Tumour necrosis factor-α increases intracellular Ca\(^{2+}\) and induces a depolarization in cultured astroglial cells

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Summary
Tumour necrosis factor (TNF)-α, a strong immune mediator, is released within the brain during inflammatory diseases and contributes to immunological activation of glial cells. Here we report that, in astrocytes, TNF-α also affects the intracellular Ca\(^{2+}\) homeostasis and basic electrophysiological properties such as the membrane potential. Using the Ca\(^{2+}\) indicator dye fura-2 in a cell culture model, we found that TNF-α (10-1000 U ml\(^{-1}\)), but not interleukin 1 or 6, induced a slow but more than two-fold increase of the intracellular Ca\(^{2+}\) concentration, which could be blocked by Co\(^{2+}\) (1.0 mM), verapamil (100 μM) or omission of external Ca\(^{2+}\). This intracellular Ca\(^{2+}\) increase was accompanied by a marked decrease of the membrane potential by 35 mV. CSF of patients with bacterial meningitis, known to contain large amounts of TNF-α, induced a similar depolarization of astrocytes, which was markedly reduced by a neutralizing anti-TNF-α antibody. We conclude that TNF-α induces an increase of intracellular Ca\(^{2+}\) and a depolarization in astrocytes with the consequence of disturbing voltage-dependent glial functions such as regulation of local ion concentrations and glutamate uptake. During inflammatory CNS diseases this immuno-electrical coupling may contribute to an impairment of neuronal function.

Keywords: tumour necrosis factor α; cultured astrocytes; intracellular calcium; meningitis; cerebrospinal fluid

Abbreviations: CSF-CTRL = CSF from patients with non-inflammatory neurological diseases; CSF-M = CSF from patients with bacterial meningitis; Ig = immunoglobulin; IL = interleukin; TNF = tumour necrosis factor

Introduction
Among pleiotropic cytokines, TNF-α plays a central role in the immunological cascade during inflammatory diseases of the central nervous system. It contributes to the immunopathogenesis of such different diseases as multiple sclerosis (Cannella and Raine, 1995; Hartung et al., 1995; Riekmann et al., 1995), bacterial meningitis (Arditi et al., 1990; Sharief et al., 1992) and AIDS-associated encephalopathy (Glass et al., 1993; Epstein et al., 1993; Wilt et al., 1995). Elevation of TNF-α titres in CSF correlates with the degree of disruption of the blood–brain barrier (Sharief and Thompson, 1992; Sharief et al., 1992) and also with electrophysiological dysfunction (Arditi et al., 1990) in patients. The cellular mechanisms of this immuno-electrical coupling are unclear.

Experimental studies have revealed that cytokines may affect neuronal excitability either directly (Sawada et al., 1990, 1991; Plata-Salaman and ffrench-Mullen, 1992, 1993) or indirectly via an alteration of neuron–glia interaction. TNF-α also induced a delay of cortical visual evoked potentials after experimental intra-ocular injection (Brosnan et al., 1989) and inhibited long-term potentiation in hippocampal slices (Tancredi et al., 1992). In previous studies we found that basic electrophysiological properties of astrocytes can be strongly affected by injurious molecules like leukotrienes and lipopolysaccharides (Köller and Siebler, 1993; Köller et al., 1993; Köller et al., 1994a, b). Here we report that TNF-α specifically induces an increase of intracellular Ca\(^{2+}\) and a depolarization in astrocytes. Parts of this study have been published in abstract form (Köller et al., 1995).
Material and methods

Cell culture procedures
Serum-free cell culture procedures for rat neurons, astrocytes and electrophysiological techniques using the whole cell recording mode of the patch clamp technique were identical to those described previously (Köller et al., 1993, 1994a). Neurons were prepared from cortices of embryonic Wistar rats at embryonic day 15. After short trypsinization and trituration with fire polished Pasteur pipettes, cells were washed by centrifugation and plated on glass coverslips, previously coated by poly-L-lysine (0.1 mg ml⁻¹, 24 h, 4°C) and laminin (10 µg ml⁻¹, 24 h, 4°C). They were grown in serum-free media, which were conditioned by a monolayer of spatially separated astrocytes for ≥3 days prior to use with neuronal cultures. Cells were identified as neurons by displaying action potentials upon depolarization by intracellular current injection as well as by immunohistochemical staining with the monoclonal mouse antimicrotubule-associated protein 2 antibody from Sigma (Stichel and Müller, 1992).

