The detection of plasma levels of connective tissue growth factor in rheumatoid arthritis patients

Sirs,

Connective tissue growth factor (CTGF) is a member of the CCN family (CCN2) that was discovered due to its cross-reactivity with the platelet-derived growth factor (PDGF) antiserum. It is a single 38 kDa polypeptide secreted by cultured human umbilical vein endothelial cells (HUVECs). CTGF has functions in several biological processes such as fibrosis, tumourgenesis, angiogenesis, and endochondral ossification (1-3). Our recent proteomice analyses revealed that infliximab, a tumour necrosis factor (TNF)-alpha antibody, dramatically changes the concentration of CTGF in the serum and plasma of rheumatoid arthritis (RA) patients (4). We have also found that TNF-alpha induces CTGF in RA patient-derived synovial cells and that CTGF promotes the aberrant activation of osteoclasts, which results in bone destruction in RA patients (5). These findings suggested that CTGF strongly contributes to the development of RA. We herein investigated the blood levels of CTGF in RA patients.

Blood samples were collected from RA patients (n=31, male; 8, female; 23, age; 57±12.1, disease activity score 28 = DAS28; 3.34±0.94) and normal volunteers(n=13, male; 6, female; 7, age; 33±7.9), and all of the participants provided written informed consents. The CTGF present in blood is an N-terminal fragment (N-fragment) consisting of modules 1 and 2 (M1,2), whereas the CTGF in platelets is the full-length molecule (full-length) consisting of four conserved domains (modules 1–4; M1,2,3,4). To avoid contamination with full length CTGF derived from platelets and to accurately the detect CTGF (N-fragment) in blood, we examined the plasma levels of CTGF in RA patients using a recently developed two sandwich enzyme-linked immunesorbent assay (ELISAs; M1/M2 ELISA and M1/M2/M3/M4 ELISA) (6). The M1/M2 ELISA measured the total CTGF levels, including both the N-terminal CTGF fragment (M1,2) and full-length CTGF (M1,2,3,4). The M1/M2/M3/M4 ELISA measures only the full length CTGF (M1,2,3,4) levels. The plasma N-terminal CTGF level is then determined by subtracting the full-length CTGF levels from the total CTGF levels.

Briefly, plasma samples were added to 96 well plates (Nunc 475094) which were coated with a monoclonal antibody (mAb) against human CTGF modules (anti-CTGF module 1 in both the M1/M2 ELISA and M1/M2/M3/M4 ELISA; Nosan Corporation, Yokohama, Japan) for 12 hours at 4°C. A biotin–labeled anti-human CTGF mAb (Nosan Corporation) was added (anti-CTGF module 2 in the M1/M2 ELISA, and anti-CTGF module 4 in the M1/M2/M3/M4 ELISA) for 1 hour, and a peroxidase-conjugated streptavidin antibody (Jackson Immuno Research, PA, USA) was added for an additional 1 hour. The optical density at 450 nm was read to measure the CTGF concentration (6). Data regarding the C-reactive protein (CRP) concentration (normal range; n <0.3 mg/dl), the erythrocyte sedimentation rate (ESR) (n <10 mm/1hour), the white blood cell (WBC) count (4,000 <n <8,000 mm^3), and matrix metalloproteinase-3 (MMP-3)(36.1 <n <121 ng/ml) levels were obtained by routinely laboratory examinations. The serum levels of cartilage oligometric matrix protein (COMP), which is known to be derived from damaged cartilages (7), were measured by an ELISA system (Abnova, Taipei City, Taiwan). The cut-off point of CTGF or COMP was obtained from the mean value plus 3 standard deviation of the normal controls, and the normal ranges were considered to be those less than the cut-off point. The normal ranges of CTGF and COMP are <29.55 ng/ml and <687.64 ng/ml, respectively.

As shown in panel A, the CTGF (N-terminal fragment) levels were higher in RA patients in comparison to the normal controls (Student's *t*-test, $p<0.05$). Our data also indicated that the serum levels of COMP in RA patients (634.08±19.27 ng/

