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Role of Interleukin-4 in Regulation of Age-related Inflammatory Changes in the Hippocampus*

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Yvonne Nolan‡§, Frank O. Maher‡§‖, Darren S. Martin‡¶, Rachael M. Clarke‡, Miriam T. Brady‡‡, Anthony E Bolton¶, Kingston H. G. Mills‡‡, and Marina A. Lynch‡§§**

From the ‡Physiology Department, Trinity College Institute of Neuroscience, the ‡‡Biochemistry Department, Immune Regulation Research Group, Trinity College, Dublin 2, Ireland, ¶Vasogen Ireland Limited, Shannon, Ireland

It is well documented that long term potentiation (LTP) is impaired in the hippocampus of the aged animal. Among the changes that contribute to this impairment is an increase in hippocampal concentration of the pro-inflammatory cytokine interleukin-1β (IL-1β), and increased IL-1β-induced signaling. In this study we investigated the possibility that these changes were a consequence of decreased concentration of the anti-inflammatory cytokine, IL-4, and decreased IL-4-stimulated signaling. We report that functional IL-4 receptors are expressed on granule cells of the dentate gyrus and that receptor activation results in phosphorylation of JAK1 and STAT6. Hippocampal IL-4 concentration was decreased with age, and this was accompanied by a decrease in phosphorylation of JAK1 and STAT6. The evidence indicates that IL-4 modulates expression of IL-1β mRNA and protein and that it attenuates IL-1β-induced impairment of LTP and phosphorylation of JNK and c-Jun. We argued that, if a decrease in hippocampal IL-4 concentration significantly contributed to the age-related impairment in LTP, then restoration of IL-4 should restore LTP. To test this, we treated rats with VP015 (phospholipid microparticles-incorporating phosphatidylserine), which increases IL-4 concentration in hippocampus. The data indicate that the VP015-induced increase in IL-4 concentration in hippocampus of aged rats and lipopolysaccharide (LPS)-treated rats was accompanied by a reversal of the age-related and LPS-induced impairment in LTP in perforant path granule cell synapses. We propose that interplay between pro-inflammatory and anti-inflammatory responses impact significantly on synaptic function in the hippocampus of the aged rat.

Inflammatory changes contribute to the deficits that accompany several neurodegenerative conditions, and, in animal models, it has been repeatedly shown that inflammation is associated with deficits in synaptic function and consequently impairments in cognitive function. For example, peripheral administration of lipopolysaccharide (LPS[1]), which increases pro-inflammatory cytokine interleukin-1β (IL-1β) concentration in the brain, is accompanied by evidence of cell stress and neuronal cell death (1, 2) as well as impairment of hippocampal-dependent learning and memory (3). Deficits in long term potentiation (LTP) have been reported in aged rats (4) and in other experimental conditions in which hippocampal IL-1β concentration is increased (5–9). Significantly, administration of anti-inflammatory agents restores synaptic function in LPS-treated animals in which IL-1β concentration is enhanced (6, 10, 11).

Inflammatory responses are regulated in part by anti-inflammatory cytokines, including IL-4 and IL-10, which are produced by T cells and cells of the innate immune system. T helper type 2 (Th2) cells secrete IL-4, IL-5, and IL-10 and are responsible for reciprocally regulating the T helper type 1 (Th1) subtype, which mediate cellular and inflammatory responses. IL-4 also plays a role in promoting the differentiation of naïve T cells to Th2 cells. A key anti-inflammatory action of IL-4 results from its ability to inhibit release of pro-inflammatory cytokines by innate immune cells and to up-regulate the synthesis of IL-1 receptor antagonist (12, 13).

Several cells express IL-4 receptors, including hematopoietic, endothelial, and epithelial cells (14), but robust evidence indicating their presence on neurons is lacking, although expression on cultured glial cells has been reported (15–17). The study of signaling events following IL-4 receptor activation has been confined largely to hematopoietic cells where it has been shown that formation of the ligand-receptor complex leads to sequential activation of the Janus family of tyrosine kinases, JAK1 and JAK3 and the transcription factor STAT6 (14).

Because of the anti-inflammatory properties of IL-4, we hypothesized that the age-related changes in hippocampal function, which we have attributed to the increase in IL-1β concentration (4) and up-regulated IL-1β-induced signaling (18), may be exacerbated by a decrease in IL-4 concentration and down-regulation of IL-4-stimulated signaling. We addressed this hypothesis by assessing parallel age-related changes in cytokine concentration and signaling and LTP in perforant path-granule cell synapses. Our findings demonstrate the presence of functional IL-4 receptors in rat hippocampus and indicate that the age-related decrease in IL-4 concentration and the associated down-regulation of IL-4-stimulated signaling significantly con-

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§ Both authors contributed equally to this work.

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** Recipient of a Trinity College Ussher Fellowship.

§§ To whom correspondence should be addressed. Tel.: 353-1-608-1770; Fax: 353-1-679-3545; E-mail: lynchma@tcd.ie.

[1] The abbreviations used are: LPS, lipopolysaccharide; IL-1β, interleukin-1β; IL-1RI, IL-1 receptor type I; LTP, long term potentiation; Th1, -2, T helper types 1 and 2; JAK1, Janus tyrosine kinase 1; STAT6, signal transducers and activators of transcription 6; PBS, phosphate-buffered saline; NBM, neurobasal medium; ES, excretory/secretory; BSA, bovine serum albumin; JNK, c-Jun N-terminal kinase; TBS, Tris-buffered saline; NGS, normal goat serum; ANOVA, analysis of variance; p, phosphorylated; epsp, excitatory post-synaptic potential.

tribute to the deficit in LTP observed in aged rats. Significantly, restoration of IL-4 concentration to that observed in hippocampus of young rats was associated with successful maintenance of LTP.

EXPERIMENTAL PROCEDURES

Animals—Male Wistar rats (BioResources Unit, Trinity College, Dublin, Ireland) of mean age 4 months (250–350 g) or 22 months (450–550 g) were used in these experiments. Animals were housed in pairs (22-month-old rats) or groups of 4–6 (4-month-old rats) under a 12-h light schedule, ambient temperature was controlled between 22 and 23 °C, and rats were maintained under veterinary supervision throughout the study. These experiments were performed under a license issued by the Department of Health (Ireland).

VP015 Treatment Regimen—Rats were randomly assigned to four treatment groups; rats in two of these groups were injected with VP015 (150 μl, 6×10^6 particles incorporating phosphatidylserine/ml of suspension in PBS; Vasogen Inc., Mississauga, Canada) intramuscularly into the upper hind limb 14 days, 13 days, and 24 h before treatment with anesthetic and subsequent assessment of the ability of rats to sustain LTP. At corresponding times, the remaining two groups received three injections of saline and/or phospholipid microparticles-incorporating phosphatidylcholine as control. The effect of this treatment was assessed in rats treated intraperitoneally with lipopolysaccharide (LPS) or saline (see below). In a separate set of experiments, groups of young (4 months) and aged (23 months) male Wistar rats underwent the same treatment regime. Injections were given on alternate limbs and no local adverse effects were observed at any time.

Induction of LTP in Vivo—On the day of the experiment, rats were anesthetized by intraperitoneal injection of urethane (1.5 g/kg); the absence of a pedal reflex was considered to be an indicator of deep anesthesia. In those experiments where rats were challenged with LPS, intraperitoneal injection of LPS (100 μg/kg; Sigma) or saline was administered to young (4 months) rats 3 h before analysis of LTP as described previously (1). Briefly, a bipolar stimulating electrode and a unipolar recording electrode were stereotaxically positioned in the perforant path (4.4-mm lateral to lambda) and dorsal cell body region of the dentate gyrus (2.5-mm lateral and 3.9-mm posterior to Bregma), respectively. Test shocks were delivered at 30-s intervals, and recorded for 10 min before and 40 min after tetanic stimulation (three trains of stimuli; 250 Hz for 200 ms; 30-s intertrain interval). In some experiments, rats were injected intracerebroventricularly (2.5-mm posterior, and 0.5-mm lateral, to Bregma) with either 5 μl of saline, or 5 μl of IL-1β (3.5 ng/ml, R & D Systems, UK) 5 min after an intracerebroventricular IL-4 injection (5 μl, 20 μg/ml, R & D Systems, UK) and in a further series of experiments, a group of aged rats were injected intracerebroventricularly with IL-4 to assess whether acute treatment with the anti-inflammatory cytokine might attenuate the age-related deficit in LTP. In these studies, tetani were delivered to the perforant path 30 min after IL-1β or IL-4 injection.

