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14. ABSTRACT

Our addresses the critical clinical problem of posttraumatic joint stiffness, a pathology that reduces the range of motion (ROM) of injured joints and contributes to the development of osteoarthritis. The fundamental hypothesis that drives the current study is that pathological fibrotic response of injured joint tissues may be limited by targeting the formation of collagen fibrils, a main component of the fibrotic mass. Key preliminary data indicate the following: (i) in comparison to the non-treated control, deposition of newly-formed collagen fibrils in posterior capsules from injured knees of rabbits treated with the anti-fibrotic antibody is reduced significantly, (ii) treatment with the antibody-based therapeutic does not cause any side effects, (iii) the impact of the therapeutic antibody on healing of joint tissues is not significant, (iv) mechanism of action of the antibody indicates blocking collagen fibrillogenesis. Conclusion of these experiments will explain the utility and safety of the anti-collagen I antibody applied to reduce to block excessive fibrosis associated with joint injury.

15. SUBJECT TERMS

Post-traumatic joint stiffness, anti-fibrotic therapy, collagen, therapeutic antibody, fibrosis, knee joint, animal model, range of motion.

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TABLE OF CONTENTS

1. INTRODUCTION

The first annual report describes crucial activities and results associated with studies on a novel, antibody-based approach to reduce post-traumatic joint contracture. These studies are the continuation of earlier research carried out by our team to establish the efficacy of the antibody-based therapy to limit joint stiffness.

The fundamental objective of the present study is to reduce post-traumatic joint stiffness in military personnel by blocking fibrotic scarring. The central hypothesis states that limiting excessive formation of collagen fibrils will reduce post-traumatic joint stiffness but will not impact the healing of injured joint tissues in any significant way. We emphasized that testing this hypothesis is essential, mainly because of the clinical implications of our study. Specifically, we believe that applying any anti-fibrotic treatment, including the one proposed here, carries the risks of unwanted side effects. While some of the possible side effects may include tissue toxicity and off-target activity of the therapeutic compound, others may limit the healing process that depends on collagen fibrillogenesis. As collagen fibrillogenesis is the target of our antibody, referred to as the anti-collagen antibody (ACA), studies of its impact on the healing of joint tissues are essential to define any unwanted side effects.

To test the central hypothesis, we defined two Specific Aims (SA):

SA1: To determine the safety of the ACA applied short-term and long-term to reduce post-traumatic joint stiffness.

SA2: To define the effects of the ACA on the healing of joint tissues.

We pursue these aims by employing a clinically-relevant rabbit-based model of post-traumatic joint contracture. In this model, one of the knee joints undergoes injury surgically and then is immobilized using the Kirschner (k)-wire for eight weeks. During the first surgery, we also injure the patellar tendon by removing a defined tissue segment. Furthermore, we create an osteochondral bone defect to study the impact of the ACA on the healing of the subchondral bone.

Eight weeks after the initial knee-injury surgery, the rabbits undergo the second surgery to remove the k-wires, and they are sacrificed four weeks later. Next, we harvest selected tissues and organs and assay them for possible pathological changes that might occur due to extended exposure to the ACA. Besides, we collect the hind legs to perform studies of the mechanical properties of joints healed in the presence or the absence of the ACA. These tests include measurements of the contracture and mechanical properties of the patellar tendons.

In addition to the studies of a possible impact of the ACA on the healing of the joint tissues, we also analyze this antibody's mechanism of action. In brief, we first collect sera from the rabbits before injuring their knees, and then again at various stages of the healing process. Subsequently, we measure the changes in crucial markers of collagen-derived metabolites as the function of the presence or the absence of the ACA. We also utilize blood samples to analyze crucial cellular and biochemical parameters whose changes provide clues about the overall impact of the ACA on the health of treated animals.

Completion of these experiments will determine the safety and the utility of the ACA to block excessive fibrosis associated with joint injury.

2. KEYWORDS

Post-traumatic joint stiffness, anti-fibrotic therapy, collagen, therapeutic antibody, fibrosis, knee joint, animal model, range of motion.

3. ACCOMPLISHMENTS

General note: There were no significant changes in the project's content or direction.

The major goals of the project. As indicated in introduction above, we formulated two Specific Aims that define our project. In the original Statement of Work (SOW), we defined the Major Tasks and set the timelines to complete them; these parameters serve as a blueprint for reaching the Specific Aims. The following paragraphs highlight the Major Tasks and indicate their current status.

Major Task 1: "**Production and purification of therapeutic antibodies (ACA).**" In this task, we produce the ACA needed for animal studies. During our original study, we developed crucial methods to produce, purify, and characterize the ACA. Here, we continue to utilize these methods. In brief, we employ CHO cells we engineered to produce a chimeric version of the ACA consisting of mouse-derived CDRs and human IgG-derived domains. We culture these cells in a bioreactor in the absence of serum and collect conditioned media (Fig. 1).

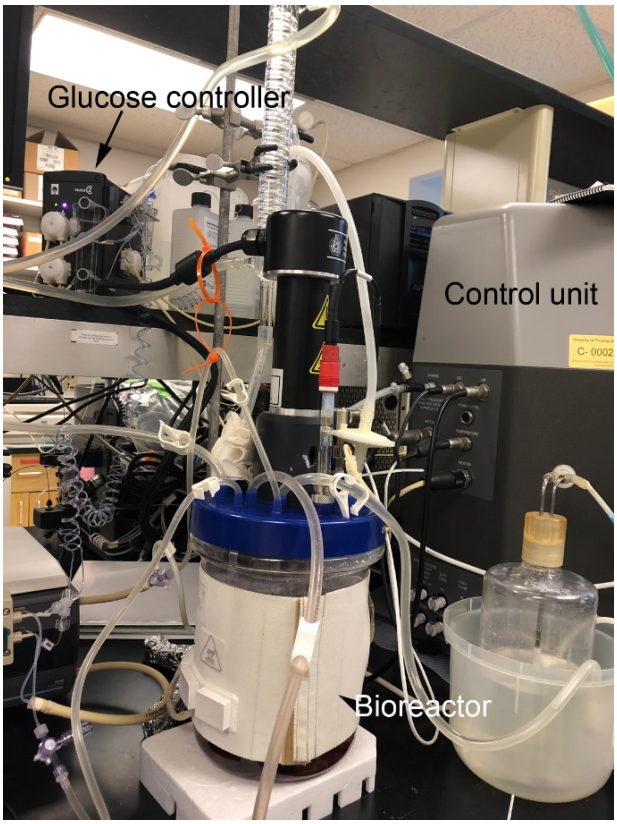


Figure 1. A picture illustrating the main elements of the bioreactor we use to produce the ACA.

Subsequently, we isolate the ACA by affinity chromatography. Each batch of ACA is then concentrated and sterilized by filtration. Moreover, the target specificity and the affinity of each batch is defined using a biosensor.

While the production of the chimeric form of the ACA in CHO cells is now a well-established technology, the main limitation we experience is a relatively low yield of the final product. This limitation is caused by cells producing low amounts of the ACA rather than with downstream processes. Consequently, we have to run multiple batches of the antibody production to generate enough material for the animal studies. This need increases the time needed to produce adequate amounts of the ACA and adds to the cost of the antibody production.

Significantly increasing the yield of a recombinant antibody is not a trivial task. To make necessary improvements without compromising the quality of the antibody, it is usually necessary to employ elaborate cell-engineering technology. Considering our long-term goals, we undertook necessary efforts independently of the research discussed here.

For our study here, we satisfy the need of generating adequate amounts of the ACA by combining a few batches of purified ACA. Accordingly, at present, we have enough ACA on hand to start surgeries and treatment of the next group of the rabbits, which will receive a higher concentration of the antibody.

Utilizing intramural funds, we introduced significant improvements to our ACA production system: (i) we purchased a glucose controller that maintains a set glucose concentration in the bioreactor, (ii) we acquired an optical-based pH-measuring system that controls the pH in the bioreactor, and (iii) we purchased an ultra-concentrator that reduces the volume of conditioned media, thereby significantly cutting the cost and time of antibody purification.

Major Task 2: "Concentration-dependent safety assays."

Major Task 3: "To determine the impact of the ACA treatment on the healing of joint tissues."

The central goal of the second task is to determine the safety of the ACA. As indicated in one of our Quarterly Reports, first, we decided to determine both the safety and the impact on the healing process of the lowest proposed concentration, which, based on our previous research, demonstrated the efficacy to reduce post-traumatic joint stiffness.

