

Brief Communication



A Potential New Mouse Model of Axial Spondyloarthritis Involving the Complement System

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Conflict of Interest

The authors declare no potential conflicts of interest.

Abbreviations

AS, ankylosing spondylitis; AxSpA, axial spondyloarthritis; CDA, clinical disease activity; CAIA, collagen antibody-induced arthritis; GPI, glucose-6-phosphate isomerase; IHC, immunohistochemistry; IVD,

ABSTRACT

Many mouse models of rheumatoid arthritis have been identified, but only a limited number are present for axial spondyloarthritis (AxSpA). Collagen Ab-induced arthritis (CAIA) is one of the most widely used mouse models of arthritis, and it is complement-dependent. We found that mice developing CAIA also developed spinal lesions similar to those found in AxSpA. To induce CAIA, mice were injected intraperitoneally at day 0 with anti-collagen Abs, followed by LPS injection at day 3. CAIA mice demonstrated a significant kyphosis through the spine, as well as hypertrophic cartilage and osseous damage of the intravertebral joints. Immunohistochemical staining of the kyphotic area revealed increased complement C3 deposition and macrophage infiltration, with localization to the intravertebral joint margins. Near Infrared (NIR) *in vivo* imaging showed that anti-collagen Abs conjugated with IRDye® 800CW not only localized to cartilage surface in the joints but also to the spine in arthritic mice. We report here a novel preclinical mouse model in which, associated with the induction of CAIA, mice also exhibited salient features of AxSpA; this new experimental model of AxSpA may allow investigators to shed light on the local causal mechanisms of AxSpA bone and soft tissue changes as well as treatment.

Keywords: Axial spondyloarthritis; Arthritis; Complement

INTRODUCTION

Spondyloarthritis (SpA) represents a group of inflammatory diseases that are characterized by inflammation and tissue damage within the spine, sacro-iliac joints and/or peripheral joints. Axial spondyloarthritis (AxSpA) involve spine but not peripheral joints. Overall, SpA has been classified into various categories such as ankylosing spondylitis (AS), AxSpA, psoriatic arthritis, reactive arthritis (RA), and enteropathic arthritis (ulcerative colitis and Crohn's disease). AS is the most common SpA, and its prevalence in the US has been estimated to be 0.2% to 0.5% (1). AS is characterized by inflammation at the entheses that is followed by osteoproliferation, or new bone formation, which often leads to fusion (ankylosis) of the affected joints. HLA-B27 is the major Class I gene associated with AS, as about 90% of patients express this allele (2).

intervertebral disc; microCT, micro-computed tomography; RA, reactive arthritis; SpA, spondyloarthritis; T-blue, Toluidine blue; WT, wild type.

Author Contributions

Conceptualization: Banda NK, Holers VM; Data curation: Banda NK, La Rosa FG; Formal analysis: Banda NK, La Rosa FG; Funding acquisition: Holers VM, Banda NK; Investigation: Banda NK, La Rosa FG; Methodology: Banda NK, La Rosa FG; Project administration: Banda NK; Resources: Banda NK, Holers VM; Supervision: Banda NK; Writing - original draft: Banda NK; Writing - review & editing: Banda NK, Holers VM, La Rosa FG.

There are 2 types of AxSpA; one is radiographic and second is non-radiographic and subjects with AxSpA can have inflammation in non-articular sites. With regard to animal models, it has been shown in HLA-B27 rats with overexpression of human β 2-microglobulin that these animals developed the clinical (arthritis and dactylitis) and histopathologic features (synovitis, spondylitis, enthesitis, fibrosis, and bony proliferation) of the arthritic disease resemble human SpA to a greater degree than has been observed in the high-copy HLA-B27/Hu β 2m (3). It has also been shown that older male DBA/1 mice develop spontaneous enthesal cell proliferation and infiltration, progressively leading to endochondral bone formation similar to human SpA (4). By using HLA-B27 transgenic mice, it has been shown that there is an increased inflammation in the collagen Ab-induced arthritis (CAIA) model, but there was no effect on bone formation in AxSpA (5); in this later study no analysis of spine tissue was reported. With regard to mechanism, recently it has been shown by using mesenchymal stem cells from AS patients that HLA-B27 misfolding activates a p-IRE1/sXBP1/RARB/TNAP pathway which contributes to the pathogenesis of bone mineralization and ectopic bone formation at inflamed entheses (6). Interestingly despite of strong association of HLA-B27 with AS, initial studies with HLA-B27 expressed in mice lacking β 2m were not reproducible (7,8) and it was shown that the frequency of spontaneous inflammatory arthritis was not increased by HLA-B27 in β 2m B6 mice (8).

Previously, SpA was normally considered an auto-inflammatory disease, but this view is evolving due in part to the reported presence of autoantibodies in a subset of SpA patients (9). In addition, studies have shown that Th17 cells might be involved in the pathogenesis of SpA (10). Targeted liver overexpression of IL-23 in B10.RIII resulted in an AxSpA phenotype as well as peripheral disease that was due to the activation of enthesal-resident non-canonical T cells (5). Nonetheless, the presence of autoantibodies is the main characteristic of autoimmune disease, and sometime these autoantibodies are even present before the development of disease. For example, anti- β 2m Abs have been reported in SpA patients (11).

