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IMMUNOTHROMBOSIS

FROM ANTI-PATHOGEN TO ANTI-HUMAN

VLIEGEN, C.N.C. (COEN)
6129919
Supervisor: Dr. Coen Maas

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Layman's Summary

The human body is constantly being attacked by invading bacteria and viruses. To prevent these intruders from harming the body, it has developed an elaborate defence mechanism. Different immune cells are programmed to kill these invaders, but also the blood system helps in the form of blood clotting. A downside to this help from the blood is that the formation of the clots can also lead to complications like lung embolisms. This clotting process during an overreaction of the immune system is called immunothrombosis.

Both the blood clotting mechanism and immune system evolved from primitive cells called hemocytes. These cells were able to regulate the blood clot formation and the killing of intruders. Over time, however, pathogens developed and therefore this defence mechanism had to as well. Different subsets from of the hemocytes were developed, resulting ultimately in the coagulation system and immune system as we know it today. Because these systems have the same ancestor there are still certain links between these systems, explaining the possible immunothrombotic outcome after infections. It is almost as if the coagulation system and immune system have developed too far as it now results in complications.

Furthermore, there are pathogens that are able to circumvent the immune system and coagulation system or actively use these mechanisms to be more infective. This could maybe help form a solution for possible immunothrombotic events after other infections.

It is almost impossible to look at the blood coagulation system and immune system separately as they are intertwined with each other. So, by completely understanding how these two systems are linked to each other by looking at the evolution, it could help determine potential treatments for preventing certain events, like lung embolisms, from happening.

Introduction

The human body has developed multiple ways to fight and defend itself against a variety of pathogens. One of these ways is the use of thrombosis. This process is used to form blood clots and prevent the spread of pathogens while recruiting immune cells as well. This system has for example been seen in arthropods like the horseshoe crab. The findings in the horseshoe crab, but also in other earlier organisms, indicate a form of primitive blood clotting with its main objective to trap and eventually lead to the removal of invading pathogens. This system probably has evolved over the years into the blood clotting system we now know in humans. However, the big difference of this blood clotting process is that its primary function has changed from protection of pathogens to the healing of wounds, although it still activates the immune system as a secondary function.

In humans the thrombus formation normally occurs as a result to different reasons such as a small trauma or rupturing atherosclerotic plaques. Through contact activation via clotting factor XII or the release of Tissue Factor (TF), the blood coagulation system is activated, resulting in the formation of a fibrin network in which blood cells, leukocytes and even bacteria are trapped while forming a clot. This clot closes the blood vessel wall preventing further blood loss.

The blood coagulation system is tightly regulated. However, blood clots can be formed intravascular without certain trauma. Activated neutrophils and monocytes interact with the coagulation cascade and platelets, leading to the formation of thrombi in the blood. Furthermore, because of different links between the two systems, an overreaction of the immune system after a viral or bacterial infection could lead to the activation of coagulation, also called immunothrombosis. A prime example of how this occurs is via the contact system, yet the platelets and neutrophils play an important role as well.

Obviously, there are physiological properties and mechanisms that are needed during infections and even are good for humans for fighting different pathogens. It is not unrealistic that there is a small immunothrombotic event happening somewhere in the microvasculature in the lungs right now and it will not be noticed because the pathogen is cleared and the body will not get sick. However, there is a chance that the immune response against a pathogen is too substantial that the immunothrombotic events are too immense as well and complications occur.

In this overview, the different properties of contact activation, platelets and neutrophils in immunothrombosis are described and how the blood coagulation system has developed from the hemocytes in the different “separated” systems they are today. This information leads to an important answer to the question of how the anti-pathogen function of immunothrombosis developed into a possible death-inducing complication. Furthermore, if this immunothrombosis is still needed in humans or if it is an unnecessary and dangerous remainder of evolution.

Evolution of the open hemolymph system

Further evolved organisms rely on both the coagulation system and the immune system to fight off invading pathogens. The role of coagulation is forming a barrier to prevent pathogens from entering the body and the immune system defends the body by attacking these invading pathogens directly. In organisms earlier in evolution, like the horseshoe crab, hemocytes could fulfil both functions. In these creatures a similarity between their coagulation and that of humans can be found. In the horseshoe crab an extracellular network is formed as well, although it is not made of fibrin. This fibrin-like clot is made of polymers from the protein coagulin[1]. These coagulin polymers can trap invading pathogens like bacteria tightly. After trapping the bacteria, components of the plasma kill most of the bacteria in synergy with the formation of the clot [2].

