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The vagal nerve as a link between the nervous and immune system in the instance of polymicrobial sepsis

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Abstract *Background:* The role of the vagal nerve in the autonomic nervous system is widely well known. Recently, an additional function was revealed serving as a connector between the nervous and immune system. This connection is called the “cholinergic inflammatory pathway.” Through stimulation of the acetylcholine receptors located upon the macrophages, the “unspecific” immune system can be directly influenced. *Methods:* The vagal nerve was completely transected directly posterior to its passage through the diaphragm. The effect of complete vagotomy was analyzed using a murine model of polymicrobial peritonitis (colon ascendens stent peritonitis, CASP). Survival and clinical course of vagotomized or sham-operated

mice were analyzed in the CASP model. *Results:* After CASP surgery, vagotomy led to a significantly increased mortality (64.7%) in comparison to sham-vagotomized animals (34%). No difference in the bacterial load of various tissues (lung, liver, spleen, blood, lavage fluid, and kidney) from septic animals with or without vagotomy was observed. Vagotomized animals reveal elevated serum cytokine levels (TNF, IL-6, IL-10, and MCP-1) 20 h after the induction of polymicrobial peritonitis. *Conclusion:* The vagal nerve is therefore an important modulator of the immune system.

Keywords CASP · Cytokines · Sepsis · Inflammation · Vagotomy

Introduction

The role of the vagal nerve in the autonomic nervous system is widely well known. Involvement of the vagal nerve in inflammation was supposed for some time now [1, 2]. Discussion has taken place pertaining not only to a role in the regulation of pyrexia [3–5] but also the service provided by afferent vagal fibers as a rapid information pathway for gut-associated inflammation [1, 6, 7]. Lately, the efferent portion of the vagal nerve became the main focus of interest. Borovikova et al. [8] described an attenuating effect of vagal nerve stimulation on endotoxin-induced systemic inflammation. They demonstrated that acetylcholine receptors upon the surface of macrophages

were influenced by acetylcholine and nicotine, thereby establishing the so-called “cholinergic inflammatory pathway” [8]. In 2004, these results were attributed to the nicotinic acetylcholine receptor subtype 7 [9]. For many models of systemic inflammation [cecal ligation and puncture, lipopolysaccharide (LPS), and injection of *Escherichia coli*], an attenuating effect of an electrical or “chemical” stimulation of the vagal nerve were described in vitro and in vivo. Survival rates post cecal ligation and puncture [9], LPS application [9], and injection of *E. coli* [10] into the abdominal cavity were elevated after the injection of nicotine or stimulation upon the cervical vagal trunk at different time points. Through the application of nicotine or acetylcholine in vitro, the amount of TNF- α ,

IL-1 β , IL-6, and IL-18 in the supernatant of stimulated human macrophages was reduced. The amount of IL-10 was kept at an almost steady state [9].

In most investigations, a unilateral, cervical transection of the vagal nerve was performed. There are, however, only few data showing the effect of a complete vagotomy on systemic inflammations. In some surgical procedures—such as gastrectomy or esophagectomy—resection of the vagal nerve is unavoidable. Unfortunately, there is a risk of developing an additional systemic inflammation after major surgery. In this study, the influence of complete vagotomy upon a simultaneously acquired systemic inflammation was examined. As a model for systemic inflammation, the colon ascendens stent peritonitis (CASP) model was used. In our experience, the CASP appears to mimic the clinical situation of a polymicrobial sepsis after anastomosis insufficiency quite well [11]. A comparison was undertaken between the survival kinetics after CASP induction to the combination of CASP and vagotomy (CASP + Vgx). If there is a direct link between the nervous system and immune system based upon the theses of the cholinergic inflammatory pathway, the combination of vagotomy and sepsis should lead to a higher mortality, increased bacterial load, and elevated systemic cytokines.

Materials and methods

Mice

For all experiments, 8- to 12-week-old female mice (weight 20–25 g) were used. C57BL/6 mice were purchased from Charles River (L'Arbresle, France). The mice were bred in a conventional animal facility. Before surgery, mice were kept for at least 2 weeks in the animal facility to recover after transport. All experimental procedures were performed according to German animal safety regulations. For all surgical procedures, Avertin (Sigma-Aldrich Chemie, Taufkirchen) anesthesia was used.