Some reports also suggest the presence of anti-type I and type II collagen Abs in SpA patients (12). In one study, serum levels of metalloproteinase-degraded collagen type I (C1M), type II (C2M), type III (C3M) and type IV (C4M2) were quantified (13). These investigators found that the best metabolite that differentiates between AxSpA and control was C3M, i.e., type III (13). Overall this study suggested that collagen degradation metabolites are higher in AxSpA and these metabolites may be disease activity biomarkers of AxSpA. In another study, Abs against collagen were analyzed and found that AS patients exhibited slightly increased levels as compared to controls (14). Thus, collagen and reactivity to it as an integral part of the cartilage might play an important role in the pathogenesis of SpA.

Our previously studies showed the important role complement system in CAIA in mice (15). The complement system is one of the major effector arms of the innate immunity and is activated by 3 different pathways, i.e., classical, lectin pathway and alternative pathway. Regardless of how these 3 pathways get activated, all of these pathways converge to cleave C3. An activated complement system generates effectors such as anaphylatoxins (C3a, C5a), opsonins (C3b, iC3b, C3d) and membrane attack complex (MAC, C5b-9) (16). Furthermore, complement activation is linked to the adaptive immune responses. One of the most important functions of the complement system is the clearance of immune complexes and injured cells as well as serving as opsonins to clear the immune complexes and other debris (16). *In vitro*, adherent immune complexes of collagen and anti-collagen Abs activate C3 in normal serum but not in C3 complement deficient mice (17). Anti-collagen Abs

directly binds to the cartilage surface to form immune complexes (18) which can activate the complement. Anti-CD74 autoantibodies are found in early and late AxSpA patients (19) but their relationship to complement activation is not known. Interestingly although there are not many studies related to the role of complement in the pathogenesis of AxSpA, it has been shown that there is complement activation in AS and elevated complement proteins such as C3, C3d and C4 are found (20-22). Additionally, inhibition of complement retards the development of an AS model (23).

There exist various mouse and rat models of SpA, but these models might not fully mirror the human disease. Regardless, these models provide opportunities to examine the localized pathobiology of this disorder. SpA mouse models include the proteoglycan (24)-induced AS (25,26) and HLA-B27/human β 2-microglobulin-transgenic model, which develop a multisystem disorder also involving spondylitis (27). HLA-B27 transgenic mice (28) and mice deficient in β 2-microglobulin develop peripheral arthritis (29). An intraperitoneal injection of Curdlan resulted in the induction of SpA-like phenotype in the SKG mouse (30).

Here we report a potential new preclinical mouse model of AxSpA as a by-product of CAIA induction, which involves the complement system. Our results also suggest that one autoimmune disease, such as inflammatory arthritis, may also lead to a secondary autoimmune disease such as AxSpA; this interesting disease association may be due to the presence of anti-CII autoantibodies, or to some alteration of the immune system that requires further investigation. The main purpose of this publication is to report our unique observation on the effect of CAIA on the spine, leading to lesions similar to AxSpA and involvement of the complement system.

MATERIALS AND METHODS

Combined CAIA and AxSpA induction

A severe arthritis was induced in 6 week old C57BL/6 wild type (WT) mice (The Jackson Laboratory, Bar Harbor, ME, USA), by injecting a mixture of 5 anti-collagen monoclonal (anti-CII mAbs) (8 mg/mouse/i.p.) followed by an injection of LPS (50 ug/mouse/i.p.) (*Escherichia coli* strain) (17). Clinical disease activity (CDA) (31) in the joints in all mice was examined blindly from day 4 to day 10 according to our published studies (17). Briefly a 3-point scale was used for each paw: 0=normal joint; 1=slight inflammation and redness; 2=severe erythema and swelling affecting the entire paw; and 3=deformed paw or joint with ankylosis, joint rigidity, and loss of function (17,32). The maximum score of twelve per animal was based on the total score for all 4 paws, i.e., 3 for each paw. We also examined all mice for the presence of bending of the spine (kyphosis) from day 4 to day 10. All mice were sacrificed at day 10. All of these studies were approved by the Institutional Animal Care and Use Committee at the University of Colorado, Anschutz Medical Campus and all guidelines handling laboratory animals have been followed (No. B-14912(11)1E).

Histopathology of AxSpA spine

At day 10, the spine was surgically removed from sacrificed mice and fixed in a 10% neutral buffered formalin and processed for histopathology (17,32). Spine sections were also examined for inflammation, intervertebral disc (IVD) damage, cartilage damage, bone erosion, excess tissue formation and ectopic chondrocyte according to previously published criteria for a mouse model of AS (33). H&E staining was done to show histopathological

changes in the spine lesion area. Toluidine blue (T-blue) staining was used to examine the proliferation of chondrocytes in the deformed areas on the spine.

Immunohistochemistry (IHC) for C3 fragment deposition and macrophages infiltration in the AxSpA mice spine

At day 10, all specimens were evaluated with IHC staining using a polyclonal goat anti-mouse C3 antisera (34); C3 deposition was assessed in the spine kyphotic and surrounding area. Similarly, the presence of macrophages were also examined in the same areas of consecutive sections by using rat anti-mouse F4/80 Ab (34).

