Transfusion and Apheresis Science xxx (2013) xxx–xxx

Contents lists available at ScienceDirect

Transfusion and Apheresis Science

journal homepage: www.elsevier.com/locate/transci

Induction of monocyte-to-dendritic cell maturation by extracorporeal photochemotherapy: Initiation via direct platelet signaling

Tyler S. Durazzo [a], Robert E. Tigelaar [a,b], Renata Filler [a], Adrian Hayday [c], Michael Girardi [a,b], Richard L. Edelson [a,b,*]

[a] Department of Dermatology, Yale University, School of Medicine, New Haven, CT, USA
[b] Comprehensive Cancer Center of Yale University, School of Medicine, New Haven, CT, USA
[c] Kings College, London, UK

ARTICLE INFO

Article history:
Received 29 July 2013
Received in revised form 12 November 2013
Accepted 20 November 2013
Available online xxxx

Keywords:
Extracorporeal photochemotherapy
Dendritic cells
Platelets
Monocytes
P-selectin

ABSTRACT

Extracorporeal Photochemotherapy (ECP) is a widely used therapy for cutaneous T cell lymphoma (CTCL). Although the mechanism of clinical action of ECP is not precisely established, previous studies have shown evidence of induction of dendritic cells (DCs). Here we show that, under flow conditions similar to those in post-capillary venules, ECP promotes platelet immobilization and activation, initiating stepwise receptor–ligand interactions with monocytes, which then differentiate into DC. These findings clarify how ECP directly stimulates DC maturation; suggest a new clinically applicable approach to the obtainment of DC; and identify a novel mechanism that may reflect physiological induction of DC.

1. Introduction

The potential for cellular immunity to control or therapeutically eliminate cancer has long aroused considerable interest [1]. For example, an important immunoprotective role for anti-tumor T cells in colorectal, melanoma and prostate cancers is supported by recent evidence [2–4]. However, once a cancer is clinically progressive, it has already overcome immunologic defenses. Therefore, the substantial challenge to the cancer immunotherapist is that a functional tolerance to the malignant cells must be broken.

Extracorporeal Photochemotherapy (ECP), a therapy with expanding use in more than 500 centers in Europe and America, induces clinical responses in the majority of immunocompetent cutaneous T cell lymphoma (CTCL) patients, including some complete and long term remissions [2–4]. Although its mechanism of action is complex and not clearly elucidated, its clinical efficacy and advantageous safety profile have attracted much investigative interest [5–11]. In ECP therapy, the patient's blood is extracorporeally routed, as a thin film, between parallel transparent plates, permitting site-focused targeting of passaged leukocytes by UVA irradiation, followed by intravenous return of the treated blood. While the initial rationale was that rapidly-dividing lymphoma cells were killed by UV-induced DNA damage, we recently reported that ECP induces extracorporeally passaged monocytes to enter the DC differentiation pathway within a single day of treatment [12].

ECP causes both clinically potent anti-CTCL T cell responses and functional DCs suggesting a linkage between the two phenomena. Since ECP's induction of monocyte-to-DC maturation does not require addition of cytokine growth factors, but merely monocyte passage through

* Corresponding author. Address: 333 Cedar Street, New Haven, CT 06511, USA. Tel.: +1 203 737 6853; fax: +1 203 785 7637.
E-mail address: redelson@yale.edu (R.L. Edelson).

1473-0502/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.transci.2013.11.008

the ECP flow chamber, we reasoned physiologic signaling is likely responsible. We further reasoned that, if the basis for that phenomenon could be deciphered and then harnessed, it might then become possible to not only enhance ECP's clinical potency for CTCL, but also to apply the underlying therapeutic principle to treatment of a broader range of cancers.

To interrogate this possibility, we hypothesized that ECP directly promotes DC differentiation. Based initially on dynamic imaging of leukocytes passing through an experimental photopheresis chamber, we have identified and report a process by which platelets adhere to the device chamber wall, become activated, engaging monocytes in a P-selectin-dependent interaction that promotes DC differentiation in a manner dependent on platelet density and shear stress. These findings support the hypothesis that ECP is a potent initiator of immune activation; they suggest a new clinically applicable approach to the obtainment of DC that does not depend on supra-physiologic concentrations of cytokines; and they identify a functional, shear-stress-dependent platelet monocyte interaction that may reflect a physiologic method of induction of DC.

