Reciprocal Regulation of the Junctional Proteins Claudin-1 and Connexin43 by Interleukin-1β in Primary Human Fetal Astrocytes

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Vertebrate tissues use multiple junctional types to establish and maintain tissue architecture, including gap junctions for cytoplasmic connectivity and tight junctions (TJs) for paracellular and/or cell polarity barriers. The integral membrane proteins of gap junctions are connexins, whereas TJs are a complex between occludin and members of a recently characterized multigene family, the claudins. In normal brain, astrocytes are coupled by gap junctions composed primarily of connexin43 (Cx43), whereas TJs have not been detected in these cells. We now show that treatment of primary human astrocytes with the cytokine interleukin-1β (IL-1β) causes rapid induction of claudin-1, with an expression pattern reciprocal to loss of Cx43. Treatment also led to protracted downregulation of occludin but no change in expression of zonula occludens proteins ZO-1 and -2. Immunofluorescence staining localized claudin-1 to cell membranes in IL-1β-treated astrocytes, whereas freeze-fracture replicas showed strand-like arrays of intramembranous particles in treated cells resembling rudimentary TJ assemblies. We conclude that in human astrocytes, IL-1β regulates expression of the claudin multigene family and that gap and tight junction proteins are inversely regulated by this proinflammatory cytokine. We suggest that in pathological conditions of the human CNS, elevated IL-1β expression fundamentally alters astrocyte-to-astrocyte connectivity.

Key words: gap junction; tight junction; cytokine; astrocyte; CNS; human

The cytokine interleukin-1β (IL-1β) is found at low levels in the CNS, where its expression is restricted to specific neuronal tracts (Breder et al., 1988). However, levels of IL-1β increase dramatically in a number of different inflammatory and degenerative conditions, with expression localized to activated microglia and macrophages. Experiments both *in vivo* and *in vitro* have strongly implicated a role for this cytokine in pathogenesis of CNS disease (for review, see Rothwell et al., 1997). IL-1β is a key activator for human astrocytes *in vitro* (Lee et al., 1993) and has been implicated in the induction of reactive astrogliosis, a common response to brain injury (Giulian et al., 1988). Studies using *in vivo* models have suggested that reactive astrogliosis is associated with the development of a CNS environment less permissive for the movements of solutes (Nicholson and Sykova, 1998), but the mechanisms underlying this are unknown. One possibility is that changes in astrocyte-to-astrocyte connectivity play a role in this response.

In the CNS, distinct specialized junctional complexes mediate cell–cell connectivity. Intercellular communication is mediated by gap junctions, which allow the direct exchange of small molecules between cells (Spray et al., 1999). Gap junctions may also form a macromolecular signaling complex, or nexus, at appositional membranes via interaction of the cytoplasmic domain with the scaffolding protein zonula occludens-1 (ZO-1) (Giepmans and Moolenaar, 1998; Toyofuku et al., 1998). In contrast, tight junctions (TJs) are complexes of proteins that mediate paracellular sealing, thereby restricting diffusion of fluid and small molecules in the extracellular space ("barrier function") and also function to establish and maintain cell polarity ("fence function") (Tsukita and Furuse, 1999). Although the first intramembrane TJ-associated protein identified was the 65 kDa protein occludin (Furuse et al., 1993), recent studies have also strongly supported a role for members of a novel family of integral membrane proteins, the claudins (Furuse et al., 1998a,b) such that TJ formation is now thought to involve an interaction between occludin and one or more of the claudins. The carboxyl terminus of claudin, in turn, associates with the second PDZ domain of ZO-1, linking the complex to a cellular scaffold (Tsukita et al., 1999).

In the normal CNS, gap junctions composed primarily of connexin43 (Cx43), with a minor contribution by other connexins such as Cx30, Cx40, Cx45, and Cx46 (Dermietzel, 1996; Spray et al., 1998), abundantly interconnect human cortical astrocytes, linking these cells into a functional syncytium. In contrast, TJs have not been noted among these cells (Massa and Mugnaini,

