Inflammatory Response in Alzheimer's Disease

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AKIYAMA, H. *Inflammatory Response in Alzheimer's Disease*. Tohoku J. Exp. Med., 1994, 174 (3), 295-303 — Microglia belong to the mononuclear phagocyte system. They represent the brain resident tissue macrophages and function as the scavenger cells in brain. In Alzheimer's disease (AD), microglia become activated. Reactive microglia aggregate around senile plaque β -amyloid and neurofibrillary tangles. Heavy accumulation of these pathological debris in postmortem, however, indicates the failure or, at best, partial success of the removal. It is supposed that continued activation of microglia in these lesions elicits a persistent inflammatory response. In fact, activation fragments of the complement system have been detected in association with β -amyloid deposits and extracellular ghost tangles. Thrombin, a central serine protease of the coagulation pathway, is also deposited in these pathological debris. Both complements and thrombin could augment the biochemical, synthetic and phagocytic capacities of microglia. Microglia, in turn, might play a major role for the activation of complement and coagulation systems in brain. The available evidence strongly suggests a significant similarity between the chronic inflammation and the tissue response in AD lesions, supporting a notion that the inflammatory process is a part of Alzheimer pathology. ——— Alzheimer's disease; inflammation; microglia

The inflammatory response is primarily a host defence reaction. It serves to degrade and eliminate the foreign invader. Components of the inflammatory response play essential roles when phagocytic cells remove microorganisms, necrotic host tissue and undesired deposits of abnormal substance. However, prolonged inflammation often destroys the surrounding host tissue. Such a damage is particularly serious if it occurs in brain, where regeneration and recovery take place only in a very limited degree. Alzheimer's disease (AD) is characterized pathologically by heavy accumulation of β -amyloid and neurofibrillary tangles. This indicates the failure or, at best, partial success of the removal of these unpleasant deposits. Senile plaques and ghost tangles could, therefore, be the site of sustained inflammatory response.

In the periphery, the process of inflammatory response is consistent regardless of the inciting agent. Following the activation of several plasma proteins such as the contact activation, complement and coagulation systems, phagocytic cells

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enter the lesioned area and engulf pathological debris. Granulocytes predominate in the initial phase of acute inflammation. Monocyte-derived macrophages replace granulocytes and govern the progress of the inflammatory response in chronic stages. In this paper, attempts will be made to point out the similarities between AD lesions and chronic inflammation. Evidence largely comes from the immunohistochemical analysis of postmortem AD brain tissue in the light of our recent understanding of the molecules involved in the inflammatory processes.

Microglia as inflammatory cells in brain

In brain, the key cell in the inflammatory processes is the reactive microglial cell. In 1919, del Rio Hortega described the third element, "el tercer elemento", that had puzzled Ramon y Cajal. With his new silver carbonate stain, he divided "el tercer elemento" into two cell types (del Rio Hortega 1919). One is oligodendroglia, the myelin forming cells of neuroectodermal origin. The other is microglia, which he believed to be phagocytic in nature and of mesodermal origin. Fig. 1A-C illustrate morphology of microglial cells at different stages of activation from 'resting' microglia (A) to brain macrophages (C) (Penfield 1925). Since the first description by del Rio Hortega, the origin and the nature of brain microglia have long been a matter of significant debate (Jordan and Thomas 1988). Some investigators, as an example, argued that microglia were of neuroectodermal origin and functionally different from 'leukocyte derived' brain macrophages. In the last decade, however, overwhelming evidence has been accumulated that microglia are the brain representative of the mononuclear phagocyte system (Hickey and Kimura 1987; Perry and Lawson 1992; McGeer et al. 1993; Akiyama et al. 1994a). The mononuclear phagocyte system is a unifying concept that comprises monocytes, macrophages, histiocytes, and other phagocytic cells distributed throughout the body (van Furth 1982).

Table 1 summarizes our results on the phenotypic resemblance of microglia

Fig. 1. CR4 staining of a control (A) and Alzheimer (B, C) brain tissue, showing the morphological transition from resting microglia (A) to brain macrophages (C).

with monocytes and macrophages (Itagaki et al. 1988; Akiyama and McGeer 1990; Akiyama et al. 1991, 1993b, 1994b, McGeer et al. 1993). In general, expression of these antigens are upregulated upon activation. Some molecules are expressed constitutively by all microglia. Leucocyte common antigen (LCA) is a family of membrane bound tyrosine phosphatases, which are expressed exclusively by nucleated cells of hematopoietic origin (Itagaki et al. 1988; Akiyama and McGeer 1990; Akiyama et al. 1994c). A more restrictive marker is the receptor for CSF-1, macrophage colony stimulating factor (Akiyama et al. 1994b). Fc γ receptors (Fig. 2A) have as their ligand the Fc chain of immunoglobulin G. Binding of immunoglobulin to microorganism or foreign particle dramatically enhances the phagocytic activity via Fc γ receptors. In other words, the expression of Fc γ receptors is an essential nature of the phagocytic cells of the immune system.

