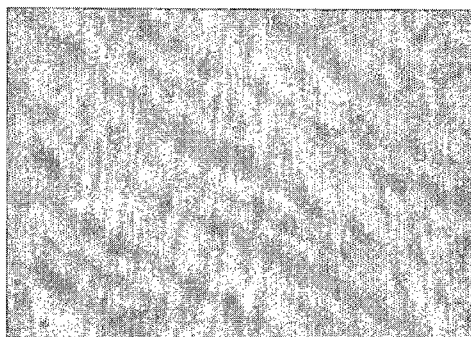
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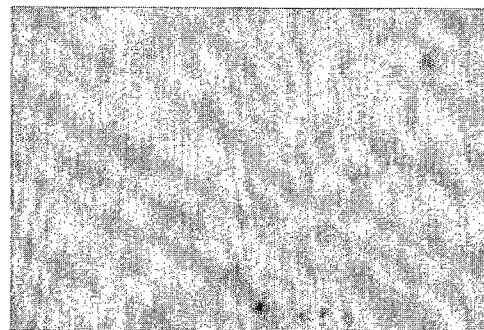
Con



LPS



IL-1F5



LPS+IL-1F5

B

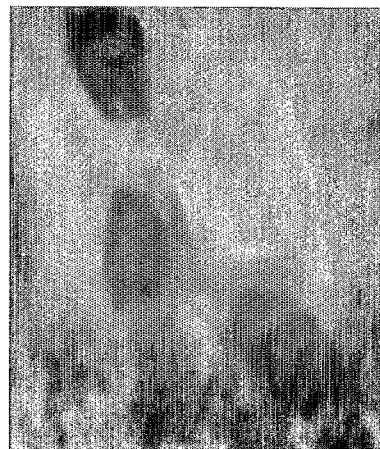
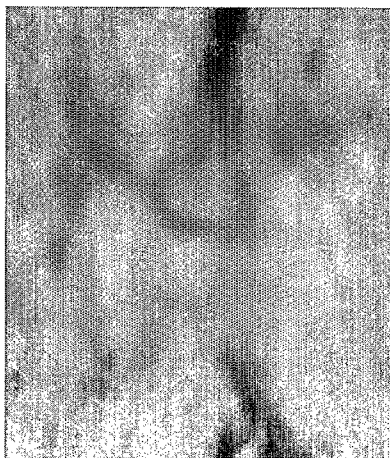


Figure 4 -LPS administration is associated with enhanced immunopositive staining for Ox-6.

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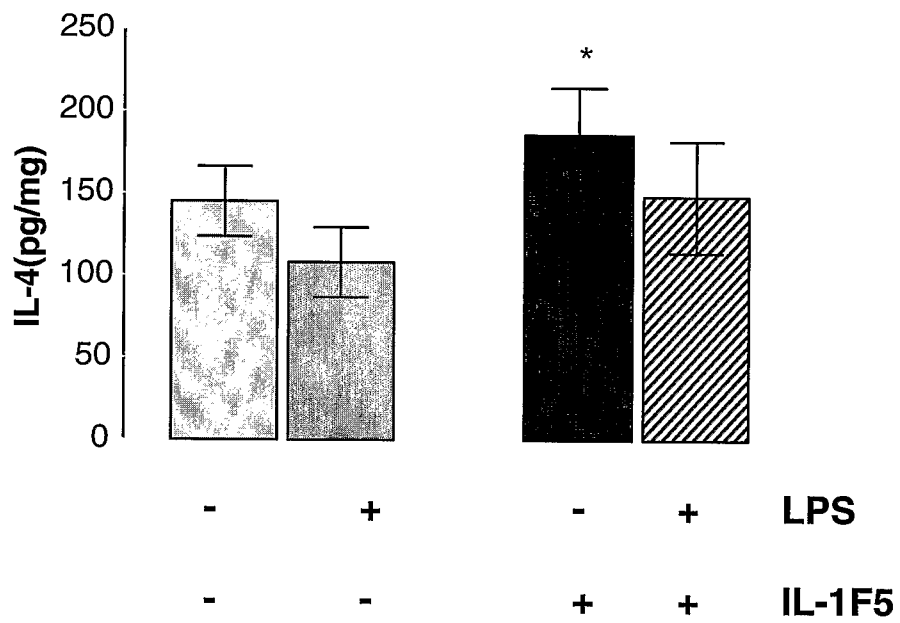


Figure 5 - IL-4 concentration is increased in hippocampus following IL-1F5 treatment

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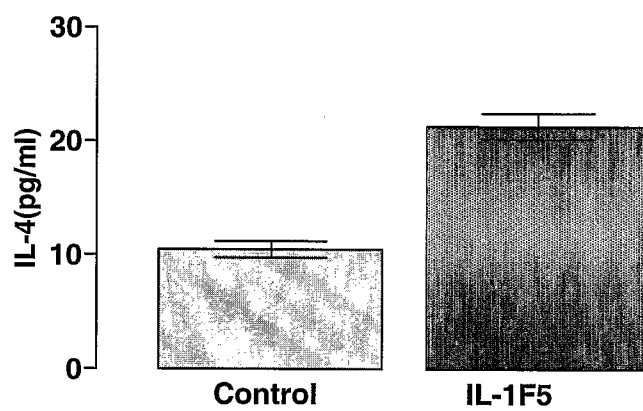


Figure 6 - IL-1F5 treatment is associated with an increase in IL-4 protein in cultured cortical glial cells

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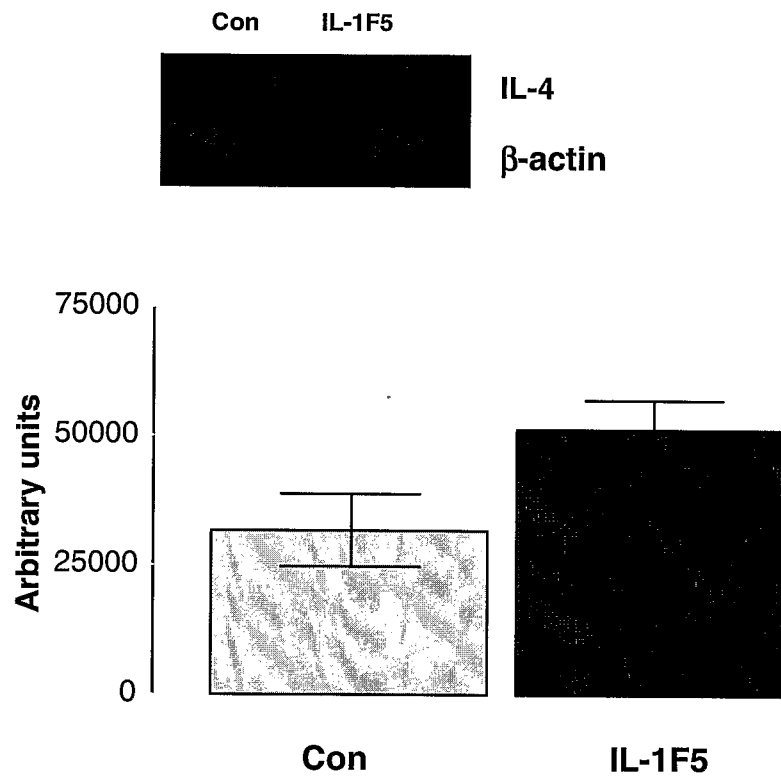