Hemocytes have the ability to initiate immune responses by identifying intruders in the body via several recognition patterns and receptors. When hemocytes are activated they release the content of their granules which contain antimicrobial peptides and thereby kill the invading pathogen. Furthermore, these hemocytes have phagocytic properties. They are known to be able to bind pathogens and internalise them and thereby protect the body from further harm. Not only do hemocytes have immune properties, they also have coagulation properties. Hemocytes are able to release procoagulation factors like protein C and G. These factors interact with other hemolymph proteins in the presence of calcium ions and form a network of cross-linked soluble proteins with different receptors.[3]

During evolution, because of the occurrence of more advanced pathogens, this system separated. This separation led to the development of different cell types like platelets and the plasmatic coagulation subsystem. Nowadays the link between the coagulation and immune system, because of its common predecessor, can still be seen and examples are given later on in this review.

The first subset that developed from hemocytes were the thrombocytes. These thrombocytes are the so-called equivalent to the mammalian platelets. Both undergo morphological changes when stimulated. Moreover, substances that stimulate aggregation by platelets have the same effect on the thrombocytes from the fish. This suggests a sequence homology of both cell types. However, there are some differences between thrombocytes and platelets. For instance, the most obvious one is that thrombocytes have a nucleus and differentiate directly from their progenitor cells originating from the bone marrow.[4] This is not the case for mammals and the development of platelets. Platelets do not contain a nucleus and according to a paper by Martin *et al.,* this has a quantitative hemostatic advantage as the smaller size results in a large increase in surface area and speed of granule secretion. The larger surface area occurs due the more platelets produced compared to thrombocytes. Furthermore, the megakaryocytes, the cells producing the platelets, can increase their DNA content. By increasing the DNA content more platelets with more organelles are produced and the receptor density and the ability to produce more prothrombotic proteins is increased.[5]

A second subset evolved from hemocytes is the plasma-based coagulation. This system relies on different coagulation factors and proteins to alter the state of blood from liquid to solid. Most of these coagulation factors are related to each other via gene duplications that occurred early on in vertebrate evolution (between the appearance of protochordates and the jawless fish). However, during this evolution, the main mechanisms of clot formation with fibrin were retained. An article by Russel Doolittle provided an overview of how the different coagulation factors evolved from each other from the protochordates up until the mammals and specifically humans. With the use of whole-genome sequencing he discovered the step-by-step evolution of vertebrate blood coagulation factors (see figure 1)[6].

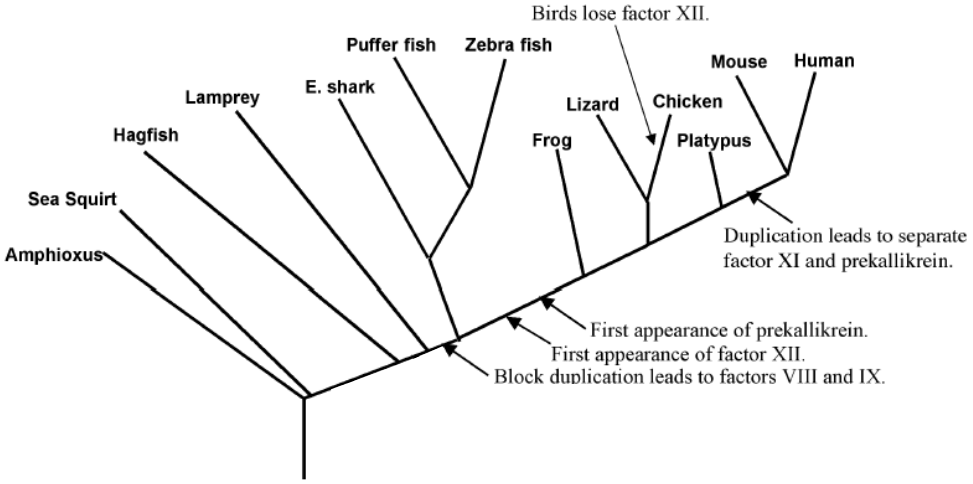

Figure 1: Time line phylogeny for appearances and or disappearances of different clotting factors in evolution. Adapted from Doolittle et al., (2009) [6]

Interesting to elaborate on is the formation of thrombin. Thrombin has next to its procoagulant function, the ability to activate the immune system by cleaving of Interleukin 1α (IL-1α). How this protein is able to do this could probably be explained by it evolution. Ponczek et al., described that in the amphioxus gene there is a protein located on scaffold 396 which has 42% homology with human thrombin. This shows the high conservation in the sequence for thrombin. Although, when back searched on *B.Floridae*, an amphioxus species, the did not find the specific regions needed for functional thrombin, they found an exon for an immunoglobulin-like (IG-like) domain between the hypothetic promotor and serine protease domain. This IG-like domain had hits up to 73% in other amphioxus domains as well. This IG like domain, although not researched, could be the reason thrombin later on in evolution has its immunological properties, as its consist partially of immunogenic genes.[7]

Contact activation

Lastly, an important part of the coagulation system we know today is the contact activation. Contact activation in humans is the recognition of artificial substances by factor XII which thereby activates factor XI and induces blood coagulation. Factor XII is part of the Kallikrein-Kinin system (KKS) and together with prekallikrein (PK) and high-molecular-weight kininogen (KH), factor XII is able to activate factor XI.

