ELSEVIER

CYTOKINE

www.elsevier.com/locate/issn/10434666

Cytokine 37 (2007) 128–137

Role of interleukin-1β in the control of neuroepithelial proliferation and differentiation of the spinal cord during development

A. de la Mano *, A. Gato, M.I. Alonso, E. Carnicero, C. Martín, J.A. Moro

Departamento de Anatomía y Radiología, Facultad de Medicina, Universidad de Valladolid, C/Ramón y Cajal 7, 47005 Valladolid, Spain

Received 28 July 2006; received in revised form 7 February 2007; accepted 12 March 2007

Abstract

Interleukin-1β (IL-1β) is an important trophic factor in the nervous system (NS). IL-1β is ubiquitously expressed from very early stages during the development of the amphibian NS and its action has been demonstrated in vitro on survival, proliferation and differentiation in mammalian embryos. In this report, we show that IL-1β is immunocytochemically expressed in embryonic spinal cord from early stages, both in rat (embryonic day 12) and in chicken (stage 17-HH), in neuroepithelial cells and nerve fibres, dorsal root ganglia, anterior and posterior roots of the spinal nerves, and in the fibres of these nerves. Our in vivo experiments on chick embryos, with microbeads impregnated with IL-1β implanted laterally to the spinal cord at the level of the wing anlage, demonstrate that this cytokine produces a statistically significant increase in nuclear incorporation of BrdU at the dorsal level and a reduction of this at the ventral level, whereas local immunoblocking with anti-IL-1β antibodies causes a dorsal reduction of BrdU incorporation and alters ventral differentiation. These data demonstrate that IL-1β plays a part in controlling proliferation and early differentiation during the development of the spinal cord in chick embryos.

Keywords: Cytokines; Rat embryo; Chick embryo; Neuroepithelium; Nervous system

1. Introduction

Interleukins are pleiotrophic molecules, traditionally implicated in the regulation of the immune system [1]. The expression of diverse cytokines in different parts of the nervous system (NS), both in the adult as well as during embryonic development, is well-known [2–9]. It has been demonstrated that numerous interleukins play a role in controlling neuronal and glial survival, proliferation, and differentiation [10–12].

Interleukin-1β (IL-1β) seems to be involved in spinal cord development. In this regard, it has been shown that IL-1β and its type-1 receptor (IL1R-1) are present in sensory neurones, primary motor neurones, and cells from dorsal root ganglia during the early development of the spinal cord in *Xenopus laevis* [13]; nevertheless, no information exists regarding their expression in other evolved species. In vitro studies indicate that IL-1β promotes neuronal survival at spinal cord level during murine foetal development [14,15]; in addition, in the adult stage, the roles of neuroprotection and neuroregeneration have been assigned to this interleukin, both in vitro and after spinal cord injury in vivo [16–18].

It has also been suggested that IL-1β could play a part in regulating neuronal proliferation in diverse locations during the development of the NS; in this regard, IL-1β increases histidine-decarboxylase activity in cultures of diencephalic cells from rat embryos, indicating a possible activation of the proliferation of these cells [19]; furthermore, IL-1β promotes in vitro proliferation of mesencephalic progenitor cells from rat embryos [20,21].

During development of the NS, IL-1β could also play a part in modulating neuronal differentiation: IL-1β stimulates in vitro differentiation of foetal mesencephalic

* Corresponding author. Fax: +34 983423022.
E-mail addresses: alamano@ah.uva.es (A. de la Mano), gato@med.uva.es (A. Gato), mialonso@med.uva.es (M.I. Alonso), moro@med.uva.es (J.A. Moro).

1043-4666/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.cyto.2007.03.004

precursor cells to dopaminergic neurones both in rat [21–23] and human [24,25]. IL-1β also induces neuronal differentiation in cultures of human neuroepithelial olfactory cells [26].

As previously mentioned, IL-1β is expressed precociously in the spinal cord during the embryonic development of *Xenopus laevis*; in addition, this interleukin seems to be involved in proliferation and neuronal differentiation processes during the development of the NS. In this report, we demonstrate, using immunocytochemistry techniques, that IL-1β is expressed precociously in spinal cord and dorsal root ganglia during the development of rat and chick embryos; moreover, by means of in vivo experiments involving local stimulation and immunoblocking, we show that IL-1β produces an increase in neuroepithelial dorsal proliferation, as well as a reduction in proliferation and enhancement of differentiation at the ventral level, in the spinal cord of chick embryos.

2. Materials and methods

2.1. Obtaining rat and chick embryos

Wistar rats, housed at a constant temperature (22 °C) and with a cycle of 12 h of light and 12 h of darkness, were employed. In calculating the embryonic age, we termed day 0 the morning when a copulation plug was found after overnight mating. The pregnant rats were sacrificed by ether inhalation, the embryos were extracted from the uterus, the enveloping membranes removed and their heads cut then the trunks were processed for histology. For our study, embryos between embryonic day 10.5 (E10.5) and 19.5 (E19.5) were employed. Five embryos from each stage were used for immunocytochemistry (a total of 50 embryos). At all times, the animals were treated according to the effective norm for handling laboratory animals (European Communities Council Directive 86/609/EEC).

To obtain chick embryos, fertile White Leghorn eggs were incubated at 38 °C in a humidified atmosphere. In our study, we used chick embryos at different Hamburger–Hamilton developmental stages, ranging from 15 to 25-HH [27]. Embryos were removed from the extra-embryonic membranes and fixed for immunocytochemical techniques. Five embryos from each stage were used for immunocytochemistry (a total of 55 embryos).

2.2. IL-1β immunocytochemistry

Rat and chick embryos were fixed in picric acid-paraformaldehyde [28] for 12 h at 4 °C. Following this, the specimens were dehydrated through a graded series of ethanol and embedded in paraffin through xylene. We performed 8 μm-thick serial transverse sections, which were mounted on glass slides. These were then deparaffinized, rehydrated and submerged consecutively in lugol and sodium metabisulphite in order to eliminate the fixing agent; next, they were washed several times in PBS and the endogenous peroxidase inhibited with a solution of methanol and 3% hydrogen peroxide for 30 min. After being washed several times in PBS, the sections were incubated in PBS/BSA for 20 min and subsequently in the primary antibody, IL-1β rabbit anti-rat polyclonal (Endogen), at a dilution of 1/100 in PBS, maintained at 4 °C overnight in a humidity chamber. Following washing in PBS, we proceeded to incubate with the secondary antibody, horseradish peroxidase-conjugated goat anti-rabbit IgG (Sigma), at a dilution of 1/100 in PBS, maintained at room temperature (RT) for 90 min. After further washing in PBS, the sections were incubated with 3,3′-diaminobenzidine tetrahydrochloride (DAB; Sigma) and H_2O_2 (DAB 10 mg/15 ml in 0.05 M Tris–HCl buffer, pH 7.4, and 0.003% H_2O_2). Haematoxylin was employed to counterstain the nuclei. In order to verify the specificity of the immunoreaction, control sections of rat and chick embryos were prepared as mentioned above but using pre-immune serum instead of the primary antibody, confirming that there was not any non-specific reaction (data not shown). The tissue sections were observed with a Nikon Microphot-FXA Photomicroscope and the most representative were photographed.