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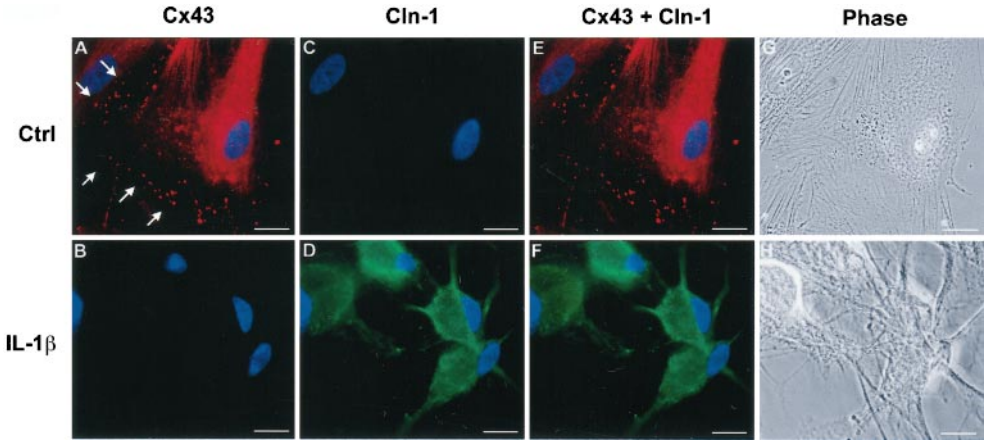

Figure 1. Immunofluorescence labeling of junctional proteins in control (*Ctrl*) and Interleukin-1β (*IL-1β*)-treated human astrocytes. Connexin43 (*Cx43*) labeling (*red*) is seen within the cytoplasm and at cell–cell junctions (*arrows*) in control cells (*A*), with cell nuclei counterstained with DAPI (*blue*). Examination of the phase image shows that the punctate staining is localized to cell–cell contacts in control cells (*G*). IL-1β treatment caused a complete loss of Cx43 (*B*). Double labeling of cells for Cln-1 (*green*) showed no staining in control cells (*C*), whereas treatment with IL-1β caused Cln-1 staining to be evident at cell membranes of treated astrocytes (*D*), although the phase image (*H*) shows that Cln-1 is not uniquely localized to sites of cell–cell contact. Note the striking change in cell morphology from flat to stellate, which is a characteristic effect of treatment with IL-1β. Overlay of the *red* and *green* channels is shown in control (*E*) and IL-1β-treated cells (*F*). Scale bar, 25 μm.

1985; Rash et al., 1996). However, we now show that IL-1β causes a rapid and robust induction of the TJ-associated protein, claudin-1 (Cln-1) with a temporal pattern reciprocal to the previously reported IL-1β-induced loss of Cx43 in the same cells (John et al., 1999). Striking morphological changes at the cell membrane accompanied this response. Thus, a switch from gap junction to TJ-associated proteins and morphology occurs under conditions in which IL-1β is elevated, which may alter intercellular and extracellular diffusion and thereby contribute to the dysfunctional state associated with inflammatory or degenerative conditions of the CNS.

MATERIALS AND METHODS

Astrocyte cultures and cytokines. Human fetal cortical astrocyte cultures were established, and purity (>98%) was determined as described previously (Lee et al., 1992). Tissue collection was approved by the Albert Einstein College of Medicine Institutional Clinical Review Committee (CCI number 94–32). Recombinant human IL-1β was a gift from National Cancer Institute Biological Response Modifiers Program (Bethesda, MD). Recombinant human IL-1Ra, the endogenous IL-1β receptor antagonist, was from R & D Systems (Minneapolis, MN).

Immunostaining. For primary antibodies (except occludin), cells were fixed 5 min in 1% paraformaldehyde, rinsed, then blocked 30 min with PBS + 10% normal goat serum + 0.4% Triton X-100. For occludin, cells were fixed in 2% paraformaldehyde incubated in ethanol and acetic acid (95:5 v:v) 20 min at −20°C and blocked with 10% goat serum in PBS 30 min at RT. Cells were stained overnight at 4°C with primary antibodies against junctional proteins (Cln-1, ZO-1, ZO-2, ZO-3, and occludin; Zymed, South San Francisco, CA; Cx43 and ZO-3, Chemicon, Temecula, CA) diluted 1:1000 in blocking solution (see above). After rinsing in PBS with 0.4% Triton X-100 (except for occludin staining, in which no Triton was used), cells were incubated 1 hr at RT with secondary antibodies conjugated to Alexa 488 or 546 (Molecular Probes, Eugene, OR), examined on a Nikon Eclipse TE300 microscope and photographed using a SPOT-RT digital camera (Diagnostic Imaging) with phase-contrast and epifluorescence optics.

SDS-PAGE and immunoblotting. Confluent astrocyte cultures (treated as described in Results) were lysed in boiling 1× SDS-PAGE loading buffer to obtain whole-cell extracts, which were then centrifuged, and pellets were discarded. For cell membrane extracts, astrocytes were lysed in 10 mM Tris-HCl buffer (4°C, pH 7.5), centrifuged, and pellets were resuspended in boiling 2× SDS-PAGE loading buffer. Proteins were separated using SDS-PAGE and transferred onto polyvinylidene difluoride membrane as described (John et al., 1999). Parallel blots were probed with polyclonal antisera for Cln-1, occludin, ZO-1, ZO-2, or ZO-3, or with monoclonal anti-Cx43 or anti-ZO-3.

