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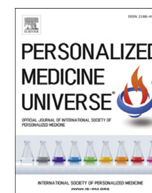
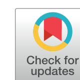


Original article

Assessing antitumor and T cell immune responses by cytokine assay in cancer patients treated with immunotherapy – A pilot study



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ABSTRACT

Clinical evaluation methods for therapeutic effectiveness based on scientific evidence are desired as tools for contributing to therapeutic strategies and advancements in cancer immunotherapy research. By quantitatively measuring multiple immune response factors responsible for T cell activity and cytotoxic activity, we can be expected to evaluate a patient's immune response. We used plasma samples of human peripheral blood and compared the T cell and antitumor immune response by measuring various immune related cytokines (TNFα, sCD137, IL-2, IL-5, INFɣ, Perforin, Granzyme A, Granzyme B, IL-10, IL-4). In this pilot study, we compared samples from healthy donors with those from cancer patients treated with multivalent dendritic cell (DC) vaccines and natural killer (NK) cell immunotherapy before and after treatment. We examined the usefulness of this method as a biomarker to assess the therapeutic effectiveness of immune cell therapy by statistical analysis and by plotting a cancer immunogram correlating patient data with clinical outcomes.

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1. Introduction

The process of an immune response against cancer cells, much like in the manner against an infection, is created by signaling between cells. Various cytokines play a key role in these signals for the immune cells to communicate and to be activated. Cytokines and other immune-related proteins are classified into interleukins (mediating between T cells), chemokines (inducing migration of T cells), and cytotoxic mediators (destruction of target cells by cytotoxic T lymphocytes (CTL) and NK cells). In many clinical studies, it is suggested that even very low levels of cytokines contribute to immune responses against infected and cancerous cells in the preclinical stages [1–5]. The central role in cytotoxic activation is conducted by Perforins (forming pores in the target cell membrane) and Granzymes (proteases into the target cells). Targeted cells such as cancer cells are attacked by Perforins and Granzymes, resulting in apoptosis and cell death.

Cancer is currently the number one cause of death in Japan and there is a varied approach to treatment methods [6]. Recently, in addition to conventional or standard therapies to cancer, immunotherapy, exploiting the bodies' own immune system to attack cancer cells, has been in the limelight. In our study, we wished to know if we could measure an immune response in patients who had received multivalent DC vaccines along with NK cell therapy. DCs are the most powerful antigen presenting cells (APC) of the immune system, and mature DCs that have been presented with tumor antigens are capable of stimulating both CD4+ helper T (Th2) cell immune responses and antigen specific CD8+ CTL tumor specific responses [7]. DC vaccines are generated from peripheral blood mononuclear cells (PBMC) and then pulsed with multiple selected over-lapping long peptide tumor antigens in order to create a personalized multivalent DC vaccine that is administered intradermally, every 2–3 weeks for a total of 4–5 times, closest to a lymph node where it migrates and interfaces with Th2 cells and CTLs for signaling [8]. In order to deal with tumor heterogeneity and downregulated expression of MHC class I molecules on the cell surface of tumor cells, NK cells which have direct cytotoxic activity on tumor or non-self cells [9] is also used synergistically in this hybrid immunotherapy treatment.

The immune system is constantly battling to eliminate abnormal and cancerous cells dynamically in vivo. The cancer-immunity cycle is a concept that shows this mechanism as a series of seven steps: 1)

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releasing of cancer cell antigens, 2) antigen presentation, 3) priming and activation, 4) trafficking of T cells to tumors, 5) infiltration of T cells into tumors, 6) recognition, and 7) killing of cancer cells [10] (Fig. 1). By quantitatively measuring, with high sensitivity, the cytokines that are responsible for each of the T cell responses and cytotoxic activity in this cycle, we wished to evaluate the immune responses induced by cellular immunotherapy.

2. Methods and study selection

This observational study was performed in accordance with the amended Declaration of Helsinki. The protocol for the present study was approved by the Ethics Committee of the Hakushin Koseikai Medical Foundation. Written informed consent was obtained from all participants included in the present study.

2.1. Patient selection

Plasma samples were collected from human peripheral whole blood. 12 samples were from cancer patients with the following types of cancer: pancreas, breast, large intestine, and stomach, and 4 samples were from healthy donors. All were obtained between March 2016 and November 2017. The cancerous pathological stages were classified according to the TNM classification of malignant tumors [11] and were obtained from patient records (Table 1).

