15 Neuroimmune Cross Talk

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Abstract: Immune system and the nervous system share the job of keeping the homeostatic control and responding to changes in external and internal environment. Similarity of their functions and common use of receptors, ligands and other cell-to-cell communication molecules by cells of nervous and immune system origin supports the idea that the two systems function in close cooperation. This chapter intends to provide an overview of the field of neuroimmune cross talk including innervation of immune system, innate immune system of the brain, commonly used mediators and transmitters, and the similarity of neuronal and immune synapse. We did not focus on autoimmune diseases of the brain and only barely touch the medical neuroimmunology because we aimed to give a survey of basic facts and principles of neuroimmune cross talk, providing a firm background for understanding medical consequences of interaction between the two systems.

List of Abbreviations: ACTH, adrenocorticotropic hormone; APC, antigen-presenting cell; BDNF, brain-derived nerve growth factor; CGRP, Calcitonin gene-related peptide; CRF, Corticotrophin-releasing factor; CRH, corticotropin-releasing hormone; ICAM, integrin-mediated cell adhesion molecules; LPS, lipopolysaccharides; MAPK, mitogen-activated protein kinase; MCP-1, monocyte chemoattractant protein; NCAM, Neuronal cell adhesion molecules; NOS, nitric oxide synthase; NGF, nerve growth factor; PVN, paraventricular nucleus; RAMP, receptor activity-modulating protein; SMAC, supramolecular activation cluster; TLR, toll-like receptor; VIP, vasoactive intestinal polypeptide

1 Introduction to Neuroimmunology

The nervous and immune systems provide a mutual function, preserving integrity and homeostasis of the organism. These two systems are composed of networks of cells, monitoring, processing, and responding to changes in external and internal environment including extracellular space of tissues. Similarity of their functions and common use of receptors, ligands and other cell-to-cell communication molecules by cells of nervous and immune system origin supports the idea that the two systems function in close cooperation. Indeed, the amount of experimental evidences for neuronal control of immune system and impact of immune response on neuronal functions is growing exponentially. The main frontier of neuroimmune cross talk is at the barrier regions such as skin, lung, and gut, where the interaction of mast cells and eosinophils with neurons is confirmed. Behavioral changes under inflammatory reactions modifying mood and motor activity as well as sleep are common experiences in infectious diseases. The severe influence of depression and other psychological disorders on immune reaction has also been extensively reported. Thus, evidently the bilateral interaction of nervous and immune systems is a rapidly growing field of molecular and cellular neuroscience (Blalock, 2005).

History of comparative studies of immune and nervous system goes back to the Neuroscience Study Program books edited by F. O. Smith and particularly to the scientific efforts of G. Edelmann. So the idea is old that immune cells and their molecular compartments have several similarities to the nerve cells. The main outcome of genome era that the number of genes is much lower than expected led us to a conclusion that same proteins can play different roles in different cell phenotypes. In fact, there are receptors, proteins in the signalosomes, and synapses which are in use in nerve cells and immune system cells, such as caveolines, cholinergic receptors, and so on. Here, we give a review of common structural elements with and without functional similarities.

The efferent part of neuroimmune cross talk is the action of neurotransmitters and modulators on immune cells. This chapter provides an insight to mechanisms of immune cell activation or depletion by neuropeptides such as calcitonin gene-related peptide (CGRP), vasoactive intestinal polypeptide (VIP), substance P. The cholinergic anti-inflammatory pathway and adrenergic control of immune cells are direct efferent actions of CNS on immune system. Nicotinic-type acetylcholine receptor is present on microglia and on some other immune system cells. As of the other side of the neuroimmune cross talk system is concerned, specific behavioral effects can be achieved by inflammatory cytokines. There are immune system elements in the brain such as microglia, the resident macrophage of the brain. Microglia is a typical immune system cell activated by surrounding neuronal and glial events. Complement system elements

expressed on astrocytes are also possible targets of peripheral immune reaction. The open areas of the blood–brain barrier (BBB) compose an interface between blood and brain. Peripheral immune response induced proinflammatory cytokines and other small blood-derived peptides can enter the brain and induce changes in nervous function by activation of endogenous cytokines and neurotransmitters.

Our aim is to call attention of neuroscientists for investigation and importance of neuroimmune cross talk and spread out the idea that neurosciences can learn novel scope and new targets of research from immunology. At the present stage of knowledge, we try to give a general scope to neuroimmune cross talk principles but we do not give a detailed, complete review of its literature; thus, we skipped several features of the topic and aimed to make a short but informative survey of neuroimmunology.

2 Immune System of the Brain; Activation by Inflammation

Brain is isolated from the blood by BBB. BBB does not allow the free penetration of blood immune cells into the brain and limits the spread of large molecules from the blood to the brain. BBB is built from epithelial cells covering the brain surface and endothelial walls of capillary blood vessels and end feet of astrocytes are also involved. Permeability of BBB depends on cell adhesion molecules, connecting the epithelial cells such as CD40, vascular adhesion molecule-1, intercellular adhesion molecule-1, E-selectin. There are open areas of BBB under physiological conditions, allowing direct communication between blood and brain tissue and penetration of large molecules even cells. Circumventricular organs are such open places locating at strategic position near to the middle ventricular system. Because of anatomical vicinity, the blood-derived molecules easily reach pineal gland, subcomissural organ, subfornical organ, and particularly reach organum vasculosum, median eminence, and hypophysis. All these structures are close to the autonomic nervous system centers in the brain. Area postrema is very close to the medullar autonomic nuclei including solitary tract (Rubin and Staddon, 1999).

