INTRODUCTION TO HUMAN VIRUSES

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Keywords: virus, replication, pathogenesis, vaccines, tropism, aging, immune senescence

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Summary

Viruses are obligate intracellular infectious agents that require the host cell machinery to complete their replication cycle and propagate. In fact, the word "virus" is derived from the Latin word for poison. The Russian scientist Dmitry Ivanosky first discovered viruses in 1892 by demonstrating that tobacco mosaic virus was capable of passing through a filter that was known to be impenetrable to bacteria and that this filtrate caused plant disease. Initially, he thought that the plant disease may have been caused by a bacterial toxin, but later work by Martinus Bejerinck, Wendall Stanley and Friedrich Loeffler demonstrated that viruses were filterable infectious agents that replicated within cells.

Viruses are found in numerous different shapes and sizes, and have different levels of complexity. Today there are over 5,000 known viruses that are grouped into families using a combination of two different classification schemes: the Baltimore Classification System (BCS) and the International Committee on Taxonomy of Viruses (ICTV) system. Baltimore classification places viruses into 7 main categories based upon their genome type and replication strategy. The Group I viruses contain double-stranded (ds) DNA genomes; Group II viruses contain positive sense (+), single-stranded (ss) DNA genomes; Group III viruses have dsRNA genomes; Group IV viruses have (+) ssRNA genomes and Group V viruses have negative sense (-), ssRNA genomes. Both the Group VI and VII viruses require reverse transcriptase (RT) activity for their replication but differ in the their genome type. Group VI viruses have an ssRNA genome whereas Group VII viruses have a dsDNA genome. Virus groups are further taxonomically separated into Order, Family, Subfamily, Genus and Species using ICTV classification based on common characteristics such as morphology, host range and type of disease caused.

In this chapter, we will describe many of the known human viruses. We will define their clinical manifestations, structure, entry mechanisms, replication strategies, and vaccines or therapeutics utilized to treat infections associated with various viruses.

1. Overview of the Immune System

The immune system must overcome daily challenges from pathogens to protect the body from infection. The success of the immune response to infection relies on its ability to sense and evaluate microbial threats, then eliminate the threat while limiting damage to host tissues. This delicate balance is achieved through coordinated action of innate and adaptive arms of the immune system. The main distinguishing characteristic of these two branches of the immune response is the way they recognize antigens. Whereas innate immunity relies on germline-encoded receptors to sense the presence of pathogens, adaptive immunity employs a highly diverse set of receptors generated through somatic mutation and recombination that are tailored to specific pathogens. The second major defining characteristic of the adaptive immune system is the development of immunological memory that manifests with increased functionality and frequency of responding cells upon re-exposure to the same antigen.

The innate immune system is the first line of defense against pathogens and its action is mediated by several immune cell subsets that include neutrophils, natural killer (NK) cells, dendritic cells (DC), and macrophages. DC and macrophages are scavenger cells that can ingest and destroy infectious agents in endosomal compartments. Since they capture infectious organisms with high efficiency, they also serve as professional antigen-presenting cells (APC), which are responsible for processing and presenting foreign antigen peptides to T cells thereby bridging innate and adaptive immunity (discussed below). DCs can be divided into myeloid (mDC) also known as conventional DCs and plasmacytoid DCs (pDCs). The main function of mDCs is to process and present pathogen-derived peptides to T cells.

On the other hand, pDCs recognize viral DNA and RNA and produce vast amounts of type I interferons, such as interferon α (IFNα), which are potent antiviral cytokines. DCs and macrophages are alerted to the presence of pathogens via recognition of microbial non-self, missing self, or altered self. Recognition of microbial entities relies on the detection of conserved molecular patterns referred to as pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs) of the innate immune system. The three best-characterized families of PRRs are: 1) the toll-like receptors (TLR); 2) the NOD-Like Receptors (NLR); and 3) the retinoic acid-inducible gene (RIG-I)-like RNA helicases (RLHs). Following PAMP encounter, these PRRs initiate signaling cascades that drive production of several anti-microbial molecules that ultimately limit pathogen replication and spread and enhance the effector functions of DCs and macrophages. For instance, activation of PRRs on macrophages activates the release of intracellular antimicrobial molecules as well as inflammatory cytokines such as IL-6, IL-8, and TNFα.