At the end of the experiment, rats were killed by decapitation, and the brains rapidly removed. The hippocampus was dissected free from the whole brain; slices (350 × 350 μm) were prepared using a McIlwain tissue chopper and stored in Krebs buffer containing $CaCl_2$ (1.13 mM) and 10% Me_2SO at −80 °C as described previously (19) until required for analysis. In some cases, untreated brains were removed whole, coated with OCT compound (Sakura Tissue-Tek, Netherlands), immersed in isopentane at −30 °C and stored at −80 °C until sections were prepared. 20-μm cryostat sections were prepared, mounted on gelatin-coated slides, air dried for 30 min, and stored at −20 °C until used for immunohistochemical analysis.

Preparation of Primary Neuronal and Glial Cultures—Primary hippocampal neurons were isolated and prepared from 1-day-old Wistar rats (BioResources Unit, Trinity College, Dublin, Ireland) and maintained in neurobasal medium (NBM, Invitrogen) as described previously (6). Tissue from three neonates was pooled and represented an individual culture. Data were obtained from a total of six cultures. Rats were decapitated, the hippocampus dissected, and the meninges removed. Tissue was incubated in phosphate-buffered saline (PBS, Sigma) with trypsin (0.25 μg/ml, Sigma) for 25 min at 37 °C, triturated in PBS containing soybean trypsin inhibitor (0.2 μg/ml, Sigma) and DNase (0.2 mg/ml, Sigma) and gently passed through a sterile mesh filter (40 μm). The suspension was centrifuged at 2000 × *g* for 3 min at 20 °C, and the pellet was resuspended in warm NBM, supplemented with heat-inactivated horse serum (10%, Sigma), penicillin (100 units/ml, Invitrogen), streptomycin (100 units/ml, Invitrogen), and Glutamax (2 mM, Invitrogen). Suspended cells were plated at a density of 0.25×10^6 cells on circular 10-mm diameter coverslips, coated with poly-L-lysine (60 μg/ml, Sigma), and incubated in a humidified atmosphere containing 5% CO_2:95% O_2 at 37 °C for 2 h prior to being flooded with pre-warmed NBM. After 48 h, 5 ng/ml cytosine-arabino-furanoside (Sigma) was added to the culture medium to suppress the proliferation of non-neuronal cells. Culture media were exchanged every 3 days and cells were grown in culture for up to 7 days prior to treatment.

Neurons were incubated in the absence or presence of LPS (100 ng/ml in NBM) with or without a 2-h pre-treatment with IL-4 (100 ng/ml in NBM). 24 h later supernatant was removed and stored for analysis of IL-1β concentration. Cells were rinsed in TBS and fixed in 4% paraformaldehyde in TBS for immunohistochemical assessment of expression of IL-4 receptor (IL-4R), pJAK1, pJAK3, pSTAT6, and IL-1 receptor type I (IL-1RI). In some cases, hippocampal neurons were incubated in the presence or absence of IL-4 (100 ng/ml for 2 h) and harvested for analysis of expression of IL-1RI and IL-4R; the protocols used for assessment of expression of IL-4R and IL-1RI are outlined below. In a separate set of experiments, total RNA was extracted from LPS- and/or IL-4-treated hippocampal neurons using TRI reagent (Sigma) according to the manufacturer's instructions and used for analysis of IL-1β and IL-1RI mRNA (see below).

Mixed glial cultures were prepared from whole brains of 1-day-old Wistar rats (BioResources Unit, Trinity College, Dublin, Ireland). In this case, dissected tissue was roughly chopped and added to pre-warmed Dulbecco's modified Eagle's medium (Invitrogen) containing fetal calf serum, penicillin (100 units/ml), and streptomycin (100 units/ml). Tissue was triturated, and the suspension was filtered through a sterile mesh filter (40 μm) and centrifuged at 2000 × *g* for 3 min at 20 °C, and the resulting pellet was resuspended in warmed Dulbecco's modified Eagle's medium. Resuspended cells were pipetted onto poly-L-lysine-coated (60 μg/ml) coverslips in 24-well plates at a density of 1.0×10^6 cells and incubated for 1 h before addition of warmed Dulbecco's modified Eagle's medium. Cells were grown at 37 °C in a humidified 5% CO_2:95% air environment, and media were changed every 3 days. Resultant cells were composed of microglia and astrocytes as visualized by OX-42 and glial fibrillary acidic protein immunoreactivity, respectively. Tissue from three neonates was pooled and represented an individual culture. Data were obtained from a total of four cultures. Glia and neurons were incubated in the presence or absence of homogenate (20 μg/ml) prepared from the helminth parasite, *Fasciola hepatica* (liver fluke homogenate) or excretory/secretory (ES) products (20 μg/ml) obtained from the parasite. Twenty-four hours later supernatant was removed and stored for analysis of IL-4 concentration.

Generation of Bone Marrow-derived Dendritic Cells—Femurs were removed from BALB/c mice (BioResources Unit, Trinity College, Dublin, Ireland) and dissected from the surrounding muscle tissue. The bone marrow was flushed out with RPMI medium (Invitrogen), and cell aggregates were disintegrated using a syringe. The resulting cells were washed, pelleted by centrifugation (1200 rpm for 5 min), and resuspended in fresh RPMI. Immature bone marrow-derived dendritic cells were prepared by culturing cells from one leg per tissue culture flask in complete RPMI supplemented with 10% of a supernatant from a granulocyte macrophage-colony-stimulating factor-secreting cell line, J558. The cells were maintained in culture for 3 days at 37 °C with 5% CO_2, loosely adherent cells were removed, and fresh medium containing 10% granulocyte macrophage-colony-stimulating factor was added. Cells were re-cultured for an additional 4 days, and the viability of these myeloid dendritic cells was assessed and found to be 80% (20). Dendritic cells were incubated in the presence or absence of liver fluke homogenate (20 μg/ml) or ES products (20 μg/ml). 24 h later supernatant was removed, and the IL-4 concentration was determined by enzyme-linked immunosorbent assay (21).

Analysis of IL-4 and IL-1β Protein Concentration—The concentration of IL-1β was assessed in supernatant obtained from neuronal culture as well as in hippocampal homogenates, whereas IL-4 concentration was assessed in hippocampal homogenate and in supernatant prepared from glia, which were treated in the presence and absence of liver fluke homogenate or ES. Analysis was carried out by enzyme-linked immunosorbent assay (R & D Systems). Hippocampal slices were thawed and rinsed three times in ice-cold Krebs solution and homogenized in ice-cold Krebs solution. Protein concentrations in homogenates were equalized (22), and triplicate aliquots (100 μl) were used for enzyme-linked immunosorbent assay. Antibody-coated (with either 1.0 μg/ml goat anti-rat IL-1β antibody or 2.0 μg/ml mouse anti-rat IL-4 antibody diluted in PBS, pH 7.3) 96-well plates were incubated overnight at room temperature, washed several times with PBS containing 0.05% Tween 20, blocked for 1 h at room temperature with blocking buffer (PBS, pH

7.3, 5% sucrose, 1% bovine serum albumin (BSA), 0.05% NaN_3), and incubated with standards (IL-1β (0–1000 pg/ml), IL-4 (0–1000 pg/ml)) or samples for 2 h at room temperature. Wells were washed with PBS, incubated with secondary antibody (350 ng/ml biotinylated goat anti-rat antibody for IL-1β or 50 ng/ml biotinylated goat anti-rat antibody for IL-4; each diluted in PBS containing 1% BSA and 2% normal goat serum) for 2 h at room temperature, washed again, and incubated in horseradish peroxidase-conjugated streptavidin (1:200 dilution in PBS containing 1% BSA) for 20 min at room temperature. Substrate solution (1:1 mixture of H_2O_2 and tetramethylbenzidine) was added, incubation was continued at room temperature in the dark for 30 min, and the reaction was stopped using 1 M H_2SO_4. Absorbance was read at 450 nm; values were corrected for protein in the case of homogenates and expressed as picograms/mg of protein.