Endothelial cells of BBB could release neurotransmitters and modulators as a response to cytokine signals from the blood. In addition, activation of BBB epithelial cells induces leakage of BBB; in turn, neurotoxic and inflammatory mediators can penetrate the brain. Penetrability of BBB is controlled by inflammatory cytokines, metal ions such as Al, organic solvents, and many other compounds. During inflammation, opening of BBB is a major factor of brain symptoms. Inflammation activates genes of cycloxygenase (COX2) and enhances transcription via nuclear factor of kappa B (NFκB) pathway. That was disclosed by using lipopolysaccharide (LPS) for induction of inflammation. LPS also activates mitogen-activated protein kinase (MAPK) pathway through soluble CD14, induces release of IL1β and TNFα from epithelial cells, and also activates nitric oxide synthase (NOS) (Wong et al., 2000). These mediators could regulate BBB so that permeability increase allows entrance of blood cytokines into the brain and enhances infiltration of macrophages (see later in this chapter). Such an effect on BBB can be attenuated by glial cells, dexamethasone, and NOS inhibitors. Cerebrovascular dysfunctions can also be observed during inflammation but changes in cerebral blood flow data are controversial (Hedley and Pippa, 2002).

For neuroimmune communication, brain should detect inflammatory mediators as well. Components of innate and adaptive immune systems are expressed in the brain in experimental endotoxin shock and expression of immune system molecules spread from the circumventricular organs to the deeper structures of the nervous system to areas controlling autonomic nervous system and neuroendocrine regulatory system. It is nicely shown by LPS receptor CD14 expression. Recently, Toll-like receptor (TLR) has been demonstrated in resting and LPS-activated brain; expression of TLR2, TLR4, and TLR9 was convincingly established on microglia, oligodendroglia, and astrocytes. TLR4 receptor mRNA has been found in the circumventricular organ, hypothalamus and medulla, but in contrast to CD14, TLR4 mRNA is downregulated in LPS infection (Opal and Huber, 2002). Data on TLR4 protein are contradictory because of the poor selectivity of TLR4 antibodies available. TLR2 and TLR9 mRNA was found in microglia. TLR and CD14 both activate NFκB pathway and enhance transcription of proinflammatory cytokines. LPS also activates NOS in the brain and increases the release of proinflammatory and anti-inflammatory cytokines synchronously suggesting a delicate immune counterimmune regulation in the brain. Prostaglandin E2 is also activated in the brain

under inflammation and its role in fever genesis is extensively studied (Blatteis et al., 2004). Finally, it has to be noted that free radicals and superoxides are generated in the brain during inflammation.

The outcome of inflammatory reaction in the brain is complex. Cytokines, NO, and prostaglandins modulate neurotransmission particularly the β-adrenergic system and production of corticotrophin-releasing factor (CRF), ACTH, and vasopressin. Autonomic nervous system is also controlled by inflammation as we will describe later in detail. Intracellular changes in brain cells under inflammatory influence are both destructive as apoptotic cell death induced by NO or TLR4 receptor activation and protective as heat shock protein expression (Rotwell, 1999). Apoptotic cell death can be induced by LPS via IL1β, TNFα. Some data support that epileptic activity in the brain is enhanced by IL1β and reduced by endogenous antagonist of it (ILR1a) (Vezzani and Granata, 2005). Investigation of inflammatory reaction and its molecular consequences in the brain is a promising field for research to understand how severe brain diseases are as neurodegenerative disease and psychiatric diseases can develop in subjects involved sustained inflammatory diseases (Kronfol and Pemick, 2000). Statistical epidemiology data support a correlation between frequency of inflammation and expectance of neurodegenerative disease and depression. On the other hand, manipulation of immune reaction and inflammatory processes via neuronal influence on the peripheral immune system is also an old experience in Chinese and other Oriental medicines.

Brain cells such as astrocytes, microglia, and oligodendroglia express complement system proteins. The classical and the alternative complement activation pathway is present in the brain and plays an important role when BBB is broken and immunoglobulins or various LPS can enter the brain under infections (Guillemin and Brew, 2004). The classical pathway, activated by IgG or IgM molecules via C1 converted to C1b by C1-convertase, is activated in neurodegenerative disorders such as Alzheimer's disease. Amyloid-β can bind the C1 receptor directly (Yasojima et al., 1999). The alternative pathway activated by membrane lipids and liposaccharides and mediated by C5 converted to C5b by C5-convertase is involved in viral and bacterial infections of the brain. The brain complement system and its endogenous inhibitors have particular therapeutic importance in medication of neurodegenerative disorders. The glial localization of complement system supports the particular importance of glial cells in pathomechanisms of neurodegeneration based on protein aggregation (Yasojima et al., 1999).

As it has been demonstrated, several functional elements of immune system such as tissue resident macrophages, cytokine-releasing cells, and the complement system are present in the brain. It is interesting to note that only few components of immune system proteins are expressed in neurons but all of them are found in different glia cells. The microglia is equipped with all immune system protein components because microglia cells are the resident macrophages of the brain as we are to describe in the next chapter. Actually, it is clear that brain and immune system communicate under inflammation and early and late immune response. The immune system proteins containing glia cells mediate the immune system actions to neurons in great extent but there are several examples of direct action of immune system on neurons. The molecular substrate of neuroimmune cooperation is the immune system of the brain on one hand and the neurotransmitter or neuromodulator receptors on the immune cells particularly on macrophages and lymphocytes on the other.

2.1 Microglia and Other Brain Macrophages

Microglia cells are resident macrophages in the nervous system. The name "microglia" was introduced by del Rio Hortega in 1919. Recently, the name of microglia remained in use but it turned out that it is more real to classify the microglia cells into the group of central nervous system macrophages. CNS contains pericytes, perivascular macrophages, and the real microglia; thus, they all form the group of resident macrophage cells. The controversial nomenclature derives from the controversial origin and function of different CNS macrophages. The most common hypothesis of the cellular origin of CNS macrophages is that they are derived from monocytes (Guillemin and Brew, 2004). In developing brain, however, the pial macrophages can be an additional source of brain macrophages. In embryogenesis, macrophages can migrate from any direction into the developing CNS but in developed brain the source of migration is restricted to the blood vessels. Differentiation of primary embryonic macrophages is governed by microenvironmental factors and finally they differentiate into four different types of brain macrophages listed earlier. There are evidences

suggesting that different brain macrophage types can be transformed to each other in certain conditions and such transformations are influenced by diseases (Guillemin and Brew, 2004).