Figure 7 - IL-1F5 treatment is associated with an increase in IL-4 mRNA in cultured cortical glial cells

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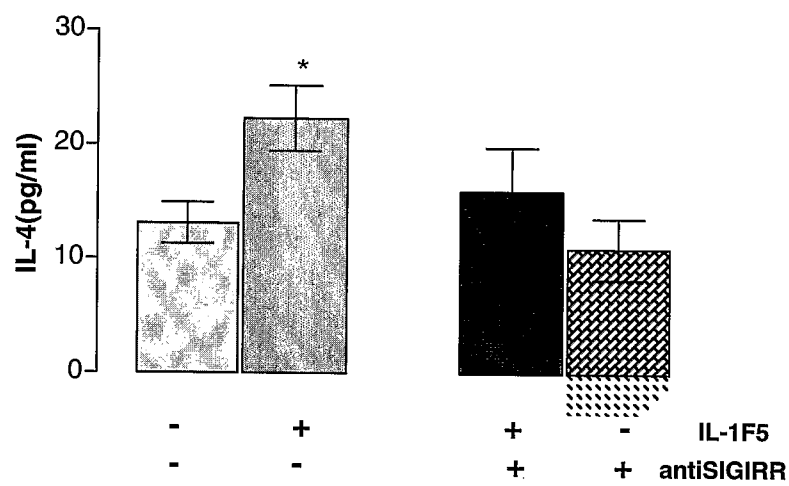


Figure 8 - Pre-incubation with an antibody for the novel IL-1 receptor SIGIRR is associated with inhibition of IL-1F5 induced increase in IL-4 in cultured cortical glia

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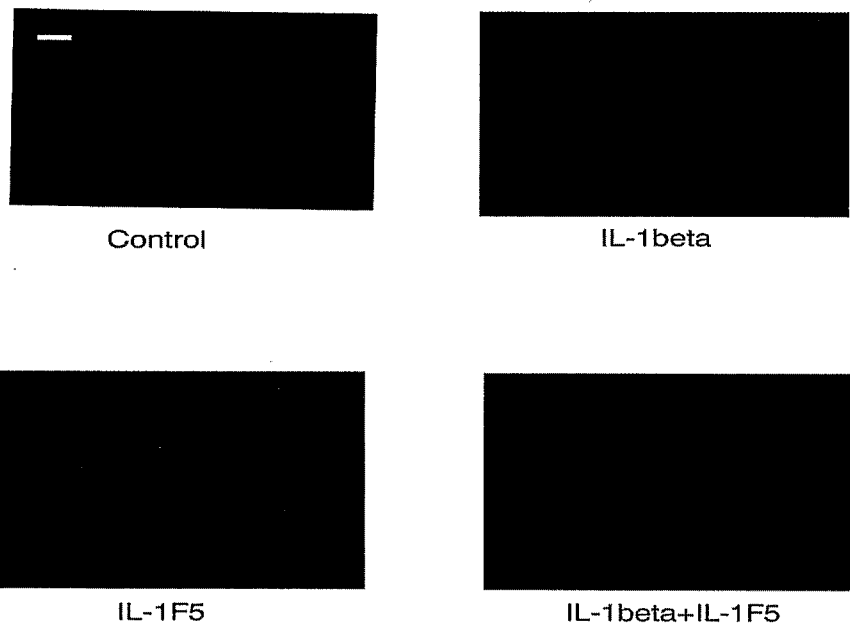


Figure 9 - IL-1F5 treatments is associated with an increased surface expression of the novel IL-1 receptor SIGIRR in cultural cortical glial cells

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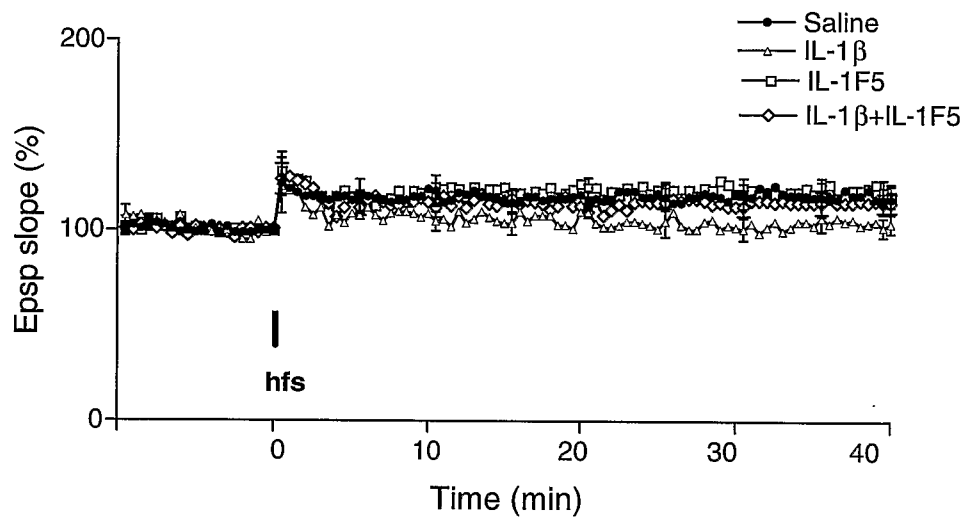


