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Phagocytic activity of neutrophil is induced by granulocyte colony stimulating factor and interleukin-15 in leukemic animal model

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Abstract

In leukemia, secondary infection is common and after chemotherapy the number of normal active neutrophil reduced significantly. Therefore, to avoid side effects of chemotherapy and to induce neutrophil activity and number, in this study, we have treated with G-CSF plus IL-15 in combination to N−N′ Ethylnitrosourea (ENU) induced leukemia in BALB/c mice. After 5 months of ENU treatment in 3−4 weeks old mice at 80 mg/kg body weight, leukemia was confirmed by histology of blood smear co-treatment started with cytokines for 5 days. The *in vitro* phagocytosis activity of neutrophil from spleen was assayed using Dalton's lymphoma ascites as target cell. Using real time polymerase chain reaction (RT-PCR), Toll like receptor 4 (TLR4) and toll like receptor 9 (TLR9) expression of neutrophil was also measured. Our data suggests that G-CSF plus IL-15 induce the phagocytic activity of neutrophil. TLR4 and TLR9 expression was induced in neutrophil after treatment with cytokines together which was significantly reduced in leukemia. In conclusion, G-CSF plus IL-15 has potential role as therapeutic immunomodulators for neutrophil activity in leukemia.

Keywords: Leukemia; Granulocyte colony stimulating factor (G-CSF); Interleukin-15 (IL-15); N−N′-Ethyl-Nitrosourea (ENU); Toll like receptor 4 (TLR4); Toll like receptor 9 (TLR9)

1. Introduction

Leukemia is a type of cancer of the blood or bone marrow characterized by an abnormal increase of immature white blood cells. These immature cells accumulate in blood and organs but all of them are not able to carry out the normal functions of blood [1]. Due to increase the abnormal blood cells in the peripheral blood the normal function of normal immune cells are altered.

Specific doses of ENU, also known as N-ethyl-N-nitrosourea (chemical formula $C_3H_7N_3O_2$), can induce mixed type of leukemia (both the myelogenous and lymphogenous) [2]. Neutrophils, the most abundant immune cell in blood quickly arrive at sites of infection and form the first line of defense following infection. The key role of neutrophils is the antimicrobial effector functions and the ability to produce cytokines to initiate inflammatory responses and chemokines to induce trafficking of immune cells [3].

During the beginning (acute) phase of inflammation, particularly as a result of bacterial infection, environmental exposure [4], and some cancers [5], neutrophils are one of the first-responders of inflammatory cells to migrate towards the site of inflammation. In leukemia, the immune system is in depressed condition and the infection by different microorganism in that condition, is prevented generally by the neutrophils. In acute leukemic condition the number of neutrophil decreased significantly [6]. Neutrophils migrate through the blood vessels, then through interstitial tissue, following chemical signals such as Interleukin-8 (IL-8) and C5a by the process called chemotaxis [7]. The neutrophils in chronic myeloid leukemia (CML) exhibit defects in several functions [8].

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The innate immune system has evolved to discriminate between self and foreign pathogens through a process that relies, to a great extent, on an evolutionary conserved family of pattern recognition receptors, including TLRs. At least 13 TLRs have been identified in mammals to date and their ligands are predominantly pathogen-associated molecular patterns, a limited set of conserved molecular patterns that are unique to microbes [9]. TLRs are type 1 transmembrane receptors that play important role in innate immune recognition of pathogens [10]. Recognition of conserved molecular patterns found on microbes by these invariant, germ line encoded receptors leads to a signal transduction cascade that results in cellular activation and cytokine release in both immune and non-immune cells.

Granulocyte colony stimulating factor (G-CSF) is a lineage specific hematopoietic growth factor that initiates the differentiation and proliferation of committed progenitor cells into mature neutrophils. G-CSF exerts its proliferative effect mainly at the stage of myeloblast-promyelocyte, and also stimulates the release of mature bone marrow neutrophils from storage pools into the peripheral circulation [11]. In vivo studies of G-CSF administration reveals enhanced adhesion on nylon wool, phagocytosis, luminal enhanced chemiluminescence, degranulation and expression of cell surface antigens [12]. One study also showed promotion of neutrophil survival *in vitro* [13]. After allogeneic stem cell transplantation the patient with invasive fungal disease also improved with G-CSF treatment [14].

