

Translational Article

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Role of Aging on Innate Responses to Viral Infections

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Older people exhibit increased morbidity and mortality after viral infections than younger people. Additionally, vaccines are less protective in older people than in younger people. As the immune system is critical for host defense to viral infections and for vaccine efficacy, the implications are that aging negatively affects immunity. The immune system is broadly categorized into adaptive and innate systems. The innate immune system acts as a first line of defense to pathogen invasion. In this review, I focus on how aging affects the innate immune response to viral infection.

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OLDER people exhibit a higher morbidity and mortality in response to viral infections than younger people. This is particularly important for influenza viral lung infection in which older people suffer more from the consequences of influenza viral lung infection than young people (1,2). These morbid consequences include secondary bacterial pneumonia, exacerbations of chronic cardiopulmonary diseases, and cerebrovascular diseases (2). As a result, hospitalization and death rates from influenza-related illnesses are increasing in older people (1). Influenza is not the only viral pathogen that exhibits age-enhanced morbidity and mortality as older people suffer more from West Nile viral infection than younger people (3). Vaccinations are less effective in older people than in younger people including those that protect from influenza viral infections, rendering older people more susceptible from subsequent influenza viral infections than younger counterparts (4–6). Given that the immune system is a critical defense against viral infections and for effective immunity induced by vaccines, the above clinical studies demonstrate that aging negatively affects the immune response. This review will focus on how aging affects the innate response to viral infections.

THE INNATE IMMUNE SYSTEM

An effective immune response is a product of a carefully orchestrated interaction between the innate and the adaptive immune systems (7). The innate immune system acts as “the first line of defense” toward pathogen invasion and responds rapidly to the presence of microbial invasion.

Typically, the innate system responds in a consistent way to repeated infections, whereas the adaptive immune system responds slower but develops a specific response to an initial infection from a pathogen. With a repeat infection by the same pathogen, the adaptive immune system exhibits immune memory by developing a rapid and specific response to the pathogen, which is the immunological basis of vaccines.

Cellular mediators of the innate system include dendritic cells (DCs), natural killer (NK) cells, and macrophages. Myeloid DCs possess strong antigen-presenting functions by responding to microbes through upregulation of certain costimulatory molecules (eg, CD40/86), chemokine receptors (eg, CCR7), and producing inflammatory cytokines (eg, interleukin [IL]-12, IL-6, and tumor necrosis factor- α). This alters the phenotype of myeloid DCs and enhances their ability to traffic to the draining lymph nodes where they “present” microbial peptides to T cells to initiate adaptive immune responses (8). Plasmacytoid DCs are important sentinel cells that alert the body to the presence of viral infections (9,10). During viral infections, these cells produce copious amounts of the type I interferons (IFNs), cytokines that are critical for inducing antiviral responses by NK cells. NK cells sense the presence of virally infected cells as virally infected cells downregulate or alter surface expression of “self” inhibitory molecules, allowing NK cells to determine the presence of viral infection in a cell via a “missing self” process (11). Once a NK cell has established a cell is virally infected, it releases cytotoxic molecules, for example, granzyme B that kills the infected cell. Macrophages respond

to pathogen invasion by also producing inflammatory cytokines and chemokines that attract other innate effector cells, such as neutrophils, which aid in pathogen clearance. Macrophages also possess potent phagocytic properties, which enable these cells to engulf dead cells or infected debris. Hence, the innate immune system consists of a variety of different cells with specialized functions that are activated in response to pathogen invasion, including viruses.

INNATE IMMUNE RECEPTORS

Innate immune cells sense the presence of a microbe by a variety of receptors. Such receptors are transmembrane or cytosolic (12,13). There are also receptors that are secreted and bind pathogens directly, for example, collectins, which activate the complement system of inflammation after pathogen binding. The Toll-like receptors (TLR) are the best-characterized transmembrane innate immune receptors (12). These receptors are expressed either on the cell surface or within endosomes. Specific TLRs are activated by different microbial components. For example, TLR4 expressed on the cell membrane is activated by lipopolysaccharide derived from the cell wall of gram-negative bacteria, and TLR5 is activated by bacterial flagellin. These TLRs are typically present on macrophages, DCs, and also on a variety of nonlymphoid cells, such as vascular smooth muscle cells, epithelial cells, and endothelial cells. The TLRs that are principally involved in viral recognition are expressed within the endosomes of cells. For example, TLR3 is activated by double-stranded RNA viruses, TLR7 by single-stranded RNA viruses, and TLR9 by double-stranded DNA viruses. TLR7 and TLR9 are highly expressed in plasmacytoid DCs, which as stated above, are important for alerting to the presence of viral infections. For some viral infections, for example, respiratory syncytial virus, multiple TLRs, including TLR2, TLR4, TLR3, and TLR7, appear important for viral recognition and immune activation (14). Hence, TLRs are well-positioned innate immune receptors that detect the presence of microbial infections at different portals of entry.

