Pro-Inflammatory Cytokines Modulate Matrix Metalloproteinase Secretion and Organic Anion Transport at the Blood-Cerebrospinal Fluid Barrier

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Abstract. Neuroinflammation and neuroinfection trigger cytokine-mediated responses that include an increase in the cerebrospinal fluid (CSF) levels of pro-inflammatory matrix metalloproteinases (MMPs) and organic anions such as leukotrienes and prostaglandins. The choroid plexus (CP) epithelium forming the interface between the blood and the CSF regulates the CSF concentration of bioactive organic anions and is involved in neuro-immune regulation. We demonstrated that both fourth and lateral ventricle CPs are a source of pro- and active MMP-2 and MMP-9 in the brain. Using a cellular model of the blood-CSF barrier, we showed that a pro-inflammatory cytokine treatment leads to an increase in the choroidal MMP secretion at either the apical or the basolateral membrane, depending on the ventricular origin of the choroidal cells. This effect was not concomitant with an alteration in the structural blood-CSF barrier. Neither was the pool of antioxidant sulfhydryls in the choroidal cells challenged. In contrast, the efficiency of the choroidal epithelium to clear the CSF from organic anions was highly reduced. Thus, during inflammation, the CPs could be one source of MMPs found in the CSF facilitate leukocyte migration by secreting MMPs into the choroidal stroma, and promote the inflammatory process by failing in its ability to clear deleterious compounds from the brain.

Key Words: Blood-brain barrier; Cerebrospinal fluid; Choroid plexus; Glutathione; Matrix metalloproteinases; Neuroinflammation; Organic anion.

INTRODUCTION

Exchanges between the brain and the external environment are strictly regulated by the blood-brain interfaces that comprise the cerebral microvessels, the choroid plexus (CP), and the arachnoid membrane. During neuroinflammatory and infectious events, activated immune cells and pathogens may contact the blood-brain interfaces and invade the CNS and inflammatory mediators are released into the cerebrospinal fluid (CSF). These include pro-inflammatory cytokines as well as other inflammatory modulators such as eicosanoids and matrix metalloproteinases (MMPs). For example, tumor necrosis factor α (TNF-α) and interleukin 1 (II-1) have been reported in the CSF of patients suffering from CNS inflammatory diseases such as multiple sclerosis (1-5). Also, potent biologically active organic anions, such as leukotriene C4 (LTC4) and some prostaglandins, participate in the inflammatory response by promoting vasoconstriction, leukocyte chemotraction, or fever. The CSF level of these eicosanoids can to some extent be regulated (6), yet may increase during infection, inflammation, or ischemia, (5, 7-9). MMPs are enzymes that catalyze the proteolytic cleavage of basal lamina components and thus are involved in the remodeling of the extracellular matrix and in cellular migration. They also participate in the regulation of cell surface signaling molecules (10). A dysregulation of MMPs, especially MMP-9 and MMP-2, and of their specific endogenous inhibitors, tissue inhibitors of metalloproteinases (TIMPs), appears to play an important role in the pathophysiology of neuroinflammatory diseases (11-15). A consistent increase in the CSF concentration of MMP-9 is a landmark of patients suffering from neuroinflammatory or infectious diseases such as multiple sclerosis, HTLV-1-induced tropical spastic paraparesis, AIDS, or bacterial and viral meningitis (16-23). This increase is also observed in the CSF of animals after induction of an infectious or inflammatory central response (24-26).