Analysis of IL-1β mRNA and IL-1RI mRNA—cDNA synthesis was performed on 1 μg of total RNA using oligo(dT) primer (Superscript reverse transcriptase, Invitrogen). The RNA was treated with RNase-free DNase I (Invitrogen) at 1 unit/μg of RNA for 10 min at 65 °C. Equal amounts of cDNA were used for PCR amplification for a total of 26 cycles. Primers were pre-tested through an increasing number of cycles to obtain reverse transcription-PCR products in the exponential range. In the case of rat IL-1β mRNA expression the following sequences of primers were used: upstream, 5′-GCA CCT TCT TTT CCT TCA TC-3′; downstream, 5′-CTG ATG TAC CAG TTG GGG AA-3′; and for rat β-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 s followed by cycles of 94 °C for 45 s, 60 °C for 45 s, and 72 °C for 90 s. The reaction was stopped by final extension for 10 min at 70 °C. These primers generated IL-1β PCR products of 447 bp and β-actin PCR products of 360 bp. In the case of IL-1RI mRNA, cDNA synthesis was performed on 1 μg of total RNA using oligo(dT) primer (Superscript reverse transcriptase, Invitrogen). The RNA was treated with RNase-free DNase I (Invitrogen) at 1 unit/μg of RNA for 10 min at 65 °C. Equal amounts of cDNA were used for PCR amplification for a total of 30 cycles. Primers were pre-tested through an increasing number of cycles to obtain reverse transcription-PCR products in the exponential range. The following sequences of primers were used: upstream, 5′-AGA TGG AAG GAC CTA TGA TC-3′; downstream, 5′-TGC AGC ATC TGA CGA CAG GA-3′; and for rat β-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 180 s, 54 °C for 60 s, and 72 °C for 180 s followed by cycles of 94 °C for 15 s, 54 °C for 20 s, and 72 °C for 60 s. The reaction was stopped by final extension for 10 min at 72 °C. These primers generated IL-1RI PCR products of 640 bp and β-actin PCR products of 360 bp. Equal volumes of PCR product from each sample were loaded onto 1.5% agarose gels, and bands were separated by application of 90 V, photographed, and quantified using densitometry. Estimation of mRNA expression was carried out using β-actin as a reference gene. No observable change in β-actin mRNA was observed in any of the treatment conditions.

Western Immunoblot Analysis of pJAK1, pJAK3, pSTAT6, pJNK1, p-c-Jun, IL-1RI, and IL-4R—Phosphorylated forms of JAK1 (p-JAK1), JAK3 (p-JAK3), STAT6 (p-STAT6), and JNK1 (p-JNK1) were assessed in homogenates obtained from hippocampus. Phosphorylated STAT6 was assessed in nuclear fraction while p-JNK, p-JAK1, and p-JAK3 were analyzed in cytosolic fractions. The effects of IL-1β and IL-4 were also assessed on p-JNK1 and phosphorylated c-Jun (p-c-Jun). Expression of IL-4R and IL-1RI was assessed in preparations obtained from cultured hippocampal neurons incubated in the presence or absence of IL-4 (100 ng/ml in NBM).

The nuclear and cytosolic fractions were prepared by homogenizing hippocampal slices in lysis buffer (composition in mM: Hepes 10 (pH 7.0), KCl 10, EDTA 0.1, $MgCl_2$ 1.5, Nonidet P-40 0.2%, dithiothreitol 1.0, phenylmethylsulfonyl fluoride 0.5). Cells were pelleted by centrifugation at 10,000 × *g* for 5 min at 4 °C and reconstituted in lysis buffer. Lysates were then centrifuged at 10,000 × *g* for 5 min at 4 °C, and supernatants containing the cytosolic extracts were removed. Pelleted nuclei were reconstituted in nuclear extract buffer (composition in mM: Hepes 20 (pH 7.9), NaCl 420, EDTA 0.1, $MgCl_2$ 1.5, glycerol 25%, dithiothreitol 1.0, phenylmethylsulfonyl fluoride 0.5). Nuclei were incubated on ice for 15 min at room temperature then centrifuged at 15,000 × *g* for 30 min at 4 °C. The supernatants were removed as nuclear extracts.

All tissue samples were equalized for protein concentration, and 10-μl aliquots (1 mg/ml) were added to 5 μl of sample buffer (Tris-HCl, 0.5 mM, pH 6.8; glycerol, 10%; SDS, 10%; β-mercaptoethanol, 5%; bromphenol blue, 0.05% w/v), boiled for 5 min and loaded onto gels (7.5% SDS for p-JAK1 and pJAK3, 12% for p-JNK1 and p-c-Jun, and 10% for p-STAT6, IL-1RI, and IL-4R). Proteins were separated by application of 30 mA constant current for 25–30 min, transferred onto nitrocellulose strips (225 mA for 75 min), and immunoblotted with the appropriate antibody. To assess expression of p-JAK1, p-JAK3, and p-STAT6, nitrocellulose strips were incubated overnight at 4 °C in the presence of a goat polyclonal IgG antibody that specifically targets either p-JAK1 (Santa Cruz Biotechnology; diluted 1:200); p-JAK3 (Santa Cruz Biotechnology; diluted 1:100); or p-STAT6 (Santa Cruz Biotechnology; diluted 1:200) in TBS containing 5% Triton X-100 and 0.1% BSA. To examine JNK1 and c-Jun phosphorylation, nitrocellulose strips were probed with a mouse monoclonal IgG raised against a recombinant protein corresponding to an amino acid sequence of p-JNK1 (Santa Cruz Biotechnology; diluted 1:300) or p-c-Jun (Santa Cruz Biotechnology; diluted 1:200) of human origin. Antibodies were diluted in TBS/Tween (0.1% Tween-20) to which 0.1% BSA was added. In the case of p-JNK1, nitrocellulose was stripped and probed with a mouse monoclonal antibody that targets total JNK (Santa Cruz Biotechnology; diluted 1:200). The primary antibodies and conditions used for analysis of IL-4R and IL-1RI were as follows: nitrocellulose strips were probed with rabbit polyclonal IgG antibodies that specifically targets IL-1RI (Santa Cruz Biotechnology; diluted 1:1000) or IL-4Rα (S-20; Santa Cruz Biotechnology; diluted 1:200) in TBS containing 0.1% BSA. Nitrocellulose strips were washed and incubated for 1 h at room temperature with secondary antibody as follows: peroxidase conjugated anti-goat IgG (Santa Cruz Biotechnology) for p-JAK1, p-JAK3, and p-STAT6, peroxidase-linked anti-mouse IgG (Sigma) in the case of p-JNK, t-JNK, and p-c-Jun and peroxidase-linked anti-rabbit IgG (Sigma) in the case of IL-1RI and IL-4R. All of the nitrocellulose strips were reprobed for β-actin expression to ensure equal loading of protein on all SDS-PAGE gels. β-Actin expression was assessed using a mouse monoclonal IgG antibody (Santa Cruz Biotechnology; diluted 1:300) in PBS/Tween (0.1% Tween-20) containing 2% nonfat dried milk, and immunoreactive bands were detected using peroxidase-conjugated anti-mouse IgG (Sigma).

