Cancer Immunology:  
From Immunosurveillance to Immunoediting

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ABSTRAK


Tanjauan pustaka ini akan merangkum interaksi antara pejamu dengan sel-sel tumor yang berakibat eliminasi, ekilibrium dan escape, yang dikenal dengan istilah 3E dari proses immunoediting.

Kata kunci: immnosurveillance, immunoescape, immunoediting

ABSTRACT

The immune system can specifically identify and eliminate tumor cells on the basis of their expression of tumor specific antigens or molecules induced by cellular stress. This process is referred to as tumor immnosurveillance, whereby the immune system identifies cancerous and precancerous cells and eliminates them before they can cause harm. The central roles of immune effector cells, such as T, B, natural killer cells (NK and NKT), IFN, perforins and granzymes have long been clarified in cancer immnosurveillance. However, despite tumor immnosurveillance, why do tumors develop and flourish in a fully functional immunocompetent host? There has been a growing recognition that immnosurveillance represents only one dimension of the complex relationship between the immune system and cancer. Recent studies has shown that the immune system may also promote the emergence of primary tumors with reduced immunogenicity that are capable of escaping immune recognition and destruction. These findings lead to the development of the cancer immunoediting hypothesis. This review will summarize the interaction of host and tumor cells leading to elimination, equilibrium and escape, which is known as the 3E of the immunoediting process.

Key words: immnosurveillance, immunoescape, immunoediting

INTRODUCTION

The immune system has three primary roles in the prevention of cancers. First, the immune system can protect the host from virus induced tumors by eliminating or suppressing viral infection. Secondly, the timely elimination of pathogens and prompt resolution of inflammation can prevent the establishment of an inflammatory environment conductive to tumorigenesis. Third, the immune system can specifically identify and eliminate tumor cells on the basis of their expression of tumor specific antigens or molecules induced by cellular stress. With the development of mouse tumor models using inbred mice with molecularly defined immunodeficiencies, it has become possible to demonstrate the existence of a cancer immnosurveillance process that can prevent primary tumor growth.

However, if tumors are immunogenic and there is immnosurveillance, why do tumors flourish in a fully functional immunocompetent host? Several studies have shown that the immune system also functions to promote

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or select variants with reduced immunogenicity, thereby providing developing tumors with a mechanism to escape immunologic detection and elimination. Cancer immunosurveillance represents only one step of the broader process, termed cancer immunoeediting, that stresses the dual host-protective versus tumor sculpting actions of the immune system in cancer. Cancer immunology is therefore the study of interactions between the immune system and cancer cells. It is a growing field of research that aims to discover innovative cancer immunotherapies to treat and retard progression of the disease. The immune response, including the recognition of specific antigens is of particular interest in this field as knowledge gained drives the development of new vaccines and antibody therapies.

**IMMUNOSURVEILLANCE**

Cancer immunosurveillance is a theory formulated in 1957 by Burnet and Thomas, who proposed that lymphocytes act as sentinels in recognizing and eliminating continuously arising transformed cells. Rapidly accumulating data have begun to elucidate the cellular basis of cancer immunosurveillance and demonstrate that lymphocytes of both the adaptive and innate immune compartment prevent tumor development. Cancer immunosurveillance appears to be an important host protection process that inhibit neoplastic nascent transformation and maintains regular cellular homeostasis. It has been suggested that immunosurveillance primarily functions as a component of a more general process of cancer immunoediting.

