Mechanisms of inflammatory neurodegeneration: iNOS and NADPH oxidase

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Abstract

Inflammation contributes to a wide variety of brain pathologies, apparently via glia killing neurons. A number of mechanisms by which inflammatory-activated microglia and astrocytes kill neurons have been identified in culture. These include iNOS (inducible nitric oxide synthase), which is expressed in glia only during inflammation, and PHOX (phagocytic NADPH oxidase) found in microglia and acutely activated by inflammation. High levels of iNOS expression in glia cause (i) NO (nitric oxide) inhibition of neuronal respiration, resulting in neuronal depolarization and glutamate release, followed by excitotoxicity, and (ii) glutamate release from astrocytes via calcium-dependent vesicular release. Hypoxia strongly synergizes with iNOS expression to induce neuronal death via mechanism (i), because NO inhibits cytochrome oxidase in competition with oxygen. Activation of PHOX (by cytokines, β-amyloid, prion protein, ATP or arachidonate) causes microglial proliferation and inflammatory activation; thus PHOX is a key regulator of inflammation. Activation of PHOX alone causes no death, but when combined with expressed iNOS results in extensive neuronal death via peroxynitrite production.

Introduction

Inflammatory neurodegeneration is neurodegeneration caused by inflammation. There is now significant evidence that brain inflammation contributes to the pathology of neurodegenerative diseases (Alzheimer's disease, Parkinson's disease, multiple sclerosis and AIDS dementia), as well as stroke, brain trauma and meningitis [1–6]. These pathologies have different causes and courses, but they all involve brain inflammation, and there is evidence that blocking inflammation can either prevent onset or reduce symptoms for each of these pathologies [1–6]. However, there are different types or modes of inflammation, and it is important to understand why inflammation is sometimes protective and at other times damaging, so that interventions can be designed to prevent one but not the other.

Brain inflammation itself does not cause neuronal death. For example, brain inflammation often occurs in a clinical setting (e.g. meningitis, malaria and viral encephalitis) without apparent neuronal death. However, it is equally clear that inflammation (or particular modes of inflammation) can cause neuronal death in conjunction with particular circumstances, even in the above named conditions. Thus it is important to identify which circumstances are necessary for neuronal death and how to block it.

Brain inflammation occurs behind the blood–brain barrier, and thus differs from inflammation in the periphery by the relative absence of leucocytes (particularly neutrophils, monocytes, macrophages, B-cells and T-cells) and antibodies. However, it is now recognized that there is a limited traffic of these factors across the barrier, and this traffic can be increased by inflammation [7]. Microglia are resident brain macrophages, and are the key cells in brain inflammation and inflammatory neurodegeneration [5]. Microglia are normally 'resting', where they appear to do little apart from monitor the brain for (i) pathogens, and (ii) damage, using pathogen and damage receptors. If they detect pathogens or damage they become 'activated': they round up, proliferate, migrate, phagocytose, present antigens to T-cells, produce oxidants and express a range of new genes and proteins, including iNOS [inducible NOS (nitric oxide synthase)], COX-2 (cyclo-oxygenase-2), MHC class II and complement (Figure 1). These activated microglia can kill and/or remove pathogens, but they may also kill neurons. The mechanisms by which they kill neurons are complex [5], but I will restrict myself to reviewing the roles of iNOS and NOX (NADPH oxidase) in this death with particular emphasis on my own research in this area.

iNOS and NOX

Most brain pathologies are accompanied by inflammation, during which the production of NO (nitric oxide; mainly from iNOS) and/or superoxide (O_2^-) plus H_2O_2 (mainly from NOX) are increased [4,5,8–10]. NO and superoxide, and derivative RNS (reactive nitrogen species) and ROS (reactive oxygen species) are, at low concentrations signalling molecules, for example regulating cell proliferation, but at high concentrations are key cytotoxic molecules of innate immune defence against pathogens [8–12]. They are also implicated in the pathology of most brain diseases,

Key words: cell death, inflammation, nitric oxide, peroxynitrite, reactive nitrogen species, reactive oxygen species.

Abbreviations used: COX-2, cyclo-oxygenase-2; IL-1β, interleukin-1β; LPS, lipopolysaccharide; LTA, lipoteichoic acid; NMDA, *N*-methyl-D-aspartate; NOS, nitric oxide synthase; iNOS, inducible NOS; NOX, NADPH oxidase; PHOX, phagocytic NADPH oxidase; TLR, Toll-like receptor; TNFα, tumour necrosis factor α.