Protein complexes were visualized using SuperSignal West Dura Extended Duration Substrate (Pierce) in the case of p-JNK, p-c-Jun, IL-1RI, and IL-4R and ECL Western blotting Detection System Reagents (Amersham Biosciences) in the case of p-JAK1, p-JAK3, and p-STAT6. Immunoblots were exposed to film (Amersham Biosciences) and processed using a Fuji x-ray processor. Protein bands were quantitated by densitometric analysis using two software packages, Grab It (Grab It Annotating Grabber 2.04.7, Synotics, UVP Ltd., UK) and Gelworks (Gelworks ID, Version 2.51, UVP Ltd.) for photography and densitometry, respectively. Gelworks provides a single value (in arbitrary units) representing the density of each blot, and the values presented here are means of data generated from several separate experiments.

Immunostaining for IL-4R—Frozen cryostat sections were thawed at room temperature, fixed in ice-cold absolute ethanol, washed in PBS, and blocked with 10% normal goat serum (NGS, Vector Laboratories)/4% BSA in PBS. Sections were incubated overnight in a humidified chamber at 4 °C in the presence or absence of a blocking peptide (1:50, Santa Cruz Biotechnology) and for a further 24 h with IL-4Rα rabbit polyclonal IgG antibody (S-20, at 1:50, Santa Cruz Biotechnology). Sections were washed in PBS and incubated with biotinylated anti-rabbit IgG antibody (1:50, Vector Laboratories) for 2 h at room temperature. Sections were placed in avidin-biotin-horseradish peroxidase solution, diluted according to the manufacturer's instructions for 1 h (Vectastain elite ABC kit, Vector Laboratories), and then reacted with 3,3′-diaminobenzidine (DAKO) and H_2O_2 for color development. The reaction was terminated by washing with double distilled H_2O, and positively stained cells were viewed under a light microscope at ×4 and ×100 magnifications. Negative control studies were performed by replacing the primary antibodies with PBS.

Fluorescent Immunostaining for IL-4R, IL-1RI, p-JAK1, p-JAK3, and p-STAT6—For analysis of IL-4R, IL-1RI p-JAK1, p-JAK3 and p-STAT6 expression in cultured hippocampal neurons, cells were washed in TBS, fixed in 4% paraformaldehyde, washed in TBS, and permeabilized in 0.1% Triton X-100 in the case of IL-4R and IL-1RI and in 0.1% Triton X-100/0.2% proteinase K in the case of p-JAK1, p-JAK3, and p-STAT6. Cells were washed again in TBS and refixed in 4% paraformaldehyde before nonreactive sites were blocked with 10% NGS in TBS in the case of IL-4R and IL-1RI and with 10% normal swine serum in TBS in the case of p-JAK1, p-JAK3, and p-STAT6. Cells were treated overnight in a humidified chamber at 4 °C with a rabbit polyclonal IgG antibody that targets IL-4Rα (S-20, at 1:200 in 2.5% NGS, Santa Cruz Biotechnology) or IL-1RI (1:200 in 2.5% NGS, Santa Cruz Biotechnology) or, with a

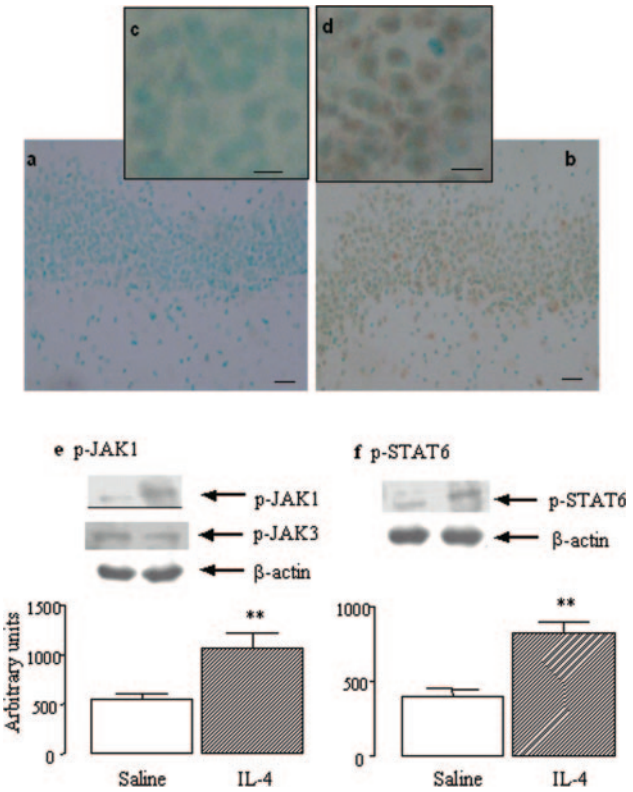

FIG. 1. **IL-4 receptor and signaling in the hippocampus.** Positive immunostaining for IL-4Rα was evident in hippocampus of 4-month-old rats (*b*) while negative control sections (in which blocking peptide was used) did not display immunoreactivity (*a*). Photomicrographs were taken at ×4 magnification. *Scale bar* = 50 μm. Intense immunoreactivity is apparent in granule cells of the dentate gyrus (*d*), which is absent in sections pretreated with blocking peptide (*c*). Photomicrographs were taken at ×100 magnification. *Scale bar* = 10 μm. Intracerebroventricular injection of IL-4 (20 μg/ml) to 4-month-old rats induced an increase in JAK1 (but not JAK3; *e*) as well as in STAT6 activity (*f*) in the hippocampus. Analysis of the mean data (±S.E.) obtained from six independent observations indicated a statistically significant difference between groups (**, $p < 0.01$, Student's *t* test for independent means). Sample Western immunoblots in each case demonstrate differences in saline treated (*left-hand lane*) compared with IL-4 treated (*right-hand lane*) rats. Sample immunoblots from the same tissue probed for β-actin are also included.

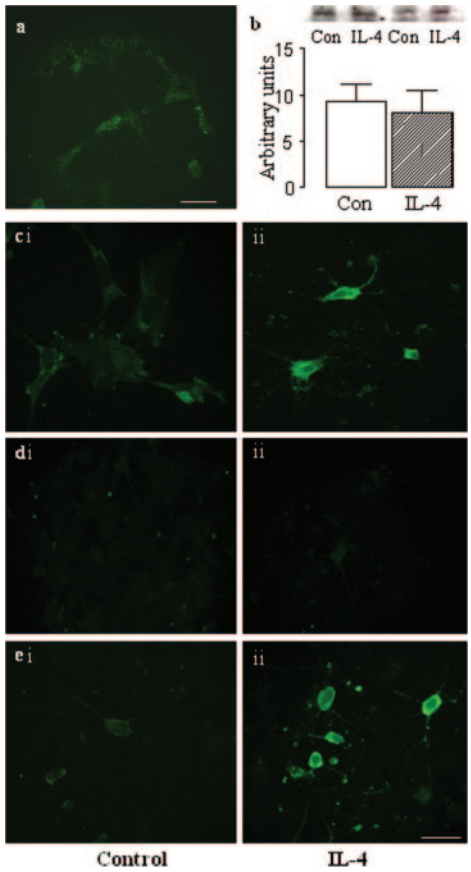

FIG. 2. **IL-4 receptor and signaling in the hippocampal neurons.** Immunoreactivity for IL-4Rα was observed in untreated primary cultured hippocampal neurons (*a*) and this was confirmed by analysis of IL-4R expression in harvested hippocampal neurons as shown by the sample immunoblot. Incubation of cells with IL-4 (100 ng/ml) did not affect IL-4R expression (*b*). In cells exposed to IL-4 (100 ng/ml) for 24 h, intense p-JAK1 (*cii*) and p-STAT6 (*eii*) immunoreactivity is apparent compared with untreated control neurons (*ci* and *ei*). IL-4 had no effect on p-JAK3 (*dii*). Photomicrographs were taken at ×40 magnification. *Scale bar* = 40 μm.

goat polyclonal IgG antibody to target p-JAK1, p-JAK3, and p-STAT6 (1:200 in 2.5% normal swine serum, Santa Cruz Biotechnology). In the case of IL-4R and IL-1RI, cells were washed in TBS and incubated in biotinylated anti-rabbit secondary antibody (1:50 in 2.5% NGS, Vector Laboratories) for 2 h at room temperature. Cells were washed again in TBS followed by incubation in the dark for 2 h with conjugated ExtrAvidin fluorescein isothiocyanate (1:50, Sigma). To reveal expression of p-JAK1, p-JAK3, and p-STAT6, cells were incubated in the dark for 2 h at room temperature in fluorescein isothiocyanate-labeled goat anti-mouse IgG or IgM (1:50 in 2.5% normal swine serum, BioSource, UK). After washing, all cells were mounted with an aqueous mounting medium (Vector Laboratories) and sealed. Slides were examined under a Zeiss fluorescence microscope at an excitation wavelength of 495 nm, and photomicrographs were taken at ×40 magnification.

