Inhibition of Src Homology 2 Domain-Containing Protein Tyrosine Phosphatase Substrate-1 Reduces the Severity of Collagen-Induced Arthritis

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ABSTRACT. ***Objective.*** To investigate whether the blockade of Src homology 2 domain-containing protein tyrosine phosphatase substrate-1 (SHPS-1) has any therapeutic effects on rheumatoid arthritis.

Methods. A functional blocking monoclonal antibody for SHPS-1 (anti-SHPS-1 mAb) was administered at various doses to collagen-induced arthritis (CIA) mice, and severity of the arthritis was evaluated by clinical and histological scores of the limbs. To clarify the mechanisms of action of the antibody, the serum concentration of anti-type II collagen antibody was measured in those mice, and *in vitro* experiments were conducted to determine the effects of the antibody on the induction of osteoclasts and the release of cytokines from mouse spleen cells.

Results. Compared with mice given control IgG, the administration of anti-SHPS-1 mAb significantly reduced the severity of inflammation and destruction of bone and cartilage in CIA mice. This therapeutic effect was observed even when the antibody treatment was started after the onset of arthritis. The appearance of anti-type II collagen antibody in CIA mice was not altered by the antibody treatment. In *in vitro* experiments, the anti-SHPS-1 mAb significantly inhibited osteoclastogenesis of bone marrow cells, and significantly reduced the release of interleukin 1ß (IL-1ß), IL-2, IL-12, interferon-γ, and tumor necrosis factor-α, but not that of IL-4 or IL-10, from the spleen cells after stimulation with concanavalin A.

Conclusion. Administration of a monoclonal antibody for SHPS-1 reduced the severity of arthritis in CIA mice. Regulation of biological functions of SHPS-1 may be a novel and potent strategy to treat patients with rheumatoid arthritis. (First Release Nov 1 2008; J Rheumatol 2008;35:2316–24; doi:10.3899/jrheum.080369)

Key Indexing Terms:
SHPS-1 RHEUMATOID ARTHRITIS THERAPEUTICS

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Rheumatoid arthritis (RA) is a chronic inflammatory disease that affects synovial joints systemically[1]. In spite of numerous investigations, there is still no fundamental therapy to treat RA. The hallmarks of this disease are synovial inflammation and destruction of articular cartilage and subchondral bone. Synovial tissue in rheumatoid joints is characterized by a marked intimal-lining hyperplasia due to increased numbers of macrophages and fibroblast-like synoviocytes. Accumulation of T cells, plasma cells, and other types of inflammatory cells in the synovial lining is also obvious[1,2]. Those cells produce cytokines such as interleukin 1ß (IL-1ß), tumor necrosis factor-α (TNF-α), and IL-6, which promote the expression of proteinases that cause tissue degradation in the joints. As well, those cytokines are responsible for the destruction of bone in the disease through the induction of osteoclasts. In joints involved in RA, osteoclasts are derived from precursor cells of the monocyte-macrophage lineage in the presence of several cytokines such as macrophage colony-stimulating factor (M-CSF), receptor activator of nuclear factor-κB ligand (RANKL), IL-1ß, and

TNF-α[3,4]. Therefore, the synovial hyperplasia and bone absorption around the joints in RA is closely correlated through the activity of those cytokines.

Src homology 2 domain-containing protein tyrosine phosphatase substrate 1 (SHPS-1), also known as signal regulatory protein α1 (SIRPα1)[5], a brain Ig-like molecule with tyrosine-based activation motifs[6], macrophage fusion receptor[7], and p84 neural adhesion molecule[8], is a transmembrane glycoprotein that belongs to the immunoglobulin superfamily. The extracellular domain of SHPS-1 consists of 3 parts, the amino-terminal Ig variable (IgV) region and 2 Ig constant (IgC) regions, although the latter may be removed by alternative splicing. The intracellular domain of SHPS-1 contains 2 immunoreceptor tyrosine-based inhibitory motifs, suggesting that it transmits signals for inactivation[9]. SHPS-1 is expressed by macrophages, dendritic cells (DC), neutrophils, and neurons[10]. CD47, another transmembrane glycoprotein belonging to the immunoglobulin superfamily, is a known ligand for SHPS-1[11-14]. CD47 is present on virtually all kinds of hemopoietic cells, including T cells, B cells, and neutrophils, as well as endothelial cells. SHPS-1 binds to CD47 via the IgV domain[11,15-18], which causes its various biological activities[19]. Interactions between SHPS-1 and CD47 are important for cellular fusion or multinucleation, processes necessary for osteoclast formation[7,11,20]. At this time, it is controversial whether CD47-SHPS-1 interaction plays a key role in the activation of T cells and the acquisition of cell-mediated immunity[21,22], or downregulates the activation of T cells by DC[16,17,23,24]. Considering that T cell activation and osteoclast formation are critical events in the pathology of RA, we speculated that inhibiting the interaction between SHIP-1 and CD47 might be beneficial in the treatment of RA.

We previously reported that an antibody against SHPS-1 effectively inhibits the migration of epidermal DC and Langerhans cells, resulting in decreased development of the delayed-type hypersensitivity response[23,24]. Using this SHPS-1 antibody, we conducted a series of *in vivo* and *in vitro* experiments to clarify the role of SHPS-1 in the pathology of RA. The results not only suggested the significance of SHPS-1 in RA, but also indicated a possibility that the administration of the anti-SHPS-1 antibody could be an effective strategy to treat patients with RA.

MATERIALS AND METHODS

Collagen-induced arthritis (CIA). Our study was performed under the approval of the Institutional Review Board of the National Hospital Organization, Sagamihara Hospital. The induction of arthritis in mice was based on a described method[25,26]. Briefly, bovine type II collagen (CII; Collagen Research Center, Tokyo, Japan) was dissolved at 2 mg/ml in 10 mM acetic acid, and was emulsified by mixing with an equal volume of Freund's complete adjuvant (Nippon BD, Tokyo, Japan). Five-week-old male DBA/1JN mice (Charles River Japan, Yokohama, Japan) were immunized by intradermal injection of the emulsion (100 μl) at the base of their tails. Twenty-one days later, the same volume of emulsion was injected again in the same manner as a booster. With this protocol, arthritis developed in 100% of mice at around 4 weeks after the initial immunization.

Treatment with anti-SHPS-1 monoclonal antibody. Hybridoma cells producing anti-mouse SHPS-1 (P84) monoclonal antibody (anti-SHPS-1 mAb) was a generous gift from Dr. C.F. Lagenaur (Pittsburgh University, Pittsburgh, PA, USA)[27]. Ascites fluid was collected from BALB/c nu/nu mice that had been injected intraperitoneally with hybridoma cells, and the p84 antibody was purified from the ascites using a protein A column[23,24]. Rat IgG1 (Sigma Diagnostics, St. Louis, MO, USA) was used as a control immunoglobulin (control IgG). The experiments were performed according to either of the following 2 protocols. In Protocol A, CIA was induced as described, and anti-SHPS-1 mAb, control IgG, or methotrexate (MTX; Wyeth, Tokyo, Japan) were given to the mice every other day from Day 21 (the day of second immunization) until Day 31, 6 times in total. The antibody or control IgG was dissolved in 200 μl phosphate buffered saline (PBS) and injected intraperitoneally. MTX was administered orally. In Protocol B, the administration of the SHPS-1 antibody, control IgG, or MTX was started on Day 29 (8 days after the second immunization) and was repeated 6 times until Day 39, on every other day.

Evaluation of arthritis. The development of arthritis was determined by the presence of redness or swelling in any of the 4 limbs. If these signs were observed in at least 1 limb, the mouse was determined to be positive for arthritis. The incidence of arthritis was defined in each treatment group by the ratio of the number of positive mice to the total number of mice in the group. The occurrence and severity of arthritis were evaluated macroscopically on each hind limb in each mouse by scores from 0 (normal) to 3 (joint deformity or rigidity). The sum of scores for both hind limbs (0 to 6) was used as the clinical score for that animal. The severity of arthritis was also evaluated by the average thickness of footpads of the right and left hind limbs, which was measured using a caliper. The body weight was recorded daily throughout the experimental period.

Histological evaluation. For histology, the mice were sacrificed and their hind limbs were amputated, fixed with 10% formaldehyde, decalcified with EDTA, and embedded in paraffin. Four-micron-thick sections of the ankle and toe joints were prepared in a sagittal plane and were stained with hematoxylin and eosin (H&E). Using a light microscope, the severity of inflammation and joint destruction was assessed semiquantitatively based on a described procedure[28]. The severity of inflammatory change was assessed as a score from 0 to 4, considering the extent of inflammatory cell infiltration, synovial lining-cell hyperplasia, and pannus formation. Further, the severity of bone destruction was evaluated by scores from 0 to 5, according to the following criteria: 0 = normal; 1 = minimal loss of cortical bone at a few sites; 2 = mild loss of cortical and trabecular bone at a few sites; 3 = moderate bone loss at multiple sites; 4 = marked bone loss at multiple sites; and 5 = marked bone loss with distortion of the profile of the remaining cortical surface.

Measurement of anti-type II collagen antibodies. The concentration of anti-CII antibodies in the sera of mice was determined by ELISA[29]. For this, 96-well flat-bottom plates (Iwaki, Tokyo, Japan) were coated with 50 μl CII (2 μg/ml in PBS) overnight at 4°C. Prior to use, the wells were blocked with PBS containing 1% (w/v) bovine serum albumin at 37°C for 1 h. Sera were then diluted appropriately in PBS containing 0.05% (v/v) Tween-20, and were added to the wells. After incubation at 37°C for 2 h, levels of CII-specific IgG2a were measured using biotin-labeled rat anti-mouse IgG2a (R&D Systems, Minneapolis, MN, USA). The amount of biotin-labeled antibody bound was determined by color reaction using streptavidin-peroxidase coupled with peroxidase substrate (Substrate Reagent Pack, Stop Solution; R&D Systems). All measurements were performed in triplicate and averages were calculated.

Effect of antibodies on osteoclast formation from murine bone marrow cells. Bone marrow cells were obtained from 6-week male Balb/c mice, and were plated in wells of 24-well plates at 1×10^6 cells per well. The cells were cultured in Dulbecco's modified Eagle medium (DMEM; Invitrogen, Tokyo, Japan) containing 10% fetal bovine serum (FBS; Invitrogen), glut-

amine, streptomycin, penicillin, macrophage colony-stimulating factor (M-CSF, 50 ng/ml; R&D Systems), and RANKL (30 ng/ml; R&D Systems). In this experiment, the effect of anti-SHPS-1 mAb was compared with that of control IgG. Immediately after plating, anti-SHPS-1 mAb or control IgG was added to the media at graded concentrations, and the cells were cultured for 5 days. The medium was then removed and the cells were fixed and stained for tartrate-resistant acid phosphatase (TRAP) using a commercial kit (Sigma Diagnostics Acid Phosphatase Kit; Sigma Diagnostics). TRAP-positive multinuclear cells that had more than 3 nuclei were counted as osteoclasts. The experiment was then repeated with an anti-CD47 monoclonal antibody, and the results were compared. The antibody against CD47 (miap301; anti-CD47 mAb) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

Effect of antibodies on cytokine production by murine spleen cells. Spleen cells were obtained from 6-week male Balb/c mice, and were plated at a density of 5×10^5 cells per well in 96-well plates. Cells were maintained in RPMI-1640 medium (Invitrogen) containing 10% FBS, glutamine, streptomycin, and penicillin. One hour after plating, anti-SHPS-1 mAb, anti-CD47 mAb, or control IgG were added to the media at the indicated concentrations. One hour after the addition of antibody or control IgG, the cells were stimulated by 5 μg/ml concanavalin A (ConA; Wako, Osaka, Japan). After 24 h, the supernatants were collected and the concentrations of IL-1ß, IL-2, IL-12, interferon-γ (IFN-γ), TNF-α, IL-4, and IL-10 in the media were determined by ELISA (R&D Systems).

Statistical analysis. For parametric data, statistical significance was determined by 2-way analysis of variance and contrast as a post hoc test. Nonparametric data were analyzed using the Kruskal-Wallis test, and the Dunn procedure was used as a post-hoc test when necessary. Log-rank test was used to determine the difference in the incidence of arthritis. The level of significance was set at $p < 0.05$.

RESULTS

Anti-SHPS-1 antibody reduces incidence and severity of CIA. Six groups of mice, each consisting of 10 animals, were prepared, and CIA was induced in 5 of those groups. The other group was maintained without any treatment and served as a non-CIA control. Each of the 5 CIA-induced groups received 6 consecutive administrations of either anti-SHPS-1 mAb (1, 10, or 100 μg), control IgG (100 μg), or MTX (3 mg), following Protocol A, in which the treatments were started on the day of the second immunization. The incidence of arthritis was significantly reduced by the SHPS-1 antibody treatment (Figure 1A). While arthritis developed in all mice treated with the control IgG, the administration of 10 μg or 100 μg anti-SHPS-1 mAb reduced the incidence by 20% and 30%, respectively, although no significant reduction was observed with 1 μg anti-SHPS-1 mAb. The decline in the incidence was significant in the 10 and 100 μg antibody-treated groups (both at $p < 0.05$). The incidence of arthritis was dramatically reduced by MTX, indicating that the immune response was profoundly involved in the development of arthritis.