Activation of TLRs leads to an inflammatory program that is characterized by the production of inflammatory cytokines, upregulation of costimulatory molecules chemokines, and in certain epithelial cells production of antimicrobial peptides. All TLRs with the exception of TLR3 signal via an adaptor MyD88 (15). Signaling via MyD88 leads to NF- κ B activation and translocation to the nucleus to induce the inflammatory response. TLR3 uses an alternative adaptor, TRIF, leading to the upregulation of IRF3 transcription factor (15). TLR4 is unique in that it can signal via either MyD88 or Trif, and these different signaling pathways influence the upregulation of costimulatory molecules and cytokine production differently in macrophages and DCs (16).

The cytosolic innate immune receptors include the retinoic acid-inducible gene I (RIG-I)-like receptors, termed RLRs (12,15). RLRs sense viral pathogens including single-stranded

RNA viruses and double-stranded DNA viruses (15). RLRs are expressed by a wide variety of immune and nonimmune cells. RLRs are activated by the presence of single-stranded RNA by recognizing the 5'-triphosphate component of these RNA species (12,15). RLRs also sense double-stranded RNA via a RNA polymerase III-dependent process (17). Signaling of RLRs occurs via an adaptor protein termed molecular mitochondrial antiviral signaling protein to activate an inflammatory signaling process that leads to transcription of NF- κ B and IRF3 transcription factors (18).

The Nucleotide Oligomerization Domain (NOD)-like receptors, which have recently been renamed as the nucleotide-binding domain and leucine-rich repeat containing receptors (NLRs), consist of more than 20 family members of intracellular receptors that detect pathogens and stress signals (19). These receptors are components of the inflammasomes, multiprotein intracellular complexes that promote caspase-1 activation and subsequent production of mature IL-1 β (20,21). Inflammasomes contain three domains including an N-terminal protein-interacting effector domain, a nucleotide-binding domain, and a C-terminal leucine-rich repeat domain (20). NLP3 is one of the best-characterized NLRs, and it is involved in recognizing both RNA and DNA viruses (22,23). NLP3 signal transduction occurs via the adaptor ASC (apoptosis-associated speck-like protein contains a caspase recruitment domain), which binds to caspase-1 and induces the induction of IL-1 β .

The above studies indicate that the innate system has three broad groups of receptors that alert the body to the presence of viral infections, TLRs, RLRs, and NLRs. However, activation of these pathways by a specific virus may not be mutually exclusive as exemplified by influenza viral lung infection. Prior experimental studies in mice have shown that influenza viral infection activates TLR7 within pDCs and RLRs within alveolar macrophages to induce type I IFN responses (24,25). However, neither TLRs nor RLRs appear critical for T-cell activation in response to influenza viral infection (20). Recent experimental studies in mice have demonstrated that IL-1 β and IL-18, T-cell activation, and subsequent clearance of influenza virus are dependent on NLP3 (26–28).

IMPACT OF AGING ON INNATE RESPONSES TO VIRAL INFECTION

West Nile virus is a mosquito borne RNA flavivirus that exhibits a higher morbidity in older people, which is manifested as encephalitis ([3] Table 1). A prior study demonstrated that TLR3 is involved in the immune response to this virus (36). In particular, lack of TLR3 led to *resistance* to the neurological complications of this infection (36). This study found that TLR3 led to the induction of tumor necrosis factor- α and subsequent breaching of the blood-brain barrier. A study conducted with macrophages from young and older people found that macrophages from older people exhibited elevations in several cytokines including tumor

Table 1. Viral Infections and Innate Immunity With Aging

Viral Infection	Impact of Aging	Study Design	Reference Citation
West Nile	↑ TNF- α , IL-6, and IFN- β ↓ type I IFN from pDCs	Human macrophages + in vitro infection	(29,30)
Herpes viral infections	↑ IL-17A from NKT cells and liver inflammation ↓ type I IFN from pDCs and ↓ viral clearance	Murine study + systemic infection with mCMV or HSV-2	(30,31)
Ectromelia viral infections	↓ NK cell migration to sites of infection	Murine experimental study + systemic infection	(32)
Influenza lung infection	A: ↓ CD8+ T-cell priming increased by transfer of young DCs. B: ↓ type I IFN from pDCs	A: Murine experimental lung infection model of influenza virus + adoptive transfer of young DCs. B: Human study of PBMCs infected in vitro with influenza virus	(33,34)
LCMV	Aged host environment exhibit = priming vs young host environment	Murine experimental model of systemic LCMV infection	(35)

Notes: DC = dendritic cells; HSV-2 = herpes simplex virus 2; IFN = interferon; IL = interleukin; LCMV = Lymphocytic Choriomeningitis Virus; mCMV = Murine cytomegalovirus; NK = natural killer; TNF- α = tumor necrosis factor; PBMCs = peripheral blood mononuclear cells.

necrosis factor- α , IL-6, and IFN- β during in vitro infection with West Nile virus (29). Elevated IL-6 responses to TLR activation have also been recently documented in aging baboons (37). The West Nile virus study associated the enhanced cytokine response of older macrophages with higher TLR3 expression, which may have been due to impaired signaling via DC sign, a C-type lectin receptor that regulates TLR3 expression. Thus, this study documents in human macrophages that aging leads to elevated cytokine responses to viral infection, which could potentially affect the clinical course of viral infection (29).

