Natural killer cells: the first defense against human papilloma virus early infection

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Abstract: Natural killer (NK) cells contribute innate immunity against transformed cells. Clinical data indicate that cancer patients benefit from the adoptive transfer therapy based on vigorous NK cells. In this review, with the aim of effective prevention and quick control of human papilloma virus (HPV) caused cervical cancer, we summarized the regulatory mechanisms of HPV on NK cells, and highlighted the important role of the artificial induced NK cells in HPV early infection.

Keywords: Human papilloma virus (HPV); natural killer cells (NK cells); early infection

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Introduction

Sexually active women are at risk of human papilloma virus (HPV) infection by 80% (1). HPV infection alone does not directly lead to cervical cancer, but other factors such as smoking, long-term oral contraceptives, maternal and immune related diseases are very potent trigger. Low-risk HPVs cause reproductive tract condyloma and low-grade cervical intraepithelial neoplasia (CIN). It will take at least three years for most (90%) HPV infected patients to completely sweep out the virus owning to impressed immune response. When body immune system of a high-risk HPV carried patient is somehow inhibited or when viral genome integrates to host DNA, abnormal cell cycle regulation will occur followed by invasive cancer.

Characteristics of HPV gene and proteins

More than 180 types of HPV have been found, of which 40 types belong to the alpha genera. They invade the stratified squamous epithelium of the genital tract and some of them are closely related to cervical cancer. Nearly 70% of cervical cancer around the world is caused by HPV subtypes 16 and 18. HPV genome contains 7,900 base pairs, and outer circumference of the virus is a polyhedral shell surrounded by proteins. Eight open reading frames cover six early genes (*E1*, *E2*, *E4*, *E5*, *E6*, and *E7*) and two late genes (*L1* and *L2*). E1 prepares for viral genome replication in host cells. E2 is an early gene transcription factor and it maintains the independence of the virus genome. E4 is widely expressed in the epithelial cells, involving in viral replication and interfering cytoskeletal structure when cell differentiation so as to help virion maturation and prolapse. E5 regulates Golgi apparatus and lysosomes, and it reduces antigen presentation effect of major histocompatibility complex (MHC) class I molecular (2,3). E6 and E7 are the main carcinogens of HPV. In low-risk HPVs, E6 and E7 are inactive or at low level of transcription (4). When combined with tumor suppressor p53, E6 blocks cell cycle at phase S and cells fail to apoptosis; E7 inactivates tumor suppressor pRB and induces malignant transformation of cervical cells (5).

HPV has strict eosinophilic epithelial characteristics. It mainly invades the transitional zone of cervical vaginal squamous epithelium and cervical canal columnar

epithelium, and its life cycle is completely dependent on basal cells differentiation. After invasion into basal cells in the transitional zone, HPV DNA replicates silently. When these cells move from the basal layer to surface, virus capsid proteins will be fully expressed again and viral DNA will be packaged in. Then this complete mature virus particle will be released to extracellular fluid.

HPV immunogenicity and host immune system

It is not easy to eliminate HPV by host immune system. It can be explained as follows: (I) HPV does not induce notable systemic symptoms and viremia, and immune cells in blood circulation fail to interact with it; (II) in the early stage of infection, HPV genome is non-integrated and stable. The virus does not destroy host cells and body inflammatory response is minimized, so the virus may survive; (III) although L1 and L2 are high immunogenic, they are only expressed in surface cells in the late stage of infection; (IV) viral transcription products regulate cytokines and chemokines expression; (V) both E6 and E7 down-regulate immune related signal transduction pathways.