In vivo imaging of arthritis joints and AxSpA spine using infrared IRDye800 dye

For *in vivo* imaging, and to localize the anti-CII mAbs, WT mice were injected i.p. at day 0 with anti-CII mAbs conjugated with IRDye[®] 800CW (50 µg/mouse; LI-COR, Lincoln, NE, USA) (18). Anti-CII mAbs or control murine IgG were labelled with IRDye800 similarly (1–2 dye molecules/IgG). At day 14, mice were sacrificed for *in vivo* imaging to localize the IRDye800-conjugated anti-CII mAbs or control mIgG IRDye800-conjugated in the entire spine and also in the joints. Mice were scanned using LI-COR Odyssey Imaging system (LI-COR) according to our previous report (18).

Statistics

To assess the significant differences between means of mice with and without AxSpA for parameters such as C3, T-blue and histopathology, the Student's *t*-test was used. The $p < 0.05$ considered significant. Similarly, arthritis development in mice with AxSpA was compared with control mice using Student's *t*-test (data not shown). All data were analyzed and graphs were plotted using statistical GraphPad Prism version 4.0 software (GraphPad, La Jolla, CA, USA).

RESULTS

Co-development of arthritis and AxSpA in mice

All mice with CAIA not only developed synovitis in the joints after 5–7 days, but also developed kyphosis consistent with AxSpA-like phenotype, whereas control mice did not (Fig. 1). The CDA in arthritis mice injected which also developed AxSpA was significantly high ($p < 0.05$) 10.4 ± 1.60 compared with the control mice (Fig. 1A). There was a significant ($p < 0.05$) percent weight loss at day 10 in mice with CAIA/AxSpA (90.19 ± 1.62) compared with mice without CAIA/AxSpA (105.93 ± 3.02) (Fig. 1B). In another cohort of WT mice (male or female) with arthritis consistently developed the AxSpA-like phenotype and there was a change in posture due to kyphosis however in mice without arthritis there was no change in the posture or AxSpA-like phenotype developed (Fig. 1C and D). At day 10, kyphosis in the spine was visible in mice with arthritis/AxSpA (Fig. 1C) but not in mice without arthritis/AxSpA (Fig. 1D). Furthermore, the kyphotic spine was clearly visible after making an incision on the skin above area with kyphosis (Fig. 1E and F). Autopsy examination at day 10 confirmed, after surgical exposure of the dorsal skin in the kyphotic area, that the spine was deformed showing kyphosis only in mice with arthritis injected with anti-CII Abs plus LPS but not in control mice (Fig. 1G and H).

Histology of the kyphotic spine area of AxSpA mice

At day 10, T-blue and also H&E staining showed that the majority of the mice with CAIA/AxSpA demonstrated chondrocyte proliferation in the kyphotic area (Fig. 2A) in contrast to the