2.3. Local treatment of IL-1β stimulation and immunoblocking

Fertile chick eggs were incubated to obtain embryos at stage 17-HH (the moment at which there was evident IL-1β expression in the spinal cord at the level of the wing region). After the opening of a small window in the eggshell, the vitellin and amniotic membranes were sectioned, and, by microdissection, a small incision was made in an intersomitic space at the level of the right wing anlage. Next, a heparin acrylic microbead (Sigma) of between 125 and 150 μm in diameter was implanted in the incision laterally to the spinal cord (Fig. 3A). These microbeads were previously impregnated, for 24 h at 4 °C, with the following: a 50 ng/ml solution of recombinant rat IL-1β (Endogen) for the stimulation experiments, a dilution 1:50 of rabbit anti-rat polyclonal IL-1β antibody (Endogen) in the immunoblocking experiments, and with Ringer solution in the control embryos. After implantation of the microbeads, the embryos were reincubated for 24 h. until reaching stage 22-HH (Fig. 3B).

2.4. Cellular proliferation study

Locally treated and non-manipulated 22-HH-stage chick embryos were intracardially microinjected with 2 μl of a 10 mM solution of BrdU (Sigma), reincubated for 60 min and then extracted and fixed in Carnoy's solution for 6 h at 4 °C. The specimens were dehydrated in an ethanol series, passed through xylene and embedded in paraffin. After transversal sectioning of the tissues, they were deparaffinated and BrdU was detected following standard procedures. The sections were incubated in a 1/100 solution of a monoclonal antibody to BrdU (Dako) for 30 min at RT. To detect the primary antibody, we used the avidin–extravidin system

conjugated to peroxidase (1/20 mouse anti-rabbit for 30 min and 1/20 extravidin for 10 min; Sigma) and we stained with DAB. We visualized and photographed the preparations using a Nikon microphot-FXA photomicroscope.

A quantitative analysis of nuclear BrdU incorporation was performed by counting the number of BrdU-positive nuclei in the spinal cord sections next to the microbead. Five embryos per experimental condition (non-manipulated control, Ringer control, IL-1β, and anti-IL-1β) were used. In every embryo, one section from three was selected (a total of 12 sections per embryo) throughout the microbead implantation zone, and the number of BrdU positive nuclei in the spinal cord was scored by quadrants: right dorsal, left dorsal, right ventral, and left ventral (Fig. 3C). Once the data was collected, they were listed in a computed system SPSS 14.0 to carry out the statistical analysis. Homogeneity of variances among the different groups was evaluated by means of the Levene test. In order to evaluate the differences in means among the groups, an analysis of variance (ANOVA) with a 95% significance level was performed ($p = 0.05$), followed by multiple comparison tests to determine which specific groups significantly differed from the others (the Bonferroni test in quadrants with equal variances, and the Games–Howell test in those with different variances). The average and the standard deviation of each experimental condition and quadrant were plotted, indicating the statistically significant differences between control and experimental groups.

2.5. Neuronal differentiation study

We detected neuronal differentiation by β3-tubulin expression. After local treatment, five chick embryos from each different experimental condition were fixed in Carnoy's solution for 6 h at 4 °C, and processed as described above for histological study. Sections were blocked in PBS/BSA and subsequently incubated in a 1/500 solution of a monoclonal anti-β3-tubulin antibody (BAbCO) at 4 °C overnight; after extensive washes, they were incubated with a solution containing an anti-mouse antibody conjugated to FITC at 1/64 (Sigma) for 1 h at room temperature. All sections throughout the microbead implantation area were visualized with a confocal microscope Zeiss LSM-310, and the most representative samples were photographed.

Since the contours of β3-tubulin positive cells cannot be accurately distinguished, the cell counting is very difficult; therefore, a qualitative analysis of neuronal differentiation was performed by comparing the intensity and extension of β3-tubulin expression among the different groups.

3. Results

3.1. IL-1β expression in spinal cord at the level of the forelimb anlage in rat embryo

We determined by immunocytochemistry the expression of IL-1β during the development of rat embryo spinal cord at the level of the forelimb implantation area. The first manifestation of IL-1β expression at this level appeared on E12 embryos, mainly in the roof plate, and to a lesser extent in the floor plate (Fig. 1A), with intense located marking. The rest of the neuroepithelium did not yet display immunomarking during this period of development. Cells were observed dorsolaterally to the spinal cord that showed intense immunoreactivity to IL-1β, and which, in view of their location, could correspond to the ventral migration pathway of the neural crest (Fig. 1A).

In E13.5 and E14.5 embryos, IL-1β immunoexpression started to appear in other areas of the spinal cord neuroepithelium (Fig. 1B); some dispersed IL-1β positive cells appeared at the level of the alar plate (Fig. 1C), and in lesser proportion and intensity on the basal plate (Fig. 1D); also, abundant immunostained cells appeared in contact with the cavity of the neural tube (Fig. 1E). Nerve fibres of the marginal layer also displayed a fibrillar pattern of IL-1β expression (Fig. 1B and D). Finally, we detected positive immunoreactivity to IL-1β during these stages in the anterior and posterior roots of the spinal nerves, as well as in the fibres of the nerves themselves (Fig. 1B), along the whole of their trajectory, and from the moment of their earliest appearance through the latest stage analyzed (E19.5).

As from E16.5, the number of IL-1β positive cells, as well as the intensity of their marking, increased considerably at the level of the basal plate, in which practically all the cells displayed immunoreactivity (Fig. 1F and G). Furthermore, at the level of the dorsal root ganglia, in which we observed no reaction previously, there began to appear marked cells on E15.5, with the number of cells as well as the intensity of their marking increasing considerably during the following stages (Fig. 1H): the highest levels were reached at the last stage studied (E19.5).

