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Understanding the human innate immune system

In-silico studies

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General Introduction

I am not young enough to know everything.

~ Oscar Wilde

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General Introduction

Imagine a primitive animal, one that is perhaps the predecessor of all humans. This animal has, thus far, lived a fruitful long life by depending on a primary defense mechanism that is capable of defending itself effectively against foreign substances that dare attack it. It does so by releasing tons of chemicals and proteins, or setting forth a battalion of phagocytotic cells aimed at wiping out pathogens¹. If this defense wall crumbles, it simply gets rid of the infected body part and regenerates a new one².

Nevertheless, confronted with an ever-changing environment that is conducive to breeding a plethora of toxins that can eradicate a line of descent of organisms challenges the survival of the primitive animal. Its rudimentary defense mechanism is blatantly inadequate. Faced with such heavy burden and the need to ensure the survival of its own species, the primitive animal goes down the line of evolution; developing a more resilient, sophisticated, and intelligent defense system that provides a lasting security; one that is highly specialized and delicately regulated and does not attack its own tissues, putting itself at risk. We call this immunity.

It takes nature hundreds of thousands, or even millions of years to craft a

sophisticated biological defense mechanism. This is done by preserving beneficial attributes and building on existing defense mechanisms that are both resilient and efficient, as well as discarding the least useful of them. What is left after the selection we call the *innate immunity*. “Innate” simply because it is coded in the germline; an intricate network of cells and molecules that has been especially chosen and molded over evolutionary time and passed down from one generation to another with only minor enhancements³.

The **human innate immune system** (HIIS) is one of the two subsystems of the *human immune system* – a biological defense system that is complex, yet delicate network of highly specialized cells, tissues and organs that work together to protect the body from potentially damaging or fatal threat. HIIS is characterized as the body’s first line of defense against insult by augmenting the protection that anatomical and physiological barriers offer. Speed is its defining feature, generating an inflammatory response within minutes of exposure to pathogens up until 4 hours. However, HIIS does not exhibit a lasting protection that can be attributed to only providing a non-specific protection. That is, HIIS is only capable of detecting the distinction between the self and non-self, triggering an immune response against the antigen that happens to exhibit this distinction.

While innate immunity has long been acknowledged, going all the way back to 1908 due to the winning efforts of Ilya Melnichnikov, it has been markedly overshadowed by the high-impact discoveries on the *adaptive immunity* – the second subsystem of the human immune response that provides a response capable of manifesting immune memory, allowing it to prominently aid the body for a more effective response to pathogens or toxins when encountered the second time, or even decades after the initial encounter⁴. Interestingly however, the dogma that the innate immune response is nonspecific has been challenged by new discoveries⁵. According to more recent findings on what HIIS is actually capable of, one thing is clear – HIIS now stands as a proud partner of the adaptive immune system⁶. HIIS is in fact emerging as a critical regulator of human inflammatory disease⁴.

In light of the critical role that the human innate immune system plays in health and disease, as it is after all the body's first line of defense against foreign invaders, this dissertation seeks to unravel the mechanisms of HIIS in response to various stimuli that can jeopardize the organism's survival. The goal of this research is not only to understand how the human innate immune system functions, that is how its various components interact with each other and work together to neutralize inflammation via a well-orchestrated and well-regulated network of complex processes, but also to pinpoint under which conditions the human innate immune system ceases to function, possibly leading to the death of the organism.

The first step in understanding HIIS is developing a robust model of the human innate immune system that predicts the dynamics of the following identified key players: resting and activated macrophages, resting and activated neutrophils, inflammation triggering moieties (ITMs), pro-inflammatory and anti-inflammatory cytokines. More specifically, we investigate how HIIS reacts to an insult that is so intense to the point that it invokes a systemic inflammation such as that experienced by patients undergoing cardiac surgery with bypass filter. Open-heart surgery with bypass filter invokes such a vigorous response from HIIS because concentrations of ITMs are not only heightened but also originate simultaneously from different sources in the body. HIIS model has been calibrated and validated against clinical trials data of patients undergoing cardiac surgery with bypass filter. A model of the human innate immune response for systemic inflammation has never before been published in literature at the time of writing of the article ⁷.

Despite being known for years for its neutralizing effect against ITMs ⁸, the enzyme alkaline phosphatase, surprisingly, has never before been associated with the human innate immune response. As a matter of fact, it was even observed that there is a prominent induction of endogenous alkaline phosphatase with continuous infusion of bovine Intestinal Alkaline Phosphatase (blAP) or supplemented AP on top of standard care protocol in cardiac surgery patients ⁹. If the induction of AP in the bloodstream is indeed

the body's approach at assisting the innate immune response during systemic inflammation, investigating how the rest of HHS behaves in different AP supplementation regimes would provide useful insights that medical practitioners could actually use, with helping patients as the end result. In **Chapter 1**, I present a complete model of the human innate immune system in response to systemic inflammation that also incorporates an equation that models the induction mechanism of alkaline phosphatase in cardiac surgery patients infused with continuous concentration of bIAP. The results of the *in-silico* experiments not only show that the infusion of alkaline phosphatase has no detrimental effects on the body, it also assists the human innate immune system by neutralizing the ITMs.

