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Systemic Inflammatory Response Syndrome in Non-human Primates Culminating in Multiple Organ Failure, Acute Lung Injury, and Disseminated Intravascular Coagulation

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Abstract

The Systemic Inflammatory Response Syndrome (SIRS) is a clinicopathological manifestation of over-exuberant acute phase inflammation caused by infectious or non-infectious etiologies. The systemic release of pro-inflammatory cytokines, chemokines, lipid and vasoactive mediators induces endothelial damage and microvascular thrombosis, potentially culminating in disseminated intravascular coagulation (DIC), acute respiratory distress syndrome (ARDS), and multiple organ dysfunction (MOD) or failure (MOF). We present five cases in the pig-tailed macaque and olive baboon where SIRS resulted in MOF, ARDS, DIC, and the Waterhouse-Friderichsen syndrome; each with gross and histological elements manifested as edema, deposition of fibrin, hemorrhage, and thrombosis. In the described cases, SIRS was the end-common pathway for multiple risk factors which parallel those documented in humans: major surgery, obstetric complications, and infection. The diagnosis of SIRS should be considered when evaluating non-human primate (NHP) cases of MOF manifesting with histological evidence of vascular leakage. Experimental manipulation of NHP models may be complicated by SIRS and accompanying rapid clinical decompensation. Such adverse events may compromise toxicological studies and should be avoided when possible.

Keywords

Primate Pathology; Inflammation; Immunopathology; SIRS (Systemic Inflammatory Response Syndrome); ARDS (Acute Respiratory Distress Syndrome); DIC (Disseminated Intravascular Coagulation); MOF/MOD (Multiple Organ Failure/Multiple Organ Dysfunction)

The systemic inflammatory response syndrome (SIRS) describes a generalized inflammatory process due to numerous and varied causes (Bone, Balk et al. 1992). It is defined clinically

in humans through a combination of two or more of the following conditions: hypo ($<36^{\circ}\text{C}$) or hyperthermia ($>38^{\circ}\text{C}$), tachypnea measured as >20 breaths per minute, $\text{PaCp2} < 32\text{mmHg}$, or alterations in blood gas, elevations in heart rate (>90 beats per minute), and white blood cell counts $>12,000/\text{cu mm}$, $<4,000/\text{cu mm}$, or with $>10\%$ immature forms (left shift). Sepsis is defined in the human literature as the systemic inflammatory response to a known infection; therefore, when a bacterial infection is diagnosed (or a positive blood culture is obtained), SIRS becomes sepsis. (Cavaillon and Annane 2006) SIRS and sepsis lead to predictable pathophysiology through similar pathogenesis, often culminating in decompensation and death. SIRS and sepsis are important clinical entities in humans, associated with a mortality rate ranging from 10-34% (Angus, Linde-Zwirble et al. 2001).

Described risk factors of SIRS include major abdominal injury (most commonly secondary to vehicular trauma), multiple fractures, thermal and chemical burns, pancreatitis, obstetrical surgery, bacterial translocation, sepsis, toxemia, and neoplasia. (Matsuda and Hattori 2006) Although varied, the risk factors for SIRS each have the potential to activate the innate immune system, resulting in a convergence of common sequella. The major effector cells of the innate immune system (monocytes, macrophages, and neutrophils), are triggered through pattern recognition receptors (PRR) which activate signaling pathways and coordinate an inflammatory response. PRR, such as toll-like receptors, recognize pathogen associated molecular patterns of infectious agents, endogenous peptides, and damage associated molecular patterns associated with cell death (Bianchi 2007).

The innate immune system protects the host from infectious organisms in a non-specific manner. While such generalized responses are initially beneficial to the host, they may become deleterious when immune stimulation is excessive or combinatorial. SIRS represents a destructive, unbalanced immune response leading to the systemic release of pro-inflammatory cytokines, chemokines, and vasoactive amines. Circulating mediators damage endothelium and compromise vasculature, triggering the clotting cascade and potentially culminating in disseminated intravascular coagulation (DIC). Tissue injury results from the combination of an over zealous inflammatory response, vascular damage, and compromise to the microcirculation, secondary to microthrombi. Although the inflammatory response is systemic, damage may manifest first, or to a greater degree in individual organs. The lungs are particularly sensitive to septic or SIRS mediated endothelial damage; vascular damage associated within the pulmonary capillaries can lead to acute lung injury (capillary leak syndrome), leakage of albumin and fibrin, alveolar flooding, reduced oxygen exchange. Such damage presents as an acute respiratory distress syndrome (ARDS). In cases which progress to ARDS, mortality can reach 40%, resulting in up to 43,000 annual patient deaths. (Frevert and Martin 2004; Hajjar, Harowicz et al. 2005; Rubenfeld, Caldwell et al. 2005) As tissue damage progresses, multiple organ dysfunction (MOD), and further, multiple organ failure (MOF) may ensue. MOF, MOD, and ARDS are common clinical sequella to SIRS.

