

## Colon irrigation causes lymphocyte movement from gut-associated lymphatic tissues to peripheral blood

Yoko UCHIYAMA-TANAKA

Yoko Clinic, 3-3-13 Takami, Yahatahigashi-ku, Kitakyushu 805-0016, Japan

(Received 21 July 2009; and accepted 19 August 2009)

### Abstract

It is well established that the intestine is an important site responsible for the local immune system. It is speculated that people suffering from constipation and carrying fecal residues in the intestine may have a decreased function of this immune system. In this study, colon irrigation, which is cleansing of the colon using a simple hydrotherapy instrument, was performed in 10 subjects with or without the disease. The number of leukocytes and their demarcation were then evaluated. The number and ratio of lymphocytes increased significantly after irrigation. This result suggested that colon irrigation might induce lymphocyte transmigration from gut-associated lymphatic tissues into the circulation, which may improve colon and immune system function.

It is well established that the intestine is an important site responsible for local immunity. It was recently reported that the intestine is a major site of extrathymic T cell differentiation (4, 6, 7). Many active and inactive lymphocytes are produced from gut-associated lymphatic tissues (GALT) such as Peyer's patches (16). Thus, it is speculated that people suffering from constipation and carrying fecal residues in the intestine may have a decreased function of such an immune system. Colon irrigation, also known as colonics, is a colon hydrotherapy performed using an instrument in combination with abdominal massage, but without drugs or mechanical pressure. Since the early history surrounding ancient Egypt, colon hydrotherapy has been practiced in its most basic form, such as enemas or clysters, and has provided people with internal cleansing as an adjunct to personal external hygiene. The Ebers Papyrus, from the 14th century BC, prescribes internal cleansing for no less than 20 gastric and intestinal complaints. In this pilot study, colon irrigation was performed using a simple hydrotherapy instrument

and lymphocyte movement was evaluated. This is the first report discussing the evaluation of lymphocyte movement in human peripheral blood following colonic irrigation.

The study procedures were in accordance with the guidelines of the 2000 Declaration of Helsinki for human experimentation. All subjects provided informed consent for participation in the study. Ten outpatients from the Yoko clinic (4 men and 6 women; mean age,  $38 \pm 6$  years; range, 27–47 years) from April to May 2009 were enrolled in this study. None of the patients had any signs, symptoms, or history of cancer or any active inflammatory disease. None of the patients were given any medicine. Blood samples were collected from patients in a sitting position before the start of and 10 min after hydrotherapy. The number of leukocytes and their demarcation (granulocytes and lymphocytes) were measured using an automatic blood measurement system (Pentra 60 LC-5000; Horiba, Kyoto, Japan) within 5 min after blood collection. This machine measures the actual cell impedance and cell counts by diffraction and optimal absorbance. Flow cytometry and a sequential measurement system were also used.

Colon irrigations were performed by the same trained physician for all patients. Therapy was performed using an intestinal irrigation machine (Colon

---

Address correspondence to: Dr. Yoko Uchiyama-Tanaka  
Yoko clinic, 3-3-13 Takami, Yahatahigashi-ku, Kitakyushu  
805-0016, Japan

Tel: +81-93-651-0880, Fax: +81-93-651-0881

E-mail: yoko-uchi-yama-tanaka@yoko-clinic.net

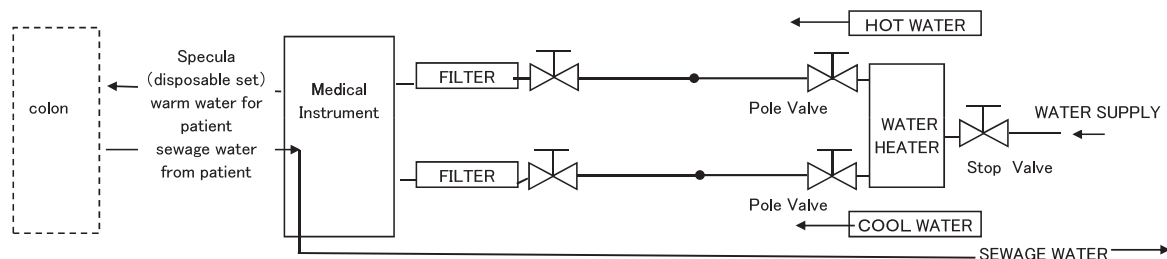
Hydromat Comfort; HERRMANN Apparatebau GmbH, Kleinwallstadt, Germany), which circulated about 30–50 liters of purified hot water (38°C) through a filter (Fig. 1). Patients lay prone on a bed and received an abdominal massage during the therapy. Values are expressed as mean  $\pm$  SD. Student's *t*-test was used for the comparisons, and *P* values less than 0.05 were considered significant.