RT-PCR for human Cln-1. Total RNA was isolated from confluent control or IL-1β-treated cultures and subjected to RT-PCR as previously described (John et al., 1999). Primers were based on reported sequence of human Cln-1: forward, 5′-AAC GCG GGG CTG CAG CTG TTG-3′; and reverse, 5′-GGA TAG GGC CTT GGT GTT GGG T-3′ (GenBank accession numbers AF115546, AF134160, and AF101051). Human β-actin primers were obtained commercially (Clontech, Palo Alto, CA). Conditions applied for PCR were: 95° for 5 min, 31 cycles of 95°C for 1 min, 71.5°C for 1 min, 72°C for 1 min, and 72°C for 7 min. Samples were separated by electrophoresis in ethidium bromide-impregnated 1.6% agarose gels. PCR product was identified by cloning and sequencing of five independent clones using methods previously reported (John et al., 1999).

Thin section electron microscopy. Human astrocyte monolayers cultured in 5% fetal calf serum were treated with 0 or 10 ng/ml IL-1β for 24 hr. Cells were fixed 1 hr at RT in 2.5% glutaraldehyde, 0.1 M cacodylate, and 0.1% tannic acid, post-fixed in osmium tetroxide, dehydrated stepwise in graded alcohols to 100%, and embedded in Epon. Ultrathin sections counterstained with lead citrate and uranyl acetate were then examined on a JEOL 100CX transmission electron microscope.

Freeze-fracture electron microscopy. Human astrocytes grown on 100 mm dishes were treated with 10 ng/ml IL-1β for 0 or 24 hr, fixed in 2.5% glutaraldehyde in 0.1% cacodylate buffer 60 min at RT, and rinsed in 0.1% cacodylate buffer (3× for 10 min). Cells were brought stepwise to 20% glycerol in 0.1% cacodylate buffer, rapidly frozen by immersion in ultracold Freon-22, and fractured in a Cressington CFE-50 Freeze Etch unit. Samples were platinum shadowed at a 45° angle, rotary shadowed with carbon, and examined on a JEOL 100CX transmission electron microscope, and stereo pairs were taken at 8° angles.

RESULTS

Immunolocalization of Cln-1 to cell membranes in IL-1β treated astrocyte cultures

Localization of Cx43, Cln-1, occludin, and ZO-1, -2, and -3 were examined in control and IL-1β-treated primary human astrocyte cultures using immunofluorescence staining. In control cultures there was robust expression of Cx43, particularly at cell membranes in which punctate staining was observed at sites of cell–cell contact (Fig. 1*A*). Conversely, in the same cultures Cln-1 staining was totally absent (Fig. 1*C*). After treatment of cultures with 10 ng/ml IL-1β for 24 hr, Cx43 was lost (Fig. 1*B*), which contrasted with the robust expression of Cln-1, particularly at cell membranes (Fig. 1*D*). The striking change from Cx43 to Cln-1 expression after 24 hr of 10 ng/ml IL-1β treatment was particularly evident when images obtained from double-labeled cells were superimposed (Fig. 1*E,F*).

Expression of occludin and ZO-1 and -2 was also observed at cell membranes in control cultures, and no substantive change was seen in their localization after 10 ng/ml IL-1β treatment, although over a period of ~30–55 hr of treatment the number of cells with occludin localized to cell membranes gradually decreased. In cells that maintained occludin expression, no change in intensity or membrane localization was seen (data not shown).

Expression of ZO-3 was not detected in either control or cytokine-treated cultures using two commercially available antibodies (data not shown).

IL-1β treatment of primary human astrocytes causes rapid and robust induction of Cln-1

The patterns of expression of Cx43, Cln-1, occludin and ZO-1, -2, and -3 were examined in control and IL-1β-treated primary human astrocyte cultures using immunoblotting of whole-cell extracts (Fig. 2*A*). No signal for Cln-1 was detected in control cultures; however, robust expression of a single 22 kDa band corresponding to Cln-1 was observed within 6 hr after addition of IL-1β (10 ng/ml) to cultures, reaching a plateau at 15 hr after treatment. The expression pattern of Cln-1 was reciprocal to the dramatic downregulation of Cx43 that was observed within 6 hr after treatment with IL-1β, and Cx43 was not detected by immunoblotting from 15 hr onward, in agreement with previous findings (John et al., 1999). After IL-1β treatment there was progressive downregulation of occludin over a more protracted time course (Fig. 2*A*). Expression of both ZO-1 and ZO-2 was detected in control cultures and did not alter after IL-1β treatment, but as with the immunolocalization studies, no expression of ZO-3 was detected (negative data not shown).