Understanding the mechanisms leading to these CSF alterations is critical to decipher neuroinflammation pathogenesis. In this context, the CPs forming the main barrier between the blood and the CSF may play an important role with regard to these alterations. The CPs are composed of a simple epithelium protruding into the ventricles of the brain and delimiting a conjunctive stroma that contains large, fenestrated blood vessels. CPs are potential gates of entry into the CNS for different pathogens and retrovirus-infected leucocytes present in the choroidal stroma in the course of infectious diseases, and for activated lymphocytes in the context of demyelinating diseases (27-29). There is a structural restriction to diffusion of numerous blood-borne compounds into the CSF accounted for by a continuous belt of interepithelial tight junctions. This epithelium also provides neuroprotective functions based on its high enzymatic and glutathione (GSH)-dependent antioxidant activities and on active...
transport systems, among which are organic anion and cation transporters that clear the CSF of various bioactive or deleterious compounds such as LTC4 (6, 29–31). Furthermore, CPs are secretory structures responsible for the delivery into the CSF of various bioactive peptides (32). With respect to neuroimmune regulation, peripheral inflammatory stimuli rapidly upregulate the expression of transcription factors, different cytokines, and adhesion molecules in the choroidal tissue, whereas the brain parenchyma displays a more restricted and delayed activation pattern, suggesting that CPs also mediate interaction and/or signaling between the peripheral immune system and the brain (5, 33–35).

In this study, we investigated the role of the CPs as a source of MMP found in the CSF during neuroinflammation and examined whether an inflammatory environment can challenge the neuroprotective functions associated with the choroidal epithelium. To achieve this goal, we used an in vitro cellular model that provides separate access to both apical (CSF-facing) and basolateral (blood-facing) membranes of the choroidal epithelium and thus allows study of transepithelial flux measurement across the blood-CSF barrier (36, 37). In this paper, we show that the CPs have the ability to secrete MMP-2 and MMP-9 in both developing and adult brain. We demonstrate that exposure to the pro-inflammatory cytokines TNF-α and II-1 increases MMP-9 secretion by the choroidal epithelium in a polarized manner, and that cytokine treatment leads to functional rather than structural changes at the blood-CSF barrier. Functional parameters include measurements of intracellular content of sulfhydryls GSH and cysteine, as an index of the antioxidant capacity of the choroidal epithelium, and also the ability of the epithelium to actively remove organic anions from the CSF compartment assessed using phenol red as a model substrate for organic anion transporters.

MATERIALS AND METHODS

Choroid Plexus Isolation and Primary Cultures of Choroidal Epithelial Cells

Animal care and procedures have been conducted according to the guidelines approved by the French Ethical Committee (Decree 87–848) and by the European Community directive 86–609-EEC. OFA males or OFA-timed pregnant rats (200–240 g) were obtained from Harlan (Gannat, France). CPs from both adult and newborn rats were sampled intact under a sterile conditions and kept in 10% fetal calf serum supplemented DMEM/F12 (1/1; Invitrogen, Paisley, Scotland) medium at 37°C for MMP secretion analysis. Alternatively, 1- or 2-day-old rat CPs, sampled in sterile conditions and kept in 10% fetal calf serum supplemented DMEM/F12 (1/1) medium, were used to prepare primary cultures of epithelial cells. Because the 4 CPs present in mammalian brain were located within different ventricles and displayed a different morphology and may not share identical functional properties, cell cultures were obtained separately from lateral versus fourth ventricle choroidal tissue. Cells were seeded on Transwell-Clear filter inserts (0.33 cm² surface, 0.4-μm pore size; Costar Plastics, Cambridge, MA) precoated on the upper side with laminin (Becton Dickinson, Bedford, MA), as described previously in detail (36). Laminin-coated inserts without cells were kept in the same conditions. Cytokine treatment was performed 4 days after cells achieved confluence. Cells were rinsed twice with serum-free medium and treated with TNF-α and either II-1-β or II-1-α (recombinant human proteins, 5 to 30 ng/ml each; R&D Systems, Abingdon, UK), or with vehicle alone for 48 hours. Two hundred fifty and 500 μl of medium were used in the upper and lower compartment, respectively. Cell culture medium was sampled during the next 48 hours in both compartments and analyzed for MMP content or phenol red concentration (active transport studies). The filters with cells were then used either for paracellular permeability studies, immunocytochemistry, or intracellular GSH and cysteine content determination.

Similar concentrations of cytokines were used for intact isolated CP incubation studies. At the end of the treatment, the choroidal tissue was transferred to fresh medium (volume identical to the incubation volume), and homogenized. Both incubation medium and homogenate were submitted to zymography.

