Cytokine Induced Killer (CIK) Cells Based Adoptive Immunotherapy

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ABSTRACT

Cytokine induced killer (CIK) cells has been increasingly used in adoptive immunotherapy against various cancers and viral infections. This review summarizes the basic overview of CIK cells as a therapeutic immunocyte. Herein, the basic concepts on CIK cells, their general characteristics, approaches in enhancing their functions, cytotoxic mechanism of CIK cells and their therapeutic benefits in tumors and viral infections are explored.

INTRODUCTION

Immunotherapy has emerged as a promising treatment option to boost immune response to defense against diseases and infections. The two immunotherapeutic options include active and passive immunotherapy. Active immunotherapy mainly refers to vaccines, immune adjuvants and cytokines which can activate endogenous immune system. On the other hand, the latter consists of immune modulating antibody-based therapy and adoptive immunotherapy which provides or strengthen immune reaction in patients by infusing antibodies or effector cells produced *in vitro*[1].

ADOPTIVE IMMUNOTHERAPY

Over the past two decades, adoptive transfer of immunocytes has been increasingly used in treatment of cancers and viral infections[2]. The immunocytes for adoptive immunotherapy falls into two distinct categories. The first included lymphokine activated killer cells (LAK cells), cytokine induced killer cell (CIK cells) and natural killer (NK cells) that recognizes antigens in major histocompatibility complex (MHC) unrestricted manner. The other group included tumor infiltrating lymphocytes (TIL) and cytotoxic T lymphocytes (CTL) that recognize antigens presented by MHC molecules[1]. Previously, TIL and LAK used to be the best candidate for adoptive immunotherapy. However, limitations to obtain sufficient number of immune effectors cells, higher alloreactivity and inability to show antitumor property effectively attracted the researchers to seek for additional and better-tolerated strategy[3]. With the initial report of Schmidt-Wolf *et al*[4] (1991) CIK cells has been identified as a promising candidate of adoptive immunotherapy that holds all the desired properties.

CYTOKINE INDUCED KILLER CELLS AND THEIR GENERAL CHARACTERISTICS

CIK cells are *ex-vivo* expanded T cells that display phenotypic and functional characteristics of both NK cells and cytotoxic T cells[5]. As these cells were generated under the influence of cytokines and mediate the potent MHC-unrestricted cytotoxicity against various types of cancer,

they were named as "cytokine induced killer" cells. Briefly, CIK cells were generated by culturing interferon gamma (IFN-γ) activated human peripheral blood mononuclear cells (PBMC) in presence of cluster of differentiation 3 (CD3) antibody, interleukin 1 (IL-1) and IL-2 for 21 days[4,6,7]. These *ex-vivo* expanded cells were used for targeted treatment of human disease[1,8].

The CIK cells consisted of heterogeneous subsets based on surface expression of CD3 and CD56 molecules. The major population positive for both CD3 and CD56 ($CD3^+CD56^+$) (40 - 80%) exhibited MHC unrestricted antitumor activity against malignant cells lines and believed to be the type II NK-T cells[9-12]. The remaining other populations included; $CD3^+CD56^-$ (T cells, 20 - 60%), and a small fraction of $CD3^-CD56^+$ (NK cells, <10%) cells[11].

$CD3^+CD56^+$ cells are rare (1% to 5%) in human PBMC. However, enhanced increase in this major effector population was achieved during *ex-vivo* expansion[13]. Moreover, the degree of expansion of $CD3^+CD56^+$ cells vary among individual patients (mostly from three to thousand folds)[4,6,9,14]. Additionally, the $CD3^+CD56^+$ cells were derived from $CD8^+$ T cells suggesting their cytotoxic nature[9].

SOURCE FOR CIK CELLS

PBMC is given the first priority to generate these cells in humans[4,15]. However, expansion of human CIK cells from cord blood had been achieved[16,17]. Moreover, CIK cells were generated from splenocytes[18] thymus[13,19] lymph nodes[20] and bone marrow[19] of murine models.

IMPROVING CIK CELL PROLIFERATION AND FUNCTION

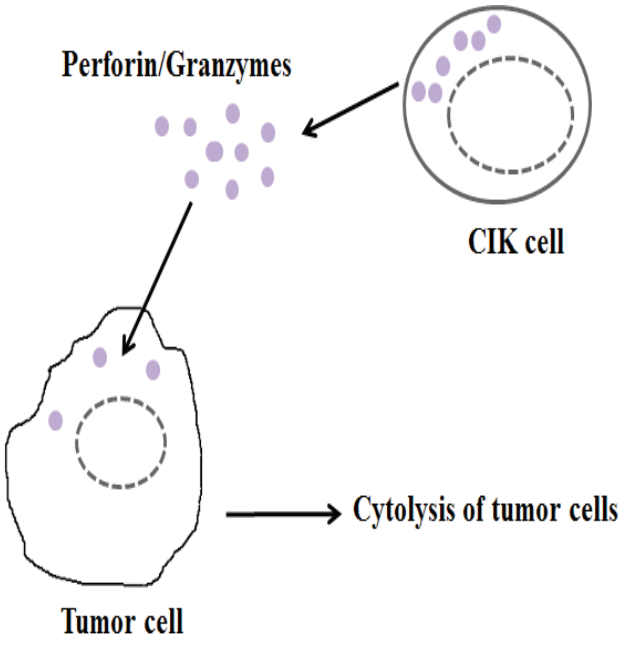
Since the first report of CIK cells by Schmidt-Wolf *et al*[4] in 1991, various groups of researchers focused on improving both the expansion and anti-tumor cytotoxicity of CIK cells. The use of cytokines other than IL-2 or the co-culture of CIK cells with dendritic cells (DCs) and even the suppression of regulatory T cells (Tregs) within the CIK cell culture are the major modification being performed[21-24]. As such, use of various cytokines and antibodies like IL-6[25] IL-15[26,27], IL-21[28], and anti-CD28[13,29]. These modifications enhanced the expansion and anti-tumor activity of CIK cells.

