Supporting Information

Cross-circulation for Extracorporeal Liver Support in a Swine Model

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SUPPLEMENTARY METHODS

Oxygen consumption calculation

Percent oxygen saturation (SaO_2), partial pressure of oxygen (PaO_2), and Hb levels were measured in blood drawn from the PV inflow cannula ('pre-organ') and from the IVC outflow cannula ('post-organ') using a point-of care blood analysis system (epoc; Heska). Because PV and HA blood are both derived from the same oxygenated source (**Figure 1**), the pre-organ sO_2 are pO_2 measurements are representative of both PV and HA levels. Oxygen consumption was calculated using the equation below.

$$Total\ organ\ O_2\ consumption\ (mL/min) = Q\ \times \left[\left(1.34 \times Hb \times \frac{\Delta SaO_{2,}}{100} + 0.0032\ \times \Delta PaO_2\right)\right.$$

Where,

Q = total organ blood flow (dL/min)
Hb = hemoglobin level (g/dL)
$\Delta SaO_2 = SaO_{2,pre} - SaO_{2,post}$ (%)
$\Delta PaO_2 = PaO_{2,pre} - PaO_{2,post}$ (mmHg)

Given differences in organ weight across experiments, oxygen consumption was normalized to every 100g of organ weight in **Figure 5B**.

Lactate clearance calculation

Pre- and post- (designated as described in *Oxygen consumption calculation* above) organ lactate levels were measured using a point-of care blood analysis system (epoc; Heska). Percent lactate clearance was calculated using the equation below.

$$lactate\ clearance\ (\%) = \frac{lactate_{pre} - lactate_{post}}{lactate_{pre}}$$

Where,

$lactate_{pre}$ = pre-organ lactate level (mmol/L)
$lactate_{post}$ = post-organ lactate level (mmol/L)

Lactate clearance is shown in **Figure 5C**.

Initiation of cross circulation

The circuit was primed with Normosol-R and donor blood. The circuit was connected to the host venous and arterial cannulas, and extracorporeal V-AV blood flow was initiated without initial inclusion of the extracorporeal liver. After confirming cannula site hemostasis and host hemodynamic stability, the circuit was clamped, briefly pausing extracorporeal blood flow. Sections of the circuit were divided, and the PV, HA, and IVC cannulas were connected to the appropriate inflow and outflow circuit components, thus introducing the extracorporeal liver into the circuit (**Figure 1A**). Clamps were removed from hepatic inflow tubing and circuit flows were gradually increased, marking the start of V-AV XC.

Blood counts and serum biochemical analyses
Complete blood counts were performed by the Tissue and Pathology Shared Resource Laboratory at Vanderbilt University Medical Center, using the Forcyte Veterinary Hematology Analyzer (Oxford Science, Inc, Oxford, CT). Clinical chemistry testing was performed by ANTECH Diagnostics (Southhaven, MS) using Beckman Coulter reagents and the Beckman Coulter AU680 clinical chemistry analyzer. Test performed by the AU680 included: albumin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, urea nitrogen, calcium, cholesterol, chloride, creatinine kinase, creatinine, glucose, potassium, sodium, phosphorus, total bilirubin and total protein.

Bile Acid Controls, Chemicals, and Calibrators
All bile acids were purchased from C.D.N. Isotopes Inc. (Pointe Claire, Montreal, PQ, Canada). High-performance liquid chromatography–grade water, acetonitrile, ethanol, methanol, ammonium acetate, and ammonia were purchased from Sigma Chemicals (St Louis, MO). Formic acid was purchased from Thermo Scientific.
To prepare 20 mL of a 2.0 nM internal standard, 250 µL of d4-GCDCAwas added to 20% (vol/vol) acetonitrile.

Sample Preparation of Bile Acids for Ultra Performance Liquid Chromatography/Electrospray Ionization Mass Spectrometry
For bile acid measurements, the following were added to 50 µL of homogenate: 200 µL of 100 mM aqueous sodium hydroxide and 50 µL of a 2-nM internal standard mix. Samples were heated at 64°C for 30 minutes, centrifuged for 10 minutes at 14,400g, and supernatant acidified to pH 7.0 with 50 µL of 0.1M hydrochloric acid. Samples were brought to final volumes of 1 mL with water and applied to 1-mL (30 mg) Oasis HLB cartridges (Waters, Milford, MA) previously equilibrated first with 1 mL methanol, then 1 mL water (Rodrigues, 1996).12 Column-bound bile acids were washed with 1 mL 5% (vol/vol) aqueous methanol, then 1 mL 2% (vol/vol) aqueous formic acid. Bile acids were eluted from the column with 1 mL 2% (vol/vol) ammonia in methanol and the eluent evaporated to dryness using a rotary evaporator at 30°C for 2 hours. Samples were resuspended in 100 µL 25% (vol/vol) acetonitrile in water.