In untreated mice, the body weight increased by 1.8 g on average between Day 21 and Day 41 (Figure 1B). Among the 5 experimental groups, the mice treated with control IgG lost 1.0 g in weight during that period, likely due to the general exhaustion associated with the arthritis. This decline in

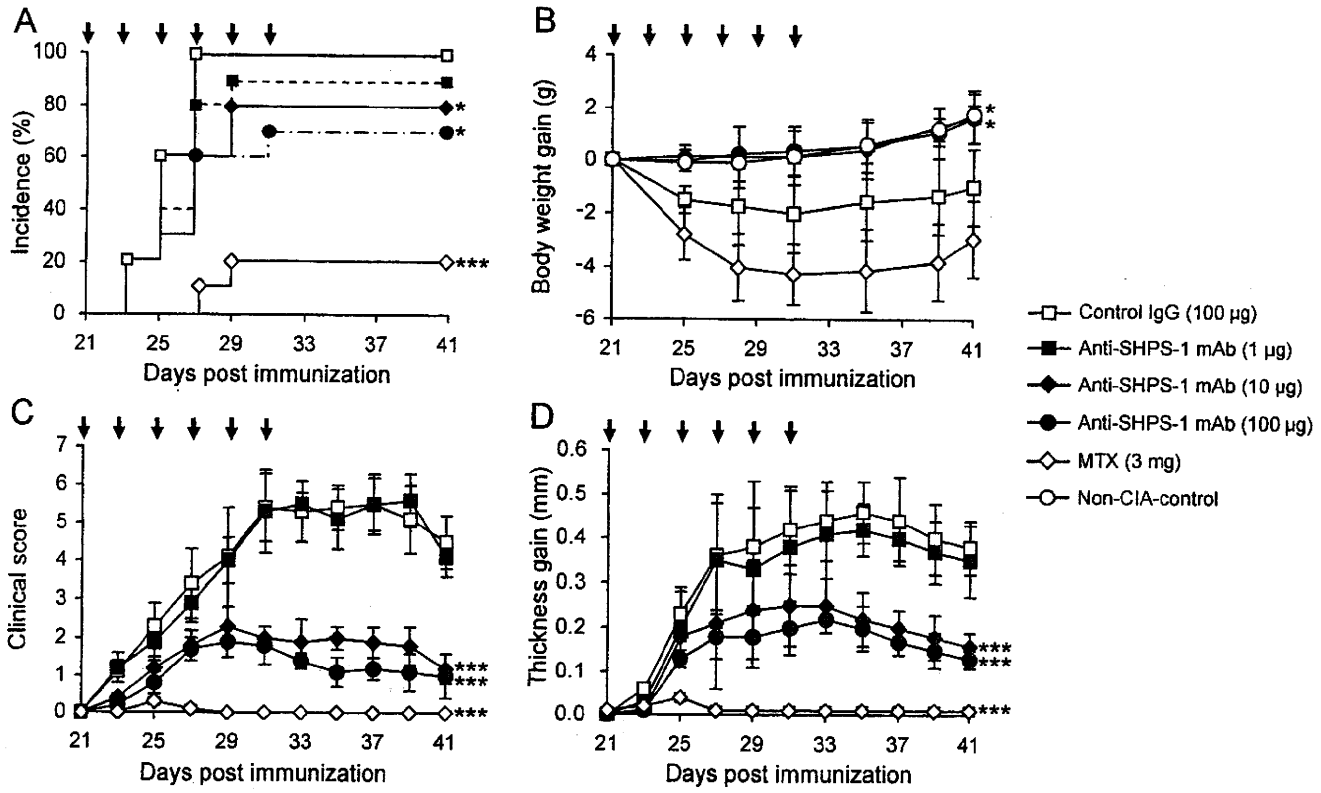

Figure 1. Incidence and severity of arthritis in CIA mice treated with anti-SHPS-1 mAb. Mice were given graded doses of antibody, and the incidence and severity of arthritis were compared with those in control IgG- or MTX-treated mice. Incidence (A), gain of body weight (B), clinical score (C), and increase of footpad thickness (D) from Day 21 to Day 41 are shown. Black arrows indicate the timing of antibody administration. Data are mean ± SD. *$p < 0.05$ and ***$p < 0.001$ compared with control IgG.

body weight was reversed by the anti-SHPS-1 mAb treatment. The administration of 10 or 100 μg anti-SHPS-1 mAb recovered the body weight almost completely to the level of non-CIA mice. The increase of body weights in those 2 groups was significantly greater than that of the control IgG group (both at $p < 0.05$). Although the development of arthritis was strongly inhibited by MTX, the mice treated with MTX lost approximately 2.9 g in body weight during the experimental period, which might be ascribed to the toxic effects of the immunosuppressant.

In the control mice, the clinical score started to increase on Day 23 and reached a maximum on Day 31 (Figure 1C). The administration of 10 or 100 μg anti-SHPS-1 mAb significantly improved the clinical score on Day 31 and later. Since improvement was not observed in mice treated with 1 μg anti-SHPS-1 mAb, the critical dose of antibody treatment for the mice was considered to be between 1 and 10 μg per injection. Interestingly, the improvement in clinical score was maintained until Day 41, 10 days after the last antibody administration. In mice treated with MTX, the development of arthritis was completely inhibited.

Consistent results were obtained by the measurement of footpad thickness (Figure 1D). In control mice, the footpad thickness started to increase on Day 25, and it continued to increase until Day 35, while no increase was observed in the MTX-treated mice. In mice treated with 10 or 100 μg anti-SHPS-1 mAb, the increase of footpad thickness was inhibited on Day 27 and later. In accord with the clinical score, 1 μg anti-SHPS-1 mAb was not enough to show the effect.

Anti-SHPS-1 antibody ameliorated the severity of established arthritis. We then investigated whether the administration of anti-SHPS-1 mAb could reduce the severity of established arthritis. In this experiment, the antibody treatment was commenced after the onset of arthritis (on Day 29), and the severity of arthritis was evaluated by the clinical score and footpad thickness of the hind limbs. The clinical score was reduced significantly as early as 2 days after the first injection of 100 μg anti-SHPS-1 mAb (Figure 2A). The reduction in clinical score became more obvious, and this was maintained until Day 45, 6 days after the completion of antibody administration.

In accord with the change of clinical score, the antibody treatment reduced the footpad thickness at 2 days and later after the initiation of antibody treatment (Figure 2B). Similar to the clinical score, the reduction in footpad thickness was maintained until Day 45.

Histological evaluation. Next, the effect of the antibody treatment was evaluated by histology. The mice were given 6 injections of 10 or 100 μg anti-SHPS-1 mAb after the onset of arthritis following Protocol B, and sacrificed 6 days after the last antibody administration. H&E-stained sections of ankle and tarsal joints were prepared, and the severity of arthritic change was evaluated by scores that were compared with those of mice treated with control IgG.

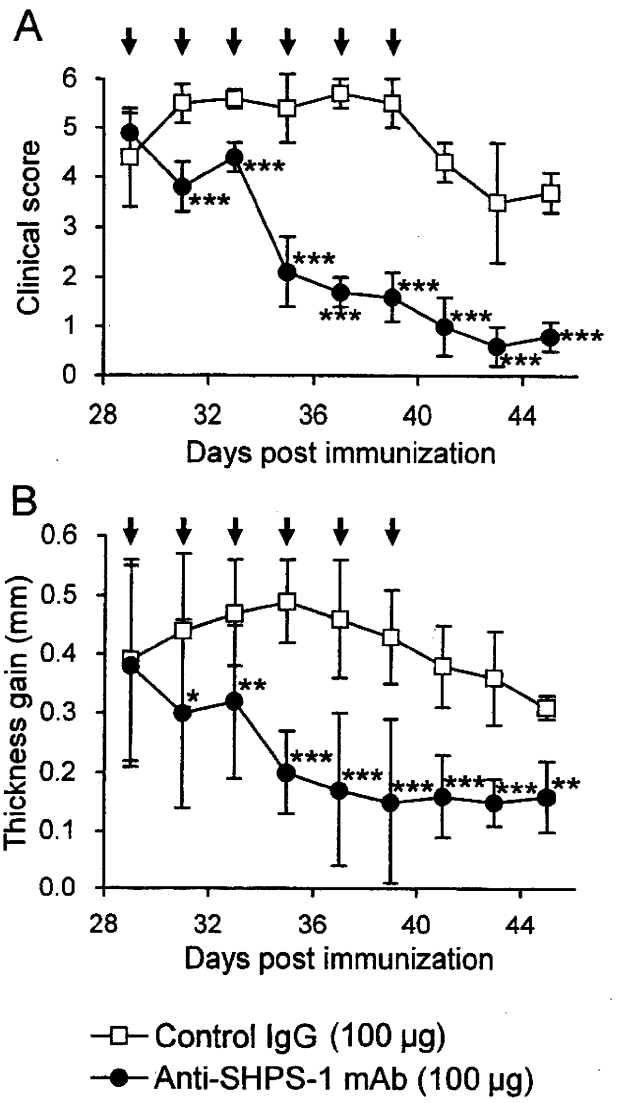

Figure 2. Effect of anti-SHPS-1 mAb treatment on severity of established arthritis. 100 μg of anti-SHPS-1 mAb or control IgG was given to mice after onset of arthritis, and severity of arthritis was evaluated by clinical score (A) and gain of footpad thickness (B), from the beginning of the treatment until 2 weeks after its end. Black arrows indicate the timing of antibody administration. Data are mean ± SD. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared with control IgG.

In control mice, severe arthritic change with obvious inflammatory cell infiltration and bone erosion was observed within and around the ankle and tarsal joints (Figure 3A-3C). Although the anti-SHPS-1 antibody was given after the onset of arthritis, the severity of arthritic change was considerably reduced in mice treated with anti-SHPS-1 mAb (Figure 3D-3F). Thus, the scores for inflammatory cell infiltration and those for bone destruction were significantly reduced in the antibody-treated mice (Figure 3G and 3H, respectively).

Anti-SHPS-1 antibody did not affect induction of anti-type II collagen antibodies. In CIA mice, arthritis is caused by

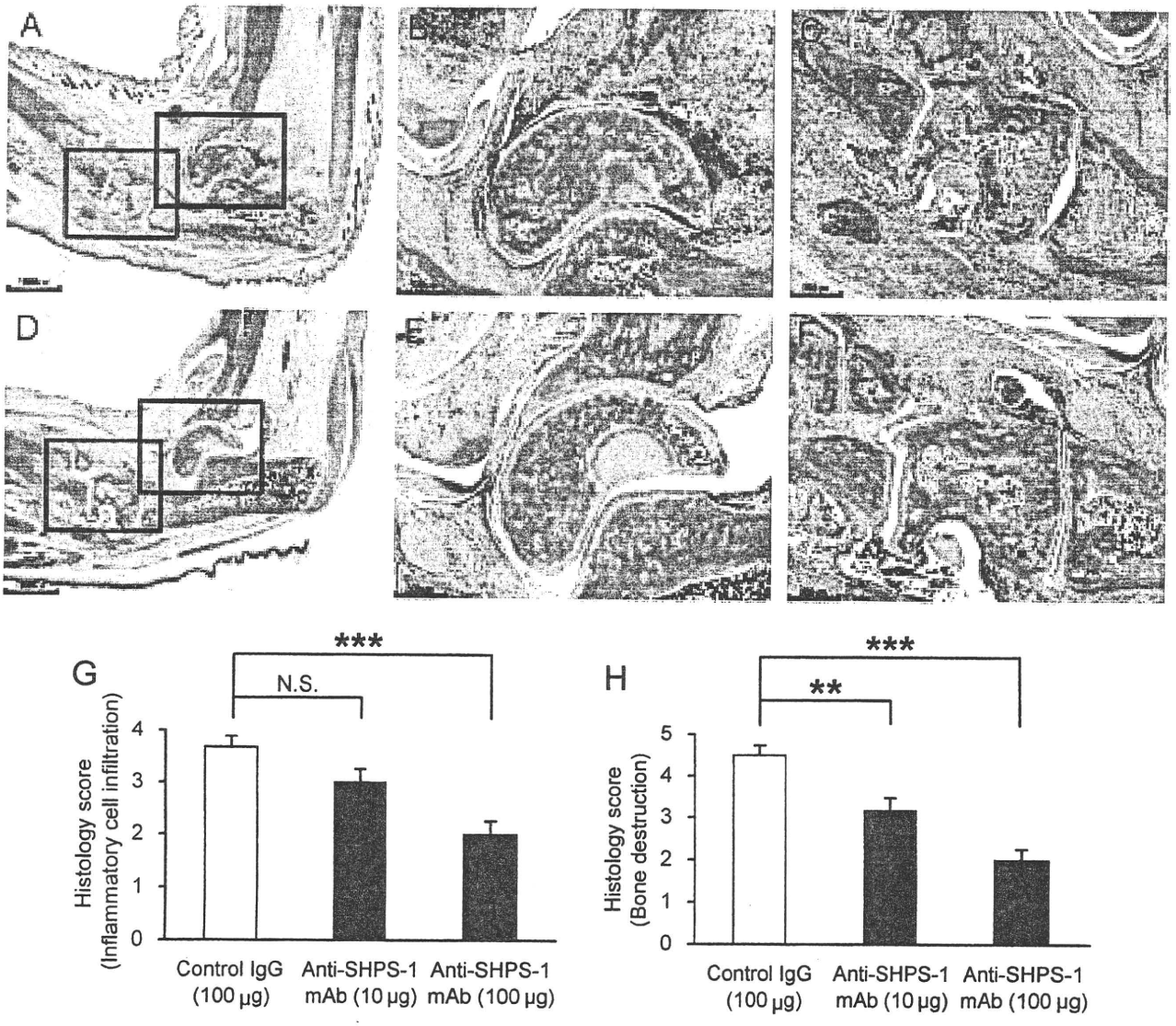

Figure 3. Histological evaluation of ankle and tarsal joints in antibody-treated and control mice. (A-C) In control mice given 100 μg control IgG, obvious synovial thickening and pannus formation was observed, together with marked inflammatory cell infiltration. Bone erosion occurred at multiple sites, which often extended deep into the subchondral bone. (D-F) In mice treated with 100 μg anti-SHPS-1 mAb, severity of synovial thickening and extent of inflammatory cell infiltration were considerably reduced. Area of bone erosion was also reduced, and rarely extended into the subchondral bone. Higher magnification images of inset areas in A and D are shown in B and C, and E and F, respectively. Scale bars are 1000 μm in A and D, and 300 μm in B, C, E, and F. H&E staining. (G and H) Histological scores for inflammatory cell infiltration (G) and bone destruction (H) in mice treated with 10 or 100 μg anti-SHPS-1 mAb are shown together with those for mice given 100 μg control IgG. Data are mean ± SD. **$p < 0.01$ and ***$p < 0.001$ compared with control IgG.