In a toxicological study, infectious disease is considered an experimental accident and a serious determinant error, compromising the experiment (Keenan 1996). SIRS and MOF, as adverse events, may occur in drug trials secondary to an infectious event (sepsis), or administration of experimental compound which is an immunomodulant/stimulant. Such adverse events must be assessed to determine relatedness to the experimental compound.

Occurrences of SIRS and MOF in non-human primates are rarely documented in the literature; the following case report details five such adverse events, giving background as to cause and experimental manipulation. It is our intention to shed light on possible etiologies of such adverse events. This manuscript describes 5 cases of SIRS in two species of NHP's (Table 1). Each case is due to distinct experimental etiologies related to 5 separate experimental protocols approved by the University of Washington's Institutional Animal Care and Use Committee. Although multiple different risk factors trigger the innate immune system, the resultant lesions overlap to a great extent.

Case 1

A 3 month post-partum, 4.5 year old female *Macaca nemestrina* underwent experimental vascular catheterization and placement of a stomach tube (transcutaneous). On two separate occasions, the gastric/epidermal seal of the gastric tube ulcerated requiring replacement tubes (45 and 62 days prior to euthanasia). Placement of a third tube (6 days prior to euthanasia) resulted in penetration of the esophagus due to alteration of gastric anatomy secondary to abdominal fibrous adhesions. Post-operative blood chemistry (24 hours post final tube placement) demonstrated a white blood cell [WBC] count of 15,000 $10^3/\text{ul}$, predominantly composed of neutrophils 11,000 $10^3/\text{ul}$. Over the subsequent days, the animal developed fibrinous pleuritis, pericarditis, serositis, and a diffuse vascular leak syndrome leading to anasarca. On day 6 post-op, the animal developed increasing respiratory distress. Chest radiographs demonstrated collapse of the left caudal lung lobes with pleural effusion. A urinalysis demonstrated moderate levels of urine protein and glucose; clinicopathological monitoring demonstrated hypoalbuminemia (1.8 g/dL) and an elevated gamma-glutamyl transferase [GGT] (122 U/L). During clinical evaluation, the animal became hypothermic and dyspneic; euthanasia was elected. Gross lesions included dependent pitting edema, pleural, pericardial, and thoracic effusions. Effusion fluids were turbid and contained protein (500mg/dL), free erythrocytes (250 Ery/ul), and glucose (100 mg/dl). Histologically, acute lung injury consistent with ARDS was evident (Figure 1: C). Histology of the adrenal glands (Figure 1: G and H) demonstrated frank hemorrhage and necrosis consistent with Waterhouse-Friedrickson syndrome (WHF) and sepsis (Cary, Kosanke et al. 2001; Adem, Montgomery et al. 2005). Sepsis secondary to translocation of enteric flora is suspected, however, cultures were not performed in this case as the animal had been administered antimicrobials (Entrofloxacin, Cephalexin, and Cefazolin) postoperatively.

Case2

A 7.7 year old female *Macaca nemestrina* on a drug safety study underwent vascular catheterization, caesarian section, gastric catheter placement, jejunal resection, and hepatic lobe resection three days prior to necropsy. Clinicopathological monitoring was performed 24 hours post-op, and again on the day of necropsy (72 hours post-op). At 24 hours post-op, the animal was anemic (hematocrit [HCT] of 25 % with a hemoglobin [Hb] of 7.6 g/dL), hypoalbuminemic (1.4 g/dL), diffusely edematous, and demonstrated elevated liver enzymes (alkaline phosphatase [Alk] 224 U/L, serum glutamic oxaloacetic transaminase [SGOT] 164 U/L, serum glutamic pyruvic transaminase [SGPT] 123 U/L, and GGT 55 U/L); the animal was transfused with 100ml of whole blood. Forty-eight hours later, on the day of necropsy,