Signaling through TLR-7 and TLR-9 expressed by pDCs results in the production of large amounts of IFNα and IL-12, and the upregulation of surface expression of CD86, which in turn increases the pDCs' ability to activate T cells. Finally, signaling through TLR-3, -4, -7, and -8 expressed by mDCs leads to the upregulation of co-stimulatory molecules CD40 and CD86, important for T cell activation. NK cells are cytotoxic innate immune cells that play a critical role in eliminating virally infected cells and tumors. They express inhibitory and activation receptors that can recognize "missing and altered self".

Recognition of normal self-MHC class I molecules is mediated by killer inhibitory receptors (KIR), which deliver an inhibitory signal to NK cells. In contrast, cell damage (due to viral infections or oncogenesis) can result in the upregulation of stress-induced molecules such as MIC-A, MIC-B, and ULBPs. These altered self-molecules function as ligands for NK cell activating receptors such as NKG2D. Last but not least, neutrophils are the most abundant white blood cell and a critical component of the innate immune system. They are recruited to sites of infection and inflammation very rapidly where they play an essential role in the elimination of pathogens via phagocytosis and respiratory burst.

The adaptive immune branch is composed of B and T lymphocytes, which, unlike cells of the innate immune system, can generate a response tailored specifically to each pathogen. This specificity is acquired through the expression of diverse, clonally distributed antigen receptors on T and B cells. The initial diversity is produced in

primary lymphoid organs (the thymus in the case of T cells and the bone marrow in the case of B cells) through a series of gene recombination events; further diversification occurs by somatic hypermutation of the B-cell receptor (BCR, antibody) and by functional diversification of effector T cells. T cells recognize antigens in the form of small peptides bound to major histocompatibility (MHC) class I or class II molecules.

As mentioned above, these peptide:MHC complexes are generated by APCs, notably DCs and macrophages. CD8 T cells, commonly known as cytotoxic T cells, recognize foreign peptide bound to MHC-I molecules and have evolved to monitor for and eliminate tumor cells and cells harboring intracellular pathogens. CD4 T cells, or helper T cells, recognize foreign peptides bound to MHC-II and secrete a broad range of cytokines, which play a crucial role in the maturation of the B cell response, activation of macrophages and the development and establishment of the CD8 T cell response. In contrast to T cells, B cells can recognize foreign proteins and pathogens and do not require antigen processing.

Upon antigen encounter, naïve antigen-specific B cells quickly differentiate into short-lived plasma cells that can immediately produce antibodies while others travel to germinal centers to undergo the longer process of becoming memory cells and long-lived plasma cells. B cells within the germinal centers undergo vigorous proliferation, isotype class switching (from IgM to IgG, IgA or IgE) and somatic hypermutation thereby differentiating into memory and plasma cells that produce high affinity antibodies. Naïve B cells are induced to class switch by T cell produced cytokines. Antibodies can combat extracellular pathogens by preventing them from infecting new cells or targeting them for destructions.

2. Group I: Dsdna Viruses

2.1. Adenoviruses

There are 56 immunologically distinct Adenovirus (AdV) serotypes found ubiquitously in the human population. They are sub-grouped into 7 different species (HAdV-A through G) that cause different disease conditions: species HAdV-B and C are associated with respiratory disease; HAdV-B and D are associated with conjunctivitis of the eyes; and HAdV-F types 40 and 41 as well as HAdV-G type 52 are associated with gastroenteritis. As such they have unique sites of infection: Group A primarily replicate in the intestines; Group B in the lung and urinary tract; Group C in the upper respiratory tract and occasionally the liver; Group D in the eye and intestine; Group E in respiratory tract; and Group F and G in the intestine. AdV are typically cleared following acute infection, however some can persist in lymphoid tissues (adenoids, tonsils and gut associated lymphoid tissues). AdVs are transmitted through respiratory droplets, fecal matter, fomites and close contact.