Zymographic Analysis

SDS-polyacrylamide gel electrophoresis zymography under nonreducing conditions on a gelatin gel was used to detect the enzymatic activities of the type IV collagenases MMP-2 and MMP-9, as described previously (11). Briefly, samples to be analyzed (20 μl) were mixed with 5 μl of loading buffer (0.1 mM Tris-HCl, pH 6.8, 4% glycerol, 2% SDS, 0.003% bromophenol blue) and electrophoresed (100V for 2 hours) on an SDS-9% (wt/vol) polyacrylamide gel containing 0.07% gelatin G2500 (Sigma, St. Louis, MO). To study the polarity of MMP secretion, the medium sampled from the upper compartment of the cell culture device was diluted twice with serum-free medium before loading to account for the volume difference between the 2 compartments. Following electrophoresis, the gel was washed twice for 20 min at room temperature with 2.5% Triton X-100 to remove the SDS and to renature the enzymes, and once briefly in water. The catalytic sites were activated by incubating the gel for 20 hours at 37°C in 100 mM Tris-HCl, 15 mM CaCl2, pH 7.4 buffer and then stained for 15 min with 0.1% Coomassie blue R250 in 30% methanol-10% acetic acid aqueous solution and destained in 30% methanol-10% acetic acid aqueous solution until clear bands corresponding to areas of gelatin degradation were seen. This technique allows visualization of 2 bands corresponding to MMP-2 (the proenzyme and the enzyme with apparent molecular weights of 72 and 65 kDa, respectively), and 2 bands corresponding to MMP-9 (the 92-kDa proenzyme and the 85-kDa enzyme). Fresh incubation medium and incubation medium from TNF-α-treated neural Dev cells (which shows a gelatinolytic band corresponding to pro-MMP-9 [38]) were used as negative and positive controls, respectively. Treating the gel with 10 mM EDTA, a metal chelator that inhibits the catalytic activity of MMPs, completely abolished all the signals (not shown).

Relative Quantification of MMP Secretion

Scans of zymograms were color reverted. Density profiles were generated for each lane, and the surface area under the

peaks was measured for all MMP bands using ImageQuant 1.2 (Amersham Biosciences, Little Chalfont, UK). Standard curves were generated by successive dilutions of MMP containing samples run on similar gelatine gels, and fitted by nonlinear regression analysis. Because the optical density is not linearly proportional to the enzymatic activity of the proteins, both low and higher signal curves were generated to allow a more precise relative quantification of MMP-9 and MMP-2 forms, respectively. The relative activities of 2 samples could thus be quantitatively compared, providing the samples were run on the same gel and the activity of each sample calculated using the same standard curve.

Immunocytochemical Analysis

The cell covered filters were rapidly washed twice with ice-cold Dulbecco’s PBS containing Ca$^{++}$ and Mg$^{++}$ (Invitrogen), and the monolayers of choroidal epithelial cells were fixed by methanol/acetone (1:1), kept at −20°C, for 90 s. After fixation, filters were washed 3 times on ice with Dulbecco’s PBS without Ca$^{++}$ and Mg$^{++}$ and stored at 4°C until immunocytochemical analysis was performed.

The distribution of tight junction proteins was studied using rabbit polyclonal antibodies raised against occludin and claudins (#71−1500 and #71−7800, respectively; Zymed, San Francisco, CA) the latter recognizing both claudin-1 and -3. The choroidal monolayers were saturated in 10% fetal calf serum containing PBS for 1 hour at room temperature. The primary antibodies against occludin or claudins were diluted (1/400 and 1/200, respectively) in the same buffer and incubated with choroidal monolayers overnight at 4°C. The immunocomplexes were labeled by incubating the cells with a biotin-coupled goat anti-mouse IgG (Jackson ImmunoResearch, West Grove, PA) diluted 1/1000 in blocking medium, then rinsed in PBS. Secondary antibody (Alexa 488-streptavidin, Molecular Probes Europe BV, Leiden, The Netherlands) used at a dilution of 1/1000 in fetal calf serum containing PBS. After washes in PBS, cell covered filters were mounted on slides with Fluoprep (Biomerieux, Lyon, France) and observed with a fluorescence microscope (Zeiss Axioplan).

