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IMMUNITY: A PICTURE OF THE BODY'S RESPONSE TO VIRUS IN CELLS

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ABSTRACT

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RESPONSE TO VIRUS IN CELLS

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Viruses are a major source of disease in the human population. During infection, viruses can hide inside the body's cells and avoid detection and eradication by the host's immune cells. However, the human body has developed several different methods of fighting infections within its cells. This thesis examines the different modes of defense used against viruses during cellular infection, as well as some of the methods viruses use to avoid or subvert the immune system. The literature on immunological defense against viruses was reviewed in order to determine the different methods of defense the human body most often exhibits. While cytotoxic T-cells are most often used to kill virus-infected cells, most cells in the body have internal receptors that allow them to react to viruses in their cells and warn surrounding cells, which initiate their own defenses.

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CHAPTER 1

INTRODUCTION

1.1 Viruses

Viruses are not themselves living, yet they still manage to infect almost all types of living things and cause a multitude of problems while doing it. Viruses cause approximately 60% of infections doctors see in their practice.[1] They can cause a runny nose and cough, as seen in a simple cold, or permanent paralysis like in Polio.[1] Some cause a lifelong and often deadly malady like HIV, or a deadly pandemic as has been seen with different strains of flu several times in history.[2] Yet we do not have methods for treating the vast majority of virally caused diseases. Most current methods for dealing with viral diseases are preventative, instead of curative. We have vaccines for some, but not for all viral diseases.[1] The process of developing treatments for viral infections requires that we understand more about them.

1.1.1 Viral Composition

Viruses are, at their simplest, composed of nucleic acid and a surrounding capsid.[3] The capsid is formed from basic capsid proteins and, in non-enveloped viruses, receptor proteins.[1] In enveloped viruses the receptor proteins are held in the envelope rather than the capsid.[1] The genetic material of a virus can be DNA or RNA, and may be double or single stranded in any combination. Viruses do not code for most cellular machinery or reproductive processes, as they replicate within cells containing all (or nearly all) of the

machinery necessary for viral reproduction.[3] This allows viruses to be small and go nearly unnoticed in many cells.

1.1.2 Viral Infection

Viruses infect cells by having proteins in their capsid or envelope bind to receptor proteins on the cell, and causing the cell to phagocytize them into the cell. With non-enveloped viruses this leaves no signature on the surface of the cell. Some enveloped viruses fuse their envelope with the cell membrane and send only the contents of the virus into the cell, leaving the proteins from their membrane to mark the cell as infected for a short time before the viral proteins degrade and disappear. However, many enveloped viruses bring their membrane all the way into the cell, leaving nothing to mark the cell surface, even for a short time.[1]

1.1.3 Viral Exit

Viruses can leave their host cell in a couple different ways. One option is to cause the cell to fall apart as its membrane pieces deteriorate and are not replaced since the cellular machinery that would normally form them is occupied forming viral proteins and genomes. This releases the virus, and all the cell contents into the body fluids, making it obvious to the immune system that the virus is present. However, many viruses simply cause the infected cell to undergo apoptosis, (programmed and normal cell death) releasing the virus copies without alerting the immune system to their presence. One of the immune system's defenses to this is to cause necroptosis (programmed cell necrosis) instead of apoptosis, which, although similar in affect, is a sign of disease and infection and allows the body to recognize a viral presence in its tissues.[4]

1.1.3.1 Enveloped Exit

Enveloped viruses have one other step in their process. If the envelope of the virus comes from the outer cell membrane then the viral proteins must be added to the cell's membrane before the virus is released, giving the immune system something to search for before the virus is released.[1] This is found by B-cells, antibodies (activating Macrophages and cytotoxic T-cells), and natural killer cells.[5] However, non-enveloped viruses, and viruses who draw their envelope from membranes within the cell do not have this weakness.