Immunoblotting of whole-cell extracts versus cell membrane fractions from control and IL-1β-treated cultures was used to determine whether Cln-1 expressed after IL-1β treatment was associated with cell membranes. As a control, these blots were also probed for Cx43. In agreement with previous work from our laboratories, levels of the 46 and 44 kDa (P2 and P1) isoforms of Cx43 were enriched in membrane fraction compared with whole-cell extract, in which the 42 kDa, or NP, isoform of Cx43 predominated (Fig. 2*B*). Neither membrane fraction nor whole-cell extract contained detectable Cx43 signal at 16 and 24 hr after treatment of cultures with IL-1β. In contrast, whereas no signal for Cln-1 was detectable by immunoblotting either in whole-cell extracts or membrane fractions from control cultures, strong Cln-1 expression was observed at both 16 and 24 hr after IL-1β treatment, and the signal was enriched in the membrane fractions.

Immunoblots of whole-cell extracts from astrocyte cultures demonstrated that the effect of IL-1β on Cx43 and Cln-1 expression was concentration-dependent and could be detected at doses as low as 10–100 pg/ml (Fig. 2*C*). In addition, the effects of the highest concentrations of IL-1β (10 ng/ml) were significantly inhibited by cotreatment with the naturally occurring selective IL-1 receptor antagonist (IL-1Ra, 500 ng/ml; Fig. 2*C*).

Induction of Cln-1 mRNA after IL-1β treatment of human astrocytes

RT-PCR using specific primers demonstrated induction of Cln-1 mRNA in human astrocyte cultures after 16 hr treatment with IL-1β (Fig. 2*D*). To confirm the identity of the PCR product, bands were excised from the gel, cloned, and sequenced. Data obtained from five clones gave sequences >97% identical to GenBank sequence of Cln-1/SEMP-1 cloned from Caco-2 and mammary epithelial cells (GenBank accession numbers AF115546, AF134160, and AF101051). No band was detected in PCR samples in the absence of reverse transcriptase. This induction of Cln-1 mRNA is directly reciprocal to our previously reported loss of Cx43 (John et al., 1999).

Freeze-fracture and thin-section electron microscopy

Control and IL-1β-treated cell membranes were examined using both transmission (TEM) and freeze-fracture (FF) electron microscopy. Membranes were well preserved, and on FF images multiple square arrays (Fig. 3*A,C*), a classic identifying marker for astrocytes (Massa and Mugnaini, 1985; Rash et al., 1996), were seen in both control and treated cells. Examination of both FF and TEM images showed a marked difference in the morphology of cell membranes between control and IL-1β-treated cells. In control cells, FF images showed multiple E- and P-face gap junctional plaques of varying sizes (Fig. 3*A*). Gap junctions were also found between astrocytes using TEM (Fig. 3*B*). In contrast, examination of cell membranes after treatment of cells with IL-1β showed no gap junctions, suggesting loss of all gap junction proteins. Instead, FF images of membranes of IL-1β-treated cells showed the presence of strand-like arrays of ~10 nm particles at multiple sites on P-face membranes (Fig. 3*C*). While these strands were clearly not classical TJs, their morphology was reminiscent of rudimentary tight junction strands. In agreement with these findings, multiple sites of close membrane apposition were observed in IL-1β-treated astrocytes using TEM, which again did not resemble classical TJs (Fig. 3*D*). These ultrastructural data indicate that treatment with IL-1β induces morphological changes in primary human astrocyte junctional membranes that parallel the switch from expression of Cx43 to Cln-1 in these cells.

DISCUSSION

This study demonstrates that treatment of primary human astrocytes with the proinflammatory cytokine IL-1β results in the specific induction of an integral TJ protein, claudin-1, over a time course similar to the reciprocal loss of the gap junction protein Cx43. Furthermore, this change in expression of junctional proteins is associated with morphological changes in the appositional astrocyte membranes, such that the gap junctions present in control cultures are apparently replaced, after IL-1β treatment, by loose particle strands reminiscent of rudimentary TJs. Although structural features of gap and TJs and expression of several protein components of these junctions are known to be susceptible to environmental stimuli (Citi, 1993; Spray et al., 1999), this is the first report of such an effect on claudin expression in CNS.