autoimmune mechanisms that involve both humoral and cellular immune responses to CII[30,31]. Thus, we next determined the effect of the anti-SHPS-1 antibody treatment on the humoral response by measuring the concentration of anti-CII antibodies in sera. In this experiment, administration of anti-SHPS-1 mAb was started on the day of the second immunization, following Protocol A, and blood was obtained 2 weeks after the end of treatment. Comparison of the results between the antibody-treated mice and those of mice given control IgG or MTX revealed that the induction of anti-CII antibodies was not affected by the antibody treatment. This implies that the therapeutic effect of anti-SHPS-1 mAb is likely through the suppression of T cell responses, rather than via the modulation of B cell function.

Anti-SHPS-1 antibody and anti-CD47 antibody inhibited osteoclast formation. The observation that bone destruction was significantly reduced by the administration of anti-SHPS-1 mAb led us to hypothesize that the antibody could inhibit osteoclast formation. We then tested this hypothesis by an *in vitro* experiment. We also examined the effect of anti-CD47 mAb on osteoclast formation. In this experiment, murine bone marrow cells were obtained and osteoclast formation was induced in the presence of anti-SHPS-1 mAb or anti-CD47 mAb. The results clearly indicated that those

antibodies both inhibited the formation of osteoclasts in a dose-dependent manner (Figure 4). The inhibition was more obvious with anti-SHPS-1 mAb. With this antibody, the number of osteoclasts was significantly reduced with as little as 0.05 μg/ml of the antibody, and osteoclast formation was almost completely abrogated at the concentration of 2.5 μg/ml. Compared with this, the effect of anti-CD47 mAb was considerably lower. The ratio of inhibition did not reach 50% even with 10 μg/ml of this antibody.

Anti-SHPS-1 antibody and anti-CD47 antibody reduced secretion of proinflammatory cytokines from ConA-stimulated murine spleen cells. In order to determine the effects of anti-SHPS-1 mAb and anti-CD47 mAb on cytokine release from lymphatic cells, murine spleen cells were stimulated with ConA in the presence of anti-SHPS-1 mAb or anti-CD47 mAb, and cytokine concentrations in the media were determined. Upon stimulation with ConA, the spleen cells released all measured pro- and antiinflammatory cytokines to the media. The effect of anti-SHPS-1 mAb on cytokine release differed among the cytokines (Figure 5A-5G). The release of IL-1ß, IL-2, IL-12, IFN-γ, and TNF-α into the media was suppressed by the antibody, while that of IL-4 or IL-10 was almost unaffected. The suppression was most obvious for IFN-γ, with as little as 0.02 μg/ml of the antibody significantly reducing its release. The IC_{50} values of IL-1ß, IL-2, IL-12, IFN-γ, and TNF-α were 0.68, 0.50, 0.16,

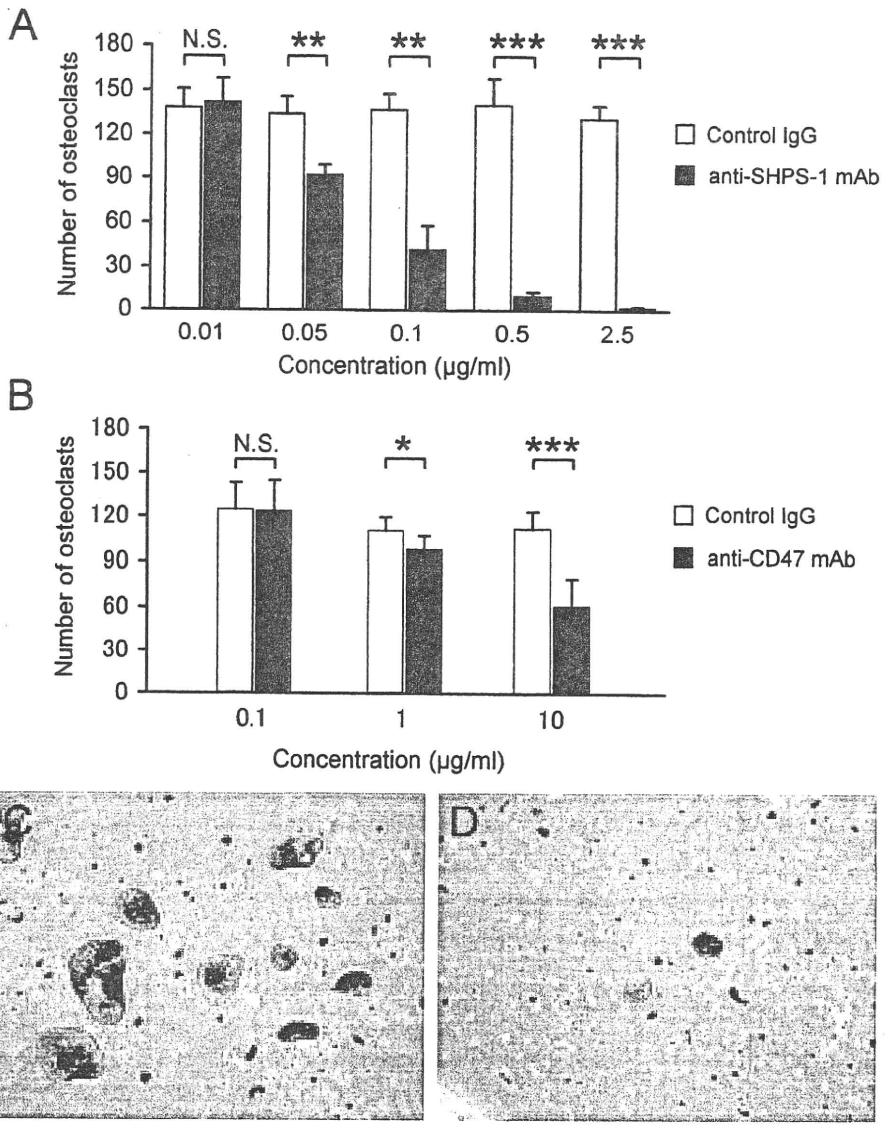

Figure 4. Effect of anti-SHPS-1 mAb and anti-CD47 mAb on osteoclast formation. (A and B) Bone marrow cells were obtained from Balb/c mice, and formation of osteoclasts was induced by M-CSF and RANKL for 5 days, in the presence of various concentrations of anti-SHPS-1 mAb or control IgG (A), and anti-CD47 mAb or control IgG (B). Number of TRAP-positive multinucleated cells in each well is shown. Data are mean ± SD. **$p < 0.01$ and ***$p < 0.001$ against control IgG. (C and D) Formation of osteoclasts in the presence of control IgG (2.5 μg/ml; panel C) or anti-SHPS-1 mAb (2.5 μg/ml; panel D). TRAP staining.

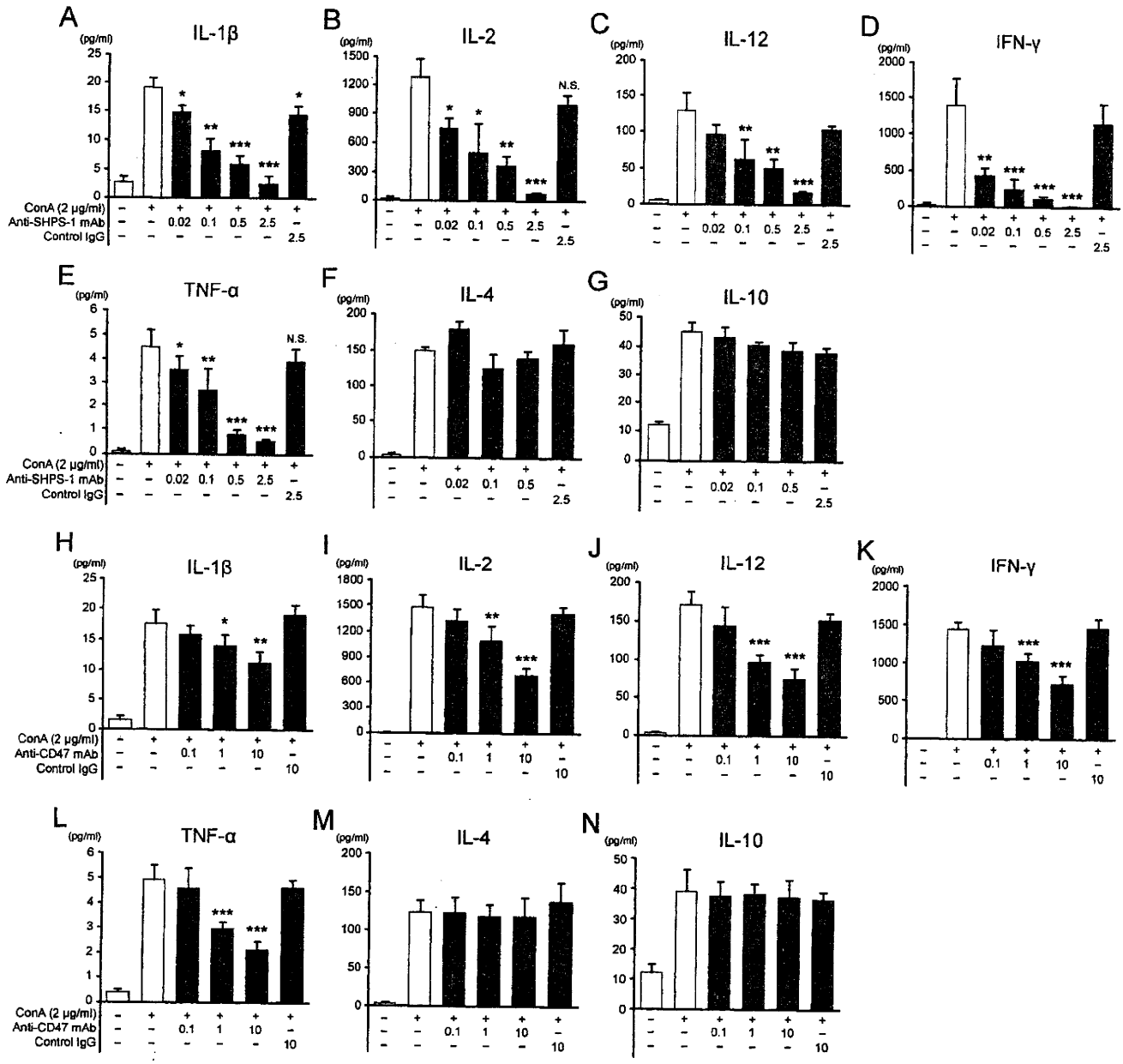

Figure 5. (A-G) Murine spleen cells were stimulated with ConA (2 μg/ml) in media containing graded doses of anti-SHPS-1 mAb or control IgG (2.5 μg/ml). 24 h later, supernatants were collected, and concentrations of IL-1ß (A), IL-2 (B), IL-12 (C), IFN-γ (D), TNF-α (E), IL-4 (F), and IL-10 (G) were determined by ELISA. (H-N) Experiments were repeated with anti-CD47 mAb, and concentrations of IL-1ß (H), IL-2 (I), IL-12 (J), IFN-γ (K), TNF-α (L), IL-4 (M), and IL-10 (N) were determined. Experiments were repeated 3 or 4 times. Data are mean ± SD. *p < 0.05, **p < 0.01, ***p < 0.001 compared with control IgG.

0.079, and 1.18, respectively. Anti-CD47 mAb showed similar effects on cytokine release (Figure 5H-5N). This antibody reduced the concentration of IL-2, IL-12, IFN-γ, and TNF-α in the media in a dose-dependent manner. However, its inhibitory effect was much lower than that of anti-SHPS-1 mAb, and the suppression was no more than 60% even with 10 μg/ml of the antibody.