3.2. IL-1β expression in spinal cord at the level of the wing anlage in chick embryo

The experimental part of the present work was carried out in chick embryos because of the technical difficulty in performing the local treatment described above (Section 2.3) in rat embryos; therefore, it was necessary to previously determine the spatial–temporal pattern of IL-1β in chick embryos during the same stages and at the same spinal cord level studied in this experimental phase.

The expression of IL-1β in chick embryo spinal cord at brachial level was first perceivable at stage 17-HH. At this stage, dispersed cells with relatively intense immunostaining could already be observed in the spinal cord neuroepithelium, but more concentrated in the future alar plate (Fig. 2A and B). In later stages, IL-1β immunomarking intensified, with the result that, by stage 21-HH, it could be observed in most of the neuroepithelial cells that formed the spinal cord wall, including the basal plate (Fig. 2C and D). We also found IL-1β expression in these developmental

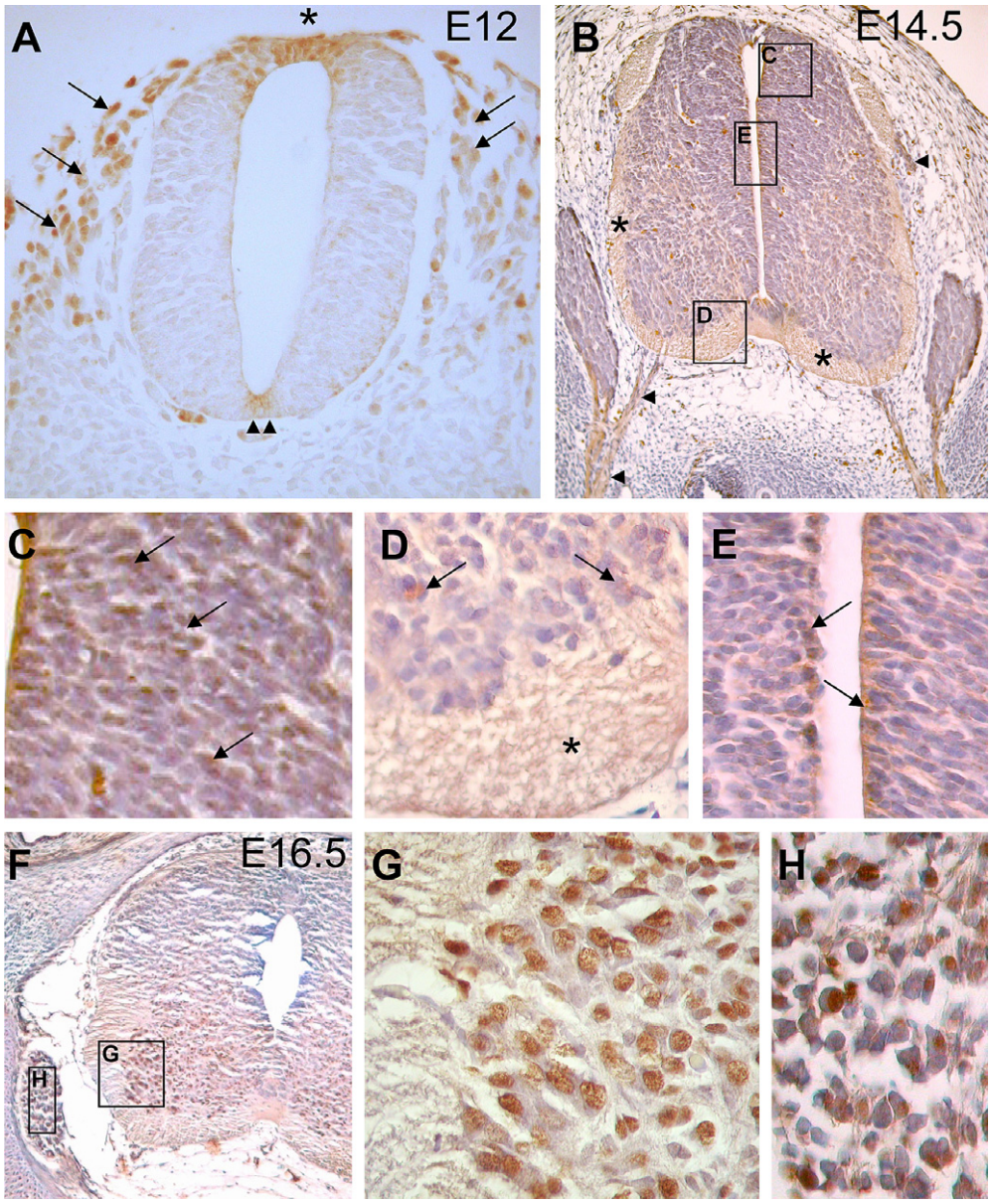


Fig. 1. IL-1β immunolabelling in the spinal cord at the level of the forelimb anlage of rat embryos. Positive IL-1β immunomarking appears in brown and haematoxilin has been used as counterstaining. (A) Transverse section of E12 rat embryo; IL-1β immunomarking can be observed in the roof plate (*), floor plate (arrow heads), and cells located laterally to the neural tube, which could correspond to neural crest cells (arrows). (B) Transverse section of E14.5 rat embryo; IL-1β immunoexpression is noticeable in the anterior and posterior roots of the spinal nerves (arrow heads) and in cells (arrows) of the alar plate (C), basal plate (D) and apical pole of the neuroepithelium (E), and in nerve fibres (*) of the marginal layer (D). (F) Transverse section of E16.5 rat embryo, showing intense IL-1β immunomarking in most of the basal plate cells (G) and in dorsal root ganglia cells (H).

stages at the level of the spinal nerve roots, as well as in cells of the dorsal root ganglia (Fig. 2C).

This expression pattern, therefore, is very similar to that described in equivalent developmental stages in rat, although the strong initial expression in the floor and roof plates that we saw in rat embryos was not detected in chick embryos.

3.3. Effect of IL-1β on spinal cord neuroepithelial proliferation

In order to assess the effects of IL-1β on neuroepithelial proliferation, we carried out local stimulation and immunoblocking experiments, by the implantation of heparin acrylic microbeads impregnated with recombinant rat IL-1β or anti-IL-1β antibodies in an intersomitic space at the level of the right wing anlage in stage 17-HH chick embryos (Fig. 3A). By this time there was already a clear expression of IL-1β in the spinal cord (Fig. 2A and B). The embryos were reincubated for 24 h up to 22-HH (Fig. 3B); for an evaluation of cellular proliferation, BrdU was microinjected intracardially and the number of positive BrdU nuclei was scored at the level of the microbead implantation zone.