The following paragraphs summarize preliminary results of the studies we have carried out thus far:

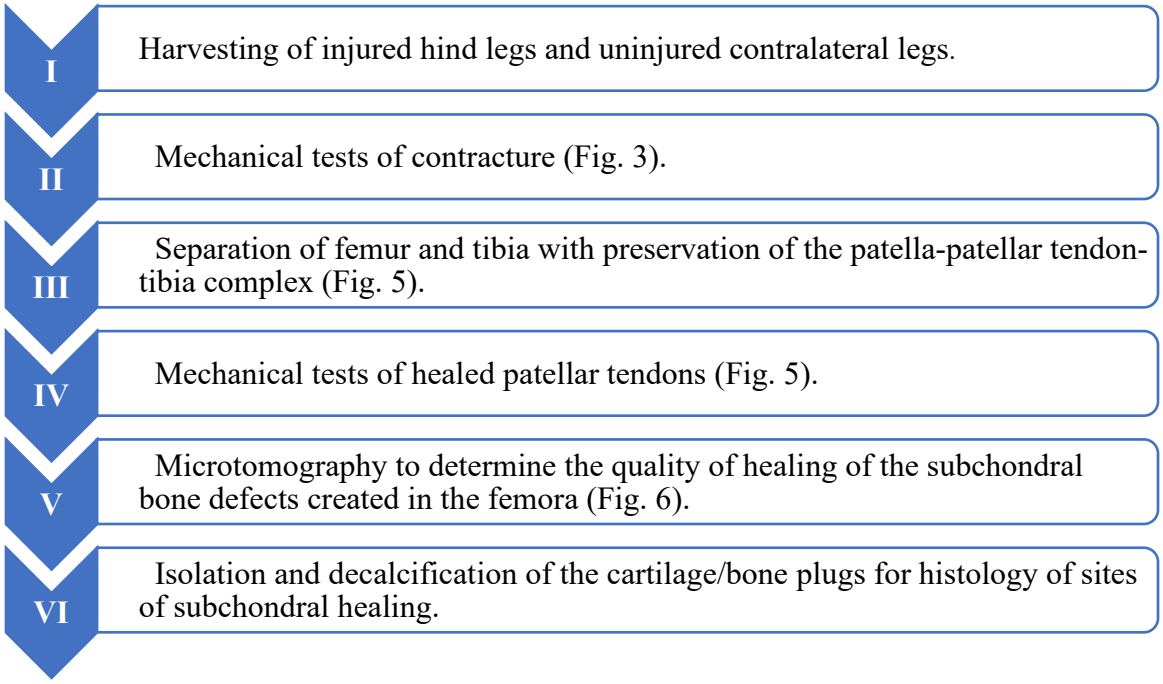
●***Rabbit surgeries***. Following the initial production of the ACA, we started surgeries to injure the rabbits' knee joints and patellar tendons. Thus far, we have operated about 30 rabbits (Fig. 2). Following the initial surgery, to damage the knee capsule, cartilage, the subchondral bone, and the patellar tendon, the rabbits are maintained for eight weeks with their operated knee immobilized with the k-wire. During this time, they receive the ACA delivered from a pump implanted subcutaneously during the initial surgery. After eight weeks, the rabbits undergo the second surgery to remove the k-wires and the pumps. Following the surgery, the rabbits recover for four weeks, and then they are sacrificed.

Figure 2. An illustration of an element of the rabbits' surgery: drilling a hole in the tibia for installation of the k-wire.

●***Tissue collection***. After sacrificing the rabbits, internal tissues and organs are collected for histology to determine the potential effects of the long-term application of the ACA. The following tissues and organs are collected: (i) brain, (ii) esophagus, (iii) stomach, (iv) intestines, (v) heart, (vi) aorta, lung, (viii) thymus, (ix) spleen, (x) liver, (xi) kidney, (xii) testis, (xiii) ovaries, (xiv) tendon, (xv) sciatic nerve, (xvi) posterior knee capsule, and (xvii) cartilage/bone plug.

Moreover, we collect five blood samples/rabbit: (i) before surgery, (ii) two weeks after surgery, (iii) four weeks after surgery, (iv) eight weeks after surgery, and (v) twelve weeks after surgery.

●***Assays of knee joint tissues***. The following diagram illustrates the flow of assays of joint tissues we perform to determine the impact of the ACA on their mechanical properties and healing.



Assays of joint contracture. We analyzed the impact of the ACA on the contracture of the injured joints. In brief, the tibia and the femur are transected about 6 cm from the knee joint. Subsequently, the ends of the bones are potted in polycarbonate cylinders with the use of polymethyl methacrylate and acrylic copolymer (Fig. 3, arrows). After securing the limb in the grips, the femur and tibia are positioned at a right angle, and then the instrument is set to 0°. Subsequently, applying the rate of loading set to 40°/min, an extension torque is applied to 0.2 Nm, and the joint extension is recorded. Values near 90° indicated maximum extension, while angles larger than 90° represented hyperextension. Flexion contracture is calculated as the difference between the angles of the control limb and the injured limb recorded at 0.2 Nm. Finally, we expressed these differences as the percent of the angle value obtained for the uninjured legs. Thus, a larger percent value indicates a more severe joint contracture.

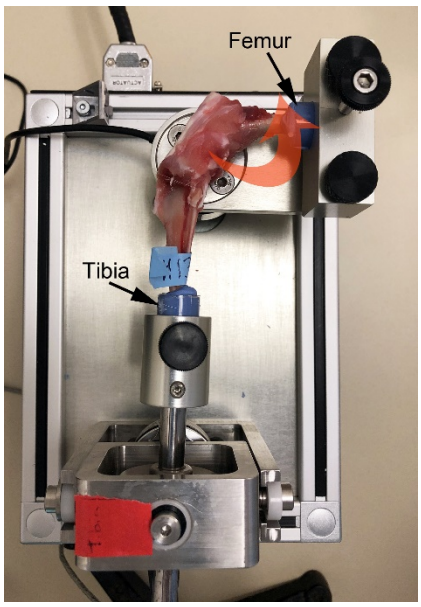


Figure 3. A depiction of a mechanical tester for measuring the joint contracture. The red arrow indicates the movement of the femur.

A two-way ANOVA was conducted to examine the effects of the sex of the rabbits and their treatment with the ACA on the contracture. Our preliminary results obtained from the rabbits operated thus far indicate that the interaction effect between sex and the treatment of the rabbits was not statistically significant, *F(1, 20)=0.521, p=0.479*. Therefore, an analysis of the main effect was performed, which indicated that there was a statistically significant main effect of the ACA treatment, *F(1, 20)=4.835, p=0.04*. Figure 4 demonstrates results of contracture measurements.

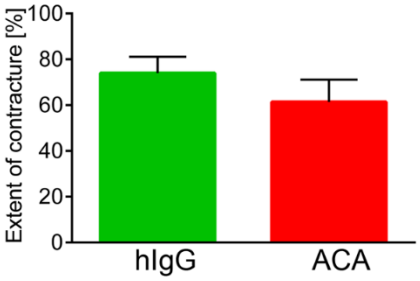


Figure 4. Measurements of the joint contracture in the rabbits treated with control antibody (hIgG) and the ACA. Results were calculated based on combined data from the males and the females. Bars represent the means, and 95% CI (confidence interval).

Assays of the impact of the ACA on healing of patellar tendon. During the initial surgery, we create a full-thickness, 3 mm x 10 mm defect in the patellar tendon to study its healing in the presence or the absence of the ACA. In contrast to the broad knee injury we create to cause joint contracture, the well-defined injury in the patellar tendon allows for more precise measurements of the impact of the ACA on the healing process.

As described in our proposal, the crucial measurements to determine this impact include assays of the mechanical properties of the healed tendons. To perform these measurements, we isolated the patella-patellar tendon-tibia complexes and analyzed them mechanically in a blinded fashion (Fig. 5). Most of the tendons from operated rabbits were already processed; at present, we continue the measurements and analyze the collected data. Besides, we will perform histological studies of the fibrous elements of the patellar scar to analyze the effects of the ACA further.

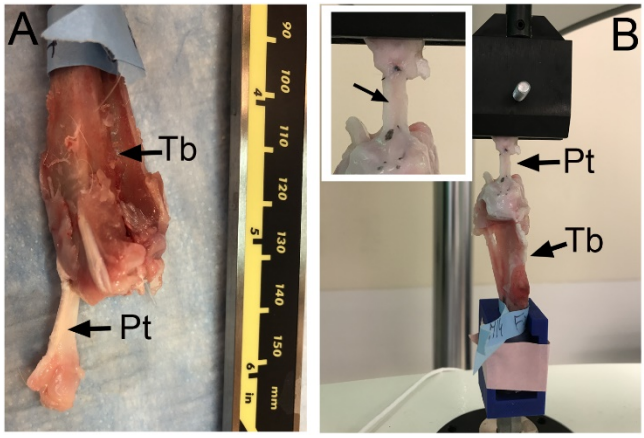


Figure 5. A. Isolation of the patellar tendon (Pt)-tibia (Tb) complex. B. A setup of the complex seen in A for the mechanical measurements. The insert depicts the Pt in which the arrow indicates the healed defect created during the initial surgery.

Assays of the impact of the ACA on healing of subchondral bone and cartilage. As cartilage and bone damage frequently occurs during traumatic joint injury, we study the impact of the ACA on the healing of these tissues. To create a model osteochondral defect, we drilled a 3-mm hole at the medial femoral condyle at the apex of the weight-bearing area.

Effect of the ACA on the healing of subchondral bone. Following collection of the injured and control femorae, we performed microtomography (mCT) to measure crucial parameters that define subchondral trabecular bone. Our primary focus was on the Bv/Tv (percent of bone volume to the total analyzed volume) and the BMD (bone

mineral density). We analyzed specific values of the Bv/Tv, and the BMD obtained from the injured regions and the matching regions of uninjured bones. Figure 6 depicts the analyzed regions.

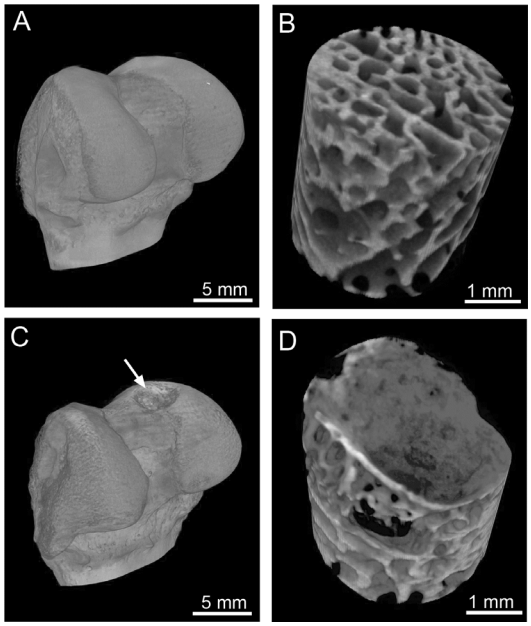


Figure 6. Representative mCT images of the uninjured (A) and injured (C, arrow) bones. Panels B (uninjured bone) and D (injured bone) indicate subchondral bone regions analyzed here to determine the extent of bone formation in the injured area (C&D).

