The American Journal of Surgery (2010) 200, 276–282

The American Journal of Surgery®

Research

The omentum is a site of stromal cell–derived factor 1α production and reservoir for CXC chemokine receptor 4–positive cell recruitment

Naveed U. Saqib, M.D.[a], Paul G. McGuire, Ph.D.[b], Thomas R. Howdieshell, M.D.[a,*]

[a]Department of Surgery, University of New Mexico HSC, MSC 105610, Albuquerque, NM 87131; [b]Department of Cell Biology and Physiology, University of New Mexico HSC, Albuquerque, NM, USA

KEYWORDS:
Omentum;
SDF-1α;
CXCR4;
Peritoneum;
Pig

Abstract

BACKGROUND: The mechanism of the omental response to injury remains poorly defined. This study investigates the omental reaction to a foreign body, examining the role of a chemokine ligand/receptor pair known to play a crucial role in angiogenesis and wound healing.

METHODS: A ventral hernia, surgically created in the abdominal wall of 6 swine, was repaired with silicone sheeting to activate the omentum. Omental thickness was determined by ultrasonography. Serial stromal cell–derived factor 1α (SDF-1α) concentrations were measured in blood, wound, and peritoneal fluids by enzyme-linked immunosorbent assay.

RESULTS: During the 14-day study period, serial ultrasonography showed a 20-fold increase in omental thickness, and enzyme-linked immunosorbent assay revealed a 4-fold increase in SDF-1α concentration in local wound fluid. Omental vessel count and vascular surface area were 8- to 10-fold higher in reactive omentum. Immunohistochemistry showed nearly complete replacement of control omental fat with CXC chemokine receptor 4 (CXCR4)-positive cells by day 14.

CONCLUSIONS: Activated omentum, important in the SDF-1α/CXCR4 axis, may serve as an intraperitoneal reservoir for recruitment of circulating bone marrow–derived cells vital to healing.

The peritoneal cavity is formed early in embryogenesis, and remains relatively isolated from the systemic circulation throughout life.[1,2] After abdominal injury or inflammation, rapid and diverse modulation of the peritoneal cell population results from expansion of resident peritoneal cells and entry of inflammatory cells from omental and mesenteric vasculature and lymphatics.[3,4]

Under normal conditions, peritoneal cells originate primarily from mesenteric and omental "milky spots."[5] These peritoneal-specific structures have been characterized as the coelom-associated lymphomyeloid tissue.[6] Omentum is the major abdominal coelom-associated lymphomyeloid tissue organ, consisting of 2 mesothelial layers, and a central connective tissue containing capillaries, adipocytes, fibroblasts, and extracellular matrix. Focal regions designated as omental milky spots are abundant in the omental adipose tissue, and are composed of numerous macrophage and lymphocyte aggregates. Omental milky spots are considered the origin of peritoneal macrophages, the first line of defense in the peritoneal cavity.[7,8]

Although its natural role is not known, the omentum has the intrinsic ability to detect, adhere to, and repair injury and inflammation in the peritoneal cavity.[9,10] It also reacts to

* Corresponding author: Tel.: +1-505-272-6441; fax: +1-505-272-0432.
E-mail address: thowdieshell@salud.unm.edu
Manuscript received April 25, 2009; revised manuscript August 31, 2009

0002-9610/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.amjsurg.2009.08.031

foreign objects and materials, encapsulating them, as if to isolate the foreign body from the abdominal viscera.[11,12] How the omentum facilitates intraperitoneal healing has not been fully elucidated. It has been postulated that omental mesothelial cells release molecular mediators into the injury site, or are themselves incorporated into the injured tissue.[13,14] An investigation of the omental reaction to a foreign body, and the factors produced in the process, might yield a greater understanding of omental activation, adherence, and growth.

Stromal cell–derived factor 1α (SDF-1α) and CXC chemokine receptor 4 (CXCR4) are an important chemokine ligand/receptor pair that play a crucial role in numerous biological processes including hematopoiesis, angiogenesis, neuronal development, and immune cell trafficking.[15] Recently, SDF-1α/CXCR4 presence has been reported in activated rodent omentum.[13,14]

We previously devised a novel, large-scale wound model in which a section of the pig abdominal wall excluding the skin is excised to create an incisional hernia.[16] The resulting defect is repaired with silicone sheeting to induce abdominal wound granulation tissue formation.[17] Moreover, as we show here, the silicone sheeting activates the omentum, creating an isolated wound fluid compartment that allows the temporal assessment of omental adherence, growth, and local mediator production.