Today, contact activation is not as necessary anymore as it seems. Patients do not show bleeding tendencies while lacking factor XII[8]. This is possible due to the fact that during evolution factor XI could be activated by thrombin as well and thereby making factor XII somewhat unnecessary. Factor XII does,

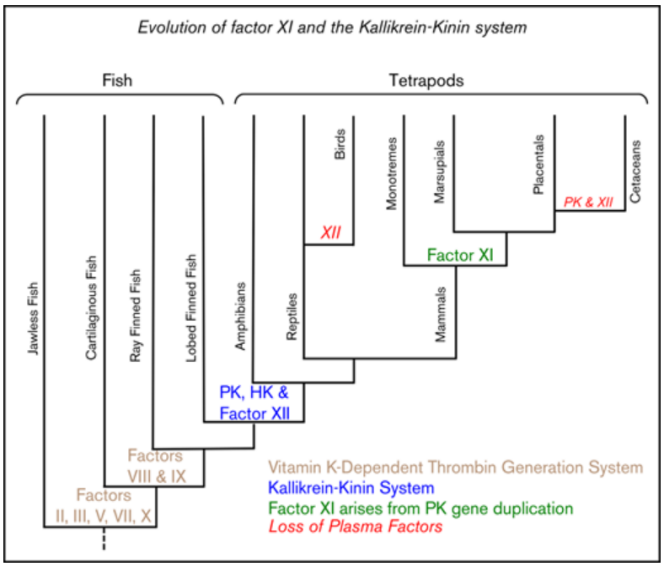

Figure 2: cladogram of vertebrate evolution. From the lower left the evolution up until the placenta and cetaceans is shown. At some of the dividers in this cladogram parts of the coagulation system is written down, which is thought to be obtained at that timepoint in evolution. First the vitamin K-dependent Thrombin Generation system came into light, with later on also the Kallikrein-Kinin system. Only from mammals factor XI arises and slowly makes the Kallikrein-Kinin System less necessary. [9]

however, play an important role in the immune system by activating the complement system. Research by Ponczek et al., shows that the KKS can be traced back to lobe-finned fish, which are the ancestors of all land vertebrates. Factor XI only originated after duplication of the PK gene in the early mammalian stage. The activation of Factor XI by thrombin came even later, namely during placental mammals (see figure 2).[9]

There are three distinct differences between PK and factor XI. First of all, factor XI is a homodimer while PK is monomer. This of course could be expected, as factor XI is a duplicated form of PK. Second, factor XI is able to more efficiently activate factor IX than PK. Lastly, thrombin is able to activate factor IX but not PK. This is because for an efficient activation of factor IX, a specific factor IX binding exosite is required, which PK does not carry[9].

Immunothrombosis

The term immunothrombosis was first introduced in 2013 by Bernd Engelmann and Steffen Massberg in a paper where they wrote about the effect of thrombi on the innate immune system. They suggest that it serves multiple physiological functions. One of them, as described before, is the capturing and isolation of pathogens circulating in the blood and thereby limiting the dissemination.[10] In an earlier study in 2010 they showed that thrombus formation prevented tissue invasion in the micro vessels. In wild type and neutrophil elastase and cathepsin G deficient (Elane$^{-/-}$;Ctsg$^{-/-}$) mice, they discovered that more *E.coli* was present in the perivascular tissue in the liver in the Elane$^{-/-}$;Ctsg$^{-/-}$ mice than in the wildtype, suggesting that the formation of thrombi leads to less tissue invasion (see figure 3). Moreover they showed that this difference in extravasation is not restricted to the liver but can even be seen in the spleen and lungs. [11]

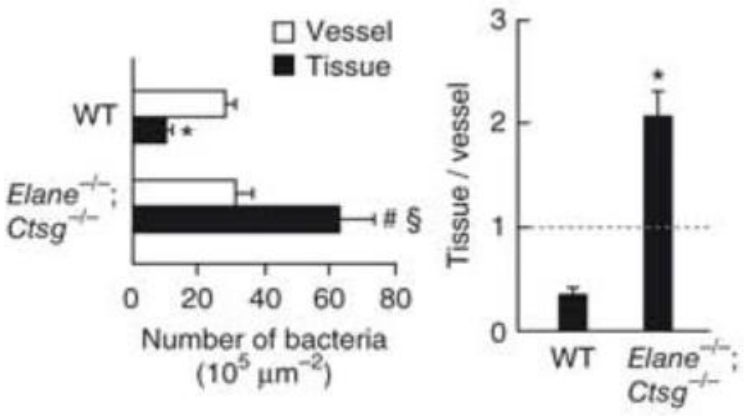

Figure 3: Number of bacteria in the vessel and in tissue and the ratio of tissue and vessel in wildtype and Elane$^{-/-}$Ctsg$^{-/-}$ mice. On the left the number of bacteria are depicted for the wildtype and Elane$^{-/-}$Ctsg$^{-/-}$ mice. With the prevalence of bacteria in the wildtype mice in the tissue is significantly lower than that in the vessel. In the Elane$^{-/-}$Ctsg$^{-/-}$ mice the total number of bacteria is higher and the prevalence in the tissue is significantly higher than that in the vessel. On the right the ratio of the number of bacteria present in the tissue compared to the vessel is visualised. The ratio is significantly higher in the Elane$^{-/-}$Ctsg$^{-/-}$ mice than that of the wildtype. Adapted from Massberg et al., (2010) [11]