AdV structure and genome organization: AdV are medium-sized (90-100 nanometers (nm)), non-enveloped icosahedral viruses. The virus is comprised of 240 hexons and 12 penton capsomers. The penton capsomer is a covalent complex of the penton base and fiber, which acts to stabilize the capsid. Interestingly, the penton capsomer is also the weakest point in the structure, making the virus sensitive to pH, temperature, trypsin and ionic strength. The penton base has Arginine-Glycine-Aspartic acid (RGD) domains that facilitate binding to cell surface integrins and aid in viral entry via clathrin coated

pits. There are four other minor capsid proteins including IIIa, VI, VIII and IX. In addition, the viral genome is associated with 6 other viral proteins including: V, VII, Mu, Iva2, TP and the viral protease. The adenoviral genome is linear, non-segmented dsDNA of approximately 30-38 Kilobase pairs (kb) in length. There is a single copy of the terminal protein (TP) associated with each end of the viral genome. The TPs act as primers during viral replication and ensure that the virus remains linear. The virus encodes 5 early transcription units (E1A, E1B, E2, E3 and E4), two delayed early units (IX and Iva2) and one major late transcript (ML). ML is processed into 5 families of late mRNAs (L1 to L5) containing about 15 different mRNA species that are involved in capsid assembly. E1A encodes two proteins that activate viral transcription and induce the host cell to enter S-phase. E1B encodes two proteins that cooperate with E1A to induce cell growth. E2 encodes three proteins involved in DNA replication (vDNA polymerase, preterminal protein primer and DNA binding protein). E3 encodes multiple proteins that modulate the host response. E4 encodes multiple proteins involved in transcription, mRNA transport and DNA replication.

AdV replication strategy: AdVs attach to the cell membrane by binding to the Coxsackie-Adenovirus receptor, and then enter the cell via clathrin-mediated endocytosis. Partial disassembly of the virion takes place and the viral genome/core protein VII is imported into the nucleus. Host cellular RNA polymerase II transcribes all of the viral genes except VA, which is transcribed by RNA polymerase III. The first gene transcribed is the immediate early E1A gene, which following translation is imported into the nucleus where it disrupts host E2f/Rb transcription complexes thereby allowing E2f to promote transcription of the viral early genes. The virus establishes a replication center in the nucleus in the ND10 regions. Additional cellular proteins are recruited to the replication complex via AdV-E4 orf3. The early genes are then transcribed and translated, which initiates viral replication creating additional genomes that are transcribed into late genes. The immature capsids are assembled from these late proteins and viral DNA is packaged. Many of the assembled late proteins (immature virion) are cleaved by the viral proteinase L3 forming the infectious particle. The progeny viruses are released from the cell during cell lysis.

AdV vaccine and anti-viral therapeutics: There are currently no vaccines or antivirals directed against AdV infections. The US military designed vaccines against two serotypes but these have not been in production for over a decade. AdV vectors have also been used in gene therapy experiments to deliver recombinant DNA or proteins, but hepatotoxicity has hampered their widespread use. AdVs are also being considered as vaccine vectors to vaccinate against viral proteins from various infectious agents including HIV.

2.2. Herpesviruses

To date, 8 human herpesviruses (HHV) have been identified. They are categorized into alphaherpesvirus, betaherpesvirus, or gammaherpesvirus subfamilies based on genome sequence and virus biology such as host range, growth kinetics, tissue tropism, and ability to transform cells. Members of the alphaherpesvirus subfamily have a relatively short reproductive cycle, efficient destruction of infected cells, and the capacity to establish latent infections primarily, but not exclusively, in sensory ganglia. Alphaherpesviruses that infect humans include herpes simplex virus type 1 and 2 (HSV-

1, HSV-2) and varicella-zoster virus (VZV). Betaherpesviruses have a long reproductive cycle, display slow spread in culture, and can establish latency in secretory glands, lymphoreticular cells, kidneys, and other tissues. Members of the betaherpesvirus family that infect humans include human cytomegalovirus (HCMV), human herpes virus 6 (HHV-6), and human herpes virus 7 (HHV-7). Gammaherpesviruses are either T cell or B cell tropic and can establish latency in lymphoid tissue. Members infecting humans include Epstein-Barr virus (EBV) and Kaposi's sarcoma-associated herpesvirus (KSHV, HHV-8).