The characteristic features of different brain macrophages are as follows: *Pericytes* are of mesodermal origin and migrate from the blood vessels at the end of vascularization. *Perivascular macrophages* are very similar to the blood-derived macrophages and they are a subpopulation of migratory macrophages. Because of their similarity to pericytes, the differentiation of them can be done only by marker proteins (Guillemin and Brew, 2004). *Infiltrating macrophages* are blood macrophages actually infiltrating through the BBB around the blood vessels. They have all markers of blood macrophages generally described, so they are not differentiated brain macrophages. *Microglia cells* are tissue-resident macrophages in CNS and their origin and functional roles are not disclosed properly. They are definitely not glia cells because of their macrophage origin. Microglia can be differentiated by characteristic marker proteins using immunostaining.

In normal brain under physiological conditions, there are resting microglia cells in all brain areas as well as perivascular macrophages and pericytes. A few blood-derived macrophages also "patrolling" and a spontaneous infiltration of macrophages through the BBB can be observed. In pathological conditions, AU1 when either a large number of cells die or inflammation of the nervous system occurs, the CNS macrophages are activating. They can proliferate and migrate in some extent and if the BBB is damaged, the number of infiltrating macrophages can increase significantly (Sharshar et al., 2005). The antigen-presenting cell (APC) of CNS is the pericyte, which can enhance constriction of blood vessels and they can convert to smooth muscle cells. Pericytes are essential elements of BBB and involved in pathomechanisms of hypoxia, hypertension, diabetic retinopathy, trauma, Alzheimer's disease, multiple sclerosis, and brain tumors. The time sequence of macrophage activation in the case of an injury is that pericytes and perivascular macrophages activate first, than blood monocytes and lymphocytes cross the BBB. The migration of macrophages is controlled by chemokines particularly the astrocyte-released monocyte chemoattractant protein-1 (MCP-1). Simultaneously, microglia cells are activated and they became capable for phagocytosis (Guillemin and Brew, 2004). That process is one of the novel hypotheses of Alzheimer's disease (Kaltschmidt et al., 1997). It has to be noted that recently several new aspects of microglia activation have been determined such as neuroprotective role of microglia activation. So the pathophysiological role of CNS inflammatory reaction is in focus of research interest.

Activated microglia cells have specific markers allowing identification of activation by immunostaining. Because it is a considerably long list of proteins available in several review papers (Graeber et al., 1999), we give only a brief survey of categories of marker proteins. One-third or even more of the microglia activation marker list contains cell adhesion molecules and membrane proteins such as integrins, CAMs, immune receptors, and so on. The others are miscellaneous proteins of cellular metabolism and transport procedures. The long list of activation marker proteins suggests that the activation process is a complex and fundamental change in cellular phenotype of macrophages. The phenotypes of activated, maturing, infiltrating macrophages are different. CNS macrophages, as other macrophages, have several phenotypes controlled by activation and microenvironmental changes (Yasojima et al., 1999). Cell culture conditions as well as other microenvironmental conditions significantly change the macrophage phenotype, so it has to be taken into account in planning experimental protocols. On the other hand, one of the major challenges in CNS immunology is to understand the phenotype–function relations of CNS macrophages. Novel methodology of laser capture microdissection and systems biology approaches including gene arrays and proteomics are promising. In addition, we should emphasize that BBB particularly the endothelial wall of blood vessels is an important interface between immune and nervous systems. The BBB epithelial cells and the participating astrocytes, macrophages are in focus of research interest for understanding the early mechanisms of neurodegenerative and autoimmune diseases of the brain. To give even a brief summary of the actual stage of BBB research, it requires a complete chapter and it has been done in excellent reviews elsewhere, so here we just attract the attention to the importance of that issue.

3 Mediator and Modulator Molecules of Neuroimmune Cross Talk

Neuroimmune mediators are small diffusible compounds released and detected by both, CNS cells and immune cells. They are the information mediator molecules and substrates of the common biochemical language of CNS and immune system. Neuroimmune modulators are hormones, cytokines, neuropeptides, and chemokines.

3.1 Neuropeptides as Neuroimmune Modulators

Neuropeptides are involved in communication between immune system and CNS. Participation of VIP, substance P, and CGRP in neuroimmune communication has been properly established but some role of other neuropeptides like neuropeptide-Y, galanin, somatostatin, and neurokinin A has also been reported. Immune cells express neuropeptide receptors and they also express neuropeptides. The main hot spots of neuroimmune communication are the open areas of the BBB, where immune cells can be in direct contact with neurons and glia cells and communicate to each other via releasing small molecules such as neuropeptides (Rubin and Staddon, 1999).

VIP is present in the immune system. It was observed in lymphocytes, mast cells, and other leukocytes. Expression of VIP gene has been described in CD4 and CD8 thymocytes and in spleen lymphocytes. Lymphocytes do only not express VIP mRNA and accumulate VIP in the cell body, but they release VIP on immune stimulation (Delgado et al., 2004). There are controversial studies on macrophages expressing VIP. Expression and secretion of VIP depends on immune stimulation of the organism by LPS or cytokines (IL1β, IL6, or TNF). Any process increasing cAMP levels or activating PKC increases VIP expression and secretion (Delgado et al., 2004). VIP and pituitary adenylate cyclase-activating peptide (PACAP) are bound to a receptor of B1 receptor subfamily expressed in immune cells and CNS cells. In CNS, the microglia is the resident macrophage and responds to VIP and expresses VIP. Microglia activation is a hallmark of Alzheimer's disease and also activated by cytokines released under CNS inflammation. Microglial activation and also the VIP secretion from microglia cells can be modulated by VIP. VIP release and expression is modulated by small dose of LPS. It has been disclosed that VIP is neuroprotective in CNS inflammatory diseases.