The patient's clinical characteristics are summarized in Table 1. Each patient underwent several colonics (1–4 times), and blood samples were collected to examine lymphocyte movement in the peripheral circulation. After colonic irrigation, the number of peripheral leukocytes increased significantly from  $5917 \pm 1215/\mu\text{L}$  to  $6610 \pm 1452/\mu\text{L}$  ( $P < 0.0001$ ) (Fig. 2). Moreover, the number of lymphocytes increased significantly from  $1788 \pm 403/\mu\text{L}$  to  $2164 \pm 430/\mu\text{L}$  ( $P < 0.0001$ ) (Fig. 2). The ratio of lymphocytes also increased significantly from  $30.9 \pm 7.1\%$  to  $34.0 \pm 6.9\%$  ( $P < 0.0001$ ) (Fig. 3). Although the number of neutrophils increased significantly from  $3442 \pm 1029/\mu\text{L}$  to  $3712 \pm 1236/\mu\text{L}$  ( $P = 0.01$ ) (Fig. 2), the ratio decreased significantly from  $58.3 \pm 8.4\%$  to  $55.1 \pm 8.2\%$  ( $P < 0.0001$ ) (Fig. 3).

This is the first report of lymphocyte movement

after a colonic irrigation. The increase in lymphocytes is suspected to be the result of lymphocytes transmigrating as intraepithelial lymphocytes (IEL) and lymphocytes in the Peyer's patch and mesenteric lymph nodes during irrigations and abdominal massage.

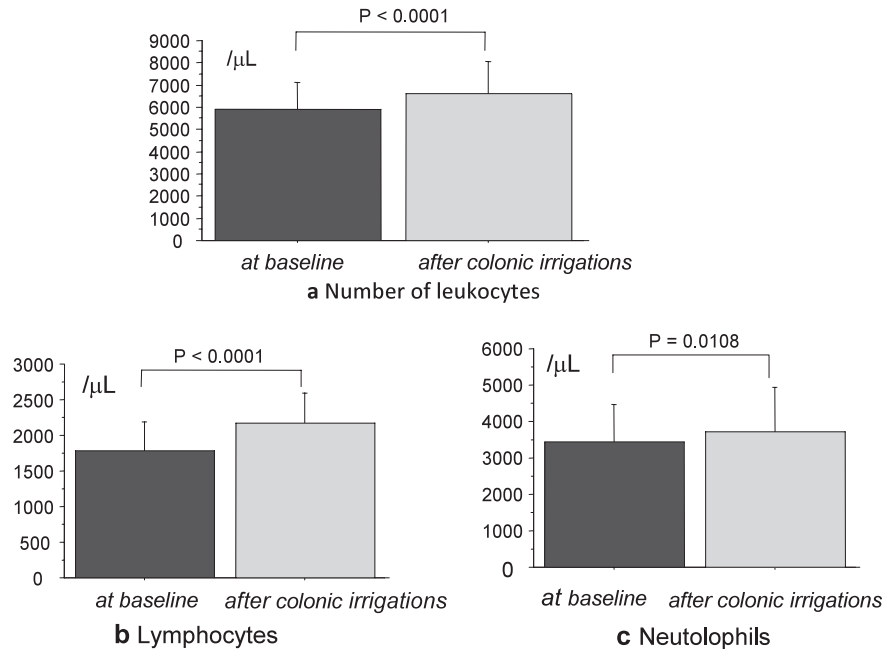
The intestinal epithelium is the first defense system to encounter intestinal pathogens or dietary antigens. It is well known that the intestine plays an important role as a local immune system. The NK T cell markers are CD56, CD57, and CD161. The CD56<sup>+</sup> T cell population resides predominantly in the liver, and the CD57<sup>+</sup> T cell population resides predominantly in the bone marrow (8, 10). CD161<sup>+</sup> T cells are cytotoxic to some cancer cell lines and preferentially accumulate in human liver and human intestinal epithelium (7). Human IEL contain many CD8<sup>+</sup> T cells (4), and the majority of CD161<sup>+</sup> T cells in humans are CD8<sup>+</sup> T cells. Further, Va24<sup>+</sup> T cells are the smallest population of human IEL CD161<sup>+</sup> T cells. When the intestine is full of feces, it is speculated that there may be reduced function of this immune system, which causes toxins to leak from the gut, and bacterial translocation from the gut to systemic circulation causes a breakdown of the intestinal wall (1). This breakdown can be



**Fig. 1** The functioning principle of Colon Hydromat Comfort. Filtered hot water and water are mixed and poured into patient's colon through the specula. The sewage water flows out by the pressure of the water filled colon.

