Journal of Pathology
J Pathol 2009; **218**: 210–221
Published online 20 January 2009 in Wiley InterScience
(www.interscience.wiley.com) **DOI:** 10.1002/path.2525

Original Paper

Disruption of tight junctions during polymicrobial sepsis *in vivo*

Qiurong Li,[1,2] Qiang Zhang,[1,2] Chenyang Wang,[1] Xiaoxiang Liu,[3] Ning Li[1] and Jieshou Li[1]*
[1] *Institute of General Surgery, Jinling Hospital, Nanjing 210002, China*
[2] *School of Medicine, Nanjing University, Nanjing 210093, China*
[3] *National Key Laboratory of Crop Genetics and Germplasm Enhancement, Nanjing Agricultural University, Nanjing 210095, China*

**Correspondence to:*
Jieshou Li, Institute of General Surgery, Jinling Hospital, No 305 East Zhongshan Road, Nanjing 210002, PR China.
E-mail: liqiurong@yahoo.com

No conflicts of interest were declared.

Received: 16 August 2008
Revised: 1 November 2008
Accepted: 6 January 2009

Abstract

The disruption of intestinal epithelial tight junctions may result in barrier function dysfunction during polymicrobial sepsis. The pathophysiology of sepsis involves breakdown of barrier integrity, which correlates with adverse outcome during sepsis. However, the mechanisms underlying loss of barrier function in sepsis remain unknown. In the present study in mice, tight junction (TJ) structure was analysed by transmission electron microscopy; intestinal permeability was assessed using molecular tracer measurement; and the distribution of TJ proteins was investigated by immunofluorescence microscopy. The membrane microdomains of TJs were isolated using discontinuous sucrose density gradients and the expression of TJ proteins in these was determined by western blot. Immunofluorescence microscopy revealed that claudins 1, 3, 4, 5, and 8 were present predominantly in the microvillous surface of epithelial cells and along the lateral membranes of the cells; in sepsis, however, labelling of these proteins was present diffusely within cells and was no longer focused at the lateral cell boundaries. Moreover, the expression of claudin-2 was markedly up-regulated in sepsis. Using western blot analysis, we found that occludin and claudins were displaced from raft fractions to non-raft fractions in membrane microdomains of TJs in sepsis. In addition, the disruption of TJ structure was accompanied by increased intestinal permeability. Our results demonstrate for the first time that redistribution of TJ proteins in TJ membrane microdomains and redistribution of claudins in epithelial cells of the colon lead to alteration of TJ architecture and TJ barrier dysfunction during the development of polymicrobial sepsis.

Keywords: **sepsis; tight junction; barrier function dysfunction; tight junction proteins**

Introduction

Despite significant advances in medical therapeutic approaches, sepsis and multiple organ failure (MOF) are still a leading cause of morbidity and mortality in the surgical intensive care unit [1]. It has been suggested that gut mucosal damage and dysfunction may play a critical role in the initiation and development of this state [2]. There is growing recognition that intestinal barrier dysfunction may contribute to the adverse clinical outcome. However, the underlying pathophysiological mechanisms of intestinal barrier disruption in sepsis are poorly understood.

Intestinal barrier function depends primarily on the integrity of the intestinal epithelium. The intestinal epithelial barrier is regulated mainly by intercellular tight junctions (TJs), which are important architectural features of the paracellular pathway [3,4]. An intact epithelial TJ barrier serves as a critical barrier against paracellular penetration of pathogenic bacteria and toxic luminal antigens including endotoxins [5,6]. The disruption of intestinal TJs causes an increase in intestinal permeability and results in a 'leaky' TJ barrier, leading to bacterial translocation [7].

TJs are specialized and complex structures composed of multiple proteins [8] including the integral membrane protein occludin [9], members of the claudin family [10], junction adhesion molecule, and zonula occludens (ZO)-1 [11]. TJs have been increasingly recognized for their role in disease states including stress, Crohn's disease, ulcerative colitis (UC), and microbial infections [12–16]. Alterations in TJ architecture are mainly attributed to redistribution of the TJ proteins in the intestinal epithelium, and epithelial barrier function is affected in UC [14]. Our previous studies have indicated that the distribution of TJ protein expression in membrane microdomains of TJs was altered by pro-inflammatory cytokines [17]. Pronounced barrier dysfunction is associated with a decreased expression and redistribution of claudins in

Figure 1. Gross morphological change of the colon in sepsis mice. Colons showing stool pellets of control animal (A); the stool pellets in the colons of sepsis mice appear smaller than those of the sham controls (B–D)

active Crohn's disease [18]. TJs have been considered as specialized lipid raft-like membrane microdomains [19]. Changes in TJ protein expression in membrane microdomains of TJs lead to altered TJ structure and barrier dysfunction [19]. *Clostridium difficile* toxins influence TJ function by modulating the redistribution of TJ proteins in the membrane microdomains [20]. These results reveal a novel insight to elucidate TJ structure and function in intestinal barrier damage.