CGRP is a 37 amino acid neuropeptide binding to its G-protein-coupled receptor. Receptor activation AU2 requires a receptor activity-modulating protein (RAMP). CGRP has neurotrophic and neurotropic effects in CNS and also regulates antigen presentation of immune system cells. On the other hand, CGRP is the most potent endogenous vasodilatator. The epidermal Langerhans cells belonging to the dendritic cell family of the immune system are controlled by CGRP-containing axons and CGRP downregulates antigen presentation by Langerhans cells (Tsatsaris et al., 2002). Thus, CGRP is a typical communication molecule having roles both in CNS and immune system.

Neurotrophins are also involved in modulation of immune system. Neurotrophins are a group of polypeptide growth factors that control development and maintain functions of the nervous system. Particularly, nerve growth factor (NGF), neurotrophins, namely NT-3, NT-4, NT-5, NT-6, NT-7, and brain-derived nerve growth factor (BDNF) are involved in immune system modulation. Neurotrophin receptors (tyrosine kinase receptor, Trk A, B, C forms) are expressed on all cells of immune system and in several components of the lymphoid system. The number of receptors increases after activation of immune cells and neurotrophins enhance cytokine release. Neurotrophins are candidate molecules for regulating communication in the immune system and also could control neuroimmune cross talk. NGF is involved in the pathomechanism of autoimmune inflammatory diseases (Vega et al., 2003).

Substance P is also involved in immune system modulation. Substance P is expressed in lymphocytes and it is a key transmitter in pain sensation. The sickness response, which is the response of brain to peripherally released cytokines, is transmitted by substance P in great extent. The sickness-induced hyperalgesia develops when central IL and TNF release is increased from astrocytes expressing substance P receptors (Hartung and Toyka, 1989). The origin of hyperalgesia is the spinal cord glutamate and substance P containing neuron population. Astrocytes expressing glutamate and substance P receptors

can be activated by substance P and in turn they release brain cytokines. Microglia is able to activate the same astrocyte population as substance P and the released substances from astrocytes further activate the microglia forming a positive feedback loop. It suggests that substance P is a key element of the molecular mechanism controlling inflammatory processes in CNS (Wiedermann et al., 1987).

3.2 Hormones in Neuroimmune Communication

Interaction of stress response with immune system has been widely studied in the past twenty years. Stress is defined as a state of disharmony in homeostasis provoked by psychological or/and environmental influences called stressors. In stress response, the neuronal and the immune systems interact forming a network of regulation of the internal and external homeostasis. Cytokines released by activated immune cells influence CNS and CNS acts on the immune system. Using hypothalamic–pituitary–adrenal axis, CNS controls the immune system by downregulating the immune response. That control is responsible for many different pathological modulations in immune reaction during and after psychiatric disorders. Interestingly, molecular mechanisms involved in immune system control of stress-induced psychiatric disorders like anxiety or depression are amazingly similar (Black, 1994).

Evidences for CNS influence on immune system derive from electrolytic lesion experiments destroying different areas of the hypothalamus. Immune suppression and facilitation were evoked by lesions depending on the hypothalamic area destroyed (Khansari et al., 1990). Hypophysectomy prevented the effects of hypothalamic lesions indicating that pituitary gland is necessary for CNS action on immune system. Molecular substrates of CNS–immune system cross talk in stress are the neurotransmitter and hormone receptors expressed in lymphocytes and macrophages as well as the cytokine receptors in hormonal system and in CNS (Black, 1994).

CRF is the main coordinator molecule of stress response and CRF containing neurons are scattered all over the brain but the highest density of CRF neurons is in the paraventricular nucleus (PVN) of the hypothalamus. PVN receives pathways from many different brain structures including limbic system and forebrain. Somatic and viscerosensory systems also send axons to the PVN, so it is a highly integrative center in the hypothalamus. Monoamine neurotransmitters such as serotonin, noradrenaline, as well as acetylcholine, glutamate transmit the information toward PVN. CRF is the key component of the stress mechanism and initiate many different processes. Some of them reach the immune system as modulator or regulator. Immune system sends a feedback to CRF releasing PVN neurons via cytokines (Licinio and Wong, 1997). Blood cytokines enter the brain and induce release of brain cytokines, initiating behavioral and sensory processing changes. Cytokine receptors are all over the CNS, so an increase in brain cytokines could result in a complex change in neuroendocrine modulation. Since most of the known hormones have some influence on the immune system, we will focus on only those which have been investigated in detail. They are as follows: corticosteroids, catecholamines, endogenous opiates, growth hormone, prolactin. It has to be noted, however, that somatostatin, cholecystokinin, oxytocin, melatonin, sex steroids, thyroxine, VIP, and arginine–vasopressin have also influences on the immune system but at the present stage of knowledge the mechanism of their action is not clear.

Corticosteroids have profound immune suppressive effect when the hormone levels increase in stress (Roy and Loh, 1996). Besides the corticosteroid action on lymphoreticular system, marked antiallergic and anti-inflammatory effects of them can be observed. Adrenalectomy or depletion of adrenaline-induced corticosteroid elevation on any other way depletes the immune-suppressive effect of stress but not entirely because some other molecular mechanisms of stress-induced immune suppression remain intact. It is supported by the fact that in aged stressed rats the tumor implantation is more successful than in young stressed rats due to the elevated corticosteroid levels in aged rats' brain.

Catecholamines, such as norepinephrine and epinephrine, also accompany molecular mechanisms of stress. They change lymphocyte, monocyte, and leukocyte activation and plasma epinephrine level is a good indicator of stress and inversely related to specific immune system functions. β-adrenergic receptors are expressed on lymphocytes and macrophages, in turn β-adrenergic agonists and antagonists down- or upregulate immune functions, respectively. β-agonists applied before initiating immune response can

increase antigen production. The late immune response, however, is suppressed by β-agonists as it is indicated by decreased antibody production and response to cytokines. Interestingly, ablation of sympathetic nervous system enhances autoimmune reaction, augmenting experimental allergic encephalitis, experimental autoimmune multiple sclerosis. It suggests that sympathetic nervous system activity generally downregulates immunity and the absence of sympathetic influence enhances autoimmunity (Pavlov and Tracey, 2004).