A two-way ANOVA was conducted to examine the effects of sex and treatment of the rabbits on the analyzed bone parameters. Our preliminary results obtained from the rabbits operated thus far indicate that the interaction effect between sex and the treatment of the rabbits on Bv/Tv was not statistically significant, *F(1, 42)=1.403, p=0.234*. Therefore, an analysis of the main effect was performed, which indicated that there was no statistically significant main effect of the ACA treatment, *F(1, 42)=2.877, p=0.097*.

Similarly, preliminary results indicate that the interaction effect between sex and the treatment of the rabbits on the BMD was not statistically significant, *F(1, 42)=0.178, p=0.675*. An analysis of the main effect indicated that there was no statistically significant main effect of the ACA treatment on the BMD, *F(1, 42)=2.207, p=0.145*.

In the ACA group and control group, the differences between the injured and the uninjured legs were significant for both parameters. For the Bv/Tv*; F(1, 42)=119.8, p<0.0005* and for the BMD *F(1, 42)=76.081, p<0005.*

Despite the lack of significance between injured legs from the ACA group and the hIgG control, the means for the Bv/Tv and the BMD measurements in the ACA group trended slightly toward smaller values when compared to the hIgG control (Fig. 7).

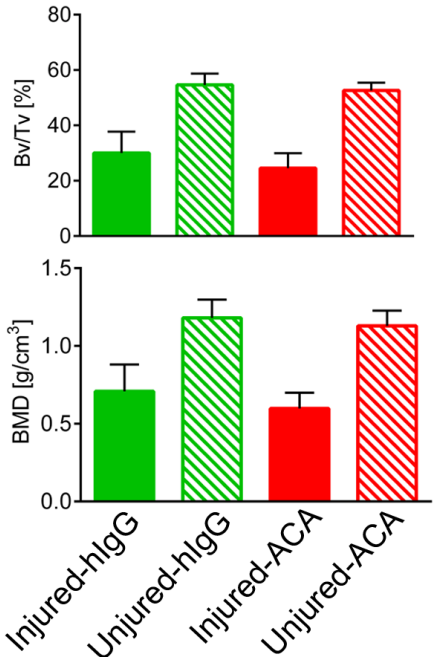


Figure 7. Graphic representation of measurements of the Bv/Tv and the BMD in rabbits treated with the ACA or hIgG; data for injured and uninjured legs are presented.

Effect of the ACA on the healing of cartilage. We initially considered performing unconfined compression mechanical tests on the healed cartilage defect to assay the effects of the ACA on the mechanical properties of the healed cartilage. After careful examination of the healed areas, we concluded that these tests are not feasible due to the complex topography of the surfaces at the defect sites and the fact that these sites were covered by fibrous tissue (Fig. 8). Consequently, we will perform detailed histological assays of the injury sites to determine the effects of the ACA. These assays will include

measurements of picrosirius red-stained collagen-rich matrices of the injury sites observed in polarized light. At present, osteochondral plugs encompassing the sites of injury undergo a decalcification process. Together with mCT assays, quantitative histology will provide substantial information on the impact of the ACA on the healing of the osteochondral defects.

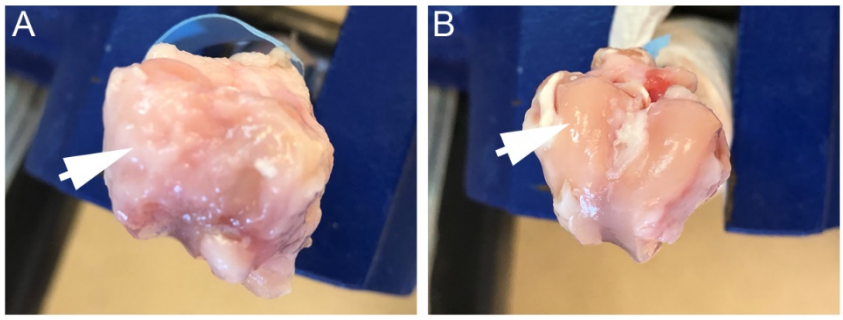


Figure 8. The appearance of the surface of injured femoral condyle after twelve weeks of healing in comparison to the contralateral uninjured counterpart. The arrow in A indicates the site of osteochondral defect made during an initial surgery, and the arrow in B indicates a similar site (uninjured) on the contralateral condyle. Please note the differences in the roughness of the cartilage surfaces in femoral condyles from the injured and uninjured legs.

●***Assays of blood***. As indicated above, we collected blood samples at five different time points. We performed a comprehensive analysis of crucial parameters to determine any possible changes of baseline values measured before surgery. The set of parameters included: (i) WBC (white blood cells count), (ii) RBC (red blood cell count), (iii) HEM (hemoglobin), (iv) HCT (hematocrit), (v) MCV (mean corpuscular volume), (vi) MCHC (mean corpuscular hemoglobin concentration), (vii) platelet count, (viii) MPV (mean platelet volume), (ix) NEUT (neutrophils), (x) LYMPH (lymphocytes), (xi) MONO (monocytes), (xii) EO (eosinophils), (xiii) BASO (basophils), (xiv) IG (immature granulocytes), (xv) NRBC (nucleated red blood cells), and (xvi) IPF (immature platelet fraction).

Furthermore, we analyzed a comprehensive biochemical panel that included: (i) ALB (albumin), (ii) ALP (alkaline phosphatase), (iii) Alt (alanine aminotransferase), (iv) AMY (amylase), , (v) TBIL (total bilirubin), (vi) BUN (blood urea nitrogen), (vii) CA (calcium), (viii) PHOS (phosphate), (ix) CRE (creatinine), (x) GLU (glucose), (xi) TP (total protein), (xii) GLOB (globuline), and (xiii), Na^+, K^+.

Analysis of all the above blood parameters done at various post-surgery time points showed no differences when compared to the baseline values in blood samples collected before injuring the knee joints. Moreover, we observed no differences between the ACA-treated group and the control group treated with hIgG.

●***Assays of production and degradation of collagen molecules to define relevant biomarkers*.** Consistent with the original SOW, we analyzed markers of collagen metabolism in the context of the ACA treatment. In particular, we selected (i) hydroxyproline (Hp), (ii) C-terminal propeptides of procollagen I (Cp), and (iii) cross-linked telopeptides (Xt). To date, we analyzed Hp and Cp in sera samples collected from all rabbits processed thus far. Assays of Xt are pending.

Assays of Hp. For each rabbit, we analyzed Hp in sera collected at five different time points (see above). We propose that changes in serum Hp in uninjured animals indicate a turnover-related natural degradation of collagenous matrices that form various connective tissues.

In the context of our study on injured animals, however, changes of serum Hp may also indicate degradation of collagen molecules newly synthesized in response to joint injury. We hypothesized that increased concentrations of Hp in serum may indicate an accelerated degradation of new collagen molecules whose incorporation into stable fibrils is blocked by the ACA.

Following measurements of the serum concentration of Hp at various postoperative time points, we plotted the results for the ACA-treated and control groups. As the baseline concentrations of Hp measured in the sera collected before injuring the knees varied among the rabbits, we calculated the Hp concentrations as a percentage of the baseline. Figure 9 presents combined data from males and females obtained at the indicated time points. Results indicate that following surgery, the concentration of Hp in the sera of the rabbits was increasing for about eight weeks. After that time, the concentration plateaued.

Rabbits treated with control antibody. To analyze changes in the Hp concentration across various time points, we employed a repeated-measures ANOVA with a Greenhouse-Geisser correction (IBM SPSS Statistics v. 26). This analysis demonstrated that, in rabbits treated with control antibody, the mean concentration of Hp differed significantly between time points, $F(2.788, 19.514)=4.467, p=0.017$. Post hoc tests, using the Bonferroni correction revealed that Hp concentration increased significantly between time 0 (day of surgery) and week-12 post-surgery ($p=0.023$).

We interpret the lack of a significant increase of Hp in serum concentration within the first four to six weeks of the active phase of scar formation as an indication of the expected incorporation of newly synthesized collagen I into insoluble fibrils that form scar tissue.

Rabbits treated with ACA. To analyze changes in the Hp concentration across various time points, we employed a repeated-measures ANOVA with a Greenhouse-Geisser correction, as indicated above. This analysis demonstrated that in rabbits treated with the ACA, the mean concentration of Hp differed significantly between time points, $F(1.979, 23.752)=6.201, p=0.007$. Post hoc tests, using the Bonferroni correction revealed that Hp concentration increased significantly between time 0 (day of surgery) and week-4 post-surgery ($p=0.005$).

We interpret the significant increase of serum Hp during the first four weeks of the active scar formation process in the rabbits treated with the ACA as an indication of reduced incorporation of newly-synthesized collagen molecules into insoluble fibrils. We propose that this observation is a result of anti-fibrillogenesis activity of the ACA due to its binding to procollagen I molecules synthesized in response to injury. This preliminary result supports our hypothesis.