IL-15 can induce phagocytosis, cytoskeleton rearrangement, gene expression, *de novo* protein synthesis and can delay apoptosis in human neutrophils [15]. Production of chemokines, cytokines and natural inhibitors is increased in IL-15 induced neutrophils, including CXCL8 (IL-8) [16], IL-1beta, IL-1RII and IL-1Ra [17]. IL-15 has also been shown to induce the redistribution of ICAM-3 and p-selectin glycoprotein ligand-1 (PSGL-1) to the uropods in neutrophil [18]. The mechanisms involved in IL-15-induced activation of human neutrophils are not fully understood. However, IL-15 was shown to activate NFk-β [19], and to induce the phosphorylation of Syk and its physical association with IL15R-α [20].

As the combined treatment approach with IL-15 and G-CSF to leukemia animal has not been well studied to evaluate their immunological response, our focus of this study was to evaluate the neutrophil immunological activity such as phagocytic activity during pre and post treatment with combinations of cytokines in ENU induced leukemia model. The molecular changes like the expression of pattern recognition molecules like TLR4, TLR9 and alteration of PTK activity in both the conditions were evaluated.

2. Materials and methods

2.1. Materials

Nitroblue tetrazolium (NBT) Hydrocloric acid (HCL), and Pyridine were purchased from SRL chemicals, India. Lipopolysaccharide (LPS), and N-ethyl-N′-nitrosourea (ENU) were purchased from Sigma Aldrich, USA. Leishman's stain was purchased from Loba chemicals, India.

Dalton's lymphoma ascites are the kind gift from Dr. Nabyendu Murmu, CNCI, Kolkata India. RPMI-1640, Fetal Bovine Serum was purchased from Hi-media, India. Recombinant mouse interleukin-15 (rmIL-15), recombinant mouse granulocyte colony stimulating factor (rmG-CSF) were purchased from ImmunoTools, Germany. Percol was purchased from GE Health Care Life Sciences, Uplasa, Sweeden. PureZOL™ RNA isolation reagent, iTaq™ universal SYBR® Green Supermix, kit, iScript™ c-DNA synthesis kit were purchased from BioRad, USA.

2.2. Animals

Healthy BALB/c male mice were obtained from National Centre for Laboratory Animal Sciences, (NCLAS), NIN, Hyderabad, India. Animals were divided into four groups having six animals in each group. Groups were divided as Mock control, ENU challenged, and control along with combination cytokine treatment and ENU along with combination cytokine treatment. These mice were kept and maintained specific pathogen free condition in Tripura University Animal House as per guidelines of Institutional Animal Ethical Committee. Food, dietary supplements and water were provided *ad libitum*.

2.3. Induction of leukemia

We have induced leukemia previously in our lab following the reference of Law et al., 2003 and detailed protocol has been published [21]. In short leukemia was induced by injecting N-ethyl-N′-nitrosourea (ENU) intraperitoneally (i.p.) to 7−10 days old mice, at the dose of 80 mg/kg body weight concentration at two times in one week interval. The peripheral blood and bone marrow slides were prepared for both control and treated group and stained with Leishman's stain. Total count and differential count of leukocytes were also done in all the four groups to confirm leukemia [21].

2.4. Cytokine treatment

ENU treated groups were followed up for leukemia induction after 4 months in one week interval. After 5 months of ENU injection leukemia was confirmed by peripheral blood smear observation. The two cytokines, rmG-CSF at the dose rate of 10 μg/kg body weight [22] and rmIL-15 at the dose of 5 μg/kg body weight [23] were injected (i.p.) in animals of one control and one ENU treated group after confirmation of leukemic induction for consecutive five days.

2.5. Isolation of splenic neutrophils

Neutrophils were isolated from the spleen as previously described [21]. In short, the splenic suspension was made by smashing the spleen gently by two frosted glass slides in a