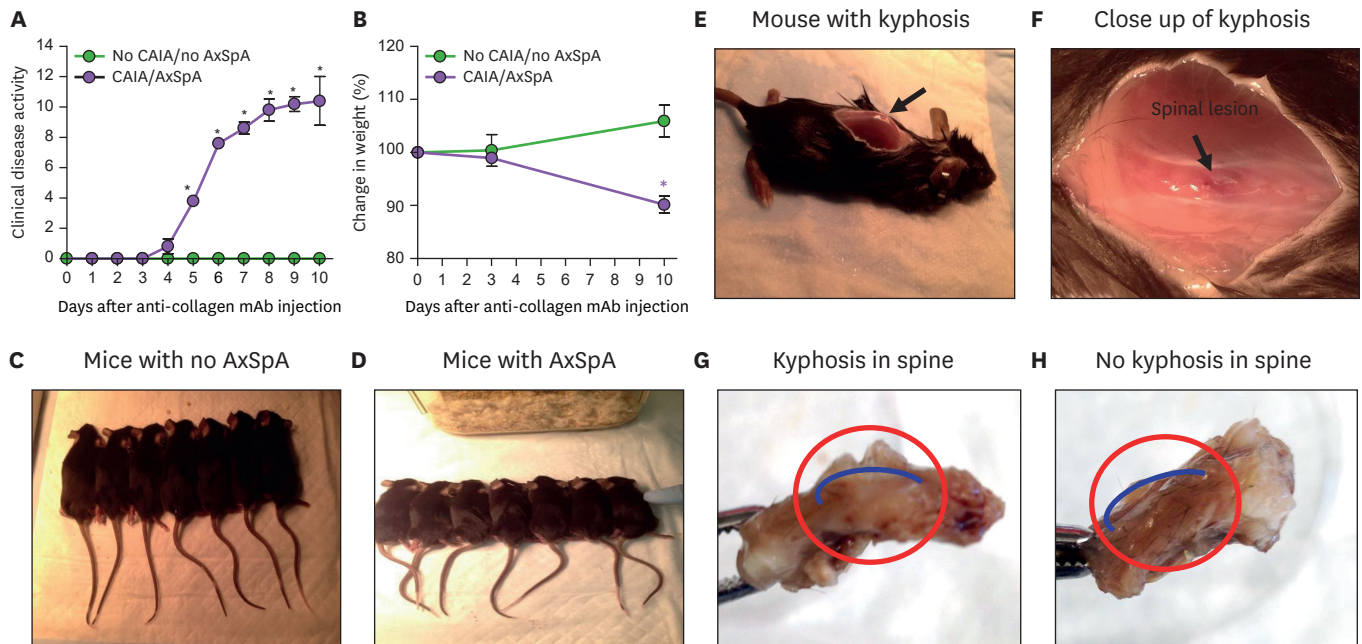


Figure 1. Mice with CAIA developed AxSpA-like phenotype and mice with no CAIA developed no AxSpA-like phenotype. All WT mice with arthritis consistently developed kyphosis and spine lesions indicating the development of AxSpA. (A) CDA in WT injected with anti-collagen Ab developed arthritis and AxSpA compared with control mice. (B) Percent change in weight in mice with CAIA/AxSpA compared with control mice without CAIA/AxSpA. (C) Representative gross images of mice with normal posture without kyphosis in a cohort of WT with no arthritis and no AxSpA. (D) Representative gross images of mice with bended backbone posture showing kyphosis in a cohort of arthritic mice co-developed AxSpA. (E) The kyphotic area in mice with CAIA/AxSpA was clearly visible at autopsy after making an incision on the overlying skin. (F) Close up view of an incision made to show the morphology of the spine lesion similar to AxSpA on a mice with induced arthritis (black arrow). (G) After dissection of the spine with arthritis the curved reddish spine area was exposed indicating AxSpA (curved blue line within red circle). (H) The same exam was performed in a mouse without arthritis; no reddish spine area was seen, and it was straight (curved blue line in the red circle). Data expressed as mean±SD.

*p < 0.05 considered significant. CDA shown in WT mice with CAIA/AxSpA (n=5) and WT control mice with no CAIA/no AxSpA (n=5). A separate cohort of WT mice with CAIA/AxSpA (n=7) or control WT mice without CAIA/AxSpA (n=7).

control mice (**Fig. 2B**). Histopathology scores for inflammation ($p < 0.001$), IVD destruction ($p < 0.05$), cartilage damage ($p < 0.05$), bone erosion ($p < 0.05$), excess tissue formation ($p < 0.05$) and presence of ectopic chondrocyte ($p < 0.007$) all show a significant increase in mice with CAIA-AxSpA with kyphosis which was not seen in control mice with no kyphosis (**Fig. 2C**).

Increased C3 staining and macrophage staining in kyphotic area of the spine of AxSpA mice

IHC staining of the kyphotic area and of the bone growth area in mice with CAIA/AxSpA revealed an increased C3 deposition in the inter vertebral region (88%) and inter-vertebral disc (65%) (**Fig. 2D**) as compared with no C3 deposition in the control mice (**Fig. 2E and F**). These data suggest that the complement system is involved in the initiation and development of AxSpA.

IHC staining of the kyphotic area in mice CAIA and AxSpA also showed a significant ($p < 0.05$) 75% increased macrophage infiltration, with localization to the inter-vertebral joint margins (**Fig. 2G**). Very little baseline macrophage staining was noticed in the mice with no CAIA and no AxSpA (**Fig. 2H and I**). These data clearly show that there is infiltration of the immune cells due to enthesitis in the kyphotic area.

At day 10, we also performed H&E staining of the spine of the kyphotic area and examined its interior surface (**Fig. 3**). We confirmed that there was an inflammation in the knee joints of