DISCUSSION

The results of our study demonstrate that the administration of an anti-SHPS-1 mAb successfully reduces the severity of arthritis in CIA mice. CIA is an animal model often used to study the pathology of RA, in which both humoral and cell-mediated immunity is necessary for the development of arthritis[31,32]. The treatment with the anti-SHPS-1 mAb virtually did not suppress the humoral immunity, since it did not alter the concentration of anti-CII antibodies in the sera of mice (Figure 6). Thus, the therapeutic effect of the anti-SHPS-1 mAb could be ascribed entirely to the suppression of the cell-mediated immune response. In *in vitro* experi-

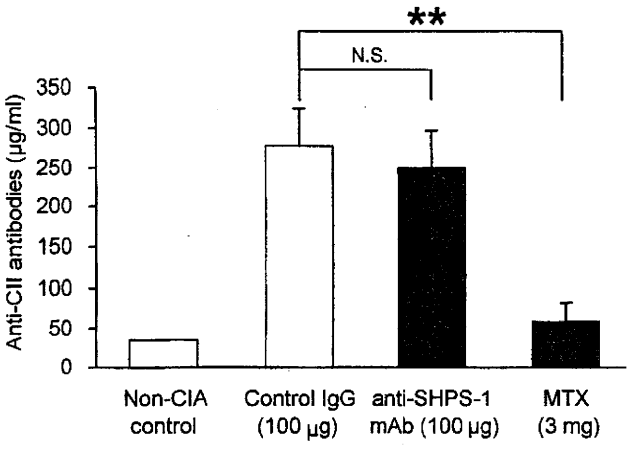

Figure 6. Concentration of anti-CII antibodies in the sera. CIA mice were treated for 11 days with control IgG (100 μg), anti-SHPS-1 mAb (100 μg), or MTX (3 mg), and the sera were obtained. Sera were also obtained from mice in which CIA was not induced, and the concentrations of anti-CII antibodies were determined by ELISA. Data are mean ± SD of 5–8 mice. **p < 0.01 compared with control IgG. NS: nonsignificant.

ments using murine spleen cells, addition of the anti-SHPS-1 mAb inhibited the release of IL-1ß, IL-2, IL-12, IFN-γ, and TNF-α into the media upon stimulation by ConA, while the release of IL-4 or IL-10 was almost unaffected. The finding that the anti-SHPS-1 mAb suppressed the release of cytokines primarily from Th1 cells but not those from Th2 cells further supports the idea that the anti-SHPS-1 mAb affects cellular immunity rather than humoral immunity. In human RA, TNF-α is profoundly involved in the progression of the disease as shown by the efficacy of anti-TNF-α therapy[33-35]. Also, IL-2 and IFN-γ are known to be involved in the catabolism in affected joints[36]. Since the pathology of arthritis in the CIA mouse closely resembles that of human RA[37], the reduction in the release of those cytokines could reasonably explain the therapeutic effects of the anti-SHPS-1 mAb observed in this work.

For such change in cytokine release, ligation of SHPS-1 by anti-SHPS-1 mAb may play a significant role, in addition to the inhibitory role of the antibody upon SHPS-1/CD47 interaction. We previously showed that SHPS-1 ligation by the antibody inhibits the migration and maturation of epidermal Langerhans cells, which suggests that DC function could be regulated by SHPS-1 engagement[23,24]. Ligation of SHPS-1 has been shown to inhibit TNF-α production by lipopolysaccharide-stimulated monocytes[38]. Thus, the observed reduction in TNF-α release by anti-SHPS-1 mAb could be ascribed, at least in part, to the suppression of TNF-α production by macrophages or DC by SHPS-1 ligation. Again, since the antibody inhibits IL-12 production by DC[16], the observed suppression of IL-12 release could be partly caused by SHPS-1 ligation. Because IL-12 is an essential cytokine for Th1 development, reduced IL-12 production favors the development of Th2 cells rather than Th1 cells. This is compatible with the finding that the production of all Th1 cytokines, but not those of Th2, was suppressed by anti-SHPS-1 mAb. The difference between anti-SHPS-1 and anti-CD47 mAb in the effects on cytokine release may be reasonable if these direct actions are assumed with the former antibody.

On the other hand, the supposed suppression of cellular immunity by anti-SHPS-1 mAb may be caused primarily by the inhibition of interaction between SHPS-1 and CD47. T cells express CD47 at a high density[39]. Since SHPS-1/CD47 interaction positively regulates T cell responses[21], it is possible that the anti-SHPS-1 mAb suppressed T cell activation by blocking that interaction. Anti-SHPS-1 mAb may inhibit proliferation of T cells via the suppression of TNF-α production by antigen-presenting cells[22]. Other studies have shown that SHPS-1/CD47 interaction may downregulate DC-T cell interaction, by reducing IL-12 production by DC and IL-12 receptor expression on T lymphocytes[16,17,39]. Reduced T cell activation by these mechanisms could be involved in the amelioration of arthritis by anti-SHPS-1 mAb.

Meanwhile, a mechanism for the reduction of bone erosion by the antibody was suggested by an *in vitro* experiment. Our current investigation and that of others consistently indicate that anti-SHPS-1 mAb and anti-CD47 mAb both inhibited induction of osteoclasts from macrophages[40]. Macrophages express SHPS-1 and CD47 abundantly, and utilize them for cell fusion, which is an essential step for osteoclast formation[7,11,20]. Therefore, it is likely that the antibodies for these molecules reduced the formation of osteoclasts through the inhibition of multinucleation. In addition to this, anti-SHPS-1 mAb might have reduced osteoclast formation through the change in released cytokines discussed above: among the cytokines whose release was suppressed by the antibody, IL-1ß and TNF-α are known to play essential roles in the formation of osteoclasts[3,4]. In our study, suppression of osteoclast formation was more obvious with anti-SHPS-1 mAb than with anti-CD47 mAb (Figure 4). This difference, again, could be ascribed to the lack of SHPS-1 ligation with the latter antibody.

Our results show that the use of anti-SHPS-1 antibody could be a promising strategy to treat patients with RA. Although our current results are based on an animal model of RA, the treatment with the antibody seems attractive because the antibody could regulate T cell immunity and osteoclast formation together, both of which are essential in treating RA[3,4,41]. Further studies are awaited to determine the feasibility of the antibody treatment.

REFERENCES

1. Koopman WJ. Arthritis and allied conditions: A textbook of rheumatology. 15th ed. Philadelphia: Williams and Wilkins; 1997.
2. Tak PP, Smeets TJ, Daha MR, et al. Analysis of the synovial cell infiltrate in early rheumatoid synovial tissue in relation to local disease activity. Arthritis Rheum 1997;40:217-25.

3. Teitelbaum SL. Osteoclasts; culprits in inflammatory osteolysis. Arthritis Res Ther 2006;8:201.
4. Goldring SR. Pathogenesis of bone and cartilage destruction in rheumatoid arthritis. Rheumatology Oxford 2003;42 Suppl:ii11-6.
5. Kharitonenkov A, Chen Z, Sures I, Wang H, Schilling J, Ullrich A. A family of proteins that inhibit signalling through tyrosine kinase receptors. Nature 1997;386:181-6.
6. Ohnishi H, Kubota M, Ohtake A, Sato K, Sano S. Activation of protein-tyrosine phosphatase SH-PTP2 by a tyrosine-based activation motif of a novel brain molecule. J Biol Chem 1996;271:25569-74.
7. Saginario C, Sterling H, Beckers C, et al. MFR, a putative receptor mediating the fusion of macrophages. Mol Cell Biol 1998;18:6213-23.
8. Comu S, Weng W, Olinsky S, et al. The murine P84 neural adhesion molecule is SHPS-1, a member of the phosphatase-binding protein family. J Neurosci 1997;17:8702-10.
9. Fujioka Y, Matozaki T, Noguchi T, et al. A novel membrane glycoprotein, SHPS-1, that binds the SH2-domain-containing protein tyrosine phosphatase SHP-2 in response to mitogens and cell adhesion. Mol Cell Biol 1996;16:6887-99.
10. Adams S, van der Laan LJ, Vernon-Wilson E, et al. Signal-regulatory protein is selectively expressed by myeloid and neuronal cells. J Immunol 1998;161:1853-9.
11. Han X, Sterling H, Chen Y, et al. CD47, a ligand for the macrophage fusion receptor, participates in macrophage multinucleation. J Biol Chem 2000;275:37984-92.
12. Jiang P, Lagenaur CF, Narayanan V. Integrin-associated protein is a ligand for the P84 neural adhesion molecule. J Biol Chem 1999;274:559-62.
13. Seiffert M, Cant C, Chen Z, et al. Human signal-regulatory protein is expressed on normal, but not on subsets of leukemic myeloid cells and mediates cellular adhesion involving its counterreceptor CD47. Blood 1999;94:3633-43.
14. Vernon-Wilson EF, Kee WJ, Willis AC, Barclay AN, Simmons DL, Brown MH. CD47 is a ligand for rat macrophage membrane signal regulatory protein SIRP (OX41) and human SIRP alpha 1. Eur J Immunol 2000;30:2130-7.
15. Babic I, Schallhorn A, Lindberg FP, Jirik FR. SHPS-1 induces aggregation of Ba/F3 pro-B cells via an interaction with CD47. J Immunol 2000;164:3652-8.
16. Latour S, Tanaka H, Demeure C, et al. Bidirectional negative regulation of human T and dendritic cells by CD47 and its cognate receptor signal-regulator protein-alpha: down-regulation of IL-12 responsiveness and inhibition of dendritic cell activation. J Immunol 2001;167:2547-54.
17. Lienard H, Bruhns P, Malbec O, Fridman WH, Daeron M. Signal regulatory proteins negatively regulate immunoreceptor-dependent cell activation. J Biol Chem 1999;274:32493-9.
18. Oldenborg PA, Zheleznyak A, Fang YF, Lagenaur CF, Gresham HD, Lindberg FP. Role of CD47 as a marker of self on red blood cells. Science 2000;288:2051-4.
19. Oshima K, Ruhul Amin AR, Suzuki A, Hamaguchi M, Matsuda S. SHPS-1, a multifunctional transmembrane glycoprotein. FEBS Lett 2002;519:1-7.
20. Vignery A. Osteoclasts and giant cells: macrophage-macrophage fusion mechanism. Int J Exp Pathol 2000;81:291-304.
21. Seiffert M, Brossart P, Cant C, et al. Signal-regulatory protein alpha (SIRP alpha) but not SIRP beta is involved in T-cell activation, binds to CD47 with high affinity, and is expressed on immature CD34(+)CD38(-) hematopoietic cells. Blood 2001;97:2741-9.
22. Patel V, Smith RE, Serra A, Brooke G, Howard CJ, Rigley KP. MyD-1 (SIRP alpha) regulates T cell function in the absence of exogenous danger signals, via a TNF alpha-dependent pathway. Eur J Immunol 2002;32:1865-72.
23. Fukunaga A, Nagai H, Noguchi T, et al. Src homology 2 domain-containing protein tyrosine phosphatase substrate 1 regulates the migration of Langerhans cells from the epidermis to draining lymph nodes. J Immunol 2004;172:4091-9.
24. Fukunaga A, Nagai H, Yu X, et al. Src homology 2 domain-containing protein tyrosine phosphatase substrate 1 regulates the induction of Langerhans cell maturation. Eur J Immunol 2006;36:3216-26.
25. Trentham DE, Townes AS, Kang AH. Autoimmunity to type II collagen an experimental model of arthritis. J Exp Med 1977;146:857-68.
26. Campbell IK, Bendele A, Smith DA, Hamilton JA. Granulocyte-macrophage colony stimulating factor exacerbates collagen-induced arthritis in mice. Ann Rheum Dis 1997;56:364-8.
27. Chuang W, Lagenaur CF. Central nervous system antigen P84 can serve as a substrate for neurite outgrowth. Devel Biol 1990;137:219-32.
28. Fishman P, Bar-Yehuda S, Madi L, et al. The PI3K-NF-kappa-B signal transduction pathway is involved in mediating the anti-inflammatory effect of IB-MECA in adjuvant-induced arthritis. Arthritis Res Ther 2006;8:R33.
29. Campbell IK, Rich MJ, Bischof RJ, Dunn AR, Grail D, Hamilton JA. Protection from collagen-induced arthritis in granulocyte-macrophage colony-stimulating factor-deficient mice. J Immunol 1998;161:3639-44.
30. Courtenay JS, Dallman MJ, Dayan AD, Martin A, Mosedale B. Immunisation against heterologous type II collagen induces arthritis in mice. Nature 1980;283:666-8.
31. Seki N, Sudo Y, Yoshioka T, et al. Type II collagen-induced murine arthritis. I. Induction and perpetuation of arthritis require synergy between humoral and cell-mediated immunity. J Immunol 1988;140:1477-84.
32. Holmdahl R, Klareskog L, Rubin K, et al. Role of T lymphocytes in murine collagen-induced arthritis. Agents Actions 1986;19:295-305.
33. Moreland LW, Schiff MH, Baumgartner SW, et al. Etanercept therapy in rheumatoid arthritis. A randomized, controlled trial. Ann Intern Med 1999;130:478-86.
34. Lipsky PE, van der Heijde DM, St. Clair EW, et al. Infliximab and methotrexate in the treatment of rheumatoid arthritis. Anti-tumor Necrosis Factor Trial in Rheumatoid Arthritis with Concomitant Therapy Study Group. N Engl J Med 2000;343:1594-602.
35. Bathon JM, Martin RW, Fleischmann RM, et al. A comparison of etanercept and methotrexate in patients with early rheumatoid arthritis. N Engl J Med 2000;343:1586-93.
36. Mosmann TR, Coffman RL. TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. Annu Rev Immunol 1989;7:145-73.
37. Brand DD, Latham KA, Rosloniec EF. Collagen-induced arthritis. Nature Protocols 2007;2:1269-75.
38. Smith RE, Patel V, Seatter SD, et al. A novel MyD-1 (SIRP alpha) signaling pathway that inhibits LPS-induced TNF alpha production by monocytes. Blood 2003;102:2532-40
39. Ticchioni M, Deckert M, Mary F, Bernard G, Brown EJ, Bernard A. Integrin-associated protein (CD47) is a comitogenic molecule on CD3-activated human T cells. J Immunol 1997;158:677-84.
40. Lundberg P, Koskinen C, Baldock PA, et al. Osteoclast formation is strongly reduced both in vivo and in vitro in the absence of CD47/SIRP alpha-interaction. Biochem Biophys Res Commun 2007;352:444-8.
41. Kremer JM, Westhovens R, Leon M, et al. Treatment of rheumatoid arthritis by selective inhibition of T-cell activation with fusion protein CTLA4Ig. N Engl J Med 2003;349:1907-15.