A third function is that the formation of thrombi in the micro vasculature favours pathogen killing. For example by the generation of antimicrobial peptides during blood coagulation or its release by the endothelium and platelets**.** [12] The fourth function is the fibrin network accumulating in the micro vessels. This accumulation leads to the recruitment of more neutrophils, further increasing the immune response.

These four immunothrombotic functions explain what the effect of thrombi formation is on the innate immune system. However, immunothrombosis can also be described as the formation of thrombi due to an overreaction of the immune response. This is what can be seen with SARS-Cov-2 in the COVID-19 pandemic with one in three patients showing thrombotic events[13]. Patients with SARS-Cov-2 show mild thrombocytopenia, prolonged prothrombin time and increased fibrinogen and D-Dimer levels[14]. There are different ways this virus can induce an overreaction by the immune system ultimately

leading to the formation of thrombi. One of them is due to inflammation. During inflammation as a result of SARS-Cov-2 infection, a cytokine storm with different cytokines like Interleukin-6 is released [15]. This cytokine storm leads to the upregulation of platelet production, activation and aggregation. Besides that, there are more prothrombotic proteins being expressed and even fibrinolysis is inhibited. Another way SARS-Cov-2 can induce thrombi is via the complement activation. The complement cascade leads to the formation of membrane attack complexes which lyse the cell. By lysing the cell microthrombi formation is initialised, increasing numbers of von Willebrand factor is produced and overall prothrombin activity is increased. Furthermore, a subunit in the complement system increases the levels of polyphosphates from platelets which results in the expression of TF [14].

Lastly, thrombotic events due to SARS-Cov-2 can also be acquired by the formation of Nuclear Extracellular Traps (NETs). The release of the chromatin from neutrophils and the ability of histones to activate platelets leads to the expression of TF and thereby blood coagulation. Additionally, elastase is able to cleave Tissue Factor Pathway Inhibitor, so there is no inhibition of TF.[14]

Neutrophils

During evolution, multiple specialised blood cells serving as antigen specific adaptive immune cells were developed. The specialised cells, like neutrophils, now play an important part in the innate immune system.

For instance, the neutrophil can secrete serine proteases which can attack different microorganisms by degrading the cell wall. Furthermore, nucleosomes can, by activation via the platelets, be exteriorises and together with the secreted proteases can inhibit the function of Tissue Factor Pathway Inhibitor (TFPI) by degrading the protein. By degrading TFPI, it enhances thrombi formation, because it normally inhibits coagulation factor Xa[16]. The specific protease that accompanies the cleaving of TFPI is elastase. Not only do elastases hydrolyse proteins in the lysosomes of neutrophils resulting in their activation, they also degrade the extracellular matrix, are able to cleave different virulence factors from for example Salmonella and Yersinia Pestis (The Plague) and cleave TFPI as mentioned[17].

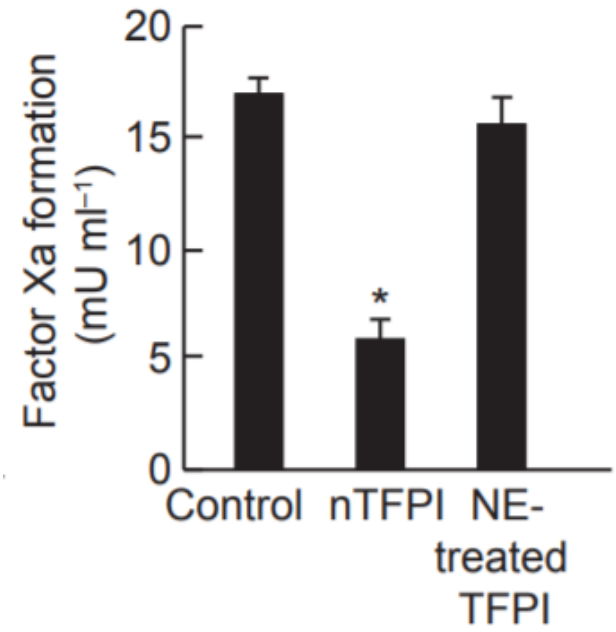

Figure 4: The factor Xa formation for native TFPI (nTFPI) and degraded TFPI by elastase (NE treated TFPI). The level of factor Xa after treatment with nTFPI showed a significant decrease in factor Xa formation, while NE-treatment didn't result in a significant decrease. The factor Xa levels after NE treated TFPI are almost the same as that of the control. Adapted from Massberg et al., (2010) [11]