Statistical Analysis—Data were analyzed, as appropriate, using either Student's *t* test for independent means or a one-way analysis of variance (ANOVA) followed by post hoc Student-Newman-Keuls test to determine which conditions were significantly different from each other. Data are expressed as means with standard errors and deemed statistically significant when $p < 0.05$.

RESULTS

We used immunohistochemical analysis to demonstrate the presence of IL-4 receptors in hippocampus of untreated 4-month-old rats. Dense staining was observed in granule cells of the dentate gyrus (Fig. 1, *b* and *d*), whereas background staining, observed when sections were pretreated with blocking peptide, was minimal (Fig. 1, *a* and *c*). Consistent with the hypothesis that these are functional receptors, is the finding that IL-4 significantly enhanced expression of phosphorylated JAK1 (p-JAK1) in a cytosolic fraction prepared from hippocampus of 4-month-old rats that were treated intracerebroventricularly with IL-4 compared with saline-treated controls ($p < 0.01$, Student's *t* test for independent means (Fig. 1*e*)). Expression of p-JAK3 was unchanged (Fig. 1*e*). The IL-4-induced increase in expression of p-JAK1 was accompanied by a parallel significant increase in p-STAT6 expression in a nuclear fraction obtained from the hippocampus of the same rats ($p < 0.001$, Student's *t* test for independent means (Fig. 1*f*)). To consolidate these observations, we prepared hippocampal neurons and demonstrate expression of IL-4 receptors on these cells (Fig. 2*a*). We also harvested hippocampal neurons, which were incubated in the presence or absence of IL-4, and assessed IL-4R expression using gel electrophoresis and Western immunoblotting. Fig. 2*b* shows the presence of the receptor and indicates that expression of IL-4R was not affected by exposure to IL-4. In an effort to assess signaling cascades activated by IL-4, we stained control-treated and IL-4-treated hippocampal neurons for JAK1 and STAT6. We report that IL-4 (100 ng/ml) up-regulated phosphorylation of JAK1 (Fig. 2*c*) and STAT6 (Fig. 2*e*) compared with untreated control neurons, whereas JAK3 was unchanged after IL-4 treatment (Fig. 2*d*).

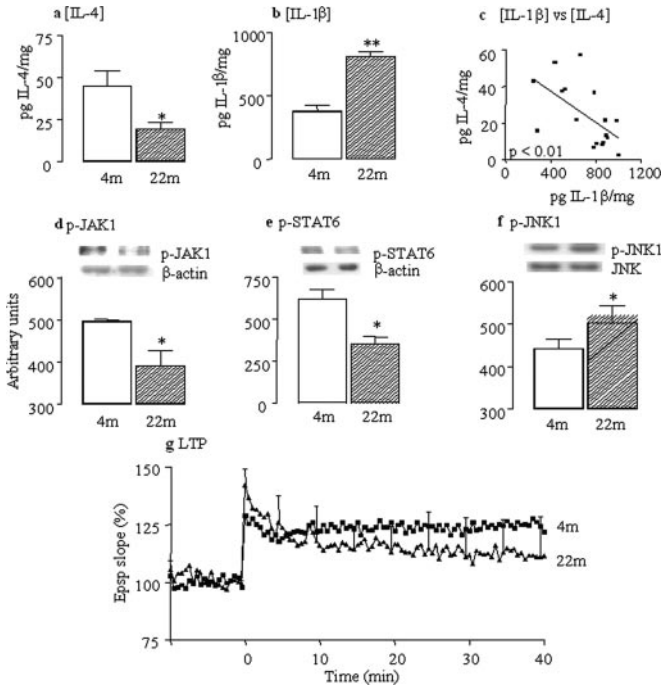

FIG. 3. **Age-related changes in IL-1β and IL-4 concentration and associated cell signaling.** IL-4 concentration was significantly decreased (*a*; *, $p < 0.05$, Student's *t* test for independent means) and IL-1β concentration significantly increased (*b*; **, $p < 0.01$, Student's *t* test for independent means) in hippocampal tissue prepared from aged (22-month-old), compared with young (4-month-old) rats. Data are expressed as means ± S.E., $n = 5–6$. There was a significant inverse relationship between hippocampal IL-1β concentration and IL-4 concentration ($p < 0.01$; *c*). Phosphorylation of JAK1 (*d*) and STAT6 (*e*) was decreased in hippocampal tissue prepared from aged rats, compared with young rats as shown by the sample immunoblots and the histograms, which represent the mean data (±S.E.) obtained from densitometric analysis (*, $p < 0.05$, Student's *t* test for independent means; $n = 6–7$). Immunoblots are accompanied by sample blots indicating that β-actin did not change with treatment. JNK1 activation was significantly enhanced in hippocampal tissue prepared from aged, compared with young rats (*f*; *, $p < 0.05$, Student's *t* test for independent means). Sample immunoblots from the same tissue probed for total JNK are also included. Values are means ± S.E. of 7 observations. Tetanus-induced LTP was inhibited in perforant path-granule cell synapses of aged rats (*triangles*) compared with young rats (*squares*; *g*). The data are expressed as the mean percent change in population epsp slope (compared with the epsp slope in the 5-min immediately prior to tetanic stimulation) of 10 aged and 6 young rats. S.E. values are included for every 10th response.

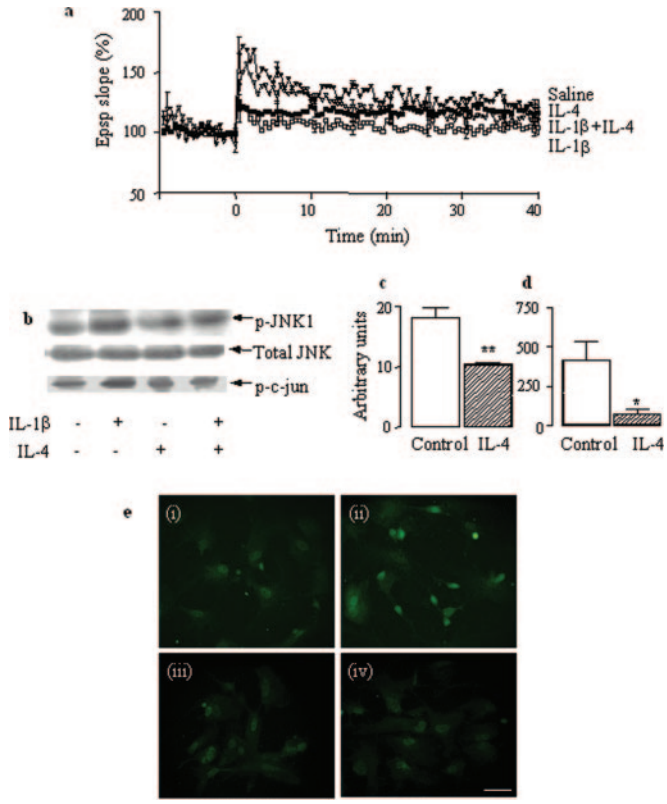

FIG. 4. **Modulation of IL-1β impairment in LTP and associated cell signaling by IL-4.** Tetanic stimulation of the perforant path induced a sustained enhancement of the population epsp slope in saline-treated 4-month-old rats (*filled triangle*; *a*). The response in IL-1β-treated rats was attenuated (*open square*). Intracerebroventricular injection of IL-4 (5 μl; 20 μg/ml) prevented the inhibitory effect of IL-1β (5 μl; 3.5 ng/ml) on LTP in perforant path-granule cell synapses (*open triangle*) while IL-4 treated control rats (*filled square*) responded in a similar fashion to saline-treated rats. Results are expressed as mean percent change in population epsp slope for six independent observations. S.E. values are included for every 10th response. IL-1β increased expression of phosphorylated JNK1 (p-JNK1) and c-Jun (p-c-Jun); these changes were reversed by IL-4, and no changes in total JNK were observed with treatment (*b*). Expression of IL-1RI protein (by Western immunoblotting) (*c*) and mRNA (by PCR) (*d*) was significantly decreased in hippocampal neurons treated with IL-4 (100 ng/ml; *, $p < 0.05$ and **, $p < 0.01$, Student's *t* test for independent means) and the LPS-induced increase in IL-1RI immunoreactivity (compare LPS-treated (*eii*) with control (*ei*)) was abrogated by IL-4 (*eiv*); photomicrographs were taken at ×40 magnification. *Scale bar* = 40 μm.

