Contusions, Blood Leakage, and the Immune Response

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Contusion to organs, in the absence of infectious agents, is a rare example of “sterile damage.” This sets up an inflammatory response that is similar but different to the response to pathogens. From there, there must be a healing response to remodel and repair the wound site. This is a vital but somewhat neglected field of immunology. Concussions and spinal cord injuries are becoming highly visible to the public due to the popularity of sports like professional American football, snowboarding, and mixed martial arts. Recent studies on Damage Associated Molecular Patterns (DAMPs) have brought much needed immunological interest in the subject. The immunological aspects of wound healing are medically important.

All organs can have traumatic but sterile contusion injuries. Most models of contusion use an engineered mechanical impactor to strike the target organ, for example a mechanical impactor to simulate traumatic brain [1], spinal cord [2], lung [3], skeletal muscle [4,5] injuries in anesthetized mice or rats. Similar systems have been used in larger animals, such as miniature pigs [6], rabbits [7], and monkeys [8]. Evaluation in human subjects remains difficult. Most basic physiological studies rely traditionally on epidemiological surveys [9] or observations of select populations such as children [10] or occupational risk in adults [11]. Advanced non-invasive imaging techniques [12,13] or sonography [14,15] may make understanding of the situation in human physiology more feasible. Further, modeling the dynamics of fluids, like blood pools, is being developed at the nexus of math and biomedical engineering [16].

There have been many recent studies looking at the cells of the innate immune response after contusion, such as macrophages [17,18] and neutrophils [19,20]. Mitochondrial derived DAMPs can also activate Y6 T cells [21]. In general, these responses are organ specific, but resident macrophages, followed by the influx of monocytes, neutrophils, and T lymphocytes all appear to play critical roles during the acute damage and repair/fibrosis phases. Of particular interest are the different activation phenotypes of T lymphocytes and macrophages. Surprisingly, in the CNS, one study found beneficial contributions to healing from the adaptive arm such as T Helper 1 lymphocytes [22]. Another found detrimental contributions by regulatory T lymphocytes [23]. One might have predicted the opposite, given the normal roles played by these cell types. Do certain subtypes of M1/M2a/M2b/M2c influence this process in different ways and in different organs [24]? For example, the study by Kumar, et al., suggests that M1 activation of microglial cells is detrimental to healing in CNS [17].

DAMPs are molecules released by damaged cells and organs. There is a growing list of DAMPs that includes the family of S100 proteins found in neutrophil, eosinophil, and mast cell granules, defensins, and components of extracellular matrix (fibronectin, hyaluronate, and heparin sulfate proteoglycans). DAMPs are recognized by members of the Toll-like receptor family, in particular TLRs 1-4 and TLRs 7-9 [25-28]. Other receptors include the purinogenic receptors P2Ys 1, 2, 4, 6, 11-14, and P2Xs 1-7. For further reading, we recommend an excellent recent review of DAMPs and their receptors in the airway by Van Crombruggen et al. [28].

In addition to damage to organ-specific cells, there is localized vasculature rupture. Components of blood also act as DAMPs. The immune response to repair the damaged vasculature, organ architecture, and removal of the dotted blood and dead cells can be quite complex in different tissues. Additionally, fibrinolysis activates many different cell types. Vascular endothelial cells produce tissue plasminogen activator (tPA) and many organ specific cells produce urokinase-like plasminogen activator (uPA) found in plasma. tPA and uPA activate plasmin to degrade fibrin into fibrin degradation products (FDPs) [29,30]. Fibrinogen, fibrin, and potentially, FDPs, can bind to TLR2 and TLR4 [30,31], as well as VLDL-R [32], VE-cadherin [33], and the integrins Mac-1 [34] and αvβ3 [35].

Red blood cells and hemoglobin can also be considered DAMPs. After blood vessel rupture, the red blood cells exit, die, and release their hemoglobin. Macrophages phagocytose and degrade the hemoglobin into hemosiderin and billiverdin [36,37]. Extravasated red blood cells and iron in the microenvironment can induce M2 phenotype in macrophages [38]. Hemosiderin is an exclusively intercellular iron-storage complex that consists of ferritin, denatured ferritin, and additional components. It is found in macrophages in areas of hemorrhage [36,37]. Unlike pro-inflammatory M1 macrophages that take up iron slowly and sequester it to make it unavailable to pathogens, alternatively activated M2 macrophages upregulate scavenger receptors (CD163). CD163 receptors allow for rapid iron uptake, and they

enable M2 macrophages to express a ferritin(hi)/ferroportin(lo) phenotype that enables a robust iron donation to the immediate microenvironment [38]. M2 phenotype is also known to secrete anti-inflammatory mediators through the heme-oxygenase-dependent heme catabolism, and is suspected of donating iron to fibroblasts for collagen synthesis, thus contributing further to collagen deposition and fibrosis [38].

Many contusion studies look at the acute phase of the response to contusion, within hours or a few days. However, there is some evidence that chronic sterile damage can lead to fibrosis in tissues such as muscle [39], spinal cord [40], heart [41], and lung [manuscript submitted]. In the lung, components of the procoagulant and anti-coagulant pathways, such as Tissue Factor upregulation, decreased anticoagulant Protein C, and thrombin generation can lead to fibrosis when chronically activated [42]. It is currently thought that pulmonary clot formation and activation of protease activated receptors PAR1 and PAR2 are early events that leads to a fibrotic cascade. Pulmonary microhemorrhages quickly lead to interstitial and alveolar fibrin deposition. Although in vitro studies have shown that the endothelium maintains an anti-thrombotic and pro-fibrinolytic profile, in vivo studies indicate that the situation is more complex, and varies with the activation status and concentration of active receptors and cytokine concentrations [43]. It is possible that repetitive chronic injury could dysregulate the balance towards procoagulant and anti-fibrinolytic state, where repetitive post-hemorrhage fibrin deposition could feed into PAR activation and Transforming Growth Factor β pro-repair activity. Alveolar/interstitial fibrin deposition contributes to provisional matrix formation which gets replaced by collagen in as little as 7 days [44].

In summary, signals from DAMPs and blood components, in the absence of infectious agents, lead to a variation of the “inflammatory” programming to control wound repair and remodeling. It is just now being understood how different subtypes of cells, such as M1 and M2 macrophages, influence this process. Further, the growing number of DAMPs should be further expanded by including the blood components, like fibrin and red blood cells, which are clearly a part of a contusion process. Finally, determining how acute damage is controlled fibrin and red blood cells, which are clearly a part of a contusion process. Further, the growing number of DAMPs should enable M2 macrophages to express a ferritin(hi)/ferroportin(lo) phenotype that enables a robust iron donation to the immediate microenvironment [38].

ACKNOWLEDGMENTS

The authors would like to thanks Jamie S. Schenkel for critical reading of the manuscript.

REFERENCES

regulatory T cells suppress the ability to withstand injury to the central nervous system. Proc Natl Acad Sci U S A. 2002; 99: 15620-15625.