The effect of degrading TFPI has been shown by Massberg et al., as well. They showed the coagulation on the basis of factor Xa formation levels with and without TFPI degradation by elastase (see figure 4). They found that the cleaving of native TFPI (nTFPI) resulted in the upregulation of blood coagulation [11]

Another import effect on the coagulation and immunothrombosis provided by neutrophils is the formation of Neutrophil Extracellular Traps (NETs). Neutrophils have different ways to attack different incoming pathogens. One of them, and maybe as a last resort is the use of NETs[18]. NET formation

means cell death for the neutrophil as the cells eject their DNA into the extracellular space after which the cell dies. This process is called NETosis. There is however a discussion if cell death is necessary as there apparently is a process called “vital NETosis”, where the neutrophils retain their viability and natural effector functions like phagocytosis[19]. Because this is not yet fully researched, only suicidal NETosis is taken into account.

During NETosis the neutrophils undergo structural changes as the nuclear membrane slowly disaggregates into vesicles and the chromatin decondenses and fills the cells. Afterwards the cells contract until the cell membrane ruptures and its content is ejected out of the cell into the blood vasculature to form NETs[18]. So not only the DNA gets ejected out of the cells but also other proteins like elastase, which effect have been described above. DNA is highly negatively charged and thereby can bind strongly to a great deal of bacteria, both gram positive and negative, thereby promoting the killing of these microbes[20]. Interestingly, some pathogens developed different defence mechanisms against the trapping and killing by the neutrophils and its NETS.

Albeit that NET formation is used as a last resort of the neutrophils to obstruct and kill microbial pathogens, an uncontrolled or excessive formation of NET contributes to thrombotic events. In an article by Elizabeth Middleton it is hypothesized that NET formation by SARS-Cov-2 may indeed explain the prothrombotic presentation the infections leads to. They examined NET in three COVID-19 lung autopsies. Soluble and cellular factor levels, which trigger NET formation, were significantly higher. Moreover, the autopsies showed NET-containing microthrombi with neutrophil infiltration. [21]

Platelets:

Platelets are an essential part in the formation of blood clots as they help to maintain primary hemostasis. Normally, during injury platelets can be activated after binding to Von Willebrand factor via GP1b-α, which results in the release of their granule content. This content leads to the activation of more platelets. Moreover, integrin αIIbβ3 is activated which leads to the binding to fibrinogen and aggregation of the platelets. This aggregation results in the formation of the hemostatic plug.[22]

What is interesting is that the platelet can be seen as an immune cell. An example of its functioning as an immune cell is its response to an influenza infection. A study by Gerard Jansen describes the clearing of the influence virus in the body. What normally could be expected from a viral infection is that the immune system gets activated and the infected cells plus the viral particles in the blood get eliminated.[23] Intriguingly, this is not the case for influenza. A common complication related to influenza infection is thrombocytopenia[24]. This already hints to a certain meddling of blood cells in the immune reaction. During an Influenza infection an increased clearance of platelets is observed. The study by Gerard Jansen described the process of how this occurs. Platelets express different types of sialo-glycans on their membrane to which different subtypes of the influenza virus can bind and afterwards enter the platelet. In the platelets, the expression of neuraminidase by the virus induces the removal of sialic acids from the membrane. The removal of the sialic acids from the platelet membrane leads to hepatic recognition and clearance (see figure 6). So the infection by the virus provokes the clearance of more platelets and removal of viral particles[24]. Furthermore, the lungs are a major site for platelets production and upon infection with Influenza, platelets show a platelet activation marker on their membrane indication the activation of the platelets. Platelet activation results in the release of its granules. Those granules contain proteins and cytokines which recruit other immune cells, but also create a possible cytokine storm related to increase of the disease phenotype[24].

