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► To cite this version:

Jérôme Gay-Quéheillard, Jean Fioramonti, Rafael Garcia Villar, Lionel Bueno. Mast cells and afferent nerves are involved in the development and sequels of intestinal inflammation in post-Nippostrongylus brasiliensis-infected rats. Digestive Disease Week / 101th Annual AGA meeting, May 2000, San Diego, California, United States. 2000, Gastroenterology 118 (4 part 2). hal-01604787

HAL Id: hal-01604787

<https://hal.science/hal-01604787>

Submitted on 4 Jun 2020

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ESSENTIAL ROLE OF EPITHELIAL SERUM AMYLOID A (SAA) IN LIPOPOLYSACCHARIDE (LPS)-ASSOCIATED MUCOSAL INFLAMMATION.

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Background/Aims: Our approach to investigate IBD-associated genes revealed that SAA was induced in colonic epithelial cells (EC) of germ-free mice by bacterial reconstitution and of IBD, ulcerative colitis (UC) and Crohn's disease (CD) (GE:114:A1054:1998, 116:A720:1999). Epithelial SAA mRNA was induced by LPS, IL-1 β and condition medium from lamina propria mononuclear cells (LPMC) in vitro. These data strongly suggest that epithelial induction of SAA is essential for mucosal inflammation particularly initiated by luminal bacteria or its product. The aim of the present study was to investigate the relevance of epithelial induction and function of SAA in mucosal inflammation. **Materials and methods:** 1) Dextran sulfate sodium (DSS) was administered to ICR mice for 5 or 10 days. Liver and colon were removed and EC were isolated. SAA mRNA and protein expression were examined by northern blot or immunohistochemistry, respectively. Serum SAA concentration was measured by ELISA. 2) Peripheral blood mononuclear cells (PBMC) were obtained from 3 healthy volunteers. LPMC were isolated from 2 control, 2 UC and 3 CD surgical specimens. Cells were cultured with graded amount of LPS (from 0 to 1 mg/ml) and recombinant SAA1 (from 0 to 50 ng/ml) alone or in combination, then supernatants were collected after 48h. We measured IL-1 β and IL-10 concentration in them using ELISA. **Results:** 1) DSS-treated mice exhibited a time-dependent severity of mucosal inflammation. Immunoreactivity for SAA was essentially absent in control EC. Surface EC exhibited cytoplasmic immunoreactivity for SAA after DSS-treatment for 5 and 10 days in parallel with SAA mRNA induction. On the contrary, mRNA expression in the liver and serum SAA was enhanced only at 10 days. 2) LPS enhanced IL-1 β and IL-10 concentration in PBMC supernatants. When recombinant SAA1 was added into cultures with LPS, SAA1 dose-dependently inhibited both cytokines. SAA1 also exhibited the inhibitory effect on LPS-stimulated IL-1 β and IL-10 secretion from LPMC regardless of the disease. **Conclusion:** Epithelial induction of SAA is relevant in various type of mucosal inflammation including human and experimental IBD. This induction was prior to elevation of serum SAA in DSS-treated mice. SAA is not only a marker of inflammation but a bioactive protein that is produced in EC and inhibit LPS-associated cytokine production. Epithelial SAA may be a member of feedback system to prevent perpetually amplified inflammation triggered by LPS, a major bacterial product.

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ADHERENCE OF LACTOBACILLUS CRISPATUS TO COLONIC EPITHELIUM IS DETERMINED BY MICROENVIRONMENTAL PH AND BACTERIAL METABOLIC SUBSTANCES.

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Adherence of *Lactobacillus* to the gut mucosa may be an important factor involved in the colonization of the intestinal tract. The ability to adhere may be determined both by bacterial metabolic substances that are released to the medium and microenvironmental factors such as pH. Our aim was to study the influence of these factors on the adherence of a strain of *Lactobacillus crispatus* (from human vaginal flora) to isolated rat colonocytes in vitro. Rat colonocytes were isolated in EMEM (Eagle's minimal essential medium) supplemented with 10% inactivated fetal calf serum and containing 1 mM dithiothreitol. Bacteria were grown in MRS (Man, Rogosa and Sharpe) broth at 37°C for 18 hours and measured by spectrophotometry at 600 nm. The number of bacteria was adjusted to achieve an adequate concentration under different conditions: 1. To evaluate the effect of bacterial metabolic factors we used different ratios of spent culture supernatant (100, 50, 25 and 0 %) in fresh MRS broth. 2. To investigate the effect of pH we used spent supernatant or fresh MRS broth fitted to different pH: 4, 6 and 7. In the adhesion assay 2×10^5 recently isolated rat colonocytes were incubated with 10^7 bacteria at 37°C in a shaking bath for 30 minutes. After incubation, cells were washed to eliminate non-adhering bacteria and examined under the microscope after Gram staining. Bacterial adherence was quantified as the number of attached bacteria per cell after counting 50 cells. **Results:** 1. Reduction of the ratio of spent supernatant decreased adherence of *L. crispatus* to colonic epithelial cells (100%: 9.3 adhering bacterial/cell; 50%: 6.1; 25%: 2.7 and 0%: 1.9). 2. Increase of pH of the medium induced a reduction of the adherence of *L. crispatus* to the cells both in spent supernatant (pH 4: 5.5 adhering bacterial/cell, pH 6: 4.3 and pH 7: 2.1). **Conclusion:** Adherence of *Lactobacillus crispatus* to colonic epithelial cells depends on both the presence of released bacterial metabolic substances and an acidic microenvironmental pH.

