Cytokine 97 (2017) 123–132

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.elsevier.com/locate/cytokine

Review article

Cytokines in immunogenic cell death: Applications for cancer immunotherapy

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ARTICLE INFO

Keywords:
Immunotherapy
Cancer
Danger-Associated Molecular Patterns (Damps)
Stress response
Chemotherapeutics

ABSTRACT

Despite advances in treatments like chemotherapy and radiotherapy, metastatic cancer remains a leading cause of death for cancer patients. While many chemotherapeutic agents can efficiently eliminate cancer cells, long-term protection against cancer is not achieved and many patients experience cancer recurrence. Mobilizing and stimulating the immune system against tumor cells is one of the most effective ways to protect against cancers that recur and/or metastasize. Activated tumor specific cytotoxic T lymphocytes (CTLs) can seek out and destroy metastatic tumor cells and reduce tumor lesions. Natural Killer (NK) cells are a front-line defense against drug-resistant tumors and can provide tumoricidal activity to enhance tumor immune surveillance. Cytokines like IFN-γ or TNF play a crucial role in creating an immunogenic microenvironment and therefore are key players in the fight against metastatic cancer. To this end, a group of anthracyclines or treatments like photodynamic therapy (PDT) exert their effects on cancer cells in a manner that activates the immune system. This process, known as immunogenic cell death (ICD), is characterized by the release of membrane-bound and soluble factors that boost the function of immune cells. This review will explore different types of ICD inducers, some in clinical trials, to demonstrate that optimizing the cytokine response brought about by treatments with ICD-inducing agents is central to promoting anti-cancer immunity that provides long-lasting protection against disease recurrence and metastasis.

1. Introduction

1.1. Cancer and the immune response

In an era of cancer treatment breakthroughs, immunotherapy emerges as a promising approach for cancers that recur and metastasize. Examples of immunotherapy include the use of monoclonal antibodies to block immune checkpoint activity, enabling anti-cancer T cell responses, and adoptive cellular therapy to prime the patient's own lymphocytes to attack cancer cells [1]. The goal of immunotherapy is to generate a robust immune response, stimulating the body's cytotoxic lymphocytes to eradicate tumor cells and ultimately achieve long-term anticancer immunity. In a typical immune response, antigens are captured by dendritic cells (DCs), which then mature and present antigenic peptide in the context of MHC molecules to T cells in lymph nodes, generating effector T cells that migrate towards sites of infection, inflammation or injury. IFN-γ and GM-CSF are central to the process of DC maturation and macrophage activation. DCs in turn release cytokines like IL-1β, IL-6, IL-12 or TNF that shape the Natural Killer cell (NK) and T cell responses. CD4+ and CD8+ T cells, with NK cells, can receive survival signals and stimulation from IL-2, leading to full effector activities, and produce additional IFN- γ. Normal immune regulation involves cytokines like IL-10 and TGF-β to limit the activity of T cells and macrophages and reduce inflammation, terminating immune responses and protecting the host from the immune-mediated damage. However, tumors hijack mechanisms of immunosuppression to evade anti-cancer immune responses, for example, preventing cytotoxic T lymphocytes (CTLs) or NKs from reaching and killing tumor cells [2]. Shifting the balance from inhibitory to activating cytokines in order to generate a protective anti-cancer response, despite tumor immune suppression, remains a major challenge for successful immunotherapy approaches.

One way that cancers evade the immune response is by being poorly immunogenic. Cancer cells can express antigens but these fail to distinguish them from tolerized self-antigens. Frequently such cancers have low mutation rates and produce few *de novo* antigens [3].

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http://dx.doi.org/10.1016/j.cyto.2017.05.024
Received 22 March 2017; Received in revised form 24 May 2017; Accepted 27 May 2017

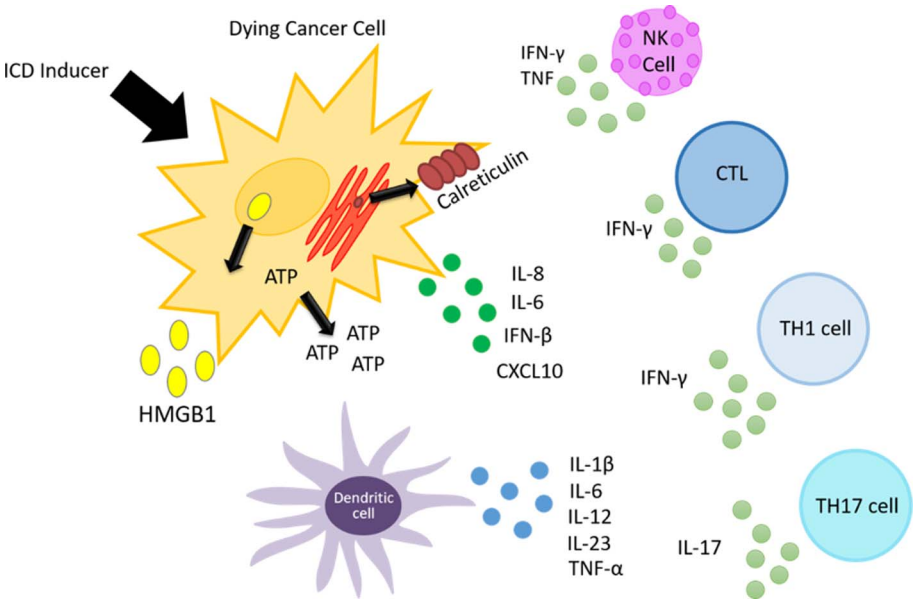

Fig. 1. Cytokines involved in ICD Induction. ICD inducers trigger the release of DAMPs (e.g. HMGB1, Calreticulin, ATP) and inflammatory cytokines from cancer cells. The DAMPs and cytokines signal to dendritic cells and Natural Killer (NK) cells that in turn release effector cytokines. T cells differentiate into cytotoxic lymphocytes (CTLs) or TH1/TH17 cells, releasing additional effector cytokines.

