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1 .Topic in Research Achievements in the Year 2006

1. Intra-articular gene transfer of cyclin-dependent kinase inhibitors (CDKI) to suppress synovial cell cycling has shown efficacy in treating animal models of rheumatoid arthritis (RA). CDKIs also modulate immune function via a CDKindependent pathway. Accordingly, systemic administration of small molecules that inhibit CDK might ameliorate arthritis. In order to address this issue, alvocidib (flavopiridol), known to be tolerated clinically for treating cancers, and also a newly synthesized CDK4/6-selective inhibitor were tested for anti-arthritic effects. In vitro, they inhibited proliferation of human and mouse synovial fibroblasts without inducing apoptosis. In vivo, treatment of collagen-induced arthritis (CIA) mice with alvocidib suppressed synovial hyperplasia and joint destruction while serum concentrations of anti- type II collagen (CII) antibodies and proliferative responses to CII were maintained. Treatment was effective even when therapeutically administered. Treated mice developed arthritis after termination of treatment. Thus, immune responses to CII were unimpaired. Similarly, the CDK4/6-selective inhibitor suppressed CIA. Both small molecule (sm) CDK inhibitors were effective in treating animal models of RA not by suppressing lymphocyte function. We believe that smCDK inhibitors hold promise as a new class of anti-rheumatic drugs that inhibit a distinct phase of rheumatoid pathogenesis.

2. Increased bone resorption mediated by osteoclasts causes various diseases such as osteoporosis and bone erosion in rheumatoid arthritis (RA). Osteoclasts are derived from monocyte/macrophage lineage, however, the precise origin remains unclear. In this study, we show that purified $CD16^-$ human peripheral blood monocyte subset, but not $CD16^+$ monocyte subset, differentiated into osteoclast by stimulation with receptor activator of NF-κB ligand (RANKL) in combination with M-CSF. Integrin β3 mRNA and αvβ3 heterodimer were only expressed on $CD16^-$ monocytes, when they were stimulated with RANKL + M-CSF. Downregulation of β3 subunit expression by small interfering RNA targeting β3 abrogated osteoclastogenesis from $CD16^-$ monocyte subset. In contrast, $CD16^+$ monocyte subset expressed larger amounts of TNF-α and IL-6 than $CD16^-$ subset, which was further enhanced by RANKL stimulation. Examination of RA synovial tissue showed accumulation of both $CD16^+$ and $CD16^-$ macrophages. Our results suggest that peripheral blood monocytes consist of two functionally heterogeneous subsets with distinct response to RANKL. Osteoclasts seem to originate from $CD16^-$ monocytes, and integrin β3 is necessary for the osteoclastogenesis. Thus, blockade of accumulation and activation of $CD16^-$ monocytes could be a beneficial approach as an anti-bone resorptive therapy, especially for RA.

2 .Publications in the year 2006

1. Nonomura Y, Nagasaka K, Hagiyama H, Sekine C, Nanki T, Tamamori-Adachi M, Miyasaka, N and Kohsaka H Cyclin-dependent kinase 4/6 directly modulates expression of rheumatoid inflammatory mediators in retinoblastoma protein-dependent and independent pathways ***Arthritis Rheum*** 54(7): 2074-2083, 2006

2. Murakami Y, Kohsaka H, Kitasato H, and Akahoshi T. Lipopolysaccharide-induced up-regulation of triggering receptor expressed on myeloid cells-1 expression on macrophages is regulated by endogenous Prostaglandin E2. ***J Immunol*** 178: 1144-115-, 2007

3. Sugihara T, Sekine C, Nakae T, Kohyama K, Harigai M, Iwakura Y, Matsumoto Y, Miyasaka N, and Kohsaka H. A new murine model defines critical mediators in the

pathology and treatment of polymyositis ***Arthritis Rheum*** (in press)
4. ○Yukiko Komano, Toshihiro Nanki, Kenji Hayashida, Ken Taniguchi, Nobuyuki Miyasaka. Identification of a human peripheral blood monocyte subset that differentiates into osteoclasts. Arthritis Res. Ther. 8(5): R152, 2006.
5. Abe T, Takeuchi T, Miyasaka N, Hashimoto H, Kondo H, Ichikawa Y, Nagaya I. A multicenter, double-blind, randomized, placebo controlled trial of infliximab combined with low dose methotrexate in Japanese patints with rheumatoid arthritis. J. Rheumatol. 2006;33(1):37-44
6. Ogawa J, Harigai M, Akashi T, Nagasaka K, Suzuki F, Tominaga S, Miyasaka N. Exacerbation of chronic active Epstein-Barr virus infection in a patient with rheumatoid arthritis receiving humanized anti-interleukin-6 receptor monoclonal antibody. Ann. Rheum. Dis. 2006;65(12):1667-1669
7. Ochi S, Harigai M, Mizoguchi F, Iwai H, Hagiyama H, Oka T, Miyasaka N. Leflunomide-related acute interstitial pneumonia in two patients with rheumatoid arthritis: autopsy findings with a mosaic pattern of acute and organizing diffuse alveolar damage. Mod. Rheumatol. 2006;16(5):316-320
8. Devi S.S, Hagiyama H, Adachi T, Miyasaka N, Tsubata T. The tumor suppressor p53 is not required for antigen receptor-mediated apoptosis of B lymphocytes. Signal Transduction 2006;6:54-61
9. Miyasaka N, Takeuchi T, Eguchi K. Guidelines for the proper use of etanercept in Japan. Mod Rheumatol. Review 2006;16(2):63-67
10. Miyasaka N. Etanercept and methotrexate for the treatment of rheumatoid arthritis. Therapy 2006;3(3):365-373
11. Miyasaka N. Issues in the usages of new anti-rheumatic drugs in Japan. Jap Med Assoc J. 2006;49(5･6):208-211

３. Abstracts in the year 2006
1. Yoshinori Nonomura, Masayasu Toyomoto, Tosiniro Nanki, Nobuyuki Miyasaka, Hitoshi Kohsaka Hypoxia Overcomes Contact-Dependent Proliferative Inhibition of Rheumatoid Synovial Fibroblasts by Downregulating N-Cadherin and Subsequent p27Kip1 Expression. American College of Rheumatology 70th National Meetings, Washington, DC, November 11-16 , 2006
2. Tetsuo Kubota, Rieko Hashimoto, Yasuko Fukuya, Toshihiro Nanki, Nobuyuki Miyasaka, Kazuo Umezawa. Suppression of Key Molecules Involved in the Thrombogenic Mechanisms of Antiphospholipid Syndrome by an NF-□B Inhibitor. 8th International Congress on SLE.2007.
3. Yoshinori Nonomura, Masayasu Toyomoto, Tosiniro Nanki, Nobuyuki Miyasaka, Hitoshi Kohsaka Hypoxia Overcomes Contact-Dependent Proliferative Inhibition of Rheumatoid Synovial Fibroblasts by Downregulating N-Cadherin and Subsequent p27Kip1 Expression. American College of Rheumatology 70th National Meetings, Washington, DC, November 11-16 , 2006
4. Toshihiro Nanki, Takeshi Shimaoka, Kenji Hayashida, Ken Taniguchi, Shin Yonehara, Nobuyuki Miyasaka. Pathogenic Role of CXCL16-CXCR6 Pathway in Rheumatoid Arthritis. Keystone symposia, Chemokines and Chemokine Receptors. 2006.