**Table 1** Clinical characteristics of the study subjects

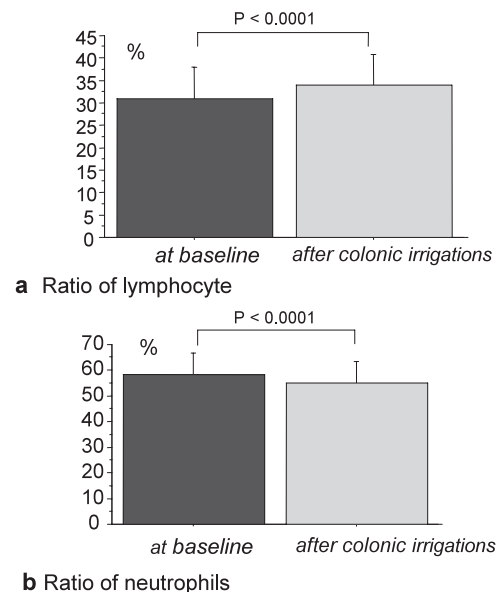
	Age	Gender	Underlying disease	Number of colonic irrigations
No. 1	39	female	diabetes mellitus	3
No. 2	38	female	constipation	4
No. 3	32	female	face eruption	3
No. 4	28	male	psoriasis	4
No. 5	45	female	ovarian cysts	3
No. 6	47	male	atopic dermatitis	3
No. 7	39	female	constipation	2
No. 8	44	male	pollen allergy	1
No. 9	38	female	constipation	3
No. 10	29	male	allergic rhinitis	3



**Fig. 2** a. The number of peripheral leukocytes increased significantly from  $5917 \pm 1215/\mu\text{L}$  to  $6610 \pm 1452/\mu\text{L}$  ( $P < 0.0001$ ). b. The number of lymphocytes increased significantly from  $1788 \pm 403/\mu\text{L}$  to  $2164 \pm 430/\mu\text{L}$  ( $P < 0.0001$ ). c. The number of neutrophils increased significantly from  $3442 \pm 1029/\mu\text{L}$  to  $3712 \pm 1236/\mu\text{L}$  ( $P = 0.01$ ). Error bar:  $\pm$  SD

caused by a variety of injuries to the body at locations far from the gut. Increased gut permeability and bacterial translocation play a role in multiple organ failure (MOF) (15). Failure of the gut barrier remains central to the hypothesis that toxins escaping from the gut lumen contribute to activation of host immune inflammatory defense mechanisms, subsequently leading to the auto-intoxication and tissue destruction observed in the septic response characteristic of MOF (1, 5, 13, 15). It is therefore believed that it is important to keep the intestine clean as a preparation for mucosal regeneration. According to a study in which glass beads were used to check the transit time of feces through the colon, the transit time was quite variable and the colon often retained some food residues from the entire preceding week (3). Thus, it is useful to irrigate the colon to remove fecal residues.

The colonic irrigations are distinguished from enemas in the following manners: (a) they are not self-administered but are administered by a professionally trained person, and (b) they are administered using a device to control water flow, to infuse the entire colon with water, in contrast to the more limited infusion of warm filtered water into the rectum. The water circulates throughout the colon, removing its contents, while the patient lies on a bed. Water temperature and pressure are closely moni-



**Fig. 3** a. The ratio of lymphocytes also increased significantly from  $30.9 \pm 7.1\%$  to  $34.0 \pm 6.9\%$  ( $P < 0.0001$ ). b. The ratio of lymphocytes decreased significantly from  $58.3 \pm 8.4\%$  to  $55.1 \pm 8.2\%$  ( $P < 0.0001$ ). Error bar:  $\pm$  SD

tored and regulated during a series of fills and releases to aid in the peristaltic action of the colon. Because this method involves a closed system, the waste materials are removed without the unpleasant

odors or discomfort usually associated with enemas.

A report by Satterlee *et al.* (14) discussed nervous system symptomatology during chronic intestinal toxemia. They noted the newly found and rapidly developing relationship between mental and nervous conditions and disturbances of the intestinal tract. The connection between the intestine and nervous system is well established. There are reflexes from the colon that affect the entire nervous system, emphasizing the importance of the “abdominal brain” or enteric nervous system (9). It is estimated that 80% of vagal fibers are visceral afferents (2). There is also a vast overlap of neuropeptide activity in the gut and brain (12). The enteric nervous system has become an active area of physiological research with over 600 articles in Medline since 1985. Modern medicine recognizes the involvement of the abdominal nervous system in several neurological disorders, including migraine, epilepsy, and autism (9). Granulocytes have mainly adrenergic receptors and lymphocytes have cholinergic receptors on their surfaces and are controlled by the autonomic nervous system (11, 17).