MMP-9 immunodetection was performed using an avidin-biotin amplification system as follows. After a blocking step for endogenous biotin (avidin/biotin blocking kit, SP-2001 Vector), nonspecific antigenic sites were blocked with PBS containing 4% BSA and 10% goat serum for 30 min. The cells were then incubated overnight at 4°C with a mouse monoclonal antibody recognizing both the pro- and active forms of rat MMP-9 (MAB 13421; Chemicon, Temecula, CA) diluted 1/30 in blocking buffer, then rinsed 3 times in PBS. The cells were then incubated for 2 hours with a biotin-coupled goat anti-mouse IgG secondary antibody (Jackson ImmunoResearch, West Grove, PA) diluted 1/1000 in blocking medium, then rinsed in PBS. The immune complexes were labeled by incubating the cells for 2 hours in Alexa 488-streptavidin (Molecular Probes Europe BV) diluted 1/1000 in PBS. After additional rinsing steps and staining of nuclei with DAPI (100 ng/ml), the cell covered filters were mounted on glass slides using fluoroprep and observed using a Zeiss fluorescence microscope.

Permeability and Transport Studies

Paracellular permeability was measured using [$^{14}$C]sucrose (Amersham Biosciences) as a paracellular marker, as previously described (36, 39). Briefly, culture inserts with and without cells were rinsed in ringer’s solution-HEPES buffer (RH) (in mM 150 NaCl, 5.2 KCl, 2.2 CaCl$_2$, 0.2 MgCl$_2$, 6 NaHCO$_3$, 2.8 Glucose, 5 HEPES, pH 7.4) once on both sides before initiating the transfer measurement. All incubations were performed on a rotating platform (250 rpm) at 37°C in RH. The clearance volume of the tracer added to the upper compartment was measured during 4 consecutive time intervals, and permeability surface area (PS) coefficients were calculated for both laminin-coated filters (PSt) and laminin-coated filters with cells (PSf). The permeability surface area (PS) coefficient of the cell monolayer was then obtained according to the following equation $1/PS_e = 1/PS_t − 1/PS_f$, and the permeability coefficient Pe (in cm−min$^{-1}$) was obtained by dividing the PSe value by the surface area of the filter.

The active transport of phenol red, a model substrate for organic anion transport at the choroid plexus, was estimated by measuring the ability of the cells to generate an imbalance in phenol red concentration between the apical and basolateral compartment. Precise volumes of culture medium containing 23 µM of phenol red were added to the upper and lower compartments of the inserts, and after 48 hours both media were collected. One hundred µl of medium was mixed with 300 µl of NaOH 0.5 N and the spectrum of the solution was recorded between 500 and 700 nm in a Carry 100 dual spectrophotometer. The difference in optical densities between 550 and 700 nm is proportional to phenol red concentration in the medium. The active clearance of phenol red was then calculated as the volume cleared from the apical to the basolateral chamber during the incubation period per surface unit (µl·cm$^{-2}$), as previously described (39).