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USEFULNESS OF INTRAVENOUS CYCLOSPORINE A (CSA) TREATMENT IN CROHN'S DISEASE (CD).

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Intravenous CsA treatment has shown its benefit in acute steroid-resistant ulcerative colitis. However, its role in the treatment of CD has not been established due to the lack of controlled trials. Only a few short series and open-label studies using i.v. CsA in either fistulizing and chronic active CD have been published. **AIMS:** To assess the outcome of a series of refractory CD patients on i.v. CsA with different indications. **METHODS:** Patients with CD admitted in our Department and treated with i.v. CsA from January 1991 to June 1999 were identified and retrospectively analyzed. Patients were grouped depending on the main indication for CsA treatment in steroid-resistant inflammatory disease (Group A), perianal disease (Group B) and enteric fistulae (Group C). All patients received CsA in the form of Sandimmune i.v. solution at a dose of 4mg/Kg/day as a continuous infusion over 24 h. or as a daily dose divided in two single 4-h. perfusions every 12 h. CsA dose was adjusted to maintain a whole blood CsA concentration within a target range of 100 to 200 ng/mL. In Group A, response was defined as improvement of clinical and biochemical parameters (CRP, ESR, platelet count and fibrinogenemia). In Groups B and C, complete response was defined as closure and cessation of fistulae drainage, while partial response was defined as a reduction in drainage or a reduction in the number of open fistulae but with persistence of some of them. **RESULTS:** 29 patients were treated with i.v. CsA: 5 patients in Group A, 16 patients in Group B and 8 patients in Group C. Demographic and clinical data were similar in the three groups, although male patients predominated in Group C and patients in Group A had received steroids for a longer period of time prior to CsA therapy than the others. Mean duration of i.v. CsA therapy was 24 days (range 10-45). All patients in Group A (n=5) achieved complete response after 10-21 days on i.v. CsA. 4 of these 5 patients were then put on AZA treatment and steroids could be discontinued. In Group B, only 3/16 patients achieved a complete response while 9 cases showed partial response. In Group C (n=8), only 1 patient showed a complete response and another one had a partial response to i.v. CsA. The incidence of adverse events was very low (only 2/29 patients) and reversible, but CsA dose had to be reduced in the majority of patients (mean final dose 2.88 mg/Kg/day). **CONCLUSIONS:** i.v. CsA seems to be particularly useful in steroid-resistant CD and may improve perianal disease in more than 50% of cases, but it has no impact on the outcome of enteric fistulae

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MAST CELLS AND AFFERENT NERVES ARE INVOLVED IN THE DEVELOPMENT AND SEQUELS OF INTESTINAL INFLAMMATION IN POST-NIPPOSTRONGYLUS BRASILIENSIS-INFECTED RATS.

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Mast cells and afferent nerves have been reported to modulate the intensity of gut inflammatory processes. In rats, *Nippostrongylus brasiliensis* (Nb) induces an inflammation of the jejunum whose intensity peaks at 10-12 d after infection and is associated with hypermastocytosis; at 30 d, the inflammation has resolved but mastocytosis persists for several weeks. **Aims.** To determine whether mast cells and afferent nerves are involved in the development and sequels of the inflammation induced by exposure of the rat jejunum to Nb. **Methods.** Twelve groups of 8 male Wistar rats (200-250 g) were used. i) Groups 1, 2, 3 were treated orally (po) with the mast cell stabilizer ketotifen (1 mg/Kg, 4 days, 2/d) and groups 4, 5, 6 with its vehicle (0.9% NaCl saline, 0.5 ml, po). After this, groups 1, 2, 4, 5 were infected with 2500 L3 larvae of Nb (in 0.5 ml saline, sc) and control groups (3, 6) were given 0.5 ml saline (sc). Ketotifen (1 mg/Kg, po, 1/d) was continued for 12 d or 35 d for Nb-infected rats and 35 d for controls. ii) Groups 7, 8, 9 were treated for 4 d with increasing doses of capsaicin (total dose 100 mg/kg, sc) and groups 10, 11, 12 with its vehicle (ethanol-tween 80-saline, 0.2 ml, sc). Seven days later, groups 7, 8, 10, 11 were infected with Nb as above; groups 9, 12 were not infected. Samples of jejunum were collected at 12 d and 35 d after infection, and at 35 d in controls for myeloperoxidase (MPO) assay (inflammation marker) and mast cell counting. **Results.** Values (mean \pm SEM) for MPO and mast cells are reported in Table 1. **Conclusion.** Mast cell stabilization decreases gut inflammation at day 12 but maintains an inflammatory state at day 35. Capsaicin-sensitive afferent nerves are not involved in the development of mast cell hyperplasia but limit the intensity of inflammation.

	MPO (U/g protein)			Mast Cells (/Imm ²)		
	Nb (d.12)	Nb (d.35)	Controls (d.35)	Nb (d.12)	Nb (d.35)	Controls (d.35)
Saline	380 \pm 55	44 \pm 11	55 \pm 14	564 \pm 51	633 \pm 29	239 \pm 19
Ketotifen	207 \pm 18*	299 \pm 41*	146 \pm 27*	656 \pm 34	861 \pm 58*	425 \pm 35*
Capsaicin vehicle	374 \pm 40	148 \pm 31	63 \pm 13	774 \pm 74	804 \pm 62	359 \pm 73
Capsaicin	667 \pm 121#	234 \pm 55#	70 \pm 25	759 \pm 76#	600 \pm 102#	317 \pm 69

*Significantly different (p<0.05) from control vehicle; #from control capsaicin