Methods

Activation of omentum by incisional hernia repair

Domestic female Landrace swine (n = 6) weighing 15 to 20 kg were induced with telazol (2 mg/kg) intramuscularly, xylazine (2 mg/kg) intramuscularly, and atropine (.2 mg) subcutaneously, and anesthetized with isoflurane (1.5%–3%) by inhalation through an endotracheal tube. Postoperative analgesia was provided by buprenorphine hydrochloride (Buprenex; Reckitt and Colman Pharmaceuticals, Richmond, VA, .01 mg/kg intramuscularly) every 9 to 12 hours for 48 hours. After maintenance of inhalation anesthesia, sterile povidone-iodine (Betadine; Purdue Frederick Company, Norwalk, CT) preparation, and draping, an infraumbilical diagnostic peritoneal lavage (DPL) was performed (Arrow DPL Kit; Arrow International, Reading, PA) by using the percutaneous Seldinger technique to obtain pre-incision peritoneal fluid for biochemical analysis. A supraumbilical midline laparotomy was performed, and an 8 $\times$ 12 cm full-thickness section of the abdominal wall, excluding skin, was surgically excised to create a hernia defect. A biopsy of control omentum was obtained for histologic and morphometric analysis. A similar-sized piece of silicone sheeting (BioPlexus Corp., Saticoy, CA) was sutured to the fascial edges with monofilament suture to effect abdominal wall closure. The skin was closed in 2 layers with interrupted sutures to cover the biomaterial and complete wound closure. Each animal was housed for 14 days postoperatively, and received water and food ad libitum.

Ultrasonography, wound and peritoneal fluid aspiration, and blood sampling

On days 2, 4, 7, 9, 11, and 14 after surgery, each animal was sedated and placed supine, and abdominal ultrasound was performed using an Acusom Ultrasound Imager with 7.5-MHz linear array and a 3.5-MHz sector probe (Acusom Corp., Mountain View, CA). At each time point, images recorded electronically for later analysis included omental thickness measured in millimeters by a cursor at multiple consistent sites. On each study day, a sample of wound fluid located in the compartment between the skin and omental-encapsulated silicone sheeting was aspirated percutaneously using a 23-gauge needle under sterile conditions with ultrasound guidance. A repeat infraumbilical DPL was performed on each study day to obtain peritoneal fluid for SDF-1α assay, and a blood sample was obtained from an ear vein. On day 14 after surgery, the animals were sedated, and an infraumbilical DPL was performed to obtain peritoneal fluid for assay before death. Each animal was killed using intravenous pentobarbital, and the abdominal wall was harvested en bloc.

Enzyme-linked immunosorbent assay measurement

SDF-1α was assayed in plasma and wound and peritoneal fluids using quantitative sandwich immunoassay kits (R&D Systems, Minneapolis, MN), according to the manufacturer's instructions. All fluids were centrifuged immediately after harvest and then frozen at -80°C until assayed. Each sample was run in duplicate, and at pertinent dilutions, with mean values reported.

Western blot analysis of wound fluid

Wound fluid was analyzed for protein concentration using the bicinchoninic acid method (Pierce, Rockford, IL). Proteins were separated on 15% sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride membrane with a wet-blotting apparatus (trans-blot; Bio-Rad, Hercules, CA) for 60 minutes. The blot was allowed to air-dry for 30 minutes and was blocked with 5% nonfat dry milk diluted in TRIS-buffered saline (blocking buffer) for 1 hour at room temperature. The blot then was incubated with primary antibody (anti–SDF-1α, FL-93, 1:200; Santa Cruz Biotechnology, Santa Cruz, CA) diluted in blocking buffer overnight at 4°C followed by 2 washes with blocking buffer at room temperature. The blot then was incubated with secondary antibody (horseradish-peroxidase–conjugated goat anti-rabbit antibody, sc-2004, 1:5,000; Santa Cruz Biotechnology) for 1 hour at room temperature, followed by 4 washes with TRIS-buffered sa-