Endogenous opiates are a large group of peptides found in the brain and pituitary gland, which have antinociceptive effect. Endogenous opiates have three groups. One is the endorphin family, the second is the enkephalin family, and the third is the dynorphin. Opiates bind to their specific mu, delta, and kappa opiate receptors. The opiate level increases in stress in conjunction with catecholamine release. The opiate effect on immune system is clearly established but the results are conflicting. Opiates are mostly immunosuppressive, as it is supported by application of opioid receptor blockers like naltrexone (Roy and Loh, 1996). Natural killer cells were depressed and interferon release from lymphocytes was also decreased by opiates and mitogenesis of lymphocytes was depleted. However, some early studies reported that opiates can be immunostimulatory. Contradictions in experimental data on opiate action on immune system are mainly derived from differences in experimentation protocols. Because of complexity of CNS control on immune system, the age and sex in in vivo or in vitro conditions can controversially influence the results.

Growth hormones and prolactin are immunoenhancing agents of pituitary gland. Immune-suppressive effect of hypophysectomy can be antagonized by growth hormone and prolactin. Growth hormone and prolactin receptors are expressed on lymphocytes and macrophages. Growth hormone receptors can influence T lymphocytes, monocytes, stem cells, whereas prolactin controls T lymphocytes and macrophages. Both hormones are essential for thymus cell differentiation via receptors of thymus epithelial cells. Prolactin and growth hormone levels elevate in early phase of stress response and it is proposed that prolactin buffers the immune-suppressive effect of stress. It is unlikely because the prolactin level decreases in late phase of the stress response to the prestress value and the immune suppression could develop. AU3 Growth hormone and prolactin release is controlled by many other hormones and neurotransmitters like serotonin, CRF, oxytocin, and so on; so this complex regulatory network of prolactin release makes investigation of endocrine influence on immune system very difficult (Williams and Frohman, 1986).

Compounds mediating CNS–endocryne–immune system cross talk are neurotransmitters, neuropeptides, and hormones. Immune system cells like lymphocytes, macrophages, killer cells, and stem cells express their receptors and several neuropeptides and neurotransmitters are synthesized and released by immune cells not only by neurons (Feltend, 1991). Neurotransmitters and modulators from the effectors of CNS mediate the controlling messages from CNS structures, mainly from hypothalamic regions to the immune cells. In spite of the immense data, many important questions of neuroimmune communication remain unsolved. We do not know how the neurotransmitters and modulators reach the targeted immune cells; there are many inconsistent data on hormonal and neuropeptide actions on immune cells. At the actual state of knowledge, however, it can be suggested that molecular mechanisms of neuronal and hormonal control mechanisms of immune system could be important targets of development of novel methods for medication of autoimmune and neurodegenerative disorders.

3.3 Brain Cytokines

Brain cytokines are the main communication molecules in neuroimmune cross talk. Cytokines are soluble protein molecules that allow for communication between cells and external environment. Cytokines are expressed in the brain and modulate neuronal survival and cell death. To date, all cytokines were found in CNS cells including neurons. In addition to CNS cytokine effects, cytokines released by immune cells during inflammation can modulate physiological, neuroendocrine, and behavioral response to inflammation (Larson, 2002). Cytokines can modulate body temperature inducing fever, enhance sleep and modulate the hypothalamo–pituitary–adrenal gland axis, and initiate sickness behavior (Dantzer et al., 1998). In addition, cytokines effect depression and anxiety and modulate cell survival in stroke and hypoxia; so the scale of cytokine action on brain is fairly wide. Cytokines bind their specific receptors expressed on neurons,

astrocytes, oligodendrocytes, and microglia cells. Neurons express receptors for IL1, IL2, IL3, IL6, IL8, TNFα, and GM-CSF. In addition, neurons express the protein of IL1, IL2, IL3, and IL6. Astrocytes express receptors for IL1, IL3, IL4, IL6, IL7, IL8, IL9, IL10, TNFα, TGFβ, and GM-CSF. Expression of cytokine proteins has been found in astrocytes including protein of IL1, IL3, IL5, IL6, IL10, IL11, IL15, TNFα, IFN, TGFβ, GM-CSF, and M-CSF. Oligodendrocytes express cytokine receptors in great extent but only a few cytokine proteins are expressed by oligodendrocytes. Oligodendrocytes have receptors for IL1, IL2, IL3, IL4, IL7, IL15, TGFβ, GM-CSF, and M-CSF. However, only IL1 and TGFβ proteins are expressed in oligodendrocytes. Microglia cells, one of the tissue resident macrophages in the brain, express receptors of IL2, IL3, IL4, IL5, IL6, IL7, IL8, IL10, TNFα, IFN, TGFβ, GM-CSF, and M-CSF. Protein of IL1, IL5, IL6, IL10, IL12, IL15, TNFα, TGFβ, and M-CSF has also been found in microglia cells.

One of the main questions of cytokine action in the brain is that how and why such large molecules as cytokines (15–20 kDa) penetrate the BBB. Peripherally released cytokines act like hormones stimulating the hypothalamo–pituitary–adrenal axis and regulate neuroendocrine functions, sympathetic nervous system responses, behavioral events like fever, sickness, and sleep (Larson, 2002). Cytokines not only simply penetrate the BBB but also cross it at penetrable spots of the barrier like the periventricular organs. Cytokines also bind their receptors on cerebral blood vessels and signal the brain via NO and prostaglandins (De Simoni and Imeri, 1998). A rapid action of peripheral cytokines can reach the brain on vagal nerve (Floto and Smith, 2003). After peripheral cytokine stimulation on CNS, the central mechanisms of stress response are activated and corticotropin-releasing hormone (CRH) and adrenocorticotropic hormone (ACTH) are released from the hypothalamus and from the pituitary gland, respectively. In turn, adrenal gland releases glucocorticoids and as a final step cortisol in humans and corticosterone in rodents are released. As a feedback regulation, glucocorticoids suppress the release of some cytokines, not all of them. IL12, TNFα, and IL1 are the most sensitive cytokines for glucocorticoid suppression (Blalock, 2005). IL6 is relatively resistant to glucocorticoids. Summarizing the data of glucocorticoid effect on cytokines, it can be stated that glucocorticoids suppress proinflammatory cytokines and stimulate anti-inflammatory cytokines such as IL4 and IL10. Thus, we can conclude that glucocorticoids play a role in physiological adjustment of immune response. The molecular mechanism of glycocorticoid action on cytokines is unknown.