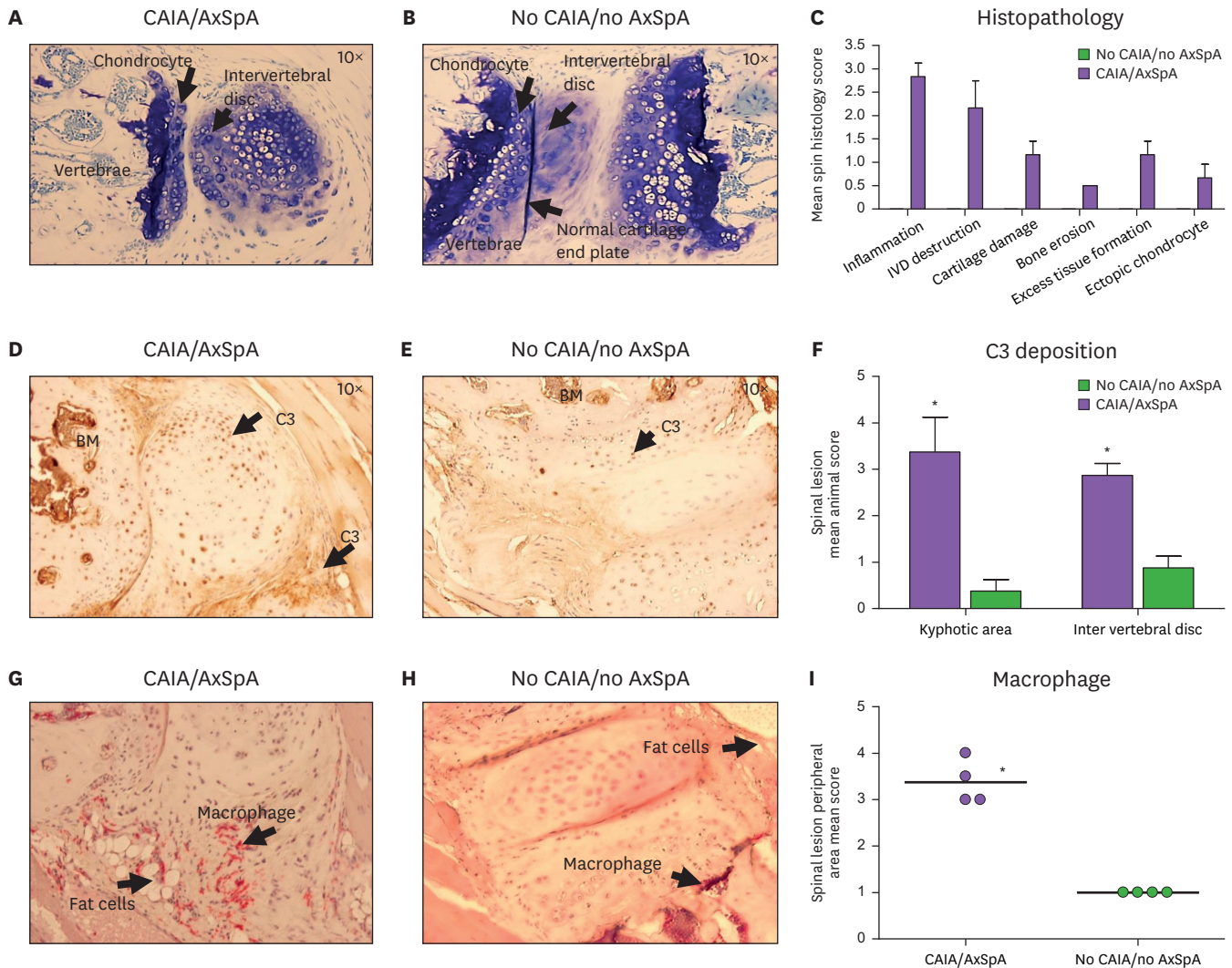


Figure 2. Histopathology, C3 deposition and macrophage infiltration of kyphotic area of spine. (A) A large and reactive chondrocyte proliferation in the kyphotic area of the vertebrae of mice with arthritis and AxSpA specifically in IVD area. (B) A normal chondrocyte growth in mice in inter vertebral disc area with no arthritis and no kyphosis. (C) Histopathological scores for inflammation, IVD destruction, cartilage damage, bone erosion, excess tissue formation and ectopic chondrocyte presence on the cartilage in the kyphotic area in mice with or without CAIA/AxSpA. (D) Increased C3 deposition score in the spine lesion and surrounding area of a treated mouse with spine lesion. (E) Minimal or no C3 deposition in spine areas of mice without arthritis and AxSpA. (F) C3 deposition in the spinal kyphotic and surrounding area as well as on the IVD. (G) Score of macrophages (red color) marked by a block arrow seen in the kyphotic area of mice with arthritis and AxSpA. (H) Few macrophages present in the spine areas of mice without arthritis and AxSpA. (I) Scores for F4/80 positivity in the spine lesion area and vertebral peripheral areas. Data expressed for histopathology scores as mean±SD (n=5 in each group). Data expressed for C3 deposition and macrophage as mean±SD. All microphotographs at 10× magnification.

BM, bone marrow.

*p<0.05 considered significant (n=4 in each group).

mice with arthritis compared with the control mice (**Fig. 3A and B**). Mice with CAIA/AxSpA demonstrated a significant hypertrophy of the cartilage as well as osseous damage of the inter-vertebral joints, as compared with no damage and no enthesitis in the untreated mice (**Fig. 3C and D**). There was multilayered pannus present similar to enthesitis on the side of inter vertebral disc (**Fig. 3E**) whereas no pannus was noticed in control mice as expected (**Fig. 3F**). Histological examination of the kyphotic area showed substantial bone damage only in the mice with arthritis and AxSpA (**Fig. 3G**) but not in control mice (**Fig. 3H**), which is an important characteristic of AxSpA. We could not confirm the fusion of the vertebrae in mice with CAIA/AxSpA-like phenotype (**Fig. 3C**).

