Introduction: Rheumatoid arthritis (RA) is characterized by chronic proliferative synovitis leading to poyarticular destruction. Analysis of the mechanism of synovial autonomous proliferation may be important to better understanding of the joint destruction and to new therapies for RA. This study provides the data that membrane-associated IL-1 (MA-IL-1) contributes to the understanding of the joint destruction and to new therapies for RA. This study has revealed both 33 kDa precursor and 17 kDa mature forms of IL-1α by immunoprecipitation of the culture media and lysates of the synoviocytes from the Tg mice. IL-1α precursor of 33 kDa, which is biologically active, can be isolated from the membrane fraction. MA-IL-1 on the PFA-fixed cells was stained simultaneously with phycoerythrin-labeled anti-hIL-1α mAb and cytokine-labeled anti-macrophage antibody (F4/80), and then analyzed by fluorescence-activated cell sorter (FACS). Cell surface localization of MA-IL-1 in the synovocytes was also studied by FACS analysis of the cells after incubation with 10 µg/ml trypsin which causes a cleavage of IL-1α as described [6], and by immunoprecipitation of the [35S] methionine-labeled synoviocyte membranes with anti-hIL-1α mAb followed by SDS-PAGE. MA-IL-1 bioactivity of the synoviocytes, which is known to be detectable using PFA-fixed synoviocytes during the final 4 hr of a 48 hr culture with or without the neutralizing antibodies against either human or mouse IL-1α. Similarly, the effect of MA-IL-1 on synoviocyte proliferation was determined by culturing the cells on PFA-fixed synoviocytes. In each experiment, synoviocytes derived from the littersmates (C3H background) were used as a control.

Results: Immunostaining of cultured synoviocytes demonstrated cell surface localization of IL-1α. Two-color FACS analysis revealed that both macrophage- and fibroblast-like cells express IL-1α when treated with or without PFA-fixation. Trypsin treatment removed the putative MA-IL-1 from the cell surface (Fig.1) and identified a 33 kDa protein immunoprecipitated from the membrane fraction: MA-IL-1 on the PFA-fixed synoviocytes was biologically active since D10.G4.1 cells proliferated when cultured on such synoviocytes. Pre-incubation with either anti-human or anti-mouse IL-1α mAb before the bioassay resulted in the significant inhibition of D10.G4.1 cell proliferation, suggesting that bioactivity of the PFA-fixed synoviocytes is due to MA-IL-1 of transgene-derived IL-1α. Importantly, MA-IL-1 on PFA-fixed synoviocytes exerted an accelerated effect on the proliferation of synoviocytes (Fig.2A). The expression of MA-IL-1 was induced earlier and persisted longer than secreted IL-1α (Fig.2B).

Discussion: The results in the present experiments indicate that the transgene-derived MA-IL-1 is expressed on the synoviocytes from IL-1α Tg mice. Since MA-IL-1 on the synoviocytes is biologically active to stimulate the cells to proliferate, it appears to play a role in synovial autonomous proliferation through cell to cell interaction. Our previous study has revealed high levels of metalloproteinases expression in synoviocytes and chondrocytes adjacent to the pannus [8] and it suggests that MA-IL-1 stimulated synoviocytes may trigger their own proliferation and induce the degradation of cartilage adjacent to the synovial pannus through cell to cell, juxtacrine mechanism during the course of arthritis in Tg mice.

References:
5) Gilles K et al. Blood 84:4242-4424,1994
9) Department of Orthopaedic Surgery, Kose University School of Medicine, Tokyo
10) Department of Pathology, Keio University School of Medicine, Tokyo
11) Lab. for Molecular Biology, Medicinal Research, Taisho Pharma Co., Ltd.