An experimental study in mice determined the importance of age-enhanced innate cytokine responses during viral infection (31). In a model in which mice are infected with herpes viruses (either human herpes simplex virus or murine cytomegalovirus) systemically, aged mice succumbed to viral infection, whereas young mice survived (31). The aged mice died of severe liver necrosis, in contrast to young mice that survived viral infection without any obvious liver inflammation. Additionally, aged mice died with rapidly elevated serum levels of IL-17A levels, which were predominantly produced by liver NK cells (NKT cells [31]), lymphocytes that express both NK and T-cell markers (38,39). IL-17A is a proinflammatory cytokine, which induces the migration of innate effector cells to sites of inflammation (40,41). Accordingly, aged virally infected mice exhibited higher levels of the neutrophil-attracting chemokine MCP-1 than young mice, and the livers of aged mice exhibited higher levels of neutrophil infiltration than young infected mice (31). Importantly, either IL-17A neutralization or neutrophil depletion at the time of viral infection reversed immune pathology and prevented the death of virally infected aged mice. Hence, this study determined that age-elevated IL-17A levels induced immune pathology with aging (31).

Aging impairs the ability of pDCs to produce type I IFNs in response to herpes viral infections (30), which are known to activate TLR7 or TLR9 in pDCs. In this study, it was found that aging impairs the upregulation of a critical transcription factor in the type I IFN signaling pathway: IRF-7

(30). Age-induced oxidative stress plays a role in this phenotype (30). The type I IFN defect in aged pDCs impairs the ability of aged mice to clear herpes viral infections (30). These findings have been translated to humans, as one study found that pDCs from older people produce reduced amounts of type I IFNs after activation with a TLR7 agonist than young pDCs (42). The same investigators also reported that pDCs from older people exhibited a reduced ability to upregulate IRF-7 in response to viral infection compared with pDCs from young people (43). Importantly, there is evidence in humans that pDCs from older people are less able to produce type I IFNs in response to influenza viral infection in vitro as compared with younger people (33).

Regarding the role of NK cells and viral infection, a recent study found that aged mice exhibited increased lethality and defective viral clearance to ectromelia virus, the murine equivalent of human smallpox, when the virus was administered via footpad inoculation (32). Although aged mice exhibited defective CD8+ T-cell proliferation after viral infection compared with young mice, this defect appeared to be T-cell extrinsic as aged and young CD8+ T cells exhibited similar antiviral responses when adoptively transferred into a young host environment, whereas young CD8+ T cells exhibited impaired antiviral responses in an aged environment. One of these T-cell extrinsic factors was defective NK cell responses (32). Specifically, virally infected aged mice manifested an impaired accumulation of NK cells in the footpad draining lymph nodes than young infected mice, and this was mainly due to defective migration of mature (ie, CD27⁻, CD11b⁺) NK cells (32). This defective migration was associated with reduced upregulation of CD62L (also known as L-selectin), which is an adhesion molecule that allows attachment of cells to high endothelial venules. Thus, this study documented that defective NK cell responses with aging influences the course of mousepox viral infection.

Altered myeloid DC priming function may contribute to the impaired CD8+ T-cell response to viral infection. One study found that the impaired CD8+ T-cell response to influenza viral infection of aged mice was augmented by the

adoptive transfer of young DCs (34). Transfer of young DCs into young mice did not alter CD8⁺ T-cell response to influenza viral infection, suggesting that the transfer of young DCs to aged hosts to augment T-cell responses bypassed a defect in aged DCs. However, this study did not directly compare the priming function of aged and young DCs after adoptive transfer and did not determine any potential explanation as to why DCs from aged mice exhibit defective priming. This study indicates that T-cell extrinsic factors, which would include the innate immune system, affect the course of viral infection with aging. However, one study found that the aged host environment allowed priming of young viral reactive CD8⁺ T cells to a similar degree as a young host environment during Lymphocytic Choriomeningitis Virus infection (35).

The reduced efficacy of the currently administered vaccines in older people has led to novel vaccine strategies to protect older people from viral infections. Because TLRs can act as adjuvants, one strategy is to link viral epitopes to TLR activators to enhance immune protection in older people. One specific vaccine platform that links viral epitopes to flagellin, a TLR5 activator, has been shown to induce protection in aged mice from subsequent influenza viral infection and to induce antiviral immunity safely in older people (44,45). A more in-depth discussion of novel vaccine adjuvants to enhance immune protection with aging will be the focus of a future review.

CONCLUSIONS AND FUTURE DIRECTIONS

Studies over the last 10 years have indicated a role for the innate immune system in altered responses to viral infection with aging. Although declining adaptive immunity with aging likely plays a large role in the higher morbidity and mortality suffered by older people infected with viruses, emerging studies have provided experimental and clinical evidence that the innate immune system contributes to the impaired response to viral infection with aging. Our understanding of how viruses are recognized by the innate immune system is rapidly evolving. It will be important for both future clinical and experimental studies to determine how aging affects some of these pathways, for example, the RIG-I and NLRs, as this knowledge may lead to improved or new therapies to reduce morbidity after viral infection in older people.

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