Biochemical and Biophysical Research Communications 443 (2014) 524–530

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Murine T cell activation is regulated by surfen (*bis*-2-methyl-4-amino-quinolyl-6-carbamide)

Jordan Warford [a], Carolyn D. Doucette [b], David W. Hoskin [a,b], Alexander S. Easton [a,b,c,*]

[a] Department of Pathology, Dalhousie University, Tupper Building, 5850 College Street, Halifax, Nova Scotia B3H 4R2, Canada
[b] Department of Microbiology & Immunology, Dalhousie University, Tupper Building, 5850 College Street, Halifax, Nova Scotia B3H 4R2, Canada
[c] Department of Surgery (Neurosurgery), Dalhousie University, Tupper Building, 5850 College Street, Halifax, Nova Scotia B3H 4R2, Canada

ARTICLE INFO

Article history:
Received 25 October 2013
Available online 6 December 2013

Keywords:
Surfen
T cell
Activation
Proliferation
Interleukin-2
Glycosaminoglycans

ABSTRACT

Surfen (*bis*-2-methyl-4-amino-quinolyl-6-carbamide) binds to glycosaminoglycans (GAGs) and has been shown to influence their function, and the function of proteoglycans (complexes of GAGs linked to a core protein). T cells synthesize, secrete and express GAGs and proteoglycans which are involved in several aspects of T cell function. However, there are as yet no studies on the effect of GAG-binding agents such as surfen on T cell function. In this study, surfen was found to influence murine T cell activation. Doses between 2.5 and 20 μM produced a graduated reduction in the proliferation of T cells activated with anti-CD3/CD28 antibody-coated T cell expander beads. Surfen (20 mg/kg) was also administered to mice treated with anti-CD3 antibody to activate T cells in vivo. Lymphocytes from surfen-treated mice also showed reduced proliferation and lymph node cell counts were reduced. Surfen reduced labeling with a cell viability marker (7-ADD) but to a much lower extent than its effect on proliferation. Surfen also reduced CD25 (the α-subunit of the interleukin (IL)-2 receptor) expression with no effect on CD69 expression in T cells treated in vivo but not in vitro. When receptor activation was bypassed by treating T cells in vitro with phorbyl myristate acetate (10 ng/ml) and ionomycin (100 ng/ml), surfen treatment either increased proliferation (10 μM) or had no effect (2.5, 5 and 20 μM). In vitro treatment of T cells with surfen had no effect on IL-2 or interferon-γ synthesis and did not alter proliferation of the IL-2 dependent cell line CTLL-2. The effect of surfen was antagonized dose-dependently by co-treatment with heparin sulfate. We conclude that surfen inhibits T cell proliferation in vitro and in vivo. When T cell receptor-driven activation is bypassed surfen had a neutral or stimulatory effect on T cell proliferation. The results imply that endogenous GAGs and proteoglycans play a complex role in promoting or inhibiting different aspects of T cell activation.

1. Introduction

Surfen (*bis*-2-methyl-4-amino-quinolyl-6-carbamide) was first described in 1938 as a component of depot insulin [1]; however, subsequent studies have revealed its efficacy in binding to glycosaminoglycans (GAGs). GAGs are composed of repeating disaccharide units (uronate:hexosamine) that vary in degree of acetylation and the pattern of *N* and *O*-sulphation; individual GAGs may contain 1–25,000 disaccharide units. All GAGs carry a net negative charge due to the presence of sulfate and carboxyl groups, and exist in soluble or cell-bound forms. When bound to the cell surface, GAGs form proteoglycans linked to a protein core by a linkage sequence (glucuronic acid–galactose–galactose–xylose) covalently bound to the protein core through serine residues. There is considerable interest in characterizing the biological function of GAGs, given their ubiquitous expression and ability to bind hundreds of proteins, including proteases, cytokines, adhesion molecules, cytokines and chemokines. The interaction is often charge-based since GAGs have a high affinity for cationic (basic) proteins; however, this is not the only mechanism because GAGs can also bind to anionic (acidic) proteins. The major classes of GAG are heparan sulfate, heparin, chondroitin sulfate, dermatan sulfate, keratin sulfate and hyaluronic acid [2].

Surfen contains four quinoline rings that contain positively charged amino or methyl groups. When characterized further, surfen was found to bind with greatest avidity to heparin, followed by dermatan sulfate, heparan sulfate and chondroitin sulfate [3]. There are now a handful of studies on the biological effects of surfen, many of which relate to its ability to block the interaction between GAGs and signaling proteins, including effects on growth

* Corresponding author at: Tupper Building, 5850 College Street, Room 11J1, Halifax, Nova Scotia B3H 4R2, Canada. Fax: +1 (902) 473 1049.
E-mail addresses: jordan.warford@dal.ca (J. Warford), carolyn.doucette@dal.ca (C.D. Doucette), d.w.hoskin@dal.ca (D.W. Hoskin), alexander.easton@dal.ca (A.S. Easton).

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.11.119

factors (fibroblast growth factor and vascular endothelial growth factor) and fibrils associated with the binding of human immunodeficiency virus (HIV)-1 to target cells [3–5].

Although GAG synthesis, secretion and cell surface expression on T cells has been documented in several studies [6–15], the effects of a GAG binding inhibitor on T cells have yet to be reported. To our knowledge this study is the first to examine the effects of surfen on murine T cell activation. The results have implications for the function of GAGs in regulating T cell activation.