The one-way ANOVA analysis for a comparison of the group averages showed statistically significant differences

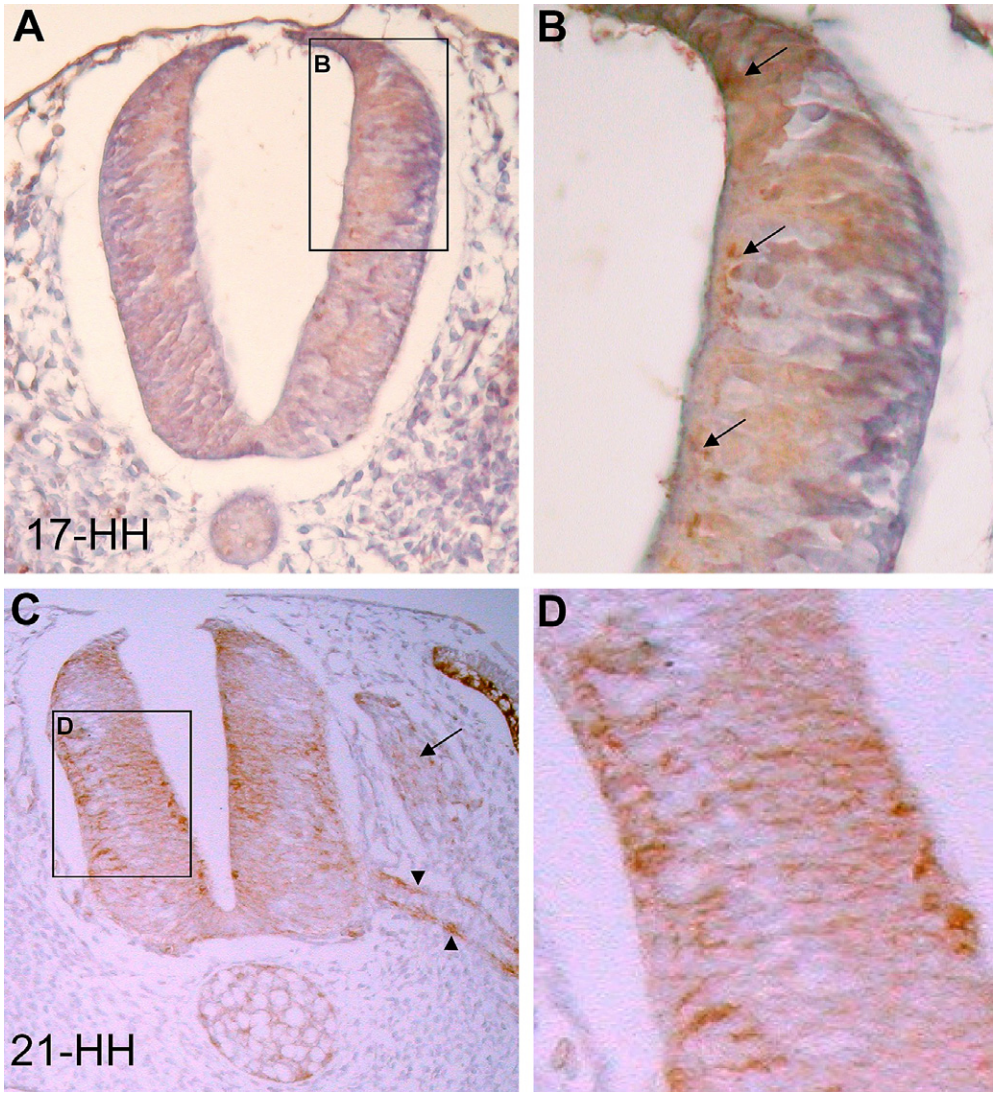


Fig. 2. IL-1β immunolabelling in the spinal cord at the level of the wing anlage of chick embryos. Positive IL-1β immunomarking appears in brown and haematoxilin has been used as counterstaining. (A) Transverse section of a stage 17-HH chick embryo. (B) Positive IL-1β immunomarking of dispersed neuroepithelial cells in the spinal cord at the level of the alar plate (arrows). (C) Transverse section of a stage 20-HH chick embryo; immunostaining in the motor root of the spinal nerve (arrow heads), as well as the presence of IL-1β-positive cells in the dorsal root ganglion (arrow) and in abundant cells throughout all the spinal cord neuroepithelium (D), can be observed.

among the different groups. According to the results of the Levene test, the variances among the four experimental groups were different in the right quadrants (the side of microbead implantation), and not in the left quadrants; therefore, a Games–Howell test in the right quadrants and a Bonferroni test in the left ones, were performed as post-hoc multiple comparison tests.

In the control embryos, which did not undergo experimental manipulation, the rate of cellular replication was visibly greater in the dorsal than in the ventral quadrants (Figs. 3C and 4). The control embryos with the Ringer-impregnated microbead implant displayed a degree of BrdU incorporation very similar to that of the non-manipulated controls (Figs. 3D and 4), and there were no statistically significant differences between both control groups. This indicates that microdissection and microbead implantation techniques do not produce appreciable effects on cellular proliferation in the spinal cord. The histological aspect of both control groups was similar in all the animals (Fig. 3C and D).

3.3.1. Stimulation experiments

In these experiments, we found statistically significant variations in the cellular replication rate in all the spinal cord quadrants, except the left ventral one. In the dorsal quadrants, embryos treated with recombinant rat IL-1β showed a statistically significant increase—between 13% and 14%—in the number of cells that incorporate BrdU in comparison with the non-handled control embryos (Fig. 4). These differences in proliferation could be appreciated in the morphologic analysis of the sections as increases in the density of BrdU positive neuroepithelial cells