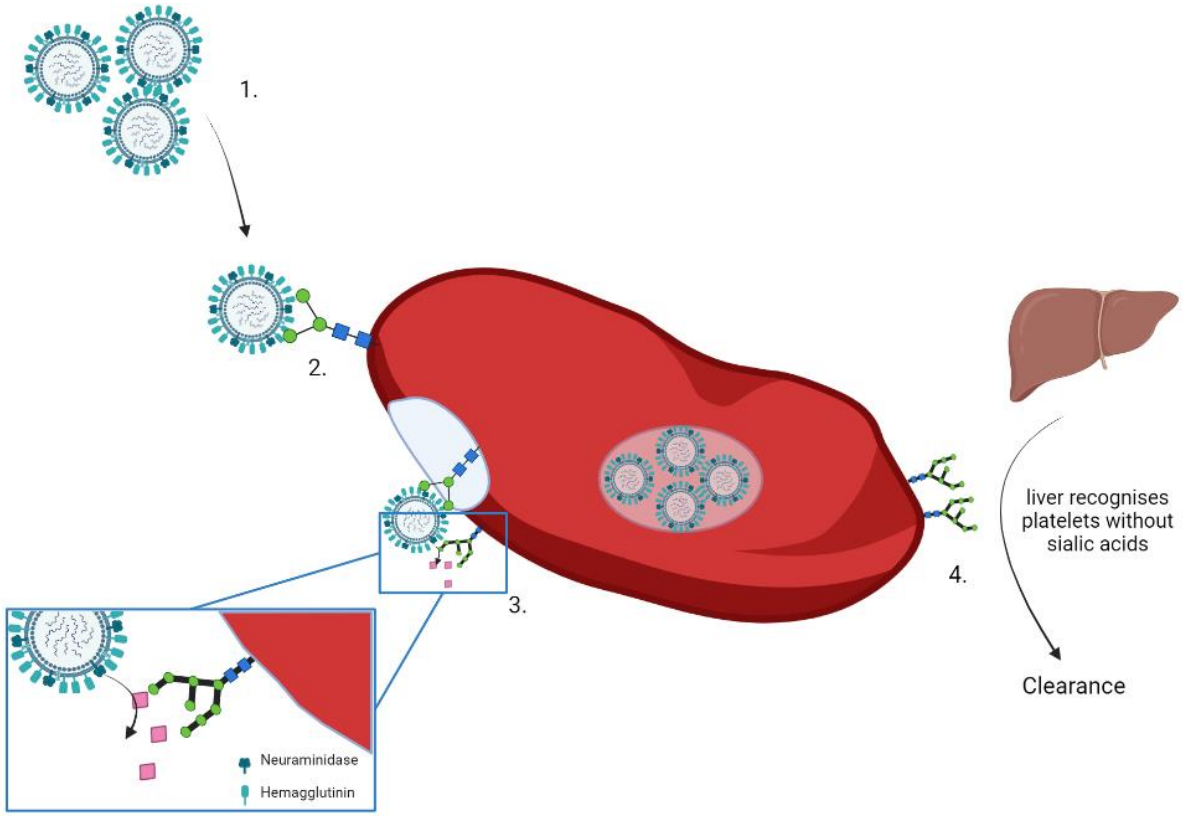

Figure 5: Overview of the platelet directed clearance of influenza. 1. The virus particles enter the bloodstream. 2. The virus particles bind to sialo-glycans. 3. During the internalization by the platelet, neuraminidase on the outer layer of the viral particle cleaves sialic acids form the glycans. 4. Liver recognises platelets without sialic acids on their glycan structure and clears them.

Not only do the platelets play as an immune cells themself in certain situations. They are also capable of activating the immune system. This is due their granule content which contain different inflammatory molecules. Furthermore, they express different Toll-like-receptors (TLR) on their membrane. These TLR's even recognise different various isoforms of lipopolysaccharide (LPS) resulting in the release of different cytokine profiles from the granules[25]. Because the platelet provides direct effector functions during infection these cells can therefore even be regarded as effector cells in the innate immune response.[26]

Regarding immunothrombosis, the platelets induce NETosis. Platelet activation via TLR-4 induces platelet binding to neutrophils and thereby activation of NETosis[27]. Furthermore the platelets release different cytokines which influence NET formation and even other integrins on the surface of the platelets can bind to the neutrophil influencing NETosis[28].

Defence mechanism immunothrombosis

Evading Neutrophil activity (NETs)

The human body has developed substantially over time and is able to tackle different types of pathogens. However, the pathogens also developed and gained ways to circumvent the host immune system. Some bacteria "ignore" the effect of the neutrophils. For example, the well-known bacterium Streptococcus Pneumiae, is an example of such bacteria. Streptococcus degrades the DNA of the NETs. Other pathogens like Staphylococcus Aureus and Mycobacterium Tuberculosis are able to degrade DNA as well.[29]

Streptococci degrade the DNA with different proteins. One of those is TatD, which is located on the bacterial extracellular vesicles. With an experiment by Jhelum et al, they showed the effect of TatD on the NET degradation and thereby the virulent effect of Streptococcus Pneumoniae. Both in the secretome and extracellular vesicles, a significant decrease in NET degradation was observed in mutant bacterial strains (see figure 6)[29]. Moreover, S. Pneumoniae also expresses antimicrobial proteins on their cell wall called endonuclease A (EndA). EndA is able to degrade NETs, but it has limited access to it. Due to the association with the cell wall, this protein can only degrade the DNA that are in proximity. The same sort of experiment for EndA was executed and the outcome looked like the results they got for TatD, however the extent of NET degradation was lower. A deficiency in TatD leads to quite the amount less degradation of NETs than a deficiency in EndA[29,30].

