RESEARCH ARTICLE

Neutrophil-mediated Treatment of Neurodegenerative and Other Inflammatory Disorders

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ABSTRACT

Conventionally, neuroinflammation has been seen as a branch of immunology linked to neurodegenerative and other inflammatory disorders. The diagnosis and treatment of these disorders are dependent on the ability of delivering diagnostic and therapeutic pharmaceuticals through the blood–brain barrier. While itself contributing to the inflammatory process, the barrier largely hinders or even forbids this delivery but allows neutrophils to pass freely through it. This article discusses the use of a nanobiotechnology-based, neutrophil-mediated treatment of neurodegenerative and other inflammatory disorders.

Key words: Dopamine, levodopa, paclitaxel

INTRODUCTION

Conventionally, neuroinflammation has been seen as a central nervous system (CNS) -centric and specific branch of immunology. Thus, a great deal of effort has been made to find immunocompetent or inflammatory cells in the brain (or spinal cord) parenchyma. The purpose of this article is to query whether neutrophils, which pass freely through the blood–brain barrier (BBB), can mediate the delivery of diagnostic and therapeutic drugs to the brain to treat neuroinflammatory processes that underlie many of the nearly 400 known neurodegenerative disorders (NDDs). I will review below more particularly the cases of three major NDDs (epilepsy, Parkinson's, and Alzheimer's) to better understand the relationship of the underlying inflammatory process with the BBB. I will subsequently discuss the application of cell (neutrophil)-mediated therapy in the case of recurring brain cancers (glioblastomas [GB] and glioblastoma multiformes [GBMs]) and its extension to NDDs.

LINKING INTRAVASCULAR INFLAMMATORY EVENTS TO NDDs

To illustrate the process linking intravascular inflammatory events to NDDs, I will use epilepsy as an example. A schematic representation of the cells and molecules involved in cerebral inflammation is shown in Figure 1. The schema summarizes the pathogenic paths that may link intravascular inflammatory events to proepileptogenic events in the brain parenchyma. The BBB and its component cells forming tight junctions are diagrammed by the oblique descending gray lines. External activated microglia and gliotic astrocytes interact with the BBB being mediated by interleukin (IL-1β, IL-6, and IL-12) and tumor necrosis factor alpha (TNF-α). Within the BBB, mast cells, monocytes, complement, and T-cells are in a grouping from which mast cells emerge from the BBB and, after interaction with a histamine, reenter the BBB. From the above grouping also emerge extravasated cells that through their CD-40 ending interact with microglia. In turn, the microglia undergoes two interactions: (a) With IL-1β, IL-6, and TNF-α to recycle into microglia and (b) become activated and interact with astrocytes through further interaction with interferon-gamma (IFN-γ). In turn, the astrocytes result in (a) gliosis, (b) the major histocompatibility complex-2, and (c) interact back with the microglia through IL-1β, IL-6, TNF-α, and the colony-stimulating factor.

Similar diagrams for other NDDs could likewise be theoretically constructed (although their existence is not currently known). These representations do not implicate

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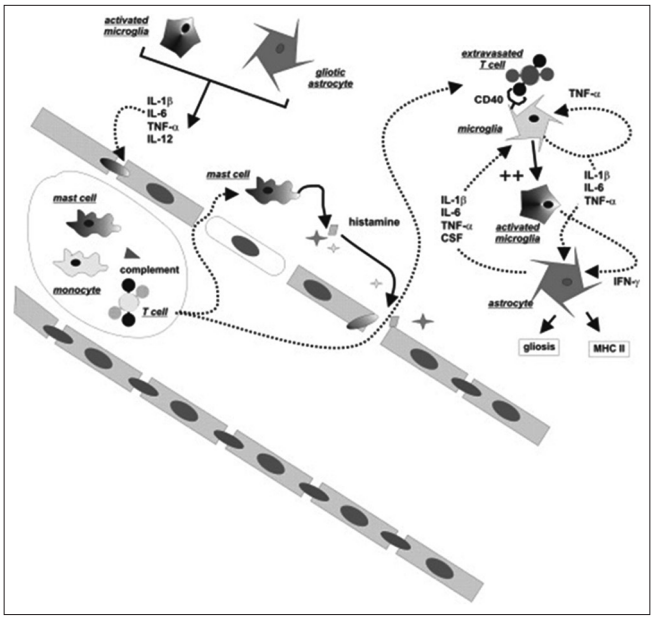

Figure 1: Linking intravascular inflammatory events to proepileptogenic events in the brain parenchyma. Source: From Oby and Janigro (1)

the presence of a particular pathogen and may actually occur under sterile inflammatory conditions.