We considered that the age-related increase in IL-1β concentration in hippocampus, which is associated with up-regulation of JNK and consequent inhibition of LTP, might be accompanied by an age-related decrease in IL-4 and IL-4-induced signaling. To assess this, aged (22-month-old) and young (4-month-old) rats were examined for their ability to sustain LTP, and hippocampal tissue prepared from these rats was investigated for concentration of IL-4 and IL-1β and for cytokine-triggered changes in protein kinases. IL-4 concentration was significantly decreased in hippocampal tissue prepared from aged, compared with young rats ($p < 0.05$, Student's *t* test for independent means (Fig. 3*a*)), and this was accompanied by age-related decreases in expression of p-JAK1 in cytosol (Fig. 3*d*) and p-STAT6 in nuclear fractions (Fig. 3*e*) prepared from the same tissue. Significantly, and consistent with previous findings, the age-related decreases in IL-4 concentration and IL-4-induced signaling was associated with age-related increases in IL-1β concentration ($p < 0.01$, Student's *t* test (Fig. 3*b*)) and expression of phosphorylated JNK1 ($p < 0.05$, Student's *t* test for independent means (Fig. 3*f*)). Fig. 3*c* shows that there was a significant inverse correlation between hippocampal concentration of IL-1β and hippocampal concentration of IL-4 ($p < 0.01$). The data presented in Fig. 3*g* indicate that age-related up-regulation of IL-1β-induced signaling, coupled with down-regulation of IL-4-induced signaling, is associated with impaired LTP. Thus, although tetanic stimulation of the perforant path induced an immediate and sustained enhancement of the population epsp slope in young rats, the response in aged rats was attenuated; the mean percent changes in epsp slope in the last 5 min of the experiment compared with those in the 5 min immediately prior to tetanic stimulation were 125.2 ± 0.48 and 112.3 ± 0.47 in young and aged rats, respectively ($p < 0.001$, Student's *t* test for independent values).

IL-4 may act to antagonize the effects of IL-1β, or, alternatively, it may act by inhibiting synthesis of IL-1β mRNA and protein. To address these possibilities, we first asked whether IL-4 suppressed the inhibitory effect of IL-1β on LTP, and Fig. 4*a* indicates that, although it failed to suppress the inhibitory effect of IL-1β on the immediate response to tetanic stimulation, IL-4 abrogated the later inhibitory effect of IL-1β on LTP; thus the mean percent changes in population epsp slope in the

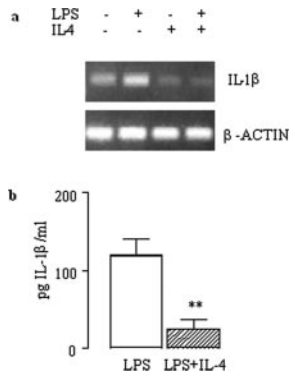

FIG. 5. **Modulation of LPS-induced synthesis of IL-1β mRNA and protein.** IL-1β mRNA expression was increased in LPS-treated cultured hippocampal neurons (100 ng/ml for 24 h (*a*; *lane 2*). Pretreatment with IL-4 (100 ng/ml) prevented this effect (*a*; *lane 4*). No change in reference gene β-actin mRNA was observed in any of the treatment conditions (*a*). IL-1β concentration in supernatant taken from LPS (100 ng/ml)-treated cultured hippocampal neurons was increased compared with untreated controls (undetectable; data not shown; *b*). In supernatant taken from cells that had been co-cultured with IL-4 (100 ng/ml), there was a significant decrease in IL-1β concentration (**, $p < 0.01$, Student's *t* test for independent means). Data are expressed as means ± S.E. of eight cultures.

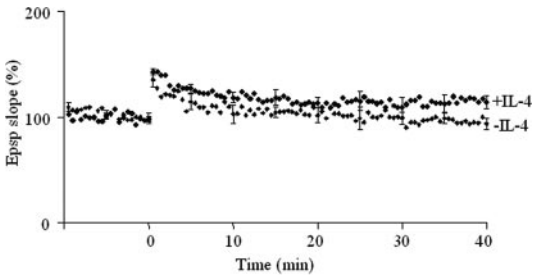

FIG. 6. **IL-4 abrogates the age-related impairment in LTP.** Intracerebroventricular injection of IL-4 (5 μl and 20 μg/ml) abrogated the age-related deficit in LTP so that population epsp slope in the IL-4-treated aged rats ($n = 4$) in the response to tetanic stimulation was significantly greater than in untreated aged rats ($p < 0.01$, Student's *t* test for independent means, $n = 7$).

last 5 min of the experiment were 118.5 ± 0.65 and 104.7 ± 0.51 in 4-month-old control rats that were treated with saline and IL-1β, respectively ($p < 0.001$, ANOVA) and 122.2 ± 0.80 and 111.1 ± 0.79 in 4-month-old saline-treated and IL-1β-treated rats that also received IL-4. Treatment with IL-4 also blocked the IL-1β-induced increases in JNK phosphorylation and c-Jun phosphorylation (Fig. 4*b*). Mean arbitrary values for expression of p-JNK were 29 ± 3 and 39.9 ± 3.3 in hippocampus of saline- and IL-1β-treated rats, and the corresponding values for p-c-*jun* were 46 ± 8.3 and 124 ± 18.8 ($p < 0.05$ in both cases, ANOVA); the values for p-JNK and p-c-Jun in rats treated with both IL-1β and IL-4 were similar to those in saline-treated samples, *i.e.* 29 ± 2.8 and 72 ± 0.8 respectively; expression of total JNK was unchanged.

One mechanism by which IL-4 may antagonize the effects of IL-1β is by down-regulating IL-1RI expression, and we addressed this possibility in two ways. First, we analyzed IL-1RI protein and mRNA expression in hippocampal neurons, which were incubated in the presence or absence of IL-4 using gel electrophoresis and Western immunoblotting, and PCR respectively. The data indicate that IL-4 significantly decreased IL-1RI protein and mRNA expression ($p < 0.01$ and $p < 0.05$, respectively, Student's *t* test for independent means (Figs. 4*c* and 4*d*)). Second, we examined IL-1RI expression on IL-4-treated hippocampal neurons, which were incubated in the presence or absence of LPS, using immunohistochemistry. We report that LPS markedly enhanced immunopositive staining of IL-1RI (Fig. 4*e*, *panel ii*), but this effect was antagonized by co-treatment of neurons with IL-4 (Fig. 4*e*, *panel iv*) so that staining in these neurons was similar to that in control-treated cells (Fig. 4*e*, *panel i*) or cells treated with IL-4 alone (Fig. 4*e*, *panel iii*). Consistent with this observation is the finding that IL-4 also inhibited the LPS-induced increase in IL-1RI mRNA (data not shown).