In this study, we evaluated lymphocyte movement after colon irrigation. The increase in the number of lymphocytes after colonic irrigation might indicate their transmigration from GALT to peripheral blood. Therefore, colon irrigation may improve colon and immune system function. This study was limited by a small population, and the fact that the patients had no immune disease and normal lymphocyte ranges at baseline. Moreover, we did not verify the lymphocyte phenotype. In a future study, we plan to enroll lymphopenic or autoimmune disease patients.

#### Acknowledgment

I appreciate the help of Dr. Toru Abo, Department of Immunology, Niigata University School of Medicine for providing useful advice during the preparation of this manuscript.

#### REFERENCES

- Alexander JW, Boyce ST, Babcock GF, Gianotti L, Peck MD, Dunn DL, Pyles T, Childress CP and Ash SK (1990) The process of microbial translocation. *Ann Surg* **212**, 496–512.
- Alverez WC and Freedlander BL (1924) The rate of progress of food residues through the bowel. *JAMA* **83**, 576–580.
- Alverez WC (1919) Origin of so-called autointoxication symptoms. *JAMA* **72**, 8–13.
- Cerf-Bensussan N, Guy-Grand D and Griscelli C (1985) Intraepithelial lymphocytes of human gut: isolation, characterization and study of natural killer activity. *Gut* **26**, 81–88.
- Garcia-Tsao G, Lee FY, Bardeb GE, Cartun R and West AB (1995) Bacterial translocation to mesenteric lymph nodes is increased in cirrhotic rats with ascites. *Gastroenterology* **108**, 1835–1841.
- Guy-Grand D, Cerf-Bensussan N, Malissen B, Malassis-Seris M, Briottet C and Vassalli P (1991) Two gut intraepithelial CD8<sup>+</sup> lymphocyte population with different T cell receptors. A role for the gut epithelium in T cell differentiation. *J Exp Med* **173**, 471–481.
- Iiai T, Watanabe H, Suda T, Okamoto H, Abo T and Hatakeyama K (2002) CD161<sup>+</sup> (NT) cells exist predominantly in human intestinal epithelium as well as in liver. *Clin Exp Immunol* **129**, 92–98.
- Ishihara S, Nieda M, Kitayama J, Osada T, Yabe T, Ishikawa Y, Nagawa H, Muto T and Juji T (1999) CD8<sup>+</sup> NKR-P1A<sup>+</sup> T cells preferentially accumulate in human liver. *Eur J Immunol* **29**, 2406–2413.
- McMillin DL, Richards DG, Mein EA and Nelson CD (1999) The abdominal brain and enteric nervous system. *J Altern Complement Med* **5**, 575–586.
- Norris S, Doherty DG, Collins C, McEntee G, Traynor O, Hegarty JE and O’Farrelly C (1999) Natural T cells in the human liver. Cytotoxic lymphocytes with dual T cell and natural killer cell phenotype and function are phenotypically heterogeneous and include Va24-JaQ and  $\gamma\delta$ T cell receptor bearing cells. *Human Immunol* **60**, 20–31.
- Panosian JQ and Marinetti GV (1983)  $\alpha$ -Adrenergic receptors in human polymorphonuclear leucocyte membranes. *Biochem Pharmacol* **32**, 2243–2247.
- Pert CB, Ruff MR, Weber RJ and Herkenham M (1985) Neuropeptides and their receptors: A psychosomatic network. *J Immunol* **135**, 820S–826S.
- Purohit V, Bode JC, Bode C, Brenner DA, Choudhry MA, Hamilton F, Kang YJ, Keshavarzin A, Rao R, Sartor RB, Swanson C and Turner JR (2008) Alcohol, intestinal bacterial growth, intestinal permeability to endotoxin, and medical consequences: Summary of a symposium. *Alcohol* **42**, 349–361.
- Satterlee GR and Eldridge WW (1917) Symptomatology of nervous system in chronic intestinal toxemia. *JAMA* **69**, 1414–1418.
- Swank GM and Deitch EA (1996) Role of the gut in multiple organ failure. Bacterial translocation and permeability changes. *World J Surg* **20**, 411–417.
- Takahashi S, Kawamura T, Kanda Y, Taniguchi T, Nishizawa T, Iiai T, Hatakeyama K and Abo T (2005) Multipotential acceptance of Peyer’s patches in the intestine for both thymus-derived T cells and extrathymic T cells in mice. *Immunol Cell Biol* **83**, 504–510.
- Toyabe S, Iiai T, Fukuda M, Kawamura T, Suzuki S, Uchiyama M and Abo T (1997) Identification of nicotinic acetylcholine receptors on lymphocytes in the periphery as well as thymus in mice. *Immunology* **92**, 201–205.