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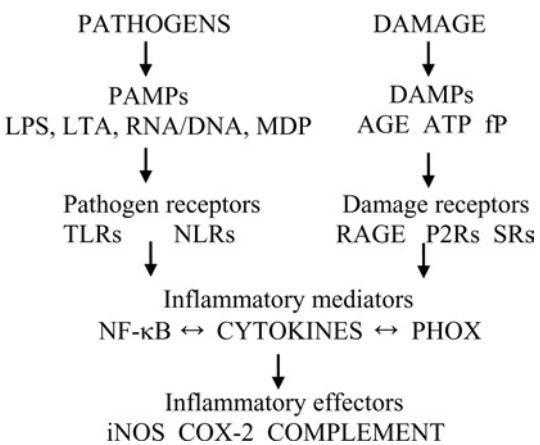
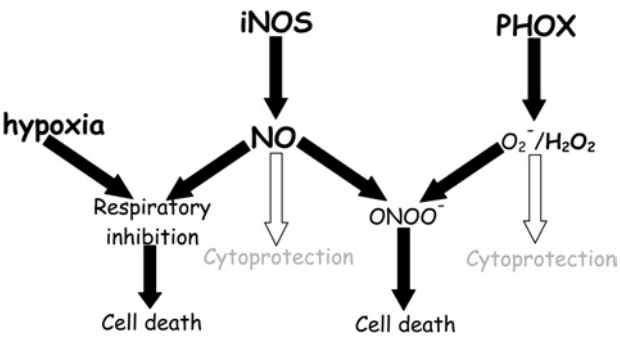
Figure 1 | How inflammation is induced by pathogens or damage
Pathogens are recognized by pathogen receptors, mainly TLRs (Toll-like receptors) or Nod-like receptors (NLRs), on host cells, which recognize pathogen-associated molecular patterns (PAMPs) on the pathogen, such as the LPS of Gram-negative bacteria or the LTA and muramyl dipeptide (MDP) of Gram-positive bacteria respectively, or bacterial/viral RNA or DNA. Damage produces damage-associated molecular patterns (DAMPs) such as advanced glycation end-product (AGE) that are recognized by the receptor for AGE (RAGE), or extracellular nucleotides such as ATP that are recognized by certain purinergic receptors (P2Rs), or fibrillar or denatured proteins (fP) that are recognized by a variety of receptors such as scavenger receptors (SRs). Engagement of receptors results in activation of inflammatory mediators, such as the NADPH oxidase (PHOX) and NF-κB (nuclear factor κB), and causes cytokine production and release, which feeds back to cause further and spreading inflammation. This in turn causes expression of inflammatory mediators: iNOS, COX-2 and complement proteins.



including inflammatory, infectious, ischaemic, traumatic and neurodegenerative diseases, as well as aging of the brain [4,5,8–10]. To take just the example of Alzheimer's disease: activated, iNOS-expressing microglia are consistently found in the neuritic plaques of Alzheimer's disease patients, iNOS-expressing astrocytes surround the plaques, and the NOX is up-regulated and activated; β-amyloid causes inflammatory activation of glia in culture including iNOS expression and activation of the NOX, and β-amyloid's killing of neurons in co-culture with glia can be prevented by iNOS inhibitors; *in vivo* in humans anti-inflammatory drugs protect against Alzheimer's disease [1,6,8,9].

iNOS is not normally expressed in the brain, but inflammatory mediators such as LPS (lipopolysaccharide) and cytokines cause its expression in microglia and astrocytes [10], and possibly in neurons [13]. Once expressed iNOS produces high levels of NO continuously. Phagocytic cells such as neutrophils, macrophages and microglia have a specific NOX known as PHOX (phagocytic NADPH oxidase), consisting of subunits gp91 (glycoprotein 91), p22, p47, p67, p40 and Rac. In the healthy, non-inflamed brain, PHOX is expressed at high levels in microglia (and possibly at low levels in astrocytes and neurons). However, PHOX is not active

Figure 2 | Mechanisms of inflammatory neurodegeneration
Hypoxia, iNOS expression or PHOX activation may be relatively benign, or even cytoprotective, when present alone, but hypoxia combined with NO results in cell death via respiratory inhibition, and PHOX activation combined with iNOS expression results in death via peroxynitrite production.



unless acutely stimulated by for example TNFα (tumour necrosis factor α), IL-1β (interleukin 1β), chemokines, arachidonate, ATP or phagocytosis, when it produces high levels of superoxide extracellularly, which dismutate to H_2O_2.