Glia cells were prepared from hemispheres of newborn Wistar rats and grown in media containing 10% foetal calf serum. After 10 days cells were disassociated to remove contaminating oligodendrocytes and neurons and plated on poly-L-lysine (0.1 mg ml⁻¹) coated glass coverslips. Cells were allowed to proliferate for 1 week until a nearly confluent monolayer was established. The media were then changed to serum-free media in order to reduce proliferation. Astrocytes were identified with immunohistochemical staining for glial fibrillary acidic protein being positive by >95% (Stichel and Müller, 1992). Electrophysiological recordings as well as Ca²⁺ measurements were performed 1–3 weeks after the change to serum-free media.

Electrophysiological recordings
Recordings were performed using the whole cell mode of the patch clamp technique (Hamill et al., 1981) at room temperature. Cells were incubated in bath solutions containing cytokines or in CSF at 37°C unless otherwise indicated. For every series of measurements 0.7–1.0 ml of CSF was used without any dilution with artificial solutions. Pipettes were drawn from borosilicate glass capillaries without filament (GC 150-50, Clark Electromedical Instruments) and filled with a solution of (in mM): KCl 140.0, CaCl₂ 1.0, MgCl₂ 2.0, EGTA 11.0, HEPES 10.0, pH adjusted to 7.4 by KOH. Electrodes had a tip diameter of about 1 µm and resistances of 5–6 MΩ. Bath solution contained (in mM): NaCl 150.0, KCl 4.0, CaCl₂ 2.8, MgCl₂ 1.0, HEPES 10.0, sucrose 10.0, pH adjusted to 7.4 by NaOH. In order to control stable electrophysiological recordings we evaluated the membrane resistance by applying depolarizing and hyperpolarizing current pulses from a holding potential of −80 mV to a potential of −100, −90, −70 or −60 mV, and calculated the resistance as the inverse of the slope conductance between −70 and −100 mV. Astrocytes with resistances below 30 MΩ or cells with a marked decrease of resistance during the experiment were excluded. Cytokines and the anti-TNF-α antibody were added to the bath solution or the CSF in amounts to reach the final concentrations as indicated. Solution changes were performed by rinsing. In the case of CSF experiments, cover slips were washed in both solutions and transferred to another CSF, because only small amounts of CSF were available and rinsing experiments were not possible.

All materials were purchased from Sigma, Deisenhoven, Germany, unless otherwise indicated. Recombinant human TNF-α and TNF-α antibody (polyclonal rabbit anti-human TNF-α with a neutralizing potency of 1000 U TNF-α per 10 µl) were purchased from Genzyme, Rüsselsheim, Germany.

Measurements of intracellular Ca²⁺
Intracellular Ca²⁺ concentrations were measured using the Ca²⁺ sensitive dye fura-2 and by determining the ratio of the fluorescence at 340 and 380 nm excitation (Grynkiewicz et al., 1985). Astrocytes were loaded with the dye by incubation in bath solution containing 5.0 µM fura-2 acetylmethylester for 30 min. Experiments were performed using a D104G/Deltascan system (Photon Technology International, South Brunswick, USA) in which excitation shutters, monochromator settings and data acquisition were controlled by a personal computer. Fluorescence measurements were performed on a small area of the monolayer containing ~10 cell bodies. Intracellular Ca²⁺ concentrations were calculated according to the method given by Grynkiewicz et al. (1985) after determining the fluorescence ratio (340/380 nm wavelengths) in Ca²⁺-free solution (0 Ca²⁺, 10 mM EGTA, 10 µM ionomycin) and in Ca²⁺-saturating solution (10 mM Ca²⁺ in presence of 10 µM ionomycin).

