Inflamed Brains: Neuroimmune Interactions in Diseases of the Nervous System
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In the past ten years, the brain’s status as “immune privileged,” that is, beyond the surveillance and influence of the immune system, has been reconsidered as important interactions between the nervous and immune systems have been recognized. Increasing attention is being paid to the role of inflammatory responses in degenerative diseases of the brain, particularly in Alzheimer’s disease, as well as in trauma and even in mental illness. The Autumn Colloque of the Fondation IPSEN (Neuroimmune Interactions in Diseases of the Nervous System) held on October 6, 1998 in Paris, was organized by P. Patterson (Pasadena, USA), C. Kordon (Paris, France), and Y. Christen (Paris, France). This meeting brought together researchers in immunology and neuroscience to discuss progress and to identify areas for future collaboration.

The healthy central nervous system does indeed lack the classic immunological antibody response for neutralizing foreign proteins found in the rest of the body. But it has long been known that, when faced with certain insults, the nervous system can produce a modified inflammatory response, as in multiple sclerosis (H. Wekerle, Max-Planck-Institut für Neurobiologie, Planegg-Martinsried, Germany). More recently, the macrophages and lymphocytes of the immune system have been shown to patrol the healthy brain and to invade it in large numbers when disease threatens.

Outside the brain, injury or infection triggers macrophages and lymphocytes to produce a complex cascade of chemical and cellular reactions that results in inflammation, which both protects against the insult and then promotes repair. In a bad infection, there may be fever and the behavior pattern associated with sickness. The acute response is usually short lasting as it is balanced by mechanisms restoring the body to health. Occasionally, for reasons that are not well understood, the restorative process fails and a chronic, pathological inflammation persists — typified by arthritis.

Attention to neuroimmune interactions leading to inflammation in the brain increased when many classes of molecule associated with the peripheral inflammatory response were discovered in association with amyloid plaques in the brains of patients with Alzheimer’s disease (AD). This may be a sign of a chronic inflammatory condition, according to P. McGeer (Univ. British Columbia, Vancouver, Canada), one pioneer of this concept. Now it seems likely that even psychiatric conditions such as major depression may have an inflammatory component.

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The peripheral immune response has a second line of defense in addition to the production of antibodies, that is, the cascade of reactions initiated by the activation of complement proteins. McGeer’s colleague and co-pioneer, J. Rogers (Sun Health Research Institute, Sun City, AZ, USA), sees complement activation as one of the seminal events in establishing inflammation in AD. Beta amyloid peptide (βA), which is produced by neurons in response to injury and is deposited in senile plaques, activates both the classical and alternative complement pathways in vitro. This reaction is likely also to take place in the brain, because virtually all the complement activation products co-localize with βA deposits. Recent studies indicate that neurofibrillary tangles may also activate complement. As a result of complement activation, several mechanisms of inflammation may be invoked, leading to further damage to neurons and thus becoming self-reinforcing.

Complement activation generates opsonins and anaphylatoxins, chemicals that may be signals attracting microglia, the macrophage-like scavenger cells of the brain, which possess complement receptors. Microglial cells in culture are recruited to βA deposits, becoming more activated and increasing their secretion of complement, as well as of cytokines and chemokines, intercellular signalling proteins involved in inflammatory responses. The complement pathways lead to formation of the membrane attack complex (MAC), which have been demonstrated on dystrophic neurites adjacent to βA deposits.

Microglia also seem to play a pivotal role in the inflammatory response in AD. They become activated by many forms of insult to the brain and in AD are found clustered around plaques. They initiate a complex, self-reinforcing cycle of intercellular signals and cellular responses, involving over 100 different molecules characteristic of acute peripheral inflammation (Rogers; S. Griffin, McClellan VA Medical Center, Little Rock; B. Cordell, Scios Inc., Sunnyvale, CA, USA). One key molecule in this cycle is the cytokine interleukin-1 (IL-1). It stimulates both neurons and astrocytes to produce amyloid precursor protein (APP), as well as molecules such as S100P and another interleukin, IL-6, all of which can promote neurite outgrowth (Griffin; Cordell). IL-1 also stimulates production of apolipoprotein E and α1-antichymotrypsin, which both increase the production of βA, nitric oxide synthase, and S100β and further stress the damaged neurons, amplifying the initial response to IL-1. In response to βA, the neurons, in turn, release macrophage colony stimulating factor (M-CSF), a protein that encourages microglia to divide. When M-CSF and βA act synergistically on a microglial cell line in vitro, there is a dramatic increase in the production of IL-1, IL-6, and nitric oxide (Cordell).