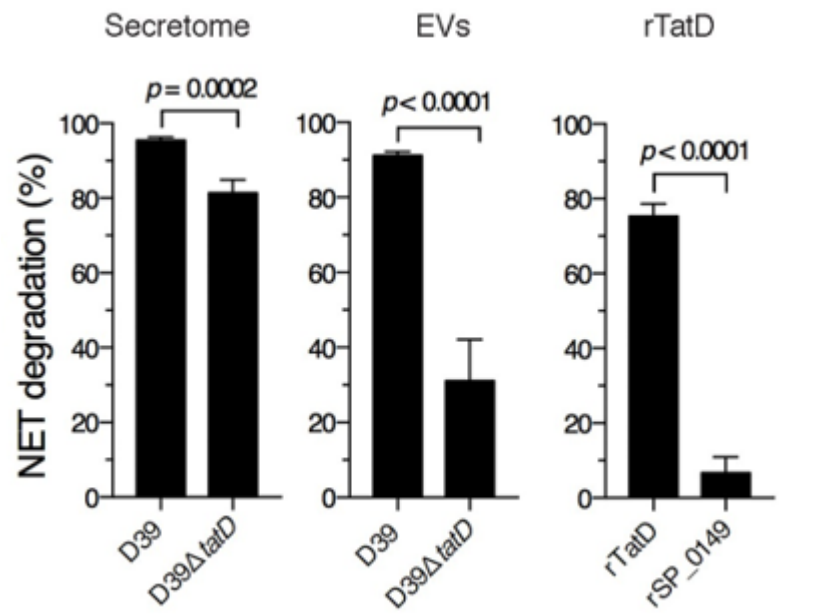

Figure 6: The effect of TatD on NET degradation in mice. In the figures of the secretome and EV, a significant decrease in the total NET degradation was found in mutated bacterium strains (D39-delta-tatD). Furthermore, as a positive control the effect of TatD was checked with a random recombinant protein of the Streptococcus (rSP_0149). rTatD has a significant higher NET degradation percentage than rSP_0149 has, meaning TatD itself has effect on the NET degradation. Adapted from Jhelum et al., (2018)[29]

On a side note, another interesting streptococcus called Streptococcus Pyogenes can avoid being caught in a blood clot as well. This streptococcus produces streptokinase which induces the activation of plasmin. The activation of plasmin leads to the upregulation of fibrinolysis. [31] Notably, streptokinase is already used as treatment for thrombosis. It is normally directly used after a heart attack[32].

The link between coagulation and the immune system

In older organisms a combined coagulation and immune system can be found. As previously mentioned the horseshoe crab for example has an open hemolymph circulatory system, which can function as a blood coagulator and immune response. These systems were linked and acted directly. Today, in more advanced mammals, some links can still be observed, but they act slower and indirect.

Where coagulation acts immediately with the activation of a protease cascade resulting in the clotting of blood, the innate immune system is slower, because it requires the recognition of pathogen related particles, such as Interleukin-1 (IL-1) to direct the inflammation and also recruitment of the adaptive immune system. IL-1 also can be found in certain marine invertebrates. The release of IL-1 leads to an increased inflammatory response via vasodilation, leukocyte recruitment. Because of its proinflammatory effect, it is tightly regulated. Common diseases due to dysregulation are atherosclerosis and rheumatoid arthritis. The link with the coagulation cascade and IL-1 is that IL-1α (part of the IL-1 family) can be activated by the cleaving of thrombin at a highly conserved site. This activation of the immune system, because of the cleaving in a highly conserved region by thrombin, suggests its importance and a possible conserved system but with different proteins.

Contact activation.

The contact activation played an important role during evolution and the coagulation cascade, by both functioning as an procoagulant and inflammatory response. While its functioning in the blood coagulation is minimal today as patients with a factor XII deficiency do not have bleeding tendencies, it still plays an important part in the activation of the immune response. It did play an important role in coagulation before placental mammalians where factor XI was not developed yet. In humans the link between the coagulation system and the immune system maybe even disappears during evolution contact activation wise.

Complement system

A distinct link between the two systems is the complement system. Its main component is the protein C3. When activated, C3 is able to activate three different pathways which lead to opsonisation, cleaving and induction of inflammatory proteins[33]. Interestingly this is system can be both activated by the immune system and the blood coagulation system. During evolution this system changed it location from intracellular to intravascular gradually[34]. Traces of an active complement system were found in the horseshoe crab as well where a functional homolog of C3 and complement mediated killing of bacteria via phagocytosis by hemocytes were observed[35]. Furthermore there is a high degree of homology found among this horseshoe crab clotting factor C, certain complement system factors, but also the vitamin-k-dependent clotting factors[36]. Both hemocytes and the complement system differentiated over time, but the complement system is still able to activate the systems. C3 plays a crucial role in this activation. Thrombin, different coagulation factors and also plasmin are able to cleave C3 and thereby activate an immune response[37]. Serine proteases from both systems can also have substrates from the other pathway, which leads to the activation of both systems during, for example, trauma[38,39]. Furthermore, complement system products are known to activate platelets and thereby induce coagulation[40].