Measurement of GSH and Cysteine Intracellular Content

Inserts were rinsed in ice-cold RH, and the cell covered filters were quickly separated from the plastic support and placed in a microcentrifuge tube and covered with 200 µl of 500 µM 5,5′-dithio-bis(2-nitrobenzoic) acid (DTNB; Sigma) solution prepared in 10 mM K-phosphate buffer, pH 7.4. After a freeze-thaw cycle, the biological material was further titurated within the tube, heated at 37°C for 10 min to complete the reaction of DTNB with SH-containing residues, and centrifuged at 8000 g for 10 min. Standard solutions of mixed cysteine and GSH were prepared and processed in a similar way. The supernatants were analyzed for content in GSH- and cysteine-derived disulfide by HPLC using a protocol modified from Komuro et al (40), which allows quantitation of both sulfhydryls separately. Analysis was performed on a LC10 Shimadzu system (Duisburg, Germany) as follows: Samples (20 µl) were applied with an autoinjector device cooled at 4°C onto an Ultraphere ODS RP-18 analytical column (5 mm, 4.6 × 250 mm; Beckman, Fullerton, CA). The elution of the disulfides was isocratic, using a mobile phase constituted by a 10/10/80 mixture of methanol/0.1 M K-phosphate buffer, pH 6/water pumped at a constant rate of 1 ml/min. After 8 min, the methanol concentration was increased from 10% to 40% over 2 min and kept at 40% for an additional 9 min to allow the elution of the unreacted DTNB. Absorbance of the effluent was monitored at 324 nm, and the retention times of the cysteine disulfide, glutathione disulfide, and DTNB were
4.9, 6.3, and 13.9 min, respectively. The free 2-nitro-5-thiobenzoic acid generated from DTNB during the conjugation reaction was eluted in the solvent front.

Quantification was performed from the calibration curve based on the peak areas obtained after injection of supernatants produced from the standard solutions. Preliminary assays showed that, in the range of concentration measured in the study, 100% of cysteine or GSH reacted with DTNB to generate a disulfide (not shown).

RESULTS

Basal Matrix Metalloproteinase Secretion by the Choroidal Epithelium

Secretion of MMPs by the choroidal epithelium was evaluated using both freshly isolated CPs over a 20-hour period and a reconstituted choroidal epithelium in primary culture over a 48-hour period. Freshly isolated CPs from lateral (Fig. 1A) and fourth ventricle (not shown) of newborn rat brain secreted MMP-2 and MMP-9. MMPs were also released from adult material, albeit at an apparent lower level (Fig. 1B). With both newborn and adult tissue, MMP-2 was secreted in a time-dependent manner during the 20-hour incubation period. Its accumulation in the medium was sufficient to generate a clear signal after only 2 hours of incubation. Both the pro- and active forms were detected. Very low levels of MMP-9 forms could be detected in the medium after 5 hours of incubation. The signal was more intense at the longest time period, indicating that MMP-9 also was continuously secreted by CPs. A similar basal secretion of MMPs was observed when using the in vitro reconstituted choroidal epithelium, a model in which apical secretion (i.e. at the CSF-facing membrane) can be differentiated from basolateral secretion (i.e. at the stroma-facing membrane) (Fig. 2A). MMP-2 in both its pro- and active forms was secreted in a time-dependent manner over 48 hours in both the apical and basolateral compartments, with no predominance or a slightly apical (Fig. 2B) predominance. MMP-9 forms became detectable only after 18 hours of accumulation, with a predominant secretion in the apical compartment (Fig. 2B). The immunocytochemical detection of MMP-9 in primary cultures showed that the protein accumulated within the intercellular clefts, thus confirming that part of the protein was secreted at the basolateral membrane (Fig. 2C).

Effect of Pro-Inflammatory Cytokine Treatment on the Secretion of Matrix Metalloproteinases by the Choroid Plexus Epithelium