Because βA deposits and neurofibrillary tangles are present from the preclinical stage of AD onward, they may provide a long-lasting stimulus for inflammation. As the disease progresses for over a decade, the inflammatory response must be relatively mild but chronic, accumulating damage over many years. Indeed, if inflammation in AD were as potent as that seen in many acute peripheral conditions, AD would probably be terminal in days, rather than years (Rogers).
Thus the normal functions of microglia and astrocytes, to rescue and repair damaged neurons, become unbalanced, and chronic inflammation ensues, resulting in the death of neurons and the clinical symptoms associated with AD. Why this happens is poorly understood. Overall, it seems that the intersection of several factors, some more significant than others, can result in a shift from the benign repair process to the vicious cycle that leads to neurodegeneration and dementia.

Many of the molecules involved have both pro- and anti-inflammatory actions, depending on the context in which they act. For instance, another cytokine, transforming growth factor β (TGF-β), is involved in organizing wound repair but can lead to fibrosis. In AD, it may contribute to plaque formation (L. Mucke, Univ. California, San Francisco, USA): there is a high correlation between βA deposits and the mRNA for TGF-β in brain blood vessels in cerebral amyloid angiopathy; and mice engineered to overproduce TGF-β in astrocytes develop amyloid plaques very young.

Factors that help to tip the balance towards chronic inflammation include the well-known familial AD gene mutations, head trauma, epilepsy, the ε4 variant of apolipoprotein E, and simply age. In older animals, microglia and astrocytes both become more easily activated and IL-1 levels are elevated (Griffin; C. Finch, Univ. Southern California, Los Angeles, USA). However, Finch demonstrated persuasively that the microglia of rats fed a reduced diet maintain a more youthful behavior — missing lunch may help us keep our brains healthy! Apolipoprotein E, which co-localizes with APP, may act as a mediator in the formation of plaques (Mucke). Apolipoprotein E-ε4, which is a well-established risk factor for AD, seems to promote degeneration, whereas the ε3 variant is protective. The difference may lie in the inability of ε4 to bind to APP; ε3 may inactivate APP and prevent it from promoting inappropriate neurite outgrowth (Mucke) or stimulating the inflammatory responses of microglia (S. Barger, Univ. Arkansas Medical Sciences, Little Rock, USA).

The pro- and anti-inflammatory balance is reflected in which signaling pathways within the cell are activated when proteins such as IL-1 or TGF-β bind to receptors on the surface of neurons, astrocytes, or microglial cells. Many of these pathways target transcription factors that regulate particular genes, leading to specific changes in the cell’s behavior, such as growing neurites, dividing, secreting certain molecules, or even dying. It is important, therefore, to identify both the members of individual signal cascades and the mechanisms of their action. In cultured microglia, stimulation of cyclic AMP, a second messenger in many intracellular pathways, depressed the production of damaging nitrogen and oxygen radicals, as well as of molecules involved in inflammation, such as interferon-γ, tumor necrosis factor and IL-12. At the same time, the production of the potentially protective neurotrophic factors, IL-10 and prostaglandin E2, were increased (G. Levi, Institute Superiore di Sanita, Rome, Italy).

The response to a stimulus may also be determined by cell-specific responses. The form of soluble APP released from APP by α-secretase (sAPPα) protects cultured neurons against death from glucose deprivation and results in the production of protective molecules, such as antioxidants. In contrast, both α and β forms of soluble APP stimulate microglia but here they lead to the induction of nitric oxide synthase and IL-1β and the
death of co-cultured neurons (Barger). Thus one factor in the balance between repair and inflammation may be the levels of sAPPα and β present, which leads back to whether APP is cleaved at the α- or β-secretase site. The distinctly different outcomes reflect substantial differences in the signalling pathways in the two cell types: in neurons, sAPPα acts through a cyclic GMP-dependent pathway to activate an NFκB-like transcription factor, which binds to DNA in an unconventional way. In microglia, sAPP indirectly activates the conventional binding of the classic p50/p65 NFκB (Barger).