2. Materials and methods

2.1. Animals

Female C57/BL6 mice (6–8 weeks of age) were purchased from Charles River Canada (Lasalle, QC) and were used for all primary T cell experiments. All mice were housed in the Carleton Animal Care Facility at Dalhousie University. Animals were fed a standard diet of rodent chow and water ad libitum. All animal protocols were approved by the Dalhousie University Committee on Laboratory Animals and were in accordance with the Canadian Council on Animal Care guidelines.

2.2. Reagents and antibodies

Unless otherwise indicated, all chemicals including surfen were purchased from Sigma Aldrich Canada (Oakville, ON). A 100 mM stock solution of surfen was prepared in DMSO and stored at −80 °C. Phycoerythrin (PE)-conjugated anti-CD25 antibody (Ab), fluorescein isothiocyanate (FITC)-conjugated anti-CD69, PE-conjugated rat IgG1 and FITC-conjugated Armenian hamster IgG Abs were purchased from eBioscience Inc. (San Diego, CA).

2.3. T cell isolation

Mice were euthanized by cervical dislocation and axillary, brachial and inguinal lymph nodes pooled using two mice per sample. Lymph nodes were homogenized and $CD3^+$ T cells were isolated by negative selection using magnetic bead isolation columns from Miltenyi Biotech (Auburn, CA). Erythrocytes were removed by osmotic shock. Total cell numbers were assessed with trypan blue dye exclusion; cell viability was typically over 95%. For all experiments, T cells were cultured at 37 °C/5% CO_2/95% humidity in RPMI-1640 medium (Invitrogen, Burlington, ON) supplemented with 5% heat-inactivated fetal calf serum (FCS), 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine and 5 mM HEPES (all from Sigma–Aldrich).

2.4. CTLL-2 cells

Cytotoxic lymphoid line-2 (CTLL-2) cells were obtained from ATCC and were cultured in HEPES-free RPMI-1640 medium supplemented with 10% heat-inactivated FCS and 30 U/ml of recombinant murine IL-2 (Pepro Tech Inc., Rocky Hill, NJ).

2.5. Tritiated-thymidine ([^{3}H]TdR) incorporation

To assess [^{3}H]TdR incorporation, T cells were cultured for 24, 48 or 72 h at 2.5×10^5 cells/well while CTLL-2 cells were cultured for 48 h at 1×10^4 cells/well in 96-well round-bottom plates with quadruplicate wells per treatment. Cells were stimulated with 5×10^4 anti-CD3/anti-CD28 Ab-coated T cell expander beads (Invitrogen) or combined phorbyl myristate acetate (PMA, 10 ng/ml) and ionomycin (100 ng/ml) with different doses of surfen or vehicle (0.02% DMSO) dissolved in serum free RPMI-1640 medium. For the last 6 h of incubation, cells were pulsed with 0.2 μCi of methyl [^{3}H]TdR (MP Biochemicals, Irvine, CA). Cells were harvested immediately onto fiberglass filter mats with a Titertek Cell Harvester (both from Skatron Instruments, Sterling, VA). [^{3}H]TdR incorporation into newly synthesized DNA was measured using a Beckman LS6000IC liquid scintillation counter (Beckman Coulter Inc., Mississuaga, ON).

2.6. Oregon Green proliferation assay

T cells were labeled with 2 μM Oregon Green 488 dye (Invitrogen) for 15 min at room temperature and then cultured in 96 well plates (1.5×10^5 cells/well) and stimulated for 72 h with 5×10^4 T cell expander beads. Cells were analyzed by flow cytometry using a FACSCaliber flow cytometer to calculate % proliferating cells.

2.7. Cytokine assays

T cells (1×10^5) were cultured in 96 well plates (200 μl/well) alone or in the presence of 5×10^4 T cell expander beads. Following 72 h incubation, culture supernatants were harvested and interleukin-2 (IL-2) and interferon-γ (IFN-γ) concentrations determined by ELISA (IL-2 kit from eBioscience, IFN-γ kit from BD Biosciences). All assays were performed in quadruplicate.

2.8. Cell viability assay

T cells were labeled with 7-ADD (0.25 μg in 5 μl) for 5 min then washed and analyzed by flow cytometry.

2.9. Staining for CD25 and CD69 expression

Cells were labeled on ice with fluorochrome-conjugated Abs or isotype matched fluorochrome-conjugated control Abs at a concentration of 0.5 μg in 50 μl FACS buffer (containing 0.2% NaN_3 and 1% BSA in PBS) for 45 min in the dark. Cells were then washed twice in FACS buffer, fixed in paraformaldehye solution and analyzed by flow cytometry.

2.10. In vivo administration of surfen

T cell activation was induced in vivo by intraperitoneal (ip) injection of each mouse with a 5 μg dose of anti-CD3 Ab (eBioscience). Mice were either treated with vehicle (0.1% DMSO) or with surfen (20 mg/kg, ip, both dissolved in serum free RPMI-1640 culture medium) by daily injection for 3 days prior to a single anti-CD3 Ab injection. They were killed 24 h later to harvest lymph nodes and obtain $CD3^+$ T cells using methods described above.

2.11. Statistical analysis

Comparisons between multiple data sets were performed with statistical software (GraphPad Prism) by one way analysis of variance with Bonferroni post-testing. Comparisons between two data sets were compared by Student's *t*-test, either paired or unpaired depending on experimental design; $p < 0.05$ was considered to be significant.

3. Results

3.1. Surfen reduces T cell proliferation in vitro and in vivo

$CD3^+$ murine T cells were stimulated with T cell expander beads, which resulted in a time-dependent increase in uptake of [^{3}H]TdR that peaked at 48 h (Fig. 1A). The co-addition of surfen during activation resulted in a dose-dependent reduction in [^{3}H]TdR incorporation. For wells stimulated for 48 h, incorporation was significantly