MedImmune

Immune complex complications in toxicology studies with large molecules

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STP-India
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Why is it important to be aware of possible immune complex formation in safety studies with large molecules?

- Immune complexes can alter pharmacokinetic (PK) parameters, lower systemic exposure, and lead to an invalid toxicology study
- Even if you do not perform studies in your facility, you may review tissues from animals on a study and encounter lesions
- It is important to understand the nature of the changes and allow a correct interpretation, or investigation, if necessary
- Be able to critique findings in study reports and ensure immune complex changes are not interpreted as a direct treatment-related effect

What has changed in the last 35 years?

- Early biopharmaceuticals human doses were in the ug/kg range and multiples of these tested in animals were in similar or low mg/kg ranges.

- With the advent of monoclonal antibody (mAb) therapies in 1986 human doses became mg/kg and 10X multiples of these doses became much larger.

- Toxicity was uncommon but immune responses to these foreign proteins was not. Doses now in the 50 to 300 mg/kg range to attempt to overcome the neutralizing effect of the anti-drug antibodies (ADA) and achieve effective test article (TA) exposure.

What factors influence the immunogenicity of the TA?

- Nonhuman primates are often the most pharmacologically relevant test species.
- These recombinant human proteins are sufficiently different to be immunogenic.
- Other factors include:
  - How different the TA is from native test species protein, e.g. mouse > chimeric mouse > humanized mAb
  - Glycosylation is affected by the production cell line
  - Excipients or protein aggregates in the formulation
  - Mechanism of action of the TA, i.e. immunosuppressive or immunostimulant?

Possible affects of ADA

- Altered pharmacokinetic or pharmacodynamic properties of the TA. This may or may not occur.
- There is <60% correlation between development of ADA in NHP in toxicology studies and human ADA to those same recombinant human therapeutic proteins in the clinic.
- Immune complex formation, clearance or deposition

Review of hypersensitivity reactions

- Type 1 :Immediate, anaphylactic reactions characterized by IgE recognition of soluble Ag, mast cell degranulation with histamine release.
- Type 2: Cytotoxic reactions with IgG and/or IgM recognition of cell-surface bound or matrix-associated Ags, complement activation, complement dependent cytotoxicity, NK cell activation and Ab-dependent cell-mediated cytotoxicity.
- Type 3: Immune complex (IC) mediated, anaphylactoid reactions due to IgG/IgM recognition of soluble Ags and complement activation
- Type 4: Delayed-type reactions with recognition of Ag by Antigen-presenting cells, T-cell activation, cytokine release and cytotoxicity

Potential consequences of antidrug antibody (ADA) formation in nonclinical toxicology studies

- Hypersensitivity reactions (HSR)
  - Type 1 Immediate, anaphylactic reactions characterized by IgE recognition of soluble antigen
  - Type 3 Immune complex (IC) mediated anaphylactoid reactions characterized by IgG/IgM recognition of soluble antigens and complement activation (NOT IgE mediated)

- IC deposition in blood vessels

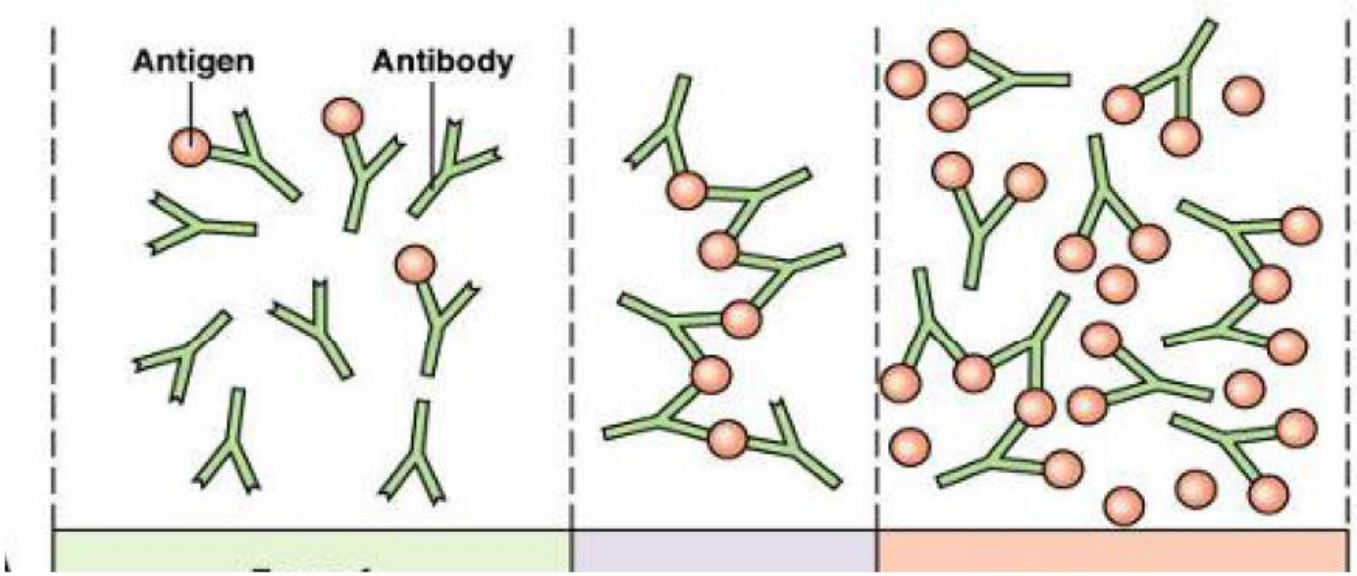
Determinants of IC characteristics

- Lattice size
- Ag concentration , valence, charge, and sites of formation and localization
- Ab concentration, valence, affinity, charge, and subclass/isotype
- Ability to fix complement
- Ag/Ab ratio

Why are IC lesions so variable within a study?

- Ag/Ab ratio is key. The ratio and balance in an individual animal determines lattice size and whether the specific requirements for deposition are achieved.
- NHPs are a heterogeneous population and each individual animal will respond differently to a given dose of Ag.
- Not necessarily a dose response

Antigen-antibody relationships immediately postdose



Low-dose	Mid-dose	High-dose
ADA > Drug	ADA ~ Drug	ADA < Drug
Drug ~BLQ	Large CICs	Small CICs easily cleared
Small CICs		No acute effects

Tissue deposition of IC

- ICs with an intermediate lattice size are most likely to deposit in tissue and activate complement
- ICs with a large lattice size activate the classical complement pathway efficiently
- C3b binds to the Fc region of the IC Abs

Tissue deposition of IC

- C3b mediates binding of the IC to complement receptor 1 on erythrocytes
- These erythrocyte-bound ICs are readily cleared by liver Kupffer cells and spleen macrophages
- This phenomenon can be seen histologically as Kupffer cell hypertrophy and splenic red pulp hyperplasia
- If complement mediated clearance is blocked or saturated large lattice ICs can also deposit in tissue

Histologic appearance of ICs

- On H&E, ICs can appear as amorphous, homogeneous, eosinophilic material.
- In the kidney, deposits may be observed in mesangial, subepithelial, and/or subendolthelial locations.
- In blood vessels, deposits are observed in the intima or media and often at the internal elastic lamina in small to medium arterioles and associated with mononuclear cell infiltrates.

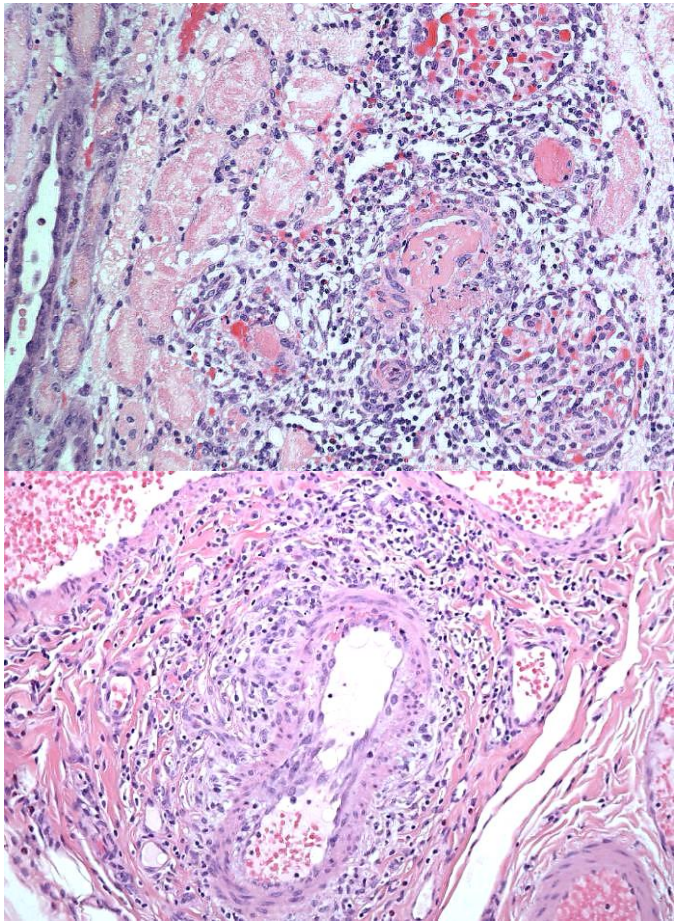
Histologic appearance of ICs

- With immunohistochemistry (IHC) granular deposits can be identified colocalized within the lesions seen on H&E, and confirmed to contain monkey antibody (IgG), the antigen (test article), and/or complement components.

- Most health regulatory authorities are familiar with the ADA/immune complex phenomenon and do not any longer require IHC confirmation, but you may rarely receive such requests.

Case example 1: Immune-complex disease in a 4-WK primate safety study caused by a biologic (mAb)

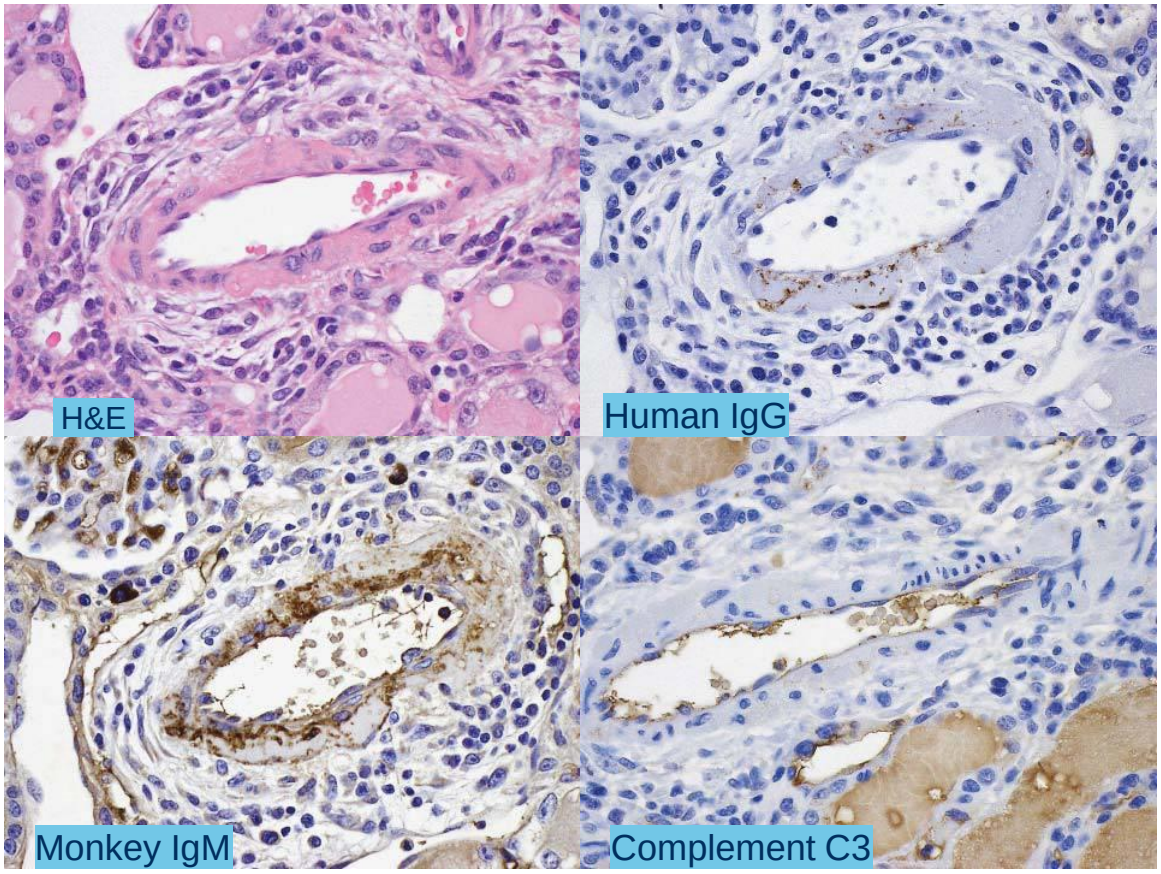
- Clinical Signs
  - **Rash** - legs, ears, arms, and head approximately 6 hours after dosing on Day 29 (5th & final dose)
  - **Red urine** observed on Days 29 and 30
  - Animal was euthanized and necropsy performed
- Histopathology
  - Kidney (renal tubules)
    - Bilateral, marked, cortical, diffuse, acute **tubular necrosis**
  - Kidney, heart (coronary artery), epididymis
    - **Arteritis** (preferred term over vasculitis)