Assays of the C-terminal propeptide (Cp) of procollagen I. For each rabbit, we analyzed Cp in sera collected at five different time points. We propose that changes in serum Cp in non-injured animals indicate a turnover-related natural biosynthesis of collagenous I-rich matrices that form various connective tissues.

It is essential, however, to comprehend that during collagen fibrillogenesis, procollagen I molecules, that include the C-terminal propeptides, associate transiently with collagen fibrils, and this association represents a mechanism that controls the fibril diameter. Thus, we expect that during normal fibrillogenesis, a bulk of the C-terminal propeptides remains bound to the fibrillar matrices before being steadily released.

Here, however, we hypothesize that due to the ACA-procollagen I binding, the amount of collagen matrix-bound procollagen molecules is reduced, despite procollagen production in response to injury. Consequently, we predict that free procollagen molecules degrade, thereby increasing the serum concentration of the C-terminal propeptides.

Following measurements of the serum concentration of Cp at various postoperative time points, we plotted results for the ACA-treated and control groups. As the baseline concentrations of Cp measured in the sera collected before injuring the knees varied among the rabbits, we calculated the Cp concentrations as a percentage of the baseline. Figure 9 presents combined data from males and females obtained at the indicated time points.

Rabbits treated with control antibody. To analyze changes in the Cp concentration across various time points, we employed a repeated-measures ANOVA with a Greenhouse-Geisser correction. This analysis

demonstrated that in rabbits treated with control antibody, the mean concentration of Cp did not differ significantly between time points, *F(1.708, 10.248)=3.911, p=0.06*.

We interpret the lack of significant differences in the Cp serum concentration as an indication of the expected steady release of the Cp processed from procollagen I molecules synthesized in response to injury.

Rabbits treated with ACA. To analyze changes in the Cp serum concentration across various time points, we employed a repeated-measures ANOVA with a Greenhouse-Geisser correction, as indicated above. This analysis demonstrated that in rabbits treated with ACA, the mean percent of baseline concentration of Cp differed significantly between time points, *F(2.137, 21.374)=10.387, p=0.001*. Post hoc tests, using the Bonferroni correction revealed that serum Cp increased significantly between time 0 (day of surgery) and week eight post-surgery (p=0.037) and between time 0 and week 12 (p=0.011).

We interpret the significant increase in serum Cp during the period of active scar formation in the rabbits treated with the ACA as an indication of poor incorporation of newly-synthesized procollagen I molecules into insoluble fibrils. We suggest that this observation is a result of anti-fibrillogenesis activity of the ACA due to its binding to procollagen I molecules synthesized in response to injury. Similar to the results for measurements of serum concentration of Hp, this preliminary result supports our primary hypothesis.

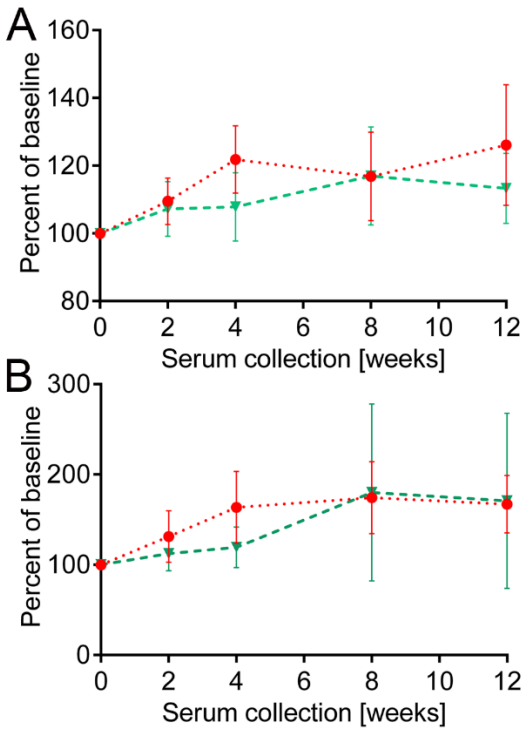


Figure 9. A graphic representation of assays of time-dependent changes in the concentration of Hp (A) and Cp (B); the circle symbols (●) represent the ACA-treated group, and the triangle symbols (▼) represent the control group.

●***Histological analysis of collected tissues***. To determine the potential long-term, chronic effects of the 8-week treatment with the ACA, we analyzed the histology of selected tissues and organs. These analyses did not reveal any significant changes in the morphology of analyzed tissues; the Appendix shows representative microscopic images of analyzed tissue samples. In the next phase of our study, we will also analyze potential acute effects of the antibody.

Opportunities for training and professional development.

Nothing to report.

Dissemination of the results.

Nothing to report.

Plans for the next reporting period.

In the next reporting period, we will continue our studies with groups of rabbits that will receive higher doses of the ACA than those applied thus far. To accomplish this goal, we will continue production of the ACA in a bioreactor. Additional groups of rabbits will also include those in which we will analyze potential acute side effects of the ACA. Moreover, we will perform specific assays of the osteochondral pugs that encompass the site of femoral injuries. Together with mCT assays of these regions, these data will provide vital information on the effects of the ACA on the healing of cartilage and subchondral bone.

We will analyze the data we collected during mechanical tests of the patellar tendon. Furthermore, we will prepare histological samples from these tendons, and then we will analyze the architecture of the collagenous matrices, the synthesis of which we try to reduce with the ACA.

We will also continue assays of collagen metabolites, in particular, cross-linked telopeptides. Combining new data with those on serum Hp and Cp will paint a clear picture of the mechanism of the ACA action.

We plan to disseminate our findings in the form of a publication and/or a conference presentation.

4. IMPACT

The impact on the development of the principal discipline(s).

The studies we carried out in the first reporting period will continue to have a significant positive impact on the principal disciplines, including fibrosis and orthopedics. By validating the anti-fibrotic target, namely collagen fibrillogenesis, we create a new paradigm for how to treat a large group of fibrotic diseases, including lung fibrosis, kidney fibrosis, ocular fibrosis, abdominal adhesions, and others. Moreover, based on our preliminary results, we expect that our anti-fibrotic approach is safe, and it does not impact the overall healing process in any significant way. Thus, our studies offer a novel approach to limit fibrotic diseases not only effectively but also in a safe manner.

As our research aims to reduce post-traumatic joint stiffness, its outcomes will have a positive impact in the area of orthopedics. As fibrotic scarring is a common problem in the area of orthopedics, including due to accidental trauma and surgery, our studies will provide a new therapeutic tool to limit the formation of unwanted scar tissue. We believe that our study will not only help to limit excessive scar formation around joints, but also around other vital elements of the musculoskeletal system, including in the spine, around peripheral nerves, within muscles, and elsewhere.

The impact on other disciplines.

As antibody-based therapies represent one of the most attractive novel approaches to treat a broad spectrum of diseases, including cancer, immune diseases, and others, the results of our studies contribute to the area of novel biologics significantly. Because our research raised the interest of the scientific community, we plan collaborations in the area of biomaterials, drug delivery, protein structure, to name a few.

Impact on technology transfer.

We believe that our research is likely to lead to technology transfer to entities in industry. We predict that with the results of our study, our patent-protected technology will reach the critical mass needed to attract a commercial partner.

The impact on society beyond science and technology.

With the direct clinical implications of our project, its impact on main stakeholders is high. If successful, our project will benefit the following groups of stakeholders:

- **Military personnel**: effective recovery and improved readiness.
- **Patients**: higher patient satisfaction and quality of life due to the reduced number of aggressive. Physiotherapy, hospital admissions, and revision surgeries, as well as fewer cases of severe disability.
- **Families**: positive outcomes of patients' recovery will have a positive impact on their families as a whole.
- **Health care system**: a significant cut in the immense costs of revision surgeries (by 50 %)
- and severe disabilities.
- **Health care professionals**: effective preventive treatment option, less burden in revision surgeries.
- **Employers**: less sick leave days, earlier return to work, and increased work longevity.

5. CHANGES/PROBLEMS

Changes in approach and reasons for change

No changes.

Actual or anticipated problems or delays and actions or plans to resolve them

Because of the need to combine batches of the ACA, production of this antibody takes longer than expected.

Changes that had a significant impact on expenditures.

None, thus far.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to report.

Significant changes in use or care of human subjects

Not applicable.

Significant changes in use or care of vertebrate animals

Nothing to report.

Significant changes in use of biohazards and/or select agents

Nothing to report.

6. PRODUCTS

Nothing to report.

Technologies or techniques

We finalized development of a technology for the pilot-scale production of the ACA and batch characterization of this antibody.

Inventions, patent applications, and/or licenses

Nothing to report.