CIK cells primed with dendritic cells (DC-CIK cells) have been shown to promote their functions. As such, metastatic non-small cell lung cancer[30] cervical cancer[31] advanced renal cell carcinoma[32] and colorectal cancer[33] patients receiving DC-CIK cells improved the immune function, reduced the recurrence rate and prolonged the survival time. This might be due to reduced expansion of Treg cells[34]; however the detailed underlying mechanism is still unknown.

CYTOTOXIC MECHANISM OF CIK CELLS

CIK cells are endowed with potent MHC-unrestricted cytotoxicity against both syngeneic and allogenic hematological and solid malignancies. The antitumor activity is mainly associated with $CD3^+CD56^+$ fraction as this fraction of cells has higher proportion of $CD8^+$ cells. The exact mechanism involved in tumor recognition and killing by CIK cells is not completely known. However, cytotoxic granules like perforin and granzymes were proposed to be a mediator of cytolysis[35] as shown in Fig 1. It is believed that CIK cells approach the target cells by chemotaxis, and then release a number of toxins and chemicals into the medium to induce the apoptosis, necrosis and lysis of target cells. That's why in the early stage (5 hours) CIK cells induce apoptosis while in later stage (14 hours) they induce necrosis for cell lysis[36].

Fig 1: Mechanism of cytolysis of tumor cells by CIK cells (Perforin and granzymes released by CIK cells in tumor environment cause lysis of the tumor cells)



CIK CELLS IN IMMUNOTHERAPY

The dual T_H1 and NK properties that can kill abnormal cells such as cancerous cells have promoted these CIK cells as a unique immunotherapeutic approach[3,18,37]. There are

numerous research groups who have proven the efficacy and safety of CIK cells to treat a variety of cancers through clinical studies. Outcome of some of those clinical trials is as shown in Table 1.

Table 1: CIK cell based oncotherapy in phase-I clinical trials

Cancer type	Cases	Clinical Response (Cases)	Ref
Colon carcinoma; Follicular lymphoma; Renal cell carcinoma (RCC)	10	Complete response	[38]
Hepatocellular carcinoma (HCC)	13	Reduced tumor volume; Improved symptoms; Decreased HBV-DNA load	[14]
Relapsed Hodgkin's disease (HD); Non-Hodgkin's lymphoma (NHL)	9	Partial response (2); Stable disease (3)	[39]
Relapsed acute myeloid leukemia (AML); Chronic myeloid leukemia (CML); HD; Acute lymphoblastic leukemia (ALL); Myelodisplastic syndrome (MDS)	11	Complete response (3); Stable disease (1)	[40]
HCC	85	Decreased recurrence rate	[41]
Resected HCC	127	Increased DFS	[42]
Metastatic RCC; HCC	12	Complete response (3); Partial response (1); Stable disease (2)	[43]
AML; ALL	5	Partial response (1)	[44]
B-cell NHL; AML; Multiple myeloma; ALL; MDS; HD; Chronic lymphoid leukemia	18	Complete response (5)	[45]
B-cell NHL	9	Complete response	[46]
Multiple myeloma	1	Complete response	[47]

Moreover, a few studies have explored the therapeutic benefit of CIK cells against viral infection that cause mortality and morbidity in immunecompromised individuals. As such, CIK cell therapy has been shown to be effective in targeted lysis of cells infected with human immunodeficiency virus[48]. Epstein–Barr virus[49] or cytomegalovirus[50]. This antiviral activity could be due to the production of IFN-γ, TNF-α, perforin and granzyme B by CIK cells.

CONTRAINDICATIONS OF CIK CELL IMMUNOTHERAPY

There are many clinical studies using the CIK cells as immunotherapy. Till date, no severe side effects have been reported after CIK treatment. The most common side-effects were fever, chills, headache, rash, nausea, and vomiting occurring during or after transfusion. These could easily be treated with symptomatic therapy in case they did not resolve on their own within 24 hours[51,52]. This indicates that CIK therapy is safe for clinical application although larger population cohorts should be investigated.

CONCLUSIONS

CIK cells were used as an adoptive immunotherapy against various cancers and some viral infections. However, the effectiveness varies with different clinical settings, mainly due to different approaches followed in their generation. Hence, further studies are warranted to enhance the expansion and cytolytic function of the CIK cells. Moreover, the beneficial use of CIK cell in various viral infections needs to be investigated.

Competing interests

We declare no competing interest.

Financial disclosure

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