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Flow cytometric detection of CD79a expression in DMBA induced mammary tumour treated with withaferin a in female Sprague-Dawley rats

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Abstract

The present study was designed to examine the expression of CD79a, total B lymphocytes in DMBA (7,12-dimethylbenz[a]anthracene) induced rat mammary carcinogenesis by flow cytometry. Twenty four female Sprague-Dawley (SD) rats were equally distributed to control, DMBA, DMBA+Tamoxifen (Standard drug) and DMBA+Withaferin A groups. Peripheral blood was collected at the 120th day from the initial dose of DMBA and CD79a, total B lymphocytes were determined by using flow cytometry. There was a significant increase in the total B lymphocytes (CD79a) in the DMBA+Withaferin A group from the DMBA+Tamoxifen group indicating the immunostimulatory effect of Withaferin A.

Keywords: CD79a B lymphocytes, DMBA, mammary carcinogenesis, withaferin A

Introduction

CD79a is one of the common and useful B-cell markers for identifying B- cell lineage in malignant neoplasms due to its structural and functional relationship with the B-cell antigen receptor and it is highly specific for B-cells (Mason *et al.*, 1991) ^[11]. It is expressed both in the earliest stage of B- cell development and also in the late stage of B-cell differentiation i.e. plasma cells (Dworzak *et al.*, 1998, Sakaguchi *et al.*, 1988 and Mason *et al.*, 1995) ^[5, 12, 11].

Withaferin A is a biologically-active steroidal lactone that is mainly localized in the leaves (Gajbhiye *et al.*, 2015) ^[7] of the ayurvedic medicinal plant *Withania somnifera* (also known as Ashwagandha, Indian ginseng or Winter cherry). Its structure resembles aromatic isothiocyanates, which are highly promising cancer chemo preventive constituents of cruciferous vegetables.

Withaferin A is known to possess therapeutic properties including anti-inflammatory, anti-angiogenic and antitumor effects (Vannden Bergh *et al.*, 2012) ^[4] but known to be a potent immunosuppressor (Furmanowa *et al.*, 2001) ^[6]. An attempt was made to study the immunomodulatory effect of Withaferin A in experimental mammary carcinogenesis and find out the expression of CD79a, total B-lymphocytes in DMBA induced mammary tumour.

Materials and Methods

Twenty four virgin female Sprague-Dawley rats (38-days-old) were obtained from the National Institute of Nutrition, Hyderabad and acclimatized for 5 days in a controlled environment under standard conditions of temperature (22±3 °C) and humidity (50±10%) with an alternating 12h light/dark cycle. The rats were randomized into four groups (n=6) with mean body weight (g) variation not exceeding 10%. All the treatments were initiated at the age of 43rd day. Animals in group 1 received basal diet and served as control. Animals in group 2 were given four weekly doses of 7,12-dimethylbenz[a]anthracene (DMBA) (5mg DMBA/rat/week) dissolved in olive oil by intragastric intubation. From the day of the first dosing of DMBA, animals in group 3 received tamoxifen (100 µg/kg BW/day/*per os*) dissolved in gingelly oil and group 4 animals were administered Withaferin A dissolved in PBS (16 mg/kg BW/thrice a week/*per os*) till the end of the 16-week study. This study was carried out after the approval of the Institutional animal ethical committee (IAEC), Madras Veterinary College (MVC), Chennai-07, India and as per the guidelines of the Committee for the Purpose of Control and Supervision of Experimentation in Animals (CPCSEA),

Government of India. Blood samples were collected on the 120th day from the lateral tail vein in EDTA vacutainers. Lymphocytes were separated from the whole blood by a density gradient centrifugation method (Boyum, 1968)^[2] using Histopaque-1083 (catalogue No. 1083-1, M/s. Sigma Aldrich Inc., USA). The cells were washed with PBS and resuspended in such a way that 10 μ L of PBS contains a cell density of approximately 10^6 cells/mL. This was followed by incubating this cell suspension with 10 μ L of mouse-anti-human CD79a-FITC monoclonal antibody (Clone: MB1, Ref. No. MCA2538PET) conjugated with lyophilized R. Phycoerythrin (RPE) (AbD Serotech, UK) in the dark and on ice for 1 h. The cells were washed with PBS and the readings

were taken using a flow cytometer (BD Biosciences, USA). The data generated were subjected to one-way analysis of variance (ANOVA) test using SPSS software version 20 for windows.

Results and Discussion

The percentage of total B lymphocytes (CD79a) in the peripheral blood of the control, DMBA, DMBA+Tamoxifen and DMBA+Withaferin A treated groups of SD rats are presented in Table-1. Flow cytometric analysis of the blood showed a significant ($P < 0.05$) increase in the CD79a total B lymphocytes in the DMBA+Withaferin A treated group (Fig. 1).