To analyze MMP secretion by the choroid plexus epithelium in an inflammatory context, we exposed the reconstituted epithelium to TNF-α/Il-1-β or TNF-α/Il-1-α treatment for up to 48 hours. When cells originated from the fourth ventricle CP, the secretion of the pro- and active forms of MMP-2 was increased only at the basolateral membrane (Fig. 3A). The secretion of MMP-9, mostly in its pro-form, was increased in both compartments; however, there was a clear predominance at the basolateral membrane of the epithelium. This polarity was consistently observed in 8 different experiments performed on batches of cells originating from different rat litters, as shown by the apical-to-basolateral ratio of secreted MMP activities, which were (in mean ± SEM) 0.65 ± 0.05 for MMP-9 and 0.41 ± 0.08 for MMP-2. When the cells originated from lateral ventricle CPs, an increase in MMP-9 and to a lesser extent MMP-2 secretion was also observed following cytokine treatment, but the secretion of both MMPs was more pronounced at the apical pole (Fig. 3B), as illustrated by the apical-to-basolateral ratio of 2.5 ± 0.2 (n = 19) for MMP-9 and 1.5 ± 0.1 (n = 19) for MMP-2. Both TNF-α and either of the II-1 proteins were needed to achieve a complete effect, but increasing the II-1 concentration from 5 to 30 ng/ml did not change the extent or pattern of MMP secretion. The increase in MMP secretion could already be detected when the media were sampled 24 hours after cytokine addition (not shown). An additional 125-kD zymographic signal was observed in medium from treated cells, which corresponds to a previously described complex of MMP-9 with lipocalin (41). Replacing II-1-β by II-1-α also led to similar increase in MMP-2 and MMP-9 secretion in both types of CP cells. Treated-to-control ratios of secreted MMP-9 in both compartments were 6.7 ± 1 [6] and 4.1 ± 0.5 [19] (mean ± SEM, [n]) when II-1-β and II-1-α were used, respectively, and were not statistically different (p > 0.05). The ratios for MMP-2, respectively 1.8 ± 0.24 [6] and 1.9 ± 0.13 [19], were also similar for both treatments. These overall ratios may be underestimated, as the experiments in which the MMP-9 signal under control conditions was too low to be accurately quantified were not included.

To confirm the data generated using the reconstituted
epithelial monolayers, freshly isolated and intact CPs were incubated with or without IL-1 and TNF-α at 37°C for 9 hours, an incubation period that was chosen to be long enough to allow an increase in MMP secretion to be detected, while minimizing exchange between the stromal compartment and the medium through the free apertures resulting from the dissection. First, we confirmed that a cytokine treatment induced MMP-9 synthesis and secretion at the CPs. In control conditions, over the 9-hour period, MMP-2 and MMP-9 were secreted from both apical and stromal sides and diffused in the medium. Following cytokine treatment, an increase in MMP-9 became detectable after 6 hours and was clearly visible after 9 hours (Fig. 3C), both in the medium (containing apically secreted proteins and part of the basolaterally secreted enzymes that diffused from the choroidal stroma) and in the CP homogenates (containing the remaining basolaterally secreted and intracellular proteins). The 9-hour period of treatment did not allow visualization of quantitative changes in MMP-2 secretion. Second, we showed that the medium-to-homogenate ratio of MMP-9 was increased in cytokine-treated versus control CPs isolated from the lateral ventricle (2 versus 0.7), but remained steady (0.7) when fourth ventricle CPs were used (Fig. 3C), in accordance with the difference in the polarity of secretion observed for MMPs in reconstituted epithelia originating from the 2 types of CPs.
Effect of Pro-Inflammatory Cytokine Treatment on the Neuroprotective Properties of the Choroidal Epithelium

In control conditions, antibodies recognizing the tight junction proteins occludin or claudin 1/3 (42) stained junctional rings with no apparent discontinuities (Fig. 4A, C). Treatment with II-1 and TNF-α did not significantly alter this typical interepithelial localization of the tight junction proteins (Fig. 4B, D), suggesting that the tightness of the barrier is not altered. This was further supported by the functional evaluation of the paracellular permeability of the epithelium. No change in permeability coefficient of [14C]sucrose was observed at either 24 hours (0.42 ± 0.03 10⁻³ cm/min for treated monolayers versus 0.39 ± 0.02 10⁻³ cm/min for control monolayers) or 48 hours (Fig. 5A) following TNF-α/II-1 (α or β) treatment. In contrast, the active clearance of phenol red, a model substrate for organic anion transport, was decreased by 50% over the 48-hour period following cytokine addition (Fig. 5B). Similar effects were obtained when either II-1-α or II-1-β was used, and for concentra-
Our data demonstrate that both newborn and adult CPs secrete MMP-2 and MMP-9 in the CSF. The CPs play an important role during brain development as a source of nutrients, trophic factors, and molecules involved in the cellular organization of the cerebral architecture (29). MMPs and TIMPs are strongly involved in brain development and brain remodeling (14). Our data, concomitant with the evidence of a high expression of MMP inhibitors, mainly TIMP-3 in the adult CP (12), hint at a new facet of CP functions in both brain development and brain plasticity by way of regulating the MMP/TIMP balance in the CSF.