Original interpretation by testing facility was that this was caused directly by this molecule, which could have led to termination of the program.

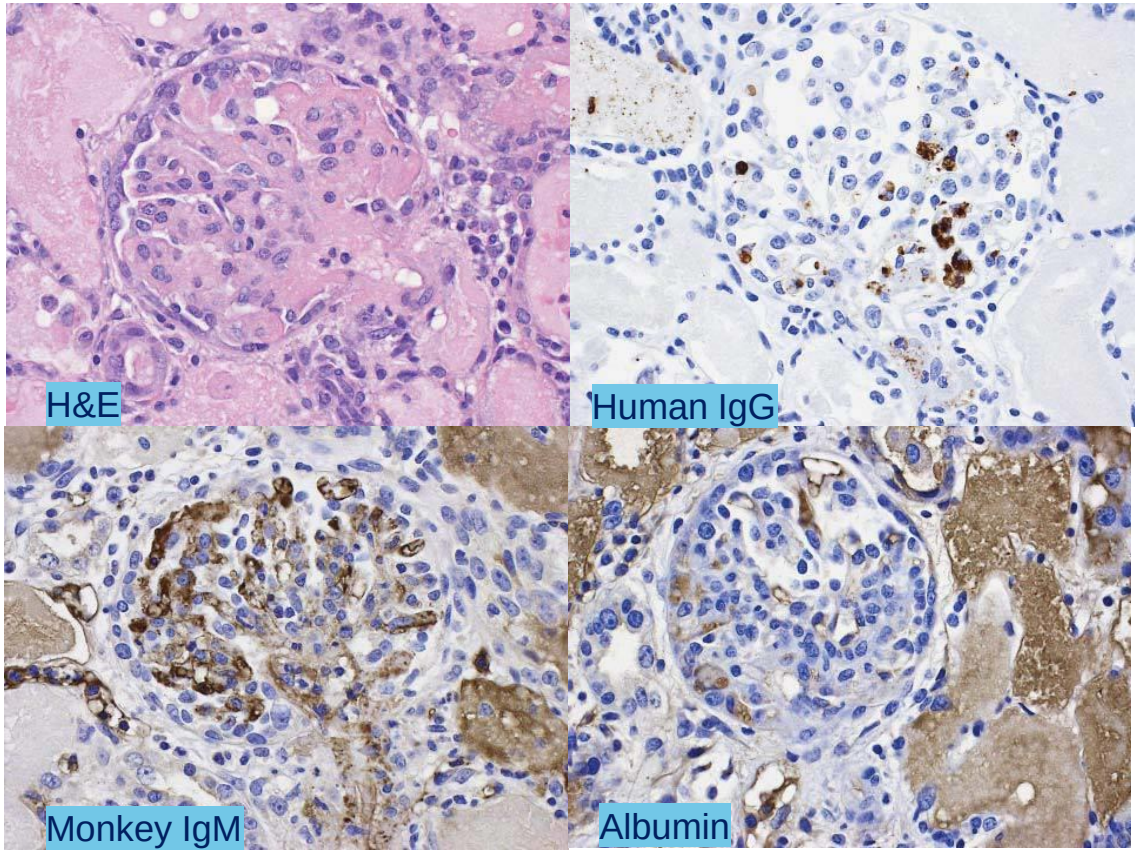
Case study 1: Immunohistochemistry study proved the lesions were due to immune complex deposition as consequence of anti-drug antibodies (ADA)

Arteriole



Case study 1: Immunohistochemistry study proved the lesions were due to immune complex deposition as consequence of ADA

Kidney



IND submitted successfully and approved by the FDA

Case 2 MEDI WWWW: anti- bacterial mAb

29-Day GLP Monkey Study Design

Group	Treatment	Dose Volume (mL/kg)	Number of Animals					
			Initial		Terminal Necropsy		Recovery Necropsy	
			M	F	M	F	M	F
1	Control	10	5	5	3	3	2	2
2	Low Dose	10	3	3	3	3	--	--
3	High Dose	10	5	5	3	3	2	2
	Total Number of Animals:		13	13	9	9	4	4

29-Day GLP Monkey Study Findings

In-life Observations:

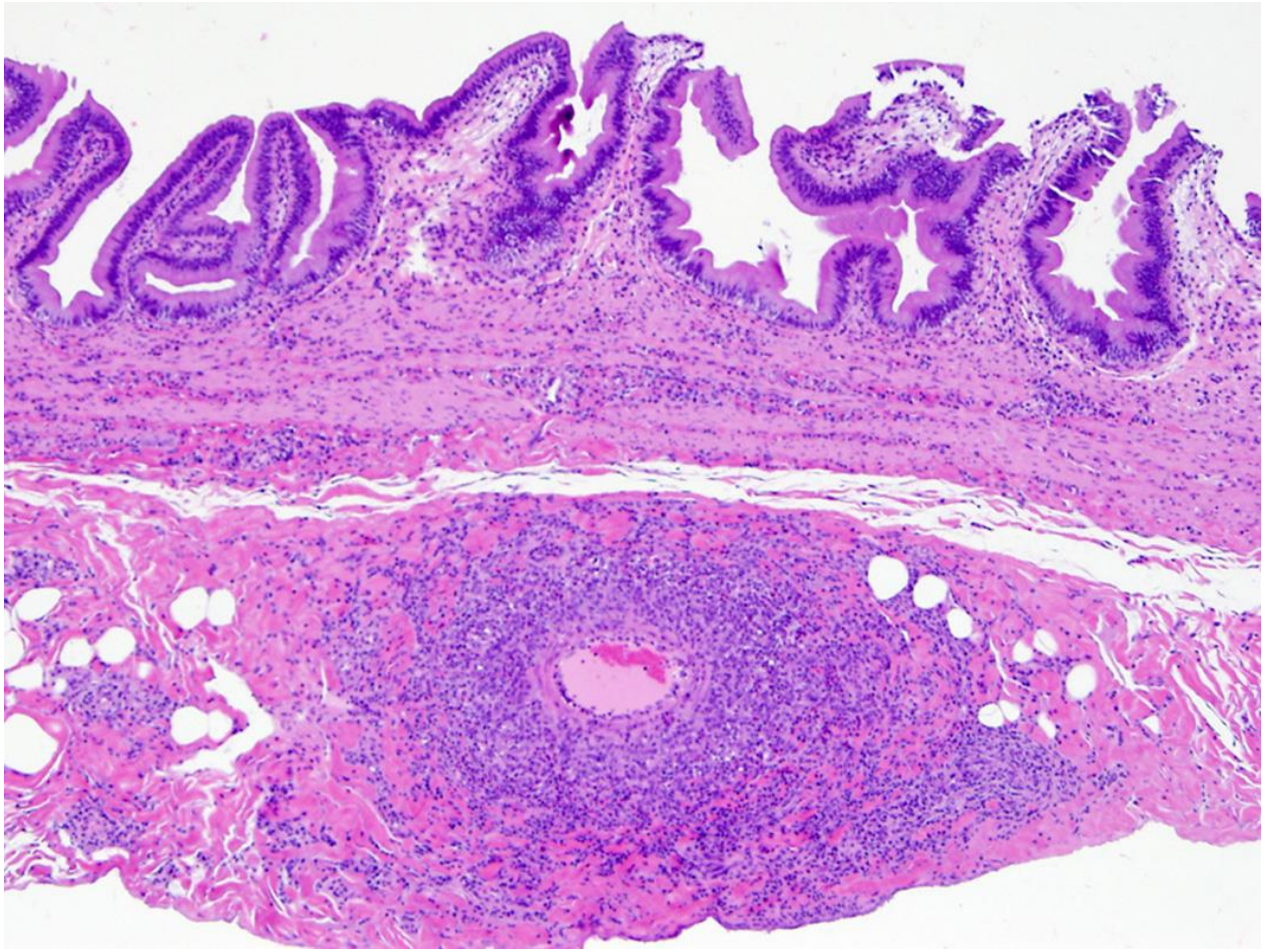
All animals successfully completed the study with no treatment-related findings in clinical observations, appetite, body weight, blood pressure, respiration rate, body temperature, ophthalmoscopic, physical, and EKG examinations.

29-Day GLP Monkey Study Findings

Histopathology Observations:

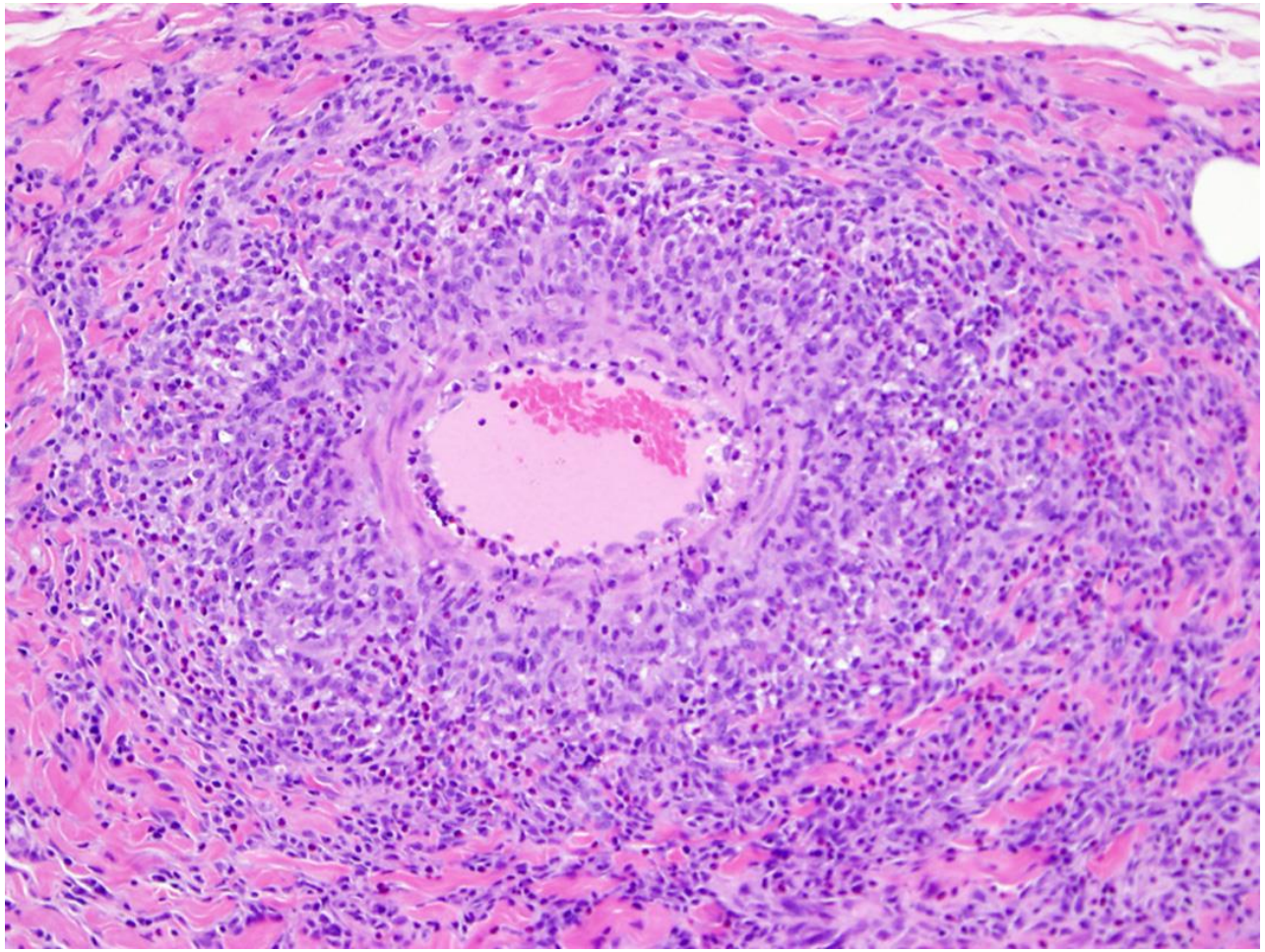
One low dose animal had the following changes.