Figure 10 - IL-1 β induced inhibition of LTP is abrogated by IL-1F5

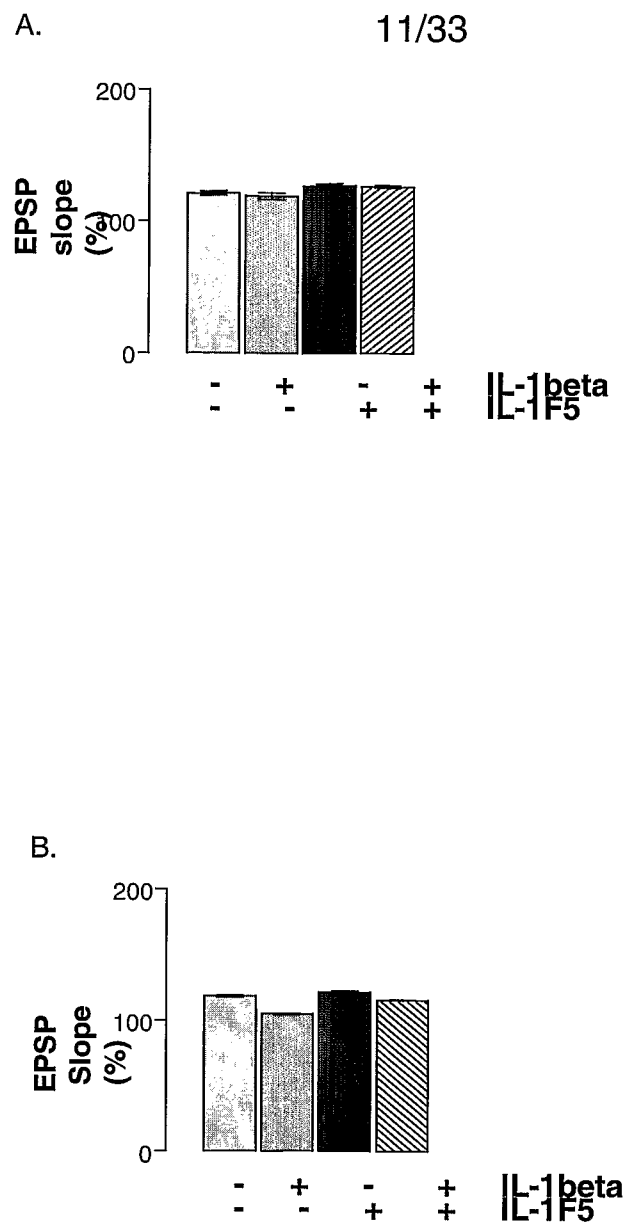


Figure 11 - IL-1 β administration affects percentage change in EPSP slope; reversal by treatment with IL-1F5

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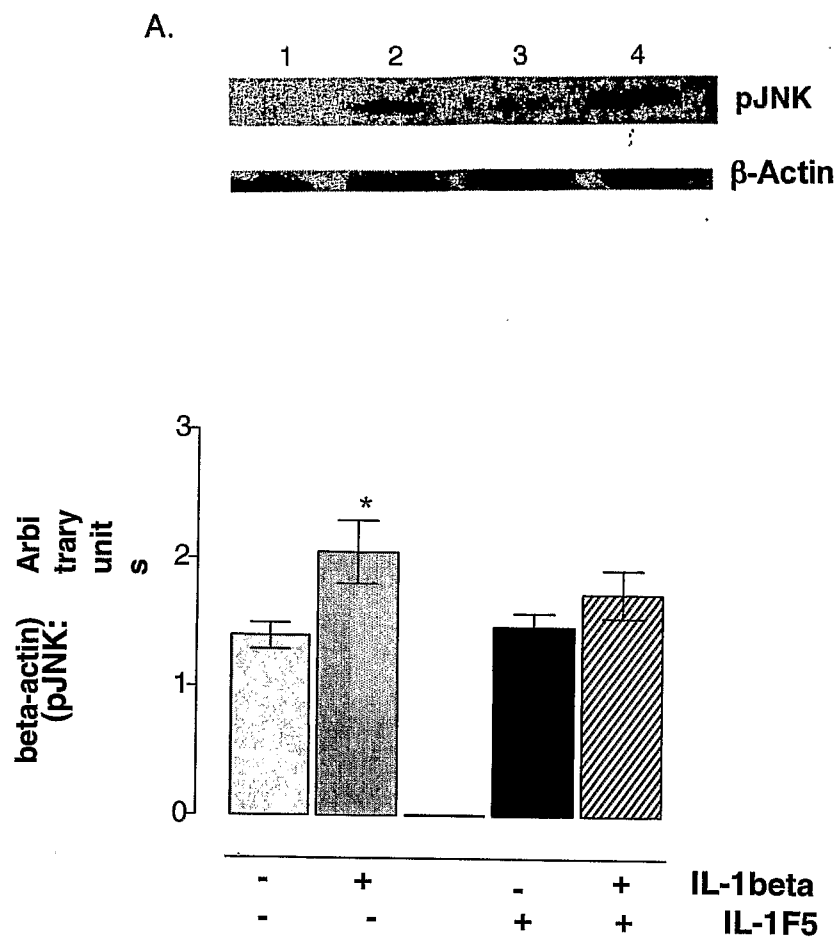


Figure 12 - IL-1 β administration is associated with an increase in JNK phosphorylation in the hippocampus

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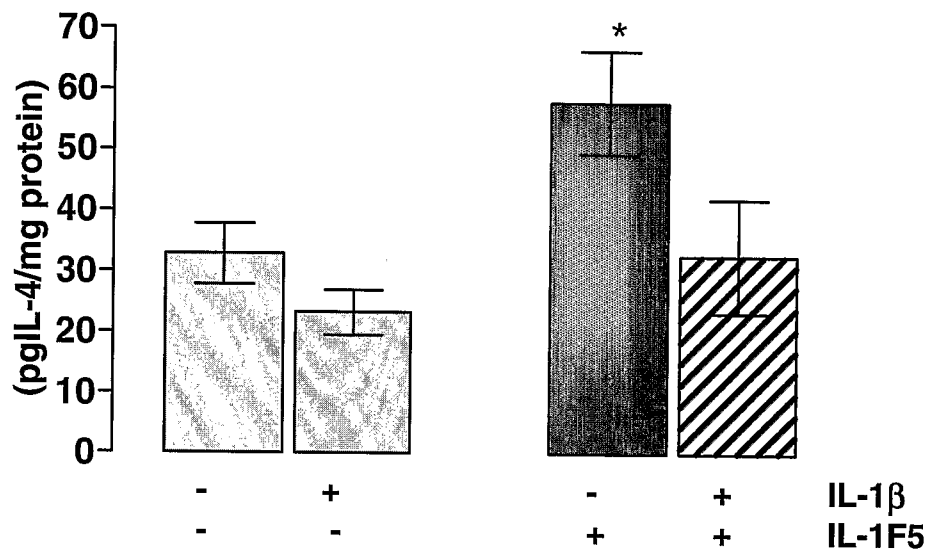


Figure 13 - IL-4 concentration is increased in hippocampus following IL-1F5 treatment