**EVIDENCE FOR CANCER IMMUNOSURVEILLANCE**

Since Ehrlich in 1909 first proposed the idea that nascent transformed cells arise continuously in our bodies and that the immune system scans for and eradicates these transformed cells before they are manifested clinically, immunosurveillance has been a controversial topic in tumor immunology. In the mid-20th century experimental evidence that tumors could be repressed by the immune system came from tumor transplantation models. The findings from these models strongly suggested the existence of tumor associated antigens and formed the basis of immunosurveillance. As was proposed by Burnet and Thomas lymphocytes are the most potent to eliminate transformed cells. Of those cells, NK, NKT and γδT cells are the most prominent. The discovery of NK cells provided a considerable stimulus for the possibility that they function as the effectors of immunosurveillance. This was elucidated by the study of Shankaran, which employed gene-targeted mice lacking the recombinase activating gene (RAG-2). The study demonstrated that lymphocytes expressing rearranged antigen receptors play critical roles in the cancer immunosurveillance process. Clinically, RAG-/- mice cannot somatically rearrange lymphocyte antigen receptors and therefore cannot produce peripheral T cells, B cells, NKT cells or gd-T cells, resulting in defect in immunosurveillance process. Other studies disclosed the role of αβ-T cells and γδ-T cells in immunosurveillance, which indicate that αβ-T cells and γδ-T cells regulate the tumor growth in a distinct fashion, and that the lack of γδ-T cells is not compensated by the presence of αβ-T cells and NK cells. It seems that gd-T cells act to inhibit initial tumor formation that converts to malignant progression, whereas αβ-T cells directly inhibit tumor progression using their cytotoxic mechanisms to kill tumor cells.

Furthermore there is also evidence for the role of effector components of the immune surveillance system in immunosurveillance, e.g. interferons (IFN-γ and type I interferon/IFN-α), perfan and Fas/FasL system. Endogenously produced interferon-γ was shown to protect the host against the growth of transplanted tumors and also the formation of primary chemically induced spontaneous tumors. IFN-γ had direct effects on the cell immunosurveillance and played an important role in promoting tumor cell recognition and elimination. Other studies identify the relevant cellular sources and targets of IFN-γ in immunosurveillance. Recent work suggest that γδ-T cells are important source of IFN-γ during the development of protective antitumor response. The role of IFN-γ in immunosurveillance against cancer was elucidated by a study employing mice treated with anti-interferon-γ monoclonal antibodies which blocked tumor rejection. IFN-γ cooperates with other cytokines to prevent tumor formation, e.g. with GM-CSF, IL-3 and other cytokines. Both type I interferon and IFN-γ have crucial roles in promoting host antitumor immunity. These cytokines are pivotal components in the cancer elimination phase.

Another critical effector function of cancer immunosurveillance is the immune system's ability to kill tumor cells. Early studies revealed perforin (pnp) as a critical cytolytic molecule in the primary host antitumor response. Cell mediated immunity attributed to CTLs and NK cells are derived from the granule exocytosis pathway or the Fas pathway. The granule exocytosis pathway utilizes pnp to direct the granules to appropriate locations in target cells, where they cleave critical substrates that initiates apoptosis. Mice lacking pnn (pnp/-) formed two to three times more tumors than wild type mice. The Fas/FasL system is responsible for activation-induced cell death but also plays an important role in lymphocyte mediated killing under certain circumstances. Subsequent studies revealed an important role for the TNF-related apoptosis-inducing ligand (TRAIL) and have underscored the importance of cytotoxicity manifested by innate immunity in immunosurveillance. TRAIL play an important role in apoptosis of target cells and is expressed constitutively on NK cells. TRAIL killing may be a critical link between target cell, genotoxic distress and immune mediated destruction.

Taken together, these data not only highlights roles for both innate and adaptive immune components in the eliminations of transformed cells, but also underline the complexity of the host's immune response to developing tumors. Specifically cancer immunosurveillance appears to be a multivariable process in which immunologic responses are influenced by a tumor's cellular origin, mode of transformation, anatomic locations, stromal response, cytokine production profile, and inherent immunogenicity.
Although above mentioned evidences are cancer immunosurveillance process in experimental animals, strong correlative clinical data has accumulated supporting that a similar process is also operative in humans.\(^3\) Three lines of evidence support this conclusion:\(^3\)

1. Several studies have shown that immunosuppressed transplant patients display a significant susceptibility to the formation of a variety of different cancers of non-viral origin.

2. A positive correlation has been made between the presence and location of T-cells – particularly CD8+ T cells – in a tumor (TILs) and the survival of patients with a variety of different cancers. Some of the most convincing evidence of the role of tumor infiltrating lymphocytes (TILs) in immune surveillance comes from the study of cutaneous melanoma, as reviewed by Dunn et al in 2002.\(^3\) Patients with abundant TILs survived one and a half to three times longer than patients in the absent TIL response group. The correlation has been refined to show that CD8+ T cells are the relevant lymphocyte population that affect survival.