MEDI WWW Arteritis



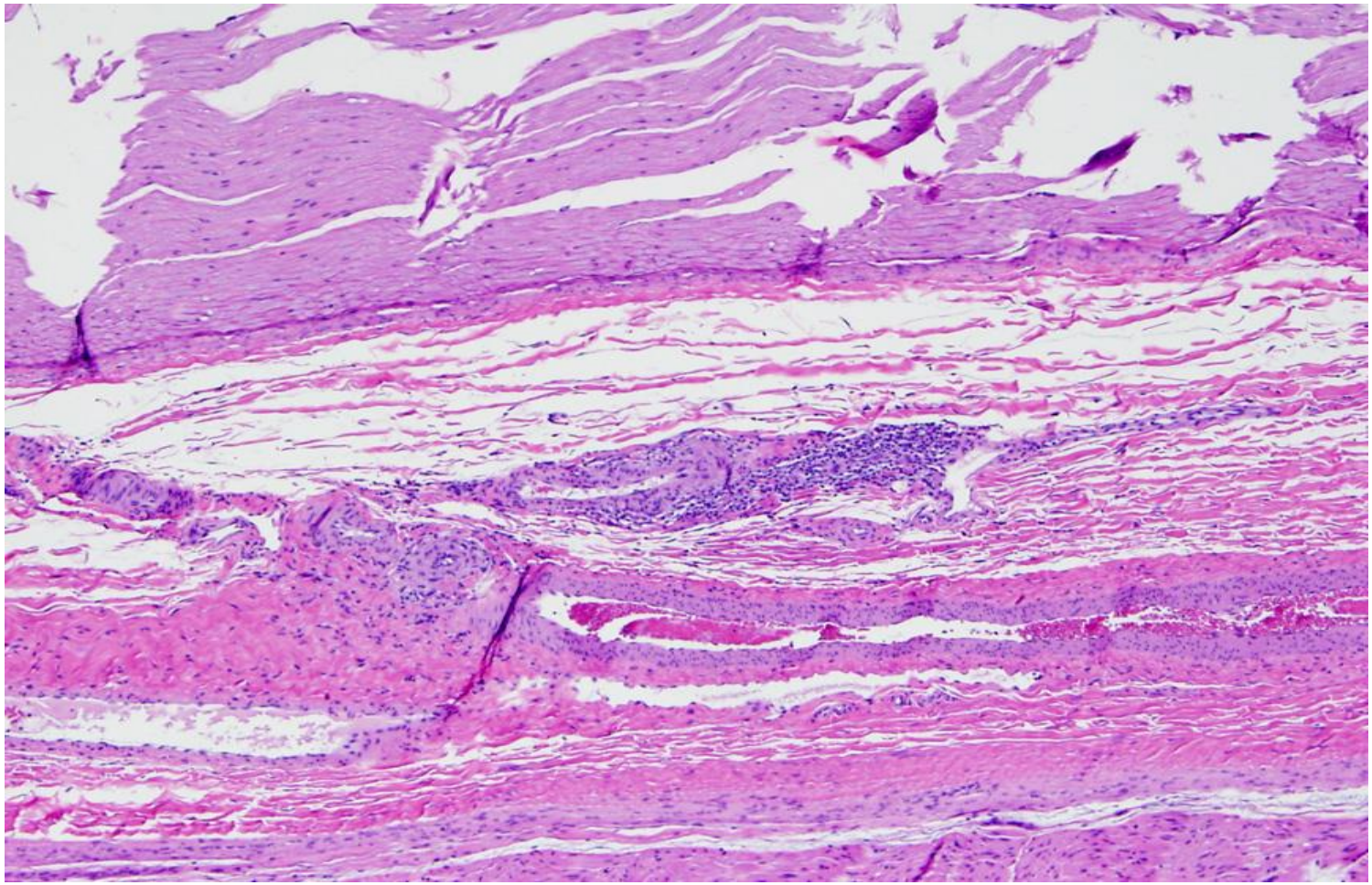
Gallbladder x 40

MEDI WWW Arteritis



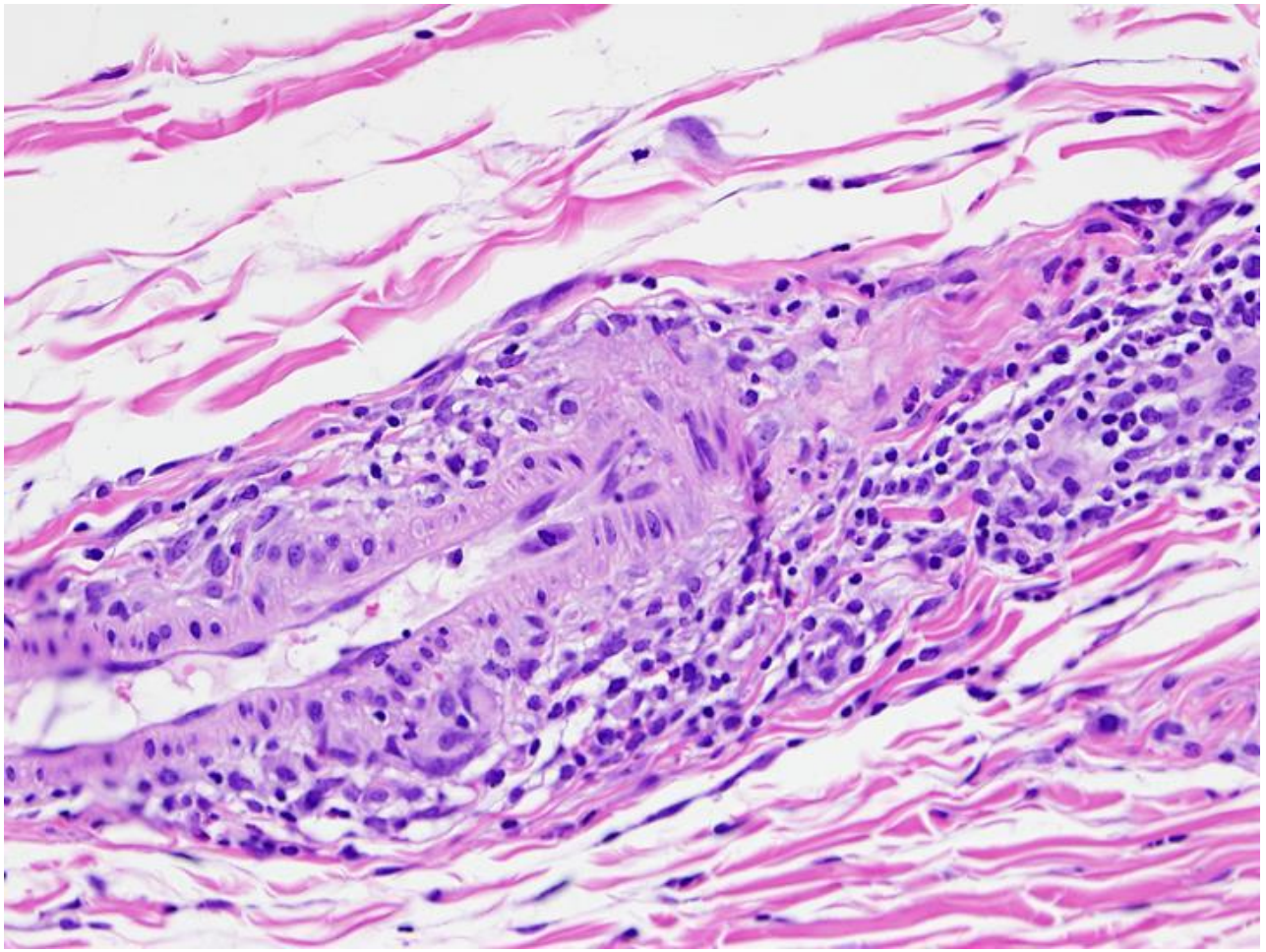
Gallbladder X100

MEDI WWW Arteritis



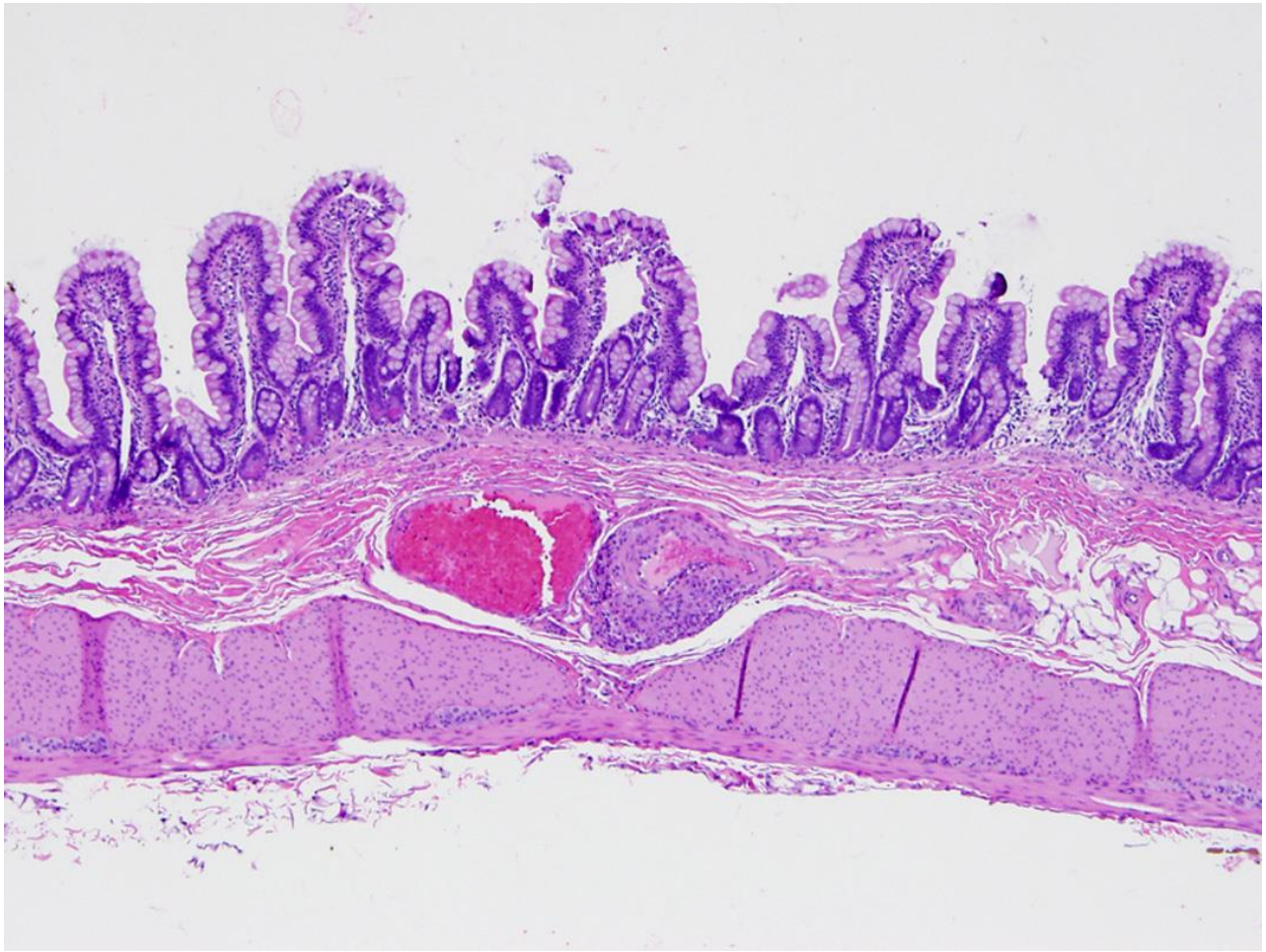
Sciatic nerve X40

MEDI WWW Arteritis



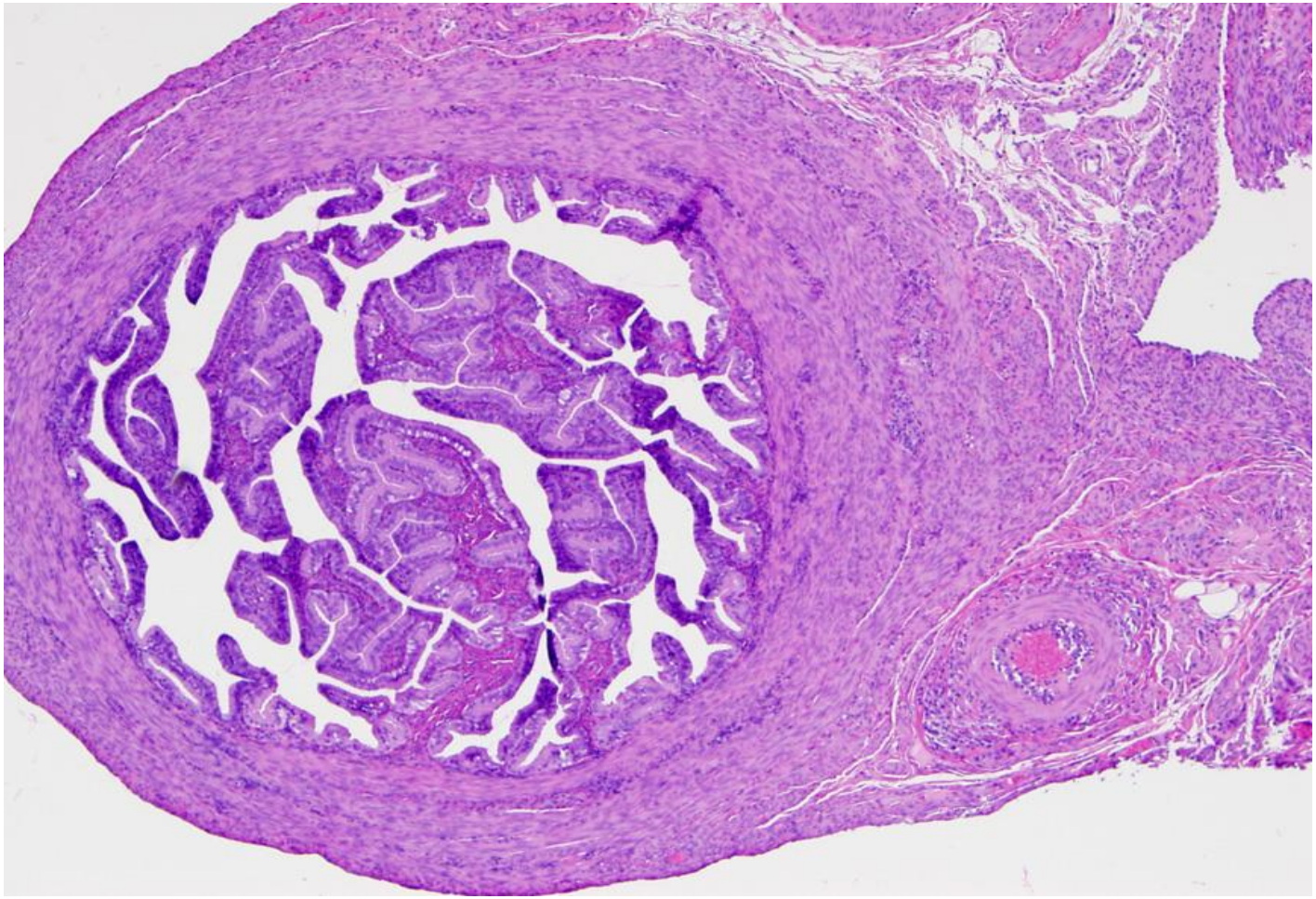
Sciatic nerve X200

MEDI WWW Arteritis



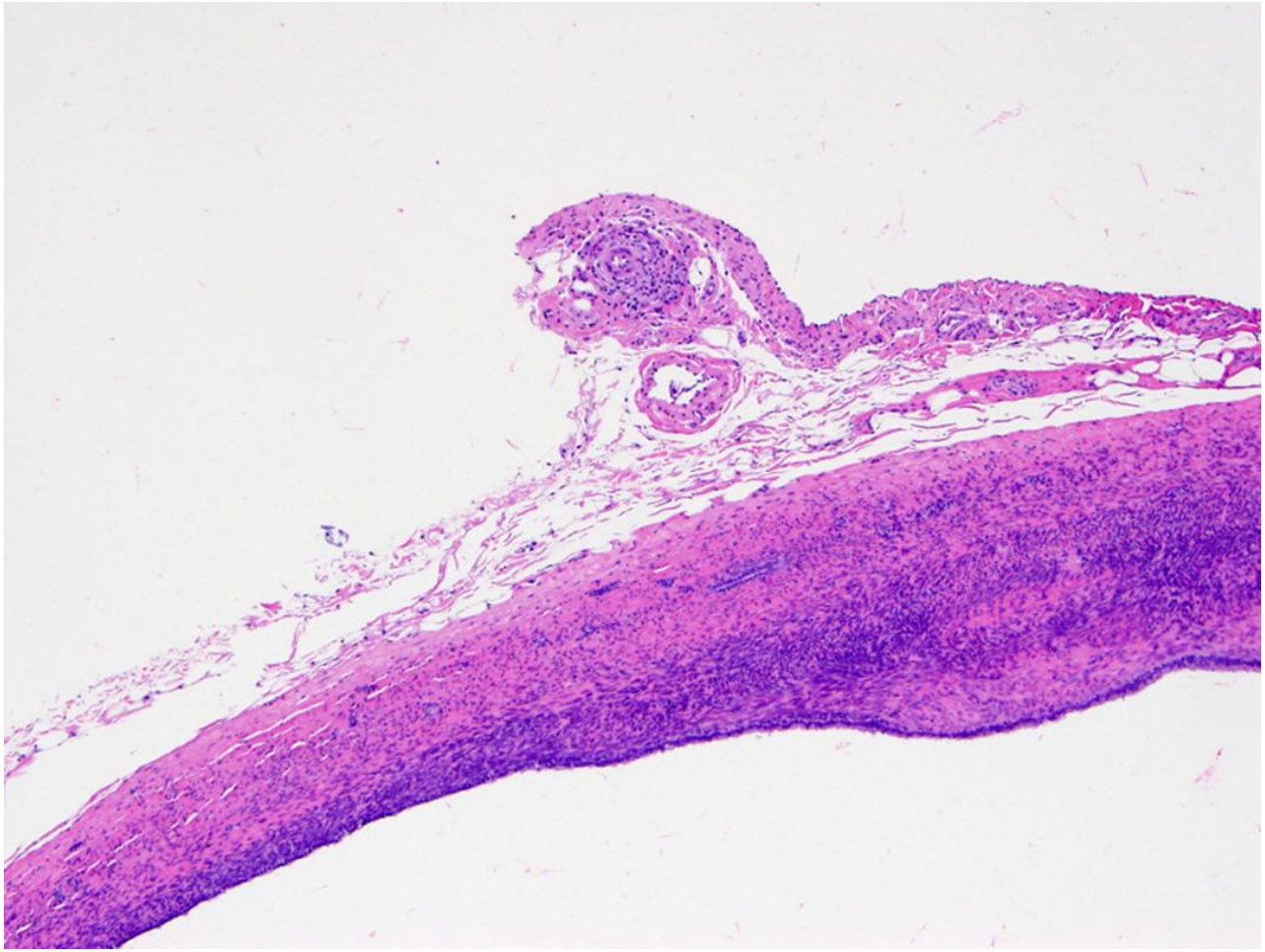
Jejunum X40

MEDI WWW Arteritis



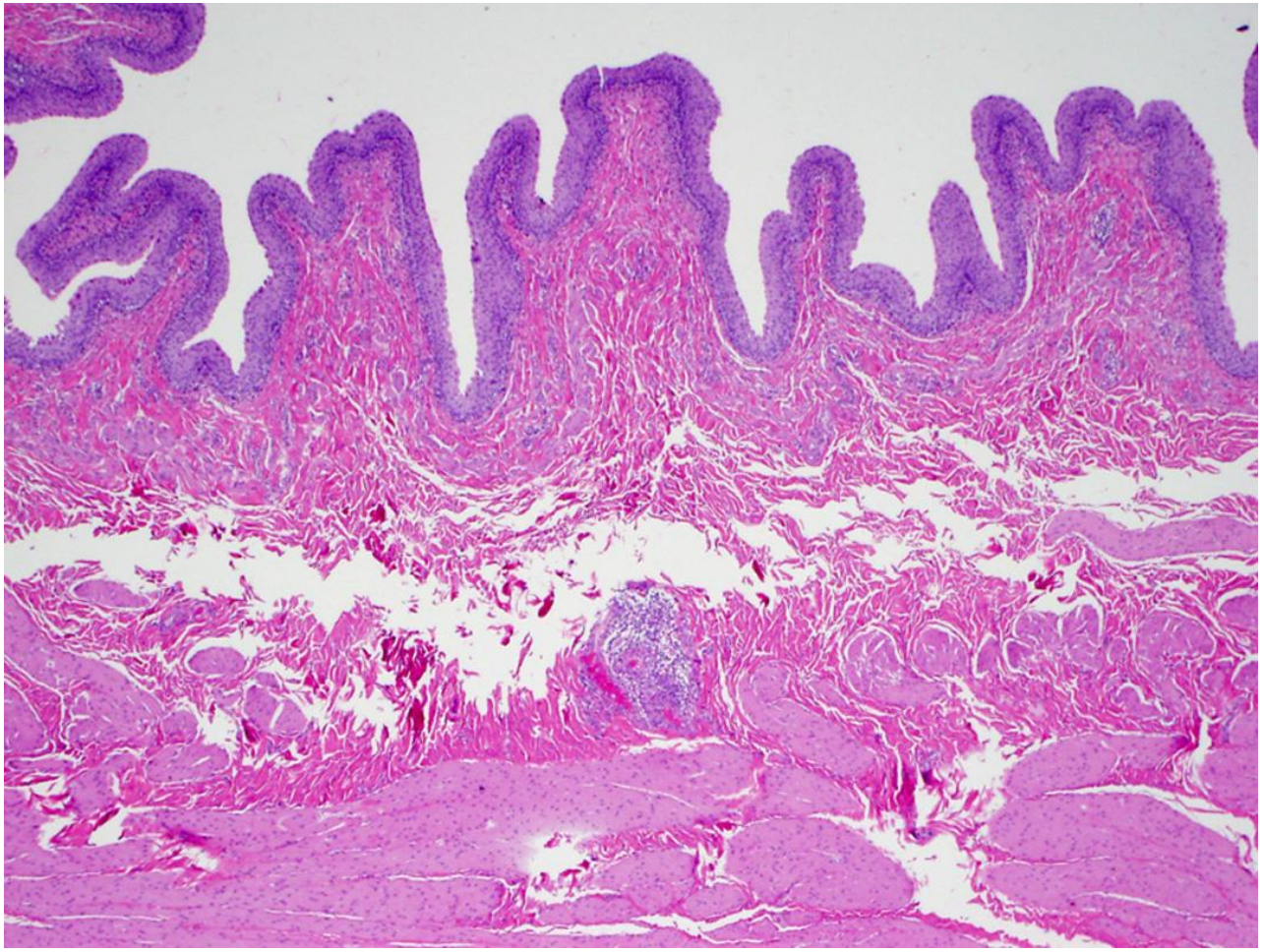
Oviduct X40

MEDI WWW Arteritis



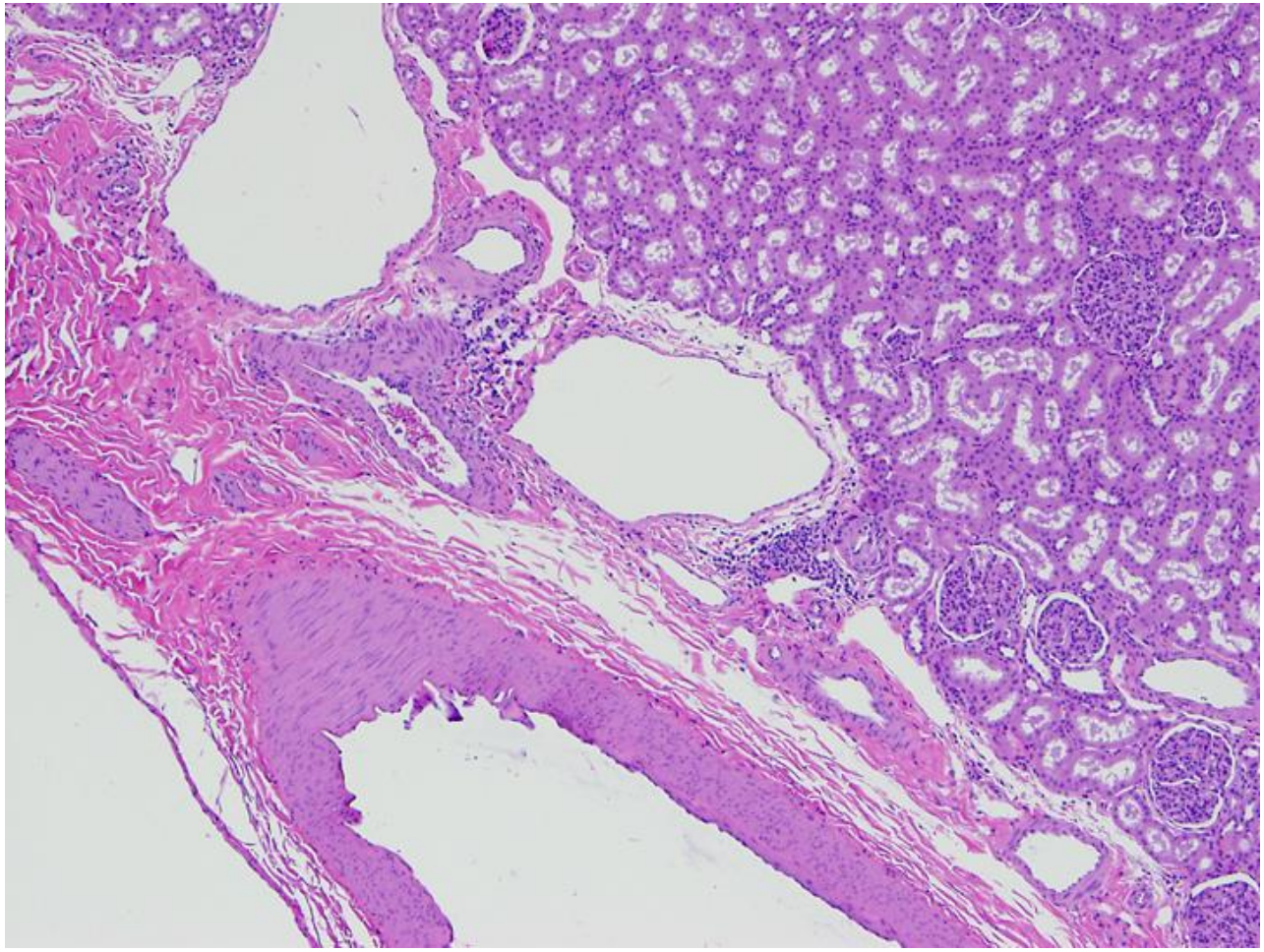
Ovary X40

MEDI WWW Arteritis



Urinary bladder X20

MEDI WWW Arteritis



Kidney X40

29-Day GLP Monkey Study Findings

- Clinical Pathology: at both doses there were
  - Mild increases in total protein, up to 17%
  - Mild increases in globulin, up to 51%
  - Concurrent decreases in albumin
  - Mild decreases in red call mass with concurrent increases in reticulocytes in all groups including controls typical of procedure-related blood sampling

Exposure at two time points in the 7 ADA positive animals

Table 5.4.1-1 Exposure after first and fourth doses for ADA positive Monkeys

Animal Number	$AUC_{Day1-Day8}$ (µg·day/mL)	$AUC_{Day22-Day29}$ (µg·day/mL)
2001	7306.22	7616.34
2002	9753.90	10823.53
2003	3675.24	3379.81
2502	7732.23	7596.58
2503	7772.89	1794.67
3003	17042.56	19212.63
3502	21924.11	17602.94

$AUC_{Day1-Day8}$= area under the curve during dosing interval from Day 1 to Day 8; $AUC_{Day22-Day29}$ = Area under the concentration-time curve from Day 22 to Day 29

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$AUC_{Day1-Day8}$= area under the curve during dosing interval from Day 1 to Day 8; $AUC_{Day22-Day29}$ = Area under the concentration-time curve from Day 22 to Day 29

29-Day GLP Monkey Study Histopathology Findings

- In a single, 100 mg/Kg , female animal
  - Microscopic findings related to immune complex deposition; inflammation in small to medium-size muscular arteries in:
    - G.I. Tract, including cecum, colon, ileum, jejunum, and stomach
    - Gallbladder and pancreas
    - Ovaries, oviducts, uterus, and vagina
    - Lung
    - Sciatic nerve
    - Spleen
    - Urinary bladder
  - Additional microscopic findings
    - Skeletal Muscle: myofiber degeneration and inflammation
    - Miscellaneous mononuclear cell infiltrates considered normal background for this age and species

29-Day GLP Monkey Study Findings

With IHC, immune complex-related granular deposits containing MEDI WWW, monkey IgM, and/or monkey IgG were observed in the intima and inner media, aligning the internal elastic lamina and/or at vascular branch points in the affected vessels.

A few granular deposits were observed to be phagocytized by monocytes/macrophages.

29-Day GLP Monkey Study Findings

Diagnosis:
Immune complex arteritis due to ADA

Case 3 Safety Assessment of MEDI XXX

T. Scott Manetz

Completed MEDI XXX Nonclinical Safety Studies For IND

- Single/Repeat IV dose non-GLP toxicology in NHP. Single dose NOAEL of 100 mg/kg. Anti-histamine treatable reaction in 1of 3 animals re-dosed on Days 37 and 50, with Day 50 most intense response.
  Anti-MEDI XXX Antibody observed (40-12,288,000).
- Single IV dose GLP PD in NHP. No toxicity noted in the 0.003 to 1 mg/kg dose range.
  Anti-MEDI XXX Antibody observed (20-2,621,440).
- Repeat IV Dose (Weekly for 4 weeks) in NHP: NOAEL at least 30 mg/kg/dose, the highest dose tested.
  Anti-MEDI XXX Antibody observed (10-83,886,080).
- Tissue Cross-Reactivity (TCR) Studies in Human and NHP tissue panels.

TCR Results

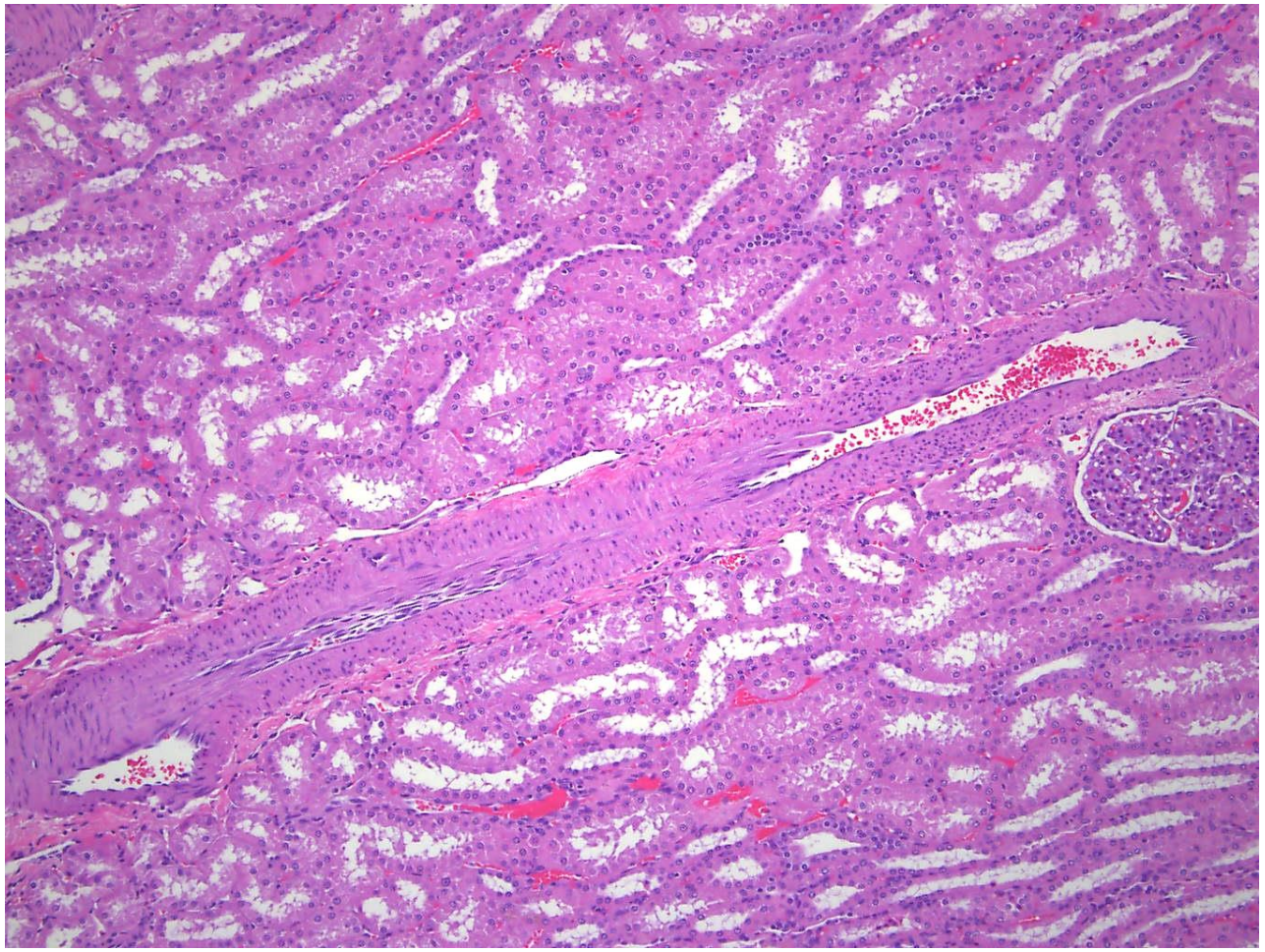
- MEDI XXX-specific staining was present in epithelium, endothelium, mesothelium, mononuclear cells, spindloid/dendritic cells, and intravascular and leaked proteinic material (serum) throughout the cynomolgus monkey tissue panel examined.