2. Materials and methods

2.1. Procurement of leukocytes and platelets

All samples were acquired from young, healthy subjects not taking medications known to influence platelet function. Samples were obtained under the guidelines of the Yale Human Investigational Review Board, and informed consent was provided according to the Declaration of Helsinki. Peripheral blood specimens were collected into syringes containing heparin, then separated using Ficoll-Hypaque (Gallard-Schlessinger). The mononuclear leukocyte fraction was collected and washed then resuspended in RPMI-1640 medium (GIBCO) to a final concentration of 5×10^6 mononuclear cells/ml.

2.2. Preparation of platelet-rich-plasma

Whole blood was centrifuged at 150*g* for 15 min at room temperature. The platelet-rich-plasma (PRP) layer was collected and centrifuged at 900*g* for 5 min, and the platelet pellet resuspended in RPMI 1640 to the desired concentration.

2.3. Preparation of parallel-plates

Two types of parallel-plate flow chambers were used to model the flow dynamics of ECP. Experiments involving the assessment of cell phenotype post-flow were conducted using the larger Glycotech system (Glycotech). This system consisted of a volumetric flow path measuring $20{,}000 \times 10{,}000 \times 254$ μm (length × width × height). The bottom plate in this system was composed of a 15 mm petri dish (BD Biosciences) separated by a gasket and vacuum-connected to an acrylic flow deck, which formed the upper plate. For experiments requiring the plates to be precoated with platelets, prior to assembling the flow chamber, 20 drops of the desired concentration of PRP was placed in the center of the petri dish and platelets allowed to settle for 20 min at room temperature. The petri dish was washed twice with 2 ml of RPMI, and the flow chamber then assembled.

Experiments not requiring the collection and phenotyping of cells post-flow were carried out using Vena8 biochips (Cellix Ltd.), a parallel-plate system made of acrylic chips with channels measuring $20{,}000 \times 400 \times 100$ μm (length × width × height). Protein coating of these chips is described in the appropriate section below.

2.4. Experiments using parallel-plates

The parallel-plate flow chamber was mounted on the stage of a phase contrast optical microscope (CK40) with a 10× objective. All runs were performed at room temperature. A uniform laminar flow field was simulated by use of a syringe pump (KD Scientific) capable of generating near-constant volumetric flow rates.

All experiments were viewed in real time, recorded at 15.2 frames per second using a DP 200 digital camera and software (DeltaPix)

2.5. Overnight culture

Cells were cultured at a concentration of 5×10^6 cells/ml in RPMI-1640 medium (GIBCO) supplemented with 15% AB serum (Gemini Bio-Products) for 18 h at 37 °C in 5% CO_2.

2.6. Immunophenotyping

Monoclonal antibodies for immunophenotyping were obtained from Beckman Coulter or Sigma and used at their pre-determined optimal dilutions. Immunofluorescence was analyzed using a FC500 flow cytometer (Beckman Coulter). Two-color membrane staining was performed using standard protocol.

2.7. Quantitative real-time PCR

Gene expression was compared between cells exposed during flow through the parallel plates to low (10 ± 5/low power field [lpf]) versus high (102 ± 32/lpf) levels of platelets, followed by overnight culture. Cell RNA was isolated using RNeasy Mini Kit columns with on-column DNase I treatment (QIAGEN). RNA yield and purity were measured using a NanoDrop ND-1000 Spectrophotometer and an Agilent 2100 Bioanalyzer. RNA was reverse transcribed to cDNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). TaqMan real-time PCR was used to detect transcripts of DC-LAMP, CD40, ADAM Decysin, Lox1, CCR7, CD80, CD83, CD86, FPRL2, and GPNMB. Primers and probes for each sequence were obtained as inventoried Taqman Gene Expression Assays (Applied Biosystems). HPRT1 was used as a reference gene.

Please cite this article in press as: Durazzo TS et al. Induction of monocyte-to-dendritic cell maturation by extracorporeal photochemotherapy: Initiation via direct platelet signaling. Transf Apheres Sci (2013), http://dx.doi.org/10.1016/j.transci.2013.11.008