Following treatment with the pro-inflammatory cytokines IL-1 and TNF-α, MMP-9 secretion by CP was strongly increased at both poles of the epithelium; however, with clear polarization that was apical (CSF-facing) for the lateral ventricle CP and basolateral (stroma-facing) for the fourth ventricle CP. This difference in the polarity of MMP release, which was observed consistently using the cells in primary culture and confirmed using freshly isolated tissue, points out that the secretion may be regulated differently in the fourth and lateral ventricle CPs. MMP-2 secretion also was induced, but to a lesser extent. The mechanisms by which the treatment with both cytokines leads to the increase in MMP secretion could involve the functional cytokine receptors TNFRI and IL-1RI because TNF-RI is expressed in the rat choroidal tissue under basal conditions and is induced following TNF-α treatment (43), and because the restricted distribution of both IL-1RI and IL-1RII receptor proteins reported for the mouse brain includes the choroidal epithelium (44, 45). In accordance with IL-1-α and IL-1-β sharing the same receptors, the use of either one or the other cytokine led to similar effects.

The apical secretion of MMPs by the choroidal epithelium seems to parallel the MMP content of CSF reported in human and animal models. MMP-2, shown to be constitutively present mostly in its pro-form in human, rodent, and dog CSF, in absence of pathology (11, 16, 18, 23–25, 46, 47), is slightly increased only in some inflammatory situations (21, 22) and is not likely to contribute to the pathogenic process. MMP-9, at low levels in control CSF, is largely increased in various neuroinflammatory and neuroinfectious diseases (16–26). In such cases, the enzyme is largely detected in its latent form (21, 23, 46). The origin of CSF-borne MMP-9 is not elucidated. A plasma origin that would result from a blood-cerebrospinal fluid barrier breakdown has been ruled out (19, 23). Infiltrating cells appear to be one source of CSF MMPs, especially in human or experimental meningitis (18, 23, 25), but MMP-9 level and either total CSF cell count or specific immune cell infiltration into the CSF are not necessarily correlated. Rather, a correlation between MMP-9 and TNF-α concentrations in the CSF has been observed in patients and in laboratory animals with bacterial meningitis (19, 48). Curiously, the potential importance of nearby CNS cells that could release MMP into the CSF has not been specifically investigated. In our study, the profile of MMPs secreted directly into the CSF by the CP epithelium, with respect to the relative occurrence of MMP-2 and MMP-9 forms (latent versus active) and upregulation in an inflammatory context, is correlated with the MMP profile observed in CSF in vivo. These results thus point out the CPs as a probable significant source of MMPs present in the CSF.
in both normal and pathological situations. In addition, although our study did not intend to make a quantitative analysis of MMP secretion, the levels of MMPs secreted for a few hours by only 0.25 mg of cells (1 filter) in 250 μl of apical medium could account for the level we previously detected in the CSF from patients with inflammatory pathologies (17), considering that the volume of CSF produced per mg of CPs per day in human is 280 μl (49).

When secreted at the basolateral membrane, epithelial MMPs may also participate in the remodeling of the basal membrane and conjunctive network forming the choroidal stroma. This will depend on their proteolytic activity, which is modulated by the extent of cleavage of the proinactive form into the active form of the protein, and by their interaction with their endogenous specific inhibitors, TIMPs. The activation of latent forms of secreted MMP-2 and MMP-9 can be catalyzed by other MMPs or plasmin (14), or via the action of reactive oxygen species often associated with inflammatory events (50). All activation patterns are likely to take place in the choroidal stroma. MMPs are not expected to directly alter the integrity of the tight junction as, to our knowledge, their potential substrates do not include occludin and claudins. However, following an inflammatory stimulus, basolaterally secreted choroidal MMPs may modulate or even alter the integrity of the basal membrane and the stromal architecture, and thus take an important part in the CNS infiltration of infected or activated immune cells by facilitating their migration within the stroma and across the choroidal epithelium. Besides, cytokine-responsive MMPs have the ability to participate to the maturation, by cleavage, of various membrane receptors and ligands, such as TNF-α and its receptor (14), involved in the cascade of events that leads to inflammation. The CPs may therefore be the site of an inflammatory amplification loop triggered by cytokines that are released in the CP stroma and/or found in the CSF (5), and working through choroidal MMPs. The pro-inflammatory cytokines TNF-α and IL-1 did not trigger an apparent impairment of the blood-cerebrospinal fluid barrier at the structural level, as visualized by the lack of effect on either the subcellular distribution of the tight junction proteins occludin and claudins, or the paracellular permeability investigated after 24 or 48 hours of cytokine treatment in our model.