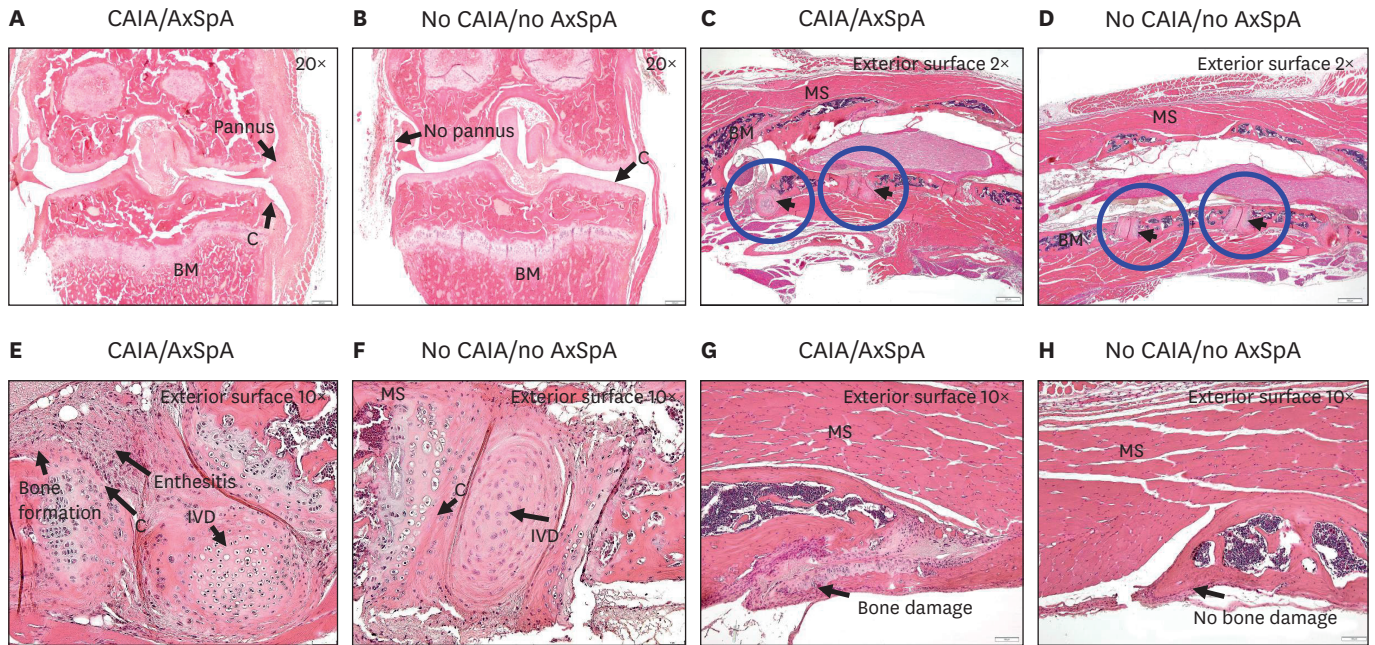


Figure 3. Comparative histological examination of H&E sections of mice with and without CAIA/AxSpA and spine lesions. (A) Knee joint of mice with CAIA/AxSpA showing multilayered synovium (pannus) and damaged cartilage (black arrow). (B) Knee joint of mice without CAIA/AxSpA with normal synovium and no cartilage damage (black arrow). (C) Interior surface of the hunched pouch showing significant cartilage growth of the vertebrae in mice with arthritis and AxSpA (marked by a black arrow in a blue circle). (D) Interior surface of the spine showing no cartilage growth of the vertebrae in a mouse with no arthritis and no AxSpA (marked by a black arrow in a blue circle). (E) Exterior surface of the kyphotic area, confirming large cartilage growth of the vertebrae in mice with arthritis and AxSpA. Enthesitis shown by a black arrow on one side of inter vertebral disc. (F) Exterior surface of the backbone in mice without arthritis and AxSpA showing no cartilage growth of the vertebrae. (G) Exterior surface of the kyphosis confirming huge spine damage in mice with arthritis and AxSpA (marked by a black arrow). (H) Exterior surface showing no bone damage in mice without arthritis and AxSpA (marked by a black arrow). CAIA/AxSpA (n=3); no CAIA/no AxSpA (n=4). (A, B) Pictures at 20× magnification (200 μm scale bar); (C, D) Pictures at 2× magnification (500 μm scale bar); and (E-H) Pictures at 10× magnification (100 μm scale bar). Scale bar shown at the bottom of right hand corner. C, cartilage; MS, muscle; BM, bone marrow.

In vivo localization of IRDye800 labeled anti-CII Abs in the arthritic joints and vertebrae of CAIA/AxSpA mice

We performed *in vivo* imaging using IRDye800 labeling to explore whether anti-CII Abs used to develop arthritis and AxSpA actually reached the joints, the kyphotic area of the spine and/or the vertebrae of the treated mice. *In vivo* imaging data show that IRDye800 labeled anti-CII Abs (ArthroGen-CIA[®]) were clearly visible (green color) not only in all the joints and fore paws (Fig. 4A) and hind paws (Fig. 4B) of mice, but also in the vertebrae (spine) of kyphotic mice (Fig. 4C). In contrast, mIgG IRDye800 labeled, as control, was not localized similarly to the labeled anti-collagen Abs, indicating that mIgG cannot bind to the cartilage surface in the joints or on the vertebrae of spine (Fig. 4D-4F). Specifically IRDye800 labelled anti-CII mAbs, were also present in the kyphotic area of spine indicating that anti-CII mAbs bound on the surface of vertebrae in mice compared with the control mIgG. The spine lesion with heavily bound anti-CII Abs was clearly visible (Fig. 4C). Localization of the only labelled IRDye 800 anti-CII mAbs not mIgG in the kyphotic area showed that these Abs might be causing damage not only to the joints but also to the spine, causing AxSpA-like lesions.