Data analysis
All recordings were stored on a personal computer. Membrane currents and potentials, as well as intracellular Ca²⁺ concentrations, were evaluated off line. Data were given as mean±standard deviation. Student’s t test was used for statistical analysis.

CSF samples
Fourteen CSF samples were tested for possible effects on the electrophysiological membrane properties of cultured astrocytes. All lumbar punctures were carried out for diagnostic reasons with the informed consent of the patients. The CSF of six patients with bacterial meningitis (CSF-M) showed a purulent pleocytosis with cell counts of 560–2600 cells µl⁻¹, mainly granulocytes, and an elevated protein content. The CSF samples from eight patients with non-inflammatory neurological diseases (CSF-CTRL) such
TNF-α increases intracellular Ca$^{2+}$

2023

TNF 100 U/ml

Fig. 1 Example of the increase of internal Ca$^{2+}$ induced by TNF-α. (A) TNF-α led to a slow increase of intracellular Ca$^{2+}$, measured with the Ca$^{2+}$ indicator dye fura-2. The Ca$^{2+}$ increase started after a delay of 20 min and reached a plateau of ~114 nM, as estimated from calibration curves (for details see Material and methods). (B) Internal Ca$^{2+}$ was not increased by TNF-α in the presence of TNF-α antibody.

Fig. 2 Time course of depolarization of astrocytes by TNF-α. The bars represent mean membrane potentials of astrocytes measured over 20 min intervals, beginning from 0 to 20 min after TNF-α application and ending >200 min later. Each bar indicates the mean membrane potential of 8–12 cells (±SD). Asterisks mark statistically significant decreases of membrane potential (Student’s $t$ test, $P < 0.001$) compared with control values (CTRL). Note that the depolarization started earlier in the presence of 100 U ml$^{-1}$ TNF-α than in the presence of 10 U ml$^{-1}$ TNF-α, but the depolarization did not reach a higher level. The solid line shows the decline of membrane potential fitted by a tanh curve. The TNF-α induced depolarization was blocked when TNF-α was applied into a bath solution containing a neutralizing polyclonal anti-TNF-α antibody (Anti-TNF-α) recorded after 120 min of incubation.

Results

TNF-α and intracellular Ca$^{2+}$ concentration in astrocytes

In concentrations of 10, 100 and 1000 U ml$^{-1}$, TNF-α led to a slow increase of astrocyte intracellular Ca$^{2+}$ from an estimated concentration of 50±16 nM to 114±8 nM ($n = 12$). An example is given in Fig. 1. This increase of internal Ca$^{2+}$ was absent if TNF-α was applied in presence of 10 μl of a neutralizing TNF-α antibody ($n = 3$), indicating that the Ca$^{2+}$ increase was induced by TNF-α and not by any contaminating compound. The increase of internal Ca$^{2+}$ started after a delay of ~20 min. The magnitude of the TNF-α-induced increase of intracellular Ca$^{2+}$ concentration was the same using different TNF-α concentrations (10 U ml$^{-1}$, 100 U ml$^{-1}$, 1000 U ml$^{-1}$). The half maximal increase, however, was reached in a dose dependent way: 10 U ml$^{-1}$ TNF-α induced a Ca$^{2+}$ increase being half maximal after 55±17 min ($n = 4$), 100 U ml$^{-1}$ after 35±16 min ($n = 4$) and 1000 U ml$^{-1}$ after 27±6 min ($n = 4$). The increase of intracellular Ca$^{2+}$ was not reversible within 3 h after removing the TNF-α containing bath solution ($n = 4$).

The TNF-α-induced increase of internal Ca$^{2+}$ was blocked in presence of Co$^{2+}$ (1 mM, $n = 3$) and in Ca$^{2+}$-free external solution ($n = 3$, data not shown). Verapamil (100 μM) also prevented the TNF-α-induced increase of intracellular Ca$^{2+}$ ($n = 3$, data not shown).