There is increasing evidence that both gap junctions and TJs are macromolecular assemblies consisting of integral membrane proteins and other proteins that bind to them. In the case of TJs, occludin, members of the claudin family, and junction adhesion molecule (JAM) appear to be the membrane-embedded proteins (Fanning et al., 1999), with zonula occludens family members (ZO-1, -2, and -3), cingulin, p130, 7H6, and possibly symplekin and ZA-1TJ being cytosolic binding partners (for review, see Mitic and Anderson, 1998). In the case of Cx43, binding by v-src and by ZO-1 have been demonstrated, suggesting that gap junctions may also form a macromolecular structure (Giepsman and Moolenar, 1998; Toyofuku et al., 1998; Loo et al., 1999). In this regard, it is interesting to note that connexin and claudin multigene families share analogous structural and physical properties. They both exist as four transmembrane domain (tetraspan) proteins with intracellular amino and carboxyl termini, and both bind the second PDZ domain of the membrane-associated guanylate kinase protein ZO-1 within the cell (Giepmans and Moolenaar, 1998; Itoh et al., 1999). Connexins are known to form hexamers

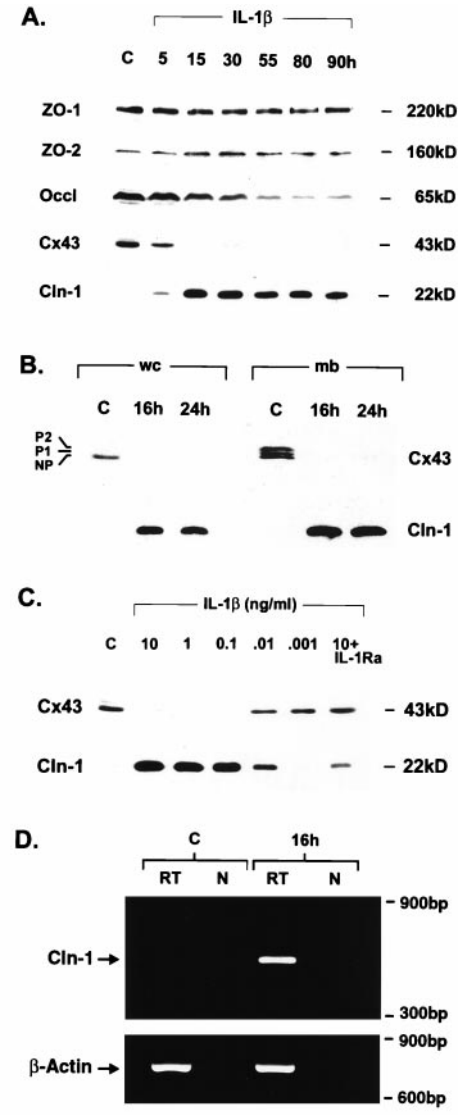

Figure 2. IL-1β induces claudin-1 expression in human fetal astrocytes. *A,* Western blot analysis of the expression of Cx43 and Cln-1 after treatment with IL-1β. Whole-cell extracts of control or IL-1β (10 ng/ml)-treated astrocytes were probed for Cln-1, Cx43, occludin and ZO-1, -2, and -3. No signal for Cln-1 was detected in control cultures; in contrast, expression of a single 22 kDa band corresponding to Cln-1 was observed within 6 hr after addition of IL-1β to cultures, with expression reaching a plateau at 15 hr. Levels of occludin were high in untreated cells but declined commencing 15–30 hr after IL-1β treatment. Treatment with IL-1β had no effect on expression of either ZO-1 or ZO-2. *B,* Western blot analysis of membrane fractions in control and IL-1β (10 ng/ml at 16 and 24 hr)-treated cells probed for Cx43 and Cln-1. No signal for Cx43 was detected at 16 hr or 24 hr after addition of IL-1β to cultures. In contrast, strong Cln-1 expression was observed at both 16 and 24 hr after IL-1β treatment (*bottom panel*). This signal for Cln-1 was enriched in the membrane fraction (*mb*) compared with whole-cell extract (*wc*). *C,* Concentration-dependent effects of IL-1β on Cx43 and Cln-1 expression as determined by Western blot analysis. Effects of IL-1β on Cln-1 and Cx43 expression was detected at a dose as low as 10 pg/ml and were significantly inhibited by cotreatment with the naturally occurring IL-1 receptor antagonist (IL-1Ra, 500 ng/ml). Analysis by RT-PCR of mRNA expression for Cln-1 in astrocytes exposed to IL-1β is shown in *D*. Total RNA from untreated (control labeled *C*) and IL-1β-treated (16 hr) human astrocyte cultures was subjected to first-strand reverse transcription followed by PCR for human Cln-1 and β-actin as described in Materials and Methods (lanes labeled *RT*). As a control, the procedure was also performed in the absence of reverse transcriptase (lanes labeled *N*). No signal for Cln-1 was detected in untreated cultures (*top panel*) although β-actin expression (*bottom panel*) was readily detected. In contrast, in astrocyte cultures treated with 10 ng/ml IL-1β for 16 hr robust expression of a single 650 bp band was detected using primers specific for human Cln-1.

surrounding a hydrophilic pore through which ions and small molecules pass from cell to cell; claudins have been proposed to form hexamers that may create paracellular pores for ion and molecular diffusion in the spaces between cells (Tsukita and Furuse, 1999).