The caecal ligation and puncture (CLP) sepsis model is considered the gold standard for sepsis research [21]. Up to now, few TJ functional and molecular data during polymicrobial sepsis have been available. In this study, we used the *in vivo* mouse sepsis model to investigate whether disruption of epithelial TJs could result in a loss of barrier function during polymicrobial sepsis. We examined possible changes of the distribution of TJ proteins in membrane microdomains to investigate mechanisms of barrier dysfunction in sepsis.

Materials and methods

Reagents

Antibodies to claudin-1, -2, -3, -4, -5, and -8 were purchased from Zymed Laboratories Inc (San Francisco, CA, USA). Secondary antibodies conjugated to horseradish peroxidase were purchased from Amersham (Piscataway, NJ, USA). Alexa Fluor-635 secondary antibodies, Alexa 488-conjugated streptavidin, and DAPI (4′,6′-diamidino-2-phenylindole) were from Molecular Probes (Eugene, OR, USA), and EZ-link

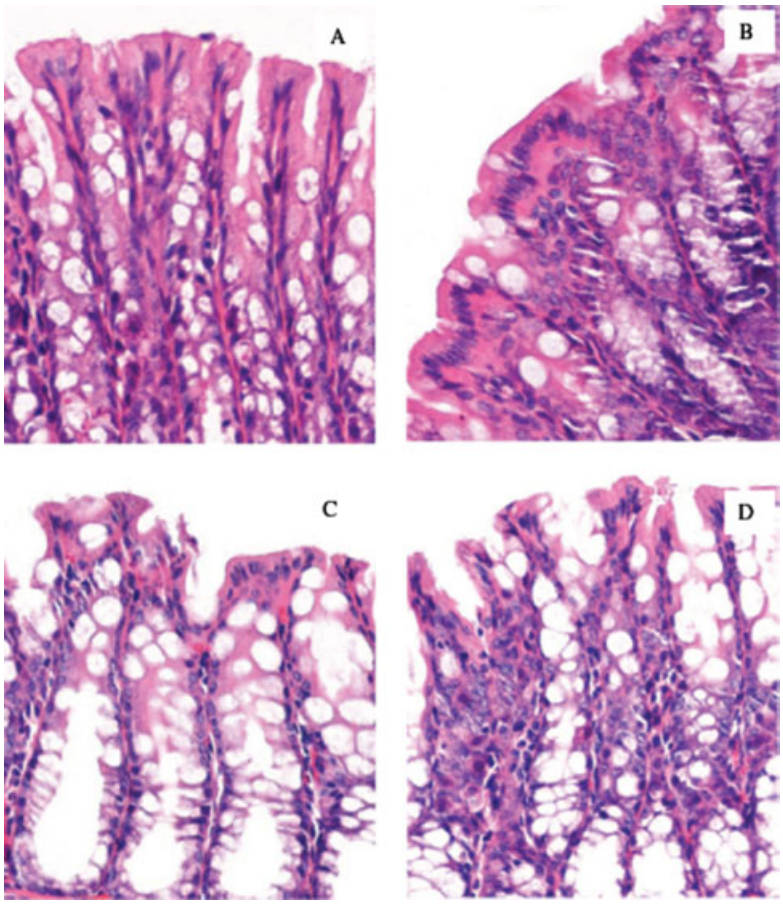

Figure 2. Histological changes in the colon of sepsis mice. In control mice, H&E staining of the colon revealed normal mucosal morphology (A) (original magnifications 200×). The colon of CLP mice showed mucosal injury and increased neutrophilic infiltration (B–D)