Other Products

We generated and preserved a vast collection of tissues relevant to the excessive scarring of joint tissues. These samples will provide opportunities to study mechanisms of post-traumatic joint contracture.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name	Andrzej Fertala
Project Role	PI
Research Identifier (ORCID ID)	https://orcid.org/0000-0001-9153-1912
Nearest person month worked	20% = 2.4 calendar months
Contribution to Project	Dr. Fertala is an expert in the structure and function of extracellular fibrous proteins with a particular focus on collagenous proteins in health and disease. He developed the concept, and then demonstrated its feasibility, that the pathological process of excessive deposition of collagenous material may be inhibited by blocking collagen fibril formation. He generated a clinically-relevant antibody characterized by the ability to block the formation of collagen-rich fibrotic deposits. Dr. Fertala oversees all aspects of the planned project and closely interacts with other members of the team. Specifically, Dr. Fertala monitors procedures for the preparative-scale production of the anti-fibrotic antibody. Dr. Fertala collaborates closely with participating clinicians and Dr. Tomlinson. Moreover, Dr. Fertala leads all analyses that are done in order to establish the safety and efficacy of the approach proposed here to limit post-traumatic joint stiffness. Besides, Dr. Fertala is responsible for coordinating analyses of all data obtained from the proposed study. In these analyses, Dr. Fertala communicates with Dr. Daskalakis, the biostatistician assigned to this project. Moreover, Dr. Fertala is responsible for communicating with the DoD.
Funding Support	Not applicable

Name	Andrzej Steplewski
Project Role	Research Associate
Research Identifier (ORCID ID)	https://orcid.org/0000-0001-6869-3999
Nearest person month worked	50% = 6 calendar months
Contribution to Project	Dr. Steplewski is an expert in connective tissue research and has extensive experience in protein engineering and preparative-scale protein production. Under the guidance of Dr. Fertala, he oversees the production and characterization of recombinant antibodies needed for the proposed study. He also participates in animal surgeries by assisting orthopaedic surgeons. In collaboration with Dr. Tomlinson, Dr. Steplewski also participates in biomechanical assays associated with Specific Aim 2. Moreover, Dr. Steplewski runs microCT assays, FTIR-based assays.
Funding Support	Not applicable

Name	Jolanta Fertala
Project Role	Research Associate
Research Identifier (ORCID ID)	https://orcid.org/0000-0001-9027-0917
Nearest person month worked	50% = 6 calendar months
Contribution to Project	Dr. J. Fertala has extensive experience in the physiology and pathology of skeletal connective tissues. She performs the histological assays and microscopic quantification of collagen-rich deposits in tissues obtained from animal groups. Moreover, she studies the architecture of scar tissue formed in the presence of the therapeutic antibody. She is also responsible for the biochemical assays of fibrotic deposits. These assays include processing crude samples, extracting collagenous proteins, determining the concentration of collagen-specific hydroxyproline, and determining the composition of collagen-rich deposits. She closely interacts with clinical partners to discuss the clinical relevance of biochemical and morphological data. She also collaborates with the analytical team that performs biochemical and histopathological tests of blood and collected organs and tissues. Moreover, she performs assays of collected sera for changes in hydroxyproline, C-terminal propeptides, and cross-linked telopeptides.
Funding Support	Not applicable

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report.

What other organizations were involved as partners?

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS

Not applicable.

9. APPENDICES.

Quad Chart (see below).

Histology of tissues and organs (see below).

Appendix

Prevention of the Posttraumatic Fibrotic Response in Joints

Insert ERMS/Log Number: OR170347
Award Number: W81XWH-18-1-0554

PI: Andrzej Fertala, Ph.D. **Org:** Thomas Jefferson University **Award Amount:** $993,637.00

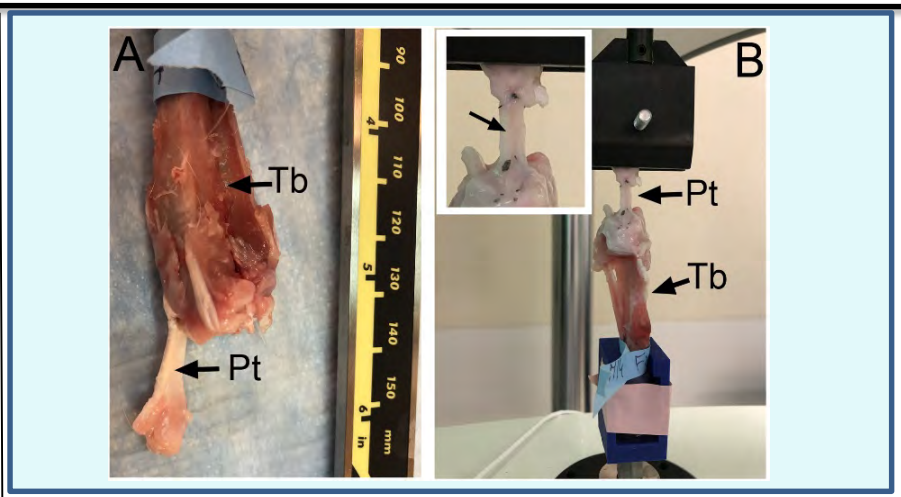
Study/Product Aim(s)

Two Specific Aims will be implemented to test antibody-mediated reduction of post-traumatic joint stiffness:

- Specific Aim 1: "To block the fibrotic process after joint injury in a rabbit-based model"
- Specific Aim 2 "To analyze long-term effects of the antibody-based inhibitor of fibrosis at the biochemical, cellular, and biomechanical levels"

Approach

The research strategy includes three key technical elements: (i) production of the inhibitory chIgG, (ii) creation and utilization of a rabbit model of post-traumatic joint contracture to test the efficacy of the inhibitory chIgG to decrease the fibrotic response and to preserve ROM, and (iii) microscopic, biochemical, and biomechanical tests of joints to determine the organ-level, tissue-level, cell-level, and molecular-level outcomes of the novel therapeutic approach in the context of relevant controls.



A. Isolation of the patellar tendon (Pt)-tibia (Tb) complex. B. A setup of the complex seen in A for the mechanical measurements. The insert depicts the Pt in which the arrow indicates the healed defect created during the initial surgery.

Timeline and Cost

Activities CY	18	19	20	21
Implement/test the model system.				
Execution of Specific Aim 1				
Execution of Specific Aim 2				
Final tests/Data analysis				
Estimated Budget ($K)	**$77**	**$319**	**$344**	**$253**

Updated: September 28, 2019

Goals/Milestones

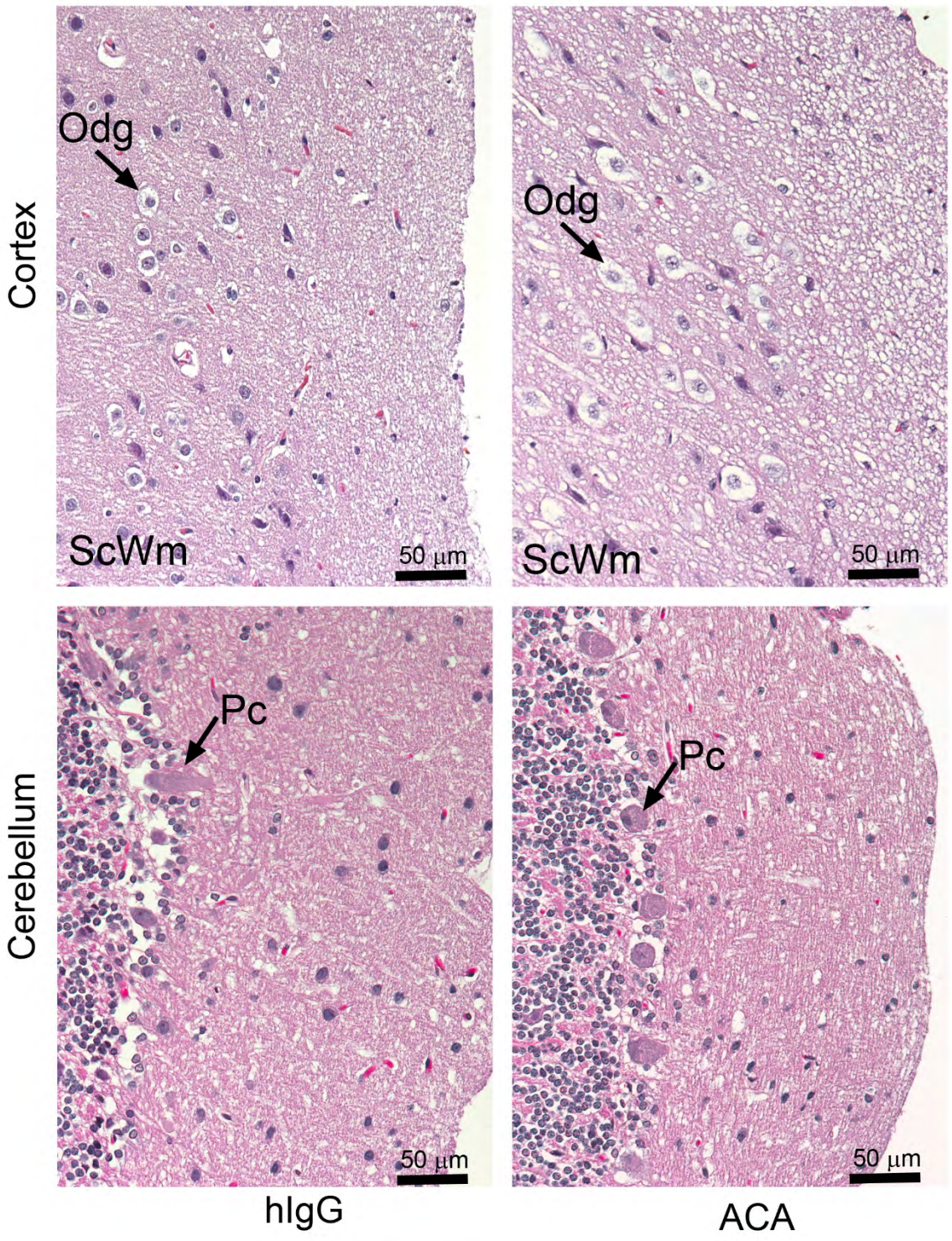
CY18 Goal – Implementation of model systems.
☑ Launch production of chIgG and create relevant animal model.
CY19 Goals-Model validation and execution of Specific Aim 1.
☑ Carry out tests of the utility of chIgG and the selected animal model.
☐Study the effects of varying amounts of inhibitory IgG on knee joints.
CY20 Goal – Execute and achieve Specific Aim 2.
☑ Analyze the biological and biomechanical characteristics of injured joints treated with anti-fibrotic chIgG.
CY21 Goal – Determine the clinical utility of anti-fibrotic chIgG antibody.
☐ Data analysis and statistical evaluation of results.