Bile Acid Analysis by High-Performance Liquid Chromatography–Mass Spectrometry
An Acquity ultra performance liquid chromatography system (Waters, Milford, MA) was used with an Acquity ultra performance liquid chromatography BEH C18 1.7-µm, 2.1 × 100-mm column (Waters), and heated to 50°C, and a binary solvent system of 20% (vol/vol) acetonitrile in water (mobile phase A) and 80% (vol/vol) acetonitrile in water (mobile phase B), both containing 1 mM ammonium acetate, were used to resolve bile acids. Mass spectrometry analysis was performed using a TSQ Quantum mass spectrometer (ThermoFinnigan) equipped with an ESI probe in negative-ion mode. The following (optimized) parameters were used for the detection of the analytes and the internal standard: N2 sheath gas, 49 psi; N2 auxiliary gas, 25 psi; spray voltage, 3.0 kV; source CID, 25 V; capillary temperature, 300°C; capillary offset, −35 V; tube lens voltage, 160 V; Q2 gas pressure, 1.5 mtor; Q3 scan width 1 m/z; Q1/Q3 peak widths at half-maximum, 0.7 m/z. Relative concentrations of individual bile acids were calculated

from peak area in the chromatogram detected with SRM relative to the appropriate internal standard.

SUPPLEMENTARY TABLES

Supplementary Table 1. Veno-arterial-venous cross-circulation circuit components.

Equipment/component	Product name	Manufacturer
Main circuit		
Centrifugal pump console	RotaFlow,	Maquet
Centrifugal pump head	RotaFlow	Maquet
Adult oxygenator	Quadrox-i Adult	Maquet
1/4” Tygon® ND-100-65 medical tubing		Saint-Gobain
3/8” Tygon® ND-100-65 medical tubing		Saint-Gobain
Straight connector with leur lock, 3/8” x 3/8”		Qosina
Straight connector with leur lock, 1/4” x 1/4”		Qosina
Y-connector 3/8” x 3/8” x 1/4"		Qosina
Y-connector 3/8” x 3/8” x 3/8”		Qosina
Blood and ascites salvage		
Cardiotomy reservoir	EL400	Medtronic
Sump suction	DLP pericardial	Medtronic
Monitors		
3/8” flow probe	Sonoflow	Sonotec
1/4” flow probe	Sonoflow	Sonotec
Pressure transducer	TruWave	Edwards Lifesciences
Data acquisition software	LabChart	ADInstruments
Host and liver cannulas		
17 Fr cannula (IJV return)	Bio-Medicus	Medtronic
19 Fr cannula (IJV drainage)	Bio-Medicus	Medtronic
12-14 Fr cannula (CFA return)	Bio-Medicus	Medtronic
24 Fr cannula (portal vein inflow)	DLP	Medtronic
10-12 Fr cannula (hepatic artery inflow)	Bio-Medicus	Medtronic
8-12 Fr cannula (bile duct outflow)	Bio-Medicus	Medtronic
36 Fr cannula (IVC outflow)	DLP	Medtronic

Supplementary Table 2. Evaluation rubric of liver injury score.

Injury score	**0**	**1**	**2**	**3**	**4**
Vacuolation/steatosis	<5%	5-33%	34-66%	>66%	
Sinusoidal dilatation	None	Rare/focal	Patchy, <50% of the tissue	Patchy, >50% of the tissue	
Congestion	None	Rare/focal	Patchy, <50% of the tissue	Patchy, >50% of the tissue	
Hepatocellular necrosis	None	Rare/focal dropout	<33% of the tissue	33-66% of the tissue (bridging necrosis)	>66% of the tissue (extensive, severe, diffuse, panlobular necrosis)
Fibrosis	None	Portal	Periportal	Bridging	Cirrhosis
Neutrophils	<10/hpf	10-50/hpf	50-100/hpf	>100/hpf	
Lymphocytic infiltration/inflammation*	None	Minimal	Mild	Moderate	severe

* assessment based on the Batts-Ludwig classification system[1]. hpf: high power field

[1] Batts KP, Ludwig J. An Update on Terminology and Reporting. *Am J Surg Pathol*. 1995;19(12): 1409-1417.