3. Cancer patients often develop spontaneous immune response to the tumor that they carry.

**PATROLLING TRANSFORMATION**

One question concerns how cells of the immunosurveillance network distinguish transformed or established tumor cells from normal cells. Studies over the last decade has begun to reveal the molecular basis of this crucial distinction particularly within the adaptive immune compartment. Specifically, CD4+ and CD8+ $\beta$ T cells recognize tumor antigens in the context of MHC class II and MHC class I respectively. Many tumor antigens have been cloned and can be segregated into five categories: 1) differentiation antigens, e.g. melanocyte differentiation antigen, tyrosinase; 2) mutational antigens, e.g. abnormal forms of p53; 3) overexpressed/amplified antigens, e.g. HER2/neu; 4) cancer-testis (CT) antigens; e.g. MAGE, and 5) viral antigens e.g. EBV and HPV. In addition to tumor antigen presented on MHC molecules, transformed cells may overexpress other molecular signalpost that can function as recognition targets in the immune surveillance process, e.g. NKG2D ligand.\(^2\)

**IMMUNOESCAPE**

Given the existence of immunosurveillance, why then do cancers occur in immunocompetent individuals? Tumors evade immune surveillance by multiple mechanisms, including the production of factors, such as transforming growth factor-β (TGF-β) and vascular endothelial growth factor (VEGF) which inhibit dendritic cells activation and impair tumor specific T cell immunity.\(^16\)

To escape attack from NK cells and CTL, tumor cells upregulate certain surface molecules (B7-H and HLA-G), downregulate others (MHC class I and Fas), and shed surface molecules in soluble form such as macrophage inhibitory cytokines (MIC)\(^17,18\). Other mechanisms known today beside down regulation of MHC class I are lack of costimulatory molecules, defective death receptor signaling, immunosuppressive cytokines, and activation of suppressor T cells.\(^14\)

Killer group 2D (NKG2D) is a lectin like receptor on the surface of NK cells, $\gamma$δ T cells and ab CD8+ T cells that, when engaged by its ligand can activate a killer program. A variety of common human cancers expressed ligands for NKG2D collectively called MHC class 1 chain related (MIC) which are not expressed in most normal tissues. MIC tumours shed soluble MIC into the blood stream, resulting in NKG2D endocytosis and marked reduction of its surface expression on large numbers of tumor infiltrating (TILs) and blood T cells, which severely impaired their responsiveness to tumor antigens. This might be one other mechanism of immune escape of tumors.\(^19\)

Recent studies revealed that Toll-like receptors (TLRs) signaling triggers tumor self protection mechanisms leading to immune evasion. TLRs which activate innate and adaptive immune responses, are thought to be restricted to immune cells. However, Huang et al in their study revealed that TLRs, including TLR4 are expressed on tumor cells from a wide variety of tissues, suggesting that TLR activation may be an important event in tumor cell immune evasion. Activation of TLR signaling in tumor cells by LPS induces the synthesis of various soluble factors and proteins including IL-6, IFNα, IL-12, and results in resistance of tumor cells to CTL attack. In addition, LPS stimulated tumor cell supernatant inhibit both T cell proliferation and natural killer cell activity. Blockade of the TLR4 pathway by TLR4 inhibitory peptide reverses tumor-mediated suppression of T cell proliferation and natural killer cell activity in vitro and in vivo and delays tumor growth.\(^20\)

Genetic instability plays a major role in the ability of tumor cells to develop escape mutants that evade immune elimination. Numerous reports indicate that tumors escape immune elimination by the selective growth of tumor cells expressing random mutations that either initiate or silence genes through point mutations, frame shift mutations, genomic translocations, insertions or deletions. Tumor cells that escape immunity due to selective T cell pressure typically display mutations induced through genomic modifications. T cells, B cells and NK cells have been reported to apply selective pressure that results in the development of tumor escape mutants.\(^21\) Another study revealed that epigenetic silencing by methylation of the IL-12Rβ2 gene may result in a novel mechanism of tumor escape for B, ALL.\(^22\)

