Tumor necrosis factor-α triggers a cytokine cascade yielding postoperative cognitive decline

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Cognitive decline following surgery in older individuals is a major clinical problem of uncertain mechanism; a similar cognitive decline also follows severe infection, chemotherapy, or trauma and is currently without effective therapy. A variety of mechanisms have been proposed, and exploring the role of inflammation, we recently reported the role of IL-1β in the hippocampus after surgery in mice with postoperative cognitive dysfunction. Here, we show that TNF-α is upstream of IL-1 and provokes its production in the brain. Peripheral blockade of TNF-α is able to limit the release of IL-1 and prevent neuroinflammation and cognitive decline in a mouse model of surgery-induced cognitive decline. TNF-α appears to synergize with MyD88, the IL-1/TLR superfamily common signaling pathway, to sustain postoperative cognitive decline. Taken together, our results suggest a unique therapeutic potential for preemptive treatment with anti-TNF antibody to prevent surgery-induced cognitive decline.

Results

Early Increase in Systemic TNF and High-Mobility-Group Box Chromosomal Protein-1. Following surgery under general anesthesia, TNF-α was the first cytokine to be released and peaked \((P < 0.05)\) at 30 min after surgery (Fig. L14); in contrast, other proinflammatory cytokines, such as IL-1β and IL-6, were not detected until 6 h post-surgery \((18)\). To assess whether the inflammatory response was preceded by systemic release of damage-associated molecular-pattern (DAMPs) molecule, we measured high-mobility-group box chromosomal protein 1 (HMGB-1), which is released after cell necrosis \((19)\). HMGB-1 blood levels were increased at 1 h \((P < 0.05)\) and peaked at 6 h \((P < 0.001)\) before returning to baseline (Fig. 1B). Significant differences in cytokine kinetics could be observed following infection (endotoxemia) \((20)\) (Fig. S1). No cytokine or DAMP changes were observed following general anesthesia only (Fig. 1C and D).

Anti-TNF Prophylaxis Prevents Neuroinflammation and Cognitive Decline. In a number of systems TNF triggers the production of proinflammatory cytokines, reported first in mouse “sepsis” \((21)\) and human rheumatoid arthritis \((22)\). We next investigated the effects of TNF-α blockade on systemic cytokine levels, neuroinflammation, and cognitive dysfunction. Preoperative administration of anti-TNF effectively reduced the amount of systemic IL-1β both at 6 and 24 h following surgery \((P < 0.01, P < 0.001)\) (Fig. 2A and B). To corroborate the findings and ascertain the kinetics and specificity of TNF-α blockade, we delayed the injection of the antibody until 1 h after surgery and then measured levels of IL-1β and IL-6; administration at this time with TNF-α blockade had no effect (Fig. 2A and B).

Although IL-1β is pivotal for hippocampal learning and memory, high levels can interfere with long-term potentiation and synaptic plasticity \((23)\). Prophylaxis with anti-TNF antibody attenuated the surgery-induced up-regulation of hippocampal IL-1β (Fig. 2C) \((P < 0.01)\). Microglia, the innate immune cells of the CNS, usually reside in the quiescent state; these cells are tightly regulated and, upon activation, release cytotoxic compounds that disrupt homeostatic processes and neuronal functions \((24–26)\). Microglia are activated following surgery, changing their small cell bodies and thin, long, ramified pseudopodia into an amoeboid morphology, with enlargement of the cell body (features described as microgliosis). Preoperative administration of anti-TNF antibody significantly reduced microgliosis after surgery (Fig. 2D) \((P < 0.01)\).

To relate the inflammatory response to cognitive behavior, we used trace fear conditioning in which mice are trained to associate a tone with a noxious foot-shock stimulation \((27)\). Contextual fear response shows reduced immobility (freezing) at postoperative day 3, revealing hippocampal-dependent memory impairment (Fig. 2E) \((P < 0.05)\). Pretreatment with anti-TNF significantly ameliorated this cognitive dysfunction \((P < 0.05)\).