1.2 Immunity

The human immune system is divided into two classes: innate and adaptive. Adaptive immunity is the most often studied, and better understood of the two. This division of the immune system is what reacts to specific foreign invaders, by recognizing and attacking molecules and pieces of molecules specific to one part of one disease-causing agent. Adaptive immunity is not pre-programmed. It learns each of the molecules it will react to by being introduced to them at some point during infection. Innate immunity lacks the ability to learn, or recognize new pathogens in order to protect against them.[6] However, innate immunity plays an enormous role in protecting the body from pathogens. Most pathogens have some piece of themselves that is common to many pathogens, but is not produced by the human body. This is one of the major areas of operation for innate immunity.[7]

1.2.1 Innate Immunity

Innate immunity comprises the vast majority of human immunity as it covers all aspects of immunity that cannot learn or adapt in a person. This includes everything from

basic barriers such as the skin, to complex signaling cascades at a cellular level. All non-specific defensive cells and non-specific molecules are included in this section of human immunity.[1] Some relevant aspects of innate immunity include cell receptors, internal and external, and cytokines, used for signaling within or between cells. A significant amount of research has been conducted into each of these aspects of human defense against pathogens.

CHAPTER 2

LITERATURE REVIEW

2.1 Recognition

Pathogen associated molecular patterns (PAMPs) are pieces of an invading pathogen recognized by the immune system. These are specific molecules that are part of or released from invaders common to many different types of pathogens.[8] Examples include sugars foreign to the human body, double-stranded RNA (dsRNA), non-methylated DNA, flagellar proteins, and many others. They are recognized by pattern recognition receptors (PRRs), which are specific to the individual PAMPs.[9] Two of the larger categories of PRRs are TLRs (Toll-like receptors) and NLRs (Nod-like receptors). These receptors are located both on the external membrane of cells and in the endosomal and lysosomal membranes. TLRs are some of the best understood and most researched of the receptor families. TLR families 3, 7, and 11 are the receptor families expressed internally. TLRs 3, 7, 8 and 9 are the receptors most commonly found to provide viral immunity.[9] TLR3 is responsible for detecting double-stranded RNA and will be the focus of this essay.[11]

2.2 Signaling

Binding a pathogen is not enough to eliminate it. The binding process must initiate an immune response that will eliminate the infected cell, or cause cells around it to be protected. This is done via signaling cascades. Every receptor has a different molecule (usually a protein) that it binds after binding the antigen to which it reacts.[1]

When the receptor binds its antigen, it also causes a change in its effector molecule, starting a chain reaction that causes the cell to change behavior and act in a way to provide immunity.[6] Some receptors can start a signaling cascade that will divide to cause multiple different effects within the cell.[11] However, these pathways can be exploited by the invading pathogen. Viruses can produce molecules that will bind a cellular protein in the cascade, and stop it's function. Or they cause another cascade to start that will interfere with the protective one.[1]

2.3 Effects

Several of the common effects of signaling cascades include the release of cytokines to message other cells, changes in DNA transcription, or necroptosis (programmed cell necrosis).[8] Cytokines are molecules that are used to communicate information between cells. Often this involves immune response processes. Interferon (IFN) and interleukin (IL) are two types of cytokines that have a vast impact on immunity.[10] IFN is responsible for activating the adaptive immune response, promoting natural killer cell function, and inducing neighboring cells to enter an antiviral state that helps prevent further infection within the cell. IL is heavily involved in the body's inflammatory response and is to some extent regulated by IFN.[10] Some of the signaling cascades result in changes to transcription, activating genes that are only used in case of infection, or by causing molecules to be formed and released that are normally non-existent (such as cytokines) that initiate the different processes of extracellular immunity, or cause neighboring cells to shut down protein formation to protect against viral infection and replication. Some of these cascades can cause the cell to undergo the process of

necroptosis, which is an apoptotic disruption that activates the immune system. Each signaling cascade can cause one or all of these effects to take place.[8]

2.4 Viral Defense

As much as human bodies have defenses against viral infection, so viruses have defense mechanisms against human immunity. This continuing battle between host and virus is known as the molecular arms race. It involves the constant adaptations of viruses to outwit host defenses and cause the immune system to either miss them completely, or attack something other than the invading virus. As viruses evolve ways to circumnavigate human immunity, the immune system finds ways to retaliate against the virus, either by adding or editing current processes so that the virus cannot operate as effectively.[8]