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Case Report

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Advanced gastric cancer showing long-term complete remission in response to S-1 monotherapy: two case reports

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Abstract

We herein report two cases showing long-term complete remission (CR) in response to S-1 monotherapy. Case 1 was a 65-year-old male diagnosed with an advanced poorly differentiated adenocarcinoma of the stomach with paraaortic lymph node metastases, which disappeared after S-1 monotherapy. Subsequently a total gastrectomy was performed, and histological CR was evident. His progress is presently uneventful without recurrence 50 months after surgery. Case 2 was a 59-year-old female who underwent a total gastrectomy with a jejunal pouch. The resected tumor was a medullary type poorly differentiated adenocarcinoma infiltrating the serosa and involving the regional lymph nodes. One year after surgery, endoscopy revealed a recurrent tumor in the jejunal pouch. After the administration of S-1, this recurrent tumor completely disappeared, and she has since maintained CR for 39 months. These cases suggest that a subgroup of patients with advanced gastric cancer may attain CR with S-1 monotherapy.

Introduction

S-1 is an oral antitumor agent that exploits the biochemical modulation of 5-fluorouracil (FU) pharmacokinetics. S-1 contains tegafur, gemistat and otastat potassium. Gemistat inhibits 5-FU degradation and maintains prolonged 5-FU concentrations. Otastat potassium alleviates the gastrointestinal toxicity induced in the host by 5-FU.[1] In phase II studies, S-1 has demonstrated high response rate for advanced gastric cancers without serious adverse reactions.[2,3] However, complete responses (CRs) with long-term survival are rare.[2,4,5] We report herein two cases of advanced gastric cancer showing long-term CR after S-1 monotherapy.

Case presentation

Case 1

A 65-year-old man complained of epigastric discomfort, dysphagia and vomiting. Endoscopic examination

showed a giant irregular tumor in the cardia of the stomach (Fig. 1), and a biopsy revealed a poorly differentiated adenocarcinoma with a medullary growth pattern (Fig. 2). Abdominal computed tomography (CT) demonstrated metastases to the paraaortic lymph nodes. There was no metastasis to liver, peritoneum or other distant organs. The tumor was clinically diagnosed as stage IV (cT3, cN3, cH0, cP0, cM0) according to the general rules of the Japanese Classification of Gastric Carcinomas.[6] S-1 (TS-1®, Taiho Pharmaceutical Co., Ltd.) at a dose of 120 mg/day was administrated orally for four weeks, followed by a two-week period of no treatment (4-week regimen). This therapeutic schedule was thereafter repeated four times. No adverse events were observed during the S-1 therapy. With the regimen, the gastric cancer remarkably decreased in size and the paraaortic lymph node metastases disappeared. A total gastrectomy with regional lymph node dissection was performed, and the removed specimen showed a scar in the cardia (Fig. 3). Microscopically, the scar consisted of regenerative mucosa and fibrosis with aggregations of histiocytes in the submucosa, partially disrupted muscularis propria and subserosa (Fig. 4). No lymph node metastases were found and some of the dissected lymph nodes (paracardial nodes and nodes along the gastroepiploic, left gastric and common hepatic arteries) showed fibrosis, indicating histological assessment to be a CR to S-1 therapy. The patient continued to be administered S-1 at a dose of 100 mg/day for two weeks, followed by two weeks' rest (2-week regimen) with 12

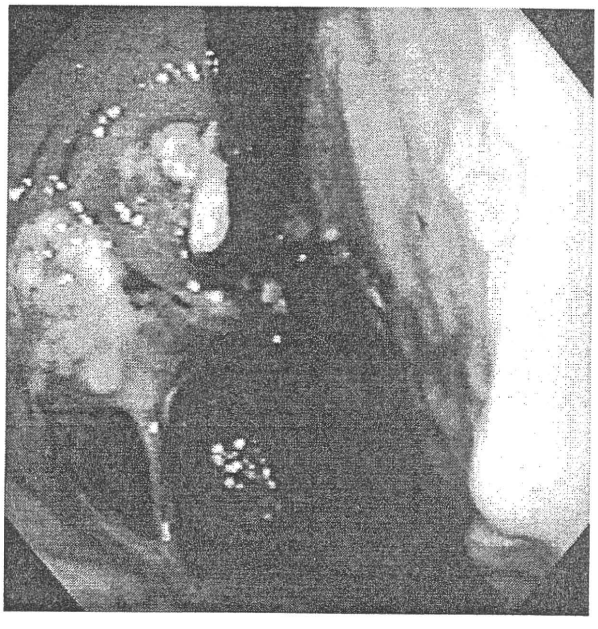

Figure 1
Gastroscopy reveals a giant tumor with ulceration in the cardia.

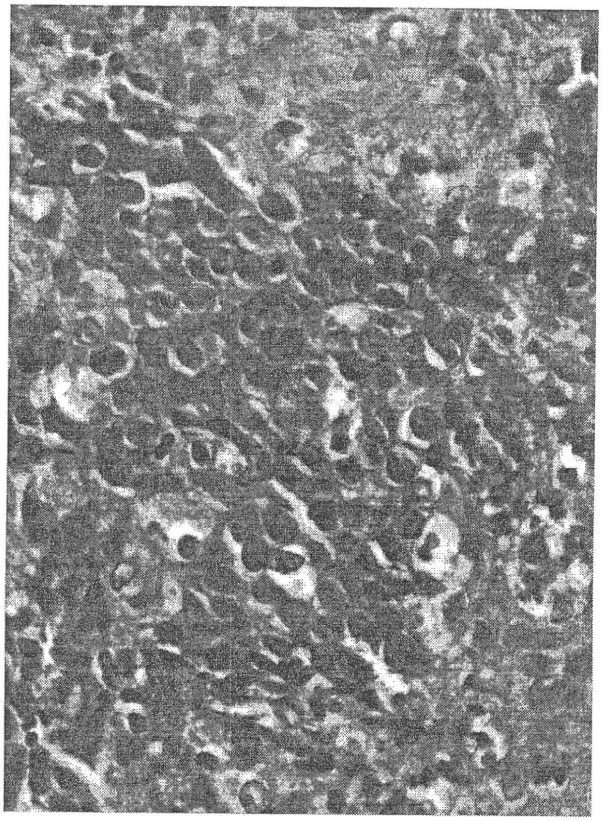

Figure 2
A biopsy specimen showing medullary growth of a poorly differentiated adenocarcinoma (hematoxylin and eosion stain, ×88).

cycles for one year after surgery in our outpatient clinic, and his progress was uneventful with neither recurrence nor metastasis 50 months after surgery.

Case 2

A 59-year-old female underwent a total gastrectomy with regional lymph node dissection and a jejunal pouch with Roux-en-Y reconstruction for a tumor (6.1 × 5.1 cm in size) in the upper corpus. Exfoliative cytology of the peritoneal lavage fluid during the operation was positive for adenocarcinoma (CY1). There was no metastasis to the liver. Microscopically, the tumor was a medullary type poorly differentiated adenocarcinoma with lymphoid stroma, infiltrating through the serosa (pT3) and involving regional lymph nodes (pN3; number of metastasis-positive per dissected lymph nodes, 9/23). Based on the surgical findings, the tumor was diagnosed as stage IV (pT3, pN3, sH0, sP0, sM0, CY1), [6] and adjuvant chemotherapy combining 5-FU (total 150 mg), methotrexate (900 mg) and leukovorin (45 mg) was subsequently performed. One year after the operation, endoscopy showed

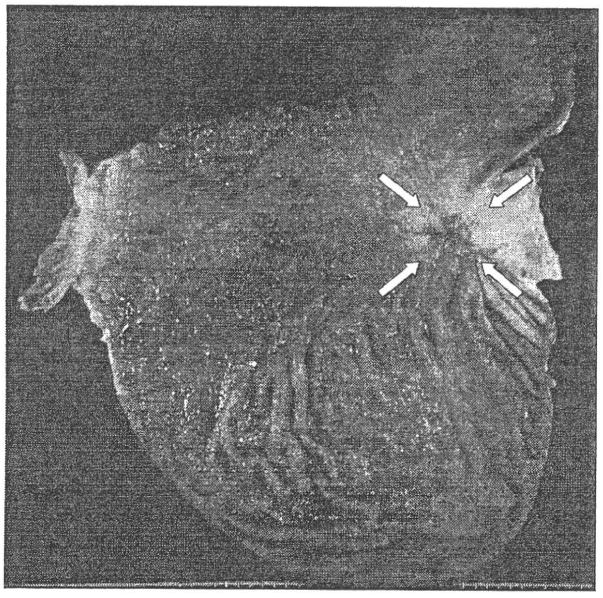

Figure 3
The scar found in the cardia of the resected stomach (arrows).

a tumor in the jejunal pouch along the suture line (Fig. 5A), and examination of a biopsy specimen revealed a poorly differentiated adenocarcinoma. CT demonstrated an intraluminal tumor in the jejunal pouch without any other recurrence. A course of chemotherapy consisting S-1 (2-week regimen) was feasible and repeated 10 times in an outpatient clinic. During the therapy, the recurrent

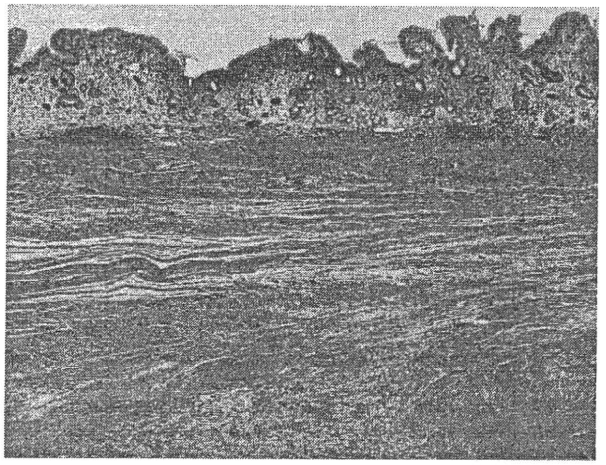

Figure 4
Histologically, regenerative mucosa and fibrosis with aggregation of histiocytes are evident in the scar without any cancer tissue (hematoxylin and eosion stain, ×5).

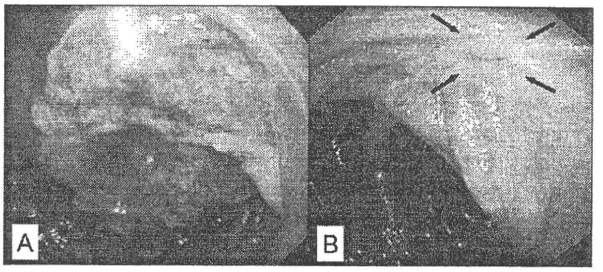

Figure 5
Endoscopic picture of the broad-based tumor in the jejunal pouch. B After S-1 chemotherapy, the recurrent tumor has disappeared and a scar is apparent in the jejunal pouch (arrows).

tumor in jejunal pouch completely disappeared (Fig. 5B), and a biopsy revealed no remnant tumor tissue. The patient has now been well without any evidence of recurrence for 39 months.

Discussion

This report documents two cases of advanced (stage IV) gastric cancer showing long-term CR to S-1 monotherapy; In case 1, CR was histologically verified in the surgically resected stomach, and in case 2 this was presented for a suture line recurrence in the jejunal pouch.