- MEDI XXX also stained myenteric plexi in the gastrointestinal tract, Meissner's plexi in the esophagus, glomerular tuft cells in the kidney, granulosa cells in the ovary, beta cells in the pancreas, chief cells of the parathyroid, endocrine cells and pituicytes in the pituitary, decidual cells in the placenta, and spermatogenic cells in the testis.

39-WK Repeat IV/SC Cynomolgus Monkey Study with MEDI XXX with a 13-WK Recovery Period

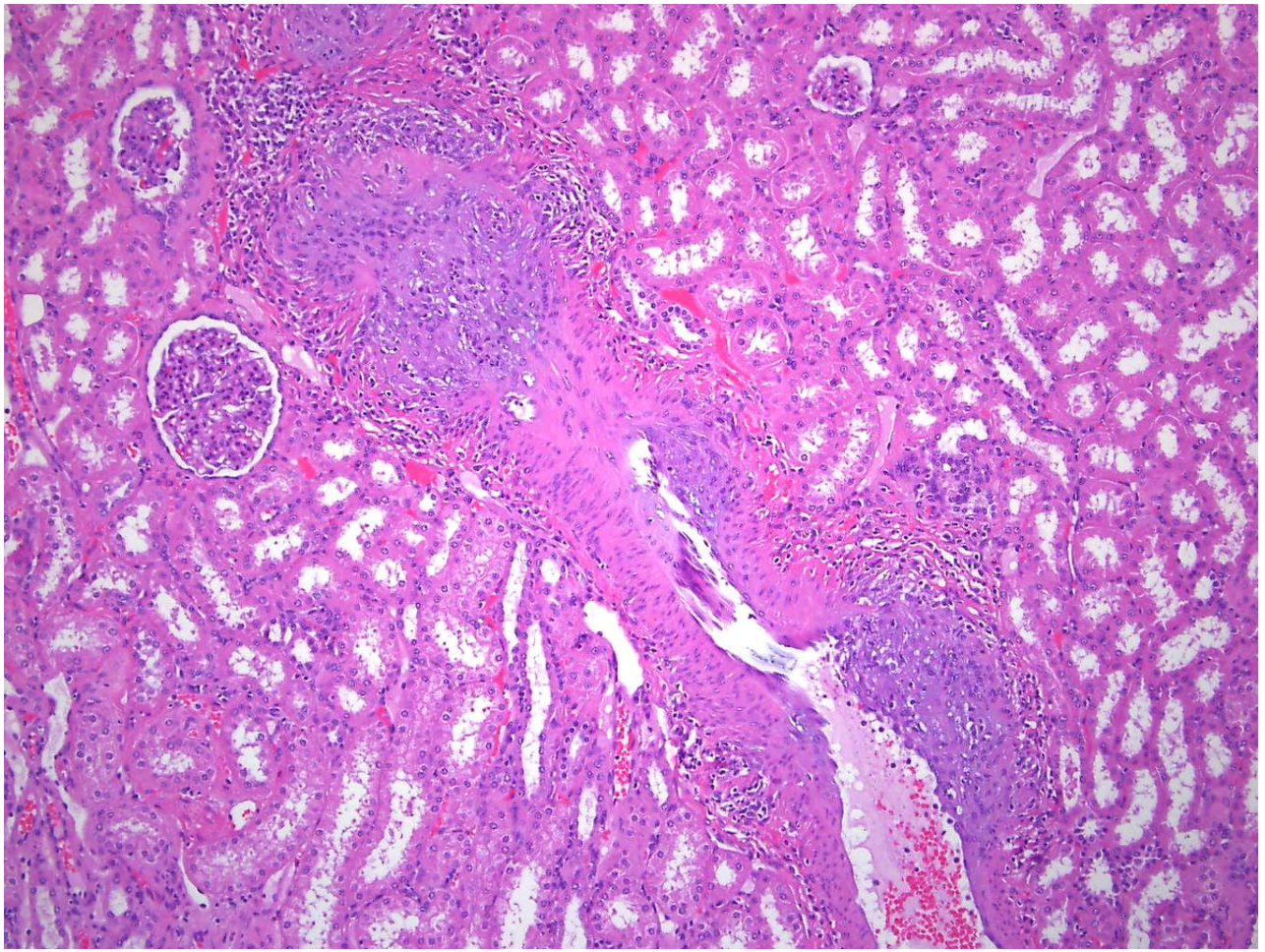
- Terminal dosing phase timepoint data after 39-Weeks of dosing
  - Animal 939 50 mg/kg IV: Grade 3 arteritis in the kidney, Grade 2 in the heart and pancreas, and Grade 1 in other locations (sciatic nerve, adrenal, thyroid, liver, gallbladder, stomach, duodenum, ileum, colon, jejunum, cecum, tongue, prostate, seminal vesicle, epididymis, lacrimal gland, spleen, mesenteric lymph node, thymus, and axillary lymph node)
  - Animal 941 50 mg/kg IV: Grade 1 arteritis in the kidney and Grade 2 in the pancreas and heart
- Arteritis was noted as accumulation of material between smooth muscle fibers, infiltration by lymphocytes, macrophages and neutrophils, and perivascular aggregates of lymphocytes and plasma cells

39-WK Repeat IV/SC Cynomolgus Monkey Study with MEDI XXX with a 13-WK Recovery Period



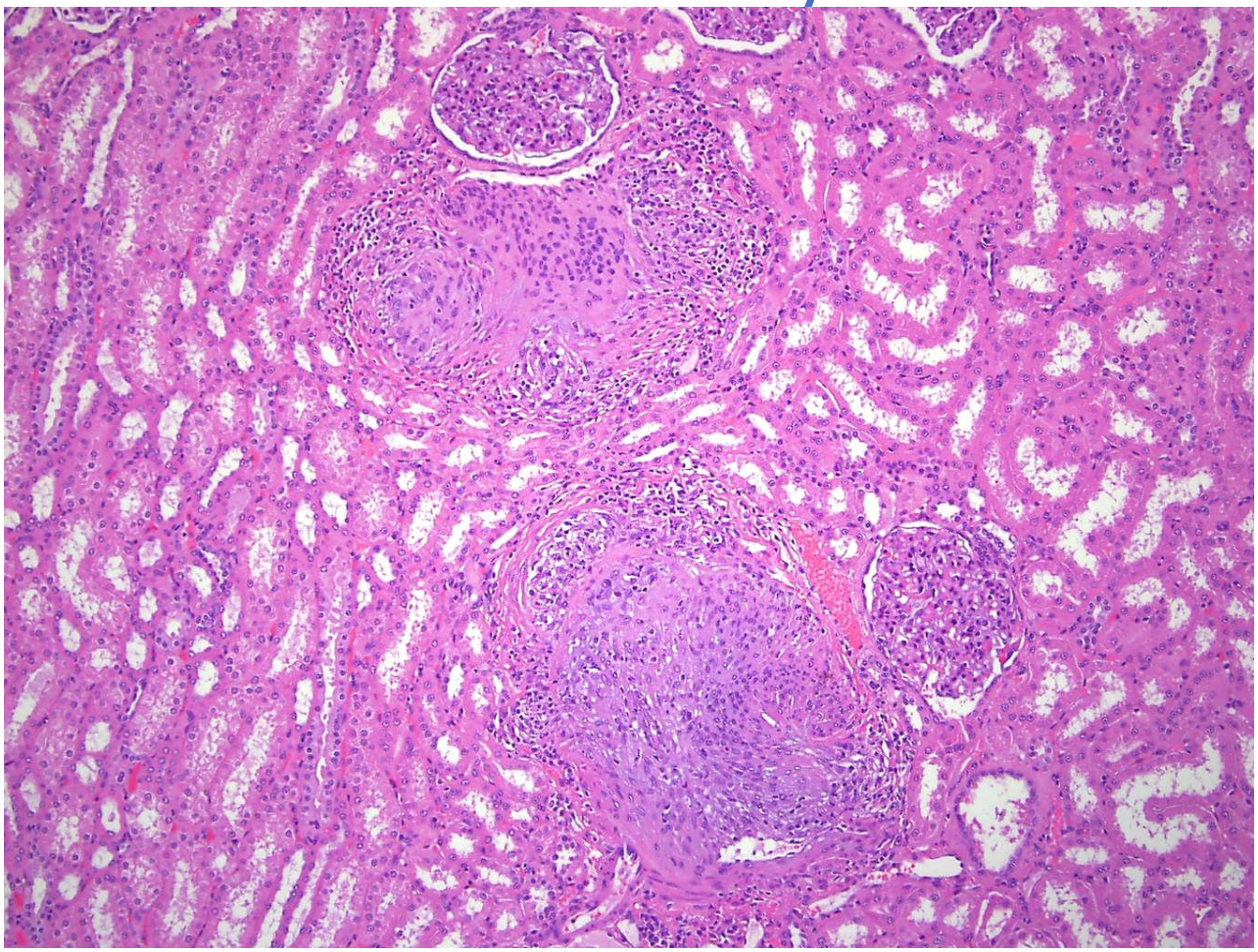
#927 Kidney Control Male Term Sac

39-WK Repeat IV/SC Cynomolgus Monkey Study with MEDI XXX with a 13-WK Recovery Period