**CYTOKINES, SICKNESS, AND DEPRESSION**

In AD the inflammatory response occurs within the tissue of the brain. Evidence is accumulating that inflammation in the periphery can also affect the way the brain functions. The sickness behavior associated with peripheral infection is now considered to be a short-term, adaptive response. The characteristic withdrawal, inactivity, eating little and sleeping a lot aids recovery and keeps the sick animal or person out of harm.

Communication between the periphery and the brain is required to initiate this change in behavior. Again the cytokines IL-1 and IL-6, released by macrophages and monocytes at the site of inflammation, seem to be the primary signals. They stimulate the brain to produce proinflammatory cytokines, mainly IL-1β and tumor necrosis factor-α, through two indirect pathways. First, they stimulate local peripheral nerves, such as the vagus, which elicit transient release of cytokines from microglia and macrophages in their terminal regions in the brain; these in turn bind to receptors on neurons and astrocytes. Second, peripheral cytokines in the circulating blood reach the circumventricular area of the brain, where they cause an increase in the release of prostaglandin E2, which results in release of IL-1 in the CNS (H. Reul, Max-Planck-Institut für Psychiatrie, München, Germany; R. Dantzer, INSERM U 394, Bordeaux, France).

Each element of sickness behavior and the recovery from it seems to be triggered by specific cytokines, so the response is precisely orchestrated and not simply a global suppression of activity (Dantzer). One effect is the release of the mood-regulating neurotransmitter serotonin (5-hydroxytryptamine) from neurons with cell bodies in the raphe nuclei in the brain stem and branches in both the forebrain and/or the spinal cord. This release is mediated specifically by IL-1 and not by TNF-α. In sickness behavior, the output to the spinal cord, which normally boosts activity in the motor circuits of the spinal cord, is silent, possibly accounting for the inactivity seen in sick animals. In contrast, extracellular levels of serotonin in the hippocampus increase markedly when an animal is challenged with a peripheral injection of the inflammation-promoting molecule, lipopolysaccharide. This challenge also increases the level of the phosphorylated form of the cAMP-responsive element binding protein gene-transcription factor, P-CREB, in the dentate gyrus of the hippocampus and parts of the neocortex (Reul).

Another area affected by centrally released cytokines is the hypothalamus, where the output of corticotropin-releasing hormone is stimulated, resulting in increased release of corticosteroid hormones from the adrenal glands. However, the various responses may interact in complex ways: in animals treated with chronic intracerebroventricular infusion of
corticotropin-releasing hormone the onset of sickness behavior is delayed and the sero-
tonin response is blunted (Reul).

The roles of corticotropin-releasing hormone and serotonin in clinical depression and stress are well established. More surprising is that major depression has recently been shown to be accompanied by activation of an “acute-phase” peripheral inflammatory response that results in increased production of proinflammatory cytokines. This implies that viral infection and other causes of inflammation may contribute to depression, which might even be a chronic manifestation of sickness behavior (M. Maes, Univ. Dept. Psychiatry, Antwerp, Belgium). Several lines of evidence support this claim. About 50% of patients with major depression have typical signs of a cell-mediated peripheral immune response, including increased levels of monocytes, macrophages, T-cells, and proinflammatory cytokines. As well as disturbing the corticosteroid pathway and serotonin metabolism, IL-1 and IL-6 produce symptoms typical of depression (Maes), as do IL-2 and IFN-α, now being tested in cancer therapy (L. Capuron, A. Ravaud, G. Goodall, and R. Dantzer, INSERM U 394, Bordeaux, France). Moreover, serum cytokine levels are elevated in students suffering from pre-examination stress and women with post-partum “blues,” whereas IL-6 in the serum of depressed patients returned to normal levels after they had received eight weeks of treatment with anti-depressants. In vitro, tricyclic and selective-serotonin-reuptake-inhibitor antidepressants suppressed the production of proinflammatory cytokines and promoted anti-inflammatory ones (Maes).

