学位論文内容の要旨
（Summary of Dissertation）

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学位論文題名
（Dissertation Title）
Development of Remote Inflammation through Interneuron Network in the Spinal Cord
(脊髄の介在ニューロンネットワークを介した遠隔炎症の発症)

Summary:

Background and purpose: Rheumatoid arthritis (RA) is a chronic inflammatory disease accompanied by joint inflammation, synovial hypertrophy, and progressive destruction of cartilage and bone, and affects 1% of the adult population worldwide. One criterion of the diagnosis and a fundamental characteristic of RA is remote inflammation, which results in widespread and severe malformation and immobility on both sides of joint. Although several studies have suggested that a neural mechanism is involved in the spreading inflammation of RA, the molecular links of inflammatory and neural pathways have not been uncovered. We previously found an amplification mechanism of inflammatory response, so called "IL-6 amplifier", in which a simultaneous activation of IL-6-STAT3 and other cytokines (e.g. IL-17, TNF-α, and growth factors)-NF-κB pathways, induces an excessive activation of NF-κB in tissue-specific non-immune cells, resulting in chronic inflammation through a local recruitment of immune cells and robust production of inflammatory cytokines such as IL-6 *per se* and chemokines. Accordingly, we have defined inflammation as an IL-6-mediated accumulation of immune cells, and/or proliferation of immune cells and regional non-immune cells, followed by the dysregulation of local homeostasis including the dysfunction of tissues and/or organs. Furthermore, we also discovered “gateway reflex” as the regulatory mechanism of IL-6 amplifier by activation of specific neural circuit. Environmental factors such as gravity, weak electricity, pain, stress, and light stimulate specific neural circuits to produce neurotransmitter locally, and induce or inhibit (only by light stimulation) IL-6 amplifier that subsequently creates a specific gateway for immune cells at blood barrier, and then promote or suppress (only by light stimulation) an onset of chronic inflammatory diseases.
Based on our original discoveries regarding IL-6 amplifier and gateway reflex, we hypothesized that a local neural regulation for bilateral inflammation may trigger initial inflammation and promotes the subsequent recruitment of immune cells by releasing attracting factors such as chemokines via the IL-6 amplifier. In my doctoral research, I aimed to clarify the underlying molecular mechanism of how specific neural circuit interconnects with bilateral inflammation for spreading pathology.

Materials and methods: To explore the molecular basis via neural pathways for spreading inflammation, F759 mice in which IL-6-mediated STAT3 activation is enhanced due to an amino acid substitution, Y759F in gp130 (IL-6 signal transducer in the IL-6R complex) at the binding site of SOCS3 for the negative regulation of IL-6 signaling, and spontaneous development of an RA-like disease in both sides of the ankle joint at around one year of age occur, were used as a representative cytokine-induced arthritis model as well as in a collagen-induced arthritis

model (CIA) throughout my study.
Since we hypothesized that spreading inflammation in RA might depend on neural pathways distributed on both sides, F759 mice were injected with IL-6 plus IL-17A, saline, or a neurotransmitter, adenosine triphosphate (ATP), with or without anti-IL-6R, anti-IL-17A, control IgG, A438079 (ATP receptor P2X7 antagonist) at unilateral or bilateral ankle joints. For some experiments, F759 mice induced arthritis by cytokine injections were deafferented the sensory neurons at one side of the dorsal root ganglions (DRGs) beside the fifth lumbar cord (L5) or spinal cord cut. These mice were then assessed for the disease severity based on two bilaterally parameters: (1) swelling of the ankle and (2) restricted mobility of the ankle joints. The severity of each parameter was graded on a scale of 0–3, where 0 indicates no change; 1, mild change; 2, medium change; and 3, severe change. Averages for a single point in one leg ankle joint from each mouse were used. Ipsilateral and/or contralateral ankle joints, DRG at third to sixth lumbar cords (L3-L6) and nineth to thirteenth thoracic cords (T9-13), were used for examining recruitment of immune cells and neural activation status, and identification of specific sensory neuron and interneuron by immunohistochemistry (IHC), for quantifying c-fos (neural activation status), cytokine, and chemokine expression levels by real-time PCR, and for measuring cytokine and neurotransmitter levels by ELISA. For assessing NF-κB activity, ankle joints from NF-κB-reporter Tg/F759 mice induced arthritis by IL-6 plus IL-17A injections were collected, and synovial tissues were homogenized in passive lysis buffer. After centrifugation, the supernatants were collected and analyzed for luciferase activities using luciferase reporter assay system. To investigate the neural networks in spinal cord that connect sensory pathways between the ankle joints, herpes simplex virus 2 (HSV2) was employed to trace neural connections regardless of the presence of synapses. The presence of HSV2 was detected by IHC and quantified by real-time PCR.

Results: The surgical ablation or pharmacological inhibition of neural pathway at one side of ankle prevented inflammation development on the other side. Mechanistic analysis showed that ATP induced by activation of the IL-6 amplifier in collagen type 1+ non-immune cells in one side of the ankle joint activates Nav1.8+ TRPV1+/- sensory neurons, which further stimulate the regional sensory neural pathway involving the lower thoracic spinal cord that contains proenkephalin+ interneurons. On the contralateral side, the response of sensory neurons in L4-L6 DRGs releases ATP, which induces inflammatory mediators, including cytokines and chemokines, by activating the IL-6 amplifier in collagen type 1+ non-immune cells, including fibroblasts and endothelial cells, through a ATP receptor, P2RX7.

Discussion: It is known that spreading inflammation is common in RA. The present study clarifies the molecular link for spreading inflammation between bilateral ankle joints in cytokine-induced and CIA models. Cytokine injections into one ankle joint of F759 mice induce ATP release from non-immune cells and subsequently activate afferent sensory pathways with c-fos induction on both sides of L5 DRG toward the contralateral ankle joint through a proenkephalin+ interneuron network in the thoracic cords, suggesting that the inflammation signal from one side activates sensory neurons in both the ipsilateral and contralateral sides. A previous report showed that RA synovial fibroblasts (RASFs) are able to migrate and contribute to the spread of the disease between bilateral synovial tissues in SCID mice. Although the authors identified RASFs as one key factor for spreading inflammation from a single joint to multiple ones, how RASFs reach the other joints remains to be elucidated. The present study suggests that the local neural regulation for bilateral inflammation may trigger the initial inflammation and promotes the subsequent recruitment of RASFs by releasing chemokines via IL-6 amplifier. Thus, a regional sensory neuron-interneuron connection between the ankle joints through the thoracic spinal cord is critical for spreading inflammation via the bilateral expression of ATP, which activates a neural pathway and enhances the IL-6 amplifier.

Conclusion: My doctoral study suggests that blockade of the sensory neuron-interneuron axis may be a therapeutic target for various inflammatory diseases including RA, in which inflammation spreads to remote positions.