Tumor escape may also be due to the activation of regulatory T cells (Treg) which are CD4+CD25+ cells. It is now well established that CD4+CD25+ T cells control key aspects of immunologic tolerance to self antigens. These cells constitutively express CD25 (IL-2 receptor α-chain) on their surface and constitute 5-10% of CD4+ T cells in humans. These cells inhibit immune cell functions either directly through cell-cell contact or indirectly through the secretion of anti-inflammatory mediators, such as IL-10 and TGF-β.\(^23\)

Recent advances in cancer immunology change our thinking about immunosurveillance and immune escape into selection process. It has been thought that the immune system functions during tumor formation to select for tumor variants that are better suited to survive in an immunologically intact environment. Many studies have shown the immuno-escape effects of transplanted tumors and the generation of tumor variants with reduced immunogenicity. Tumors are immuned by the immunologic environment in which they form. This imprinting process can often results in the generation
of tumors that are better able to withstand the tumor suppressing actions of the immune system by eliminating tumor cells of intrinsically high immunogenicity but leaving behind tumor variants of reduced immunogenicity that have better chance of surviving in the immunocompetent host. The alterations that must occur during the immunologic sculpting of a developing tumor are probably facilitated by the inherent genetic instability of tumors.\(^3\)

**IMUNOEＤİTİＮG**

Classically tumor suppression has been considered to be a cell-intrinsic program mediated by pathways that involve proteins such as p53 and Rb. These pathways coordinate tumor cells from the oncogenic and or genotoxic stimuli that can lead to malignant transformation. However, the data that support the existence of cancer-immunosurveillance process illustrate that suppression of tumor growth might also be mediated by extrinsic factors, including the immune system. Additional study has shown that tumor cells that are under the pressure of immunosurveillance behave in a way that is central to tumor biology: they escape the mechanisms that operate to suppress them. The realization that this occurs was the main motivation in changing the thinking about tumor-immune system interactions and led to the development of the term cancer immunoediting to describe the process.\(^2\,3\,5\,10\)

Much work is needed to define the molecular and cellular dynamics of cancer immunoediting. The cancer immunoediting concept is divided into 3 phases, designed elimination, equilibrium, and escape, which is called the three E's. (figure 1)\(^1\,2\,3\,5\)

**ELIMINATION (reviewed by Kim et al 5)**

The elimination phase of cancer immunoediting is exactly the same process described in the initial theory of tumor immunosurveillance, whereby the immune system detects and eliminates tumor cells that have developed as a result of failed intrinsic tumor suppressor mechanism. The process of elimination includes innate and adaptive immune responses to tumor cells. For the innate immune response, several effector cells such as NK, NKT, and γδ-T cells are activated by the inflammatory cytokines, which are released by the growing tumor cells, macrophages and stromal cells surrounding the tumor cells. The secreted cytokines recruit more immune cells, which produce other pro-inflammatory cytokines such as IL-12 and IFN-γ. Perforin, FasL and TRAIL-mediated killing of tumor cells by NK cells releases tumor antigens (TA), which lead to adaptive immune responses. In the cross talk between NK and DC's, NK cells promote the maturation of DC's and their migration to tumor draining lymphnodes, resulting in the enhancement of antigen presentation to naive T cells for clonal expansion of CTLs. The TA-specific T lymphocytes are recruited to the primary tumor site and directly attack and kill tumor cells with the production of cytotoxic IFN-γ.

![Figure 1. The three Es of cancer immunoediting (Adapted from Dunn et al.)](image)

The following four phases have been proposed for the elimination process: 1) Recognition of tumor cells by innate immune cells and their limited killing. The transformed cells can be recognized by infiltrating lymphocytes such as NK cells NKT cells, γδ-T cells, which produce IFN-γ. 2) Maturation and migration of DCs and cross priming for T cells: IFN-γ exerts a limited cytotoxicity via anti-proliferative and anti-angiogenic effects and induces apoptosis. 3) Generation of TA-specific T cells. The recruited tumor infiltrating NK and macrophages produce IL-12 and IFN-γ, which kill more tumor cells by activating cytotoxic mechanisms such as perforin, TRAIL and reactive oxygen. In the tumor draining lymphnodes (TDLNs) the migrated DCs present TAs to naive CD4+ T cells that differentiate to CD4+ T cells, which develop TAs specific CD8+ T cells that lead to clonal expansion. 4) Homing of TA-specific T cells to tumor site and elimination of tumor cells. Tumor specific CD4+ T cells and CD8+ T cells eliminate the remaining TA-expressing tumor cells, but also select for the tumor cells with reduced immunogenicity.\(^5\)