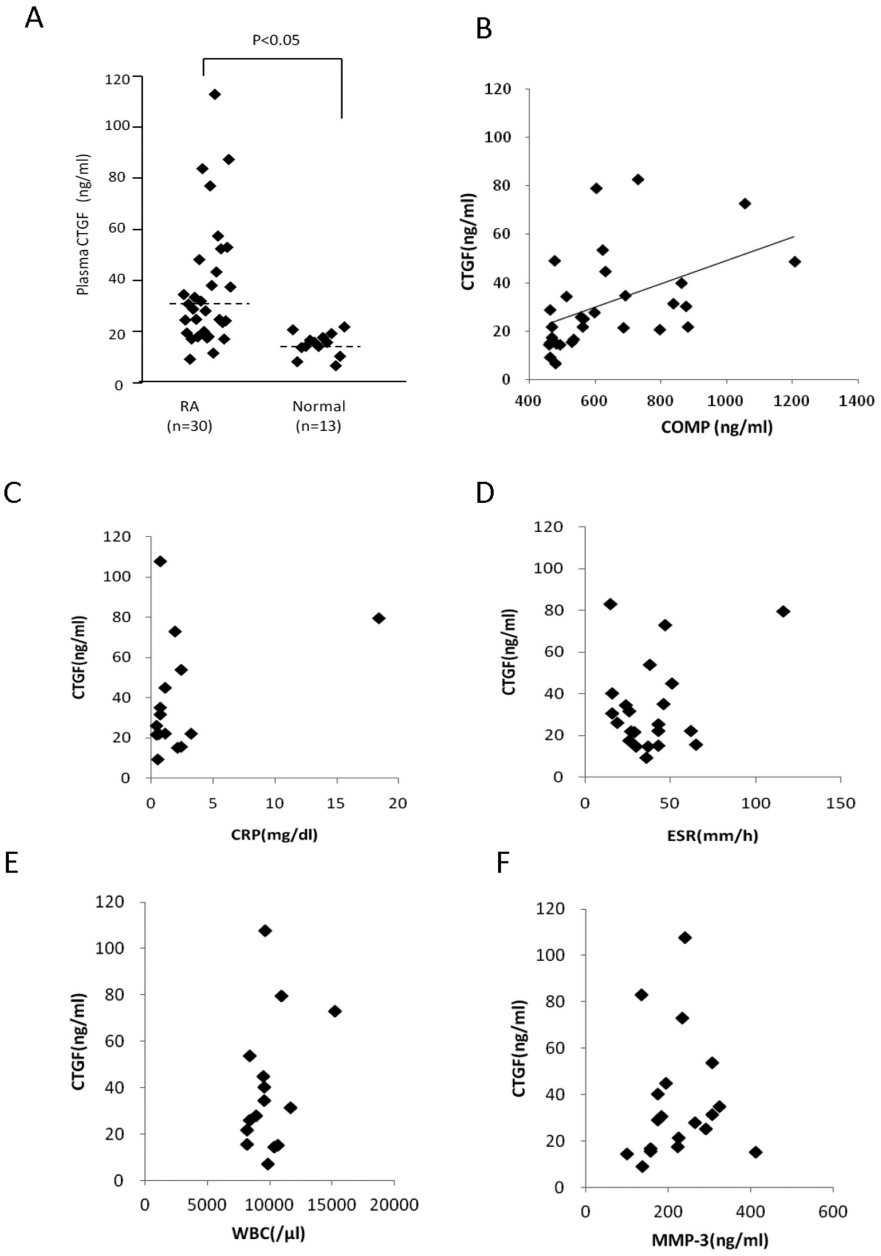

Fig. 1. Panel **A-F**: The plasma concentrations of CTGF in RA patients and normal controls (**A**), and the relationships between the CTGF levels and other RA-related biomarkers such as the COMP (**B**), CRP (**C**), ESR (**D**), WBC (**E**), and MMP-3 (**F**) levels. The dotted lines in panel **A** indicate the mean values of CTGF. (n) in panel **A** indicates the number of samples.

ml) were significantly higher in normal controls (500.07±62.29 ng/ml) ($p<0.05$), and that there was a statistically significant correlation between the concentrations of COMP and N-terminal CTGF in RA patients (Spearman's rank-correlation coefficient; n=31, r=0.6, $p=0.001$) (panel B). No statistical significantly correlations were observed between the CTGF levels and RA-related features, such as the CRP, ESR, WBC count, or the MMP-3 level in RA patients (n=31) (panel C–F). The CTGF levels in RA patients seem to reflect the degree of cartilage destruction, but not inflammation, although further studies involving large number of RA patients will required to confirm our finding. In addition, we cannot deny the possibility that a correlation exists between CTGF and COMP based on their increased production derived from activated synovial fibroblasts (panel B) (8). Regarding RA-related inflammatory markers such as CRP, ESR, and MMP-3, the average levels in patients treated with or without biologics were as follows; CRP 0.5 and 1.7 mg/dl, ESR 23 and 33 mm/h, MMP-3 184 and 172 ng/ml, respectively. Therefore, no significant differences in these markers were observed between patients treated with and without biological agents. However, our preliminary results also indicate that the plasma concentrations of N-fragment CTGF in RA patients treated with biological therapies such as infliximab were significantly lower than those in RA patients not treated with these biologics, because the CTGF levels in patients treated with (n=9) and without (n=22) biologics were 22.9±7.7 ng/ml and 38.5±26.7 ng/ml, respectively ($p<0.05$). These results may be due to the efficacy the treatments with biological agents for preventing bone destruction (9). Therefore, the CTGF level seems to be an useful new biomarker for bone destruction in RA patients, and we are now investigating the inhibitory effects of anti-CTGF antibodies on the development of RA using mouse models.

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