REPAIRING THE DAMAGE

One reason the brain was awarded its immune-privileged status is that it does not normally display molecules of the major histocompatibility complex (MHC), which are typical of T-cell-mediated immune responses in the periphery. However, many studies have now established that microglia produce MHC molecules as one of the brain’s first responses to injury, immune-mediated inflammation, infection, neoplasia, and neuronal degeneration. MHC molecules are also found on the microglia around AD plaques (Wekerle). In cultured brain slices, an inflammatory challenge stimulated glial cells to produce MHC class II molecules only when the neurons were electrically silent. The response seemed to be normally suppressed by neurotrophins, particularly nerve growth factor, presumably produced by active neurons. Neurons themselves respond to viral peptides or, when they are inactivated by tetrodotoxin, to interferon-γ by producing MHC class I molecules, which may attract cytotoxic lymphocytes to remove specifically the virus-infected cells.

Like the activated microglia in AD, microglia activated by peripheral nerve injury send out several chemical signals besides producing MHC classes I and II. These include the microglial mitogens M-CSF and GM-CSF; cytokines that activate astrocytes; and I-CAM, which attracts lymphocytes from the periphery to help repair the damage resulting from the lesion (G. Kreutzberg, Max-Planck-Institut für Neurobiologie, Planegg-Martinsried, Germany). In the periphery, nerve injury stimulates production of a neuropoietic cytokine that stands at the interface between the nervous and immune systems: leukemia inhibitory factor (LIF; named for another of its actions, on white blood cells; P. Patterson, California Institute of Technology, Pasadena, USA). Mice lacking the gene for LIF do not
mount a coordinated response to peripheral nerve injury. In the cell bodies of the damaged neurons, LIF normally regulates the production of several neuropeptides, such as VIP and substance P, that promote the regrowth of damaged axons; at the site of injury, it attracts neutrophils, macrophages and mast cells to clean up the debris of dying axons. With lesions in the brain, LIF is produced by astrocytes and seems to act in concert with IL-6 to attract inflammatory cells to the site of damage.

In contrast to its action in the nervous system, studies in mice lacking the LIF gene show that LIF is pro-inflammatory in the skin. Such results emphasize the importance of context for the actions of cytokines and underline the necessity of considering the interactions between molecules, rather than just examining single molecules in isolation.

**CLINICAL PROSPECTS**

Although this is still a young research field, several therapeutic applications are already being explored. In AD, epidemiological studies and small clinical trials indicate that non-steroidal anti-inflammatory drugs (NSAIDs) can significantly mitigate deterioration — further support for the crucial role of inflammation in the disease (Rogers). A note of caution, however: preliminary results from mice engineered to express a mutated human APP gene have shown increased inflammation around plaques after treatment with NSAIDs (Patterson). More work on the effects of these drugs in AD is clearly required.

Intervention in AD could, in the future, be aimed specifically at a particular point in the inflammation cycle, using antagonists to pro-inflammatory cytokines. The many effects of IL-1 in the cycle make it a promising target (Griffin). APOE-ε4 is another potential target for therapy and a mouse bred to produce excess ε4 may provide a tool for developing antagonistic drugs (Mucke). Depression also may respond to anti-inflammatory drugs, anti-inflammatory cytokines or antagonists to proinflammatory cytokines such as IL-1 (Maes).

But by far the most exciting prospect is a method being developed for detecting activated microglia in the human brain in situ. A compound known as 1-(2-iodophenyl)-N-methyl-N(1-methyl-propyl)-3-isoquinoline carboxamide (PK-11195) that labels activated microglia and macrophages can be detected using positron emission tomography (PET; Kreutzberg). PET scans of the brains of multiple sclerosis patients given this compound revealed the regions of inflammation with a sensitivity far greater than previous methods. This labelling should be applicable to any inflammatory condition of the central nervous system. For AD, it may offer the prospect of a tool for both accurate early diagnosis and for tracking the development of the disease.