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Permalink
https://escholarship.org/uc/item/3gn9c6n9

Journal

ISSN
2314-6133 2314-6141

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Publication Date
2014

DOI
10.1155/2014/609086

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Peer reviewed
Research Article

Anti-Inflammatory Effects of the Nicotinergic Peptides SLURP-1 and SLURP-2 on Human Intestinal Epithelial Cells and Immunocytes

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Received 18 March 2014; Accepted 17 April 2014; Published 4 May 2014

1. Introduction

A search for novel and more efficient therapeutic modalities of inflammatory bowel disease (IBD) is one of the most important tasks of contemporary clinical and experimental medicine. Both ulcerative colitis (UC) and Crohn’s disease (CD) are epidemiologically related to smoking [1–4]. Most patients with UC are nonsmokers, and patients with a history of smoking usually acquire their disease after they have stopped smoking [5–7]. Upon cessation of smoking, patients with UC experience more severe disease progression that can be ameliorated by returning to smoking [8–10]. In contrast, patients with CD experience severe disease when smoking, requiring an immediate and complete cessation of any tobacco usage [3, 11]. Nicotine administration in transdermal patches or enema inhibits inflammation associated with UC [8, 12–16]. Nicotine also exhibits a local therapeutic effect in CD [17], despite the fact that smoking worsens this disease. It is believed that the therapeutic effects of nicotine in IBD are mediated by the nicotinic acetylcholine (ACh) receptors (nAChRs) of gut immune cells that inhibit production of inflammatory mediators and correct specific alterations in cell cycle responses [18–20]. We have previously demonstrated that nicotinic agonists abrogate PHA-dependent upregulation of TNFα and IFNγ receptors (IFNγR) in the human leukemic T-cell line CCRF-CEM...
(CEM) [21] and downregulate lipopolysaccharide- (LPS-) induced production of the proinflammatory cytokines IL-6 and IL-18 but upregulated IL-10 in human macrophage-like U937 cells [22]. On the other hand, recent research has conclusively demonstrated that dysregulation of intestinal epithelial cells (IEC) plays an important role in the pathogenesis of IBD [23], but the therapeutic modalities that can effectively correct function of these cells remain unknown. An important role of IEC response to nicotine drugs in IBD has been suggested by the presence of fully developed, functional ACh axis in the intestinal epithelium, with its nicotinic arm controlling intestinal absorption, permeability, mucociliary activity, and mucin secretion, as well as IEC viability, proliferation, migration, and cohesion [24–38]. Therefore, modulation of the nicotinergic anti-inflammatory pathway is considered as a novel therapeutic target for IBD [12, 39–41]. Clinical trials of nicotine formulations, however, revealed severe side effects from therapeutic doses of nicotine [12, 42], which prompted a search for nontoxic nicotinergic agents that can mimic anti-inflammatory effects of nicotine in patients with IBD.

A novel paradigm of cell regulation via nAChRs has been discovered in studies of the autosomal recessive disease palmoplantar keratoderma featuring mutation of secreted mammalian Ly-6/urokinase plasminogen activator receptor-related protein- (SLURP-) 1 and impaired T-cell activity [43]. SLURP-2 expression was also discovered in the skin [44]. While various subtypes of nAChRs can be involved in the physiological regulation of cell functions by SLURPs, the biological effects of SLURP-1 are predominantly mediated by α7 nAChR and those of SLURP-2 by non-α7 nAChRs [45]. Cell function and gene expression studies [46, 47] suggested that SLURPs may play important roles in regulating both epithelial cells and immunocytes. Since nicotine has been shown to alter expression of SLURP-1 in IEC [48], we hypothesized that auto/paracrine action of SLURPs on IEC may, in part, mediate the anti-inflammatory activities of nicotine in IBD.

In this study, we analyzed the roles of SLURP-1 and -2 in the physiological regulation of the key elements of the pathobiology of IBD controlling intestinal inflammation and facilitating healing of intestinal ulcers. The results demonstrated that SLURPs can abolish expression of the IBD-related mediators of inflammation in both IEC and immunocytes. Learning the pharmacology of the SLURP-1 and -2 actions on enterocytes, colonocytes, T-cells, and macrophages may therefore help develop novel effective treatments of UC and CD.