Discussion

During evolution from invertebrates to higher vertebrates the hemolymph system has developed into a more difficult system or even two 'separate' systems. The hemocytes, the coagulation and immune response regulators in lower organisms, evolved over time and eventually formed platelets. From the hemocytes also plasma based coagulation was developed . Different new blood clotting factors were made, mostly via gene duplications. Despite these new blood factors, the main mechanism remained being the formation of clots via fibrin. Fibrin is formed by cleaving of fibrinogen by thrombin. Thrombin is developed later on during evolution.

Scientists have tried to make a clear distinction between the immune system and the coagulation system, but they were not able to. This is because these systems, although developed, are still linked to each other. An important player in this is the platelet. The platelets, as described, regulate both the immune system and the coagulation. Although its main function can be seen as initiating coagulation due to tissue damage, it kept a broad repertoire of immune system regulators. Platelets even have the ability to clear certain pathogens as can be seen with Influenza.

The development of the hemocytes into the advanced regulatory blood coagulation and immune system has led to the ability to kill off most of the invading pathogens. However, in some diseases, interplay between the two systems can also lead to complications called immunothrombosis. The immune response of the body against invading pathogens results in the formation of thrombi. This can be seen during a SARS-Cov-2 infection. After infection with this virus, a cytokine storm is generated. This leads to the upregulation of platelet production, activation and aggregation. Furthermore, other prothrombotic events like inhibiting fibrinolysis, expression of more prothrombotic proteins, stimulation of NET release by neutrophils and activation of the complement system are occurring at the same time. These factors lead to one third of the people infected by SARS-Cov-2 experiencing thrombotic events. Even the vaccine, the preventive treatment for this disease showed that thrombotic events may occur although with lower prevalence. This shows the importance of understanding the complete blood coagulation and immune system as a whole.

Not only SARS-Cov-2 shows prothrombotic events after infection, also other viruses or bacteria can induce an overreacting immune response leading to immunothrombosis. It can be described as the human body overreacting in certain situations, leading to other systems to react as well. A main question was if the evolution of immunothrombosis is still doing its job as it did previously in earlier organisms by helping the clearance of pathogens. It can be seen that the thrombi formation does not quite help the body with protection against the disease but even worsens the severity of the illness by the infection. The function of forming thrombi in cooperation with the immune defence to prevent dissemination for example is still functional and does help, but the extent to which it helps nowadays is debatable. Furthermore, there are pathogens that are able to circumvent the blood clot formation. For example the Staphylococcus Pyogenes upregulates plasmin activation and thereby fibrinolysis. With this infection, blood coagulation is halted and the infection can spread. A positive side for this particular infection is that the protein responsible for this evasion of the blood coagulation, streptokinase, is already used as a medicine in patients suffering from a stroke. It can even possibly be used as a preventive medicine for certain infections capable of inducing immunothrombosis, but that would depend on the pathogen as there additional possible infection improving capabilities, when dissemination is not inhibited.

The way the hemocytes evolved into the blood coagulation system and the immune system and the findings described in this review suggest that immunothrombosis mainly has a negative effect on most humans. Furthermore via research we learned that there are also multiple pathogens that use

immunothrombosis as a way to protect itself against the immune system. This would mean that immunothrombosis has indeed changed from anti-pathogen to anti-human. However, what needs to be thought about is that in articles and research conducted we mostly see the negative sides of biological systems. Evolution, nonetheless, works in a way as described by Charles Darwin as “Survival of the fittest”. Somehow the evolution of our blood and immune system made the human body more adapt to the environment then and also now. The incidence in which most immunothrombotic events occur after infection are often not high. It is still important to do research on these few extreme situations, but we have to remember that the human body is constantly under threat by pathogens that try to infiltrate the body. So it is important to keep in mind that this evolved coagulation and immune system do indeed function well, and that it is not right to write off immunothrombosis as an unnecessary and dangerous remnant of evolution.

All in all, Immunothrombosis can be seen as a possible death-inducing product of evolution. However that is what we see when we look at the many articles describing situations where immunothrombosis leads to complications. However, immunothrombosis, has prevailed during evolution which also indicates in physiological usefulness instead of its pathophysiological properties. Understanding the function of both the blood coagulation and immune system, but more importantly these two systems combined, could help develop different treatment strategies for treating the pathophysiological properties of immunothrombosis. This is the case because it is almost impossible to neglect one of the two systems in finding possible treatments. Looking back in evolution helps understand where these links between the two systems come from. Pathogens have evolved and will continue to do so over time and vertebrates usually will do the same to overcome these new forms of pathogens. More research about the link between the coagulation system and the immune system could lead to possible therapies for immunothrombotic events, like during the SARS-Cov-2 infection.

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