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Phosphatidylserine's role in Ebola's inflammatory cytokine storm and hemorrhagic consumptive coagulopathy and the therapeutic potential of annexin V

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ABSTRACT

The phosphatidylserine (PS) molecule is present in cell membranes where it is actively kept on their inner leaflets but when cells are damaged it moves to the surface and become a signal for their removal, the platform upon which the coagulation cascade takes place and a ligand that activates a feedback cycle of inflammatory cytokine secretion and initiates the wakeup call for the innate immune response. These are physiologic responses to PS but the Ebola virus displays PS molecules on its membrane's surface and the huge numbers of viruses cause a pathologic inflammatory cytokine storm and a hemorrhagic consumptive coagulopathy. Annexin V is an innate molecule that can cloak membrane displayed PS and prevents its Th1 cell's inflammatory cytokine generation and cascade thrombin generation. The hypothesis presented is that its administration will cloak PS and prevent Ebola's consumptive coagulopathy and its cytokine storm.

Introduction/background

Phosphatidylserine (PS) molecules are present in cell membranes where they are actively kept on their inner leaflets. In senescent and otherwise damaged cells PS moves to their surface where it becomes a signal for their phagocytic removal [1], the platform upon which the coagulation cascade takes place [2], a ligand for the TIM-1 receptor on T helper one (Th1) cells that activates its inflammatory cytokine secretion [3] and a ligand that binds to TIM receptors on mononuclear immune cells to provide the wakeup call for the innate immune response [4]. Billions of cells become apoptotic daily and display PS on their surface but they are rapidly removed by phagocytes that recognize it [5] and the Th1inflammation [3], cascade generated blood coagulation [2] and the innate immune activation do not take place [4]. In Ebola PS on the surface of its virus activates Th1 inflammatory cytokine secretion and the PS exposure is so great that a cytokine storm is produced [3] and all the thrombin generated on the cascade's PS platforms generates enough thrombin to consume coagulation components and produce a hemorrhagic consumptive coagulopathy [2]. Macrophages and dendritic cells are phagocytes that secrete inflammatory cytokines when TLRs on their surface recognize foreign or damage associated molecular patterns but they also display TIM-1 receptors and in Ebola those receptors bind to and phagocytize the PS+ viruses and they become infected [6]. When infected these cells are prime viral replicators that generate viruses to infect other cells. In addition, the infected dendritic cells may no longer be able to MHC present antigenic pathogen peptides to immune cells in the adaptive immune response producing immunologic depression. In 2017 it was discovered that PS on the Ebola virus could bind to the TIM-1 receptor on Th1 cells and independently produce a lethal cytokine storm in mice [3]. This was proven when an Ebola infection in knockout TIM-1$^{-/-}$negative did not produce a cytokine storm and the mice survived even though the viral load was only minimally affected [3]. The hypothesis presented here is that annexin V's therapeutic administration in Ebola can prevent its Th1 cell generated inflammatory cytokine storm, stop the cascade generated hemorrhagic consumptive coagulopathy and prevent macrophage and dendritic cell infection. Unfortunately when annexin V cloaks PS the cascade can't function and hemostasis will not be possible so let us very briefly examine the components of inflammation, blood coagulation and annexin V with regard to the possibility that its administration can prevent Ebola's cytokine storm and coagulopathy without causing bleeding.

Inflammation

Inflammation begins when pathogen or damage associated molecular patterns are recognized by TLRs on macrophages and dendritic cells and cause their inflammatory cytokine secretion and it is

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continued in a late mediated inflammatory response when some of the cells damaged by those cytokines become necrotic causing high mobility group box one (HMGB1) molecules release by nuclear disruption. The HMGB1 molecules then bind to the TLR4 receptors on dendritic cells and cause their inflammatory cytokine secretion [7]. The PS exposed on the surface of cells damaged by the innate and late mediated inflammatory responses binds to the TIM-1 receptor on Th1 cells and they begin a feedback cycle of inflammatory cytokine secretion that expose more PS and amplifies the inflammation [3]. This amplified inflammation damages and kills both pathogens and healthy cells and the PS displayed on the latter activate the innate immune response when an infection is present and make hemostasis possible when a vascular wall is breached. This physiologic PS feedback generation contrast with the pathologic cytokine storm and hemorrhagic consumptive coagulopathy in Ebola where membrane displayed PS is on every virus.

Blood coagulation

For blood coagulation to begin thrombin must be present and its generation begins when cells are damaged and the procoagulant molecules tissue factor (TF) and PS are exposed. In Ebola the inflammatory storm damages many cells and the large numbers of TF and PS molecules exposed cause intravascular blood coagulation that consumes coagulation components and leads to a hemorrhagic consumptive coagulopathy. TF initiates blood coagulation but PS is the platform upon which the coagulation cascade's thrombin generation produces the consumptive coagulopathy. In Ebola PS is not only exposed by inflammatory cell damage it is also on every virus.

TF begins blood coagulation by activating factors X and IX. Activated factor Xa changes prothrombin to thrombin and factor IXa is an essential component of the coagulation cascade. TF generated thrombin directly or indirectly activates all coagulation components including the coagulation cascade. Once activated the cascade generates thrombin in a feedback cycle of factor X activation in its tenase complex and by a catalytic generation of thrombin in its prothrombinase complex [8]. In the tenase complex activated factor VIIIa and IXa bind to membrane displayed PS and are joined there by factor X to be activated [9]. The activated factor Xa then joins factor Va on PS in the prothrombinase complex and the factor Xa, factor Va and PS amalgam catalytically begins changing prothrombin to thrombin and exponentially increases its generation making hemostasis possible [10]. In hemophilia factors VIII and/or IX are not available and so the cascade can't function and hemostasis is not possible even though TF thrombin generation is not affected [11].

