Institute of DNA Medicine
Department of Molecular Immunology

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General Summary

Our research interests have focused on the analysis of the basic immune system to protect us from diseases and of immune disorders, such as hypersensitivity diseases and autoimmune diseases.

Research Activities

The role of CRTH2 in Th1 and Th2 inflammatory reactions

Many reports support the concept that chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2) mediates the proinflammatory effects of prostaglandin (PG) D_2 in Th2-related inflammatory reactions. However, little is known about the Th1-related inflammatory reactions. The aim of this study was to investigate the role of CRTH2 in various inflammatory conditions. To induce Th2-related inflammatory reactions, BALB/c mice were infected with *Nocardia brasiliensis,* and serum IgE levels, numbers of eosinophils, and the antigen-specific Th2-cytokine production of spleen cells were measured 2 weeks after infection. To induce Th1-related inflammatory reactions, BLAB/c mice were given subcutaneous injections of complete Freund adjuvant, and interferon γ production was measured for 72 hours in a culture of draining lymph node cells stimulated with purified protein derivative. To analyze the role of CRTH2, ramatroban, a selective CRTH2 antagonist, was orally administered to some mice to inhibit CRTH2 signaling *in vivo.* In addition, CRTH2-knockout mice were also used for further analysis of the role of CRTH2. In mice infected with *N. brasiliensis,* ramatroban administration inhibited IgE production, eosinophilia, and Th2 cytokine production. In contrast, in mice treated with complete Freund adjuvant, ramatroban administration enhanced interferon γ production. Similar results were obtained in CRTH2-knockout mice. These results indicate that CRTH2 mediates the proinflammatory effects of PGD_2 in Th2 inflammatory reactions and mediates the anti-inflammatory effects of PGD_2 in Th1 inflammatory reactions.

Enhancing activity of N-glycosylation for constitutive proteins secretions in nonpolarized cells

Several fusion proteins of mouse (m) interleukins (ILs) and the enhanced green fluorescent protein were expressed in fibroblasts and epithelial cells. Among these proteins, the mIL-31 derivative was the most efficiently secreted into the medium in a N-glycosylation—dependent manner. Analysis of deletion mutants revealed that the minimal structure for constitutive secretion consisted of a signal peptide and N-

glycosylation. Introduction of the signal sequence from mIL-31 to human p53 protein failed to lead to the secretion of the products, but further addition of the N-glycosylation site resulted in constitutive secretion of biologically active p53 protein into the medium in the N-glycosylated form. This report has shown the importance of N-glycosylation for constitutive protein secretion, especially in nonpolarized cells.

Alteration of inflammatory cytokine production in the injured central nervous system of tenascin-deficient mice

Although tenascin-C (TN) is highly up-regulated during the proliferation of reactive astrocytes, little is known about the function of TN at injury sites in the central nervous system. We investigated the function of TN-expressing astrocytes in the injured brain by analyzing TN-deficient mice with stab-wound injuries of the cerebral cortex. Expression of glial fibrillary acid protein was down-regulated earlier after injury in TN-deficient mice than in wild-type (WT) mice. To evaluate immune responses in the injured central nervous system in the absence of TN, inflammatory cytokine production was examined after unilateral stab injuries of the cerebral cortex in TN-deficient and WT mice. The expression of IL-1 beta, tumor necrosis factor-alpha, and IL-6 was higher in TN-deficient mice, whereas levels of IL-4 and granulocyte colony-stimulating factor were lower in TN-deficient mice than in WT mice. Our findings suggest that TN helps to regulate the production of inflammatory cytokines in the injured brain.

Publications

Akiyama N, Ohno Y, Fukuda T, Manome Y, Saito S. Enhancing activity of *N*-glycosylation for constitutive proteins secretions in non-polarized cells. *Biochem Biophys Res Commun* 2009; **381:** 612–8.

Yamada K*[1]*, Akiyama N, Yamada S*[1]*, Tanaka H*[1]*, Saito S, Hiraoka M*[1]*, Kizaka-Kondoh S*[1] *(*[1]Kyoto Univ).*** Taip2 is a novel cell death-related gene expressed in the brain during development. *Biochem Biophys Res Commun* 2008; **369:** 426–31.

Ikeshima-Kataoka H, Shen JS, Eto Y, Saito S, Yuasa S. Alternation of inflammatory cytokine production in the injured central nervous system of tenascin-defeicient mice. *In Vivo* 2008; **22:** 409–13.

Wakahara K*[1]*, Tanaka H*[1]*, Takahashi G*[1]*, Tamari M*[1]*, Nasu R*[1]*, Toyohara T*[1]*, Hirohisa Takano*[1]*, Saito S, Inagaki N*[1]*, Shimokata K*[1]*, Nagai H*[1] *(*[1]Gifu Pharm Univ).*** Repeated instillations of *Dermatophagoides farinae* into the airways can induce Th2-dependent airway hyperresponsiveness, eosinophilia and remodeling in mice: effect of intratracheal treatment of fluticasone propionate. *Eur J Pharmacol* 2008; **578:** 87–96.

Reviews and Books

Saito S, Akiyama N. Pleiotropic function of IL-31 (in Japanese). *Rinshoumeneki・Arerugi-ka* 2008; **50:** 640–3.