Expression of β_2 -integrins and their ligands

Another important instrument for the immune cells is the cell adhesion molecules. β_2 -integrin is a family of cell adhesion molecules that are expressed by cells of leukocyte lineage. So far, three molecules have been identified; leucocyte function associated antigen (LFA)-1, CR3 (Mac-1), and CR4 (p150,95). All members of β_2 -integrin appear to play significant roles in the pathological processes of Alzheimer's disease (Akiyama and McGeer 1990). In the cerebral cortex of normal subjects, resting microglia are stained weakly for LFA-1. In

TABLE 1. *Microglia phenotype*

Leucocyte common antigen (LCA, CD45) [CD45RA- /RB+, RO+*]
IgG Fc γ receptor I (CD64)
II (CDw32)
Integrins
LFA-1 (CD11a/18)
CR3 (complement receptor-3, Mac-1, CD11b/18)
CR4 (complement receptor-4, p150,95, CD11c/18)
vitronectin receptor (CDw51/?)*
L-selectin*
CSF-1 (M-CSF) receptor (<i>c-fms</i>)
MHC class I antigens (HLA-A, B, C)*
MHC class II antigens (HLA-D)
CD4*
CD68 (EBM11)
MRP14* (calcium binding protein rich in myeloid cells)
α interferon*
Plasminogen activator*
Plasminogen activator inhibitor-2*

*Resting microglia are either negative or faintly stained.

Fig. 2. A: Resting microglia constitutively express IgG Fc γ receptor. B: LFA-1 staining of the cerebral cortex from an Alzheimer patient. C: ICAM-1 staining of the cerebral cortex from an Alzheimer patient.

Alzheimer brain, LFA-1 expression by reactive microglia is dramatically enhanced. Such reactive microglia tend to agglomerate in the centre of senile plaques (Fig. 2B) (Itagaki et al. 1989). A major ligand for LFA-1 is ICAM-1, a cell adhesion molecule which belongs to the immunoglobulin gene superfamily. In Alzheimer brain, senile plaque amyloid is stained intensely for ICAM-1. In addition, fine fibrous structures extend beyond such amyloid deposits (Fig. 2C) (Akiyama et al. 1993a). Double immunostaining for GFAP and ICAM-1 has revealed that reactive astrocytes marginating senile plaques express ICAM-1. Thus, in brain, reactive microglia and astrocytes seem to communicate via the LFA-1 and ICAM-1 mediated cell-cell adhesion.

Other members of β_2 -integrin are complement receptors CR3 (Mac-1) and CR4. Both CR3 and CR4 are constitutively expressed by microglia (Akiyama and McGeer 1990). In Alzheimer's disease, the expression of CR3 and CR4 is again upregulated. The major ligands for CR3 and CR4 are complement fragments generated by the activation of complement C3. Complement activation takes place through sequential cascade-like activation of serine proteases. During the activation processes, cleaved fragments of complement proteins such as C3a and C5a, called anaphylatoxins, mediate features of the inflammatory response. Some other fragments mediate the process of opsonization, in which foreign particles are prepared to be phagocytosed through recognition by receptors for these opsonins on phagocytic cells. Complement proteins of the classic activation pathway have been detected in lesions of Alzheimer's disease (McGeer et al. 1989). Fig. 3A shows the staining of AD cortex for C4d, a post-activation fragment of C4. The β -amyloid deposits and extracellular neurofibrillary tangles are stained positively. In double immunostaining for C4d and HLA-DR, HLA-DR positive

reactive microglia aggregate around the β -amyloid deposits (Fig 3B) and extracellular ghost tangles (Fig. 3C). These pathological debris appear to be opsonized by complements so that they are phagocytosed by activated microglial cells which vigorously express the complement receptors. CR3 (Mac-1) also mediates microglial activation, suggesting multiple roles of the complement system in brain lesions (Akiyama et al. 1994a).

Coagulation system

There is evidence for an involvement of other humoral elements of the inflammatory process in Alzheimer lesions. The blood coagulation system was discovered originally as a mechanism to control bleeding at the site of vascular injury. However, it is also an important component of the immune and inflammatory reaction. Interestingly, at an earlier stage of evolution, a common

Fig. 3. A: C4d staining of the cerebral cortex from an Alzheimer patient, showing the positive staining of the diffuse β -amyloid deposits, consolidated senile plaques and extracellular neurofibrillary tangles. B: Double immunostaining for C4d (lighter shading) and HLA-DR (darker shading). Microglia agglomerate in the centre of senile plaques. C: Layer Pre- α of the entorhinal cortex, where all tangles are extracellular in eventually all Alzheimer patients. HLA-DR positive microglia appear to be attacking C4d coated tangles.

ancestor of complement and coagulation proteins is known to form a more general defence system protecting the organism from infection and tissue injury. Central to coagulation is the generation of thrombin from its enzymatically inactive precursor, prothrombin. This highly active serine protease has multiple biological activities on a variety of cells. Thrombin causes rapid neurite retraction of cultured neurons. Cultured astrocytes become round and start proliferation after thrombin stimulation. Thrombin works as chemoattractant and mitogen for monocytes and macrophages, and, perhaps, for microglia. In *in vitro* studies, thrombin has been reported to upregulate the synthesis of β -amyloid precursor protein (APP) by platelets and to cleave APP to generate a fragment which contains the β -amyloid protein sequence.