2. Materials and Methods

2.1. Cells and Reagents. Human IEC: the small intestine enterocyte cell line CCL-241 and the colonocyte cell line CCL-248, human lymphoblastoid T-cell line CEM, and human monoblastoid tumor cell line U937 were purchased from ATCC (Manassas, VA) and grown in the respective ATCC complete growth media at 37°C in a humid, 5% CO2 incubator. To differentiate into macrophages, the U937 cells were treated with 200 nM PMA (Sigma-Aldrich Corporation, St. Louis, MO) and allowed to adhere to tissue culture plate for 3 days [49]. The full length recombinant (r)SLURP-1 and rSLURP-2 were manufactured at Viruses Corporation (Sykesville, MD), as detailed elsewhere [50]. The previously characterized anti-SLURP-1 and -2 monoclonal antibodies 336H12-IA3 and 341F10-1F12, respectively [46, 47], were from Research and Diagnostic Antibodies (North Las Vegas, NV). Normal mouse IgG (NlG) was obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Primary mouse antibodies to human ICAM, IL-1β, IL-6, IL-10, TNFα, and IFNγ receptor (IFNγR) and ELISA kits for measuring human IL-6 and CXCL10 were purchased from R&D Systems (Minneapolis, MN). The IL-8 ELISA kit was from BD Biosciences (San Jose, CA). Both recombinant IL-1β and IFNγ were from R&D Systems and both E. coli DNA and LPS from E. coli K12 strain (LPS-EK) were purchased from InvivoGen (San Diego, CA).

2.2. Quantitative Immunocytochemical Assay (QIA). The QIA (a.k.a. in-cell western), a high throughput quantitative assay of cellular proteins, was performed in situ, as described in detail elsewhere [46], using the reagents and equipment from LI-COR Biotechnology (Lincoln, NE). The CCL-241, CCL-248, CEM, or U937 cells, 1 × 10^6/well of a 96-well plate, were incubated in respective growth media with or without rSLURPs for 16 h, fixed in situ, washed, permeabilized with Triton solution, incubated with the LI-COR Odyssey Blocking Buffer for 1.5 h, and then treated overnight at 4°C with a primary antibody. The cells were then washed and stained for 1 h at room temperature with a secondary antibody, and expression of the protein of interest was quantitated using the LI-COR Odyssey Imaging System. Sapphire700 (1:1000) was used to normalize for cell number/well.

2.3. Statistical Analysis. Results were expressed as mean ± SD, and statistical significance was determined by ANOVA with Dunnett's posttest using the GraphPad Prism software (GraphPad Prism Software Inc., San Diego, CA). The differences were deemed significant when the calculated P value was <0.05.

3. Results

3.1. Anti-Inflammatory Effects of rSLURP-1 and -2 on IEC. In in vitro experiments utilizing cultured human enterocytes and colonocytes, CCL-241 and CCL-248, respectively, we recreated an aspect of IBD pathophysiology involving the proinflammatory action of IL-1β, IFNγ, and Toll-like receptor 4- (TLR4-) and TLR9-ligands (i.e., LPS-EK and E. coli DNA, resp.) on intestinal epithelium [51–53]. TLR4 and TLR9 regulate cytokine secretion, cell survival, and intestinal barrier function, and their expression on IEC is upregulated in IBD [52–57]. We hypothesized that, in response to these mediators, CCL-241 and CCL-248 cells would express proinflammatory molecules eliciting mucosal homing of T-cells and recruiting other types of inflammatory cells. Exposed
IEC indeed showed upregulated expression of IL-6, IL-8, CXCL10, and ICAM-1 (Figure 1).

Next, we sought to determine if rSLURP-1 or -2 can inhibit production of these proinflammatory molecules. rSLURP-1 significantly (P < 0.05) diminished the TLR9-dependent secretion of IL-8 by CCL-241, but not CCL-248, and the IFNγ-induced upregulation of ICAM-1 in both types of IEC (Figure 1). rSLURP-2 inhibited the IL-1β-induced secretion of IL-6 and TLR4- and TLR9-dependent induction of CXCL10 and IL-8, respectively, in CCL-241. The specificity of these effects was demonstrated by ability of anti-SLURP antibodies to abolish the inhibitory activity of corresponding rSLURP. A mixture of both nicotinergic peptides almost completely inhibited upregulated expression of all tested inflammatory molecules in both types of IEC (Figure 1), which is in keeping with the synergistic mechanisms of their biological action [58, 59].

3.2. Anti-Inflammatory Effects of rSLURP-1 and -2 on Immunocytes. rSLURP-1 significantly (P < 0.05) decreased production of TNFα by CEM, downregulated IL-1β and IL-6 secretion by U937 cells, and moderately upregulated IL-10 production by both types of immunocytes (Figure 2). rSLURP-2 significantly (P < 0.05) downregulated TNFα and IFNγR in CEM and reduced IL-6 production by U937 cells (Figure 2). Combining both rSLURPs amplified their anti-inflammatory effects.
that could be activated by rSLURP-2. Activation of inhibits immunoreactivity [72, 73].

may be due to their predominant action at distinct nAChR uniquely express α4 cells also express α7 and α9 nAChRs (data not shown), which

activation of the predominant types of nAChRs mediating anti-inflammatory effects of each SLURP protein on IEC and immunocytes should help elucidate the intracellular signaling pathways.

Conflict of Interests
The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgment
This work was supported, in part, by internal funds from University of California-Irvine School of Medicine.