Table 1: Percentage of total B lymphocytes (CD79a) in the peripheral blood of control, DMBA, DMBA+ Tamoxifen and DMBA+ Withaferin A treated SD rats (Mean \pm SE)

S. No.	Groups (n=6)	CD79a (%)
1	Control	1.67 ^{ab} \pm 0.76
2	DMBA	2.31 ^{ab} \pm 0.70
3	DMBA+ Tamoxifen	0.93 ^a \pm 0.25
4	DMBA+ Withaferin A	5.39 ^b \pm 2.05

Mean with same superscripts with in a column do not differ from each other ($P < 0.05$)

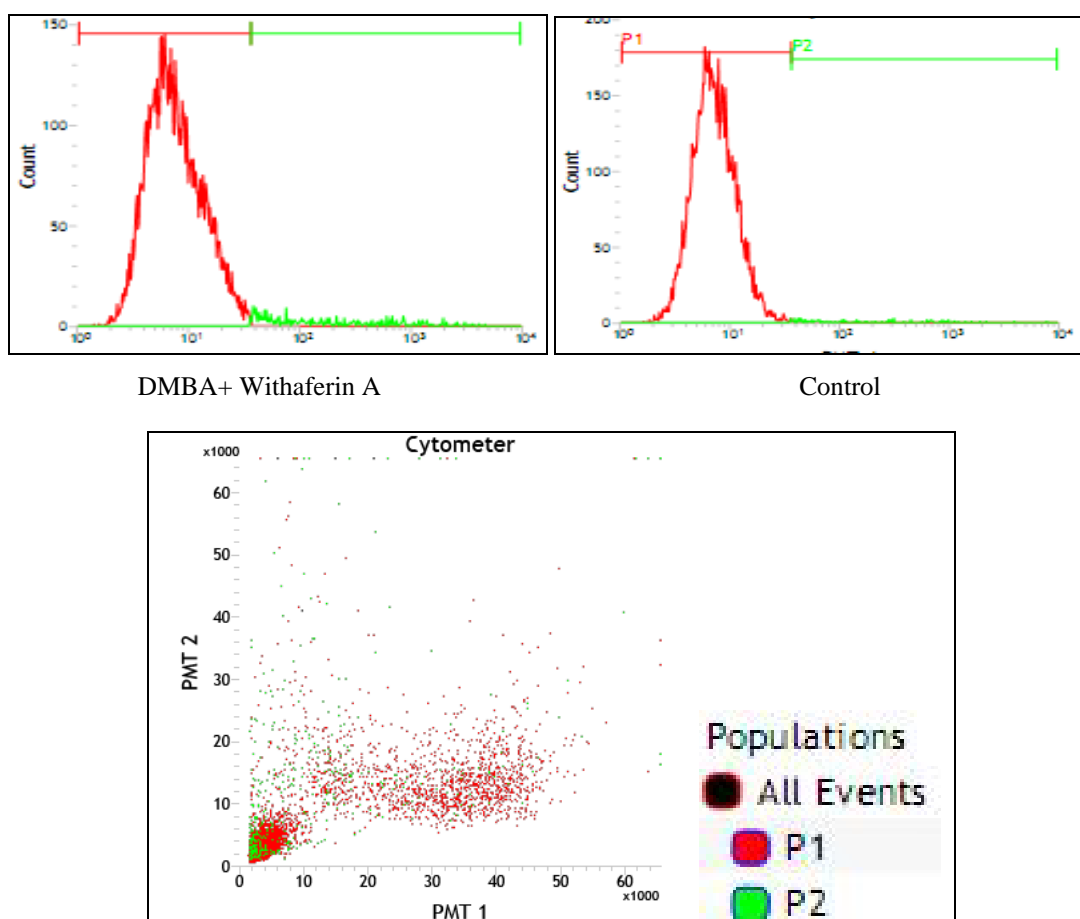


Fig 1: Flow cytometric analysis of the peripheral blood total B- lymphocytes (CD79a) P1- Total lymphocytes (Unstained cells), P2- CD79a Total B- lymphocytes (stained cells)

No significant differences were observed in the DMBA, DMBA+Tamoxifen and DMBA + Withaferin A fed rats from that of control group. DMBA, a potent carcinogen, produces more extensive and persistent B cell suppression (Burchiel *et al.*, 1988)^[3] as well as alters the host resistance to tumours (Ward *et al.*, 1984)^[15] and this chemical induced modulation of immunity is necessary for tumourigenesis. However, the carcinogenic effect of the chemical carcinogen need not

coincide with its immunosuppressive effect (Dunn *et al.*, 2004)^[4]. There was a slight increase in the percentage of B cells in the tumour control group (DMBA) compared to that of control group. This might be due to the ability of tumour to trigger the acquired humoral immunity showing that the appearance of auto antibodies as a part of defensive immune response against a developing tumour (Joseph *et al.*, 2014)^[9]. Earlier studies of tamoxifen failed to reveal any influence of

drug on immune function (Joensuu *et al.*, 1986) [8]. But the recent *in vitro* and *in vivo* studies proved the immunomodulatory effects of tamoxifen, which appears to be independent of the estrogen receptor (Behati & Frank, 2009) [1]. In this present study, DMBA+Tamoxifen showed decrease in the percentage of B cells compared to all the other three groups which requires further detailed investigations.

There was a significant ($P<0.05$) difference in the DMBA+Withaferin A treated group from that of standard DMBA+Tamoxifen group, which showed immunostimulatory effect of Withaferin A and it is contrary to the findings of Shohat *et al.* (1978) [13], who reported specific immunosuppressive effect of Withaferin A on human B & T lymphocytes and on mice thymocytes.

The tumour microenvironment contains a heterogenous population of B-cells with functionally distinct subsets, contributing to both pro- as well as anti-immune responses and balance of these responses may determine whether B cells serve as a pro- or an anti-tumorigenic function. In conclusion, the present study showed increased B cells in the peripheral blood circulation of DMBA+ Withaferin A group indicating a stimulatory immune response, which requires further detailed investigations to understand its role in the immune response.

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