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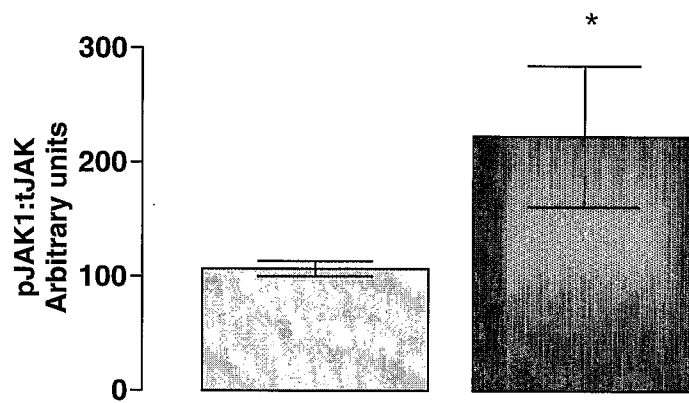


Figure 14 - IL-1F5 treatment is associated with an increase in JAK1 phosphorylation in hippocampus

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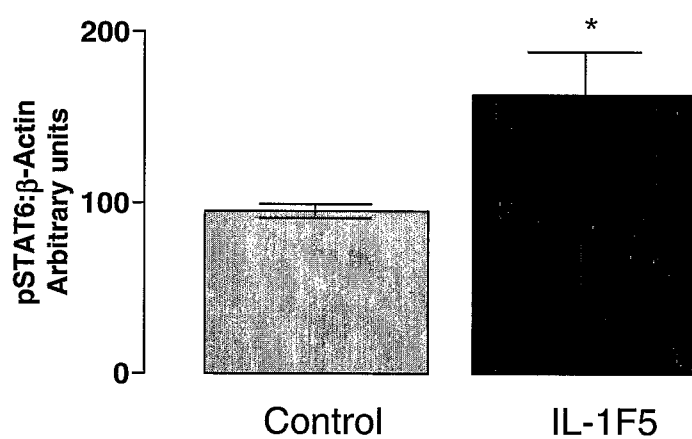
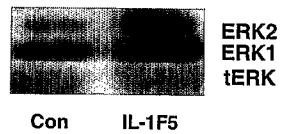


Figure 15 - IL-1F5 treatment is associated with an increase in STAT6 phosphorylation in hippocampus

16/33

A



B.

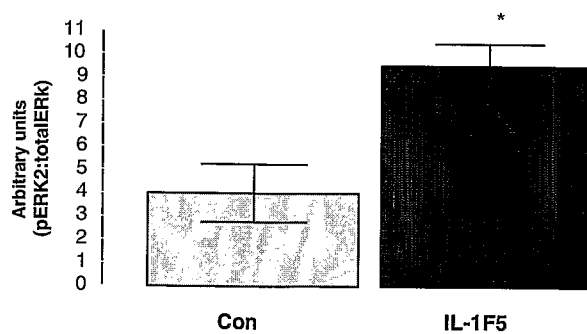
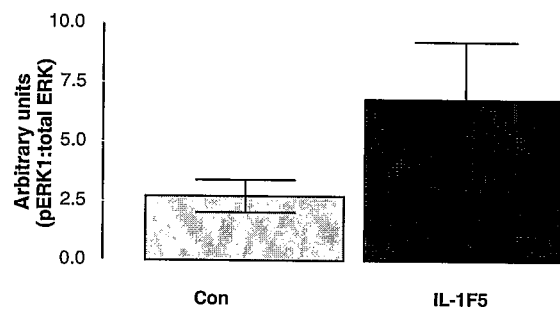


Figure 16 - IL-1F5 treatment is associated with an increase in ERK1 and ERK2 phosphorylation in hippocampus

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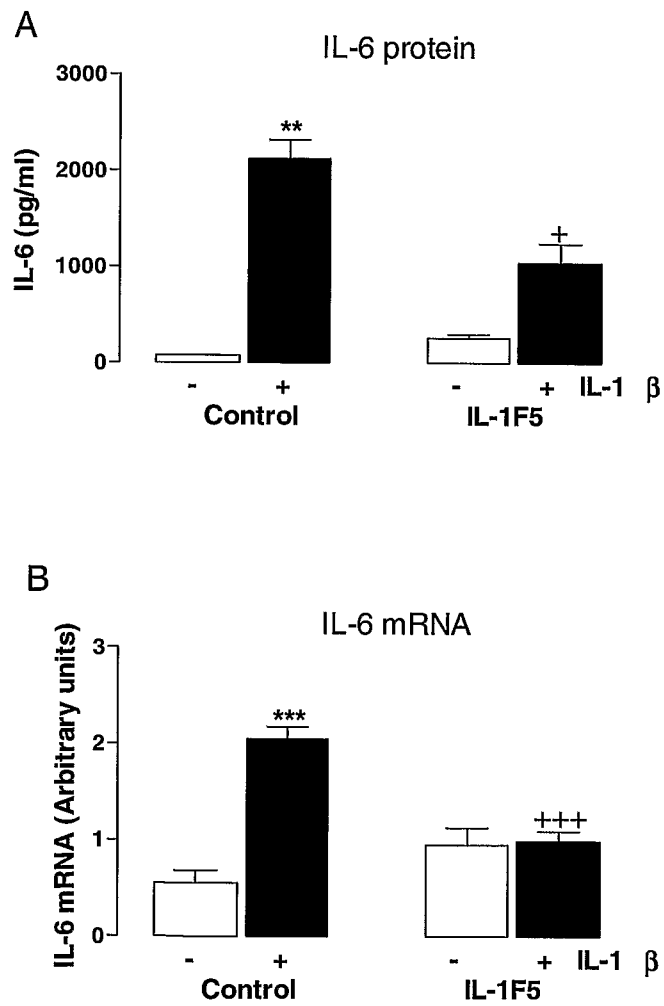


Figure 17 - IL-1F5 attenuates IL-1 β induced IL-6 protein and mRNA expression in mouse glia

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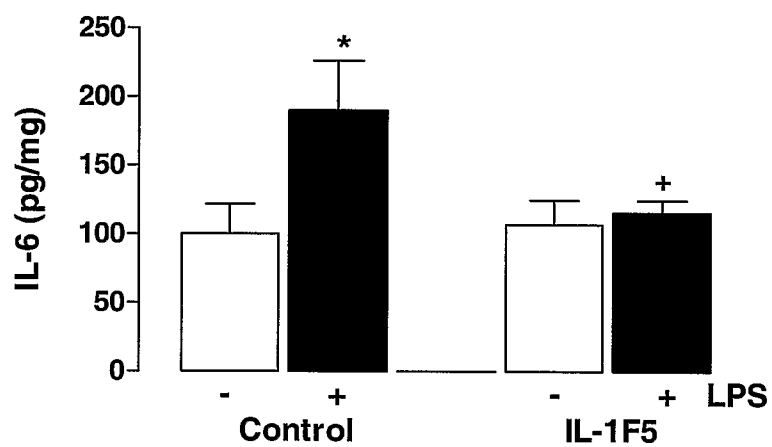


Figure 18 - intracerebroventricular injection of IL-1F5 attenuates LPS induced IL-6 in rat hippocampus