Vagotomy

Under complete anesthesia and disinfection, the upper abdominal wall was opened through a transverse incision using a thermocauter (Labo-Med GmbH, Leipzig, Germany). The esophagus was exposed by carefully keeping costal arc, liver, and stomach out of sight. Further preparation was done using a surgical microscope (20 \times magnification, Leica M651, Bensheim, Germany). The ventral branch of the vagal nerve was exposed and about 3 mm was resected. After its passage of the diaphragm, the esophagus was mobilized on its hepatic side and lifted. Using diaphanoscopy from the left the dorsal branch of the vagal nerve could be exposed beneath the esophagus. The

vagal nerve was then isolated and approximately 3–5 mm was resected. Fluid resuscitation of animals was performed by flushing 0.5 ml of sterile saline solution into the peritoneal cavity before closure of the abdominal walls (single layer; 4/0 Polyester, Catgut, Marktneukirchen, Germany). For control purposes, sham operations without transection of the nervus vagus were performed.

CASP surgery

The surgical procedure for CASP was performed as previously described [12]. Shortly before surgery, a venous catheter (16-gauge, Venflon; BOC, Ohmeda AB, Sweden) was prepared. Under complete anesthesia and after disinfection of the abdomen, according to the vagotomy surgery the abdominal wall was opened through a transverse incision of the upper abdomen. After exposure of the ascending colon, the prepared catheter was stitched through the antimesenteric wall into the lumen of the ascending colon and fixed with two stitches (7/0 Ethilon thread; Johnson–Johnson, Brüssel, Belgium) and placed approximately 10 mm from the ileocecal valve. Consecutively, the inner needle of the stent was removed and the stent was cut at the prepared site. To ensure proper intraluminal positioning of the stent, stool was milked from the cecum into the ascending colon and the stent until a small amount appeared. Fluid resuscitation of animals was performed by flushing 0.5 ml of sterile saline solution into the peritoneal cavity before closure of the abdominal walls (single layer; 4/0 Polyester, Catgut). For control purposes, sham operations without puncturing the colonic wall were performed.

Bacteriology

For the detection of the bacterial load in liver, lung, kidney, and spleen, these organs were removed 20 h after surgical treatment and placed in 5 ml of an ice-cold sterile salty solution (0.9%). The organs were homogenized for 30 s (2,500 turns/min) by using an ultra-turrax (Ultra-Turrax T25 basic, IKA, Staufen, Germany). Homogenates were plated at different dilutions on blood containing agar (Columbus 5% SB, Becton Dickinson Bioscience, Heidelberg, Germany) in petri dishes. The petri dishes were incubated for 18 h at 37°C. The number of colony forming units (CFU) relating to the whole organ were calculated. For the detection of the bacterial load, whole blood was collected in EDTA containing vials (Becton Dickinson Bioscience) to inhibit the coagulation. Ten microliters of whole blood were plated. Bacterial load relating to 1 ml of whole blood was calculated after an incubation period of 18 h. For the detection of the bacterial load in lavage fluid, 6 ml of sterile saline solution were instilled into the abdominal cavity. Four milliliters of this fluid were

aspirated and 10 μ l were plated. Bacterial load was subsequently calculated relating to 1 ml of fluid.

Cytokines

IL-6, IL-10, TNF-alpha, and MCP-1 were detected in the serum 20 h after surgical treatment. Serum was separated by centrifugation of whole blood (10 min, 16,100 \times g; Centrifuge 5415R, Eppendorf, Germany). A commercially available CBA-detection kit (BD cytometric bead array mouse inflammation kit, Becton Dickinson Bioscience) was used following the instructions for customers. Each group contained at least five animals.

Statistical methods

Statistical analysis was performed using GraphPad Prism for Windows software (GraphPad Software, San Diego, CA). Statistical differences in survival were assessed using log-rank test. Results from bacterial cultures and serum cytokine levels were analyzed using the two-tailed Mann-Whitney U test for nonparametric probes. A significance level of 0.05 was determined for all calculations.