Neither interleukin-1 (IL-1, 1000 U ml$^{-1}$, $n = 6$) nor IL-6 (1000 U ml$^{-1}$, $n = 6$) induced a change in the intracellular Ca$^{2+}$ concentration within a recording time of 150 min (data not shown).

Effect of TNF-α on astrocyte membrane potential

In presence of TNF-α (10 U ml$^{-1}$) astrocytes depolarized, starting after 60 min and reaching a plateau phase after 160 min (Fig. 2). With elevated concentrations of TNF-α (100 U ml$^{-1}$, Fig. 2) the depolarization started earlier, but reached the same membrane potential. The magnitude and velocity of depolarization was not increased after incubation of astrocytes with 1000 U ml$^{-1}$ TNF-α. The half maximal depolarization was reached after an incubation period of ~110 min (10 U ml$^{-1}$ TNF-α) or 90 min (100 or 1000 U ml$^{-1}$ TNF-α, Fig. 2). The TNF-α-induced
The TNF-α-induced depolarization of astrocytes depends on Ca²⁺ influx

TNF-α failed to depolarize the astrocytes in presence of Co²⁺ (1.0 mM) or Ni²⁺ (1.0 mM), which are divalent cations known to block Ca²⁺ channels. The TNF-α-induced depolarization was also prevented by verapamil (100 µM). In Ca²⁺-free solution astrocytes depolarized slightly but TNF-α (100 U ml⁻¹) did not induce a further depolarization (Table 2).

TNF-α and cultured cortical neurons

The intracellular Ca²⁺ concentration of cultured cortical neurons was not increased by TNF-α (1000 U ml⁻¹; n = 5; data not shown) and TNF-α did not induce a depolarization in neurons (Köller et al., 1996). Even after 90 min of incubation with TNF-α (1000 U ml⁻¹), IL-1 (1000 U ml⁻¹) or IL-6 (1000 U ml⁻¹) resting membrane potential of neurons was unchanged and neuronal Na⁺ and K⁺ currents were unaffected (Köller et al., 1996).

CSF-M and TNF-α-mediated depolarization of astrocytes

We tested, whether the TNF-α-induced depolarization occurs in the presence of CSF as well; we added TNF-α (100 U ml⁻¹) to CSF from patients who did not suffer from inflammatory CNS disease (n = 4). The astrocyte membrane potential decreased significantly from −76.2 ± 4.1 mV (n = 23) to −55.6 ± 4.9 mV (n = 28, P < 0.001 versus CSF without TNF-α, Table 3). After changing to TNF-α-free CSF, the depolarization was not reversible. The depolarization was not induced, when TNF-α was applied in CSF in the presence of a neutralizing anti-TNF-α antibody.

It is known that TNF-α is present in very high amounts (up to 850 U ml⁻¹; Sharief et al., 1992) in CSF-M. We therefore tested, whether astrocytes depolarize after incubation in CSF from patients CSF-M. Astrocytes depolarized markedly after 90 min of incubation in CSF-

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**Table 1 Effects of cytokines on the membrane potential of astrocytes**

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>n</th>
<th>Membrane potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No cytokine</td>
<td>20</td>
<td>−80.3±4.7</td>
</tr>
<tr>
<td>IL-1 (1000 U ml⁻¹)</td>
<td>11</td>
<td>−84.5±7.0</td>
</tr>
<tr>
<td>IL-6 (1000 U ml⁻¹)</td>
<td>9</td>
<td>−76.9±7.4</td>
</tr>
<tr>
<td>TNF-α (10 U ml⁻¹)</td>
<td>22</td>
<td>−48.2±12.3*</td>
</tr>
<tr>
<td>TNF-α (100 U ml⁻¹)</td>
<td>11</td>
<td>−48.8±7.0*</td>
</tr>
<tr>
<td>TNF-α (1000 U ml⁻¹)</td>
<td>15</td>
<td>−47.4±8.3*</td>
</tr>
<tr>
<td>Anti-TNF-α+TNF-α (100 U ml⁻¹)</td>
<td>23</td>
<td>−82.2±6.5</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard deviation. *Significantly different from membrane potential without cytokines (P < 0.001, Student’s t test).