Neutrophils are one of the armies of immune cells that are mobilized first to the site of inflammation in response to the signals relayed by messenger proteins called pro-inflammatory cytokines. Pro-inflammatory cytokines, which are produced by macrophages upon the phagocytosis of ITMs at the site of inflammation, open up the endothelial barrier and recruit resting neutrophils that are circulating in the bloodstream into the site of inflammation, which is normally the tissue. Neutrophils are then activated when they cross the endothelial barrier and proceed to neutralize inflammation at the site of insult. After executing its functions, the neutrophils then go into a programmed death called *apoptosis*. However, if the stimulus is too intense or persistent, neutrophils take on a different death pathway called *necrosis* that involves spilling all its contents into the surrounding tissue, thus further aggravating inflammation. Paradoxical as it may seem, the risk of deliberately aggravating the current level of inflammation induces, through several processes, the recruitment of more neutrophils to the site of inflammation.

This delicate balance between apoptosis and necrosis in response to the scale of insult can be regarded as the fine work of nature itself; carefully picking the optimized balance between the cost (local tissue damage that necrosis inflicts on surrounding tissue) as well as benefit (anti-inflammatory effect of apoptosis) of picking either death pathway through the course of evolutionary

time. In this line of work, I use a mathematical framework called *evolutionary game theory* that is used as an application of the mathematical theory of games to the context of evolving biological systems. Game theory was originally developed for applications in economics¹⁰ and was later on adapted for applications in biology, resulting in the field of evolutionary game theory. Just as evolution challenges strategies for their ability to ensure the survival of the population, the results of the game shows how good the strategy really is in terms of maximizing the so-called payoff or fitness of the individuals.

In **Chapter 2**, I take on an innovative and fresh approach in modeling the choice of death pathways in neutrophils, that is, as far as I know, applied for the first time on an essential aspect of the human innate immune response. By utilizing the concept of evolutionary game theory in explaining the emergence of apoptosis and necrosis with respect to different levels of insult, I present a high-level take on a field that is by far immensely dominated by experimental, clinical, and mechanistic modeling approaches. I regard the so-called *neutrophil entities* (composed of a single neutrophil as well as the tissue it occupies), as players of the game, and apoptosis and necrosis as their choice of strategies. The players are then made to play the game and the resulting percentage of apoptotic and necrotic population are validated against data. My model of the choice of death pathway of neutrophils confirms the hypothesis that indeed, neutrophils behave seemingly with the payoff of the entire organism in mind. That is, as if these entities are controlled by a central system that enables a single entity to “sense” the overall scale of insult before a strategy is made. An interesting question surfaces: *when does this so-called global cooperation crumble?* I explore the specific conditions when this central system shifts into defection or non-cooperation by building on top of a framework developed by Helbing and Yu¹¹ in¹². Here I show that non-strategic or random migrations made by entities undermines the survival of the organism. This in fact has been well-studied where it was confirmed that the failure of neutrophils to migrate is commonly observed in a systemic inflammatory response called sepsis^{13,14}.

When the body's first line of defense ceases to function, does HIIS exhibit indicators prior to a shift in paradigm, nudging the state of the system from health to disease, or at worst, to death? This is the central question I try to answer in **Chapter 3**. To date, several dynamical systems have been shown to exhibit these so-called *critical transitions*, where the systems exhibit significant changes in the statistical properties of their measurements. These observed deviations, as the system progresses towards a critical point, can potentially serve as early warning signals prior to the system's collapse, thereby increasing the chance of prevention, or at the least stalling the incoming fiasco minutes or even hours before it strikes. This phenomenon, however, has yet to be investigated in the human innate immune system.

In order to approach this problem, I utilized the human innate immune system model developed in **Chapter 1** to generate virtual patients that serve as data. I then designed various scenarios that could potentially lead to HIIS' collapse, which happens when HIIS is no longer capable of neutralizing the ITMs in the system. With the use of well-known early warning signals (EWS) ¹⁵, I show that critical transitions can be detected in concentrations of several key players in the human innate immune system model (alkaline phosphatase and ITMs). However, more data is needed to fully support this claim. This is because the current version of the HIIS model was only calibrated against the median of the population of patients going into cardiac surgery. Critical patients, or those who died after surgery, might have demonstrated a slightly different dynamics in their timeseries data, as suggested by the large variability in the clinical trials dataset.

With this in mind, I further assess the capability of EWS in detecting critical transitions on the clinical trials dataset that was used to calibrate and validate the HIIS model. The dataset reveals that 3 out of 53 patients died after surgery. Hence, the task here is clear: can EWS successfully segregate critical patients from the non-critical ones? Results show that the blood parameter with the highest F1 score (blood parameter where EWS is most successful in labeling critical and non-critical patients) turn out to be platelet count. Coincidentally, it

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was shown that platelet count is a significant indicator of post-surgery bleeding¹⁶. Bleeding can be considered a serious complication of surgery as it may lead to morbidity and mortality, where severe bleeding occurs in approximately 7%^{17,18}. Uncontrolled bleeding is in fact the cause of 30-40% of trauma-related death in trauma patients¹⁹.

This thesis aims to provide a solid foundation in understanding how the human innate immune system works in the case of systemic inflammation with the use of ordinary differential equations, evolutionary game theory, and early warning signals. Indeed, there is so much more to be done, but I hope that this thesis becomes a stepping stone to explore more of HHS' elaborate mechanisms.