As will now be seen, whether the BBB is in its normal integral state or has been disrupted by inflammatory or/and other processes would have important diagnostic and therapeutic implications.

BBB INTEGRITY OR DISRUPTION AND DIAGNOSTIC AND THERAPEUTIC IMPLICATIONS

The brain has five protective barriers that describe the five main interfaces between the CNS and the periphery. They include the BBB proper that extends down the spinal cord, the brain cerebrospinal fluid (CSF) barrier, the brain - inner CSF barrier, the brain - outer CSF barrier, and the brain retinal barrier. All these interfaces are physical and metabolic barriers that serve to regulate and protect the microenvironment of the brain. Composed of a monolayer of brain capillary endothelial cells, they are formed by tight junctions that limit access to the brain to small non-polar molecules by passive diffusion or catalyzed transport of large and/or polar molecules. We shall mostly be concerned with the BBB.

Under normal conditions, an intact BBB separates the immune system from the CNS parenchyma. Trafficking of white blood cells is restricted to specific regions of the vasculature (the Virchow–Robin space above the leptomeningeal fusion and the subarachnoid space). The neutrophils are such cells that pass freely through the BBB. On the other hand, when the BBB is breached, both molecular and cellular players may extravasate. The mechanism of this BBB attack by intravascular agents implicates metalloproteinases and other molecules released by activated blood cells. The abnormal permeation across the barrier results in further, and perhaps distal, disruption of tight junctions, this time mediated by release of inflammatory mediators by both extravasated blood cells and activated microglia. Frank cellular immunoagression occurs if and when histocompatibility mechanisms are activated and antibody-mediated reactions occur. It is now clear that virtually every class of brain cells has some potential or propensity to replicate immunological or inflammatory processes.[1]

Delivery of pharmaceuticals (diagnostic, therapeutic agents) to the brain and the design of the corresponding carriers depend on the state of integrity or disruption of the BBB. As stated earlier, interest here is with the neutrophils which pass freely through the BBB. The links between the BBB and NDDs will now be explored.

LINKS BETWEEN THE BBB AND NDDS

The links between the BBB and NDDs will be illustrated in three major cases: Epilepsy, Parkinson's disease (PD), and Alzheimer's disease (AD).

Epilepsy and other seizure disorders

Oby and Janigro[2] have proposed links between the BBB and epilepsy [Figure 1]. A compromised BBB has been associated with seizures in a number of disorders, not just epilepsy. Not only congenital defects such as GLUT1 deficiency but also acquired deficiencies, like those resulting from brain tumors, head trauma, etc., often result in seizure disorders. More recently, systemic and immune triggers have been implicated in a leaky BBB and epileptic neuroinflammation. As it turns out, the BBB is intimately interconnected with the cause, effect, and treatment of seizures. These relationships continue to move toward the forefront of epilepsy research and offer a distinctive opportunity to further our understanding of the disease. In addition, with the constant refinement of existing technologies and the development of new technologies (e.g., nanotechnology), our ability to image, manipulate, and explore the BBB will only improve, thereby enabling the next generation of advances. Understanding the intimate nature of the role of the BBB in these disorders is imperative in the treatment of the disease, but the fundamental question of whether the compromised integrity of the BBB is a component of the etiology of epilepsy or a consequence of seizures still remains unanswered.[3,4]

Parkinson and other movement disorders

While the extent of our body of knowledge of PD and other movement disorders, including inflammation as a mediating factor, and the number of treatment drugs available is both vast, we still have no cure for these chronic and relentlessly progressive diseases. The motor symptoms of PD are known to result from reduced dopamine production in the brain's basal ganglia. Unfortunately, dopamine does not cross the BBB so it cannot be taken as a medicine to boost the brain's depleted levels of dopamine. However, a precursor of dopamine, levodopa, can pass through this barrier to the brain where it is readily converted to dopamine. Administration of this drug temporarily diminishes the motor symptoms of PD. Unfortunately, only 5–10% of the drug crosses the barrier with much of the remainder being metabolized to dopamine elsewhere in the body, where it causes a variety of side effects.[5,6] While inflammation plays a role in PD, its exact role has not been clearly detailed, as far as is known.

Alzheimer's and other cognitive diseases

According to latest research findings,[7-10] AD is not truly a disease *per se* but the brain's protective immune response from three metabolic and toxic threats (themselves having dozens of contributors): (1) Inflammatory insults resulting in an overdrive production of amyloid-beta plaques to ward-off the attack, (2) suboptimal neurotrophic levels (brain and body nutrients, hormones, and other brain-supporting molecules), and (3) neurotoxic exposures and compounds (including biotoxins). Of interest here is the inflammatory threat. Table 1 summarizes the corresponding biochemical markers.