In phase II studies of S-1 in patients with advanced gastric cancer, the overall response rate has been approximately 40–50%.[2,3] A retrospective analysis of single-agent chemotherapy of S-1 for patients with advanced gastric cancer revealed it to be modestly effective with a 26–38% in response rate.[4,5] However, CR was rare with an incidence of only 2–4%[2,4,5] and histological verification in surgically resected stomachs was extremely rare. Mori et al. reported a patient with histological CR after a 2-week regimen of S-1 as single-agent chemotherapy for an advanced cancer.[7] In that case; the biopsy specimen featured a signet-ring cell type of poorly differentiated adenocarcinoma. The response rate for poorly differentiated (diffuse type) adenocarcinomas is reported to be higher than for well differentiated (intestinal type) lesions.[3] S-1 is also effective against the two present cases diagnosed as medullary subtype of poorly differentiated histology.

Few reports have documented advanced gastric cancer with long-term remission after neoadjuvant chemotherapy with S-1 alone; two patients with advanced or metastatic gastric cancer, who responded to S-1 monotherapy and demonstrated clinical CR for about 4 years.[8,9] In another report, a very short course of S-1 alone achieved long-term CR of metastatic gastric cancer.[10] Curative surgery following downstaging with S-1 monotherapy has

also been successfully performed for metastatic disease patients with long-term CR after surgery.[11]

Kimura et al. devised an alternative dosing regimen for S-1, i.e. 2-week regimen, and conducted a retrospective study to evaluate the efficacy and feasibility of this schedule in comparison with the 4-week regimen.[12] In their study, the incidence of adverse reactions tend to be lower in the 2-week regimen group (77%) than in the 4-week group (93%), with response rates of 23% and 21%, respectively. In the present case 1, the standard 4-week regimen was well tolerated, and in case 2, the 2-week regimen was more feasible because of toxicity at the standard dose; both cases fortunately showed long-term CR.

Jejunal pouch recurrence after gastrectomy for gastric cancer has rarely been described.[13,14] Interestingly, the earlier tumors, like the current case, were medullary type poorly differentiated adenocarcinomas characterized by a location in the upper part of the stomach, grossly expansive growth, frequently vascular permeation, and simultaneous liver metastasis, but not jejunal pouch recurrence.[15] The cause of pouch recurrence is speculated that exfoliated cancer cells were intraluminally implanted at the jejunal mucosa, or extraluminally transplanted by the stapling device.[13] Alternatively, our speculation of the cause is lymphatic theory because of the fact that the tumor of the present case 2 had extensive lymph node metastasis.

In conclusion, the two documented cases of advanced gastric cancer showed long-term CR in response to S-1 monotherapy. At present, a standard neoadjuvant strategy for advanced gastric cancer has not been established, but oral intake S-1, which is desirable in the outpatient setting because of its feasibility and mild toxicity, might prove to be considered as a possible alternative chemotherapeutic regimen for such patients, but we definitely need large randomized controlled trial.

Consent

Written informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Hiroyuki Mitomi: pathological examination for this case. Ichiro Kishimoto: surgeon and clinical follow-up for the patient. Akifumi Amemiya, chief surgeon for the patients. Goro Kaneda, assistant surgeon for the patients. Ken Adachi, chief gastroenterologist for preoperative examinations of the patient. Takuya Shimoda, assistant gastroenterologist for preoperative examinations of the patient. Masakazu Takigawa: chief radiologist for radiological examinations of the patient. Naoshi Fukui: conclusive discusser for the case. Yasuo Ohkura: main consultant for pathological findings of the case.

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References

1. Shirasaka T, Shimamoto Y, Ohshimo H, Yamaguchi M, Kato T, Yonekura K, Fukushima M: **Development of a novel form of an oral 5-fluorouracil derivative (S-1) directed to the potentiation of the tumor selective cytotoxicity of 5-fluorouracil by two biochemical modulators.** *Anti-Cancer Drugs* 1996, **7**:548-57.
2. Sakata Y, Ohtsu A, Horikoshi N, Sugimachi K, Mitachi Y, Taguchi T: **Late phase II study of novel oral fluoropyrimidine anticancer drug S-1 (1 M tegafur-0.4 M gimestat-1 M otastat potassium) in advanced gastric cancer patients.** *Eur J Cancer* 1998, **34**:1715-20.
3. Koizumi W, Kurihara M, Nakano S, Hasegawa K: **The S-1 Cooperative Gastric Cancer Study Group. Phase II study of S-1, a novel oral derivative of 5-fluorouracil, in advanced gastric cancer.** *Oncology* 2000, **58**:191-7.
4. Yonemori K, Shimada Y, Goto A, Ura T, Arai T, Hamaguchi T, Muro K, Yamada Y, Shirao K: **Retrospective analysis of clinical results and predictors of response in chemo-naïve patients with advanced gastric cancer treated with S-1, an oral fluoropyrimidine derivative, as single-agent chemotherapy.** *Gastric Cancer* 2004, **7**:204-10.
5. Kawai H, Ohtsu A, Boku N, Hamamoto Y, Nagashima F, Muto M, Sano Y, Mera K, Yano T, Doi T, Yoshida S: **Efficacy and safety profile of S-1 in patients with metastatic gastric cancer in clinical practice: results from a post-marketing survey.** *Gastric Cancer* 2003, **6**:19-23.
6. Japanese Gastric Cancer Association: **Japanese classification of gastric carcinoma. 2 nd English ed.** *Gastric Cancer* 1998, **1**:10-24.
7. Mori S, Kishimoto H, Tauchi K, Higuchi K: **Histological complete response in advanced gastric cancer after 2 weeks of S-1 administration as neoadjuvant chemotherapy.** *Gastric Cancer* 2006, **9**:136-9.
8. Suzuki Y, Kawasaki N, Ishibashi Y, Takahashi N, Kashiwagi H, Koba K, Urashima M, Yanaga K: **A case of stage IV gastric cancer: long-term remission achieved with S-1 monotherapy.** *JMAJ* 2006, **49**:219-23.
9. Ueda Y, Yamagishi H, Yamashita T, Itoh N, Itoi H, Shirasaka T, Ajani JA: **S-1-induced, prolonged complete regression of lung metastasis from gastric cancer refractory to 5'-DFUR: a case report with pharmacokinetic study.** *Jpn J Clin Oncol* 2004, **34**:282-6.
10. Schöffski P, Chollet P, Ganser A, Wiese K-H, Rambusch E, deVries MJ, Hanauske A: **EORTC Early Clinical Studies Group. Complete response of a gastric primary after a short but toxic course of 'S-1.** *Ann Oncol* 1999, **10**:1117-20.
11. Iwazawa T, Kinuta M, Yano H, Matsui S, Tamagaki S, Yasue A, Okada K, Kanoh T, Tono T, Nakano Y, Okamoto S, Monden T: **An oral anticancer drug, TS-1, enabled a patient with advanced gastric cancer with Virchow's metastasis to receive curative resection.** *Gastric Cancer* 2002, **5**:96-101.
12. Kimura Y, Kikkawa N, Iijima S, Kato T, Naoi Y, Hayashi T, Tanigawa T, Yamamoto H, Kurokawa E: **A new regimen for S-1 therapy aiming at adverse reaction mitigation and prolonged medication by introducing a 1-week drug-free interval after each 2-week dosing session: efficacy and feasibility in clinical practice.** *Gastric Cancer* 2003, **6**:34-9.
13. Miyoshi K, Fuchimoto S, Ohsaki T, Sakata T, Takeda I, Takahashi K, Ohkawa T, Tanaka K, Matsumoto T, Takakura N, Motoi M: **Suture**

line recurrence in jejunal pouch replaced after gastrectomy for gastric cancer. *Gastric Cancer* 1999, **2**:194-7.
14. Nishimura M, Honda I, Watanabe S, Nagata M, Souda H, Miyazaki M: **Recurrence in jejunal pouch after proximal gastrectomy for early upper gastric cancer.** *Gastric Cancer* 2003, **6**:197-201.
15. Adachi Y, Mori M, Maehara Y, Sugimachi K: **Poorly differentiated medullary carcinoma of the stomach.** *Cancer* 1992, **70**:1462-6.

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Addition of an arch support improves the biomechanical effect of a laterally wedged insole

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Gait analysis

ABSTRACT

In order to examine if the addition of an arch support could improve the biomechanical effect of the laterally wedged insole, three-dimensional gait analysis was performed on 20 healthy volunteers. Kinetic and kinematic parameters at the knee and subtalar joints were compared among the following four types of insoles; a 5-mm thick flat insole, a flat insole with an arch support (AS), a 6° inclined laterally wedged insole (LW), and a laterally wedged insole with an arch support (LWAS). The knee adduction moment averaged for the entire stance phase was reduced by the use of LW and LWAS by 7.7% and 13.3%, respectively, from that with FLAT. The difference in knee adduction moment between LW and LWAS was most obvious in the late stance, which was ascribed to the difference in the progression angle between those insoles. The analyses also revealed that LW tended to increase step width, and that such an increase was completely eliminated by the addition of an arch support to LW. This reduction of step width could be another mechanism for the further reduction of the moment with LWAS. The analyses of biomechanical parameters at the subtalar joints suggested that LWAS allowed the subject to walk in a more natural manner, while exerting greater biomechanical effects than LW. Thus, the addition of an arch support to the laterally wedged insole reduced knee adduction moment more efficiently, possibly through the elimination of potential negative effects of the laterally wedged insole.

1. Introduction

Osteoarthritis (OA) of the knee joint is the most prevalent joint disease among the elderly. Loading of the knee has been shown to play a key role in the development and progression of the disease [1]. Loading while walking is particularly important, because walking is the most frequently performed activity. During walking, the load is not equally distributed between the medial and lateral compartments of the joint. In a normal gait, the peak force on the medial compartment is almost 2.5 times that on the lateral compartment [2]. This uneven loading may account for the high susceptibility of the medial compartment to OA. Once OA changes are initiated, the magnitude of the medial load is associated with the severity of symptoms and progression of the disease [1,3]. Therefore, load reduction within the medial compartment could be critical in the management of patients with medial knee OA.

The load transferred through the medial and lateral compartments during walking can be estimated on the basis of the external knee adduction moment measured during three-dimensional gait analysis [2]. Using this parameter, both the symptoms and progression of medial knee OA were shown to correlate with the magnitude of load transferred through the medial compartment [1,3]. Knee adduction moment has also been used to evaluate the effects of treatments directed to reduce the medial load [4–8].

Laterally wedged insoles are used to treat patients with medial knee OA in its earlier stages [9,10], and successful results have been reported [11,12]. However, its efficacy may be limited [9,13–15], possibly because the insole fails to reduce knee adduction moment in certain individuals [7,16]. Furthermore, its effectiveness may be reduced by the discomfort caused by its use [17]. In an attempt to relieve that discomfort, we modified the insole by adding an arch

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support. Unexpectedly, the use of the modified insole not only reduced discomfort but also enhanced the clinical results [18]. This result led us to consider whether the addition of an arch support may improve the biomechanical effect of the laterally wedged insole. The present study was conducted to examine this hypothesis.

2. Methods

2.1. Subjects

This study was performed on healthy volunteers under the approval of the institutional review boards. Sample size was determined by a published nomogram [19], based upon our previous data [7,20,21]. The result indicated that a sample size of 20 would be enough to detect a 5% difference in peak knee adduction moment or peak subtalar abduction moment, with a statistical power of 80% and a 5% level of significance. Thus, 20 healthy volunteers (11 males and 9 females) who had no known history of symptoms with their back and lower extremities were enrolled in this study. Informed consent was obtained in writing from all volunteers. Prior to gait analysis, lower limbs were clinically examined and weight-bearing antero-posterior radiographs were obtained to confirm that there was no abnormality. Details of the subjects are shown in the supplementary data (Supplementary Table 1).

2.2. Data acquisition system

Three-dimensional gait analysis was conducted with a 12-camera optoelectronic motion analysis system (Vicon 512; Oxford Metrics, Oxford, UK) combined with eight force platforms (Kistler 9281C; Kistler Instrument, Winterthur, Switzerland) as described previously [7,20,21]. Details of the data acquisition system are given in the supplementary data (Supplementary Method).

2.3. Experimental protocol

Four types of insoles were tested in this study (Fig. 1). A laterally wedged insole (LW) had the size of the entire sole and was inclined medially at an angle of 6° along the full length of the insole. A laterally wedged insole with an arch support (LWAS) was used to evaluate the effect of that added arch support. A 5-mm thick, flat insole without inclination (FLAT) and a flat insole with an arch support (AS) were used as the controls. All insoles and arch supports were made of ethylene vinyl acetate (EVA 8200, Toyo Sponge, Tokyo, Japan), which had an elasticity coefficient of 100–300 kg/mm^2. The insole size and the shape and height of the arch support were adjusted to fit each subject. The insoles were directly attached to the subjects' soles bilaterally with double-sided adhesive tape, and the subjects were requested to walk on the walkway with the insoles, without wearing shoes. The insoles were tested at a self-selected, natural walking speed. To keep the gait velocity constant during measurement, a metronome was first set to the subject's cadence, and the subject was requested to walk with the insoles at that cadence. The four insoles were tested sequentially during a single measurement session in a randomized order. For each type of insole, the first or second trials were used as accommodation trials, while the data of five subsequent trials were employed for the analysis.

2.4. Data analysis

Data were analyzed by a previously described method [7,20,21]. The rotation of the subtalar joint was defined as the rotation of the calcaneus relative to the lateral and medial malleoli. All joint moments were expressed as external moments and were normalized to the subject's body weight and height and expressed as a percentage of body weight × height (%Bw × Ht).