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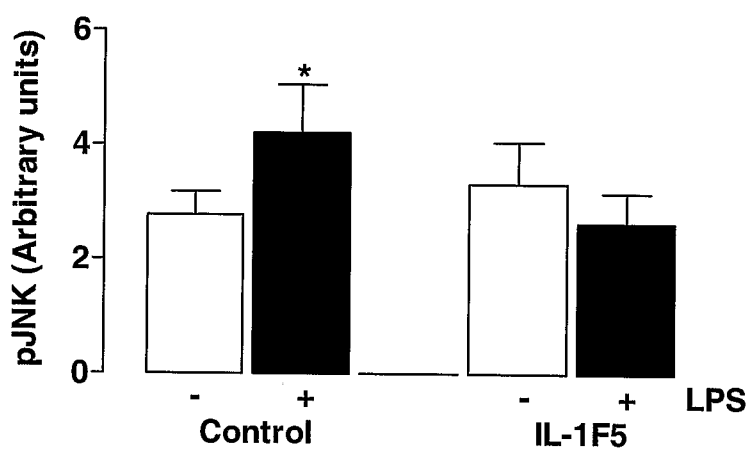


Figure 19 - intracerebroventricular injection of IL-1F5 attenuates LPS-induced JNK phosphorylation in rat hippocampus

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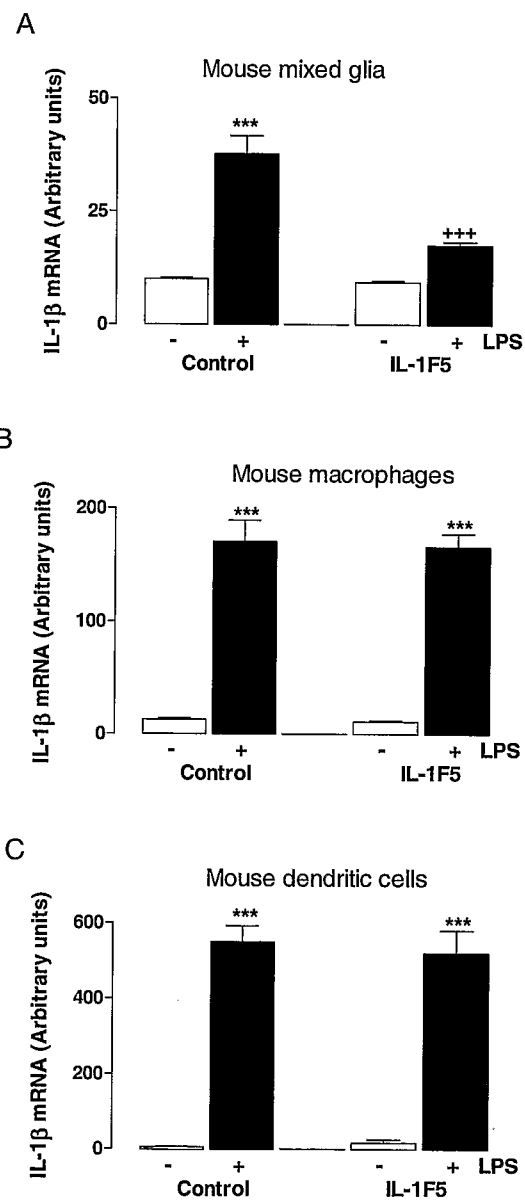


Figure 20 - IL-1F5 suppresses LPS-induced IL-1 β production by mouse mixed glia cells but not by murine macrophages or dendritic cells

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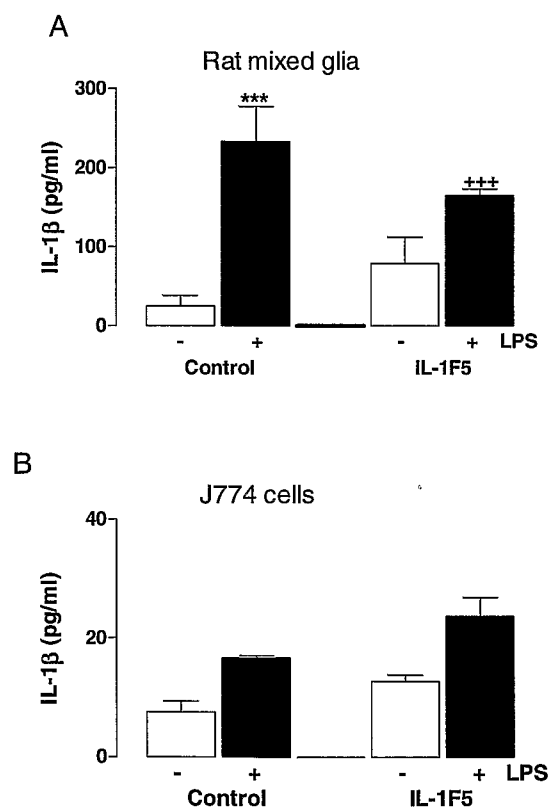


Figure 21 - IL-1F5 suppresses LPS-induced IL-1 β protein production by mixed glia cells but not by macrophages

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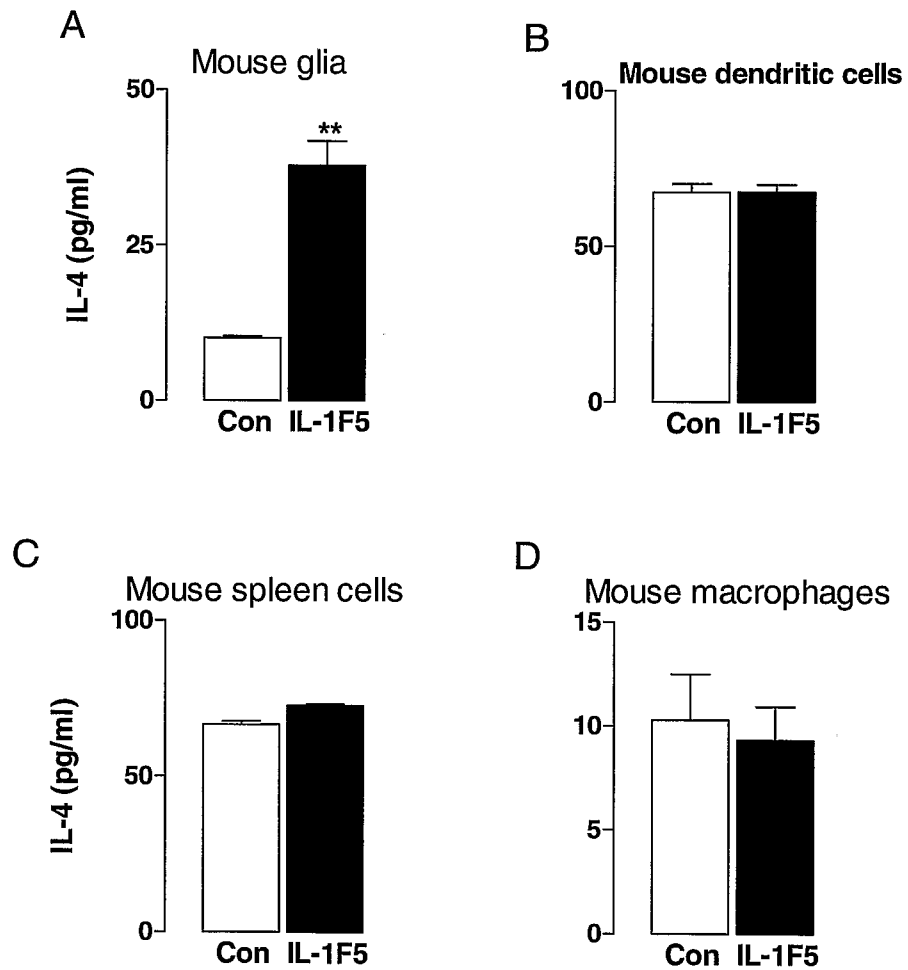