Fig. 4 illustrates immunohistochemical localization of thrombin in Alzheimer brain. Residual blood plasma in the vessels and senile plaque β -amyloid are stained positively (Fig. 4A). The immunostaining for thrombin is completely abolished by absorption of the primary antibody with purified thrombin but not with its proenzyme, prothrombin (Akiyama et al. 1992). Thrombin is also present in diffuse β -amyloid protein deposits and extracellular ghost tangles (Fig. 4B). These results are consistent with a report by Wagner et al. (1989) showing the reduction of protease nexin (PN)-1 in Alzheimer brain. PN-1 is a potent inhibitor of thrombin and extremely rich in brain.

Complement activated oligodendroglia was first reported by Yamada et al. in 1990. These complement-coated oligodendroglia occur in many degenerative neurological diseases. These oligodendroglia are considered to be under a degenerative process being opsonized by complements for removal. Complement activated oligodendroglia are stained positively for thrombin. Co-localization of thrombin and complement proteins in a variety of brain lesions suggests an interaction between these systems. This is an issue to be addressed in future

Fig. 4. Immunostaining of Alzheimer brain tissue for thrombin. A: Senile plaques and diffuse amyloid deposits are stained in addition to the residual blood plasma in vasculature. B: Neurofibrillary tangles in layer Pre- α of the entorhinal cortex of an AD case are stained intensely for thrombin.

studies, however.

In a classic scheme of the blood coagulation system, two activation pathways are known. One is the intrinsic pathway which starts from the contact activation system. The other is the extrinsic pathway which is initiated by an exposure of the membrane protein 'tissue factor' to the extracellular milieu. The key step in the cascade is the cleavage of prothrombin to generate thrombin by a complex of activated factor V and factor X. This complex is called prothrombinase complex, in which activated factor X (FXa) is an active protease and factor Va, being embedded in the phospholipid membrane, serves as a receptor for FXa. In the periphery, macrophages as well as platelets are known to provide phospholipid membrane surface where the prothrombinase complex is formed. It has yet to be determined whether reactive microglia carry the active prothrombinase complex on their surface or not. Tissue factor, an initiator of the extrinsic coagulation cascade has been shown to be concentrated in senile plaques (McComb et al. 1991). FX is also detected immunohistochemically in senile plaques. An antibody to FV stains granular structures within senile plaques. These granules appear to be located in microglia. Such results suggest that, in brain, thrombin is generated via the classic extrinsic pathway. However, an alternative mechanism for the factor X activation may exist. As previously mentioned, microglia constitutively express CR3 (Mac-1), a member of β_2 -integrin. Evidence is available indicating that CR3 is bound to factor X and activates it in the absence of factor V. There might be multiple pathways for the activation of factor X on microglial cell surface.

Fibrinolysis system

Physiological and pathological roles of the extravascular fibrinolysis are now issues of extensive investigations in many organs. As a member of the mononuclear phagocyte system, microglia are supposed to regulate plasminogen activator (PA) and plasmin activity on their cell surface. Cultured microglia from fetal rat brain have been shown to secrete urokinase type-PA. Microglia in postmortem human brain tissue are immunostained for urokinase type plasminogen activator. Microglia are also positive for type-2 plasminogen activator inhibitor (PAI-2) (Akiyama et al. 1993b). Again, the expression of these molecules are upregulated by reactive microglia located in senile plaques.

Conclusion

In summary, senile plaques are comparable in many respect with a site of persistent inflammation. Reactive microglia, a brain representative of the mononuclear phagocyte system, agglomerate in the centre of β -amyloid deposits. Astrocytes interact with microglia and demarcate these inflammatory loci. Both the complement and coagulation cascades are activated to cooperate with these cellular elements. Proteases which belong to the fibrinolysis system appear to

play a role in microglial function. These proteins were originally discovered in plasma. However, the majority are now known to be synthesized and secreted by activated macrophages. Furthermore, in vitro studies have indicated that some of these proteins are produced by microglia, astrocytes or neurons. Activation of these systems, therefore, could take place without the breakdown of the blood-brain barrier. Detection of the components of these major self defence mechanisms, both humoral and cellular, supports a notion that inflammatory processes are involved in Alzheimer lesions. Formation of dystrophic neurites in senile plaques, and the deposition of β -amyloid in ghost tangles might be a sequence of such persistent inflammatory responses in Alzheimer brain.

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