CHAPTER 3

METHODOLOGY

3.1 Research Methods

Human defense against viral infection within the cell was chosen as the subject of research for this thesis. The UTA library database was searched for relevant articles using search terms such as "viruses AND immunity/immunology", "cellular immunity" and "viral defense". The articles found were read for relevance to the topic. Several specific aspects of immunity were chosen for further research. These topics included cytokines, interferon, interleukin, TLRs and NODs. Each topic was reviewed for deeper information, and TLR3 and its function in cellular necroptosis was chosen as the basis for this essay. Articles on the function on TLR3 were assessed, and an investigation was made into the continuing work on TLRs and their function. Information on each of the secondary topics has been included as a framework for the study.

3.2 Study Methods

Experimental mice were used with TRIF (C57BL/6J-Ticam1^{Lps2}), Rip3$^{-/-}$, Rip1$^{+/-}$, Tnf$^{-/-}$, and Casp8$^{+/-}$ mutations were used for this experiment. "L929, NIH3T3, 3T3-SA, SVEC4-10, J774, and primary MEFs were maintained in DMEM" throughout the experiment. "Pooled bone marrow cells from flushed tibias and femurs were harvested into PBS containing 0.5 mm EDTA" to create the bone marrow-derived macrophage culture, or BMDM. IFNβ and TNF were added to stimulate cell cultures where indicated in the results. In this experiment "necrostatin (Nec)-1 (Calbiochem); Z-VAD-fmk (Enzo Life

Sciences); bafilomycin A_1 and cycloheximide (Sigma); poly(I:C) (GE Healthcare); and LPS, Pam3CysK, and CpG DNA (Invivogen)" and Flagellin were used. "Selective small molecule RIP3 kinase inhibitors GSK'843 and GSK'872 were identified through compound screening and optimization efforts. Control, RIP1, and MLKL siRNA ONTARGET SMART pools were obtained from Thermo Scientific, and transfection employed Lipofectamine RNAi Max (Invitrogen)." Open Biosystems provided the RIP3 shRNA constructs that were employed in this study.[11]

"Following preparation of cell extracts, immunoprecipitation, and electrophoretic separation on denaturing polyacrylamide gels followed by transfer, immunoblot analysis was performed on the following antibodies: mouse anti-β-actin (clone AC-74; Sigma); rabbit anti-Casp8 (cell Signaling); rabbit anti-MLKL (Abgent); mouse anti-RIP1 (clone 38; BD Biosciences); rabbit anti-RIP3 (Imgenex); goat anti-RIP3 (clone C-16; Santa Cruz Biotechnology); rabbit anti-IκBα (Santa Cruz Biotechnology); and anti-mouse IgG-HRP and anti-rabbit IgG-HRP (Vector Laboratories)." Immunoprecipitation analyses used two proteins: goat anti-RIP3 anti-body and A/G-agarose. All cell cultures "were seeded into Corning 96-well tissue culture plates." The "Cell Titer-Glo luminescent cell viability assay kit (Promega)" was used to measure ATP in the cells and assure viability of each culture.[11]

"Total RNA was prepared from siRNA-treated 3T3-SA cells at 48 h post-siRNA transfection using Ambion's miRVana miRNA isolation kit."[11] Specific primers were used for binding the mRNA from the cell cultures during PCR analysis. These primers were included in forward and reverse. Here the forwards are listed, and the reverses implied. MLKL: GGATTGCCCTGAGTTGTTGC and β-actin: BCTGTATTCCCCTCC-ATCGTG. "Experiments were carried out in triplicate and normalize to β-actin mRNA."[11]