In our experiments, the patterns of expression of Cln-1 and Cx43 protein in control and IL-1β-treated cultures showed minimal overlap, with Cx43 rapidly being replaced by Cln-1 after IL-1β treatment. The observation that the expression pattern of Cln-1 was reciprocal to that of Cx43 suggests that Cln-1 may substitute for Cx43 at the scaffolding complex localized to astrocyte cell membranes. Together with our findings that levels of ZO-1 do not change in IL-1β-treated astrocytes, this suggests the interesting possibility that exposure to IL-1β may result in the replacement of one tetraspan hexamer composed of connexons with another tetraspan hexamer composed of claudins and/or occludin at the same location in the scaffold localized to astrocyte cell membranes.

The switch in expression from one type of junctional protein to another is expected to result in profound effects on intercellular communication and extracellular diffusion in the CNS under inflammatory conditions. Under normal conditions, astrocytes are highly coupled by gap junctions formed primarily of Cx43, allowing for passage of information directly between cells. However, under inflammatory conditions this pathway could also allow the passage of apoptotic signals leading to widespread cell death (Lin et al., 1998). Thus, IL-1β induced loss of Cx43 could provide a protective mechanism by which apoptosis is minimized in the damaged CNS. Alternatively, the signaling domain associated with Cx43 in normal astrocytes may be damaging to the cell under pathological conditions.

Although astrocytes are not normally connected by TJs, our results indicate that they express the tight junction-associated proteins ZO-1, ZO-2, and occludin, but lack expression of Cln-1. The electron micrographs of astrocyte appositional membranes after Cln-1 induction by IL-1β treatment showed small regions of close membrane apposition, and in FF we observed short linear particle arrays in P face membrane, but we did not observe membrane fusions or the elaborate tight junctional strands reported in Cln-1 transfected fibroblasts (Furuse et al., 1998b). The fact that induction of Cln-1 in IL-1β-treated astrocytes did not form complete tight junctional strands suggests that some component of the tight junction strands may have been missing or nonfunctional in these cells. This possibility is further suggested by the decline in occludin expression after IL-1β treatment, which began to occur at about the same time that Cln-1 expression was induced. Because it has been suggested that the tight junction complex may contain occludin in addition to claudin, it is possible that the mature tight junctional strands require this or another integral membrane protein.

We speculate that the rudimentary tight junction strands detected in human astrocytes after IL-1β treatment may perform a function of paracellular sealing, thereby contributing to the reduced effective volume of the extracellular space and the increased tortuosity observed after inflammatory lesions (Nicholson and Sykova, 1998). Alternatively, or in addition, the junctional assemblies seen in astrocytes after IL-1β treatment may represent specialized microdomains that constitute a signalsome with novel roles in intercellular or extracellular signaling or adhesion (Denker et al., 1996; Saha et al., 1998).

We conclude that the junctional complexes in human astrocytes are dynamic structures that can be modified by environmental