However, IL-4 may also exert its anti-inflammatory action by inhibiting IL-1β mRNA and/or protein; therefore, we assessed both measures in hippocampal neurons treated with LPS (to stimulate IL-1β synthesis) in the presence or absence of IL-4. LPS increased expression of IL-1β mRNA (Fig. 5*a*), and this effect was abrogated by co-treatment with IL-4. Mean arbitrary values (±S.E.) were 4.5 ± 0.13 and 6.02 ± 0.16 in untreated and LPS-treated cells, respectively ($p < 0.01$, ANOVA); the mean value in cells treated with LPS and IL-4 was 4.6 ± 0.27, which was similar to that in untreated cells. No changes in expression of the reference gene, β-actin, were observed. In parallel with these findings, IL-4 significantly decreased the LPS-induced increase in IL-1β protein ($p < 0.01$, ANOVA (Fig. 5*b*)). IL-1β released from untreated and IL-4-treated cells was undetectable.

If the age-related decrease in IL-4 concentration is a critical factor in attenuating LTP, then it must be argued that restoration of IL-4 will rescue LTP. We set out to investigate this possibility (*a*) by investigating whether a single intracerebroventricular injection of IL-4 might exert any effect on the deficit in LTP observed in aged rats and (*b*) by investigating whether administration of VP015, a treatment that we have observed can increase hippocampal IL-4 concentration under specific circumstances, might exert a comparable effect. Fig. 6 shows that there was a significant difference in the response of aged control rats and aged IL-4-treated rats to tetanic stimulation ($p < 0.01$, Student's *t* test for independent means). Analysis of the changes in the 2 min immediately following the tetanus and in the last 5 min of the experiment revealed that both the early and later responses to tetanic stimulation (expressed in terms of mean percent changes in population epsp slope) were significantly greater in IL-4-treated rats compared with control rats (139 ± 1.4 (mean ± S.E.) *versus* 125.9 ± 3.54 ($p < 0.05$, Student's *t* test for independent values) and 119.1 ± 1.4 *versus* 93.3 ± 0.9 ($p < 0.01$, Student's *t* test for independent values) at the two time intervals, respectively).

We report that treatment of rats with VP015 significantly enhanced IL-4 concentrations in hippocampus of both aged rats and 4-month-old LPS-treated rats compared with control-pre-treated aged rats, and control-pre-treated LPS-challenged rats, respectively ($p < 0.05$ in both cases, ANOVA (Figs. 7*a* and 7*b*)). However, VP015 treatment exerted no significant effect on hippocampal IL-4 concentration in young untreated rats (data not shown). Consistent with the data presented in Fig. 3*a*, hippocampal IL-4 concentration was also decreased in hippocampal tissue prepared from this cohort of aged rats ($p < 0.05$, ANOVA (Fig. 7*a*)), whereas the effect of LPS treatment on IL-4 concentration was not significant (Fig. 7*b*). The action of VP015 was not confined to its effect on IL-4, because we observed that VP015 treatment abrogated the statistically significant increase in IL-1β concentration with age ($p < 0.05$, ANOVA (Fig. 7*c*)) and LPS ($p < 0.05$, ANOVA (Fig. 7*d*)). We demonstrate that LTP in VP015-treated aged rats and VP015-treated LPS-injected rats was similar to that in the respective control groups (Fig. 7, *e* and *f*). Thus the mean percent changes in population epsp slope in the last 5 min of the experiment were 103.3 ± 0.6 and 119.7 ± 0.7 in control-treated and VP015-treated aged rats ($p < 0.001$, ANOVA) and 132.3 ± 1.0 and 131.7 ± 0.66 in control-treated and VP015-treated young rats.

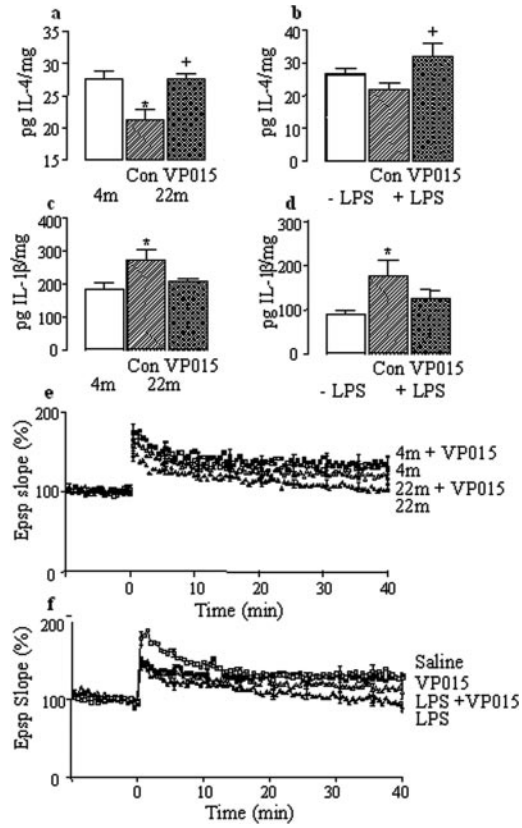

FIG. 7. **VP015 treatment increases hippocampal IL-4 concentration and reverses age and LPS-induced impairment of LTP.** IL-4 concentration was significantly decreased in aged (22-month-old) compared with young (4-month-old) rats (*, $p < 0.05$, ANOVA). Concentration of IL-4 measured in hippocampus of aged rats pre-treated with intramuscular injection of VP015 (6×10^6 particles/ml) was significantly increased compared with control pre-treated aged rats (+, $p < 0.05$, ANOVA, $n = 6$; *a*). Pre-treatment with VP015 also induced a significant increase in the concentration of hippocampal IL-4 in LPS (100 μg/kg)-challenged 4-month-old rats compared with rats receiving LPS alone (+, $p < 0.05$, ANOVA, $n = 6$; *b*). Mean IL-1β concentration was significantly increased in hippocampus of aged (*c*) and LPS-treated (*d*) rats ($p < 0.05$ in each case, ANOVA), and these changes were not observed in aged rats or LPS-treated rats, which were treated with VP015. Data are expressed as means ± S.E. in both cases ($n = 6$). VP015 pre-treatment exerted no significant effect on concentration of IL-4 or IL-1β concentration in young rats and control-treated rats (data not shown). Age (*e*; *filled triangle*) and intraperitoneal injection of LPS (100 μg/kg) to 4-month-old rats (*f*; *filled triangle*) exerted an inhibitory effect on LTP in perforant path-granule cell synapses, which was abrogated by pre-treatment with VP015 (*open triangle*). VP015 pre-treated young/control rats (*filled square*) responded in a similar fashion to saline-pre-treated young/control rats (*open square*) in both cases. Data are the means of six animals in each group, S.E. values are included for every 10th response, and values are expressed as the percent change in population epsp slope after tetanic stimulation (compared with mean value immediately prior to tetanic stimulation).

The equivalent values in the LPS experiment were 94.27 ± 2.34 and 120 ± 0.1 in control-treated and VP015-treated rats that received LPS ($p < 0.001$, ANOVA) and 130.8 ± 2.96 and 127.6 ± 1.3 in control-treated and VP015-treated rats that received saline injections.

We argued that if IL-4 exerts endogenous effects on neurons, then it must also be released from resident cells in brain. The primary source of IL-4 in the periphery is Th2 cells, but it may also be secreted by innate immune cells. It has been shown that infection of mice with the helminth parasite, *F. hepatica*, induces Th2 cells (21) and that injection of excretory/secretory (ES) products of the parasite also induces Th2 responses, possibly through the stimulation of IL-4 from an unidentified innate immune cell. Here we report that ES products significantly increased IL-4 secretion from bone marrow-derived dendritic cells ($p < 0.05$, ANOVA (Fig. 8*a*)). Because microglia, like dendritic cells, are members of the macrophage lineage of innate immune cells, we also stimulated a primary mixed glial culture with liver fluke homogenate and ES products and found that both preparations induced a significant increase in release of IL-4 ($p < 0.05$, ANOVA (Fig. 8*b*)). Liver fluke homogenate and ES products did not induce significant release of IL-4 from cultured hippocampal neurons (data not shown).