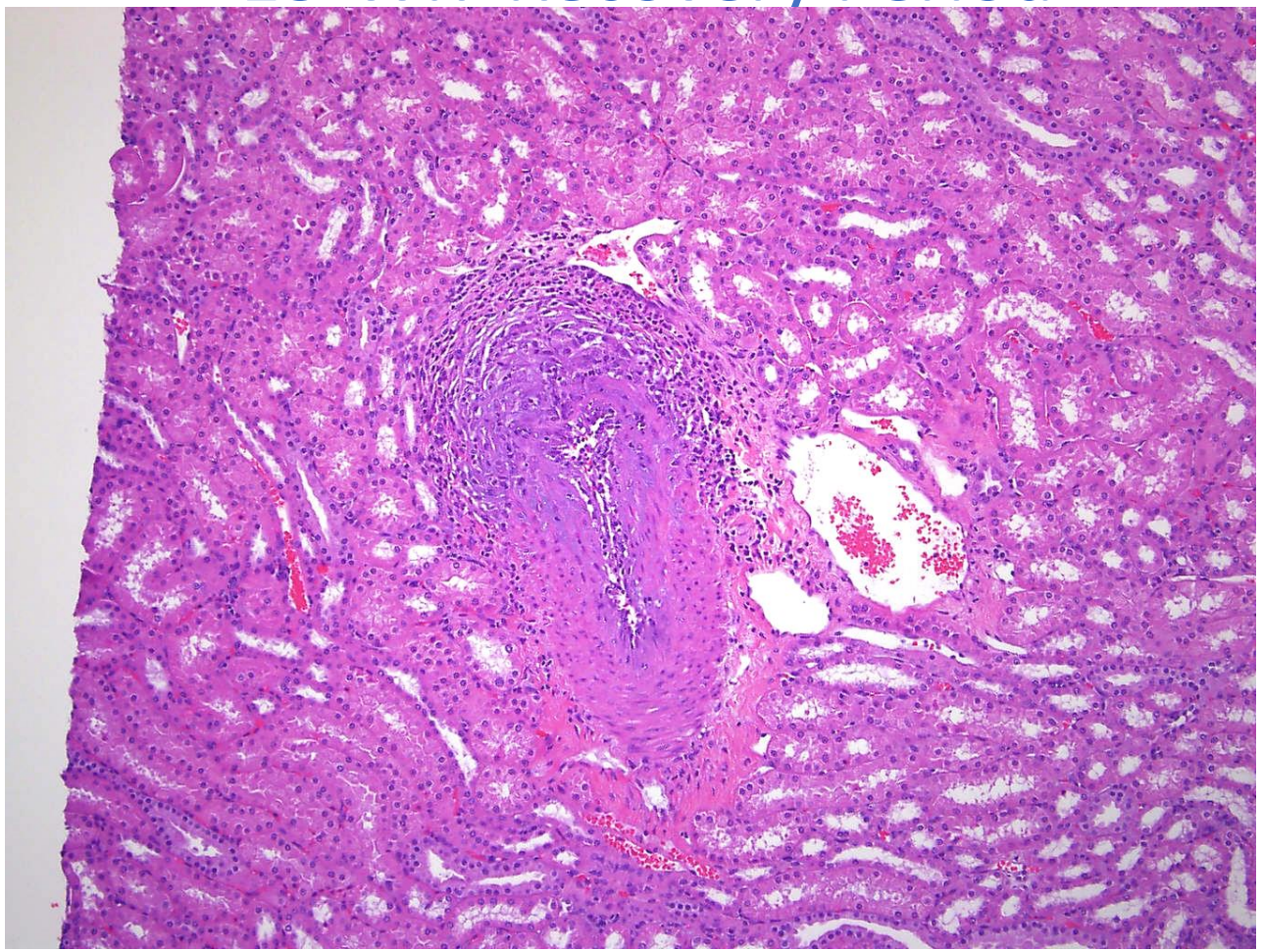
#939 Kidney High IV Dose Male Term Sac

39-WK Repeat IV/SC Cynomolgus Monkey Study with MEDI XXX with a 13-WK Recovery Period



939 Kidney High IV Dose Male Term Sac

39-WK Repeat IV/SC Cynomolgus Monkey Study with MEDI XXX with a 13-WK Recovery Period



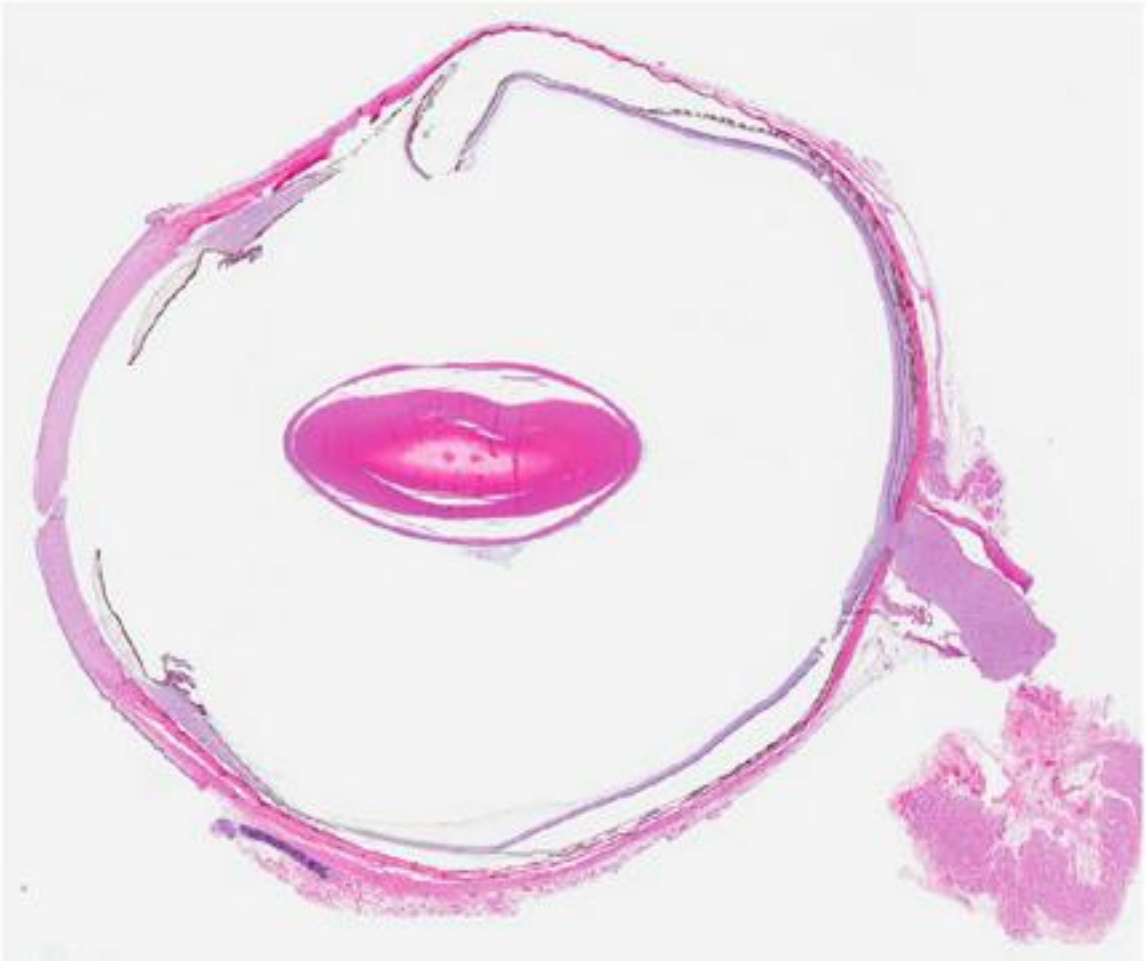
939 Kidney High IV Dose Male Term Sac

39-WK Repeat IV/SC Cynomolgus Monkey Study with MEDI XXX with a 13-WK Recovery Period

- "Recovery" data after 13-Weeks of no dosing
  - Animal 936 5 mg/Kg IV: Grade 2 arteritis in one eye only
  - Animal 944 50 mg/Kg IV: Grade 1 arteritis in kidney, gut, and pancreas
  - Animal 955 60 mg/Kg SC: Grade 2 arteritis in heart, and Grade 1 in liver, kidney, and cecum

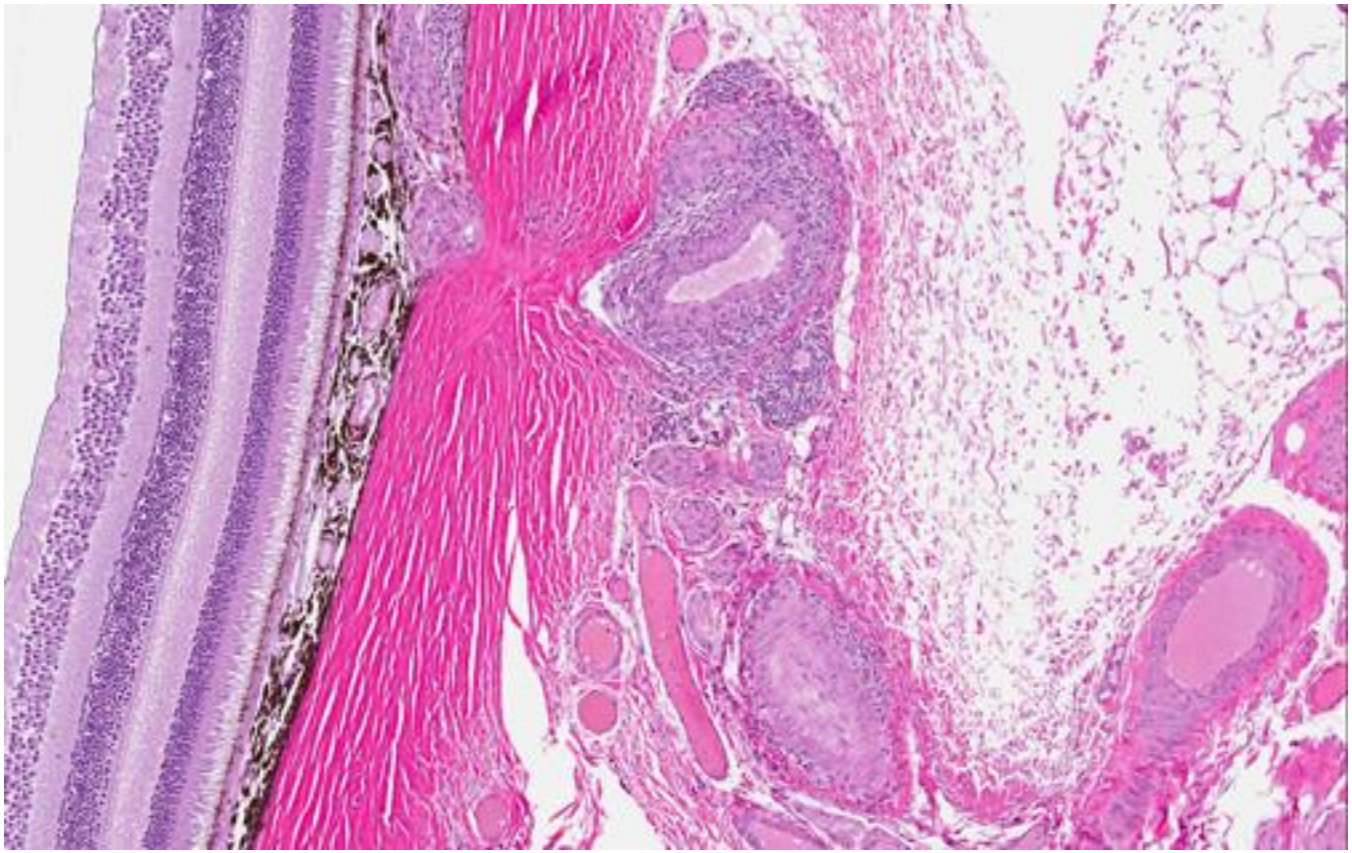
- Arteritis noted as primarily intramural and perivascular infiltrates of lymphocytes and macrophages. In some locations plasma cells and neutrophils and occasional karyorrhectic debris were also present

39-WK Repeat IV/SC Cynomolgus Monkey Study with MEDI XXX with a 13-WK Recovery Period