NO-induced neuronal death

We [4] and others [5] have identified various modes and mechanism by which inflammatory-activated glia kill neurons in culture (Figure 2). The first mode is mediated by high levels of iNOS expression in glia [4]. We showed that resulting high levels of NO induce neuronal death by causing inhibition of mitochondrial cytochrome oxidase in neurons [14,15]. NO inhibition of neuronal respiration caused neuronal depolarization and glutamate release, followed by excitotoxicity via the NMDA (*N*-methyl-D-aspartate) receptor [15–19]. This excitotoxicity may be potentiated by a second mechanism, as NO from iNOS results in glutamate release from astrocytes via calcium release from intracellular stores stimulating exocytosis of vesicular glutamate [20]. Thus inflammatory-activated astrocytes maintained a higher extracellular glutamate level [20], which was probably insufficient to induce excitotoxicity alone, but may well be sufficient if, in addition, neuronal respiration is inhibited so that NMDA receptor is activated by both depolarization and glutamate [21].

However, the above mechanism requires relatively high levels of NO or iNOS expression, and iNOS can be expressed *in vitro* [22] or *in vivo* [23] apparently with little or no neuronal death. Indeed NO from iNOS may be protective by blocking brain cell death [24,25]. On the other hand, low levels of iNOS expression may synergize with other conditions to induce cell death [26]. For example, hypoxia strongly synergizes with NO or iNOS expression to induce neuronal death via respiratory inhibition [27]. This is because NO inhibits cytochrome oxidase in competition with oxygen [14,27], so that NO greatly increases the apparent K_m of neuronal respiration for oxygen. This sensitization to hypoxia is potentially important in stroke, trauma, vascular

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dementia, Alzheimer's disease and brain aging, where both inflammation and hypoxia may coexist.

PHOX

A second mode that we identified was the dual-key (iNOS and PHOX) mechanism of inflammatory neurodegeneration [22]. We found that activation of iNOS or PHOX alone caused little or no neuronal death, but when both were activated together, this caused massive neuronal death mediated by peroxynitrite. We showed that inflammatory neurodegeneration induced by TNFα, IL-1β, prion peptide, LPS, IFNγ (interferon γ), arachidonate, ATP and/or PMA was mediated by this mechanism under particular conditions [22]. Simultaneous activation of PHOX and iNOS in microglia resulted in the disappearance of NO [28], appearance of peroxynitrite [22,28], and massive death of co-cultured neurons that was prevented by inhibitors of iNOS or PHOX or by scavengers of superoxide or peroxynitrite [22].

Importantly however, activation of PHOX alone caused no neuronal death [22], but did activate the microglia to proliferate [29] and release TNFα and IL-1β in response to fibrillar β-amyloid [30]. PHOX has been shown by many other laboratories to be a key regulator of inflammatory activation of microglia [5], and thus potentially a target for anti-inflammatory strategies.

LTA (lipoteichoic acid) is a cell wall component of Gram-positive bacteria, which may trigger inflammatory neurodegeneration in bacterial meningitis. We showed that LTA induces iNOS in glia via TLR2 (Toll-like receptor 2) [31], and results in loss of glial–neuronal co-cultures that is prevented by PHOX inhibitors, and scavengers of superoxide or peroxynitrite [32]. However, intriguingly, there is no apparent neuronal death in these cultures, only neuronal loss, suggesting phagocytosis [32]. The potential mechanisms of this are described in the accompanying paper.

NO and oxidants

In the absence of PHOX, NO from iNOS may kill cells in combination with oxidants from other sources [33]. NO itself can induce oxidative stress by a variety of means [33]. NO inhibition of cytochrome oxidase stimulates superoxide and H_2O_2 production from mitochondria [33,34]. NO inactivation of mitochondrial complex I also stimulates mitochondrial oxidant production [35], and this may be particularly relevant to Parkinson's disease where complex I is known to be inhibited. NO acutely and reversibly inhibits catalase [36], which is the normal route for disposal of high levels of H_2O_2. NO and H_2O_2 react on superoxide dismutase to produce cytotoxic levels of peroxynitrite [37]. Thus NO-induced death of some cells may be mediated by H_2O_2 [34], and this may well be the case in astrocytes where high sustained levels of NO induce Bax-mediated apoptosis via activation of p53 [38]. NO may in general induce apoptosis via oxidative stress, but this can switch to necrosis if both respiration and glycolysis are inhibited by the NO [39–41].