Supplementary Table 3. Safety and stability of host swine during 12 hours of cross-circulation support: analysis of host biochemistry, coagulation, and electrolytes.

	Timepoint (h)			
Parameter	Pre-XC	0	6	12
Vitals				
Heart rate (bpm)	92 ± 7	110 ± 5	90 ± 4	84 ± 5
Systolic BP (mmHg)	105 ± 13	90 ± 7	94 ± 4	95 ± 8
Temperature (°C)	37.8 ± 0.5	37.1 ± 0.7	38.3 ± 0.5	38.0 ± 0.2
Arterial blood gas				
pH	7.44 ± 0.02	7.48 ± 0.04	7.50 ± 0.02	7.46 ± 0.03
O_2 tension (mmHg)	358 ±61	485 ± 59	485 ± 51	389 ± 89
CO_2 tension (mmHg)	51 ± 4	46 ± 3	46 ± 3	50 ± 4
Bicarbonate (mmol/L)	34 ± 2	34 ± 1	35 ± 1	35 ± 1
Lactate (mmol/L)	1.7 ± 0.2	3.1 ± 0.9	1.2 ± 0.1	0.9 ± 0.1
Glucose (mg/dL)	134 ± 14	152 ± 27	190 ± 8	240 ± 18
Blood counts				
WBC (10^9/L)	18.3 ± 2.3	12.6 ± 1.6	19.0 ± 2.5	15.9 ± 0.9
% Neutrophil	58 ± 8	57 ± 6	81 ± 2	76 ± 1
% Lymphocytes	40 ± 7	40 ± 6	17 ± 2	19 ± 2
Platelets (10^9/L)	388 ± 39	287 ± 66	258 ± 67	263 ± 59
Hemoglobin (g/dL)	10.0 ± 0.3	10.1 ± 0.3	9.2 ± 0.5	8.5 ± 0.5
Chemistries				
Sodium (mmol/L)	138.5 ± 1.9	138.3 ± 2.4	140.2 ± 1.7	141.5 ± 1.6
Potassium (mmol/L)	4.34 ± 0.04	4.2 ± 0.1	4.8 ± 0.2	4.7 ± 0.1
Calcium (mg/dL)	9.8 ± 0.6	9.8 ± 0.2	9.6 ± 0.2	9.9 ± 0.2
BUN (mg/dL)	11.5 ± 1.3	13.5 ± 1.7	24.5 ± 2.4	33 ± 3.5
Creatine (mg/dL)	1.0 ± 0.1	1.0 ± 0.1	1.1 ± 0.3	1.0 ± 0.2
Albumin (g/dL)	3.2 ± 0.2	3.0 ± 0.2	3.0 ± 0.1	3.2 ± 0.1
AST (U/L)	16 ± 6	163 ± 54	163 ± 33	173 ± 50
ALT (U/L)	24 ± 6	37 ± 3	38 ± 2	38 ± 4
ALP (U/L)	115 ± 16	135 ± 8	120 ± 3	136 ± 6
LDH (U/L)	315 ± 58	370 ± 56	432 ± 24	630 ± 133
Coagulation studies				
PT (s)	10.2 ± 1.0	15 ± 4.1	10.7 ± 0.4	9.7 ± 0.2
D-dimer (ng/mL)	17 ± 1	293 ± 179	74 ± 50	19 ± 3
Fibrinogen (mg/dL)	146 ± 27	116 ± 13	142 ± 17	200 ± 12

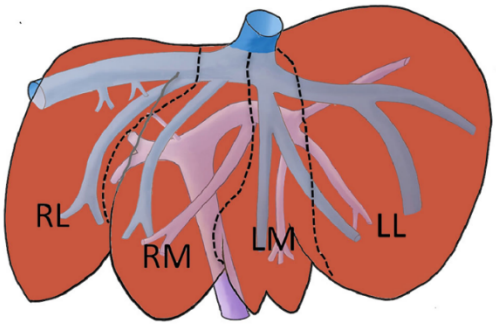
Values are presented as mean ± standard error of the mean. BP, Blood pressure; WBC, white blood cells; Hgb, hemoglobin; Hct, hematocrit; BUN, blood urea nitrogen; AST, aspartate transaminase; ALT, alanine transaminase; PTT, partial thromboplastin time; PT, prothrombin time.

Supplementary Table 4. Histopathologic evaluation of additional host and donor tissues.