Results

Survival analysis

CASP and the combination of CASP + Vgx were performed as described and survival kinetics were analyzed. In the CASP + Vgx group, CASP and vagotomy were performed at one time point. Each group contained at least 33 individual experiments. The observation period was 10 days (240 h). The results are depicted in Fig. 1. CASP surgery alone led to a mortality of approximately

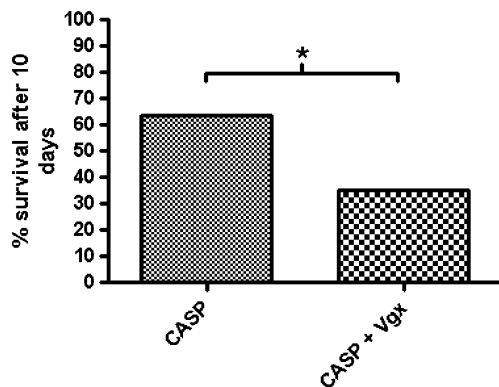


Fig. 1 Ten-day survival after CASP and CASP + Vgx: There is a higher mortality after CASP + vagotomy compared to CASP alone. CASP/CASP + Vgx $n \geq 33$

34%. The combination of vagotomy and CASP showed a significantly higher mortality (64.7%, $p=0.047$).

Analysis of bacterial cultures after CASP and CASP + Vgx

To find a rationale for the higher mortality of CASP + Vgx in comparison to CASP alone, the bacterial load in different tissues and compartments was analyzed. Twenty hours postperitonitis induction, liver, lung, kidney, and spleen were explanted. The organs were homogenized and plated on blood agar containing petri dishes. Lavage fluid and whole blood were treated as described and also plated on petri dishes. After an incubation period of 18 h at 37°C, the number of CFU was detected. CFU were related to the whole organ or to 1 ml of fluid or blood. Each group consisted of at least seven animals. The results are shown in Fig. 2. No significant differences with respect to the bacterial load could be observed in the tested organs and compartments in CASP and CASP + Vgx.

Systemic cytokine response after CASP and CASP + Vgx

To characterize the cytokine response to CASP and CASP + Vgx 20 h after surgery, the whole blood of the animals was collected in EDTA containing vials. Serum was isolated as described. The amount of the cytokines IL-6, IL-10, TNF-alpha, and MCP-1 was detected by using a commercially available cytometric bead array. IL-12 and IFN-gamma could not be detected in affordable amounts (data not shown). Twenty hours after surgery, the CASP + Vgx group showed significantly elevated serum cytokine levels for TNF-alpha,

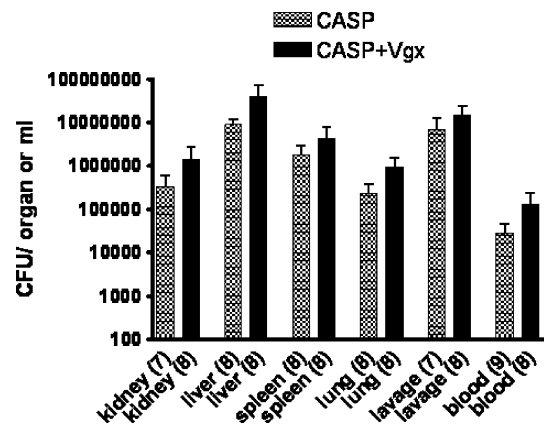
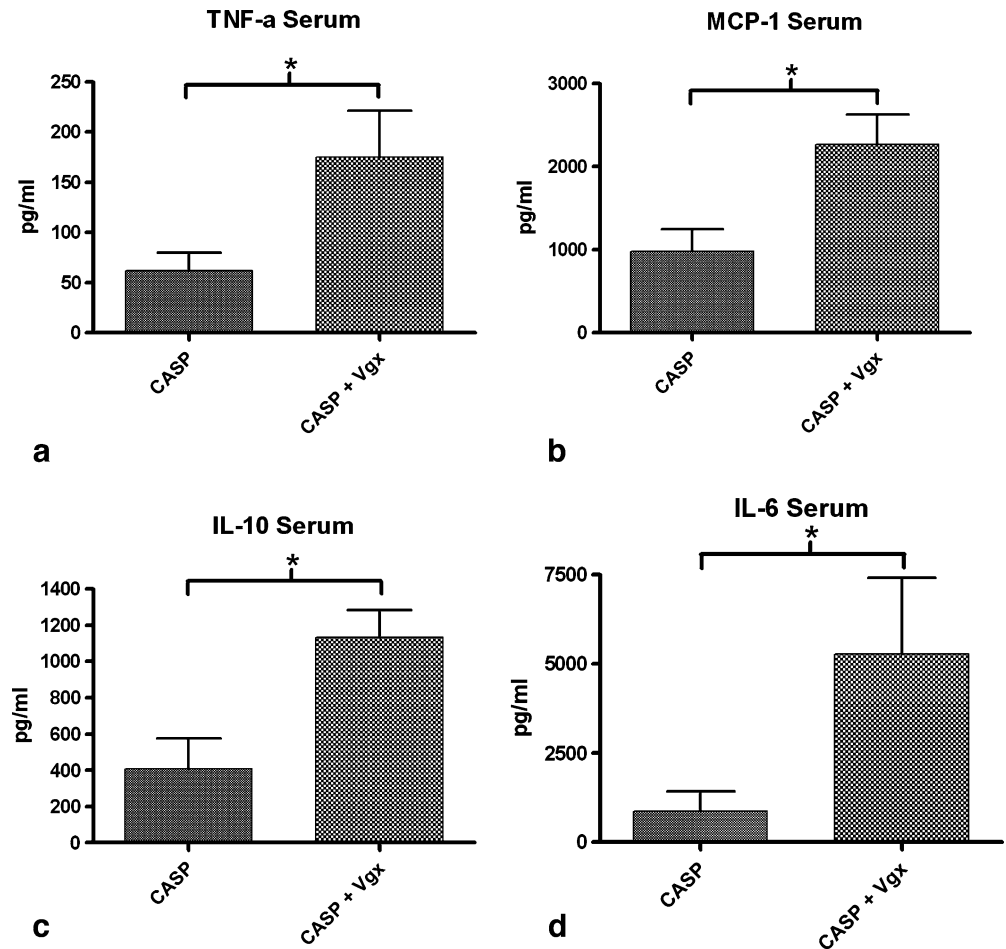