edema had progressed to anasarca; clinicopathological monitoring demonstrated elevated hepatic enzymes (Alk 285 U/L, SGOT 294 U/L, SGPT 246 U/L, GGT 55 U/L), azotemia (blood urea nitrogen [BUN] 6mg/dL, and creatinine [Crt] 1.9mg/dL), an elevated erythrocyte sedimentation rate (27 mm/hr), hypoproteinemia (4.0 g/dL), hypoalbuminemia (<1.0 g/dL), and a WBC count of 13,000 10^3 /ul with 10,400 neutrophils 10^3 /ul. Despite supportive veterinary care, the animal became hypothermic (94.9° F) and lethargic with a loss of distal limb pulses and deep pain response; euthanasia was elected. Grossly, tissues were edematous; there was approximately 100ml of ascitic fluid within the abdomen. Multiple organs demonstrated gross hemorrhage including the liver, kidneys, gastrointestinal tract, and adrenal glands. The lungs were atelectic. Histology confirmed multiple organ hemorrhage and necrosis involving the liver (hepatic necrosis with disassociation of hepatic chords), kidney (glomerular capillary microthrombosis consistent with DIC, Figure 1: E and F), gastrointestinal (with concurrent vascular infarction), and adrenal glands (WHF). At necropsy, abdominal ascitic fluid, liver, spleen, and uterus were cultured; a heavy growth of *Escherichia coli* was isolated from all samples despite post-operative prophylactic administration of Cefazolin.

Case 3

A 4.7 year old female *Macaca nemestrina* underwent experimental total body irradiation and bone marrow reconstitution. Eleven months post irradiation and bone marrow engraftment, the animal was treated with 06-benzylguanine (BCNU), a chemotherapeutic agent to select for labeled engrafted cells. Despite supportive therapy, over the subsequent 72 days, the animal developed thrombocytopenia (decreased from 300 10^3 /ul pre-BCNU, to 310³/ul), anemia (HCT decreased from 36 % pre-BCNU, to 16%, and Hb decreased from 11.1 g/dL to 5.1 g/dL), and renal failure (BUN elevation from 19 mg/dL pre-BCNU to a maximum of 74 mg/dL post-BCNU, and creatinine elevation from 0.91 mg/dL pre-BCNU to 2.0 mg/dL post-BCNU). A urinalysis performed 56 days post-BCNU demonstrated a large amount of occult blood, trace protein, and a specific gravity of 1.003, confirming clinical renal failure. The animal was given palliative care including blood transfusions, fluid therapy, and prophylactic antibiotics and antifungal drugs. At 72 days post BCNU treatment, euthanasia was elected. Grossly, the lungs, gastrointestinal tract, and kidneys contained multifocal regions of parenchymal hemorrhage. Histological renal glomerular vasculature microthrombosis, consistent with DIC, correlated with clinical renal failure. Acute lung injury with alveolar proteinosis and hyaline membrane formation correlated with clinical ARDS (Figure 1: A and B). Blood and tissue cultures were not performed due to prophylactic administration of antibiotics and antifungal drugs.

Case 4

A 3.1 year old male *Papio cynocephalus* underwent experimental aorto-iliac engraftment. Seventy-two hours post engraftment, circulation was compromised as evidenced by cool, immobile hind limbs, lameness, and a markedly reduced femoral pulse. The graft was surgically repaired; an aorto-iliac saddle thrombus was removed. Upon recovery from the surgical repair, the femoral pulses were noted to be weak despite fluid therapy. The following morning, approximately 84 hours post experimental engraftment and

approximately 12 hours post surgical repair, the animal was found dead. Grossly, there was a mild to moderate amount of subcutaneous, mesocolic, and retroperitoneal hemorrhage. The lungs were diffusely edematous and emphysematous with lobar hemorrhage and consolidation. Histologically, there was subacute, suppurative, fibrinohemorrhagic vasculitis within the aorta and iliac arteries adjacent to the engrafted region. The right atrium contained a peracute thrombus. Acute lung injury, consistent with ARDS, was evidenced by alveolar flooding, proteinosis, and hyaline membrane formation (Figure 1: D). Cultures were not obtained as the post-mortem window was unknown.