Major capsid protein L1 has good immunogenicity. But owing to the diversity of its amino acid sequence, it barely causes cross-reaction between HPV subtypes. One study compared amino acid sequence of L1 protein of dozens HPV subtypes, and indicated a conserved sequence containing 30 amino acid residues and an amidating enzyme interacting site (Leu-Gly-Arg-Lys) at their C terminus. Once mice and rabbits were immuned by designed short peptides based on the conserved sequence, corresponding IgG could be detected in the peripheral blood respectively. But their concentration were extremely low in reproductive tract and immune T cell proliferation effect was weak, suggesting that immunogenicity of these short peptide is weak and barely induces effective immune response (6). Currently, one of the cervical cancer preventive HPV vaccine officially licensed in Europe and the United States—*Cervarix*™ is consisted of L1 virus-like particles of HPV subtypes 16 and 18 and adjuvant system AS04. Following studies have revealed its antiviral mechanisms: (I) stimulates humoral immune response, produces virus antigen specific IgG and virus neutralizing antibody; (II) induces cellular immune response, promotes proliferation and activation of virus antigen specific $CD4^+$ T cells; (III) activates dendritic cells in transitional zone to enhance adaptive immunity. Although serum IgG neutralizes free virus, inhibits virus to adhere epithelial cells and has positive effect on virus re-infection, its low concentration in the cervical local microenvironment is unvoidable. Moreover, clinical data revealed that compliant antibodies in patients with persistent infection were actually negative. Therefore, it is thought that humoral immune status should not be used as an indicator of infection regression. Instead, "sentinel cells" [such as dendritic cells, Langerhans cells, natural killer (NK) cells and macrophages in transitional zone] in body mucosa directly eliminate infected cells at the very beginning.

NK cell, innate immune and HPV

MHC plays an important role in foreign peptide recognition and antigen presentation. Studies indicate that E7 not only inhibits MHC I molecule expression (7), but also escapes immune response of cytotoxic T cells by interacting with antigen processing associated transport protein (TAP)-1 to prevent intracellular antigen peptide delivery and following antigen presentation of HPV infected cell (8). Nevertheless, NK cells, as a key compartment of innate immune system, have extraordinary efficacy on viruses (such as herpesvirus) which specifically down-regulates MHC molecules to escape cytotoxic T cells surveillance. NK cells are biological barriers. When encounter with healthy cells expressed normal MHC molecules, they are in a resting state; when dangerous cells with abnormal expression of MHC molecules are nearby and being surrounded by enough activating ligands, they immediately identify and eliminate virus-infected transformed cells via particle dependent cytotoxicity, a target cell apoptosis pathway and antibody dependent cytotoxicity (ADCC) (9), or via cytokines and chemokines (such as IFN-γ) secretion to activate other immune cells (10). At present, there are three kinds of NK cell recognition hypothesis (11): (I) loss of self-recognition. NK cells activity is strictly regulated by inhibitory and activating receptors on the cell surface. MHC molecules of neighboring cells combine with inhibitory receptors (such as NKG2A) to inactivate NK cells. When these MHC molecules are low expressed, NK cells will be activated; (II) stress induced self-recognition. Some activating receptors recognize their corresponding ligands on infected or transformed cells. For example, constitutive expressed NK group protein 2 family member D (NKG2D), whose ligands are MHC I related gene A/B(MICA/MICB); (III) non-self-recognition. Activating receptors do not recognize endogenous ligands, but they specifically interact with foreign pathogen encoded proteins. For example, NKp46

and NKp44 bind to blood cell lectin of influenza virus and NKp30 binds to Cytomegalovirus shell protein pp65. Receptor-ligand interaction eventually activates NK cells.

Function-related receptors of NK cells are down-regulated after HPV infection (12). NKp46 and NKp30 in patients with cervical cancer and precancerous lesions were significantly decreased, which was consistent with NK cells dysfunction. NKG2D plays an important role in body resistance to pathogen invasion by interacting with its ligand soluble MICA (sMICA) to activate cytotoxic signaling pathways and to stimulate cytokine secretion. Results of *in vitro* experiments showed that when NK cells co-cultured with cervical cancer cell lines (such as HeLa, SiHa or C33A), NKG2D expression level was down-regulated. Co-incubation of above NK cells and HPV infected HeLa cells, cytocidal effect of NK cells was significantly weakened (13). Matrix metalloproteinase-dependent proteolysis induces MICA shedding from infected cell surface into sMICA. sMICA decreases NKG2D surface abundance by mediating its rapid endocytosis and lysosomal degradation, and leads to immune surveillance escape of the infected cells. Serum sMICA level will increase when cervical cancer progresses. Drugs that reduce sMICA concentration facilitate NK cells target MICA positive cells and effectively kill them. Hydralazine and valproate administration up-regulated MICA expression in cervical cancer cell line CaSki, prevented it from shedding to the cell culture supernatant and improved NK cells recognition; on the contrary, CaSki cells without drug stimulation were insensitive to NK cells (14). Moreover, HPV escapes from NK cells survelliance through immune enzyme such as indoleamine-2, 3-dioxygenase (IDO) (15). *In vitro*, RNA interference of *IDO* in CaSki cells led them more vulnerable to NK cells attack. Subcutaneous implant of IDO-knockout CaSki cells in immune efficiency BALB/c mice, more NK cells accumulated around the injection sites (16).