In addition to the neurohumoral routes of modulation of immune system by CNS, there are some more direct routes because autonomic and peripheral nervous systems innervate the immune system. Dense innervations can be observed in spleen, thymus, bone marrow, and lymph nodes (Mignini et al., 2003). In these innervations, the immune cells and the nerve cells are in close apposition, so they could form synapse like communication contacts (see more details in the following chapters). In such a close contact neuropeptides, neurotransmitters could exert effects on immune system. Molecular mechanisms of that borderline of immune and nervous systems are unknown and we are to give brief summary on the immunological and neuronal synapses later to shed some light to the relevance of ideas on neuroimmune synaptic communication.

There are evidences of interferences between neuropeptidergic communication and cytokine release. For example, VIP can enhance release of IL3, GM-CSF, IL6, TNFα, IFN, IL1β, and IL1α from astrocytes (Delgado et al., 2002). So the given variety of neuropeptides and cytokines in a small microenvironment containing neuronal endings and immune cells raises the possibility of complex interactions to be disclosed in near future. As we are just getting understand the importance and basic principles of neuroimmune cooperation in maintenance of homeostasis of the body, it is an open area of investigation. We have already understood that when the hypothalamus–pituitary–adrenal axis is damaged or altered in function, the subjects are enormously sensitive for inflammatory diseases while overstimulation of endocrine system can lead to either impaired immune function or increased susceptibility for infectious diseases. Depression of hormonal stress reaction was associated with autoimmune diseases like allergy, inflammatory diseases like rheumatoid arthritis, posttraumatic stress disorder, chronic fatigue, and atypical depression (Lechin et al., 2002).

Recently, clinical psychiatry was attracted by neuroimmune cross-talk principle because of the effects of cytokines on brain cells changes in psychiatric disorders like major depression, Alzheimer's disease, and schizophrenia. From early 1980s, several authors published observations on stress and cytokine receptor mRNA changes and changes in concentrations of different cytokines in humans. The stress induction and subjects were very different from examination stress to negative emotions and from volunteered students to

multiple sclerosis patients. The cytokines changes varied by experimental conditions and also by subject groups. The conclusion of these studies is that negative emotional stress and any kind of stress-inducing anxiety fair suppress immune response and decrease cytokine secretion (Lechin et al., 2002). Positive emotions and positive stress like examination stress of students could enhance immune response and cytokine release. Some cytokines have been tested in clinical practice and some of them induced psychiatric adverse effects. Interferons induced fatigue, depression, cognitive impairments, psychosis, and suicidal ideation (Guttereman, 1994). IL1 induced somnolence, confusion, and delusions. TNFα induced anorexia and fatigue. In schizophrenia, IL2 receptor concentration and IL6 secretion increase in the blood (Kronfol and Pemick, 2000). So it is an accepted idea that some types of schizophrenia are related to viral or bacterial infections.

One of the novel ideas of Alzheimer's disease pathophysiology is that cytokines play a critical role in its development and progression. Cells associated with amyloid plaques can produce cytokines; amyloid-β by itself can stimulate microglia to secrete proinflammatory cytokines. IL1, IL6, TGFβ, and TNFα have been associated with Alzheimer's disease progression. TNFα and TGFβ are present in senile plaques and contribute to the lesions around the plaques. IL1 level is elevated in the brain tissue of Alzheimer's disease patients. IL1 induces increased release of neurite extension factor S100β from activated astrocytes and in turn, the enhanced neurite growth promotes neuritic plaque formation. IL1 also regulates heparin sulfate proteoglycan synthesis, which is an important promoter factor of amyloid-β aggregation (Tarkowski et al., 2003).

4 Synaptic Innervation of Immune System by Autonomic Nervous System

Autonomic nervous system innervates all organs in the body including immune system. Accumulating evidences support that immune system is modulated by stress induced by the environment or by the pathological change in the internal environment under different diseases. Autonomic nervous system has two distinct parts: the sympathetic and the parasympathetic system. In physiological conditions, the balance of the two subsystems maintains the homeostasis, stabilizes blood pressure, heart rate, and so on. Sympathetic nervous system uses noradrenaline for neurotransmission and parasympathetic nerves are cholinergic. The involvement of autonomic nervous system in modulation of inflammatory and immune response is an exciting new field of research.

4.1 Cholinergic Anti-Inflammatory Pathway

The cholinergic anti-inflammatory pathway was discovered recently when the anti-inflammatory role of vagal nerve was demonstrated in endotoxemia and shock (Pavlov et al., 2003). It is a previously unrecognized cholinergic pathway for neuronal inhibition of inflammatory reaction and a novel interface between brain and immune system. The immune-to-brain communication can use two distinct mechanisms: one is neuronal and the other is humoral. Vagal nerve afferent fibers can be activated directly by cytokines released by dendritic cells, macrophage-associated immune cells, or indirectly via chemosensitive cells (Steven et al., 1998). The vagal afferents project to the solitary tract nucleus (NTS) and the connections of NTS are able to activate the PVN, in turn that activation reaches one of the main regulatory centers of hormonal system. Physiological evidences support that vagal nerve fails to suppress high-dose endotoxin-induced inflammation but it is efficient in suppression of reaction to mild or moderate doses. So the vagal cholinergic pathway has a particular importance in certain types of moderate inflammatory reactions (Pavlov et al., 2003).