Three kinematic parameters were also acquired from the measurements (Fig. 2). Progression angle was the angle between the direction of gait progression and the foot axis at midstance. Step width was the distance between the centers of pressure (COPs) of the right and left foot across the direction of gait progression, and step length was the distance traversed by a single step.

The kinetic and kinematic parameters of knee and subtalar joints were first compared with the four types of insoles for the entire stance phase. Then the stance phase was divided into three sections of equal length, and the parameters for the four insoles were compared in the respective sections. The results are shown by the mean +, −, or ± standard error of means (S.E.M.).

2.5. Statistical analysis

Statistical analysis was performed with the Dr. SPSS-II software (version 11.01.1J, SPSS Japan, Tokyo, Japan). Data were initially analyzed by repeated measures one-way analysis of variance (ANOVA) and, when necessary, Dunnett's multiple comparison was employed as a post hoc test. The level of significance was set at $p < 0.05$.

3. Results

3.1. External adduction moment at the knee joint

The time–distance parameters and ground reaction force that could affect the kinetics and kinematics of the knee and subtalar

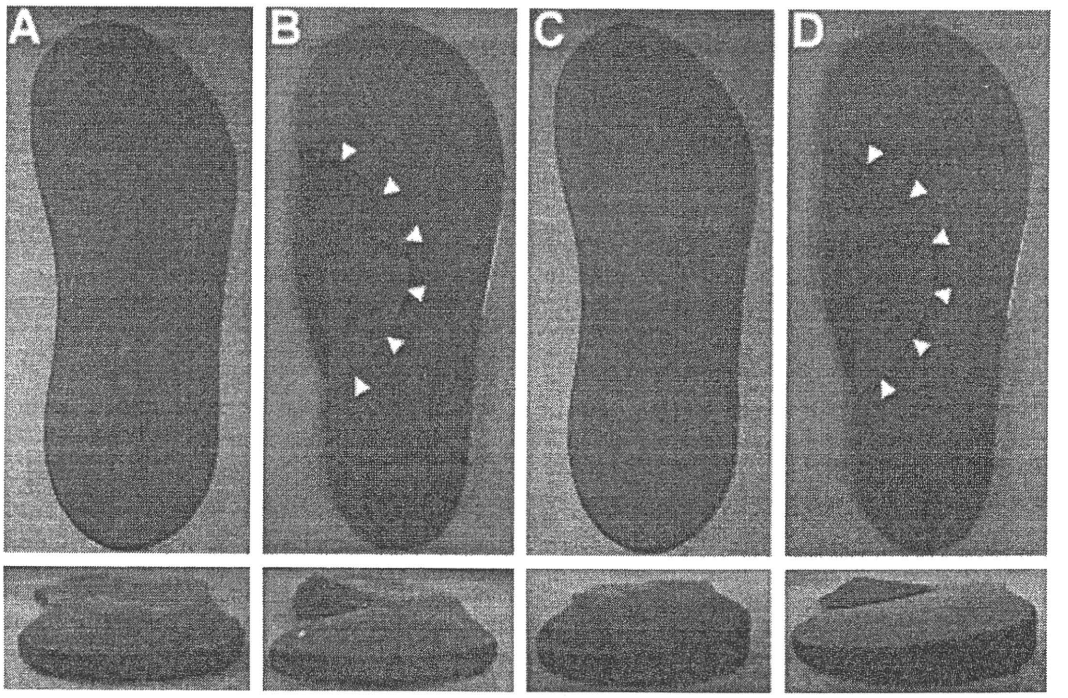

Fig. 1. Insoles used for this study. Upper (upper panels) and posterior views (lower panels) of FLAT (A), AS (B), LW (C), and LWAS (D) are shown. In (B) and (D), white arrowheads indicate arch supports added to the insoles.

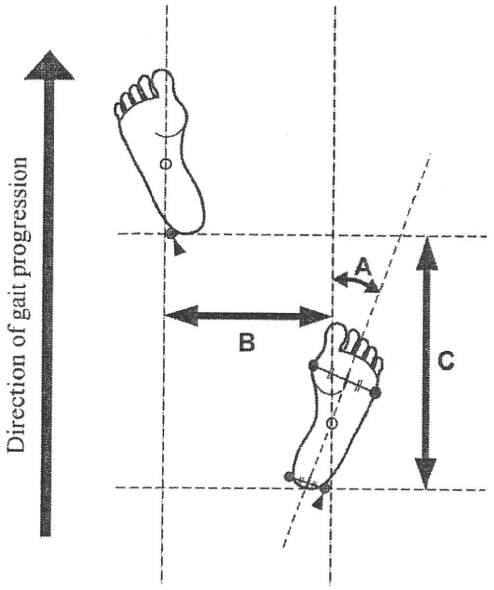

Fig. 2. Three kinematic parameters evaluated. Progression angle (A) is the angle formed by the direction of gait progression and the foot axis, which is an assumptive line passing through the middle of the first and fifth metatarsal heads and the middle of the calcaneal tuberosity at the midstance (red dotted line). Step width (B) is the distance between the COPs of right and left feet at midstance vertically across the direction of gait progression. Step length (C) is the distance of right and left feet in a single step along the direction of gait progression obtained from the positions of markers on the lateral aspects of calcaneal tuberosities at heel strike (solid arrowheads). Black dotted lines are assumptive lines drawn parallel to the direction of gait progression passing through the COPs, while blue ones are those drawn vertically to the direction of gait progression, passing markers at the lateral aspects of calcaneal tuberosities. Red circles indicate markers placed at the metatarsal heads, and blue ones denote those at the lateral and medial aspects of calcaneal tuberosities. Open circles represent COPs. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

joints were not significantly different among the insoles (Supplementary Table 2).

In accordance with previous studies [5,8,22–25], external adduction moment of the knee joint presented a two-peak pattern during the stance phase (Fig. 3A). Considering this, the stance phase in the current study was divided into three parts of equal length (early, middle, and late sections), and the effect of the insole was evaluated in respective sections as well as for the entire stance phase.

Among the four types of insoles, the peak knee adduction moment was the highest with FLAT (Fig. 3B). While the moment with AS was similar to that with FLAT, the peak moment was significantly reduced with LW and LWAS compared to FLAT ($p = 0.010$ and $p = 0.034$, respectively). The adduction moment averaged for the entire stance phase showed a similar change with the insoles (Fig. 3C). The mean moment was highest with FLAT, followed by AS and LW, and lowest with LWAS. The change of the mean moment with the insoles differed from that of the peak moment in that the reduction was more obvious with LWAS than with LW. The reduction of the mean moment with LW and LWAS was 7.7% and 13.3%, respectively, compared to that with FLAT. The mean moment with LWAS was significantly lower than that with LW ($p = 0.002$). Next, the knee adduction moment was averaged in each of the three stance phase sections, and compared among the insoles (Fig. 3D). In the early section, the moments with LW and LWAS were slightly reduced compared to that with FLAT, but the reduction was not significant for either insole. In the middle section, the moment was significantly reduced with LW and LWAS compared to that with FLAT ($p = 0.003$ and $p < 0.001$, respectively). In the late section, the moment was obviously reduced with LWAS, which was found to be significantly lower than that with LW ($p < 0.001$) as well as that with FLAT ($p < 0.001$). Although the moment with LW was lower than that with FLAT, the reduction in this section did not reach the level of significance ($p = 0.053$).

3.2. Valgus angle at the knee joint

In order to examine whether the observed difference in the adduction moment was related to the change in the kinematics of the knee joint, the valgus knee joint angle was compared among the four insoles, and no significant difference was found among any of them (Supplementary Figure). Therefore, it is unlikely that the change of knee adduction moment with the insoles was caused by any difference in knee joint kinematics.

3.3. External abduction moment and valgus angle at the subtalar joint

Compared with FLAT, the peak abduction moment at the subtalar joint was significantly higher with LW and LWAS than with FLAT ($p < 0.001$ for both), while it was almost unchanged with AS (Fig. 4A). The level of increase was similar for LW and LWAS. A similar trend was observed when the moment was evaluated in each of the three sections during the stance phase (Fig. 4B). That is, the moment was not altered with AS in either section, but was equally increased with LW and LWAS compared to FLAT in all three sections ($p \leq 0.003$ for LW and $p < 0.001$ for LWAS).

The valgus angle of the subtalar joint was averaged for each section of the stance phase and compared among the insoles (Fig. 4C). Throughout the sections, the valgus angle was lowest with FLAT, followed by AS and LWAS, and highest with LW.

From these results, the addition of the arch support to LW may indeed tend to reduce the change of subtalar valgus angle, while exerting a similar level of abduction moment at the joint to that with LW.

3.4. Progression angle and step width

The progression angle and step width were compared for the four insoles. The progression angle was lowest with LW, and highest with LWAS (Fig. 5A). The difference in the angle between those two insoles was significant ($p = 0.037$). This result indicates that the use of LW tended to induce a toe-in gait, but this trend was completely reversed by the addition of an arch support to LW. Meanwhile, the step width increased most with LW, and declined most with LWAS (Fig. 5B). The difference in width between those two insoles was statistically significant ($p = 0.033$). Comparison between LW and LWAS revealed that the step width tended to increase with LW, but that increase was completely eliminated by the addition of an arch support. These changes in the progression angle and step width imply that the gait pattern could be altered by the use of LW, but it may be normalized by the addition of an arch support.

4. Discussion

In our study, the peak knee adduction moment was reduced by approximately 8.8% by the use of LW. This level of reduction was similar to those in previous reports [5,6,26], which would support the validity of our measurements. Although the peak moment was not changed by the addition of an arch support to LW, the knee adduction moment averaged for the entire stance phase was significantly reduced by it (Fig. 3B and C). This reduction of the moment was most obvious in the late stance (Fig. 3D). Our current

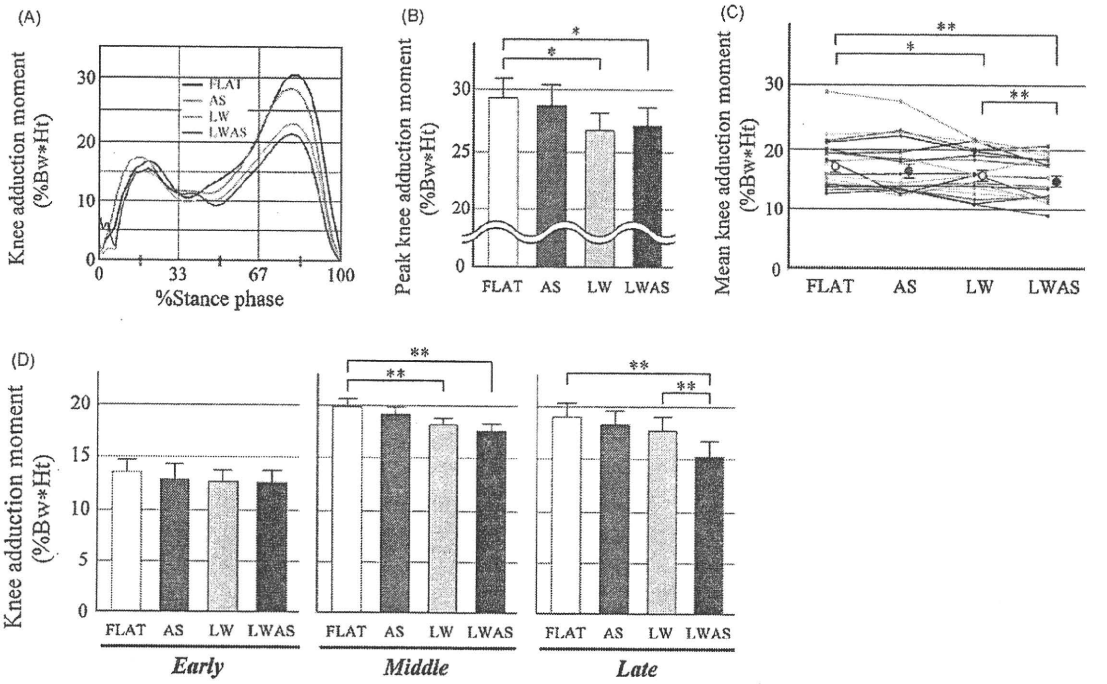

Fig. 3. External adduction moment of the knee joint with the four types of insoles. (A) Knee adduction moment with the four types of insoles during the stance phase. Representative result of a single subject is shown. Data are averages of five measurements. (B) Peak values of knee adduction moment with the four types of insoles. (C) Adduction moment averaged for the entire stance phase. Results of respective subjects are shown by lines of different colors together with the mean values of all subjects. (D) Adduction moment was averaged in respective sections of the stance phase and compared among the insoles. In (B)–(D), values are the mean + or ± S.E.M. $^{*}p < 0.05$ and $^{**}p < 0.01$, respectively.

analysis also revealed that the use of LW reduced the progression angle, and that such a change in the angle was fully reversed by the addition of an arch support (Fig. 5A). The increase in progression angle has been shown to decrease the knee adduction moment in the late stance [4,27,28]. Therefore, it is very likely that the arch support added to LW reduced the knee adduction moment through the increase in the progression angle. Meanwhile, the finding that the progression angle decreased with the use of LW implies that