An alteration of the tight junction protein organization has been described in the choroidal epithelium in experimental acute encephalomyelitis (51), or following intracerebroventricular phorbol ester injection (52). Our data indicate that, although an early transient effect cannot be excluded, such alteration in tight junction organization is not mediated by the cytokines per se. The mechanism is, therefore, more complex and involves other effectors that are likely to include recruited immune cells, such as secreting neutrophiles, as suggested by in vivo studies (53, 54).

In contrast to the absence of marked effects on tight junctions, inflammatory stimuli significantly impaired the active organic anion efflux capacity of the choroidal epithelium, as visualized by the strong decrease in phenol red active transcellular transport from the apical to basolateral side of the epithelial layer. At the CP, at least 2 families of apically located transporters, Slc22 (OATs/OCTs) and Slc21 (oatps), are involved in organic anion and cation efflux from CSF. Inflammatory mediators, such as leukotrienes and prostaglandins, are known to be
MMP secretion, CPs may represent an important source of neuroinflammatory processes. Through the elevated CSF barrier showing the potential implication of the CPs in the decrease in organic anion efflux at the blood-kine environment, the increase in choroidal MMP secretion in a pro-inflammatory cytokine environment, the increase in choroidal MMP secretion at the blood-CSF barrier neuroprotective function of the choroidal epithelium (36). The latter would reflect further impairment of the blood-CSF barrier neuroprotective potential.

Finally, the increase in the GSH content within the epithelial cells in response to the inflammatory stimulus could indicate an enhancement of GSH-mediated protective functions. The underlying mechanism may involve an increase of γ-glutamylcysteinyl synthase, as shown in alveolar epithelial cells following TNF-α activation (55). Alternatively, the concomitant increase in cysteine and GSH intracellular concentrations suggests a reduction of sulfhydryl commitment in cellular metabolic and transport pathways. This may be due to the inactivation of organic anion transport proteins such as Slc21 members, which utilize GSH as an antipporter (56), or possibly the inactivation of GSH-mediated protective enzymes such as glutathione-S-transferases, which are strongly expressed at the CP epithelium (36). The latter would reflect further impairment of the blood-CSF barrier neuroprotective potential.

In conclusion, although the structural characteristics and GSH-dependent antioxidant function of the choroidal epithelium seem unaffected in a pro-inflammatory cytokine environment, the increase in choroidal MMP secretion and the decrease in organic anion efflux at the blood-CSF barrier show the potential implication of the CPs in neuropathological consequences of this choroidal functional impairment. In addition, the cerebral bioavailability of some anti-inflammatory agents also known to be cleared from the CSF by CP organic anion transporters (30) may be modified.

Fig. 6. Enhancement of the choroidal epithelium antioxidant capacity following a pro-inflammatory cytokine treatment. Intracellular glutathione and cysteine have been measured by HPLC 48 hours following treatment initiation. Mean ± SD, n = 3. Closed bars: control cells; dashed bars: treated cells.

of these proteins in the CSF and also perturb the interaction between the extracellular matrix and cells within the choroidal stroma, possibly facilitating leukocyte migration into the CSF. Furthermore, they may promote inflammation by failing in their ability to clear cerebral inflammatory mediators. A pharmacological control of organic anion transport activity or MMP secretion at the level of the CP could be relevant to diseases with a neuroinflammatory facet.

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