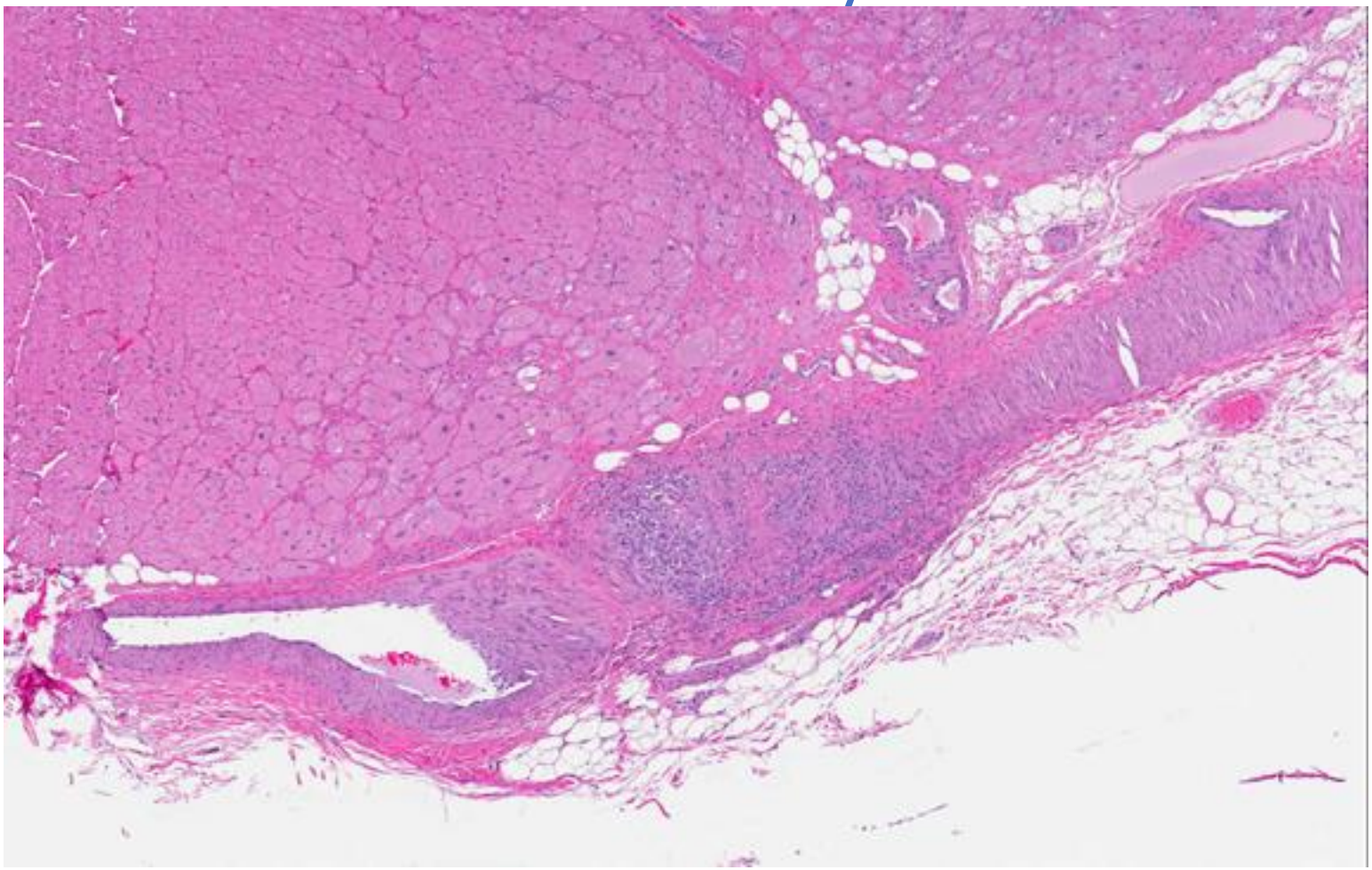
#936 Eye Low IV Dose Male Recovery Sac

39-WK Repeat IV/SC Cynomolgus Monkey Study with MEDI XXX with a 13-WK Recovery Period



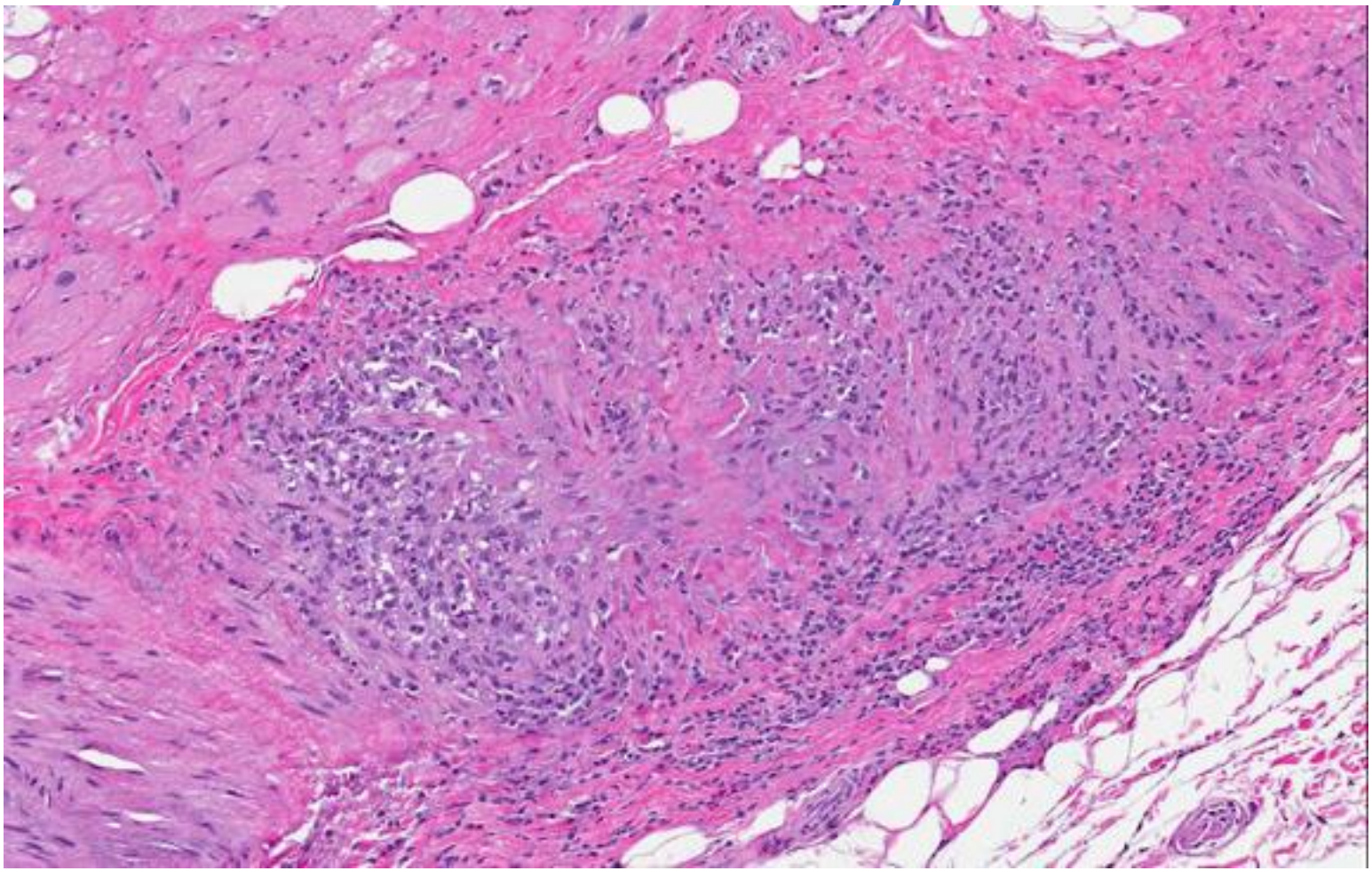
#936 Eye Low IV Dose Male Recovery Sac

39-WK Repeat IV/SC Cynomolgus Monkey Study with MEDI XXX with a 13-WK Recovery Period



#955 Heart High SC Dose Male Recovery Sac

39-WK Repeat IV/SC Cynomolgus Monkey Study with MEDI XXX with a 13-WK Recovery Period



#955 Heart High SC Dose Male Recovery Sac

39-WK Repeat IV/SC Cynomolgus Monkey Study with MEDI XXX with a 13-WK Recovery Period

- Key Characteristics Noted for this study and Diagnostic Considerations
  - Within a given tissue section, there were normal arteries, and others with the finding
  - Generally segmental within an artery section
  - Observed in smooth muscle and perivascular area of small to medium sized muscular arteries
  - Cellular Infiltrate consists of lymphocytes, macrophages, and plasma cells
  - Notable absence of necrosis, giant cells, eosinophils, and granulomatous inflammation
  - Most common organs affected were kidney, heart and pancreas
  - Kidney glomeruli appeared unaffected
  - Observed only in male animals
  - No apparent infectious agent (clinical pathology and clinical obs negative)
- Combined incidence in MEDI XXX dosed males was 5/24 (21%; no findings in females).
- Historical incidence from CRO with studies of $\geq$ 26 weeks in duration : 2/167 animals (1.2%) with diagnosis "Inflammation, vascular". Other literature suggests 1-6% incidence in cynomolgus monkeys.

Summary of Histopathology Findings in Male Cynomolgus Monkeys

		Incidence of Findings in Groups 1, 2, 3, 4, 5 (Severity Grade)	
Treatment Related Microscopic Findings		**Terminal (Day 277)**	**Recovery (Day 356)**
Various Tissues	Inflammation, Arterial	0 mg/kg 5 mg/kg IV 50 mg/kg IV 2 (1-3) 15 mg/kg SC 60 mg/kg SC	0 mg/kg 5 mg/kg IV 1 (2) 50 mg/kg IV 1 (1) 15 mg/kg SC 60 mg/kg SC 1 (1)

- While evident in more animals, the inflammatory changes in the recovery phase males were less pronounced and generally less widespread than in those of dosing phase males.

39-WK Repeat IV/SC Cynomolgus Monkey Study with MEDI XXX with a 13-WK Recovery Period

- Comparison to known human vasculitidies
  - There are more than eight described types of immune-mediated vascular disease in humans, affecting different size vessels, with different histologic features, and categorized according to various classification schemes
  - The arterial lesions seen in this monkey study do not look like any of the described human conditions

IHC Analyses

Mark Mense, Meggan Czapiga, Karma Dacosta, William Iverson,
Jennifer Ryoko (CRL, PAI)

- Analyses for MEDI XXX, C3, and possible anti-MEDI XXX antibody performed using IHC on frozen tissues collected on study. H and E slides prepared to confirm sites of lesions prior to IHC analyses.

- C3 – does not necessarily need to be localized with MEDI XXX or anti-MEDI XXX antibody as it may deposit proximal to the site of activation.

MEDI XXX IHC Conclusions

- Human IgG (MEDI XXX) demonstrable - Lack of broad/uniform staining argues against target based MOA effect
- No increase in Monkey IgG
- Increased focal deposits Monkey IgM
- C3b in some cases (but not all) may localize with monkey IgM
- Granular appearance of C3b suggests a role in formation of the changes

Background incidence data

- Chamanaza, R et al. Spontaneous lesions of the cardiovascular system in purpose bred laboratory nonhuman primates. Toxicologic Pathology 34, 357–63. (CRL Edinburgh)

  Perivasculitis and vasculitis were the most frequently encountered lesions affecting blood vessels. These were largely localized lesions characterized by perivascular or vascular wall infiltration with lymphocytes without fibrin deposition or extensive necrosis of the tunica media. The most commonly affected organs in decreasing order of frequency were: kidney, lungs, meninges (brain and spinal cord), heart, urinary bladder and sciatic nerve. However, perivasculitis of the meningeal blood vessels is a relatively common finding in cynomolgus monkey studies.