Comments/Challenges/Issues/Concerns

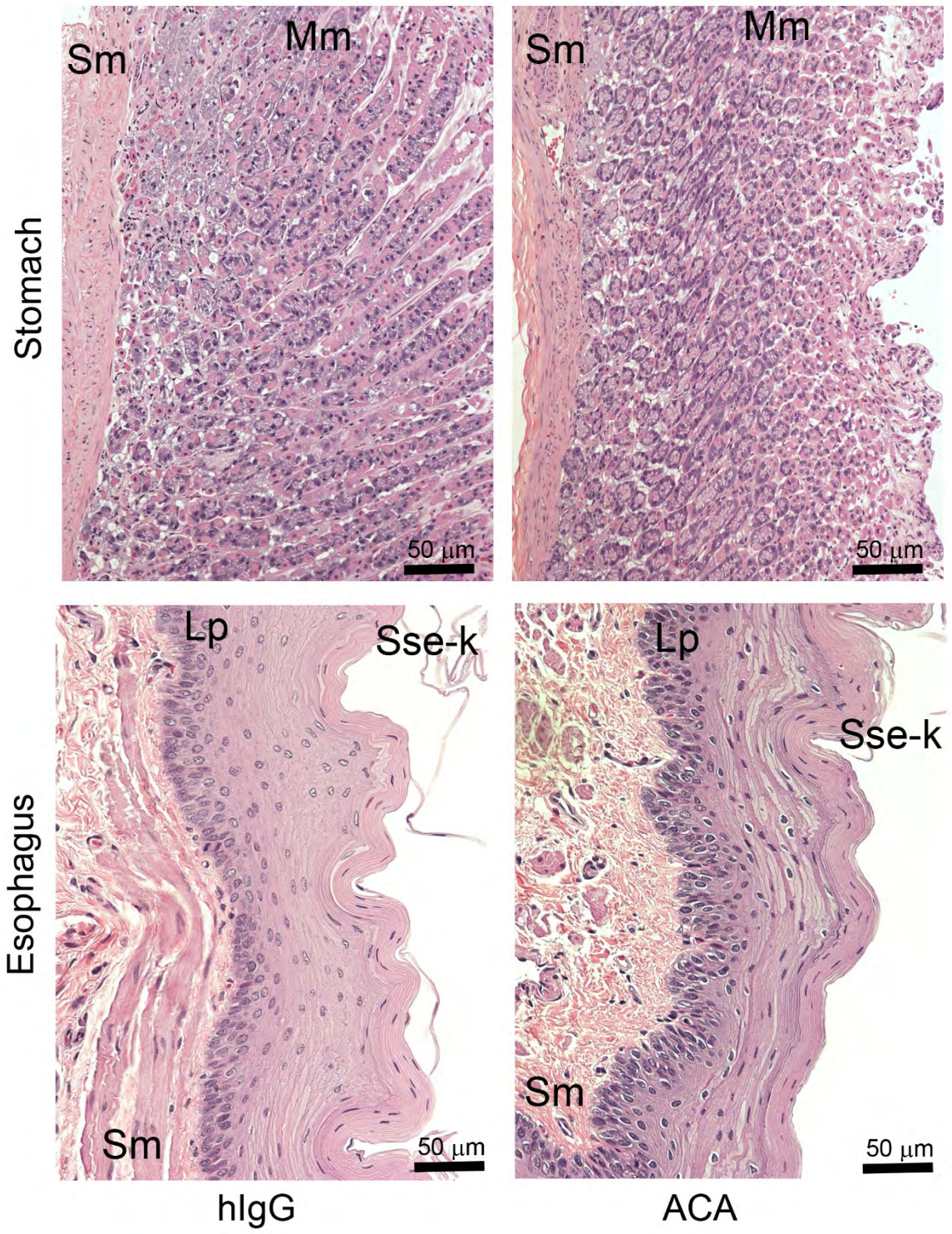
- If timelines change, comment here: N/A at current stage of the project
- If off by more than one quarter in spending, comment here.

Budget Expenditure to Date

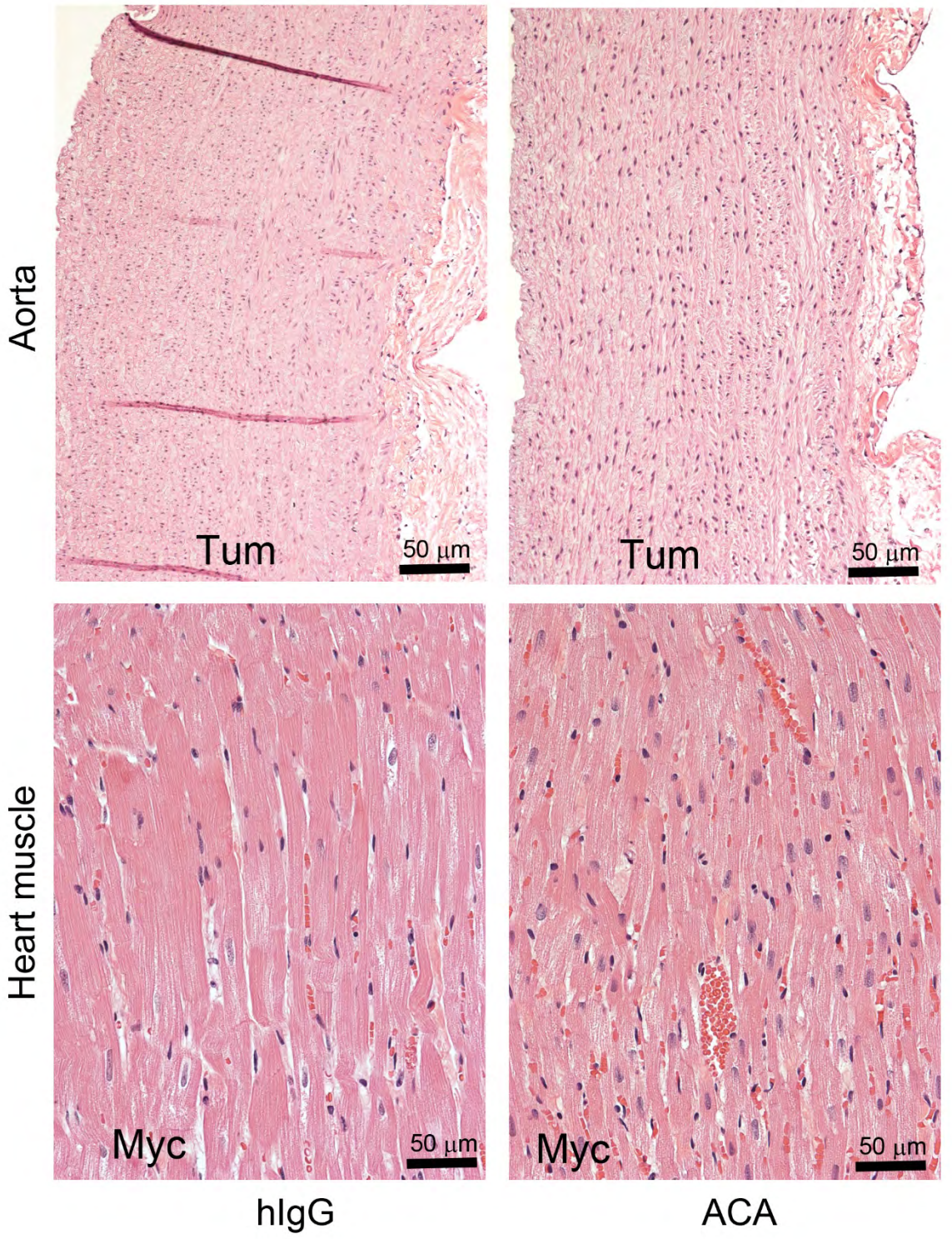
Projected Expenditure: $309,419
Actual Expenditure: $366,881