Factor IXa is required for cascade thrombin generation but TF can't supply enough of it for cascade function so cascade generated thrombin activates factor XI and it activates factor IX. This increases thrombin generation but not exponentially. This is a good thing because though fibrin generation is desirable during cellulitis intravascular blood coagulation is not. For the cascade's exponential thrombin generation to take place factor XII must activate factor XI so it can activate factor IX and to do this factor XII must be activated by binding to sulfatide on activated platelets [12]. This is not possible intravascularly because activated platelets secrete a factor XII activation inhibitor [13]. It is possible when a vascular breach is present and collagen is exposed because platelets displaying sulfatide and PS on their surface bind to the collagen and the blood flow washes away the inhibitor. In a vascular breach TF and PS are only exposed at the breach site and factor IX is activated there by factor XIIa making the cascade's exponential increase in thrombin generation possible. The same thing happens when an atherosclerotic plaque ruptures and collagen is exposed.

The physiologic feedback cycles of PS exposure that cause inflammation and thrombin generation are needed to assure a rapid innate immune response to an infection and to rapidly control bleeding when a vascular wall is breached but such cycles, like atomic chain reactions, are dangerous and require controls and it is proposed that annexin V, like factor IXa is one of them. The following 2016 study by Park supports the hypothesis that annexin V can block cytokine storms and consumptive coagulopathies [14]. In this study gram negative septicemia was induced in mice where the lipopolysaccharide (LPS) molecule on those bacteria generated a lethal cytokine storm and generated excess fibrin (not a coagulopathy). LPS generates thrombin by binding to TLR4 on dendritic cells. It was found that a single injection of annexin V stopped the cytokine storm, reduced fibrin generation and enabled the mice survival [14]. The annexin V did this by cloaking PS on damaged cells and by blocking the binding of LPS to TLR4. Let us now take a brief look at annexin V.

Annexin V

Annexin V is an innate molecule found in cells and in nano molecular amounts in plasma where its levels increase during inflammation and blood coagulation [15]. What it does in cells and how it gets from there to plasma is uncertain [16]. It is a small protein molecule that is rapidly removed by kidneys causing it to have a half-life of minutes in plasma [17]. It has a great affinity for PS and because of this its labeled molecules are used in the lab and clinically for its identification but it has no therapeutic application. When annexin V binds to PS on a cell's membrane it crystalizes and this forms a two dimensional PS cloaking shield that can internalize it into the cells cytoplasm [16]. When annexin V cloaks PS the Th1 cell's inflammatory cytokine secretion that causes Ebola's cytokine storm [3] and the cascade thrombin generation that produces its hemorrhagic consumptive coagulopathy [2] can't take place. This will also prevent hemostasis. Will this prevent its therapeutic use?

Therapeutic annexin V

Using annexin V to treat the pathologic aspects of inflammation and blood coagulation will only be possible if its therapeutic benefits are rapid and long lasting, its pathologic ones transitory and the physiologic recovery from its PS cloaking is rapid. All seem possible. When annexin V cloaks exposed PS this will instantly and permanently prevent those PS's ability to activate the Th1 cell's inflammatory cytokine secretion [16] and the blood coagulation that PS makes possible [2,14]. Annexin V's half-life of minutes [17] means it will be rapidly eliminated so that PS exposed by a subsequent vascular breach will be met by breach exposed TF generating thrombin and by PS and sulfatide on activated platelets adhering to the collagen. Regarding annexin V's blocking of the innate immune cell activation [4], annexin V will only be given during an infection so that the innate immune response will already have been initiated.

The ability to monitor the effect of annexin V administration would be desirable and since radio and florescent labeled annexin V molecules are used in the lab and clinically to detect PS+ cells it is possible that they can be used to quantify the PS+ cells that produce the inflammation and blood coagulation.

Could conditions other than Ebola be considered for annexin V treatment? The same inflammatory and coagulation pathology is present in severe septicemia as is present in Ebola and as in Ebola it has little effective treatment, annexin V may be effective there. Coronavirus SARS and severe influenza infections are other lethal conditions to consider for annexin V therapy. In them their lethal respiratory failure may be the result of the inflammation and blood coagulation caused by the PS exposure on infected alveolar and bronchiolar cells resulting in the consolidation of the air space by the massive infiltration of mixed mononuclear/neutrophilic cells, edema and fibrin deposits [18].

The administration of annexin V in Ebola and in other critically ill patients should probably be by intravenous bolus so that its cloaking of PS will instantly stop further Th1 cell feedback secretion of inflammatory cytokines and coagulation cascade thrombin generation

that is causing the inflammatory organ damage and the thrombotic coagulopathy. In Ebola its bolus administration may also prevent further macrophage and dendritic cell infection. In non-critical SARS and influenza infections monitoring the levels of PS+ cells could be used to determine the necessity for annexin V use and when it is needed the PS + cell count can be used to monitor its continuous intravenous administration.

In conclusion

Annexin V's ability to cloak PS and block its roles in inflammation and blood coagulation is well documented but the therapeutic possibilities presented are speculative and require animal studies for their verification. At present there is little or no effective treatment for these lethal conditions so those studies would seem to be indicated.

Declaration of Competing Interest

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Appendix A. Supplementary data

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