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**Table 2 Effect of Ca²⁺-free external solution and Ca²⁺ channel blockers on TNF-α-induced depolarization of astrocytes**

<table>
<thead>
<tr>
<th>External solution</th>
<th>TNF-α (U ml⁻¹)</th>
<th>n</th>
<th>Membrane potential of astrocytes (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bath solution</td>
<td>0</td>
<td>20</td>
<td>−80.3±4.7</td>
</tr>
<tr>
<td>Bath solution</td>
<td>100</td>
<td>11</td>
<td>−48.8±7.0*</td>
</tr>
<tr>
<td>Ca²⁺-free bath solution</td>
<td>0</td>
<td>13</td>
<td>−61.7±9.1*</td>
</tr>
<tr>
<td>Ca²⁺-free bath solution</td>
<td>100</td>
<td>21</td>
<td>−65.6±15.6*</td>
</tr>
<tr>
<td>Bath solution (as above)</td>
<td>100</td>
<td>11</td>
<td>−48.8±7.0*</td>
</tr>
<tr>
<td>Bath solution +Co²⁺ (1.0 mM)</td>
<td>100</td>
<td>20</td>
<td>−78.7±5.5*</td>
</tr>
<tr>
<td>Bath solution +Ni²⁺ (1.0 mM)</td>
<td>100</td>
<td>23</td>
<td>−75.7±6.8*</td>
</tr>
<tr>
<td>Bath solution +verapamil (100 µM)</td>
<td>0</td>
<td>8</td>
<td>−73.5±9.4*</td>
</tr>
<tr>
<td>Bath solution +verapamil (100 µM)</td>
<td>100</td>
<td>10</td>
<td>−70.9±8.2*</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard deviation. *Significantly different at the P < 0.001 level (Student’s t test) when compared with the first result in the same pair/group.

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**Table 3 Effect of TNF-α applied to non-inflammatory CSF on the membrane potential of astrocytes**

<table>
<thead>
<tr>
<th>Solution</th>
<th>n</th>
<th>Membrane potential of astrocytes (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF (TNF-α-free)</td>
<td>23</td>
<td>−76.2±4.1</td>
</tr>
<tr>
<td>CSF+100 U ml⁻¹ TNF-α</td>
<td>28</td>
<td>−55.6±4.9*</td>
</tr>
<tr>
<td>CSF+100 U ml⁻¹ TNF-α+anti-TNF-α</td>
<td>25</td>
<td>−74.5±4.5*</td>
</tr>
</tbody>
</table>

*Significantly different from the result with CSF (TNF-α-free) at the P < 0.001 level (Student’s t test). Not significantly different from the result with CSF (TNF-α-free).
Electrical coupling (Kettenmann and Ransom, 1988) with the way. The precise mechanisms of the depolarization, however, the expression or the unmasking of cation channels in a similar resembling neuronal L-type channels, detectable within cultured astrocytes are difficult due to the high degree of 2+ channels in astrocytes, authors observed voltage gated Ca (Kagan et al., 1992). These short time upon stimulation (Barres et al., 1989). These previously that astrocytes are able to express Ca 2+ increase was sensitive to the omission of intracellular Ca 2+ transporters (Pitts, 1979; Takuma et al., 1990). The TNF-α-induced increase of internal Ca 2+ is not electroneutral but coupled to a decrease of Ca 2+ uptake. Impairment of membrane properties of astrocytes could be due to the activation of Ca 2+ transporters (Pitts, 1979; Takuma et al., 1994), Ca 2+ release from internal stores (Finkbeiner, 1993) or opening of transmembrane cation ionophores (MacVicar, 1984; Barres et al., 1989; Corvalan et al., 1990). The TNF-α-induced intracellular Ca 2+ increase was sensitive to the omission of extracellular Ca 2+ and to the addition of Co 2+ and verapamil, thereby resembling the characteristic features of transmembrane Ca 2+ currents. It has been demonstrated previously that astrocytes are able to express Ca 2+ channels in a short time upon stimulation (Barres et al., 1989). These authors observed voltage gated Ca 2+ channels in astrocytes, resembling neuronal L-type channels, detectable within 15 min of incubation with cAMP. Thus, TNF-α may induce the expression or the unmasking of cation channels in a similar way. The precise mechanisms of the depolarization, however, are unclear so far, since direct measurements of ion currents in cultured astrocytes are difficult due to the high degree of electrical coupling (Kettenmann and Ransom, 1988) with the consequence of reduced voltage clamp facilities. The value of uncoupling procedures such as using octanol in adequate concentrations is limited due to a possible impairment of intracellular mechanisms and membrane currents (Sontheimer et al., 1991; Lee et al., 1994).