Cytokines interaction network is complex in innate immune system. Mutual coordination and antagonism co-exist. If one or multiple interacting nodes changed, local microenvironment would be out-of-balance and it significantly weakened cellular immune response. Results from cytokine concentration detection of cervical and vaginal lavage fluid in patients had been infected with high-risk HPVs and later became CIN or cancer showed that interleukin (IL)-12 and IFN-γ were significantly decreased (17). IL-12 is a powerful inducer of NK cells differentiation and IFN-γ production. IFN family is one of the key cytokine against virus invasion and it triggers immune system. E6 induced high expression of IL-10 was in a positive correlation with HPV persistent infection and IL-10 significantly inhibited *IFN-γ* and *IL-12* transcription (18). E7 blocked the JAK-STAT signal transduction pathway and interfered NK cells response to IFN-α followed by impaired infected cell dissolving effect (19). These data indicate that HPV indirectly affects the antiviral function of NK cells by altering cytokines distribution in lesion microenvironment.

Summary

The vaccine *Cervarix*™ indirectly induces NK cells functional phenotype through dendritic cells expressing IL-15 and tumor necrosis factor (TNF)-α (20). Another authorized HPV vaccine of cervical cancer in America, *Gardasil*™, has been confirmed that it increases local number of NK cells and up-regulates its receptor expression level such as NKG2D, NKp30 and NKp46, suggesting that NK cells function gets improved after vaccination (12). Practically, therapies based on NK cells are more economical compared to that of T cells. In our country, researchers use genetic-related NK cells to prevent hepatocellular carcinoma recurrence after liver transplantation (ClinicalTrials.gov. NCT02399735). In Germany, adoptive immunotherapy of NK cells has been applied in clinical experiments on non-small cell lung cancer patients (NCT02118415).

Traditionally, NK cells are classified into the innate lymphoid cells as they do not participate in cell reprogramming and do not produce antigen-specific receptors after pathogen stimulation. Recent studies indicate that human and mouse NK cells also behave a feature of "memory". When Cytomegalovirus, Hantaan virus or Chikungunya virus infected, the number of NKG2C positive NK cells increased sharply. Compared with naive NK cells, they produced more IFN-α to destroy infected cells (21). Researchers obtained NKG2C positive NK cells from serum of CMV^+ and CMV^- patients respectively, injected separately to recipients followed by CMV infection. It turned out that NKG2C positive NK cells stimulated by CMV were more powerful than unstimulated cells, suggesting that early sensitization induces memory NK cells (22). IL-12, IL-18 and IL-15 were added to the supernatant of NK cells culture supernatant *in vitro*, memory NK cells phenotype could also be induced (23). In addition, NK cells maturation is tightly controled by epigenetics. Expression of IFN-γ, perforin and granular enzyme are affected by NK cells methylation status. Several signal transduction molecules and transcription factor

promoters of memory NK cells were highly methylated after Cytomegalovirus infection, and their expression level were relatively low (24).

Research on NK cells compared to other immune cells is still inadequate. Recovery of valid biological function of NK cells in the early stage of cervical local lesion is vital for HPV resistance. With all progress have been made so far, we may wonder, is there any difference between high-risk and low-risk subtypes on NK cells regulation? Whether it is possible to develop HPV subtype-specific vaccine? It is a feasible direction for future vaccine development and it certainly will uncover new ways of HPV prevention and treatment.

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Footnote

Conflicts of Interest: Both authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/jphe.2016.12.06). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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