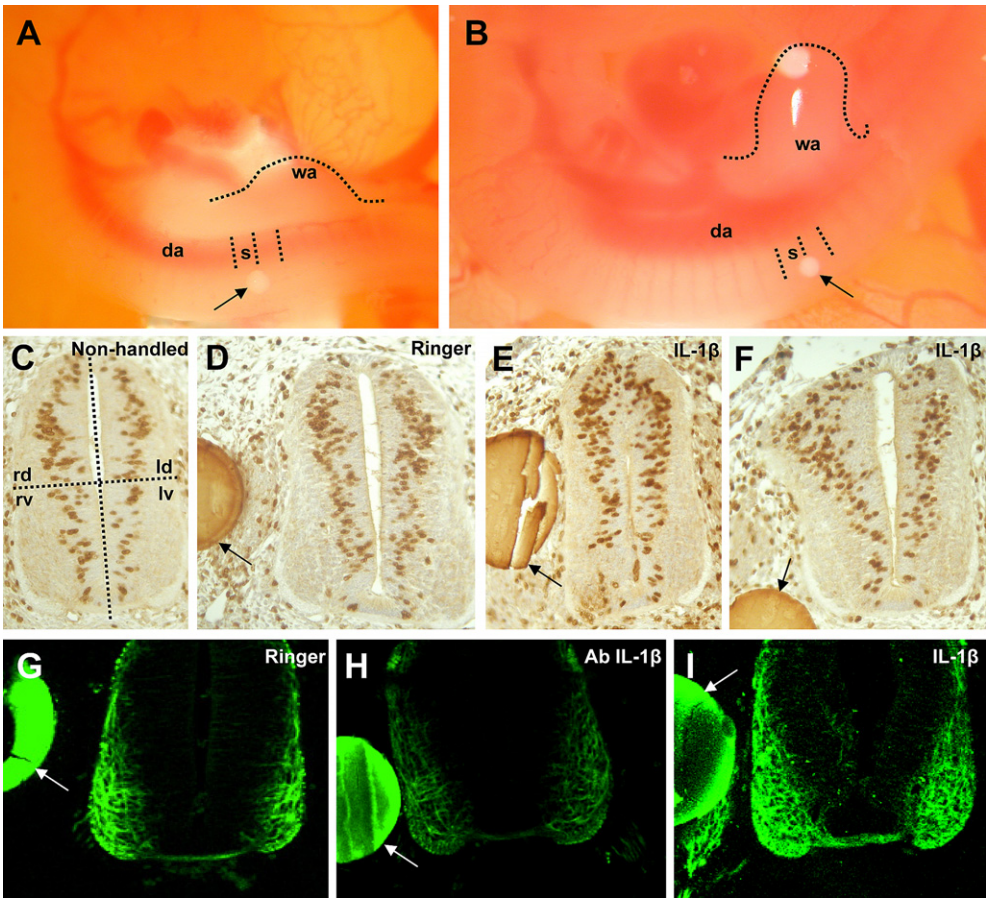


Fig. 3. Implantation of microbeads impregnated with IL-1β or anti-IL-1β antibodies. (A) The microbead (arrow) was implanted in stage 17-HH chick embryos, between two somites (s) at the level of the right wing anlage (wa); da: dorsal aorta. (B) The embryos were reincubated for 24 h until reaching stage 22-HH. (C–F) Analysis of cellular proliferation by means of BrdU incorporation in the spinal cord of chick embryos without previous manipulation (C), after the implantation of acrylic microspheres (arrows) impregnated with Ringer solution (D) or with recombinant IL-1β (E,F); BrdU-positive nuclei appear in brown. For the statistical study, the spinal cord was divided (C) in dorsal–ventral (d–v) and right–left (r–l) quadrants. Treatment with recombinant IL-1β induced increases in the density of BrdU-positive nuclei in the dorsal quadrants (E) and, on occasion, thickenings (F) of the neuroepitelial wall in the right dorsal quadrant. (G–I) Neural differentiation monitored by β3-tubulin immunostaining in chick embryo spinal cord after the implantation of acrylic microspheres (arrows) impregnated with Ringer solution in control embryos (G), with anti-IL-1β antibodies (H) or with recombinant IL-1β (I). The control embryos (G) displayed intense β3-tubulin immunomarking in the basal plate, whereas the ependymal layer did not show signs of neuronal differentiation; local treatment with anti-IL-1β antibodies considerably reduced the degree of neuronal differentiation in the basal plate (H), whereas local stimulation with recombinant IL-1β produced a slight increase in β3-tubulin immunomarking at the level of the basal plate (I).

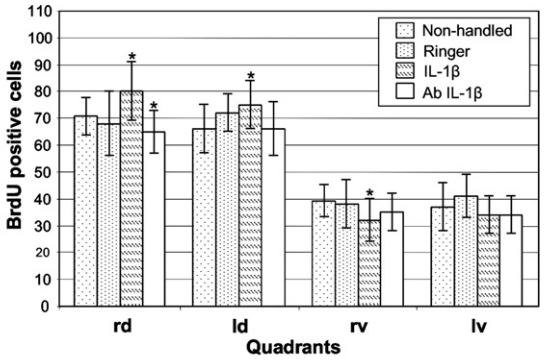
(Fig. 3E), and occasionally there appeared thickenings of the neuroepitelial wall in the right dorsal quadrant (Fig. 3F), corresponding to the side of microbead implantation.

In the ventral quadrants, local treatment with IL-1β paradoxically produced a reduction in the cellular replication rate, which was statistically significant only on the right side. An 18% decrease in the number of BrdU positive nuclei in the right ventral quadrant was observed following treatment with IL-1β (Fig. 4).

3.3.2. *Immunoblocking experiments*

Local treatment with anti-IL-1β antibodies produced fewer visible effects on cellular replication than stimulation experiments. We found statistically significant differences in the cellular replication rate only in the right dorsal quadrant (Fig. 4), closest to the microbead; at this level, the anti-IL-1β antibody produced a 9% decrease in the number of BrdU positive nuclei compared with the same quadrant in non-handled control embryos. The differences in the rate of replication observed in the rest of the quadrants were not statistically significant. These data suggest that, although the antibodies used by us were able to interfere in the biological action of IL-1β, altering the cellular replication in the right dorsal quadrant, the absence of effects in the rest of the quadrants could be due to their limited diffusion because of the high molecular weight of IgGs; this could cause an insufficient concentration in the other quadrants. We cannot rule out the possibility that higher antibody concentrations, or treatment with the receptor antagonist (IL-1Ra), could also produce significant effects in right ventral, left ventral, and left dorsal quadrants.

Our data on stimulation and immunoblocking experiments indicate that IL-1β influences cellular proliferation



Quadrants	rd	ld	rv	lv
Non-handled	71 ± 7	66 ± 9	39 ± 6	37 ± 9
Ringer	68 ± 12	72 ± 7	38 ± 9	41 ± 8
IL-1β	80 ± 11	75 ± 9	32 ± 8	34 ± 7
Ab IL-1β	65 ± 8	66 ± 10	35 ± 7	34 ± 7

Fig. 4. Quantitative analysis of neuroepithelial cells synthesizing DNA measured by the number of BrdU-positive cells in the spinal cord at the wing anlage level. Values plotted in the chart show the mean of the BrdU-positive nuclei per quadrants ± the standard deviation. Control embryos in which a Ringer-impregnated microbead was implanted (Ringer) displayed a degree of BrdU incorporation very similar to that of the non-handled controls. In dorsal quadrants, embryos treated with recombinant rat IL-1β (IL-1β) showed a statistically significant increase in the number of cells that incorporate BrdU compared with non-handled control embryos. In the ventral quadrants, local treatment with IL-1β produced a reduction in the cellular replication rate, which was statistically significant only on the right side. Local treatment with anti-IL-1β antibodies (Ab IL-1β) produced statistically significant differences in the cellular replication rate only in the right dorsal quadrant. Asterisks denote values that differed significantly from controls according to the one-way ANOVA test ($^{*}p < 0.05$). Quadrants: rd, right dorsal; ld, left dorsal; rv, right ventral; lv, left ventral.

control in the spinal cord during embryonic development, producing an increase in BrdU incorporation at the level of the alar plate and a reduction at that of the basal plate.