Herpesviruses are characterized as large (120-260 nm in diameter), enveloped particles that possess linear dsDNA genomes in the range of 120-250 kb. Herpesvirus genomes possess terminal and/or internal repeat regions that flank or interrupt unique regions containing the vast majority of open reading frames (ORFs). Herpesviruses are composed of three structural layers: 1) a nucleocapsid; 2) tegument; and 3) envelope. The icosahedral capsid is approximately 125 nm in diameter and encloses the dsDNA genome. A structured layer composed of viral and cellular proteins as well as RNAs, termed the tegument, surrounds the capsid. The tegument is enclosed in a lipid bilayer envelope, derived from modified cellular membranes and studded with viral glycoproteins.

In addition to their structural features, herpesviruses share four biological properties: 1) they encode enzymes involved in nucleic acid metabolism, DNA synthesis, and protein processing; 2) the nucleus is the site of viral DNA synthesis and assembly of capsids, but completion of the infectious virion occurs in the cytoplasm; 3) production of infectious progeny causes irreversible cellular damage or death; and 4) they enter a latent phase wherein the viral genomes become closed, circular molecules associated with chromatin. During latency only a limited subset of viral genes are expressed, which are generally not involved in viral replication and new progeny are not produced. Herpesvirus infections are life-long and virus is able to reactivate from latency over the lifetime of the host ensuring spread through the population. The immune response to herpesvirus infections is typically sufficient to prevent or suppress disease, but virus is never cleared from the infected host.

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Bibliography

David M. Knipe and Peter M. Howley (Editors), (2006) *Fields Virology* (5th edition)., IBSN-13: 978-0781760607, Lippincott Williams & Wilkins [This is a two volume classic book that has been established for the last 25 years *as the definitive virology reference*. More than 100 world-renowned investigators provide encyclopedic coverage of every aspect of contemporary virology, including the pathogenesis of

viral diseases and the molecular biology, replication, and clinical significance of all known virus families.]

Editors: S.J. Flint, L.W. Enquist, V.R. Racaniello, and A.M. Skalka, (2009) *Principles of Virology*, 3rd ed., IBSN-13: 978-1555814434, American Society of Microbiology. [This book is organized into thematic sections on the fundamental nature of viruses, their growth in cells, their interactions with the host organism and their role as agents of human disease.]

William E. Paul, (2003) *Fundamental Immunology* (5th edition), IBSN-0-7817-3514-9, Lippincott Williams & Wilkins. [This textbook has defined the field of immunology since 1984. It is a comprehensive of interest to graduate students, post-doctoral fellows, basic and clinical immunologists, microbiologists and infectious disease physicians, and any physician treating diseases in which immunologic mechanisms play a role.]

Arvin A, Campadelli-Fiume G, Mocarski E, Moore P.S., Roizman B., Whitley R., and Yamanishi K (editors) (2007). *Human Herpesviruses*: *Biology, Therapy, and Immunoprophylaxis.* Cambridge: Cambridge University Press. [This comprehensive account of the human herpesviruses provides an encyclopedic overview of their basic virology and clinical manifestations. Another important area covered within this volume concerns antiviral therapy and the development of vaccines.]