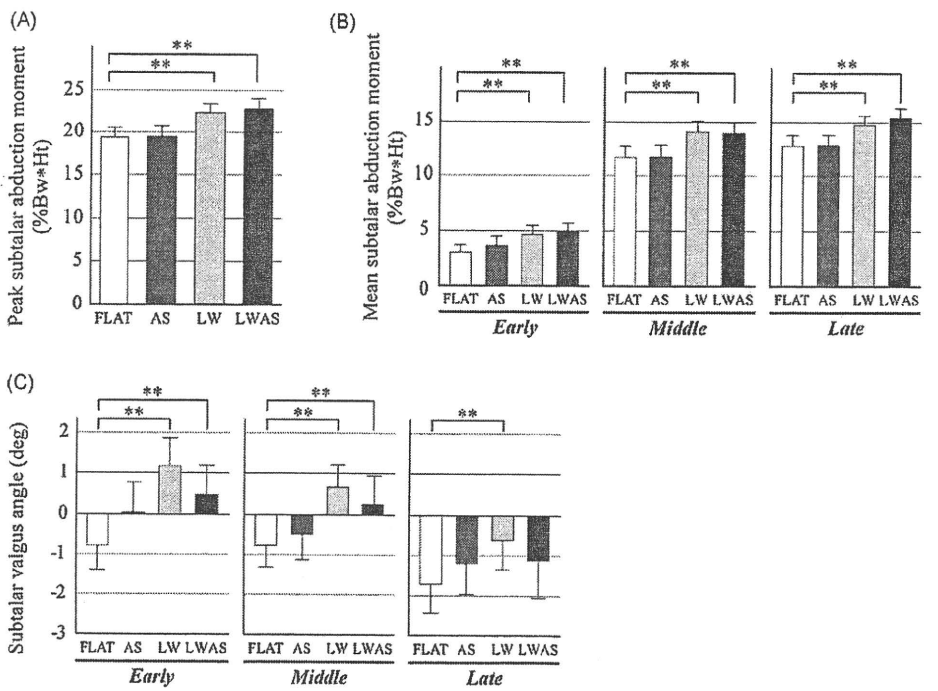

Fig. 4. Abduction moment and valgus angle at a subtalar joint with the four types of insoles. (A) Peak values of abduction moment with respective insoles. (B) Abduction moment averaged in respective sections of the stance phase. (C) Valgus angle averaged in respective sections of the stance phase. Values are the mean + or – S.E.M. $^{*}p < 0.05$ and $^{**}p < 0.01$, respectively.

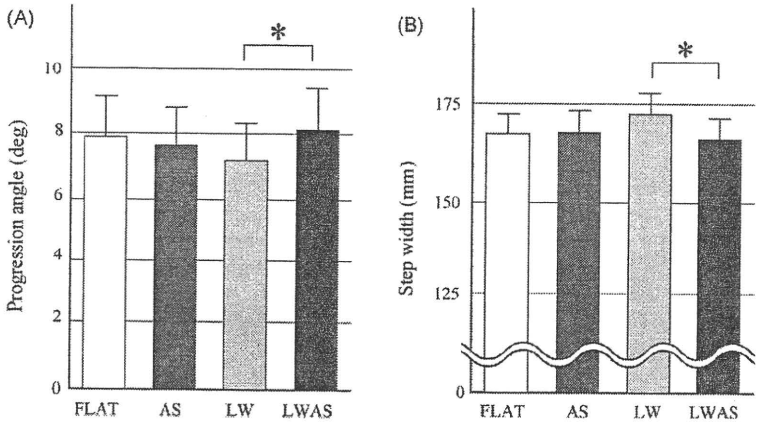

Fig. 5. Progression angle (A) and step width (B) with the four types of insoles. Values are the mean + S.E.M. $^{*}p < 0.05$.

the effect of LW in reducing the knee adduction moment could be impaired to some extent by a toe-in gait induced by it. A reduction of the progression angle by LW was reported in another study [22]. This would pose a potential drawback with that type of insole. As shown here, this drawback may be completely eliminated by the addition of an arch support. Reduction of the progression angle increases the risk of progression of medial knee OA, probably through an increase of the knee adduction moment in the late stance [29]. Therefore, such changes of progression angle should be considered carefully when insoles are used to treat knee OA patients.

The present study also revealed another potential problem with the conventional laterally wedged insole. Our current observation and those of others have consistently indicated that the use of LW increased step width (Fig. 5B) [8,12]. The wider the step width becomes, the more lateral the position of the ground reaction force would be from the center of gravity of the body, and this would increase knee adduction moment. Therefore, in addition to the change of progression angle, the increase in step width may be yet another factor limiting the effect of LW in reducing the knee adduction moment. Since the step width with LWAS was smaller than that with the control insole, the addition of an arch support to LW appeared to completely eliminate this second possible drawback of the conventional wedged insole.

Another advantage of the additional arch support was suggested by an analysis of the kinetic and kinematic parameters at the subtalar joint. We previously reported that a laterally wedged insole alters the kinetics and kinematics of the subtalar joint [7,20,21]. In accordance with those results, the use of a laterally wedged insole increased the abduction moment and valgus angle at the subtalar joint. The addition of an arch support to LW tended to reduce the valgus angle of the joint (Fig. 4C), while keeping the abduction moment equal to the level of LW (Fig. 4A and B). During the measurements, some subjects complained of instability or foot discomfort when wearing LW, but that feeling was considerably relieved with LWAS. The discomfort associated with the use of LW may be surmised to have stemmed from over-abduction of the subtalar joints. This may have been alleviated by the addition of an arch support, which reduced the degree of abduction.

Thus, the addition of an arch support to the laterally wedged insole changed all of the progression angle, step width, and valgus angle at the subtalar joint closer to the levels of the control insole. Therefore, it may be reasonable to assume that the addition of an arch support to LW allowed the subjects to walk in a more "natural" manner, while increasing the effect of the wedged insole in reducing the knee adduction moment.

A significant limitation of this study is that the biomechanical effect of insoles was investigated in healthy volunteers but not in the actual OA patients. The differences in the types of wedged insoles is another issue that was not addressed in this study, as insoles with shorter wedging or other inclinations may be used in clinics [13,14,30]. Since insoles are used more often within the shoes, our measurement without shoes may not reflect the actual situation of their use. This could also be a limitation of this study. These points should be addressed in future studies.

Medial knee OA deteriorates in a vicious circle of increasing varus angulation and loading of the medial compartment. The use of a laterally wedged insole is expected to prevent further disease progression by breaking this circle [5]. However, at present, insole therapy has not yet become a common treatment for knee OA, primarily because of its limited efficacy [7,9,13–16]. We hope that our current findings will be useful in modifying the conventional lateral wedged insole to obtain better clinical results.

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Conflict of interest statement

None of the authors has any conflict of interest regarding this study.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.gaitpost.2008.08.007.

References

[1] Miyazaki T, Wada M, Kawahara H, Sato M, Baba H, Shimada S. Dynamic load at baseline can predict radiographic disease progression in medial compartment knee osteoarthritis. Ann Rheum Dis 2002;61:617–22.
[2] Schipplein OD, Andriacchi TP. Interaction between active and passive knee stabilizers during level walking. J Orthop Res 1991;9:113–9.
[3] Hurwitz DE, Ryals AR, Block JA, Sharma L, Schnitzer TJ, Andriacchi TP. Knee pain and joint loading in subjects with osteoarthritis of the knee. J Orthop Res 2000;18:572–9.
[4] Wang JW, Kuo KN, Andriacchi TP, Galante JO. The influence of walking mechanics and time on the results of proximal tibial osteotomy. J Bone Joint Surg Am 1990;72:905–9.
[5] Crenshaw SJ, Pollo FE, Calton EF. Effects of lateral-wedged insoles on kinetics at the knee. Clin Orthop Relat Res 2000;375:185–92.
[6] Kerrigan DC, Lelas JL, Goggins J, Merriman GJ, Kaplan RJ, Felson DT. Effectiveness of a lateral-wedge insole on knee varus torque in patients with knee osteoarthritis. Arch Phys Med Rehabil 2002;83:889–93.

[7] Kakihana W, Akai M, Nakazawa K, Takashima T, Naito K, Torii S. Effects of laterally wedged insoles on knee and subtalar joint moments. Arch Phys Med Rehabil 2005;86:1465–71.
[8] Shimada S, Kobayashi S, Wada M, Uchida K, Sasaki S, Kawahara H, et al. Effects of disease severity on response to lateral wedged shoe insole for medial compartment knee osteoarthritis. Arch Phys Med Rehabil 2006;87:1436–41.
[9] Brouwer RW, Jakma TS, Verhagen AP, Verhaar JA, Bierma-Zeinstra SM. Braces and orthoses for treating osteoarthritis of the knee. Cochrane Database Syst Rev 2005;CD004020.
[10] Krohn K. Footwear alterations and bracing as treatments for knee osteoarthritis. Curr Opin Rheumatol 2005;17:653–6.
[11] Sasaki T, Yasuda K. Clinical evaluation of the treatment of osteoarthritic knees using a newly designed wedged insole. Clin Orthop Relat Res 1987;221:181–7.
[12] Keating EM, Faris PM, Ritter MA, Kane J. Use of lateral heel and sole wedges in the treatment of medial osteoarthritis of the knee. Orthop Rev 1993;22:921–4.
[13] Maillefert JF, Hudry C, Baron G, Kieffert P, Bourgeois P, Lechevalier D, et al. Laterally elevated wedged insoles in the treatment of medial knee osteoarthritis: a prospective randomized controlled study. Osteoarthritis Cartilage 2001;9:738–45.
[14] Pham T, Maillefert JF, Hudry C, Kieffert P, Bourgeois P, Lechevalier D, et al. Laterally elevated wedged insoles in the treatment of medial knee osteoarthritis: a two-year prospective randomized controlled study. Osteoarthritis Cartilage 2004;12:46–55.
[15] Baker K, Goggins J, Xie H, Szumowski K, LaValley M, Hunter DJ, et al. A randomized crossover trial of a wedged insole for treatment of knee osteoarthritis. Arthritis Rheum 2007;56:1198–203.
[16] Fisher DS, Dyrby CO, Mundermann A, Morag E, Andriacchi TP. In healthy subjects without knee osteoarthritis, the peak knee adduction moment influences the acute effect of shoe interventions designed to reduce medial compartment knee load. J Orthop Res 2007;25:540–6.
[17] Andriacchi TP, Mundermann A. The role of ambulatory mechanics in the initiation and progression of knee osteoarthritis. Curr Opin Rheumatol 2006;18:514–8.
[18] Hiraoka H, Nakajima K, Oda H. Non-surgical treatments for gonarthrosis. Clin Calcium 2002;12:92–7.
[19] Atman D. Practical statistics for medical research. London: Chapman & Hall; 1991.
[20] Kakihana W, Akai M, Yamasaki N, Takashima T, Nakazawa K. Changes of joint moments in the gait of normal subjects wearing laterally wedged insoles. Am J Phys Med Rehabil 2004;83:273–8.
[21] Kakihana W, Torii S, Akai M, Nakazawa K, Fukano M, Naito K. Effect of a lateral wedge on joint moments during gait in subjects with recurrent ankle sprain. Am J Phys Med Rehabil 2005;84:858–64.
[22] Nester CJ, van der Linden ML, Bowker P. Effect of foot orthoses on the kinematics and kinetics of normal walking gait. Gait Posture 2003;17:180–7.
[23] Teichtahl AJ, Wluka AE, Morris ME, Davis SR, Cicuttini FM. The relationship between the knee adduction moment and knee pain in middle-aged women without radiographic osteoarthritis. J Rheumatol 2006;33:1845–8.
[24] Thorp LE, Sumner DR, Block JA, Moisio KC, Shott S, Wimmer MA. Knee joint loading differs in individuals with mild compared with moderate medial knee osteoarthritis. Arthritis Rheum 2006;54:3842–9.
[25] Hunt MA, Birmingham TB, Giffin JR, Jenkyn TR. Associations among knee adduction moment, frontal plane ground reaction force, and lever arm during walking in patients with knee osteoarthritis. J Biomech 2006;39:2213–20.
[26] Rosenbaum D, Rodl R, Entrup M, Klein D. Shoe modification or inserts can effect relief of the knee joint. Z Orthop Ihre Grenzgeb 2002;140:579–80.
[27] Andrews M, Noyes FR, Hewett TE, Andriacchi TP. Lower limb alignment and foot angle are related to stance phase knee adduction in normal subjects: a critical analysis of the reliability of gait analysis data. J Orthop Res 1996;14:289–95.
[28] Hurwitz DE, Ryals AB, Case JP, Block JA, Andriacchi TP. The knee adduction moment during gait in subjects with knee osteoarthritis is more closely correlated with static alignment than radiographic disease severity, toe out angle and pain. J Orthop Res 2002;20:101–7.
[29] Chang A, Hurwitz D, Dunlop D, Song J, Cahue S, Hayes K, et al. The relationship between toe-out angle during gait and progression of medial tibiofemoral osteoarthritis. Ann Rheum Dis 2007;66:1271–5.
[30] Yasuda K, Sasaki T. The mechanics of treatment of the osteoarthritic knee with a wedged insole. Clin Orthop Relat Res 1987;215:162–72.