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Herpes simplex virus-1 infection of colonic explants as a model of viral-induced activation of Crohn's disease ☆

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Abstract

The exogenous triggers responsible for Crohn's disease (CD) relapses are not often identified. Cytomegalovirus and other members of the herpesvirus family have been implicated in precipitating relapses. However, the role of viral infections in the immunopathogenesis of CD remains poorly understood. We describe an *ex-vivo* model of primary viral infection of CD tissue with *Herpes Simplex Virus* type I (HSV-1). IL-6 and CD68 served as markers for CD inflammation, type I IFNs for viral infection. Colonic explants obtained from CD resections were infected via the luminal or the submucosal compartments with HSV-1 or mock virus solution, at varying concentrations for up to 20 h. Serial tissue sections were assayed for expression of HSV-1 specific antigens, CD-68, IL-6 and DC-SIGN. Culture supernatants were tested for IL-6 and type I IFN production. Positive immunostaining for HSV-1 specific antigens was consistently detectable using 11×10^6 PFU from 13 h onwards, mainly on cells located in the submucosa, and in the perivascular area. CD68 was up-regulated in lamina propria macrophages from mildly and non-inflamed CD tissue after HSV-1 infection. IL-6+ cells in the infected tissues were mainly submucosal DC-SIGN+ dendritic cells. IL-6 and IFN-β levels were higher in the supernatants from HSV-1-infected explants compared to controls after 20 h of culture ($p<0.01$). These data show increased expression of inflammatory markers during the initial stages of HSV-1 primary infection using CD colonic explants.

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This *in vitro* model appears promising to study the immunoregulatory changes induced by microbial infection in reactivation of CD.

1. Introduction

Crohn's disease (CD) is a chronic inflammatory bowel disorder (IBD) whose etiology and pathogenesis remain largely unknown. Increasing evidence implicates interplay between genetic, environmental and immune factors.[1] Epidemiological evidence indicates that clinical relapses in IBD are often associated with viral infections, typically involving the respiratory tract.[2,3] In some cases, DNA, genes and antigens of the herpesvirus family have been identified in inflamed IBD tissue.[4–9] However, the role of these viral infections in the immunopathogenesis of CD is not well understood.[10]

Herpes simplex virus type-1 (HSV-1) is a double-stranded DNA herpesvirus belonging to the subgroup *Alphaherpesvirinae*. It is characterized by a rapid life cycle and spreading, subsequent destruction of infected cells, and the ability to establish latent persistence in some cells after the initial primary infection.[11] Primary infection of cells with HSV-1 has been reported to result in the production of a number of cytokines, including type I IFN and IL-6 as part of an antiviral host response for the clearance of the infection.[12–18] Viruses such as HSV-1 that are capable of persistently infecting host tissues have also developed different strategies to evade the antiviral immune response by down-regulating the production of cytokines and cell surface markers, such as CD83 on dendritic cells (DC), inhibiting their ability to initiate the host immune response.[19–21]

Scant information is available about the intestinal target cells, cytokines, and immune response induced by primary or persistent viral infections involved in exacerbations of IBD.[5–8] We previously reported that IL-6 and CD68 are highly expressed in inflamed CD tissue when compared to uninvolved bowel segments.[22] The main aim of this study was to demonstrate that *in vitro* primary infection of CD tissue with HSV-1 up-regulates these molecules, as well as INF-β. The explant culture method of colonic tissue provides a promising model to study the immunopathogenesis of CD activation induced by microbial infection such as viruses.

2. Materials and methods

2.1. Patients and tissue specimens

Proximal colonic specimens were obtained from surgical resections in 9 pediatric patients with CD (5 males, 4 females; mean age 15.7 ± 2.9 years). The diagnosis of CD was based on established clinical, radiological, endoscopic and histopathological criteria. Indications for bowel resection were the presence of a symptomatic intestinal stenosis or therapy-resistant Crohn's colitis. Patients (8/9) were receiving various standard medications at the time of surgery (corticosteroids, 6-mercaptopurine, methotrexate and/or 5-ASA). Informed written consent was obtained from all patients, and the study protocol and consent forms were approved by the Research Ethics Committee of the Sainte-Justine Hospital Research Center.

2.2. Reagents

Anti-human CD68 mAb (mouse IgG), polyclonal antibody (rabbit) specific to HSV-1 early antigens, biotinylated anti-goat (rabbit IgG), biotinylated anti-mouse (goat IgG), streptavidin-alkaline phosphatase, fast red substrate system, biotin blocking system, streptavidin-peroxidase (LSAB + System-HRP), antibody diluting buffer, 3,3-diaminobenzidine tetrahydrochloride (DAB), and peroxidase labeled polymer anti-mouse-anti-rabbit (EnVision system) were obtained from Dako (Mississauga, ON, Canada). Universal blocking solution was from Lab Vision Corporation (Fremont, CA, USA). IL-6, IFN-α, IFN-β and IFN-ω ELISA kits, and anti-human IL-6 mAb (MAB206 IgG mouse) were purchased from R&D Systems (Minneapolis, MN, USA). Fetal bovine serum (FBS), CMRL-1066 medium and diaminobenzidine were obtained from Sigma Chemical Co. (St Louis, MI, USA), and PBS, Leibolitz L-15 medium, RPMI-1640 medium, and penicillin–streptomycin from GIBCO BRL (Life Technologies, Burlington, ON, Canada).

2.3. Virus preparation

Cell-free HSV-1 (McIntyre strain) was prepared from the supernatant of HSV-1-infected Vero cells, as previously described.[23] The viral preparations were titrated by plaque-forming assay, as reported elsewhere.[24] The viral stock solution used for these experiments, containing 2.86×10^8 PFU/ml, was diluted in CMRL-1066 medium to obtain virus concentrations of 1, 10, 11 and 12×10^6 PFU per 60 μl. To prepare non-infectious HSV-1 mock solution, viral preparations were irradiated with a UV source (400 μJ/s for 30 min). Loss of infectivity was confirmed by plaque-forming ability on Vero monolayers. The viral and mock preparations were aliquoted and stored at −80 °C until use.

2.4. Colonic explant cultures

All surgical specimens were washed 3 times in CMRL-1066. Multiple colonic mucosal explants (5 mm in diameter) that included both lamina propria and submucosa in similar proportions were taken using a jumbo colonoscopy forceps from the same macroscopically inflamed and/or non-inflamed areas of each resection. The severity of inflammation was confirmed histologically by an experienced pathologist using an additional specimen taken from the same area, as we previously reported.[25] Explants for culture were immediately embedded in ice-cold CMRL-1066 solution and supplemented with antibiotics: 200 U penicillin–streptomycin, 100 μg gentamicin (Schering Canada Inc.), and 0.5 μg amphotericin B (Bristol-Myers Squibb Canada Inc.). To expose the colonic tissue to HSV infection, each explant was examined