Case 5

A 2.1 year old male *Papio cynocephalus* underwent total body irradiation and was reconstituted 48 hours later with partially mismatched (mother/offspring) bone marrow. To ameliorate the potential graft immune response, graft-versus-host disease (GVHD), a CD28 antibody was administered to the host in conjunction with the bone marrow graft. Over the subsequent 10 days, the animal developed elevated liver enzymes (Alk elevation from 910 U/L to 6869 U/L, GGT elevation from 39 U/L to 175 U/L, SGPT elevation from 52 U/L to 316 U/L, and total bilirubin elevation from 0.3 mg/dL to 7.8 mg/dL), azotemia (BUN elevation from 9 mg/dL to 81 mg/dL and Cr_t elevation from 0.5 mg/dL to 3.2 mg/dL), thrombocytopenia (decrease from 369 10³/ul to 1.0 10³/ul), hypoproteinemia (decrease from 5.4 g/dL to 3.4 g/dL), hypoalbuminemia (decrease from 2.9 g/dL to 1.2 g/dL), and leukopenia (WBC decrease from 7.3 10³/ul to 1.4 10³/ul), as evidenced through clinicopathological monitoring. As the animal developed progressive edema and anasarca in combination with hepatic and renal failure; euthanasia was elected 11 days post bone marrow administration. Grossly, the animal was cachectic with subcutaneous and tissue edema, thoracic, pericardial, and abdominal effusions. There was hemorrhage and edema of multiple organs including the adrenal glands, pancreas, thyroid, epidermis, gastrointestinal tract, reproductive tract, central lymph nodes, and kidneys. There was histological evidence of diffuse vasculitis and multiple organ necrosis involving the liver (hepatocellular degeneration), kidney (diffuse tubular necrosis), and CNS (cerebral edema and hemorrhage). Additionally, acute lung injury was evidenced by alveolar flooding, hemorrhage, and accumulation of fibrin. GVHD was evidenced by lymphohistiocytic inflammation of the liver, vasculature, colon, and skin (in combination with epidermal necrosis). Blood and tissue cultures were not performed due to experimental post-irradiation prophylactic administration of antibiotics and antifungal drugs.

Discussion

The innate immune response is an evolutionarily conserved first line of defense against pathogens. Conservation of genetic code across species dictates a similar host response to various stimuli. Triggering innate immunity results in clinicopathological outcomes which overlap to a great extent due to the limited repertoire of available host responses. For example, in humans and the two NHP species examined, the triggers of SIRS are varied and not directly associated with a specific outcome, yet culminate in similar end-state pathophysiology including vascular leakage, DIC, and multiple organ dysfunction.

NHP's represent the best animal model of many human diseases due to their close phylogenetic linkage. In the described cases, NHP's model the triggers, clinicopathology, and possible outcomes of SIRS. Case 1 demonstrates the combinatorial effect of surgical complications and trauma which culminated in SIRS and systemic vascular leakage/edema. Placement of multiple gastric catheters likely led to introduction of bacteria into the peritoneal and thoracic cavities. Case 2 demonstrates the combinatorial effect of obstetrical surgery, abdominal surgical complications, and sepsis resulting in MOF. Hepatic necrosis occurring in this case was likely secondary to SIRS, however, the contribution of experimental compounds and repeated hepatic surgery is unknown. Case 3 demonstrates SIRS presenting as ARDS and DIC with elective euthanasia occurring prior to overt MOF. Case 4 demonstrates the role that vascular surgery and ischemia/reperfusion injury play in the development of SIRS and acute lung injury (Sayers 2002, (Reviewed in Matute-Bello, 2008)). In this case, the development of acute lung injury most likely resulted from reperfusion of ischemic tissue in the lower limbs following surgical treatment to remove an aorto-iliac saddle thrombus. Ischemia/reperfusion injury is often associated with activation of endothelial cells, increased oxygen radicals, release of inflammatory mediators (e.g. TNF α and complement activation), leukocyte activation, and vascular leak (Carden 2000). The development of SIRS and lung injury occurs in people and in rodents following the cross-clamping of the abdominal aorta (Sayers 2002). The findings in this case suggest animals should be closely monitored for development of SIRS and multi-organ failure when ischemia-reperfusion injury is suspected.

Case 5 demonstrates SIRS and MOF due to a host immune response to a foreign antigen. GVHD is caused by donor T cells reacting to host alloantigens; which, in this case, led to severe immunosuppression. Similar to Case 1, antibiotic therapy in cases 3 and 5 precluded cultures; however, in the latter two cases, bone marrow failure would predispose to opportunistic bacterial infections.