The humoral mechanism of NTS activation by immune system is mediated by blood cytokines crossing the BBB entering the cerebrospinal fluid at circumventricular organs. Area postrema, one of the penetration sites, is very close to NTS and so the entering cytokines could activate NTS neurons. Cytokines can also bind to the capillary endothelial cell cytokine receptors and enhance release of different neurotransmitters as NO (Bianchi et al. 1995).

Brain-to-immune communication is the efferent part of cholinergic anti-inflammatory pathway. The humoral pathway is the hypophysis–adrenal gland axis, which reduces inflammation via releasing glucocorticoids and catecholamines. Cholinergic fibers participate in PVN–pituitary communication. Parasympathetic fibers also innervate immune system and vagal nerve fibers reach the thymus. This direct effect of vagal efferents on immune cells has not been properly established yet. Vagal efferents are able to suppress the release of inflammatory cytokines such as IL1β, IL6, IL18, and TNFα (Steven et al., 1998).

Acetylcholine has also a direct influence on immune cells. It turned out that immune cells express nicotinic acetylcholine receptor proteins (Hunyady et al., 1997). Lymphocytes not only express the receptor but also synthesize acetylcholine as well. Acetylcholine is immune-suppressive in vitro, decreasing levels of ILs and TNF. Vagotomy results in increased immune reaction, suggesting that vagal tone is important for immune homeostasis. Vagal stimulation has anti-inflammatory effect in local inflammation models such as carrageenan-induced inflammation in rodents (Tracey, 2002). It is a fast immunomodulatory effect, much faster than humoral regulation of immune system. Using pharmacological agents, anti-inflammatory effect of CNI-1493 requires vagal nerve; bilateral vagotomy eliminates all effects of it. Nicotinic acetylcholine receptor-α7 subunit is the essential component of the receptor necessary for cholinergic anti-inflammatory reaction (Pavlov et al., 2003). Clinical implications of cholinergic anti-inflammatory pathway include the application of implanted vagal nerve stimulator for suppression of immune response in autoimmune diseases, application of cholinergic agonists in endotoxemia and sepsis, and new methods for the treatment of autoimmune diseases.

4.2 Nonneuronal Cholinergic System and Immune Modulation

A nonneuronal cholinergic system is a network of nonneuronal cells producing acetylcholine. Such cells are widely expressed in the upper airway. Epithelial cells lymphocytes, macrophages, and mast cells could produce acetylcholine and they also express cholinergic receptors (see Wessler and Kirpatrick, 2001). The cholinergic gene encodes expression of vesicular acetylcholine transporter and choline–acetyl transferase protein and contains the regulatory sequences for controlling their expression. When a cell becomes cholinergic, the concentration of choline and acetyl CoA is about 50 and 5 μM, respectively. The acetyl group is derived from pyruvate. Interestingly, almost all mammalian cells have the components of acetylcholine synthesis but the concentrations of the enzymes are not sufficient for high quantity acetylcholine synthesis. The main functional role of the nonneuronal cholinergic system is that it could enhance the neuronal cholinergic influence locally.

5 Synaptic Communication is not the Privilege of Neurons

Cells all over the body communicate through the extracellular space by specific communication molecules having receptors on the surface of communicating cells. The extracellular space is not a space in the common sense, but it is filled up by special protein molecules forming the extracellular matrix. At particular spots of the membrane, the communication is more intensive than on the rest of the cell surface. The communication "hot spots" were named as synapses in the nervous system. In the last decade, it turned out that synapses are adhesive junctions transmitting information by directed secretion. As it has been revealed, synapses have a complex but amazingly uniform molecular construction. Comparison of immune system with nervous system disclosed that both systems use specific molecular recognition events between discrete cells, cell–cell adhesion, positional stability, and directed secretion for communication necessary for their responsive functions. Both systems have sophisticated molecular mechanisms for information storage. The neuronal synapse concept is >100 years old. The immune system synapse has been determined only recently. The function of the synapse in the immune system is to make the freely spreading small compounds direction specific; they coordinate cell migration and antigen recognition during immune response. The two synapses were approached so differently that it seems very promising to compare them to understand the functions of the synaptic communication.

The critical difference between immune and nervous synapses is in their function and in the wiring of the cellular networks behind. CNS is hard-wired, keeping the main wiring intact throughout the life. The immune system is more flexibly wired. In CNS, the cells are anchored to each other after the early ontogenesis preventing cell migration. The axons and dendrites are long; in turn, most of the CNS synapses act at a large distance from the cell body and nucleus. CNS synapses can be translocated and diminished but the dendrites and axons remain in place. One of the important consequences of that is the absence of direct translational control on CNS synapse and the synaptic transmission feedback to the transcription is slow and late. CNS synapses can alter their efficacy by clustering and remodeling their receptor sets (Dustin and Colman, 2002).

In contrast the immune system operates by rapidly migrating T cells and by their partner cells, the dendritic cell (DC). Each T cell expresses a different antibody but the spot where the antigen enters the body to associate with the DC cell cannot be predicted. Therefore, it is essential for T cells to make as many random contacts with DC cells as possible to find the matching DC cell and from a synapse (Dustin and Colman, 2002). Migrating T cells are similar to the growth cone of the neurons in early ontogenesis but much faster. By other words, T cells and DC cells cover greater distances than any neuron, but when they AU4 form a synapse they remain attached and proximal to the gene transcriptional machinery of the nucleus. DC cell is an APC destructing the antigen to peptides binding to the major histocompatibility gene complex molecule (MHC) and 300 MHC-p complexes on an APC can activate T cell via T-cell receptors and induce T-cell proliferation. One of the daughter cells of the T cell that became a memory cell can be activated by 50 MHC-p molecules. T-cell synapse is the best studied immunological synapse and it can be compared with the neuronal synapse in many aspects (Shaw and Allen, 2001). Prototypic synapse we have to create criteria for synapse differentiating synapse from other cell–cell contacts.