3.4. Effect of IL-1β on spinal cord neuronal differentiation

In order to determine whether IL-1β influences neuronal differentiation in the spinal cord, we carried out an immunocytochemical study of the expression of β3-tubulin in chick embryos after local treatment with recombinant rat IL-1β, or antibodies against IL-1β, in the wing anlage region.

In the control embryos, implanted with a microbead impregnated with Ringer solution, intense β3-tubulin immunomarking was observed at stage 22-HH in the basal plate, whereas the ependymal layer did not show signs of neuronal differentiation (Fig. 3G). The absence of immunomarking in the dorsal region of the spinal cord indicated that neurogenesis in the alar plate had not yet taken place.

In embryos locally treated with the antibody against IL-1β, a noticeable reduction in the intensity of β3-tubulin immunomarking was observed, indicating a delay or a diminution in the process of motor neural differentiation (Fig. 3H). These effects were observed only in the vicinity of the microbead.

With regard to the stimulation experiments, in embryos treated with recombinant rat IL-1β, a slight increase in β3-tubulin immunomarking at the level of the basal plate was observed, and some β3-tubulin positive cells were observed in the ependymal layer (Fig. 3I); these did not appear in the control embryos (Fig. 3G).

As mentioned above (Section 2.5), although a quantitative analysis was not carried out, the evident differences of β3-tubulin expression between groups indicate that IL-1β could play a role in motor differentiation of the spinal cord during embryonic development.

4. Discussion

4.1. IL-1β expression in the spinal cord

We detected early expression of IL-1β, mainly in neuroepithelial cells in the basal and alar plates, floor and roof plates, and sensory ganglia, in rat and chick embryos. We also observed expression of this interleukin in the nerve fibres that form the marginal layer and roots of the spinal nerves. In this context, it has been demonstrated that there is early expression of IL-1β and its type-1 receptor in sensory and motor neurones, and cells of the spinal ganglia, during the development of the spinal cord of *Xenopus laevis* [13]. This seems to indicate that medullar expression of IL-1β during development is evolutionarily conserved.

Although the effects of IL-1β on the development of the spinal cord in vivo are not known, most studies conducted in vitro demonstrate that IL-1β stimulates neuronal survival in cultures of developing spinal cord [15], and that the administration of IL-1β-blocking antisera produces an increase in cellular death in neuronal cultures of mouse spinal cord and dorsal root ganglia in the foetal period [14].

Furthermore, this interleukin may play a key role in regeneration in the adult stage. In this regard, an immediate increase in the expression of IL-1β and its type-1 receptor has been demonstrated after in vivo spinal cord injury; furthermore, it has been verified that this expression is not only the result of the inflammatory cells invading the injury site, but that it has also a neuronal and glial origin [17,18]. IL-1β also promotes neurite outgrowth and regeneration after neural section in cultures of sensory neurones from adult rat; this neurotrophic action is probably caused by the stimulus of the local production of NGF by IL-1β [29]. The same effects have been verified in cultures of rat sensory neurones during the postnatal period [16].

4.2. Role of IL-1β in neuroblastic proliferation

We verified in vivo, by the implantation of heparin acrylic microbeads impregnated with recombinant rat IL-1β in chick embryos, that this interleukin produced a statistically significant increase in neuroepithelial cellular

proliferation in the dorsal portion of the spinal cord (alar plate), and simultaneously reduced proliferation at ventral level (basal plate). Immunoblocking with antibodies against IL-1β moderately reduced proliferation in the alar plate, without the basal plate being significantly affected.

Data does not exist with regard to in vivo actions of IL-1β on neuroblastic proliferation in the spinal cord; however, its in vitro effect on proliferation has been verified in other regions of the NS, such as the diencephalon [19] or the mesencephalon [20,21]. A stimulating effect of IL-1β on glial proliferation has also been demonstrated, both in vivo after nervous injury [30] and in vitro during pre- and postnatal periods [31,32].

Although, it is known that IL-1β could play a part in the activation of multipotential neural progenitor cells in the course of diverse pathological processes [33], we have not found data related to its action on cell proliferation during normal embryonic development of the spinal cord; nevertheless, it has been demonstrated that IL-1β increases neuronal survival in dissociated cultures from foetal mouse spinal cord [14]. A correlation between high levels of IL-1β and an increase in astroglial proliferation in vitro in the spinal cord of mutant mice with motor disorders (wobbler mouse disease) during pre and postnatal periods has been also described; this effect can be reduced by the administration of neutralizing antibodies against IL-1β [31].

It is also known that IL-1β produces effects on neuronal proliferation in other regions of the CNS; specifically, IL-1β increases histidine-decarboxylase activity in primary cultures of diencephalic cells from rat embryos, which suggests that it has a role to play in the proliferation of these cells [19]. In the same line, it is known that IL-1β promotes in vitro proliferation of mesencephalic progenitor cells of rat embryo, which finally differentiate into dopaminergic neurones, and that these effects are not mediated by glial cells, but are the direct neurotrophic effects of IL-1β on this neuronal population [20,21].

In relation to the action of IL-1β on the glial population, its stimulating effect on the proliferation of these cellular types has been demonstrated after in vivo injury of mouse substantia nigra [30]; IL-1β also halts in vitro proliferation and stimulates differentiation of oligodendrocyte precursors, as well as enhancing the maturation and survival of those in the differentiation phase [32].

4.3. Role of IL-1β in neuronal differentiation

Our results show that local IL-1β immunoblocking produced a diminution of β3-tubulin expression at the level of the basal plate of the spinal cord in chick embryos. Nevertheless, stimulation experiments with recombinant rat IL-1β did not produce very visible effects, although there seemed to be a slight increase in β3-tubulin expression in the basal plate. This indicates that IL-1β could be involved in motor neuronal differentiation.