Fig. 2 Colony forming units (CFU) in different organs and compartments 20 h after CASP and CASP + vagotomy. The number of animals per group is given in brackets

Fig. 3 Cytokine levels in serum are elevated in CASP + Vgx compared to CASP alone. **a** TNF-alpha, **b** MCP-1, **c** IL-10, and **d** IL-6; $n \geq 5$



IL-6, MCP-1, and IL-10 compared to the CASP-group (Fig. 3a-d).

Discussion

The nervus vagus is a cranial nerve possessing a wide spread within the abdominal cavity. Activation of the vagal nerve or stimulation with the “agonist” nicotine showed a reduced mortality in different animal models for inflammation [9, 10]. In all these experiments, the reverse was never tested sufficiently. Therefore, the role of a complete subdiaphragmatic vagotomy on the survival kinetics within period of 10 days was investigated. The vagal nerve was transected subdiaphragmatically due to the fact that a “higher” place of transection would lead to a loss of diaphragm function and a more peripheral transection would not completely block the vagal innervation of the abdominal cavity. In our study, we could detect a higher mortality in CASP + Vgx in comparison to CASP. These results underline the protective role of the vagal nerve in systemic inflammation. This effect was not caused by an

asymmetric distribution of the bacterial load in organs and compartments between the compared groups.

In vitro acetylcholine, the main transmitter of the parasympathetic nervous system, and its agonist nicotine lead to a reduced release of the proinflammatory cytokines IL-1 β , IL-6, and IL-18 in human macrophages after LPS stimulation [9]. Secretion of the antiinflammatory cytokine IL-10 was unaffected [9]. In contrast, our in vivo results of serum cytokines after vagotomy showed elevated levels of the proinflammatory cytokines IL-6, TNF-alpha, and MCP-1. The antiinflammatory cytokine IL-10 was, in contrast to the described in vitro experiments [9], also elevated after CASP + Vgx. During experimental peritonitis, IL-10 itself is mainly produced by Kupffer cells and is important in the survival of mice experiencing polymicrobial sepsis [13].

In the CASP model, kinetics of the serum cytokines follow a time-dependent course. The peak in concentration of cytokines in serum can be seen at about 18 h after CASP [11]. It is not clear, however, whether CASP + Vgx lead to higher peaks in serum cytokine levels compared to CASP or if CASP + Vgx leads to a slower reduction of elevated

cytokine levels. This will be investigated in subsequent experiments.