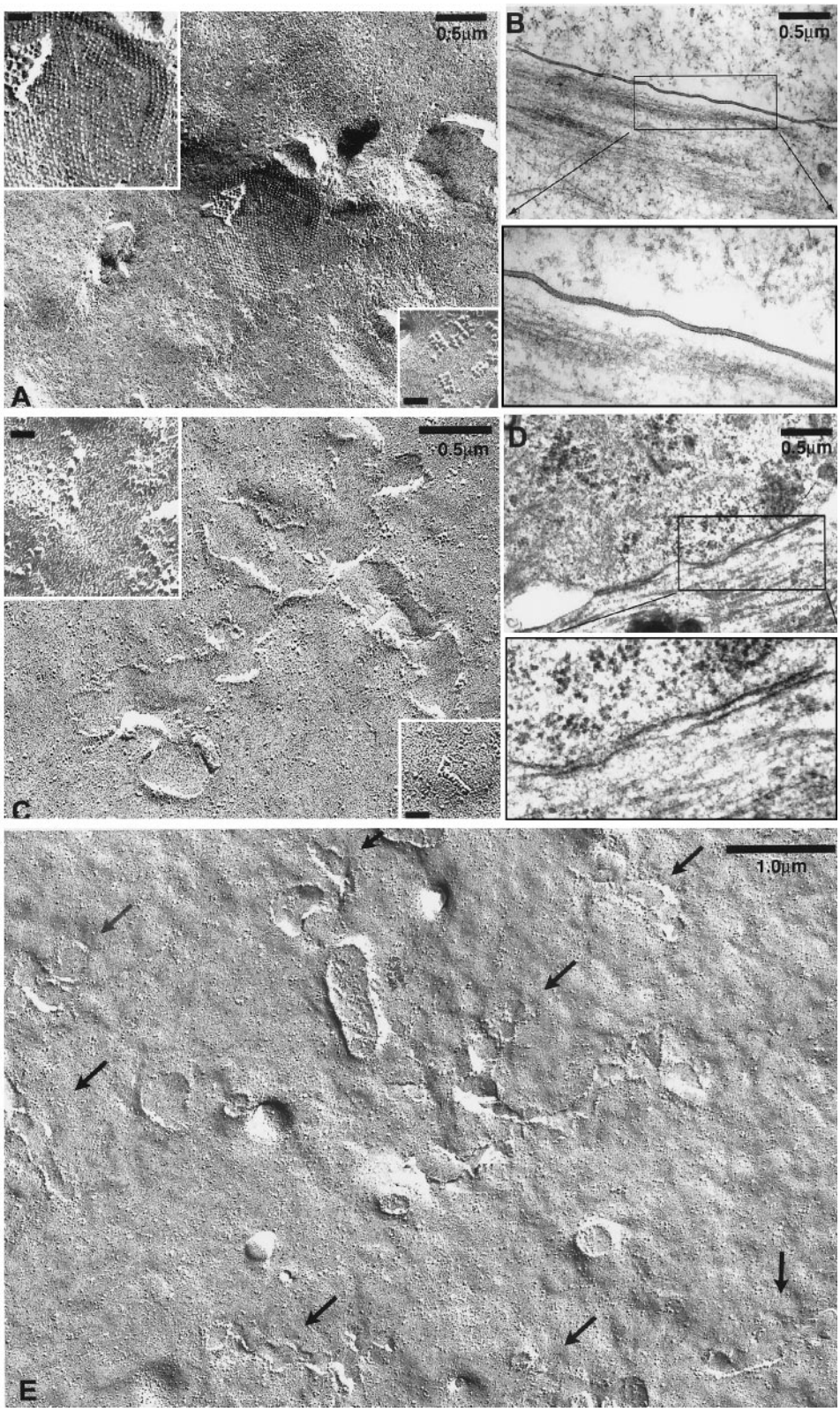

Figure 3. Ultrastructural examination of control and IL-1β-treated primary human astrocytes. Control (*A, B*) and IL-1β treated (*C, D*) astrocytes were examined by FF (*A, C*) and TEM (*B, D*). Astrocytes were identified by the presence of well preserved square particle arrays on their P-faces (*A, C, bottom right insets*). Extensive gap junctions were found between control cells by both FF (*A,* gap junction shown at higher magnification in *top left inset*) and TEM (*B*). After IL-1β treatment no gap junctions were seen. Instead, FF images showed strands of 10 nm intramembranous particles reminiscent of rudimentary tight junctions (*C,* higher magnification in *top left inset*) and TEM revealed areas of close membrane apposition (*D*). A lower power view of a membrane from an IL-1β-treated cell is seen in *E*, demonstrating the large numbers of particle strands found on these cells. Scale bars: *A* and *C insets*, 0.05 μm.

stimuli, including exposure to inflammatory cytokines and that in pathological conditions of the human CNS, elevated IL-1β expression significantly alters glial connectivity. The combined action of IL-1β on Cx43 and Cln-1 would be expected to reduce intercellular coupling and to decrease bulk fluid movement in the extracellular space. Both actions could contribute to local confinement of inflammatory responses, thereby limiting spread to surrounding uninvolved tissue.

REFERENCES

Breder CD, Dinarello CA, Saper CB (1988) Interleukin-1 immunoreactive innervation of the human hypothalamus. Science 240:321–324.