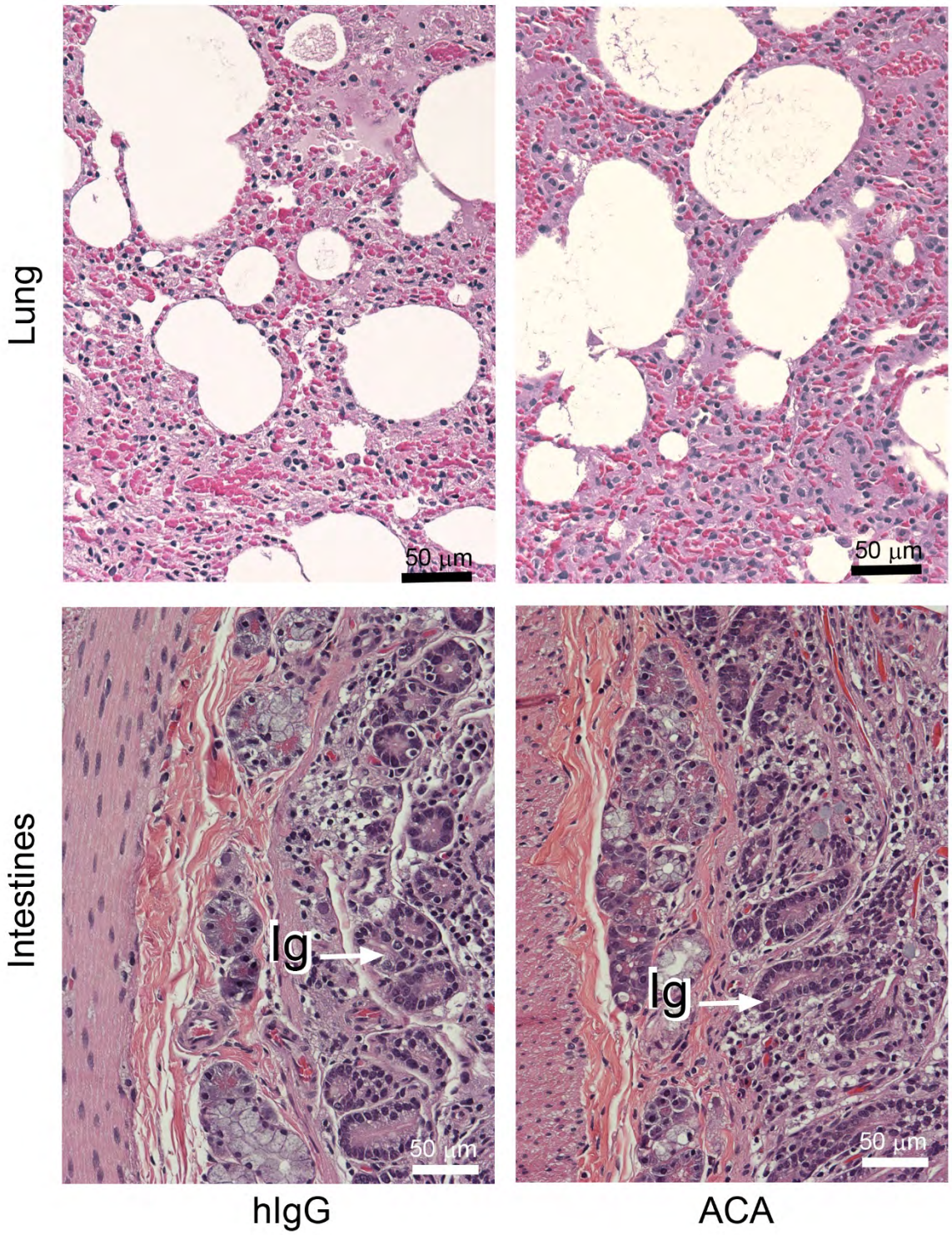
Histology of brain tissue. Symbols: ScWm; subcortical white matter, Odg; oligodendroglia, Pc; Purkinje cells, hIgG; rabbits treated with control IgG, ACA; rabbits treated with ACA.



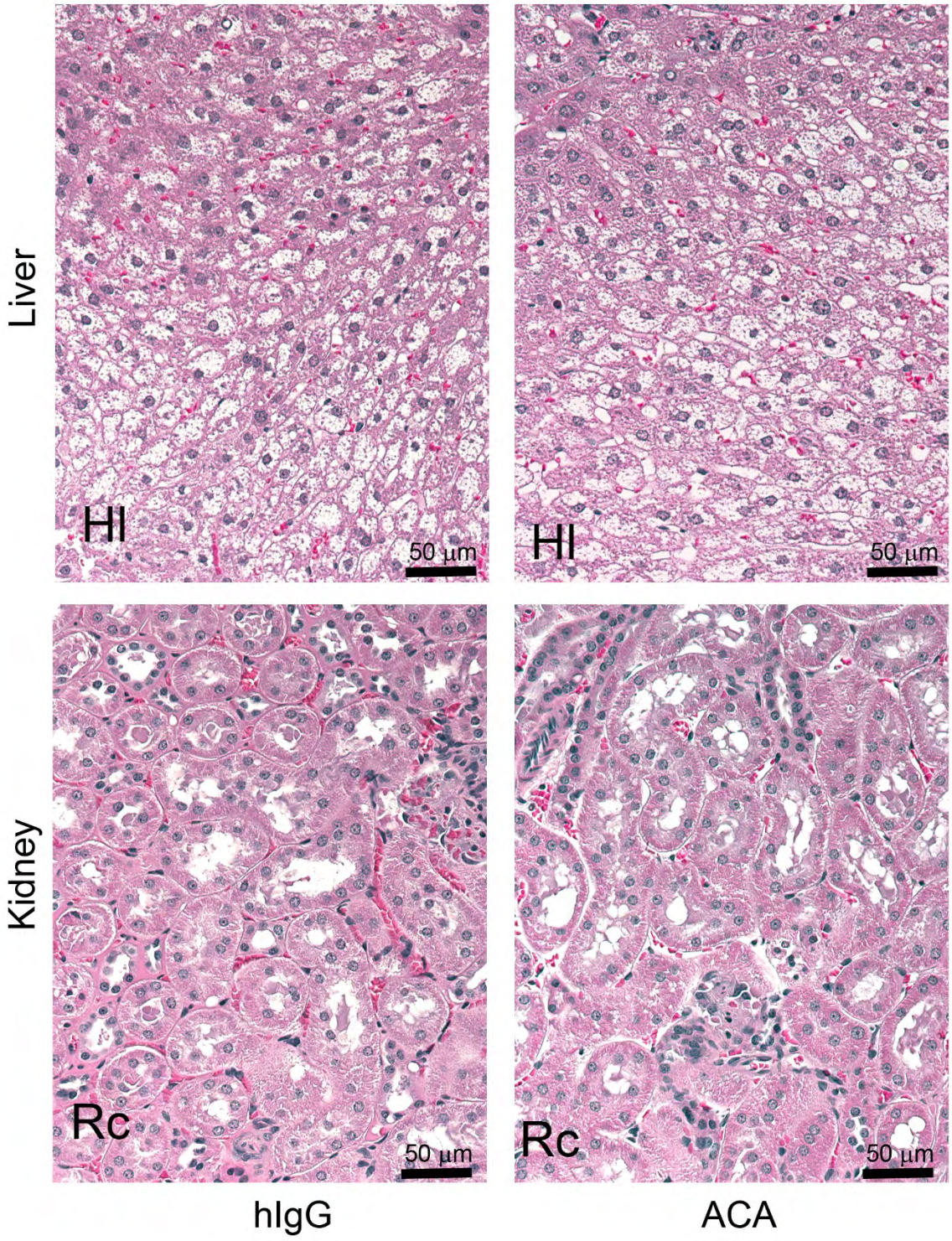
Histology of stomach and esophagus. Symbols: Mm; mucous membrane, Sm; submucosa, Lp; lamia propia, Sse-k; stratified squamous epithelium-keratinized, hIgG; rabbits treated with control IgG, ACA; rabbits treated with ACA.



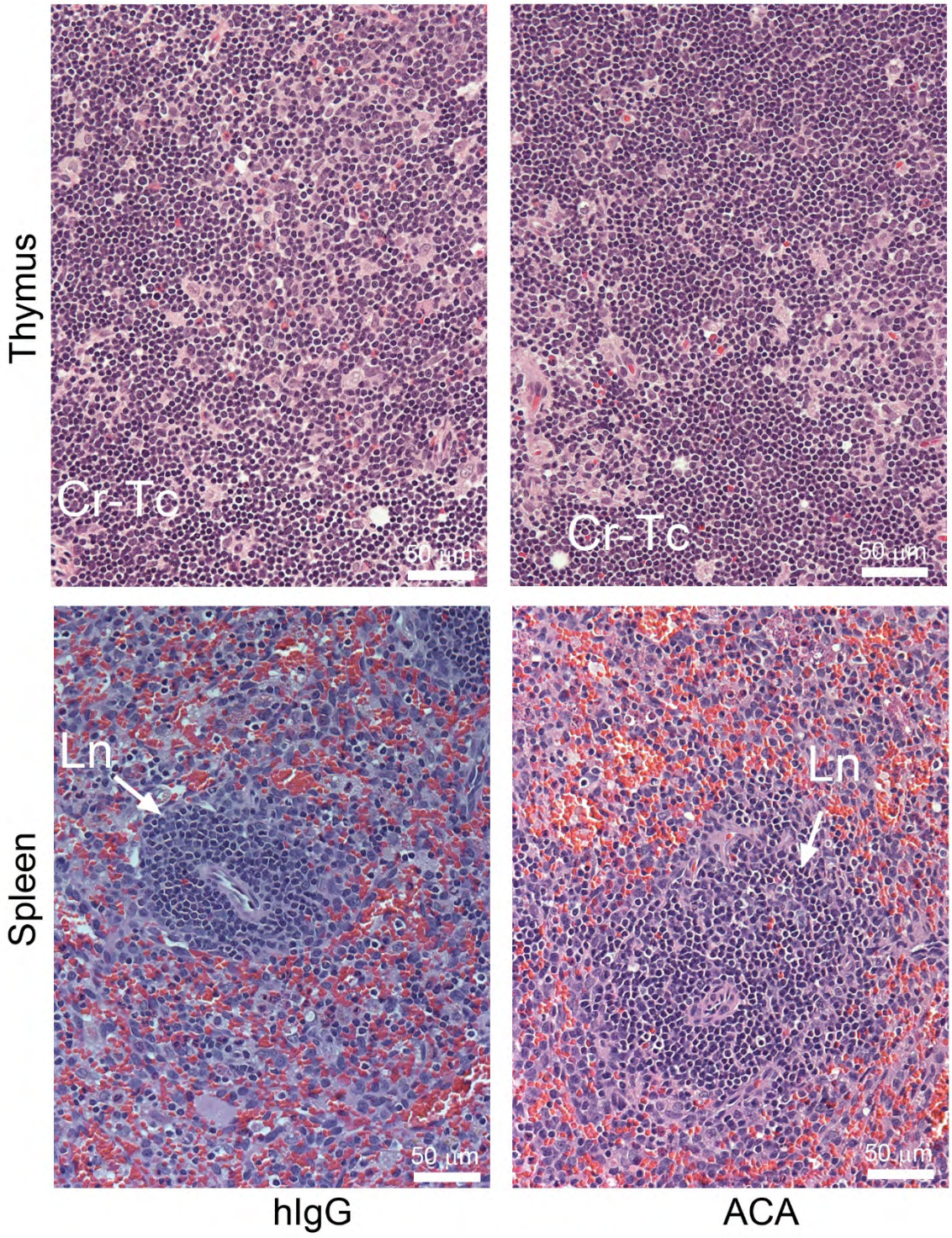
Histology of aorta and heart muscle. Symbols: Tum; tunica media, Myc; myocardium, hIgG; rabbits treated with control IgG, ACA; rabbits treated with ACA.