2.2. Sample preparation

Plasma samples were obtained with an anti-coagulant from blood in the usual manner within 12 h after blood collection and stored at −20 °C or lower. Freezing and thawing was avoided more than twice, and the stored sample was thawed at 4 °C, mixed well and then centrifuged at 14,000 rpm for 10 min to remove particulates.

2.3. Detection and analysis of plasma cytokines

Immune responses were detected by using multiplex assays MILLIPLEX® MAP Human Cytokine/Chemokine panel (Catalog No. HCD8MAG-15K17PMX, Merck Ltd.). The following immune related cytokines were detected: TNFα, sCD137, IL-2, IL-5, INFɣ, Perforin, Granzyme A, Granzyme B, IL-10, IL-4. Each were tested for quality control and standardization, according to the kit recommended protocol. Each sample quantity used was 25μL/well, run in duplicate. The panel was analyzed using Luminex® 200 equipment and xPONENT® 3.1 software. Measured concentrations of cytokines were found in each sample, and the mean value was reported.

2.4. Statistical analysis

Statistical analysis was carried out by student's *t*-test using JMP® software. Using the mean resultant measured concentrations of cytokines found in each sample, we used this data as a base to plot our version of a cancer immunogram [13] for the cancer-immunity cycle.

3. Results

3.1. Measured immune related cytokines in plasma

We measured immune related cytokines in the plasma of both healthy donors and cancer patients. The high performance of this particular assay compared to others was supported by several academic papers; we found that it performed to optimization and evaluation, and displayed good precision and reproducibility [14,15]. We also found that it was able to pick up a broad range of measured concentrations, with a minimum concentration of 0.31 pg/mL and a maximum of 36149.35 pg/mL.

We compared the measured concentrations of the following cytokines (TNFα, sCD137, IL-2, IL-5, INFɣ, Perforin, Granzyme A, Granzyme B, IL-10, IL-4) from healthy donors and cancer patients before and after treatment. Plasma samples were differentiated by four different immunotherapy treatment categories groups 1) healthy donors 2) cancer patients before treatment 3) cancer patients after treatment and 4) cancer patients 2–3 months after treatment ended in order to investigate the T cell immune responses (Table 2, Fig. 2)

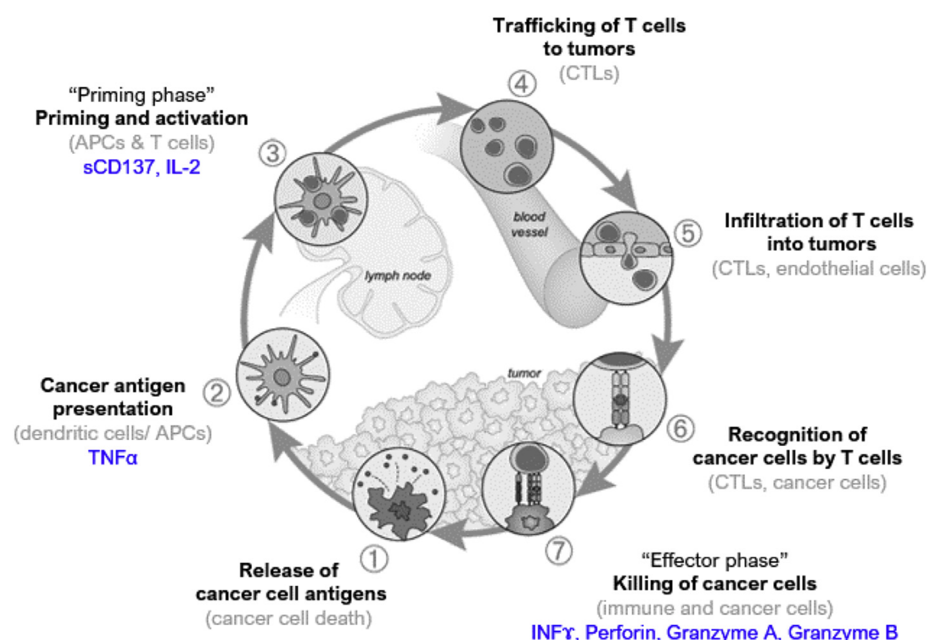

Fig. 1. The cancer-immunity cycle and stimulated cytokines. Based on Chen DS, Mellman I. Oncology meets immunology: the cancer-immunity cycle. Immunity. 2013.

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