Acheson, Nicholas H,(2007) *Fundamentals of Molecular Virology*, ISBN-13: 978-0-471-35151-1, John Wiley & Sons. [The text provides a thorough presentation of basic and contemporary concepts in virology for a student's first exposure to the field. This book is also a valuable, up-to-date source of information for graduate students, postdoctoral fellows and research scientists working with viruses.] http://www.who.int. [WHO is the directing and coordinating authority for health within the United Nations system. It is responsible for providing leadership on global health matters, shaping the health research agenda, setting norms and standards, articulating evidence-based policy options, providing technical support to countries and monitoring and assessing health trends. http://www.cdc.gov. [The CDC is one of the major operating components of the Department of Health and Human Services. CDC′s Mission is to collaborate to create the expertise, information, and tools that people and communities need to protect their health – through health promotion, prevention of disease, injury and disability, and preparedness for new health threats.]

Biographical Sketches

Dr. Daniel Streblow received his Bachelor in Science in Pharmacology/Toxicology from the University of Wisconsin-Madison in 1992. He graduated from the University of Wisconsin-Madison with a Ph.D. in Viral Pathogenesis in 1997 before joining the Department of Molecular Microbiology and Immunology at Oregon Health & Science University for a postdoctoral fellowship. He is currently an Assistant Professor at the Vaccine and Gene Therapy Institute and Department of Molecular Microbiology and Immunology studying the role of CMV in organ transplant rejection. More recently, Dr. Streblow's laboratory has been investigating chikungunya infections in both mouse and rhesus macaques models.

Dr. Mark Asquith received his Bachelor's degree (BSc Biology) from Nottingham University, his Master's degree (MSc Immunology of Infectious disease) from the London School of Hygiene and Tropical Medicine and his PhD from the University of Oxford. He is currently a postdoctoral fellow at Oregon Health Science University. He has taught both undergraduate and graduate students on the subject of infection and immunity. He is a viral immunologist particularly interested in virus-host dynamics, and the differences between acute and chronic viral immune responses.

Kristen Haberthur received her Bachelor of Science in Biochemistry from the University of Nevada, Reno in 2006. She is currently a graduate student in the Department of Microbiology and Molecular Immunology at Oregon Health and Science University. She is completing her pre-doctoral thesis project in the laboratory of Ilhem Messaoudi, Ph.D., where her goal is to uncover underlying the mechanisms underlying poor control of viral infection in older persons.

Dr. Christine Meyer received her Bachelors of Science degree from the University of Washington in vertebrate Zoology. She started her career in research studying human papilloma virus and cervical cancer as a research assistant at University of Washington. She then attended and earned her Doctoral degree from Oregon Health and Science University, studying viral transcription and regulation in a rat model of human cytomegalovirus. Currently, Dr. Meyer is a post-doctoral fellow and is continuing to study viral pathogenesis in a rhesus macaque model for varicella zoster virus.

Flora Engelmann: received a bachelor of science in Botany from the University of Maine in 2001. Since graduating she worked as a quality control technician certifying exports for the FDA at the Wheat Marketing Center in Portland Oregon. In 2007, she joined the Vaccine and Gene Therapy Institute Oregon Health and Sciences University as a research assistant where she studies pathogen-host interactions during yellow fever and influenza infection in nonhuman primate models.

Dr. Ilhem Messaoudi is an assistant scientist at the center and at the Vaccine and Gene Therapy Institute, at the Oregon Health and Science University. She holds a joint appointment in the division of pathobiology and immunology at the Oregon National Primate Research Center. She received her B.Sc. in Biochemistry from Lafayette College (Easton, Pennsylvania) in 1996. She obtained a joint doctorate degree in immunology from The Weill Graduate School of Medical Sciences of Cornell University and Memorial Sloan Kettering Cancer Center in 2001. She carried out her post-doctoral training at Oregon Health & Science University, Oregon National Primate Research Center before obtaining her independent faculty position. Her research focuses on the characterization of age-related changes in immune function and the impact of these changes on viral infection with special emphasis on Flaviruses (yellow fever virus), Togaviruses (Chickungunya), Orthomyxoviruses (influenza A) and herpesviruses (rhesus rhadibovirus, simian varicella virus and varicella zoster virus).