Citi S (1993) The molecular organization of tight junctions. J Cell Biol 121:485–489.
Denker BM, Saha C, Khawaja S, Nigam SK (1996) Involvement of a heterotrimeric G protein alpha subunit in tight junction biogenesis. J Biol Chem 271:25750–25753.
Dermietzel R (1996) Molecular diversity and plasticity of gap junctions in the nervous system. In: Gap junctions in the nervous system (Spray DC, Dermietzel R, eds), pp 13–38. Georgetown, TX: R.G. Landes.
Fanning AS, Mitic LL, Anderson JM (1999) Transmembrane proteins in the tight junction barrier. J Am Soc Nephrol 10:1337–1345.
Furuse M, Hirase T, Itoh M, Nagafuchi A, Yonemura S, Tsukita S (1993) Occludin: a novel integral membrane protein localizing at tight junctions. J Cell Biol 123:1777–1788.
Furuse M, Fujita K, Hiiragi T, Fujimoto K, Tsukita S (1998a) Claudin-1 and -2: novel integral membrane proteins localizing at tight junctions with no sequence similarity to occludin. J Cell Biol 141:1539–1550.
Furuse M, Sasaki H, Fujimoto K, Tsukita S (1998b) A single gene product, claudin-1 or -2, reconstitutes tight junction strands and recruits occludin in fibroblasts. J Cell Biol 143:391–401.
Giepmans BN, Moolenaar WH (1998) The gap junction protein connexin43 interacts with the second PDZ domain of the zona occludens-1 protein. Curr Biol 8:931–934.
Giulian D, Woodward J, Young DG, Krebs JF, Lachman LB (1988) Interleukin-1 injected into mammalian brain stimulates astrogliosis and neovascularization. J Neurosci 8:2485–2490.
Itoh M, Furuse M, Morita K, Kubota K, Saitou M, Tsukita S (1999) Direct binding of three tight junction-associated MAGUKs, ZO-1, ZO-2, and ZO-3, with the COOH termini of claudins. J Cell Biol 147:1351–1363.
John GR, Scemes E, Suadicani SO, Liu JS, Charles PC, Lee SC, Spray DC, Brosnan CF (1999) IL-1beta differentially regulates calcium wave propagation between primary human fetal astrocytes via pathways involving P2 receptors and gap junction channels. Proc Natl Acad Sci USA 96:11613–11618.
Lee SC, Liu W, Brosnan CF, Dickson DW (1992) Characterization of primary human fetal dissociated central nervous system cultures with an emphasis on microglia. Lab Invest 67:465–476.
Lee SC, Liu W, Dickson DW, Brosnan CF, Berman JW (1993) Cytokine production by human fetal microglia and astrocytes. Differential induction by lipopolysaccharide and IL-1 beta. J Immunol 150:2659–2667.
Lin JH, Weigel H, Cotrina ML, Liu S, Bueno E, Hansen AJ, Hansen TW, Goldman S, Nedergaard M (1998) Gap-junction-mediated propagation and amplification of cell injury. Nat Neurosci 1:494–500.
Loo LW, Kanemitsu MY, Lau AF (1999) In vivo association of pp60v-src and the gap-junction protein connexin 43 in v-src-transformed fibroblasts. Mol Carcinog 25:187–195.
Massa PT, Mugnaini E (1985) Cell-cell junctional interactions and characteristic plasma membrane features of cultured rat glial cells. Neuroscience 14:695–709.
Mitic LL, Anderson JM (1998) Molecular architecture of tight junctions. Annu Rev Physiol 60:121–142.
Nicholson C, Sykova E (1998) Extracellular space structure revealed by diffusion analysis. Trends Neurosci 21:207–215.
Rash JE, Duffy HS, Dudek FE, Bilhartz BL, Whalen LR, Yasumura T (1996) Grid-mapped freeze-fracture analysis of gap junctions in gray and white matter of adult rat central nervous system, with evidence for a "panglial syncytium" that is not coupled to neurons. J Comp Neurol 388:265–292.
Rothwell N, Allan S, Toulmond S (1997) The Role of Interleukin 1 in acute neurodegeneration and stroke: pathophysiological and therapeutic implications. J Clin Invest 100:2648–2652.
Saha C, Nigam SK, Denker BM (1998) Involvement of Galphai2 in the maintenance and biogenesis of epithelial cell tight junctions. J Biol Chem 273:21629–21633.
Spray DC, Vink MJ, Scemes E, Suadicani SO, Fishamn GI, Dermietzel R. (1998) Characteristics of coupling in cardiac myocytes and CNS astrocytes cultured from wildtype and Cx43-null mice. In: Gap junctions, pp 281–285. Amsterdam: IOS.
Spray DC, Duffy HS, Scemes E (1999) Gap junctions in glia. Types, roles, and plasticity. Adv Exp Med Biol 468:339–359.
Toyofuku T, Yabuki M, Otsu K, Kuzuya T, Hori M, Tada M (1998) Direct association of the gap junction protein connexin-43 with ZO-1 in cardiac myocytes. J Biol Chem 273:12725–12731.
Tsukita S, Furuse M (1999) Occludin and claudins in tight-junction strands: leading or supporting players? Trends Cell Biol 9:268–273.
Tsukita S, Furuse M, Itoh M (1999) Structural and signalling molecules come together at tight junctions. Curr Opin Cell Biol 11:628–633.