**EQUILIBRIUM (reviewed by Kim et al 5)**

The next step in cancer immunoediting proceeds to the equilibrium phase in which a continuous sculpting of tumor cells produces cells resistant to immune effector cells. This process leads to the immune selection of tumor cells with reduced immunogenicity. These cells are more capable of surviving in an immunocompetent host, which explains the apparent paradox of tumor formation in immunologically intact individuals. Although random gene mutations may occur within tumors that produce more unstable tumors, those tumor cell variants are less immunogenic, and the immune selection pressure also favours the growth of tumor cell clones with a non-immunogenic phenotype. Different deficiencies of effector molecules leads to various degrees of immune selection pressure. Since the equilibrium phase involves the continuous elimination of tumor cells and the production of resistant tumor variants by immune selection pressure, it is likely that equilibrium is the longest of the three processes in cancer immunoediting and may occur over a period of many years. In this
process, lymphocytes and IFN-γ play a critical role in exerting immune selection pressure on tumor cells. During this period of selection, many tumor variants from the original are killed but new variants emerge carrying different mutations that increase resistance to immune attack.5

ESCAPE (reviewed by Kim et al 5)

Tumors escape the immune system by several mechanisms; three critical mechanisms will be discussed below.

ALTERATIONS IN SIGNAL TRANSDUCTION MOLECULES ON效应因子CELLS

Given the lack of TA recognition, which is mediated by alterations of effector molecules and which is important for the recognition and activation by the immune system, the loss of signal transducer CD3-ζ chain of TILs has been attributed to immune evasion in the cooperation of immune suppressive cytokines and local impairment of TILs. The loss of CD3-ζ is reported to be correlated with increased levels of IL-10 and TGF-β and down regulation of IFN-γ. The CD3-ζ chain is located as a large intracytoplasmic homodimer in the TCR that forms part of the TCR-CD3 complex, which functions as a signal transducer upon antigen binding. Since the TCR signal transduction through the formation of the CD3 complex is one of three important signals for initiating a successful immune response as well as the expression of tumor antigen and TCR-1 polarization, any alterations in the CD3-ζ chain that are associated with the absence of p56lck tyrosine kinase, produces the changes in the signaling pathway for T cell activation. Given that the TCR/CD3-signaling led to lymphocyte proliferation, the poor proliferative responses of TILs could be explained by the defect in TCR-ζ expression TILs underwent marked spontaneous apoptosis in vitro, which was associated with down-regulation of the anti-apoptotic bcxl and bcI2 proteins. Furthermore, because TCR-ζ is a substrate of caspase-3 leading to apoptosis, tumor cells can trigger caspase dependent apoptosis cascade in lymphocytes which are not effectively protected by bcI2.

TUMOR DERIVED SOLUBLE FACTORS. (reviewed by Kim et al 5)

A variety of tumor derived soluble factors contribute to the emergence of complex local and regional immunosuppressive networks, including vascular endothelial growth factor (VEGF), IL-10, TGF-β, prostaglandin E1, soluble phosphatidyl serine, soluble Fas and soluble MICA. Although deposited at the primary tumor site, these secreted factors can extend immunosuppressive effects into local lymphnodes and the spleen, thereby promoting invasion and metastasis. VEGF plays a key role in recruiting immature myeloid cells from the bone marrow to enrich the microenvironment as tumor associated immature DCs (TiDCs) and macrophages. Accumulations of TiDCs may cause roving DCs and T cells to become suppressed. VEGF prevent DCs differentiation and maturation by suppressing the NF-κB in hemopoetic stem cells. Blocking NF-κB activation in hemopoetic cells by tumor derived factors is considered to be a mechanism by which tumor cells can directly down-regulate the ability of the immune system to generate an immune response. The increased serum levels of VEGF in cancer patients is reported to be correlated with poor prognosis, which involves not only its angiogenic properties but also its ability to induce immune evasion leading to tumor progression.