Figure 22 - IL-1F5 induced IL-4 production from murine mixed glial cells but not from dendritic cells, spleen cells or macrophages

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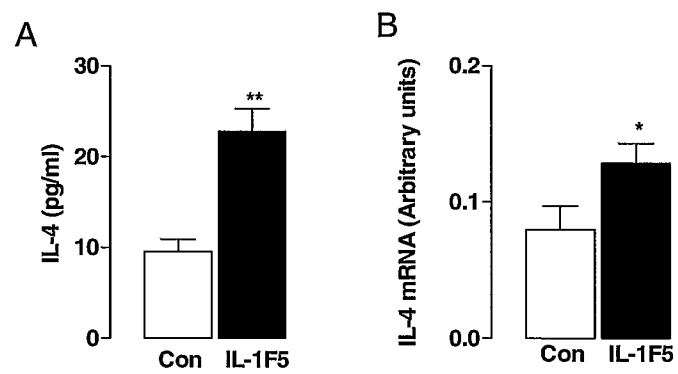
Rat mixed glia

Figure 23 - IL-1F5 induces IL-4 protein and mRNA expression in rat mixed glial cells

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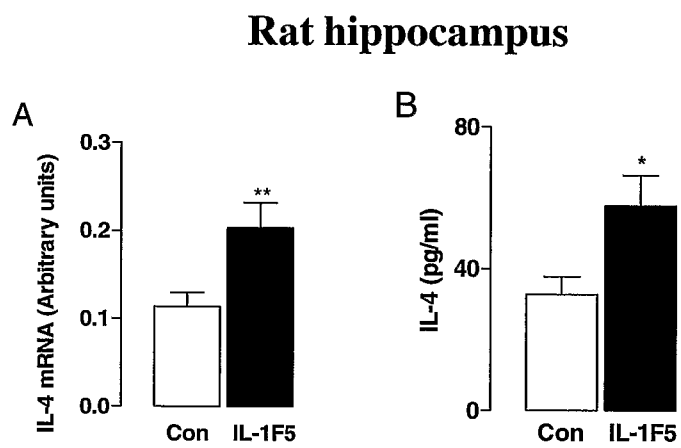


Figure 24 - intracerebroventricular injection of rats with IL-1F5 significantly increased IL-4 expression in the hippocampus

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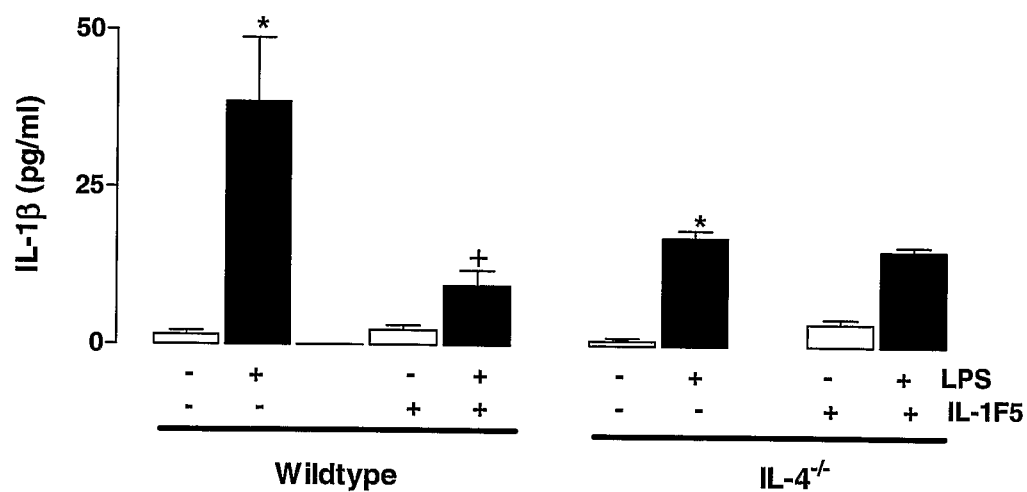


Figure 25 - IL-4 mediates the IL-1F5 attenuation of LPS-induced IL-1β production from glial cells

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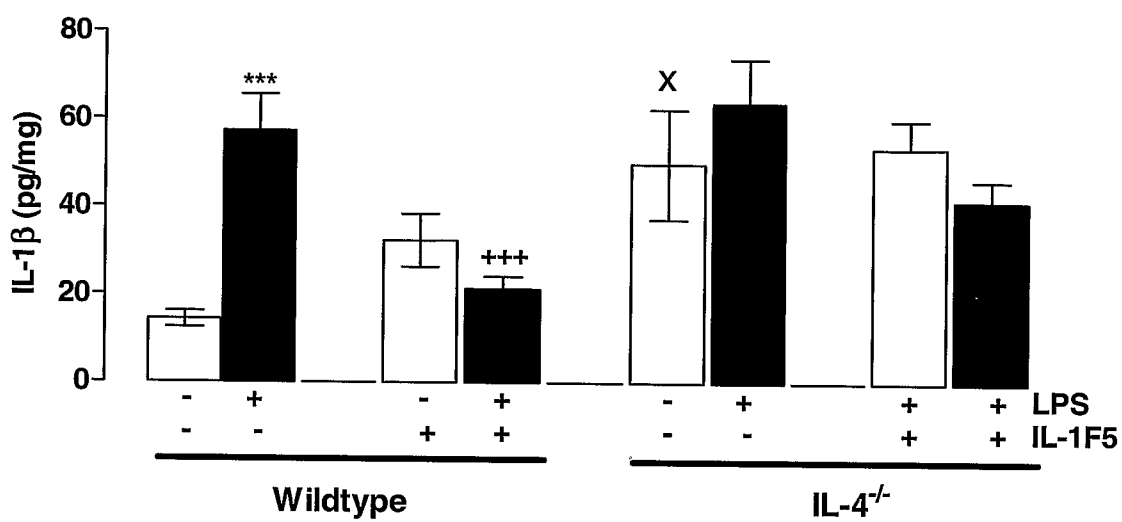