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MyD88 Mediates Cognitive Decline Following Surgery. We had previously shown that IL-1 perpetuates the inflammatory response in our model, and is prevented by blockade of IL-1 by IL-1 receptor antagonist pretreatment (18). To assess the relationship between the TNF- and the IL-1-dependent pathways, we studied the effects of surgery in mice lacking MyD88, the key signaling receptor antagonist pretreatment (18). To assess the relationship between the TNF- and the IL-1-dependent pathways, we studied the effects of surgery in mice lacking MyD88, the key signaling
adaptor of the IL-1 and TLR superfamily, but not involved in TNF signaling (28). Compared with wild-type, the systemic cytokine response to surgery was reduced, but not obliterated, in MyD88−/− both at 6 and 24 h (Fig. 3 A and B) (P < 0.01, P < 0.01). However, when MyD88−/− were administered with anti-TNF antibody, surgery-induced incremental change in IL-1β and IL-6 were abrogated, although TNF-α levels were unaffected (Fig. 3 A and B). Neither signs of neuroinflammation (Fig. 3 C and D and Fig S2) nor a reduction in freezing behavior was observed in MyD88−/− following surgery (Fig. 3E).

**Anti-TNF Prevents Surgery-Induced Inflammation in Tlr4−/− Mice.** Toll-like receptor 4 (TLR4) is involved in a large proportion of known danger signals, and more are emerging, such as tenasin-C in inflamed joints (29). Having established pivotal roles for TNF-α and MyD88 signaling in surgery-induced inflammation and cognitive dysfunction, we targeted TLR4 to understand whether this receptor could account for a MyD88-dependent signal in POCD (30). Surgery in Tlr4−/− induced similar systemic and central inflammation, as well as memory decline, as that seen in the wild-type, indicating that TLR-4 signaling is not required for establishment of cognitive decline (Fig. 4A) (P < 0.05). When Tlr4−/− mice were pretreated with anti-TNF 18 h before surgery, the systemic cytokine response, hippocampal inflammation and memory impairment were abrogated (Fig. 4 B–E).

**Discussion**

Postoperative cognitive decline is a poorly understood disorder, very common even after noncardiac surgery, which can be mimicked by surgery in mice. We have explored the inflammatory pathways and these results, taken together, demonstrate that TNF-α is very important in this process. TNF-α acts upstream of IL-1β and initiates the peripheral cytokine cascade leading to cognitive decline (Fig. 2). Prophylaxis with anti-TNF antibody prevents postoperative cognitive decline. TNF-α blockade successfully reduces further elaboration of IL-1, which has been extensively reported to modulate symptoms of “sickness behavior,” including memory dysfunction (20, 31, 32). With a single dose of anti-TNF monoclonal antibody, we have also effectively interfered with the IL-1-dependent amplification mechanism and cognitive function (freezing) was normalized.

Cytokines are central mediators of inflammatory events following all types of events, including peripheral trauma or infection, and have broad physiological effects both on the periphery and in the CNS (33). TNF-α has a pivotal role in the initiation and amplification of the inflammatory cascade; it is involved in regulating chemokines and cytokines release, oxidative stress, recruitment of immune cells and adhesion molecules, apoptosis, healing, and tissue-specific repair mechanism. This function has been extensively documented during the analysis of the pathogenesis of rheumatoid arthritis (34). TNF-α also exerts neuro-
modulatory functions, in particular in regulating microglia and astrocytes activation in the brain (35).

Systemic cytokines exert effects in the CNS both via direct and indirect pathways. TNF-α has been shown to enter the brain through relatively permeable areas in the blood–brain barrier (BBB) (36). In our model, it is possible that transient changes in BBB permeability, either caused by the systemic inflammatory response or other factors including anesthesia (37), could enable direct access to the brain. Neural afferents may also account for signs of CNS inflammation (38). Because of the changes in the peripheral inflammatory effects that were noted in anti-TNF treated surgically injured animals, and with the lack of evidence of acute changes in the BBB postsurgery, we consider it likely that the antibody exerts its ameliorative action in the periphery. This finding suggests a pivotal role for systemic inflammation in producing neuroinflammation and cognitive decline. Various indirect routes for cytokine effects need to be excluded, and the key site of anti-inflammatory effects needs further elucidation.

In the brain, microglia can actively secrete cytokines and neurotoxins upon activation (39). Using another model of peripheral organ inflammation, peripheral TNF-α was necessary to stimulate microglia activation and to allow recruitment of circulating monocytes into the brain (40). With this model of surgical trauma we are yet unable to define the precise origin of this neuroinflammatory response, in particular whether it is mediated by peripheral monocytes or resident microglia. Resulting neuroinflammation accounts for behavioral changes, in particular in vulnerable areas, such as the hippocampus (41). Local inflammation directly interferes with the processes of memory consolidation, long-term potentiation, synaptic plasticity, and neurogenesis, resulting in what has been termed “sickness behavior”; inflammatory blockade has been reported to restore hippocampal neurogenesis (42).