Tissue	Summary of pathologic commentary
Host tissue	
Liver parenchyma	Mild sinusoidal lymphocytic inflammation
Kidney	Normal
Lung	Mild interstitial chronic inflammation
Lymph node	Near normal lymph nodes with focal acute inflammation
Spleen	Normal
Thymus	Normal
Donor tissue	
Bile duct	Mild chronic inflammation, focal epithelial denudation
Hepatic artery	Minimal acute inflammation
Portal vein	Normal

SUPPLEMENTARY FIGURES

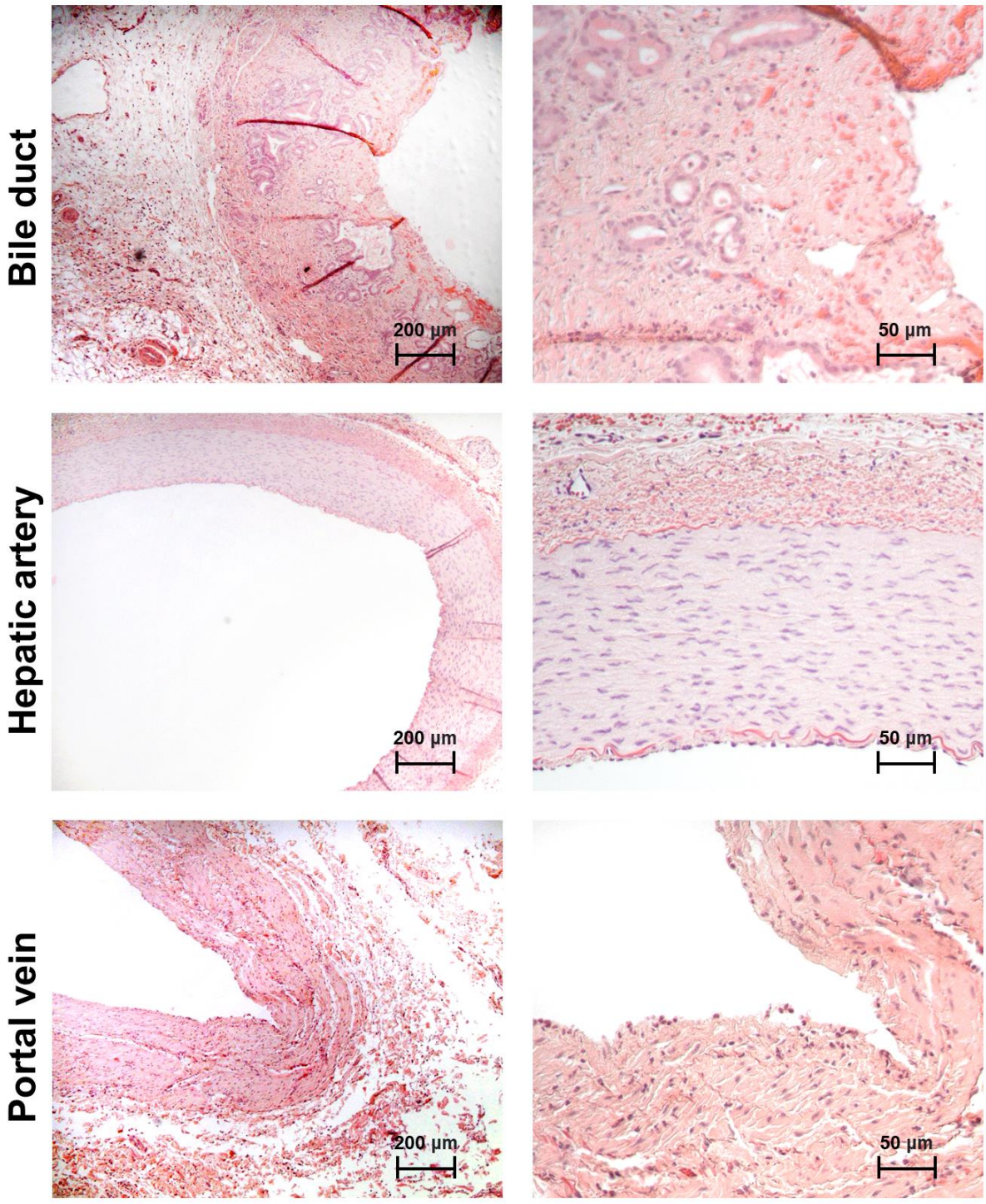
A



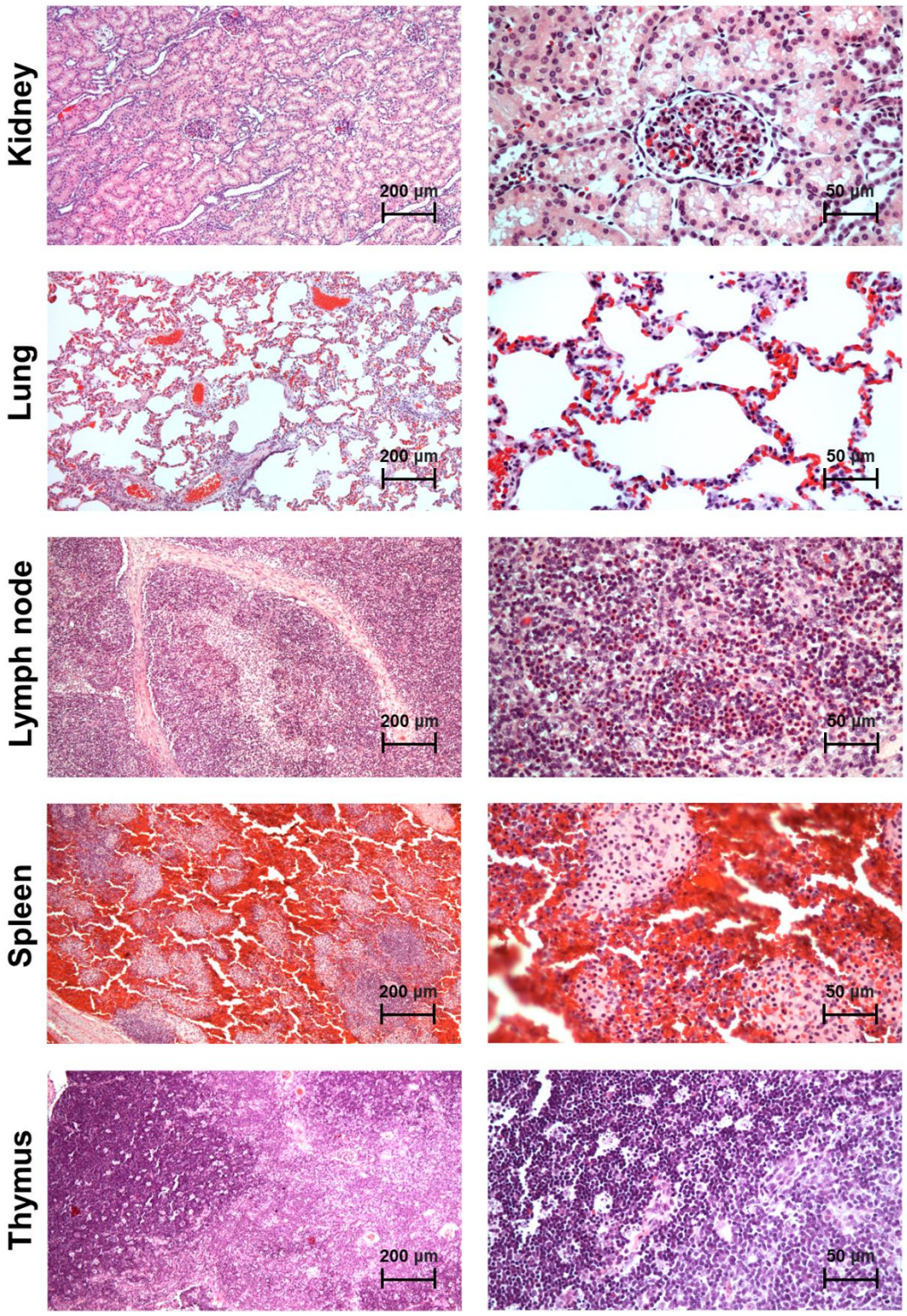
B

Experiment	Tissue sampling location	
	Baseline	12h
1	RL	RL, RM
2	LM	LL, RM
3	LM	LM, RL
4	LL	LM, RL

Supplementary Figure 1. Randomized donor liver tissue sampling. **A,** Lobar divisions used for randomized tissue sampling showing porcine livers divided into 4 major lobes**.** **B,** tissue sample locations at each time point. RL, right lateral lobe; RM, right medial lobe; LL, left lateral lobe; LM left medial lobe.



Supplementary Figure 2. Histology of additional donor tissues. Low- and high-magnification evaluation of donor bile duct, hepatic artery, and portal vein.



Supplementary Figure 3. Histology of host tissues. Low- and high- magnification evaluation of host liver, kidney, lung, lymph node, spleen, and thymus.