AD has also been associated with a disruption or breakdown of the BBB.[12] The delivery of anti-inflammatory drugs to the brain for the treatment of brain cancers and NDDs will now be considered.

NEUTROPHIL-MEDIATED DELIVERY OF ANTI-INFLAMMATORY DRUGS TO THE BRAIN

GB, or GBM, is the most common and most aggressive primary brain tumor in adults. The standard treatment consists of surgery followed by radiochemotherapy together with concomitant chemotherapy and, once radiochemotherapy is complete, an adjuvant chemotherapeutic treatment. Other treatments include boron neutron therapy, intensity-modulated proton beam therapy, antiangiogenic therapy, alternating electric fields, vaccines, palliative therapies, and even lifestyle changes. All these treatments cannot eradicate all tumor cells. Thus, surgery is often insufficient given the diffuse nature of the disease. Even when tumors have been surgically removed, deeply infiltrated cancer cells often remain and contribute to relapse; chemotherapy has major limitations because most drugs cannot cross the BBB, and penetration into brain cells is limited. In addition, the cells in brain tumors are greatly heterogeneous, which limits the treatment efficacy and explains the high rate of progression of the disease.

In a recent study,[13] a neutrophil-mediated anticancer nanotechnological drug delivery was designed for the suppression of post-operative malignant glioma recurrence.[14-16] Neutrophils are white blood cells in the granulocytic series of blood cell development [Figure 2].

Formed by myelopoietic tissue of the bone marrow and released into the circulating blood, where they normally

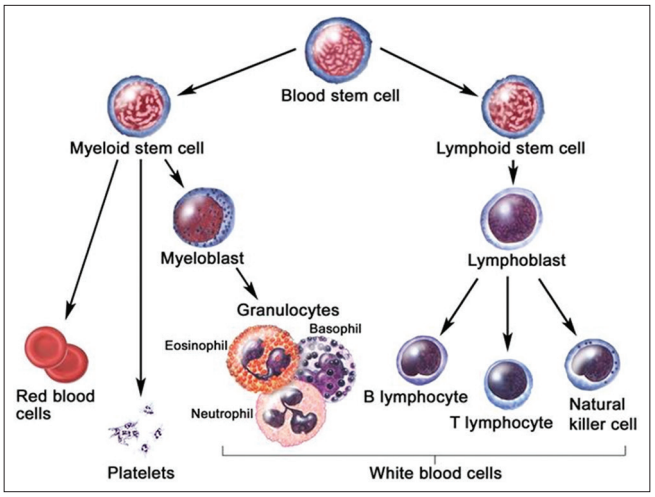

Figure 2: Blood cell development. Source: National Cancer Institute Archives

Table 1: Biochemical markers of the inflammatory subtype of Alzheimer's disease

Subtype	Biochemical markers	Notes
Inflammatory	1. Increased C-reactive protein 2. Decreased (albumin: globulin) ratio 3. Increased IL-6 4. Increased TNF 5. Abnormal metabolism and hormones 6. Increased homocysteine	1. A measure of inflammation caused by infectious agents (bacteria, viruses, fungi), radicals, AGE products, trauma, damaged proteins, damaged lipids (ox-LDL), etc. 2. Albumin is a key blood protein; globulin is a catchall name for~60 blood proteins 3. IL-6 rises with inflammation 4. TNF (another protein) rises with inflammation 5. Insulin resistance

Sources: Bredesen[9] and Fymat.[11] IL-6: Interleukin-6, TNF: Tumor necrosis factor, AGE: Advanced glycosylation end product, Ox-LDL: Oxidized low-density lipoprotein

represent 54–65% of the total number of leukocytes, they penetrate inflamed brain tumors. Prior animal and human studies had reported that neutrophils can cross the BBB, and although they are not typically attracted to GBMs, they are recruited at sites where tumors had been removed in response to post-operative inflammation. Taking advantage of the characteristics of these innate immune cells, Xue and his research team at the China Pharmaceutical University manufactured liposome capsules that encased paclitaxel (PTX), a traditional chemotherapy drug, with lipids, loaded them into neutrophils and injected them in the blood of three mouse models of GBM after surgical resection. When the treatment was applied following surgical removal of the main tumor mass, the neutrophil-carrying drugs were able to penetrate the BBB, destroy residual cancer cells, and slow the growth of new tumors. Inflammatory factors released after tumor resection guided the movement of the neutrophils into the inflamed brain. The highly concentrated inflammatory signals in the brain triggered the release of liposomal PTX from the neutrophils, allowing delivery of PTX into the remaining invading tumor cells. This neutrophil-mediated delivery of drugs efficiently slowed the recurrent growth of tumors, significantly improved survival rates, but did not completely inhibit the regrowth of tumors. While tumor recurrence was not completely prevented, overall, mice receiving this treatment lived significantly longer than controls.