Another hypothesis reported in the literature suggests that TNF-α by itself may insert into the membrane and form a cation channel. Kagan et al. (1992) described the intrinsic channel-forming activity of TNF-α in U937 histiocytic lymphoma cells. After insertion into the membrane, a pH-dependent, voltage-dependent channel appeared with a preferential Na + permeability. However, Kagan et al. (1992) observed an increase of transmembrane currents within seconds, whereas the Ca 2+ influx in our experiments appeared after 15–20 min. If TNF-α was inserted directly into the cell membrane to form a cation channel we would have expected neurons also to depolarize, but this was not the case (Köllner et al., 1996).

Two previous electrophysiological experiments testing TNF-α both revealed a striking delay in the onset of the TNF-α-induced effects, which resembles the delay of TNF-α-induced depolarization in our study; Brosnan et al. (1989) reported that visual evoked potentials in rabbits were delayed during the 3 h after intraocular injection of TNF-α and Tancredi et al. (1992) found an inhibition of long-term potentiation in hippocampal slices after a prolonged incubation (50 min).

Astrocytes are sensitive to TNF-α in many respects. They proliferate in response to stimulation by TNF-α (Selmaï et al., 1990). Reactive astrogliosis is increased after additional stimulation by cytokines including TNF-α (Balasingam et al., 1994). TNF-α also stimulates the production of IL-6 (Norris et al., 1994), phospholipase A2 (Oka and Arita, 1991) and nerve growth factor (Gradient et al., 1990). The role of Ca 2+ in these immunological effects is not fully understood. However, it is known that some of the effects of TNF-α, e.g. IL-6 production, can be mimicked by artificially increasing the intracellular Ca 2+ concentration with the Ca 2+ ionophore A23187 (Norris et al., 1994). Glial differentiation, proliferation and DNA synthesis can also be associated with increases of cytoplasmic Ca 2+ (reviewed by Finkbeiner, 1993) suggesting that Ca 2+ may serve as a second messenger. However, according to our experiments, the TNF-α-induced increase of internal Ca 2+ is not electroneutral but coupled to a decrease of the membrane potential. The marked depolarization interferes with basic glial cell functions, such as maintaining a stable ionic microenvironment (Walz, 1989). In addition, neurotransmitter uptake systems have been found to be voltage-dependent (Kimelberg et al., 1989; Flott and Seifert, 1991). Depolarized astrocytes are impaired concerning K + buffering and glutamate uptake. Impairment of membrane properties of astrocytes could indirectly affect neuronal excitability due to neuron–glia interaction (Barres, 1991; Sontheimer, 1995). Very recently, Largo et al. (1996) showed that a selective poisoning of glial cells is associated in vivo with an impairment of maintenance of K + homeostasis and pH and a disturbance of synaptic transmission. Alteration of external ionic composition, e.g.

![Fig. 3](http://brain.oxfordjournals.org/)

**Fig. 3** The anti-TNF-α antibody prevents the depolarization induced by CSF from patients with bacterial meningitis (CSF-M). Astrocytes depolarized significantly in CSF-M (P < 0.001 versus CSF from patients with non-inflammatory CNS diseases (CSF-CTRL) recorded after 90 min of incubation). The depolarization-inducing activity was significantly reduced (P < 0.001) after addition of a neutralizing anti-TNF-α antibody to the CSF-M. The data from recordings using CSF taken from the same original CSF samples, with and without an anti-TNF-α antibody, are connected by a solid line.