Having defined a role for TNF-α following surgery, we investigated whether MyD88 signaling, important for the TLR and IL-1 families, was involved in surgery-induced cognitive dysfunction. There was no surgery-induced freezing impairment, as a marker of cognitive decline in MyD88−/− (Fig. 3E). This finding is in keeping with our recently reported role of IL-1p in the same model (18). Because the Tlr4−/− mice were not protected in any way from surgery-induced inflammation or POCD, it appears that TLR4 has redundant role in this disease pathway. DAMPs, such as HMGB-1, are recognized by multiple receptors (e.g., RAGE, as well as Tlr4 and Tlr2) and one of the other receptors might be able to induce signaling in the absence of the other (43). Administration of anti-TNF to MyD88−/− or Tlr4−/− completely abrogated the surgery-induced IL-1β response and downstream IL-6 production, suggesting a perpetuating role for both these cytokines in the inflammatory response and, possibly, synergism with one another.

Anti-TNF antibody was the first cytokine-selective therapy that was shown to offer clear benefits in the setting of common disease in rheumatoid arthritis, being both effective and acceptably safe (44). Based upon our data, prophylaxis with anti-TNF antibody is a feasible therapeutic option that is ready to be exploited in the elective surgical setting. There have been attempts to use anti-TNF in acute indications in bacterial sepsis almost 20 years ago (45). Sepsis contrasts markedly with POCD, as therapy with anti-TNF, effective prophylactically in septic mice, could not be given early in humans because of the clinical reality of sepsis. This finding is in marked contrast to the preemptive manner in which this biologic can be used before surgery. Because other injured states, such as major trauma and especially chemotherapy, lead to similar states of cognitive decline as POCD, it is possible that similar up-regulation of cytokines may underpin these clinical syndromes, and this is worth investigating. It is possible that by administering anti-TNF antibody surgical patients may be at higher risk for opportunistic infections and other postoperative complications; however, most long-term randomized clinical trials have not reported any differences in the incidence of infections in patients treated with anti-TNF. Tuberculosis relapse is the chief exemption, with long-term treatment increasing its prevalence (46). For POCD, anti-TNF would be very short term: a single injection. Another possible target to prevent postoperative cognitive decline may be HMGB-1, which was significantly increased following surgical trauma (Fig. 1B) (47). Overall, we can conclude that TNF-α acts upstream of IL-1β and initiates the peripheral cytokine cascade that results in cognitive decline. Prophylaxis with a single dose of anti-TNF antibody attenuates the downstream activation of both MyD88-dependent and independent inflammatory pathways. Therapy with TNF-α inhibitors is clinically well established and already offers beneficial effects in inflammatory conditions, such as rheumatoid arthritis, Crohn’s disease, and ankylosing spondylitis (22, 48), and may thus be useful for the prevention of postoperative cognitive decline in susceptible individuals.

Methods

Animal Experiments. Experiments were performed in accordance to the United Kingdom Home Office-approved license. We randomly grouped 12- to 14-wk-old male C57BL/6 mice and assigned them to a specific experiment. Homozygous MyD88−/− on a C57BL/6 background were provided by the the
were checked on a daily basis and if they evidenced poor grooming, huddling, piloerection, weight loss, back arching, and abnormal activity, they were eliminated from further consideration. Investigators who treated animals knew the treatment groups and collected samples, which were then analyzed by other investigators blinded to the specific treatment.

**Surgery.** We performed an open tibial fracture as previously described (51). Briefly, we anesthetized mice with 2.1% isoflurane and analgesia with buprenorphine (Buprenex, 0.1 mg/kg i.m.). A middle incision was performed to expose the tibia. Mice were subjected to vehicle (saline) or buprenorphine (Buprenex, 0.1 mg/kg s.c). A middle incision was performed to expose the tibia. We subjected mice to vehicle (saline) or buprenorphine (Buprenex, 0.1 mg/kg s.c). An incision was made through the skin and fascia, and the tibia was fractured with a bone clamp. The wound was closed with 4-0 Vicryl suture. The mice were maintained in a warm environment and allowed to recover for 2 weeks before the behavioral testing. We performed the injury procedure on 51 mice, and the recovery phase lasted for 2 weeks. The mice were then subjected to various behavioral tests to assess their cognitive function.

**Data Analysis.** We used GraphPad v3.0 (GraphPad Software) to calculate the mean, SD, and SEM, and perform statistical tests. We analyzed multiple group means by one-way analysis of variance, followed by Newman-Keuls post hoc test wherever appropriate. The nonparametric test of Kruskal-Wallis followed by the Dunn’s multiple comparison test was used for categorical data. P values less than 0.05 were considered significant.

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