The stimulating effect of IL-1β on cell survival and neurite outgrowth has been previously described in cell cultures of mouse spinal cord between E12 and E14 [14]. It is also known that IL-1β is involved in neuronal differentiation processes in other regions of the nervous system outside the spinal cord: IL-1β stimulates in vitro differentiation of foetal mesencephalic precursor cells to dopaminergic neurones in rat [21–23] and human [24,25], and it promotes neuronal differentiation of cultured human neuroepithelial cells [26]. In addition, IL-1β and other interleukins such as IL-6 stimulate neurite outgrowth in rat dorsal root ganglia cells when cultured with fibroblast-like cells [16].

Altogether, our results on proliferation and differentiation during early stages of spinal cord development show that IL-1β had a very noticeable effect on proliferation in the (sensory) alar plate, which could be blocked with antibodies. In the (motor) basal plate, however, there was overall a decrease in the proliferation and stimulus of neuronal differentiation; the latter was clearly inhibited by immunoblocking treatment, but did not increase with local stimulation, suggesting that it could be a 'saturated' phenomenon in these stages, in which practically all the cells capable of differentiating do so.

4.4. How may IL-1β regulate spinal cord proliferation and differentiation?

The effects of IL-1β on CNS may be due to a direct effect, or to stimulation of production and release of diverse growth factors that act on neural progenitor cells. In this regard, it has been shown that there is a co-expression of IL-1β and the neural growth factor (NGF) in sensory and motor neurons of the spinal cord and sensory ganglia of cranial and spinal nerves during the embryonic development of *Xenopus laevis* [34]; this suggests that IL-1β contributes to neuronal growth and differentiation interacting with NGF. Similarly, it has been demonstrated, on cultures of adult rat spinal cord astrocytes, that IL-1β induces the synthesis of NGF, the fibroblast growth factor-2 (FGF-2) and the hepatocyte growth factor (HGF), and that these factors promote survival of cholinergic neurones [35]. This inductive effect of IL-1β on NGF and FGF-2 has been reproduced in vivo after intraventricular injection of IL-1β in other regions of the CNS, such as the hippocampus, striatum, corpus callosum, or the ependymal layer of the third ventricle [36–38]. NGF is an important neurotrophic factor in the development of cranial and spinal sensory ganglia, [39–41], and FGF-2 is one of the main factors involved in regulating the proliferation and differentiation of neuronal precursors in early stages of development [42,43].

Indirect actions of IL-1β mediated by other neurotrophic factors have also been described: the Insulin Growth Factor (IGF) regulates proliferation and neuronal differentiation, acts as a mitogenic factor on astrocytes and

promotes the development of oligodendrocytes, whilst its synthesis can be induced by IL-1 [44]; IL-1 and the Tumour Necrosis Factor (TNF) increase expression of the Colony-Stimulating Factor type-1 (CSF-1) in cultures of rat astrocytes, and this factor stimulates the proliferation and differentiation of microglia [45].

Acknowledgments

We thank Prof. Edgar Martínez for statistical assistance, and Prof. David Rixham for the linguistic revision of the text.