Hyaline membranes were not a consistent finding in the lungs of the described cases. Alveolar proteinosis, with marked accumulation of fibrin and hemorrhage was found in each of the 4 cases in which pulmonary pathology was noted. The pathophysiological progression of alveolar hyaline to hyaline membranes requires time and is aided by positive pressure ventilation. As the described cases were euthanized at the onset of clinical signs, or succumbed early in disease, there may have been insufficient time for the development of "classic" hyaline membranes associated with ARDS in man.

The final outcome of an insult is determined by host immunity, health, and genetics. The immune response is to some degree a genetic phenotype which predisposes certain animals to succumb to a similar insult other animals may recover from (Figure B). Similar to humans (Wurfel, Park et al. 2005), NHP populations have been shown to contain high and low immune responders; small subsets of animals will produce up to 100 fold greater cytokines (Interleukins 1b, 6, 8 [IL-1 β , IL-6, IL-8] and tumor necrosis factor-alpha [TNF- α]) in response to infectious stimuli. High responders may be more susceptible to developing SIRS when exposed to a given risk factor than would an animal with a less vigorous cytokine response.

When an animal presents with clinical signs of SIRS or sepsis, rapid clinical evaluation must be made to determine the progression of disease. In advanced cases, timely euthanasia should be considered. In early cases, before advanced clinical signs, the diagnostic work-up should include a blood chemistry panel (including fibrin split products), a coagulation panel, CBC, blood gases, blood culture, chest radiographs, blood pressure, heart rate and respiratory rate. In cases of pulmonary distress, sedation may not be indicated, thereby reducing diagnostic tests available in animals which can not be manipulated awake. For research purposes, further diagnostic work-up may include running a cytokine panel to determine elevation of key mediators such as IL-1 β , IL-6, IL-8, and TNF- α . Further, comparison of the immune response of animals succumbing to SIRS to those surviving similar insults is necessary to delineate the impact of host genetics and immune response to development of SIRS.

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Abbreviations

Alk	alkaline phosphatase
ARDS	acute respiratory distress syndrome
BCNU	06-benzylguanine
BUN	blood urea nitrogen
Crt	creatinine
DIC	disseminated intravascular coagulation
GGT	gamma-glutamyl transferase
GVHD	graft-versus-host disease
Hb	hemoglobin
HCT	hematocrit
IL-1β	Interleukin 1 beta
IL-6	interleukin 6
IL-8	interleukin 8
MOF	multiple organ failure
NHP	non-human primate
NHPs	non-human primates
PRR	pattern recognition receptor
SGOT	serum glutamic oxaloacetic transaminase
SGPT	serum glutamic pyruvic transaminase
SIRS	systemic inflammatory response syndrome
TLR	toll-like receptor
TNF-α	tumor necrosis factor
WBC	white blood cell
WHF	Waterhouse-Friderichsen syndrome

Figure 1.

Figure 2.

Table 1
Summary of the six NHP cases describing signalment, etiology of SIRS, and organs affected.

Case	Animal			Suspected Risk Factors	Organs Affected				
	Age	Sex	Species		Lung	Liver	Kidney	Vasculature	Adrenal
1	7.7yr	Female	Mn ^a	Surgical trauma, vascular surgery, sepsis suspected	ARDS	N/A	N/A	Vascular leak Vasculitis	WHF
2	4.8yr	Female	Mn	Obstetrical, abdominal, vascular surgery, Sepsis confirmed	N/A	Necrosis	DIC	Vascular leak Vasculitis Thrombosis	WHF
3	4.5yr	Female	Mn	TBI ^c with failed engraftment, BCNU ^d toxicity	ARDS	N/A	Renal Failure, DIC	Thrombosis	N/A
4	3.1yr	Male	Pc ^b	Vascular surgery, reperfusion injury	ARDS	N/A	N/A	Vasculitis Thrombosis	N/A
5	2.1yr	Male	Pc	TBI with failed engraftment, GVHD ^e	ARDS	Necrosis	Necrosis	Vascular leak Vasculitis, cerebral edema	N/A

^aMn- *Macaca nemestrina*
^bPc- *Papio cynocephalus*
^cTBI- Total body irradiation
^dBCNU-06-benzylguanine chemotherapeutic
^eGVHD- Graft-versus-host disease