One strength of the above method is, as stated earlier, that neutrophils are the most abundant white blood cells, so they can be collected in significant amount from a patient's blood. However, there are at least three limitations of this method: (1) It used approximately 10 times the number of neutrophils found in normal mouse circulation. Thus, if that same proportion must be maintained in patients, the blood amount needed for this type of procedure could be quite substantial, (2) two of the mouse models used are disliked by the neuro-oncology community because they elicit an immune rejection response, and (3) the third mouse model is based on a human GBM cell line that is different from the original tumor source. Nonetheless, although additional studies are necessary to further validate the method, the strategy of using neutrophils to deliver drugs across the BBB is novel. It could be applied to human GBMs, neurodegenerative diseases (NDDs), other inflammation-mediated disorders, and any other diseases that naturally attract neutrophils. Further, validation of this method and the initiation of clinical trials would be required. While conducting such trials, any immune rejection response should be monitored, evaluated, and possibly regulated such as suggested in Fymat.[17]

CONCLUSION

The use of a neutrophil-mediated anticancer nanotechnological drug delivery designed for the suppression of post-operative malignant glioma recurrence represents a paradigm change in the treatment of GB. It can also be applied to NDDs, other inflammation-mediated disorders, and any other diseases that naturally attract neutrophils. Further, laboratory and clinical trial validation of this method would be required, including consideration of any immune rejection response and its possible regulation.

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Glossary

Basophils = A cell with granules that stain specifically with basic dyes

B-cell (or B-lymphocyte) = A type of white blood cell that makes antibodies. B-cells are part of the immune system and develop from stem cells in the bone marrow. It is also called B-lymphocyte.

Eosinophils = A type of immune cell that has granules (small particles) with enzymes that are released during infections, allergic reactions, and asthma. An eosinophil is a type of white blood cell and a type of granulocyte.

Granulocyte = A type of immune cell that has granules (small particles) with enzymes that are released during infections, allergic reactions, and asthma. Neutrophils, eosinophils, and basophils are granulocytes. A granulocyte is a type of white blood cell. It is also called granular leukocyte, PMN, and polymorphonuclear leukocyte.

Lymphoblast = A lymphocyte that has gotten larger after being stimulated by an antigen. Lymphoblast also refers to an immature cell that can develop into a mature lymphocyte.

Lymphoid = Referring to lymphocytes, a type of white blood cell and also refers to tissue in which lymphocytes develop.

Myeloblast = A type of immature white blood cell that forms in the bone marrow. Myeloblasts become mature white blood cells called granulocytes (neutrophils, basophils, and eosinophils).

Neutrophils = Mature white blood cells in the granulocytic series formed by myelopoietic tissue of the bone marrow (sometimes also in extramedullary sites) and released into the circulating blood, where they normally represent 54–65% of the total number of leukocytes. When stained with the usual Romanowsky-type of dyes, neutrophils are characterized by (1) a nucleus that is dark, purple blue, lobated (3–5 distinct lobes joined by thin strands of chromatin) and has a rather coarse network of fairly dense chromatin and (2) a cytoplasm that is faintly pink (sharply contrasted with the nucleus) and contains numerous, fine or tiny, pink, or violet-pink granules, i.e., not acidophilic or basophilic (as in oesinophils or basophils). The precursors of neutrophils of increasing maturity are as follows: (1) Myeloblasts, (2) myelocytes, and (3) metamyelocytes or "juvenile" forms, including the "stabkernige" or staff cells (also known as stabs or band forms). Although the terms neutrophilic leukocytes and neutrophilic granulocytes include younger cells in which neutrophilic granules are recognized, the two expressions are frequently used as synonyms for neutrophils, which are mature forms unless otherwise indicated by a modifying term, such as immature neutrophil. Neutrophils manifest no special affinity for acid or basic dyes, i.e., the cytoplasm dyes approximately equally with either type of dye.

(Reference: Stedman's Medical Dictionary).

Natural Killer cells (NK cells) = Blood lymphocytes from humans which lyse (breakup, disintegrate) target cells (tumor or virus-affected cells) without involvement of antibody or complement. The cells seem to be pre-T-lymphocyte, but the mechanism involved in their killing ability is not clear; some, but not all, have surface Fc receptors, and IFN seems to play an as yet unexplained role.

T-cell (or **T-lymphocyte) =** It is a type of white blood cell. T cells are part of the immune system and develop from stem cells in the bone marrow. They help protect the body from infection and may help fight cancer. It is also called T-lymphocyte and thymocyte.