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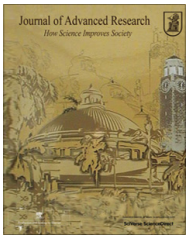

ORIGINAL ARTICLE

Acute inflammation induces immunomodulatory effects on myeloid cells associated with anti-tumor responses in a tumor mouse model

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ABSTRACT

Given the self nature of cancer, anti-tumor immune response is weak. As such, acute inflammation induced by microbial products can induce signals that result in initiation of an inflammatory cascade that helps activation of immune cells. We aimed to compare the nature and magnitude of acute inflammation induced by toll-like receptor ligands (TLRLs) on the tumor growth and the associated inflammatory immune responses. To induce acute inflammation in tumor-bearing host, CD1 mice were inoculated with intraperitoneal (i.p.) injection of Ehrlich ascites carcinoma (EAC) (5×10^5 cells/mouse), and then treated with i.p. injection on day 1, day 7 or days 1 + 7 with: (1) polyinosinic:polycytidylic (poly(I:C)) (TLR3L); (2) Poly-ICLC (clinical grade of TLR3L); (3) Bacillus Calmette Guerin (BCG) (coding for TLR9L); (4) Complete Freund's adjuvant (CFA) (coding for TLR9L); and (5) Incomplete Freund's Adjuvant (IFA). Treatment with poly(I:C), Poly-ICLC, BCG, CFA, or IFA induced anti-tumor activities as measured by 79.1%, 75.94%, 73.94%, 71.88% and 47.75% decreases, respectively in the total number of tumor cells collected 7 days after tumor challenge. Among the tested TLRLs, both poly(I:C) (TLR3L) and BCG (contain TLR9L) showed the highest anti-tumor effects as reflected by the decrease in the number of EAc cells. These effects were associated with a 2-fold increase in the numbers of inflammatory cells expressing the myeloid markers $CD11b^+$-$Ly6G^+$, $CD11b^+Ly6G^-$, and $CD11b^+Ly6G^-$. We concluded that Provision of the proper inflammatory signal with optimally defined magnitude and duration during tumor growth can induce inflammatory immune cells with potent anti-tumor responses without vaccination.

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Introduction

For many years, treatment of cancer was primarily focused on surgery, chemotherapy and radiation, but as researchers learn more about how the body fights cancer on its own, antitumour immunotherapies have been developed. With this regard, recent preclinical and clinical studies have been focusing on designing antitumor treatment strategies based on induction of specific anti-tumor immune responses [1]. Unfortunately, however, these immunotherapeutic approaches have not reached the optimal efficiency against tumor [2]. In addition, they require the identification of certain tumor antigens and tumor-reactive T cells, which are not available in many of cancer settings. As such, immunotherapeutic approaches that depend on induction of non-specific immune responses could be advantageous to the approaches since they do not need requirements. Therefore, exploring and developing non specific immunotherapies is of paramount significance in the clinical application of cancer therapy.

One approach for non specific immunotherapy could be by the induction of inflammation in particular acute inflammation with agents that code for danger signals [3]. Microbial products, which bind to toll like receptors (TLRLs) on immune cells in general and innate immune cells in particular, are the optimal candidate to induce acute inflammation since they code for danger signals that are known to activate immune cells [4]. TLRL are a class of transmembrane signaling proteins that play a critical role in the innate and adaptive immune response against invading pathogen by recognizing various protein, carbohydrates, lipids, and nucleic acids of invading microorganisms [5]. They are expressed by different types of leukocytes or other cell types [6,7]. TLRL expression profiles differ among tissues and cell types. TLRL are predominantly expressed on antigen-presenting cells (APCs), such as macrophages or dendritic cells, and their signaling activates APCs to provoke innate immunity and as a consequence adaptive immunity [8,9]. TLRL are mainly located on the plasma membrane with the exception of TLR3, TLR7 and TLR9 which are localized in the endoplasmic reticulum (ER) [8–10].