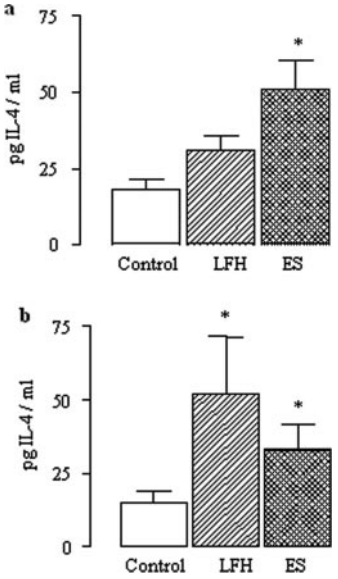

FIG. 8. **Liver fluke homogenate (*LFH*) and excretory/secretory (*ES*) products of *F. hepatica* induced IL-4 secretion from bone marrow-derived dendritic cells and glial cells.** ES products significantly increased IL-4 secretion from bone marrow-derived dendritic cells (*a*; *, $p < 0.05$, ANOVA). Data are expressed as means ± S.E. of three cultures. Primary mixed glial culture stimulated with LFH and ES induced a significant increase in release of IL-4 into culture medium (*b*; *, $p < 0.05$, ANOVA). Data are expressed as means ± S.E. of three cultures. LFH and ES did not induce significant release of IL-4 from cultured hippocampal neurons (data not shown).

DISCUSSION

In this study we demonstrate that functional IL-4 receptors are expressed in rat hippocampus and that receptor activation leads to sequential phosphorylation of JAK1 and STAT6. These findings identify a mechanism by which IL-4 exerts its anti-inflammatory effects in hippocampus. We report that hippocampal IL-4 concentration is decreased and IL-4-stimulated signaling is down-regulated with age and conclude that these changes, coupled with an increased IL-1β concentration and IL-1β-induced signaling, are responsible for the deficit in LTP, which is a consistent finding in aged rats. To date, there has been little or no evidence indicating that resident brain cells are a source of IL-4; here we demonstrate, for the first time, that glia are capable of secreting IL-4 and propose that the secreted IL-4 activates IL-4 receptors located on neurons to exert its anti-inflammatory effect in brain.

Immunohistochemical analysis revealed the expression of IL-4R in hippocampus, with particularly dense staining on granule cells of the dentate gyrus; neuronal expression of IL-4R was confirmed by immunostaining of hippocampal neurons *in vitro* and by analysis of receptor expression by Western immunoblotting. While previous studies have suggested that IL-4 receptors are expressed in cultured glial cells (15, 16), to our knowledge, there are no reports indicating receptor expression in brain tissue *in situ*, or receptor expression on neurons.

We observed that intracerebroventricular injection of IL-4 increased phosphorylation of JAK1 and STAT6; in the case of

STAT6, IL-4 resulted in increased phosphorylation in a nuclear fraction prepared from hippocampus indicating that STAT6 activation was associated with translocation to the nucleus. These findings were backed up by similar observations *in vitro*; thus treatment with IL-4 also induced activation of JAK1 (but not JAK3) and STAT6 in cultured hippocampal neurons. These data indicate that the signaling cascades induced by IL-4R activation in hippocampus are similar to those previously reported in the periphery (14). One of the most significant ramifications of these findings is that we have identified a mechanism by which anti-inflammatory effects of IL-4 might be mediated in brain. Although IL-4 was shown to inhibit LPS-induced sickness behavior in rats (23), the mechanism by which this was achieved was not elucidated. The present data suggest one possible sequence of events: IL-4 interacts with its receptor that is expressed on neurons, initiates signaling through the JAK-STAT pathway, and results in IL-4-responsive gene expression.

Previous studies have indicated that increased IL-1β concentration and IL-1β-induced signaling, including phosphorylation of JNK and c-Jun, represent a significant contributory factor leading to the age-related impairment in LTP (4, 18, 24). The present findings identify the fact that these changes are coupled with an age-related decrease in hippocampal IL-4 concentration and associated phosphorylation of JAK1 and STAT6. Indeed a significant inverse correlation between hippocampal concentrations of IL-1β and IL-4 was observed. Therefore, it is likely that the age-related deficit in LTP is a consequence of the coincident up-regulation of pro-inflammatory influences linked with down-regulation of anti-inflammatory influences. These findings identify an interesting parallel between age-related changes peripherally and centrally, because it has been demonstrated that peripheral Th2 cell-mediated responses, which result in production of IL-4, are attenuated, and Th1 cell-mediated responses are accentuated in the aged mouse (25).

One of the arguments advanced to support the idea that increased hippocampal concentration of IL-1β contributes to the age-related deficit in LTP is that IL-1β treatment also inhibits LTP (7); if a decrease in IL-4 concentration is a significant additional factor, then it might be predicted that IL-4 should be capable of suppressing the effects of IL-1β. In support of this contention are the findings that indicate that intracerebroventricular injection of IL-4 abrogates the inhibitory effect of IL-1β on LTP and its stimulatory effect on activation of JNK. These effects of IL-4 may be due to increased IL-1 receptor antagonist production that has been observed in neutrophils (26) and monocytes (13) and/or increased expression of IL-1RII, which has been reported in polymorphonuclear cells (27). However, we propose that the action of IL-4 may be a consequence of its ability to modulate expression of IL-1RI, because the data presented here indicate that IL-4 abrogates LPS-induced up-regulation of IL-1RI. This is supported by the observation that IL-4 down-regulated IL-1RI expression at the protein and mRNA level. We considered that IL-4 might also act by down-regulating IL-1β synthesis, and the data show that IL-4 suppressed LPS-induced IL-1β mRNA and protein as described previously in human peripheral blood mononuclear cells (13). Thus IL-4 modulates IL-1β expression in hippocampus, and any alteration in homeostasis affecting IL-4 synthesis and/or secretion is likely to impact on IL-1β; consequently it might be proposed that the age-related decrease in IL-4 contributes to the coincident increase in IL-1β concentration and subsequent up-regulation of IL-1β-induced signaling.

Proof of this principle relies on demonstrating that any strategy that will enhance hippocampal IL-4 concentration will antagonize the effects of increased hippocampal IL-1β concentration, specifically IL-1β-induced impairment in LTP. To challenge this principle, we selected two models in which hippocampal IL-1β concentration is increased, the aged rat and the LPS-treated rat, and we assessed the effect of VP015, a strategy which we have shown to enhance IL-4 concentration at least in some circumstances (28). Here we confirm our previous finding that VP015 increased IL-4 concentration in the hippocampus of LPS-treated rats (28) and extend it to show that it also increases IL-4 concentration in hippocampus of aged rats. Treatment of aged and LPS-injected rats with VP015 reversed the deficits in LTP observed in these experimental conditions. In addition to these findings, we also demonstrate that intracerebroventricular injection of IL-4 abrogates, at least to some extent, the deficit in LTP observed in aged rats. These data provide consistent and powerful support for our hypothesis: that IL-4 modulates IL-1β expression and IL-1β-stimulated cell events, which lead to impaired LTP in the aged and LPS-treated rat.

To date there is no evidence to suggest that IL-4 is produced in the brain or by brain cells. Here we demonstrate for the first time that glial cells prepared from hippocampus are capable of producing IL-4 in response to pathogen molecules. Products of the helminth parasite *F. hepatica*, which induces IL-4 producing Th2 cells *in vivo*, were shown to stimulate IL-4 production from dendritic cells. Dendritic cells, like microglial cells, are cells of the innate immune system that act as antigen presenting cells, but also play a critical role in directing naive T cells to Th1 or Th2 effector cells. The production of IL-4 by innate immune cells has direct anti-inflammatory activity, but also promotes the differentiation of Th2 cells, which in turn secrete IL-4 and IL-10, thus amplifying the anti-inflammatory response.

The data presented identify an endogenous source of IL-4 in brain and provide a mechanism by which IL-4 exerts its anti-inflammatory action in the brain. On the basis of our findings, which highlight the importance of integration of pro-inflammatory and anti-inflammatory responses in brain, we propose that balance between these responses significantly impacts on synaptic function and plasticity in the hippocampus of the aged rat.

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