Figure 26 - attenuation of LPS-induced IL-1 β production by IL-1F5 in the hippocampus is mediated through IL-4

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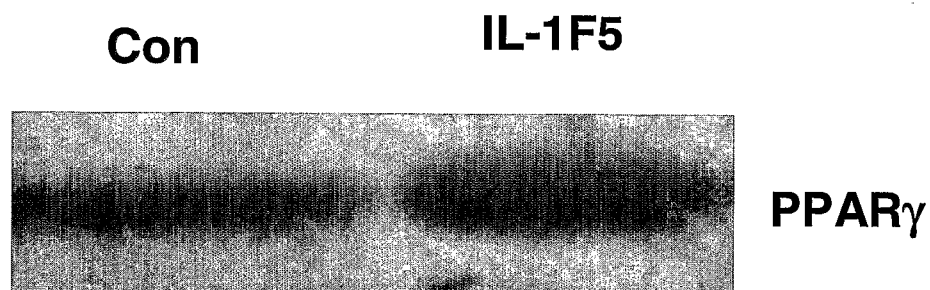


Figure 27 - PPARgamma protein expression levels in primary cultured cortical glia following treatment with IL-1F5 (24 hour incubation)

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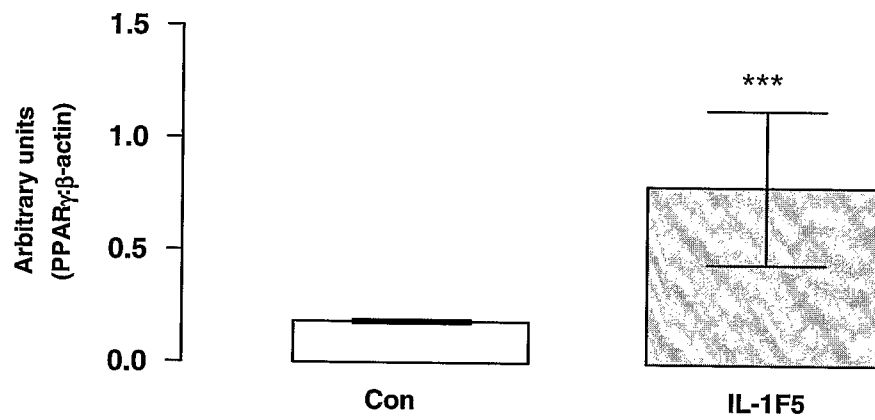


Figure 28 - PPARgamma expression is increased in cultured cortical glia following treatment with IL-1F5

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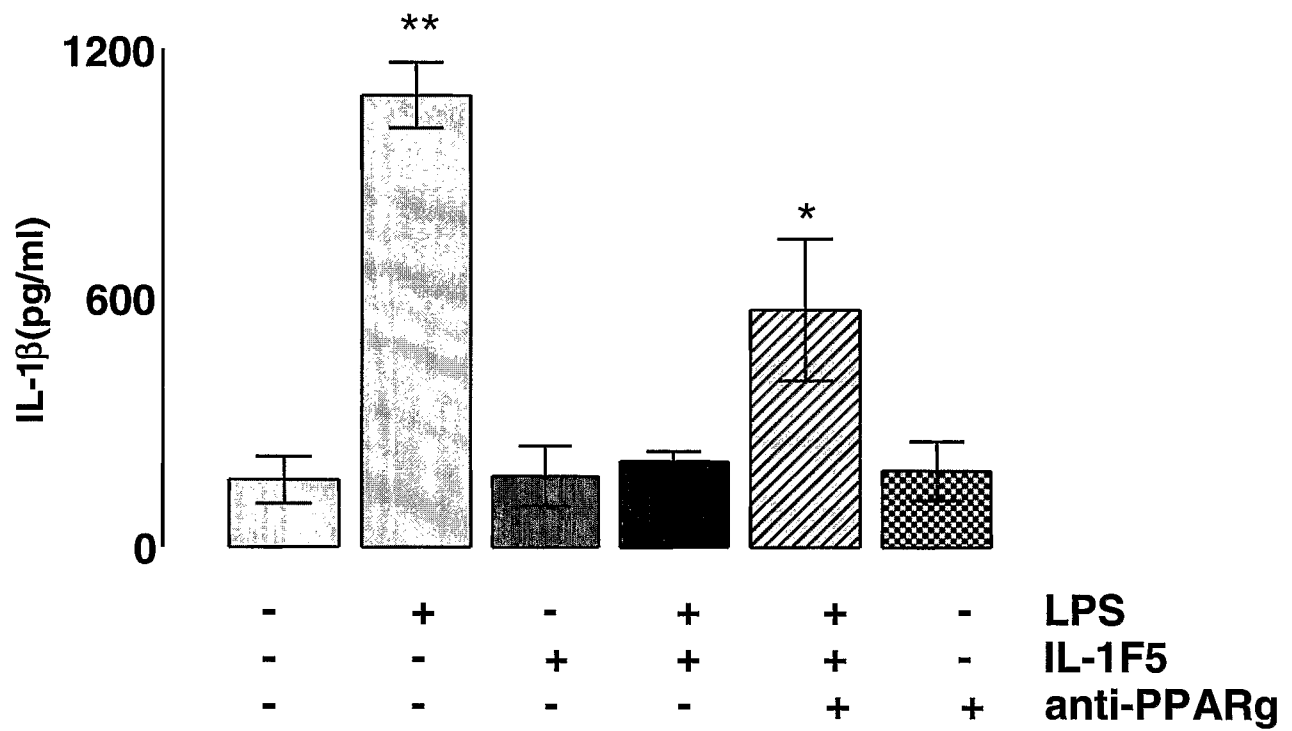


Figure 29 - Pre-incubation with a PPARgamma antagonist is associated with a reduction in IL-1F5 induced inhibition of IL-1beta in cultured cortical glia.