Mammalian TLRL include a large family consisting of ten to thirteen different types of toll-like receptors named simply TLR1 to TLR13. To date, ten human and thirteen murine TLR have been identified, TLR1–TLR9 are conserved between the human and mice [11]. However, there are TLRL found in humans and not present in all mammals, for example, TLR10 in humans is present in mice [12]. It has been found that each TLR has been shown to recognize specific microbial component and that TLR have common effects, including inflammatory cytokine or up-regulation of co-stimulatory molecule expression, but also have their specific function such as production of IFN-β [13]. TLR are substances that bind to and activate TLR. The latter constituent in different types of organisms at the cell surface or at the internal cell compartments.

The most common TLRLs that have been used in induction of potent acute inflammation is poly(I:C) which is a synthetic double-stranded RNA that mimics virus and binds to TLR3 [5]. Poly-ICLC (Hiltinol®) is a clinical grade of poly(I:C) which is a synthetic, nuclease-resistant, hydrophilic complex of poly(I:C) and stabilized with poly-L-lysine and carboxymethyl cellulose [14]. BCG is an inflammatory signal to macrophage, lymphocytes, granulocytes, and dendritic cells [15]. It contains cytidine phosphate guanosine (CpG) which is known to bind to TLR9 [16]. BCG can be used alone or integrated into IFA to form CFA.

EAC cells increased via rapid cell division during the proliferating phase and in the load peritoneal cavity. Ascites fluid accumulation occurred in parallelism with the proliferation of tumor cells [17].

In this study, we aimed to determine the impact of the nature, magnitude, and timing of different inflammatory stimuli on the host anti-tumor activity. Our hypothesis is that provision of the proper inflammatory signal with optimally defined magnitude and duration during cancer growth can induce inflammatory cells with potent anti-tumor responses leading to significant decreases in tumor growth even in the absence of vaccination.

Material and methods

Mice

All experiments were carried out on adult female Swiss albino mice 20 g and aged between 8 and 16 weeks. The mice were purchased from Theodore Bilharz Research Institute, Giza, Egypt. Mice were acclimatized at least two weeks before experimentation and randomly divided into the experimental groups, ten or twelve mice for each. Mice were maintained at regular light and dark cycles, and provided with standard food and water *ad libitum*. *This work was conducted based on the guidelines for the use of experimental animals in research at Department of Zoology, Faculty of Science, Tanta University, Egypt.*

Tumor cells

All experiments in this study were performed using the breast tumor cell line Ehrlich ascites carcinoma (EAC). EAC is a transplantable, poorly differentiated malignant tumor which appeared originally as a spontaneous breast carcinoma in a mouse. It grows in both solid and ascitic forms [18]. The parent cell line was purchased from The National Cancer Institute, Cairo University, Egypt. The tumor cell line was maintained by serial intraperitoneal (i.p.) transplantation of 2.5×10^6 viable tumor cells in 0.3 ml of saline into female swiss albino mice (8–10 weeks old).

Reagents

Polyinosinic-polycytidylic acid (poly(I:C)), purchased from Sigma Chem. Co., (St. Louis, Mo., USA), was stored at 4 °C in dark until use. Poly(I:C) was dissolved in saline (0. 9%). Poly-ICLC is kindly gifted by Dr. Salazar Andres (Oncovir, Washington, DC, USA). All reagents were obtained in suspension form and stored at 2–8 °C. Poly-ICLC was diluted in saline (0.9%). Complete Freund's adjuvant (CFA) was purchased from Sigma to Aldrich, USA. Incomplete Freund's Adjuvant (IFA) was purchased from Sigma Aldrich, USA. Bacillus Calmette Guerin (Immune BCG-T) was purchased from the vacsera company, Giza, Egypt. It is a suspension of a live attenuated mycobacterium *Bacillus calmette Guerin* is a stabilizing medium. For injection each vial containing 90 mg/3 ml was suspended in 50 ml (0.9%) saline.