M (Fig. 3; P < 0.001). The depolarization of astrocytes induced by the CSF-M was significantly reduced by a neutralizing anti-TNF-α antibody (10 μl, n = 6; Fig. 3).

**Discussion**

Our results clearly indicate (i) that TNF-α induced a slow increase of intracellular Ca 2+ in astrocytes which depended on extracellular Ca 2+ and could be blocked by Ca 2+ channel antagonists, (ii) that TNF-α slowly depolarized the cells and (iii) the depolarization was also induced by CSF-M and could be blocked by a neutralizing anti-TNF-α antibody.

The increase of internal Ca 2+ could be due to the activation of Ca 2+ transporters (Pitts, 1979; Takuma et al., 1994), Ca 2+ release from internal stores (Finkbeiner, 1993) or opening of transmembrane cation ionophores (MacVicar, 1984; Barres et al., 1989; Corvalan et al., 1990). The TNF-α-induced intracellular Ca 2+ increase was sensitive to the omission of extracellular Ca 2+ and to the addition of Co 2+ and verapamil, thereby resembling the characteristic features of transmembrane Ca 2+ currents. It has been demonstrated previously that astrocytes are able to express Ca 2+ channels in a short time upon stimulation (Barres et al., 1989). These authors observed voltage gated Ca 2+ channels in astrocytes, resembling neuronal L-type channels, detectable within 15 min of incubation with cAMP. Thus, TNF-α may induce the expression or the unmasking of cation channels in a similar way. The precise mechanisms of the depolarization, however, are unclear so far, since direct measurements of ion currents in cultured astrocytes are difficult due to the high degree of electrical coupling (Kettenmann and Ransom, 1988) with the consequence of reduced voltage clamp facilities. The value of uncoupling procedures such as using octanol in adequate concentrations is limited due to a possible impairment of intracellular mechanisms and membrane currents (Sontheimer et al., 1991; Lee et al., 1994).

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due to glial cell dysfunction, is one mechanism supposed to generate seizures (Taylor, 1988).

**Implications for inflammatory CNS diseases**

Assuming, that TNF-α evokes a similar depolarization of astrocytes in vivo, neuronal function may become indirectly impaired in inflammatory CNS diseases: the occurrence of seizures in bacterial meningitis, which correlates with the TNF-α titres in the CSF (Arditi et al., 1990), may be caused by a breakdown of local ion homeostasis due to glial dysfunction. In MS, locally released TNF-α may contribute to the pathogenesis of functional deficits. This view is supported by a recent study of Moreau et al. (1996); in association with increased levels of circulating TNF-α and interferon-γ after infusion of humanized monoclonal antibodies for MS treatment, they observed a transient exacerbation of pre-existing MS symptoms, lasting for several hours. In human immunodeficiency virus (HIV)-associated encephalopathy, TNF-α is released chronically within the brain (Tyor et al., 1992) and some data suggest that there is a correlation between TNF-α mRNA levels and HIV-associated dementia (Glass et al., 1993). In addition to the reported damage of oligodendrocytes (Wilt et al., 1995), TNF-α-induced impairment of glial functions may contribute to the pathogenesis of neurological dysfunction in inflammatory CNS diseases.

In summary, the TNF-α-induced changes of Ca²⁺ homeostasis and electrophysiological function of astrocytes may provide a cellular basis for disturbances of neurological function in CNS disorders associated with acute and chronic TNF-α release.

**Acknowledgements**

The authors thank Dr C. Schmalenbach, Düsseldorf, for preparing the neuronal and glial cell cultures, Professor Dr H. Steinmetz and Professor Dr H. Luhmann for critical reading of the manuscript. This study was supported by grants from the Deutsche Forschungsgemeinschaft (SFB 194 / B7).

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Kölle H, Buchholz J, Siebler M. Bacterial endotoxins impair...
TNF-α increases intracellular Ca²⁺ 2027


Received February 2, 1996. Revised May 23, 1996. Accepted June 25, 1996.