DISCUSSION

We have serendipitously found a murine model of AxSpA-like lesions and sought to characterize some of the unique features of this model, using clinical and histopathological

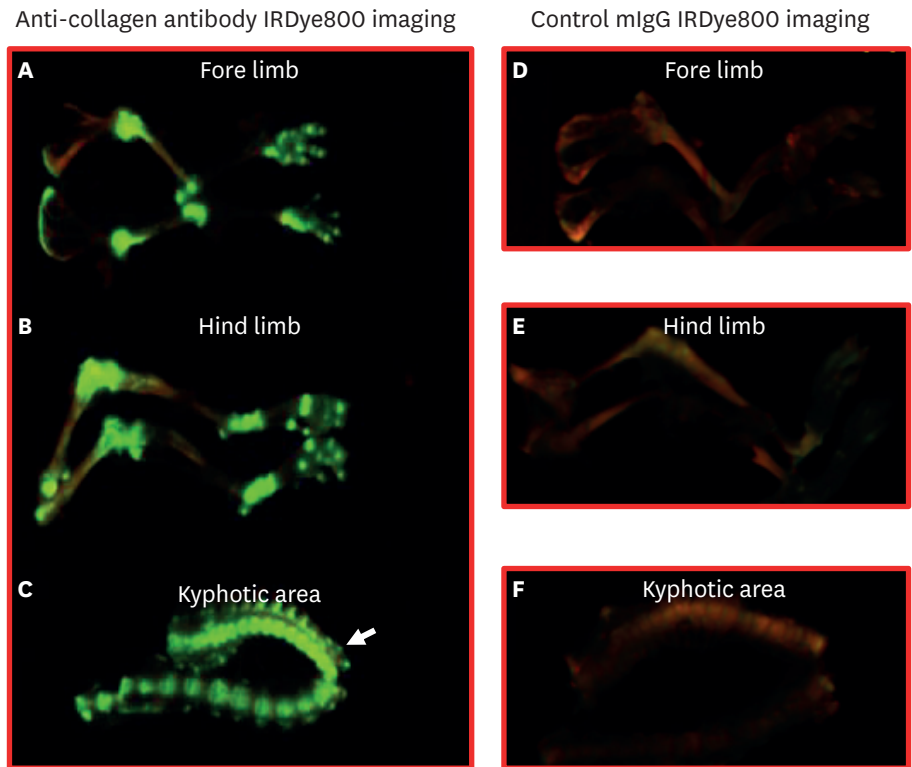


Figure 4. *In vivo* imaging of IRDye 800 labelled anti-CII mAbs and control mIgG at day 14. *In vivo* imaging of IRDye 800 labelled anti-CII mAbs actually not only reached joints but also to the spine during the development of AxSpA lesions. (A) IRDye800 labelled anti-CII mAbs localized in forelimbs (green color). (B) IRDye800 labelled anti-CII mAbs localized in hind limbs (green color). (C) Localization of the labelled anti-CII mAbs localized in spine (green color) indicating specifically anti-CII mAbs caused spine lesion similar to the AxSpA (white arrow). (D) IRDye800 labelled control mIgG not localized in the forelimbs (no green color). (E) IRDye800 labelled control mIgG not localized in hind limbs (no green color). (F) No localization of the IRDye800 labelled control mIgG in spine (no green color). Only fluorescence background is seen in mice injected with IRDye800 mIgG. One of the representative images of each from 2 independent, *in vivo*, experiments have been shown.

examinations. In this observational study, we first found that mice with arthritis consistently developed kyphosis. Second, mice with inflammatory arthritis not only showed joint damage but surprisingly also showed enthesitis, excessive bone growth and active chondrocyte proliferation in the kyphotic areas. This was demonstrated by histopathology of the backbone which confirmed the growth of new bone between the vertebrae. Third, there was C3 deposition in the kyphotic area, suggesting the involvement of the complement system in AxSpA in a manner similar to inflammatory arthritis. Interestingly, there was infiltration of the macrophages in the kyphotic area indicating a very active inflammatory process. Finally, *in vivo*, infrared IRDye800 imaging data confirmed the specific location of the anti-CII Abs in the vertebrae area (kyphotic area) and within the joints of mice that developed AxSpA and inflammatory arthritis.

SpA can cause some of the vertebrae in the spine to fuse, therefore making the spine less flexible and resulting in a hunched-forward posture. As a consequence, mice with arthritis resulted in a hunched posture and difficulty in walking (**Fig. 1A and C**). Histopathology data show that there was enthesitis in the kyphosis in mice with inflammatory arthritis as well as bone damage (**Fig. 3**). Notably, in the enthesitis a multilayered pannus appeared to be destroying one side of the IVD in mice with arthritis in contrast to the mice with no CAIA. This is one of the most important pathological features of AxSpA (**Fig. 3A and C**) and

fusion of the vertebrae might be the late event in the pathogenesis of this disease. We also asked whether anti-collagen Abs deposited to regions of the spine where there is no initial inflammation. In that study, we found that IRDye800 labeled anti-collagen Abs consistently deposited in all joints and in the entire spine. We then examined all other areas of the spine without histopathology after autopsy, and we could not locate inflammation and/or lesions except in 3–4 vertebrae in arthritic mice. Surprisingly, these lesions on the spine were consistent from one cohort after another.