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References

1 Klegeris, A., McGeer, E.G. and McGeer, P.L. (2007) Curr. Opin. Neurol. **20**, 351–357
2 Zipp, F. and Aktas, O. (2006) Trends Neurosci. **29**, 518–527
3 Lucas, S.M., Rothwell, N.J. and Gibson, R.M. (2006) Br. J. Pharmacol. **147** (Suppl. 1), S232–S240
4 Brown, G.C. and Bal-Price, A. (2003) Mol. Neurobiol. **27**, 325–355
5 Block, M.L., Zecca, L. and Hong, J.S. (2007) Nat. Rev. Neurosci. **8**, 57–69
6 Wyss-Coray, T. (2006) Nat. Med. **12**, 1005–1015
7 Engelhardt, B. and Ransohoff, R.M. (2005) Trends Immunol. **26**, 485–495
8 Floyd, R.A. (1999) Free Radical Biol. Med. **26**, 1346–1355
9 Zekry, D., Epperson, T.K. and Krause, K.H. (2003) IUBMB Life **55**, 307–313
10 Murphy, S. (2000) Glia **29**, 1–13
11 Davies, K.J. (1999) IUBMB Life **48**, 41–47
12 Forman, H.J. and Torres, M. (2002) Am. J. Respir. Crit. Care Med. **166**, S4–S8
13 Heneka, M.T. and Feinstein, D.L. (2001) J. Neuroimmunol. **114**, 8–18
14 Brown, G.C. and Cooper, C.E. (1994) FEBS Lett. **356**, 295–298
15 Bal-Price, A. and Brown, G.C. (2001) J. Neurosci. **21**, 6480–6491
16 McNaught, K.St.P. and Brown, G.C. (1998) J. Neurochem. **70**, 1541–1546
17 Stewart, V.C., Heslegrave, A.J., Brown, G.C., Clark, J.B. and Heales, S.J. (2002) Eur. J. Neurosci. **15**, 458–464
18 Golde, S., Chandran, S., Brown, G.C. and Compston, A. (2002) J. Neurochem. **82**, 269–282
19 Jekabsone, A., Nehrer, J.J., Borutaite, V. and Brown, G.C. (2007) J. Neurochem., doi:10.1111/j.1471-4159.2007.04765.x
20 Bal-Price, A., Moneer, Z. and Brown, G.C. (2002) Glia **40**, 312–323
21 Novelli, A., Reilly, J.A., Lysko, P.G. and Henneberry, R.C. (1988) Brain Res. **451**, 205–212
22 Mander, P.K. and Brown, G.C. (2005) J. Neuroinflamm. **2**, 20
23 Han, H.S., Qiao, Y., Karabiyikoglu, M., Giffard, R.G. and Yenari, M.A. (2002) J. Neurosci. **22**, 3921–3928
24 Takuma, K., Phuagphong, P., Lee, E., Mori, K., Baba, A. and Matsuda, T. (2001) J. Biol. Chem. **276**, 48093–48099
25 Cho, S., Park, E.M., Zhou, P., Frys, K., Ross, M.E. and Iadecola, C. (2005) J. Cereb. Blood. Flow Metab. **25**, 493–501
26 Borutaite, V. and Brown, G. (2005) Biochem. Soc. Trans. **33**, 1394–1396
27 Mander, P., Borutaite, V., Moncada, S. and Brown, G.C. (2005) J. Neurosci. Res. **79**, 208–215
28 Bal-Price, A. and Brown, G.C. (2002) J. Neurochem. **80**, 73–80
29 Mander, P.K., Jekabsone, A. and Brown, G.C. (2006) J. Immunol. **176**, 1046–1052
30 Jekabsone, A., Mander, P.K., Tickler, A., Sharpe, M. and Brown, G.C. (2006) J. Neuroinflamm. **3**, 24
31 Kinsner, A., Boveri, M., Hareng, L., Traub, S., Brown, G.C., Coecke, S., Hartung, T. and Bal-Price, A. (2006) J. Neurochem. **99**, 596–607
32 Kinsner, A., Pilotto, V., Deininger, S., Brown, G.C., Coecke, S., Hartung, T. and Bal-Price, A. (2005) J. Neurochem. **95**, 1132–1143
33 Brown, G.C. and Borutaite, V. (2006) Biochem. Soc. Trans. **34**, 953–956
34 Borutaite, V. and Brown, G.C. (2003) Free Radical Biol. Med. **35**, 1457–1468
35 Borutaite, V. and Brown, G.C. (2006) Biochim. Biophys. Acta **1757**, 562–566
36 Brown, G.C. (1995) Eur. J. Biochem. **232**, 188–191
37 McBride, A.G., Borutaite, V. and Brown, G.C. (1999) Biochim. Biophys. Acta **1454**, 275–288
38 Yung, H-W., Bal-Price, A.K., Brown, G.C. and Tolkovsky, A.M. (2004) J. Neurochem. **89**, 812–821
39 Brown, G.C. and Borutaite, V. (2002) Free Radical Biol. Med. **33**, 1440–1450
40 Leist, M., Single, B., Naumann, H., Fava, E., Simon, B., Kuhnle, S. and Nicotera, P. (1999) Exp. Cell Res. **249**, 396–403
41 Bal-Price, A. and Brown, G.C. (2000) J. Neurochem. **75**, 1455–1464

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