Examples are glioblastoma [4], ovarian cancer [5], and other cancers that lack stimulatory cancer neoantigens and/or promote an immunosuppressive tumor microenvironment by producing anti-inflammatory cytokines [2,6]. This problem is compounded by the fact that some treatments for cancer cause apoptotic cell death that may be immunologically silent and can also weaken the immune system, enabling cancer recurrence [6]. However, in the recent years, a group of chemotherapeutics has emerged that brings about a form of apoptosis known as immunogenic cell death (ICD), alerting the immune system to the presence of dying cancer cells. The induction of ICD could potentially turn these dying cancer cells into "vaccines" to stimulate anti-cancer immunity through the maturation of DCs and activation of CTLs [7] as well as enhancing the cytotoxic activity of NK cells.

1.2. The basic principles of ICD

ICD is characterized by the release of molecules with danger-associated molecular patterns (DAMPs). The DAMPs most commonly associated with ICD are membrane-bound calreticulin (CRT) and the secretion of high mobility group box 1 (HGMB1) protein from the nucleus [8]. Heat shock proteins (HSPs) 70 and 90 have also been found on the cell surface during ICD [9]. CRT, and the like, function as "eat me" signals for phagocytes like DCs, enhancing the uptake of antigen and maturation of DCs [8]. Normally, CRT is located in the endoplasmic reticulum (ER) and maintains calcium ion (Ca^{2+}) homeostasis [10,11]. Composed of 3 domains with variable affinities for calcium-binding, CRT also has a segment for retention in the ER lumen. Functions of CRT include chaperoning proteins, calcium release and storage, as well as regulation of cell adhesiveness through integrins [10]. CRT also has important immune functions, such as antigen processing and presentation as well as protection from anoikis [11]. The mechanism of CRT exposure after the induction of ICD is unknown but may involve the loss of a functional ER retention domain. While CRT is a pre-mortem signal, another DAMP triggered by ICD, HMGB1, is released post-mortem from dying or stressed cells. HMGB1 is normally found in the nucleus with some cytoplasmic localization due to shuttling. Macrophages can also secrete HMGB1, which acts in a cytokine-like manner to bind to the surface of APCs, inducing the release of proinflammatory cytokines [8,12]. When released from dying cancer cells, HMGB1 stimulates toll-like receptor (TLR) signaling, leading to protective immunity [13]. Another ICD marker, HSPs, are chaperones involved in protein folding, which can be upregulated when cells undergo stress such as heat shock, as a protective response [14,15]. While there are several families under the heat shock category, the release of HSP70 and HSP90 is principally associated with ICD. In a process less well understood, HSPs can be exposed on the cell surface and act as signals to attract phagocytes [16] and activate NK cells [17]. Another possible DAMP is the release of ATP from the cell which activates the $P2RX_7$ receptor on DCs leading to the formation of the NLRP3 inflammasome. This complex activates caspase-1 which cleaves pro IL-1β into IL-1β for secretion [18].

ICD is linked to the induction of ER stress, which can trigger a signaling network called the unfolded protein response (UPR). PERK, IRE1, and ATF6 are mediators of three different UPR pathways that are activated by phosphorylation, oligomerization or cleavage during a stress response and either prevent further stress-related damage or, under prolonged stress, cause apoptosis [19]. PERK attenuates protein translation by directly dephosphorylating the initiator of the mRNA translation machinery, eIF2, which can result in cell cycle arrest [20]. ATF6 is a basic leucine zipper transcription factor that upregulates the expression of genes encoding proteins involved in the UPR [21]. IRE1 activates the transcription factor, XBP1 (Xbox binding protein) that further induces UPR "stress" gene expression [22]. While all three pathways involve pro-apoptotic proteins, IRE-1 and PERK are best known for stimulating the JNK pathway and the Bcl-2 inhibitor, CHOP, respectively.

Treatments that *indirectly* initiate an ER stress response are considered to be type I ICD inducers, such as the anthracyclines that target cytosolic or nuclear proteins, causing ER stress as a downstream effect. Treatments that are *directly* linked to ER stress are type II ICD inducers, such as photodynamic therapy (PDT) or oncolytic viruses that target the ER to trigger cell death [8]. Cytokines detected during and after ICD induction (Fig. 1) can be pro-inflammatory, for example TNF, IL-6 and IL-1β, increasing MHC class I expression on antigen presenting cells (APCs) and promoting T cell differentiation and NK cell activation [23,24]. Dying tumor cells treated with ICD inducers can also release cytokines that modulate the immune response, such as IL-8, IL-6 and others [6,25,26], while critical effector cytokines produced by lymphocytes include IFN-γ made by TH1 cells and CTLs and IL-17 released from TH17 cells [23]. The functionality of NK cells is enhanced by cytokines produce by activated DCs, such as IL-12, and by other innate immune cells, such as IFN-α/β, leading to the secretion of IFN-γ and TNF [27]. This review highlights the immunity-promoting properties of different ICD inducers by demonstrating that the release of cytokines from activated immune cells plays a critical role in the stimulation of protective anti-cancer immune responses. A better understanding of the