CHAPTER 4

RESULTS

4.1 TLR

TLR3 is the TLR that response to dsRNA. This receptor causes multiple effects in the cell including a proliferation of cytokines causing inflammation. However, the focus of this study was on the process by which TLR3 initiated necroptosis in the cell and the mechanisms that could be used by viruses to block this occurrence. TLR4 was also studied along with its mechanisms for inducing necroptosis. One of the main differences between TLR3 and TLR4 is where they initiate their cellular defenses. TLR3 is bound in vacuolar membranes and senses internal pathogens, whereas TLR4 is bound to the cell membrane, allowing it to sense extracellular pathogens. However, the focus will remain on TLR3 and internal defenses rather than TLR4.[11]

4.2 Signaling Cascades

TLR 3 can initiate multiple signaling cascades. It can activate NF-κB, Initiate apoptosis, or cause necroptosis. The necroptotic pathway has a series of steps, intertwined with the down regulating of other molecules that exist in the cell as a normal part of cellular function. One of the down-regulated molecules is caspase 8. Caspase 8 is used to prevent necrosis in a healthy cell, but can cause apoptosis via the TLR3 pathway. If this molecule decreases in concentration when TLR3 is activated it causes necroptosis. As caspase 8 decreases the concentration of RIP3 kinase increases. RIP3 kinase is a part of the signaling

cascade common in most instances of necroptotic pathways. Caspase 8 inhibits this process. The exact method of inhibition is unknown. TLR 3 and 4 both activate RIP3 directly. Most other TLRs use an indirect method of activation. The pathway used for this process is the DAI-RIP3-MLKL pathway. MLKL is the execution step for necroptosis. TRIF is the molecule directly affected by TLR 3 and 4 to initiate this pathway. TLRs 3 and 4 cause rapid cell death, as opposed to several other TLR receptors, whose effects are delayed by several hours.[11]

4.3 Experiments

It was shown that cells underwent necrosis more rapidly when they had been exposed to IFN, which up-regulates the TLR3 receptor content in the cells. Also demonstrated was the fact that RIP3 is not an optional part of the signaling cascade. It is absolutely required for the TLR3- TRIF cascade to complete. Those cells in which RIP3 had been removed did not undergo necrosis. However, RIP3 does not influence the other signaling cascades initiated by TLR3 (up-regulating IL and IFN). GSK'843 and GSK'872 inhibit RIP3 kinases, ending the TLR3 necroptosis cascade by preventing the chemical changing of RIP3 that profligates the cascade. The necroptotic pathway is also slightly different in different types of cells. Macrophages require RIP1 to complete the pathway, whereas other cell types do not. This makes sense, as macrophages are the cleaners of the cell and need to have an extra/unique check to prevent necroptosis in these cells.

MLKL was knocked out of cells to see if it had an effect on the necroptotic pathway. The lack of MLKL action in cells was universally preventative of necrosis. RIP3 directly phosphorylates MLKL, which is the effector in the necroptotic pathway from TLR3.[11]

4.4 Final Notes

Caspase 8 prevents cell necrosis, however, it initiates cell apoptosis, so in absence of TLR3 activation and the initiation of the necroptotic pathway, caspase presence indicates the cell will soon undergo apoptosis. Therefore in a normally functioning cell caspase 8 is not present in high concentrations. Up-regulation of caspase 8 prevents necrosis and causes apoptosis of the cell. So, whether via caspase 8 or RIP3 the cell still dies in the end, eliminating the virus that is infecting it. This shows that RIP3 may be a backup for the caspase 8 pathway.[11]

CHAPTER 5

DISCUSSION

5.1 TLRs

TLR3 and its effects have a vast impact on defense against dsRNA-based viruses. The main function of which is to have the cell die before the virus leaves, preventing the spread of virus into nearby cells. This specific pathway has a backup path installed into it, showing resistance to viruses' attempts to prevent the pathway from reaching its conclusion. As further investigation is made into different pathways, more such checks and double action points may be found.