Criterion 1. It is that cells remain individuals. It means that synapse is a contact but there is no continuity between cells. The synapse is formed by two opposing membranes and a fluid in the synaptic cleft in between.

Criterion 2. It is the adhesion. Cells at the early stage of synapse formation recognize each other and they make an adhesion contact. The pre- and postsynaptic membrane regions are tightly locked together.

Criterion 3. It is the stability. Cell adhesion molecules clamp the pre- and postsynaptic membrane together. This clamp is very stable in time but some adhesion molecules can change conformation like N-cadherin during functioning of the synapse; but in spite of that, the synaptic structure remains considerably stable.

Criterion 4. It is the directed secretion. At the presynaptic side, a specific secretory apparatus is assembled and activated by signaling events. On the postsynaptic side, specific receptors are clustered and a molecular machinery transforming the secretory signal to a relevant intracellular signal. A special zone of the synaptic microdomain surrounds the communicating central zone of the synapse limiting the lateral spread of secreted molecules. That microdomain could change in response to secretory activity.

In CNS synapse, the pre- and postsynaptic membrane is connected to the synaptic scaffold. It is the two apposed parallel plates of the membranes attached by filamentous material spanning the synaptic cleft. The pre- and postsynaptic thickenings are also attached to a cytomatrix and that complex molecular scaffold recovers in synaptosome preparations after treatment with detergents (Loscher et al., 1985).

The CNS synapse has neurotransmitter machinery integrated with the scaffold and its interaction with the scaffold is not understood. We do not concern with the synaptic mechanism of neurotransmitter release in this chapter since we did it earlier. Several groups of recognition and adhesion molecules are in the scaffold. Cadherins are the major adhesion molecules of CNS synapse, but some others such as integrins are also important in synaptic adhesion. It is suggested that cadherins are important factors in recognition process in the recognition phase of synapse formation and later they clamp together the synaptic membranes. Neuronal cell adhesion molecules (NCAM) are also important elements of the synaptic scaffold. Presynaptic density proteins are a protein family involved in the synapse formation (Dustin and Colman, 2002).

The immune synapse construction is different from the CNS synapse but there are several features of CNS synapses like receptor clustering, or quantal release of transmitters, which can be interesting aspects for immunologists and oppositely, some lines of investigations on the immune synapse can be very promising in CNS synapse research. After the discovery of T cells, APCs, and lymphocyte adhesion

molecules in the early 1980s, it became clear that T cell–APC cell communication passes all criteria of synaptic communication. When T cell and APC cell interact, they remain as independent cells. Cell adhesion molecules clamp them together and MHCp–T cell interaction is a stop signal stabilizing the connection for long term. Secretion of immune cells is vectorial, so the immune synapse has all principal properties of a CNS synapse but it works differently to some extent and there are surprising similarities as well (Dustin and Colman, 2002).

The immune synapse is at the micron scale and it is a supramolecular activation cluster (SMAC) of particular proteins. The interaction zone between T cell and APC cell has a central SMAC surrounded by a ring of integrin-mediated cell adhesion molecules (ICAM) and microtubules are radially connected to the SMAC (Dustin and Colman, 2002). When the immune synapse is immature, the antigen presentation for T cell is at the peripheral zone of the synapse and the center is dominated by adhesive protein interactions. The first stage of immune synapse is dominated by adhesion molecule–receptor interactions and each molecule pair has their own distance determining the synaptic gap (Dustin and Colman, 2002). The SMAC formation is less known but it is hypothesized that an actin–myosin-dependent capping process forms the SMAC. There are no direct evidences for that.

The function of immune synapse is complex. One role is the induction of T-cell proliferation via integration of signals. Synapse can sustain signal patterns from APC cells in the early stage of the synapse. Later, the synapse became active and the SMAC zone is involved in secretion of small molecules such as cytokines and cytotoxic agents. The small and soluble molecules are held by SMAC, so they cannot diffuse freely away from the synaptic area. Comparing the immune synapse with CNS synapse in immune synapse integrins forms the gasket of the synapse while cadherins have the same role in the CNS synapse. Immune synapse changes functions within minutes and it is clearly demonstrated that in the early stage of synapse formation the molecules of the synaptic region play a different role dominantly in cell recognition and adhesion, later the secretion is the main function of the synapse (Shaw and Allen, 2001). As the synapse formation is faster in the immune system than in CNS and the size of the immune synapse is big, immune synapse is an ideal model for studying the early phase of synapse formation.

Recently, immunologists verify more and more CNS synapse proteins in the immune synapse. Agrin has a crucial role in neuromuscular synapse as clustering protein keeping the receptor molecules at the middle of the synapse. It has been identified in the immune synapse between T cells and APC cells (Mossman et al., 2005). That finding stimulates synaptologists and suggests that CNS and immune system synapses are variations of the same theme and organized by the same principles. The linkage of receptors to the synaptic scaffold by rapsyn, the importance of supramolecular structure of the synapse in general, the functional significance of central or peripheral location of a certain receptor molecules, and so on are common principles in both synapses. The scaffolding proteins are the same in the two synaptic structures. Postsynaptic density protein 95 (PSD95), gephyrin, GRIP, homer, Pick1, GABARAP are scaffolding proteins identified in CNS synapse first than in immune synapse (Mossman et al., 2005). Although the actual state of knowledge about CNS synapse is considerably more detailed compared with what it is known about immune synapse but immunologists found some important new aspects of synapse formation. One is that synapses can be formed rapidly and their lifetime can be as short as some minutes. It was disclosed because immunologists focused on synapse formation. In addition, the importance of protein charge and size in structure of membrane was studied by the immunologists in detail. Immune synapse develops quickly, so immunologists disclosed many novel mechanisms of molecular arrangement in cell contacts which could help in understanding neuronal development of CNS synapses. In conclusion, immunologists and neurobiologists can learn from each other about the synaptic communication and this is a promising new interdisciplinary research area for the future.

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