Soluble Fas.L products also play important roles in immune evasion, by inhibiting Fas-mediated and NGK2D-mediated killing of immune cells. Soluble phosphatidyl serine acts as an inducer of an anti-inflammatory response resulting in the release of anti-inflammatory mediators such as IL-10 and TGF-β, that inhibit immune responses of DCs and T cells. Thus it is likely that tumor derived soluble factors (TDSFs) play pivotal roles in constituting immunosuppressive networks that aid tumor progression and metastasis.5

IMMUNOLOGICAL IGNORANCE AND TOLERANCE IN TUMORS

(reviewed by Kim et al 5)

A tumor immune response is regulated by tumor antigen levels and maturation stages of antigen presenting cells such as DCs. Many solid tumors express tumor specific antigens that can serve as targets for immune effector T cells. Nevertheless, the overall immunosurveillance against such tumors seems relatively inefficient. Tumor cells are capable of inducing a protective cytotoxic T-cell response if transferred as a result of the constitution of the immunosuppressive network in tumor makes it more difficult to provoke an immune activation to eliminate a single-cell suspension. However, if they are transplanted as small tumor pieces, tumors readily grew because the tumor antigen level can be modulated in the tumor microenvironment. Thus, tumor cells are surrounded by non-tumor cells, including iDCs, fibroblasts, endothelium and ECM. The ECM binds tumor antigen, and fibroblast and endothelial cells compete with DCs for the antigens, while many macrophages are downregulated, thereby allowing tumor progression. Furthermore, the stromal cells increase interstitial fluid pressure in the tumor, resulting in escape from immune attack by effector cells. In these situations, insufficient levels of tumor antigen are largely ignored by T cells, even though T cell function is suppressed by iDCs in the tumor microenvironment. In addition, iDCs stimulate CD4+CD25+ regulatory T cells, which inhibit T cell activation. Even in the presence of sufficient levels of tumor antigens iDCs inhibit the maturation of DCs and T-cell activation, resulting in immunological tolerance. It is likely that tumor immune evasion is mediated not only by immunological ignorance as a result of decreased levels of tumor antigen but also by immunological tolerance because of inhibition of T-cell activation by iDCs.5

IMMUNOEPIGENETICS: THE UNSEEN SIDE OF IMMUNEDITING

The core of immunediting theory as described above embrace the concept that the immune system on the one hand protects the body from cancer and on the other it shapes the immunogenicity of these cancers, thus presents a persuasive rationalization of the resistance of tumors against the immune response. It has been widely accepted that DNA mutations of immune genes create a rather polymorphic conditions, their frequency is much lower than that of other genetic events. Of these, epigenetic alterations gave rise to perceptible changes which can reach up to 100% per locus. Bearing in mind that cancer is characterized by a tremendous amount of epigenetic aberrations, in both gene and global level, it is reasonable to postulate that, for the same unknown
causes, analogous aberrations could affect the immune genes. Should this be the case, the relations between oncogenesis and the immune system appears more dynamic and complex. Such an epigenetic approach to carcinogenesis could improve our understanding of a series of common cancer related aspects, such as environmental risk factors, effectiveness of demethylating agents, failure of current immunotherapies, etc. The immunoepigenetic paradigm will take the current perception of the immune system and cancer interrelation further and beyond, constituting that the immunoresistant cancer cell phenotype is not shaped by the immune system acting as a steady and rigid evolutionary pressure, but rather as an extremely dynamic variable.24

SUMMARY

There is no doubt for the existence of cancer immunoediting from immune surveillance to immunoevasion. Cancer cells are gradually able to gain several mechanisms of immune evasion during tumor progression, even though they are being pursued by the initial and continuing phases of immunosurveillance. Immunological sculpting contributes to immune selection pressure, which produces tumor cell variants that are resistant to immune effector cells. In advanced cancers, the marked shifting to immunosuppressive conditions

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