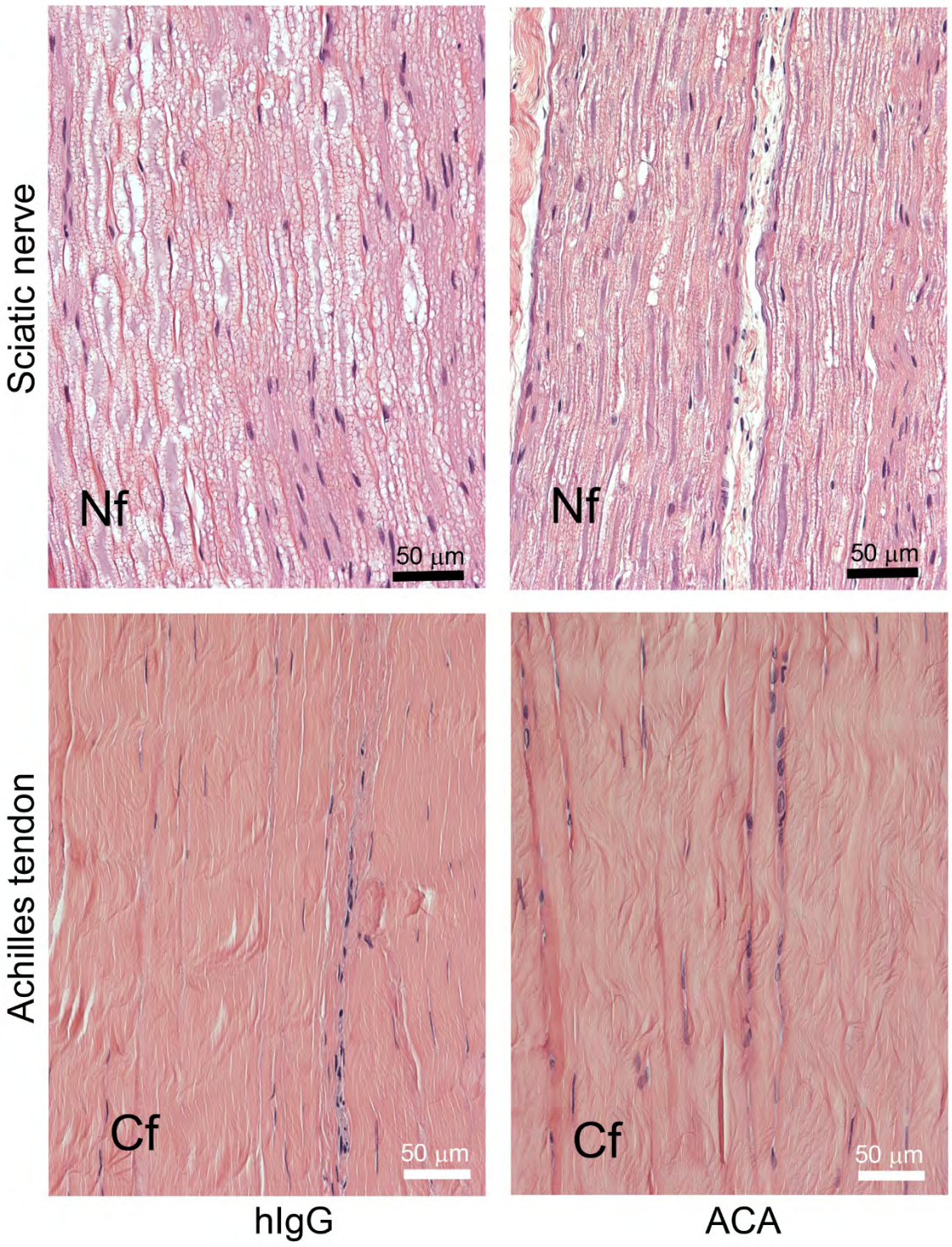
Histology of the lung tissue and intestines. Symbols: Ig; intestinal glands, hIgG; rabbits treated with control IgG, ACA; rabbits treated with ACA. Note: Because the lungs were not inflated prior to the fixation, the lung tissue appears compact.



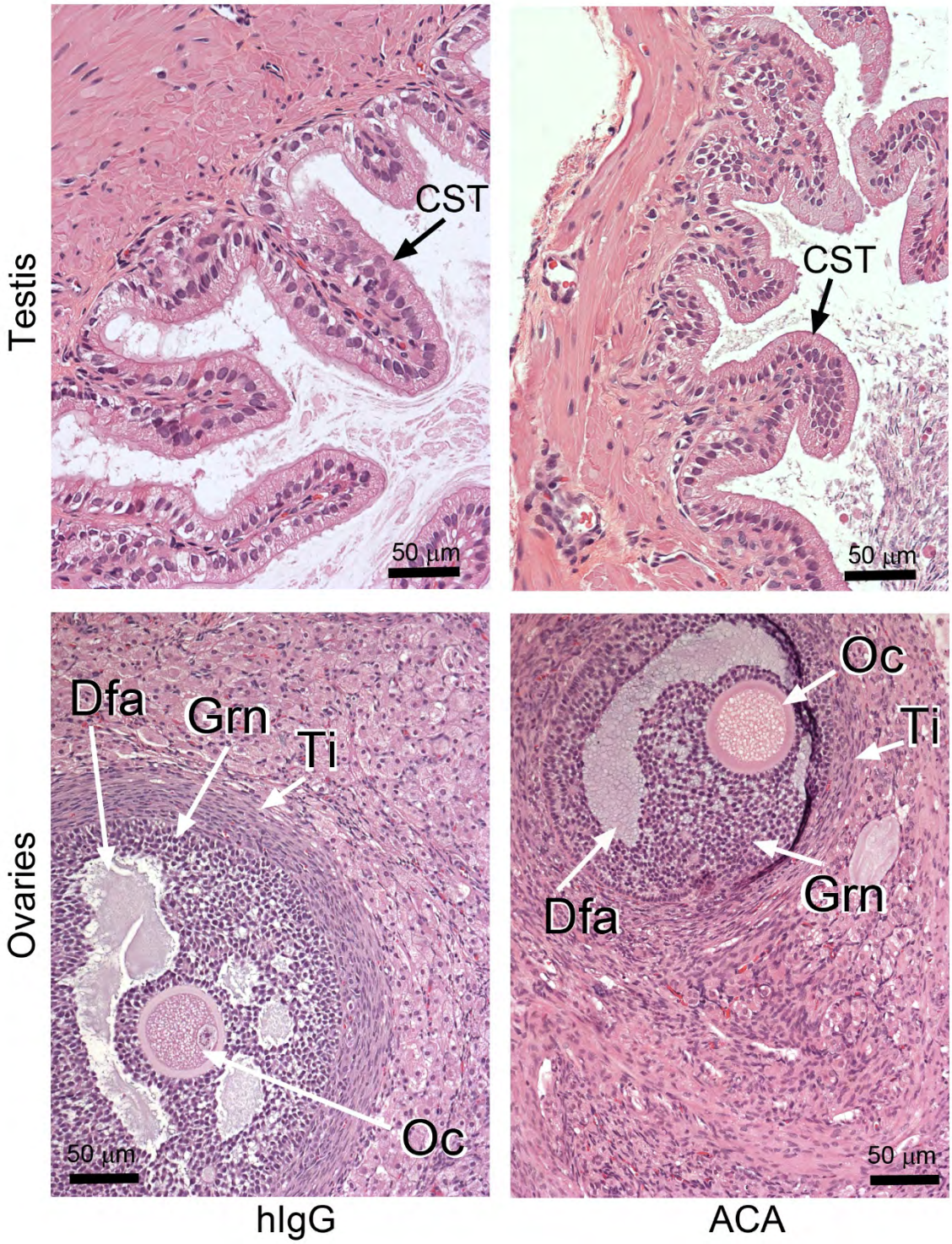
Histology of liver and kidney. Symbols: Hl; hepatic lobule, Rc; renal cortex, hIgG; rabbits treated with control IgG, ACA; rabbits treated with ACA.



Histology of spleen and thymus. Symbols: Cr-Tc; cortex thymic cells, Ln; lymphoid nodule, hIgG; rabbits treated with control IgG, ACA; rabbits treated with ACA.



Histology of the sciatic nerve and the Achilles tendon. Symbols: Nf; neural filaments, Cf; collagen fibers, hIgG; rabbits treated with control IgG, ACA; rabbits treated with ACA.



Histology of testis and ovaries. Symbols: CST; convoluted seminiferous tubules, Dfa; developing follicular antrum, Grn; granulosa cells, Ti; theca interna, Oc; oocyte, hIgG; rabbits treated with control IgG, ACA; rabbits treated with ACA.