Although the role of HLA-B27 in AS is still be debated, one study has shown that HLA-B27 transgenic rats also showed more severe arthritis compared with WT mice in the CAIA model (27,35), indicating that 2 diseases can coexist. Nevertheless, the coexistence of RA and AS in the same patient is rare, but it has been reported (36).

Our data also suggest a role for complement in this model of AxSpA due to deposition of C3 in the IVD and surrounding areas of kyphosis (**Fig. 2F**). The complement system has been shown to be involved in earlier studies of Proteoglycan-induced AS in mice (23,37). Our data are also consistent with activation of complement C3 locally in the fused vertebrae area. However, which pathway of the complement system is involved in this mouse model of AxSpA is not clear. We do suggest that the AP of the complement system is involved because CAIA is dependent on the AP (32).

We do recognize limitations in this observation study. One of the limitations of our study is that we have not used an established scoring method to examine CDA for AxSpA, also we have not performed micro-computed tomography (microCT) (X-ray imaging characterization) of the kyphotic areas; for both areas, further validation is needed. Since microCT was not performed, therefore, we could not determine the presence of syndesmophytes around the vertebral rim area; which are an important characteristic components of spine pathology in AS. Histological characteristics such as hyperplasia, neo-angiogenesis, and infiltration of synovial sub lining with inflammatory cells between rheumatoid arthritis and SpA has been reported earlier (38,39). We have also not examined sacroiliac joints because sacroiliitis is essential for axial lesions, and many studies mentioned above as limitations are planned the near future. Another limitation of our study is that we have used only the C57BL/6 mouse strain, but we think that AxSpA associated with CAIA may not be strain-specific, since the reactivity of anti-CII Abs is not strain specific. One of the most important questions is that whether or not C57BL/6 WT mice treated with K/BxN serum will develop AxSpA? We do not know, but it might be dependent on the effector function of specific autoantibodies involved. Spontaneous development of arthritis in K/BxN mouse model is dependent on pathogenic Abs to glucose-6-phosphate isomerase (GPI) protein and anti-GPI Abs binds to it (40). Interestingly anti-GPI-Abs mAbs of the IgG1 isotype (41,42) induce arthritis in the joints bypassing T and B cells (41). Anti-collagen Abs we used to inducing CAIA are a mixture of 5 monoclonal Abs containing A2-10 (IgG2a), F10-21 (IgG2a), D8-6 (IgG2a), D1-2G (IgG2b), and D2-112 (IgG2b) without the IgG1 isotype. In addition to differences in targets recognized, different isotypes have different effector functions such as triggering Fc γ Rs, Ab-dependent cell mediated cytotoxicity, and activating the complement system (43). We speculate that if anti-GPI Abs can bind to the cartilage or vertebrae directly similar to IgG2a or IgG2b isotypes as we have shown, there is a possibility that serum transfer from K/BxN mice can induce AxSpA in C57BL/6 WT mice since the pathogenic effects of both Abs are identical.

Our data shows that mice injected with anti-CII Abs not only developed arthritis but also developed hunched pouch defects, from which we conclude that AxSpA in mice is potentially a by-product of inflammatory arthritis (44). We also showed that LPS by itself played no role in the co-development of AxSpA, since mice injected with LPS only developed a mild and transient arthritis or no arthritis (45); however, both anti-CII Abs and LPS injections were required to induce disease.

Regarding the pathophysiological mechanism to explain how an autoimmune disease such as RA can give rise to the initiation of another auto-inflammatory disease such as AxSpA, our data is very clear. We found that both diseases can be induced and coexist after the induction of anti-collagen Abs; furthermore, we observed that both spine lesions could be the by-product of underlying inflammatory arthritis. The correlation between chondrocyte proliferation in the kyphotic area of spine and CDA, i.e., arthritis phenotype was + 0.51 but not statistically significant. Furthermore, some studies demonstrate that there is an increase in the levels of anti-collagen Abs in AS patients (14,46) and their role in AxSpA initiation can't be completely ruled out. Whether or not anti-CII Abs lead to the development of AxSpA is still an open question, and more studies are needed, but our current observation demonstrate that they not only induce arthritis but triggered spine lesions very similar to those in AxSpA. Interestingly anti-CII autoantibodies or anti-citrullinated protein Abs are also not a typical biomarker of AS compared with RA (47,48). However, autoantibodies to citrullinated type II collagen were detected in 78.5% of RA patients (49), as well as in 1 of 31 systemic lupus erythematosus patients and in 2 of 55 patients with osteoarthritis. Overall, many scholars agree that Abs with different auto-reactivities can be found in many autoimmune diseases, which may or may not be the direct cause of a specific disease, but they may play a role in the pathogenesis across autoimmune diseases.

In conclusion, none of the mouse models of AxSpA fully mirror human AxSpA but here we have found a new mouse model of AxSpA with many characteristics of enthesitis, IVD damage and chondrocyte proliferation as well as bone erosion along with inflammatory immune cell infiltration and complement activation. This mouse model of AxSpA might allow one to test complement based therapeutic drugs not only for RA but also for AxSpA.

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