The results presented herein show, for the first time, a survival disadvantage of vagotomized subjects during severe systemic infection. It is well known that patients undergoing esophagectomy or transhiatal gastric resection, whereby vagal resection is unavoidable, experience elevated perioperative mortality compared to other abdominal surgery [14]. The data presented in this communication suggest that the increased mortality may not be solely due to the higher surgical trauma but may indeed result from an altered and unbalanced immune response as a consequence of vagotomy. If this hypothesis is true, pharmacologic interventions to combat the cholinergic

inflammatory pathway may become a valuable tool for the upper GI surgeon.

Conclusion

Within the model of experimental sepsis, vagotomy represents a risk factor for increased mortality due to an altered and unbalanced immune response suggesting that the vagal nerve can act as a link between the nervous and immune system.

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References

1. Goehler LE, Gaykema RPA, Nguyen KT, Lee JE, Tilders FJH, Maier SF, Watkins LR (1999) Interleukin-1beta in immune cells of the abdominal vagus nerve: a link between the immune and nervous systems? *J Neurosci* 19 (7):2799–2806
2. Ge X, Yang Z, Duan L, Rao Z (2001) Evidence for involvement of the neural pathway containing the peripheral vagus nerve, medullary visceral zone and central amygdaloid nucleus in neuroimmunomodulation. *Brain Res* 914(1–2):149–158
3. Simons CT, Kulchitsky VA, Sugimoto N, Homer LD, Székely M, Romanovsky AA (1998) Signaling the brain in systemic inflammation: which vagal branch is involved in fever genesis? *Am J Physiol* 275:R63–R68
4. Roth J, de Souza GEP (2001) Fever induction: evidence from responses to systemic or local cytokine formation. *Braz J Med Biol Res* 34(3):301–314
5. Romanovsky AA (2000) Thermoregulatory manifestations of systemic inflammation: lessons from vagotomy. *Auton Neurosci* 85(1–3):39–48
6. Wang X, Wang BT, Zhang XJ, Duan XL, Guo X, Ju G (2004) Fos expression in the rat brain after intraperitoneal injection of Staphylococcus enterotoxin B and the effect of vagotomy. *Neurochem Res* 29 (9):1667–1674
7. Marquette C, Linard C, Galonnier M, Van Uye A, Mathieu J, Gourn P, Clarencon D (2003) IL-1beta, TNF-alpha and IL-6 induction in the rat brain after partial-body irradiation: role of vagal afferents. *Int J Radiat Biol* 79 (10):777–785
8. Borovikova LV, Ivanova S, Zhang M, Yang H, Botchkona GI, Watkins LR, Wang H, Abumrad N, Eaton JW, Tracey KJ (2000) Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. *NAT* 405:458–462
9. Wang H, Liao H, Ochani M, Juxiniani M, Lin X, Yang L, Al-Abed Y, Wang H, Metz C, Miller EJ, Tracey KJ, Ulloa L (2004) Cholinergic agonists inhibit HMGB1 release and improve survival in experimental sepsis. *Nat Med* 10 (11):1216–1221
10. van Westerloo DJ, Giebelen IA, Florquin S, Daalhuisen J, Bruno MJ, de Vos AF, Tracey KJ, van der Poll T (2005) The cholinergic anti-inflammatory pathway regulates the host response during septic peritonitis. *J Infect Dis* 191(12):2138–2148
11. Maier S, Traeger T, Entleutner M, Westerholt A, Kleist B, Hüser N, Holzmann B, Stier A, Pfeffer K, Heidecke CD (2004) Cecal ligation and puncture versus colon ascendens stent peritonitis: Two distinct models for polymicrobial sepsis. *Shock* 21:505–511
12. Zantl N, Uebe A, Neumann B, Wagner H, Siewert JR, Holzmann B, Heidecke CD, Pfeffer K (1998) Essential role of gamma interferon in survival of colon ascendens stent peritonitis, a novel murine model of abdominal sepsis. *Infect Immun* 66:2300–2309
13. Emmanuilidis K, Weighardt H, Maier S, Gerauer K, Fleischmann T, Zheng XX, Hancock WW, Holzmann B, Heidecke CD (2001) Critical role of Kupffer cell-derived IL-10 for host defense in septic peritonitis. *J Immunol* 167(7):3919–3927
14. Birkmeyer NJ, Goodney PP, Stukel TA, Hillner BE, Birkmeyer JD (2005) Do cancer centers designated by the National Cancer Institute have better surgical outcomes? *Cancer* 103:435–441