References

[1] Benveniste EN. Cytokines: influence on glial cell gene expression and function. In: Blalock JE, editor. Neuroimmunoendocrinology. Birmingham: Karger; 1999. p. 106–53.
[2] Ben-Hur T, Ben-Menachem O, Furer V, Einstein O, Mizrachi-Kol R, Grigoriadis N. Effects of proinflammatory cytokines on the growth, fate, and motility of multipotential neural precursor cells. Mol Cell Neurosci 2003;24:623–31.
[3] Li M, Sendtner M, Smith A. Essential function of LIF receptor in motor neurons. Nature 1995;378:724–7.
[4] Michaelson MD, Mehler MF, Xu H, Gross RE, Kessler JA. Interleukin-7 is trophic for embryonic neurons and is expressed in developing brain. Dev Biol 1996;179:251–63.
[5] Morikawa Y, Nishida H, Misawa K, Nosaka T, Miyajima A, Senba E, et al. Induction of synaptosomal-associated protein-23 kD (SNAP-23) by various cytokines. Blood 1998;92:129–35.
[6] Qiu L, Bernd P, Fukada K. Cholinergic neuronal differentiation factor (CDF)/leukemia inhibitory factor (LIF) binds to specific regions of the developing nervous system in vivo. Dev Biol 1994;163:516–20.
[7] Richards LJ, Kilpatrick TJ, Bartlett PF, Murphy M. Leukemia inhibitory factor promotes the neuronal development of spinal cord precursors from the neural tube. J Neurosci Res 1992;33:476–84.
[8] Tamura S, Morikawa Y, Senba E. Localization of oncostatin M receptor β in adult and developing CNS. Neuroscience 2003;119:991–7.
[9] Tham S, Dowsing B, Finkelstein D, Donato R, Cheema SS, Bartlett PF, et al. Leukemia inhibitory factor enhances the regeneration of transected rat sciatic nerve and the function of reinnervated muscle. J Neurosci Res 1997;47:208–15.
[10] Araujo DM, Cotman CW. Trophic effects of interleukin-4, -7 and -8 on hippocampal neuronal cultures: potential involvement of glial-derived factors. Brain Res 1993;600:49–55.
[11] Chavany C, Vicario-Abejón C, Miller G, Jendoubi M. Transgenic mice for interleukin 3 develop motor neuron degeneration associated with autoimmune reaction against spinal cord motor neurons. Proc Natl Acad Sci USA 1998;95:11354–9.
[12] Sei Y, Vitkovic L, Yokoyama MM. Cytokines in the central nervous system: regulatory roles in neuronal function, cell death and repair. Neuroimmunomodulation 1995;2:121–33.
[13] Jelaso AM, Acevedo S, Dang T, Lepere A, Ide CF. Interleukin-1β and its type 1 receptor are expressed in developing neural circuits in the frog, *Xenopus laevis*. J Comp Neurol 1998;394:242–51.
[14] Brenneman DE, Schultzberg M, Bartfai T, Gozes I. Cytokine regulation of neuronal survival. J Neurochem 1992;58:454–60.
[15] Mehler MF, Kessler JA. Hematolymphopoietic and inflammatory cytokines in neural development. Trends Neurosci 1997;20:357–65.
[16] Edoff K, Jerregård H. Effects of IL-1beta, IL-6 or LIF on rat sensory neurons co-cultured with fibroblast-like cells. J Neurosci Res 2002;67:255–63.
[17] Wang XF, Huang LD, Yu PP, Hu JG, Yin L, Wang L, et al. Upregulation of type I interleukin-1 receptor after traumatic spinal cord injury in adult rats. Acta Neuropathol 2006;111:220–8.
[18] Yang L, Blumbergs PC, Jones NR, Manavis J, Sarvestani GT, Ghabriel MN. Early expression and cellular localization of proinflammatory cytokines interleukin-1beta, interleukin-6, and tumor necrosis factor-alpha in human traumatic spinal cord injury. Spine 2004;29:966–71.
[19] Niimi M, Yamamoto Y, Takemori H, Uno A, Kondo Y, Yamatodani A. Lipopolysaccharide and interleukin-1beta augmented histidine decarboxylase activity in cultured cells of the rat embryonic brain. J Neurochem 1997;69:851–8.
[20] Akaneya Y, Takahashi M, Hatanaka H. Interleukin-1 beta enhances survival and interleukin-6 protects against MPP+ neurotoxicity in cultures of fetal rat dopaminergic neurons. Exp Neurol 1995;136:44–52.
[21] Carvey PM, Ling ZD, Sortwell CE, Pitzer MR, McGuire SO, Storch A, et al. A clonal line of mesencephalic progenitor cells converted to dopamine neurons by hematopoietic cytokines: a source of cells for transplantation in Parkinson's disease. Exp Neurol 2001;171:98–108.
[22] Johansson S, Stromberg I. Guidance of dopaminergic neuritic growth by immature astrocytes in organotypic cultures of rat fetal ventral mesencephalon. J Comp Neurol 2002;443:237–49.
[23] Ling ZD, Potter ED, Lipton JW, Carvey PM. Differentiation of mesencephalic progenitor cells into dopaminergic neurons by cytokines. Exp Neurol 1998;149:411–23.
[24] Storch A, Paul G, Csete M, Boehm BO, Carvey PM, Kupsch A, et al. Long-term proliferation and dopaminergic differentiation of human mesencephalic neural precursor cells. Exp Neurol 2001;170:317–25.
[25] Riaz SS, Theofilopoulos S, Jauniaux E, Stern GM, Bradford HF. The differentiation potential of human foetal neuronal progenitor cells in vitro. Brain Res Dev Brain Res 2004;153:39–51.
[26] Vawter MP, Basaric-Keys J, Li Y, Lester DS, Lebovics RS, Lesch KP, et al. Human olfactory neuroepithelial cells: tyrosine phosphorylation and process extension are increased by the combination of IL-1β, IL-6, NGF, and bFGF. Exp Neurol 1996;142:179–94.
[27] Hamburger V, Hamilton HL. A series of normal stages in the development of the chick embryo. J Morphol 1951;88:49–92.
[28] Somogyi P, Takagi H. A note on the use of picric acid-paraformaldehyde-glutaraldehyde fixative for correlated light and electron microscopic immunocytochemistry. Neuroscience 1982;7:1779–83.
[29] Horie H, Sakai I, Akahori Y, Kadoya T. IL-1β enhances neurite regeneration from transected-nerve terminals of adult rat DRG. Neuroreport 1997;8:1955–9.
[30] Parish CL, Finkelstein DI, Tripanichkul W, Satoskar AR, Drago J, Horne MK. The role of interleukin-1, interleukin-6, and glia in inducing growth of neuronal terminal arbors in mice. J Neurosci 2002;22:8034–41.
[31] Ait-Ikhlef A, Hantaz-Ambroise D, Jacque C, Belkadi L, Rieger F. Astrocyte proliferation induced by wobbler astrocyte conditioned medium is blocked by tumor necrosis factor-alpha (TNF-alpha) and interleukin-1beta (IL-1beta) neutralizing antibodies in vitro. Cell Mol Biol 1999;45:393–400.
[32] Vela JM, Molina-Holgado E, Arévalo-Martín A, Almazán G, Guaza C. Interleukin-1 regulates proliferation and differentiation of oligodendrocyte progenitor cells. Mol Cell Neurosci 2002;20:489–502.
[33] Klassen HJ, Imfeld KL, Kirov II, Tai LQ, Gage FH, Young MJ, et al. Expression of cytokines by multipotent neural progenitor cells. Cytokine 2003;22:101–6.
[34] Jelaso AM, DeLong C. NGF and IL-1β are co-localized in the developing nervous system of the frog, *Xenopus laevis*. Int J Devl Neurosci 2005;23:575–86.
[35] Albrecht PJ, Dahl JP, Stoltzfus OK, Levenson R, Levison SW. Ciliary neurotrophic factor activates spinal cord astrocytes, stimulating their production and release of fibroblast growth factor-2, to increase motor neuron survival. Exp Neurol 2002;173:46–62.

[36] Spranger M, Lindholm D, Bandtlow C, Heumann R, Gnahn H, Naher-Noe M, et al. Regulation of nerve growth factor (NGF) synthesis in the rat central nervous system: comparison between the effects of interleukin-1 and various growth factors in astrocyte cultures and in vivo. Eur J Neurosci 1990;2:69–76.
[37] Rivera S, Gold SJ, Gall CM. Interleukin-1 beta increases basic fibroblast growth factor mRNA expression in adult rat brain and organotypic hippocampal cultures. Brain Res Mol Brain Res 1994;27:12–26.
[38] Ho A, Blum M. Regulation of astroglial-derived dopaminergic neurotrophic factors by interleukin-1 beta in the striatum of young and middle-aged mice. Exp Neurol 1997;148:348–59.
[39] Levi-Montalcini R. The nerve growth factor. Ann NY Acad Sci 1964;118:149–70.
[40] Levi-Montalcini R. The nerve growth factor 35 years later. Science 1987;237:1154–62.
[41] Hallbook F, Ayer-Lelievre C, Ebendal T, Persson H. Expression of nerve growth factor receptor mRNA during early development of the chicken embryo: emphasis on cranial ganglia. Development 1990;108:693–704.
[42] Tropepe V, Sibilia M, Ciruna BG, Rossant J, Wagner EF, Van der Kooy D. Distinct neural stem cells proliferate in response to EGF and FGF in the developing mouse telencephalon. Dev Biol 1999;208:166–88.
[43] Panchision DM, McKay RD. The control of neural stem cells by morphogenic signals. Curr Opin Genet Dev 2002;12:478–87.
[44] Merrill JE. Tumor necrosis factor alpha, interleukin 1 and related cytokines in brain development: normal and pathological. Dev Neurosci 1992;14:1–10.
[45] Shafit-Zagardo B, Sharma N, Berman JW, Bornstein MB, Brosnan CF. CSF-1 expression is upregulated in astrocyte cultures by IL-1 and TNF and affects microglial proliferation and morphology in organotypic cultures. Int J Dev Neurosci 1993;11:189–98.