5.2 Post-Study Research

Since the time of the focal study other research has been done on TLR3 and its effects. TLR3 has been shown to be a major source of defense against many viruses, and lack of this receptor has been linked to vastly increased susceptibility to encephalomyocarditis, hepatitis B, some HIV strains, and different coronaviruses.[2] Research has also been conducted into the process of forming and moving the different TLRs to their functional locations. It has also been found that TLRs can have a role in autoimmune diseases.[9]

5.3 The Bigger Picture

TLRs are often the source of initiating reactions to viruses and creating a proper immune response, whether that is death of the cell, signaling nearby cells, or beginning to activate the adaptive immune system to respond to the infection. There is a potential that

in the future it may be possible to add receptors that bind to different PAMPs by genetic engineering. We may still be a decade away from that, but studies into how these TLRs function could prepare us for improving the human immune system with these in the future. Also, as viruses and bacteria have found ways of disturbing the processes of cell signaling in the body, and cutting short the signaling cascades in the cells, studying each of these steps may provide information on how to circumnavigate viral attacks on these areas. Perhaps we will be able to investigate and create antiviral treatments that reinstate pathways the viruses are blocking, allowing the body to undergo its normal immune response effectively.

Immunity is the difference between life and death, and that is not an exaggeration. TLRs and their pathways are one of many different ways in which the immune system reacts to invading pathogens, viral or otherwise. The human body has derived many methods for surviving the world we all live in. But it is not perfect. People die from diseases every day. Some die for lack of treatment, some because there is no current treatment option. The more we learn about immune processes, the more likely we will be to cure or prevent disease in the future. Knowledge is power, and the deeper our understanding of every aspect of immunity the greater our likelihood of producing viable alternatives to chronic, acute, or fatal illnesses.

REFERENCES

1. Shors, Teri. *Understanding Viruses*. 2nd Edition., Jones & Bartlett Learning, LLC, 2013.
2. Zimmer, Carl. *A Planet of Viruses*. University of Chicago Press, 2011.
3. Goudsmit, Jaap. *Viral Fitness: The next SARS and West Nile in the Making.* Oxford University Press, 2004.
4. Carter, John, Saunders, Venetia. *Virology: Principles and Applications.* John Wiley & Sons, Ltd., 2007.
5. Zimmer, Jacques. *Natural Killer Cells: At the Forefront of Modern Immunology.* Springer-Verlag Berlin Heidelberg, 2010.
6. Freitas, Antonio. *Tractus Immuno-Logicus: A Brief History of the Immune System.* Landes Bioscience, 2009.
7. Coleman, Robert, Lombard, Mary, Sicard, Raymond. *Fundamental Immunology.* 2nd Edition., Wm. C. Brown Publishers, 1992.
8. Lok-Yin Roy Wong, Pak-Yin Lui, Dong-Yan Jin. "A molecular arms race between host innate antiviral response and emerging human coronaviruses." *Virologica Sinica*, vol. 31, no. 1, 2016, pg. 12-23.
9. Sandra N, Lester Li, Kui Li. "Toll-Like Receptors in Antiviral Innate Immunity." *Journal of Molecular Biology*, vol. 426, no. 6, 2014, pg. 1246-1264.

10. Azhar Abbas, Ida Gregersen, Sverre Holm, Isabelle Daissormont, Vigdis Bjerkeli, Kirsten Krohg-Sorensen, Karolina R Skagen, Tuva Dahl, David Russell, Trine Almas, Dorte Bundgaard, Lars Holger Alteheld, Azita Rashidi, Christen P Dahl, Annika E. Michelsen, Erik A. Biessen, Pal Aukrust, Bente Halvorsen, Mona Skjelland. "Interleukin 23 Levels Are Increased in Carotid Atherosclerosis Possible Role for the Interleukin 23/Interleukin 17 Axis." *Stroke*, vol. 46, no. 3, pg. 793-799.

11. William J. Kaiser, Haripriya Sridharan, Chunzi Huang, Pratyusha Mandal, Jason W. Upton, Peter J. Gough, Clark A. Sehon, Robert W. Marquis, John Bertin, Edward Mocarski. "Toll-like Receptor 3-mediated Necrosis via TRIF, RIP3, and MLKL." *The Journal of Biological Chemistry*, vol. 288, no. 43, pg 31268-31279

BIOGRAPHICAL INFORMATION

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