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541    tcccgagaat ggtggctgga atgcccccat cacagacttc tacttcagc agtgtgacta
601    gggcaacgtg cccccagaa ctccctgggc agagccagct cgggtgaggg gtgagtggag
661    gagacccatg gcggacaatc actctctctg ctctcaggac cccacgtct gacttagtgg
721    gcacctgacc actttgtctt ctggttccca gtttgataa attctgagat ttggagctca
781    gtccacggtc ctccccact ggatgggtgt actgctgtgg aatcttgtaa aaacatgtg
841    gggtaaactg ggaataacat gaaaagattt ctgtggaggt ggggtggggg agtggtggga
901    atcattcctg cttaatggta actgaccagt gttaccctga gcccgcagg ccaacccatc
961    cccagttgag ccttataggg tcagtagctc tccacatgaa gacctgtcac tcaccactat
1021   gcaggagagg gaggtggtca tagagtccag gatctatggc ccttgccca gcccacctc
1081   cttcccttta atcctgccac tgtcatatgc tacctttcct atctcttccc tcatcatctt
1141   gttgtgggca tgaggaggtg ctgatgtcag aagaaatggc tcgagctcag aagataaaaag
1201   ataagtaggg tatgctgac ctcttttaaa aaccaagat acaatcaaaa tcccagatgc
1261   tggctctctat tcccatgaaa aagtgtcat gacatattga gaagacctac ttacaaagtg
1321   gcatatatatt caatttattt taattaaaag atacctattt atatatttct ttatagaaaa
1381   aagtctggaa gagtttactt caattgtagc aatgtcaggg tgggtggcagt ataggtgatt
1441   tttcttttaa ttctgttaat ttacctgtat ttcctaattt ttctacaatg aagatgaatt
1501   ccttgataaa aaataagaaa agaaattaat cttgaggtaa gcagagtaga catcatctct
1561   gattgtcctc agcctccact tcccagagt aaattcaaat tgaatcgagc tctgctgctc
1621   tggttggttg tagtagtgat caggaaacag atctcagcaa agccactgag gaggaggctg
1681   tgctgagttt gtgtggctgg aatctctggg taaggaaactt aaagaacaaa aatcatctgg
1741   taattctttc ctagaaggat cacagccctt gggattccaa ggcattggat ccagtctcta
1801   agaaggctgc tgtactggtt gaattgtgtc cccctcaaat tcacatcctt cttggaatct
1861   cagtctgtga gtttatttgg agataagggtc tctgcagatg tagttagtta agacaagggtc
1921   atgctggatg aaggtagacc taaattcaat atgactggtt tccttgatg aaaaggagag
1981   gacacagaga cagaggagat gcggggaaga ctatgtaaag atgaaggcag agatcggagt
2041   tttgcagcca caagctaaga aacaccaagg attgtggcaa ccatcagaag cttggaagag
2101   gcaaagaaga attcttccct agaggcttta gagggataac ggctctgctg aaaccttaat
2161   ctcagacttc cagcctcctg aacgaagaaa gaataaattt cggctgtttt aagccaccaa
2221   ggataattgg ttacagcagc tctaggaaac taatacagct gctaaaatga tcctgtctc
2281   ctogtgttta cattctgtgt gtgtcccctc ccacaatgta ccaaagttgt ctttgtgacc
2341   aatagaatat ggcagaagtg atggcatgcc acttccaaga ttaggttata aaagacactg
2401   cagcttctac ttgagccctc tctctctgcc acccaccgcc cccaatctat cttggctcac
2461   tcgctctggg ggaagctagc tgccatgcta tgagcaggcc tataaagaga cttacgtggt
2521   aaaaaatgaa gtctcctgcc cacagccaca ttagtgaacc tagaagcaga gactctgtga
2581   gataatcgat gtttgttgtt ttaagttgct cagttttggt ctaacttgtt atgcagcaat
2641   agataaataa tatgcagaga aagagaaaaa aaaaaaaaaa aaaaaaaaaa

```

Figure 30 - The defined nucleotide sequence of human IL-1F5

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```
1  mvlsgalcfr mkdsalkvly lhnqllagg lhagkvikge eisvvpnrwl daslspvilg
61  vgggsqclsc gvgqepthtl epvnmelyl gakesksftf yrrdmgtss fesaaypgwf
121 lctvpeadqp vrltqlpeng gwnapitdfy fqgcd
```

Figure 31 - The defined amino acid sequence of human IL-1F5

32/33

```
1   ggcacgaggg gagcctgctt tctacttagg tctcaaattt tccagccttg tctttgccta
61  aaatttcctg ctgtttattt caaaataggg tctacatact gtggagctca tgatggttct
121 gagtggggca ctatgcttcc gaatgaagga ttcagccttg aaggtagctg atctgcacaa
181 taaccagctg ctggctggag gactgcacgc agagaaggtc attaaagggtg aggagatcag
241 tgttgtccca aatcggggcac tggatgccag tctgtccctt gtcctcctgg gcgttcaagg
301 aggaagccag tgcctatctt gtgggacaga gaaagggcca attctgaaac ttgagccagt
361 gaacatcatg gagctctacc tcggggccaa ggaatcaaag agcttcacct tctaccggcg
421 ggatatgggt cttacctcca gcttcgaatc cgtgcctac ccaggctggg tcctctgcac
481 ctaccgggaa gctgaccagc ctgtcaggct cactcagatc cctgaggacc ccgcctggga
541 tgctcccatc acagacttct actttcagca gtgtgactag ggctgcgtgg tccccaaaac
601 tccataagca gaggcagagt aggcagtggc ggctcctgat agaggataga gagacagagg
661 agctccacag taggtggctt actcctctcc ttccctactg gactcccgtt tctgacctaa
721 ggcacacaga cactctcttc tcctgcatcc cagtgcctgg aaatcttctg gtatttgagg
781 ctcaatgtgt agattctttc agattggatg gtactacctc tgggtgtggaa cccaatagaa
841 accacgtagg accaacaag agcaacataa aagattcttg ggtgaagaag aggtgggaac
901 tgttcataca tagtaagatc tgacacagta cctcagaagt cctgccattc cttatgttct
961 ggagaaagtg gagggggggg caccaagact ttctctgggt ggctggggcc tttccctcaa
1021 cctttctgac atctgcagcc tctctcattc ttgccttcac tctctggccc tgaaccgaga
1081 ggggtgatatc aggatagctg acagaagatg accaggcaca ctgtcctggg ttgaaaccag
1141 aggggacaaat aaaaaaccct gattctgggtc tctactcaca taaaaagaag cttgtgaaca
1201 ttaagtggga agagattgct actaaataac ataccttgta atttcatctt aattaaata
1261 tacttctcta tattatatat tttta
```

Figure 32 - The defined nucleotide sequence of murine IL-1F5

33/33

```
1  mmvlsgalcf rmkdsalkvl ylhnnqllag glhaekvikg eeisvvpnra ldaslspvil
61  gvqggsqcls cgtekgpilk lepvnimely lgakesksft fyrrdmglts sfesaaypgw
121 flctspeadq pvrltqiped pawdapitdf yfqgcd
```

Figure 33 - The defined amino acid sequence of murine IL-1F5