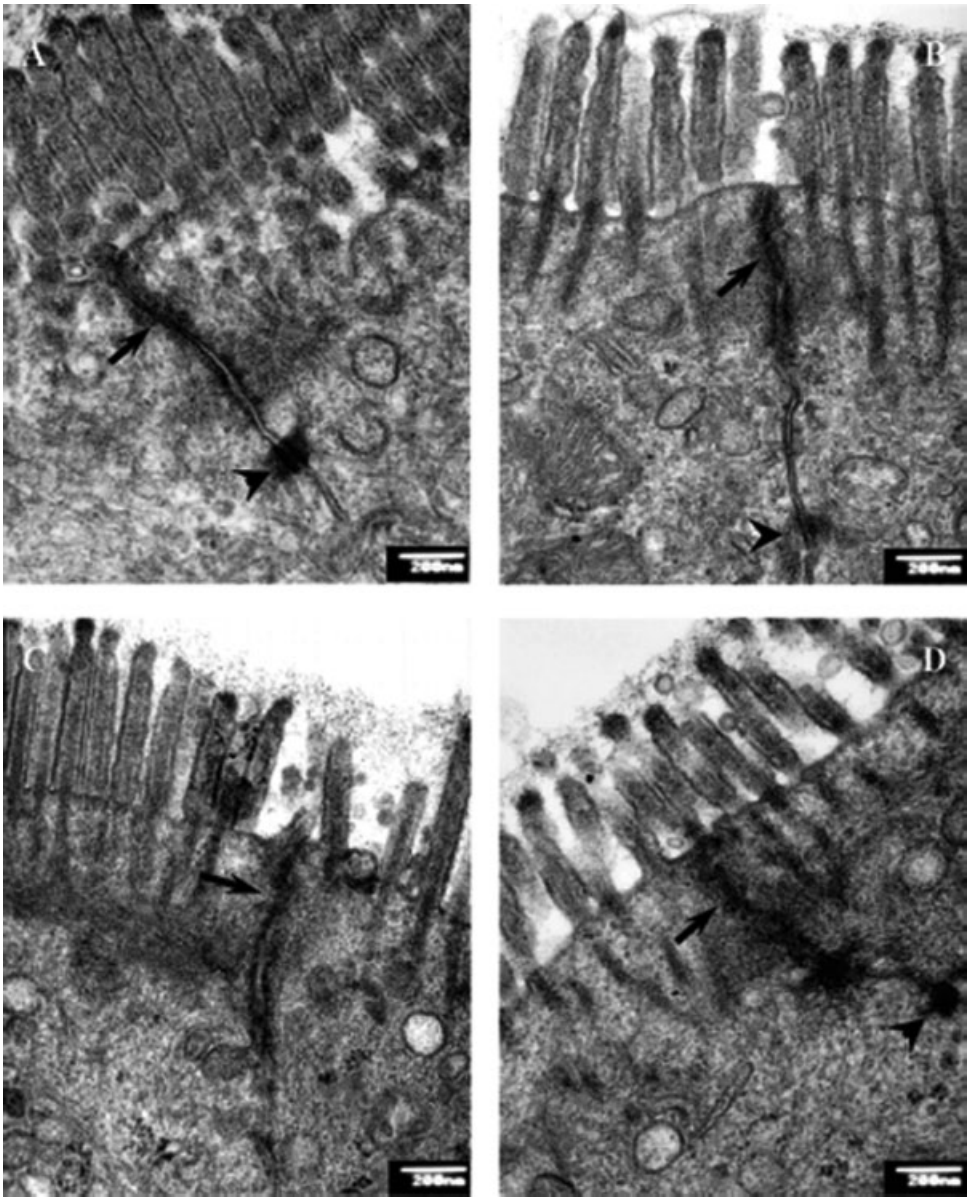

Figure 3. Ultrastructure micrographs of tight junctions (TJs) in the colon of sepsis mice. In sham-treated mice, the TJ and desmosomes are intact (A). The electron-dense materials diminished in sepsis mice (B–D), indicating disruption of TJ morphology. Arrows indicate the location of the TJs and arrowheads indicate the desmosomes. Scale bar = 200 nm

Sulfo-NHS-Biotin was from Pierce Chemical Co (Illinois, USA).

Induction of polymicrobial sepsis

Four- to six-week-old female C57BL/6 mice were used in this study. Animals were left undisturbed for 1 week to equilibrate to their environment before experiments. The study was approved by the Institutional Animal Care and Use Committee of Nanjing University, and the Principles of Laboratory Animal Care (NIH publication No 86-23, revised in 1985) were followed.

For induction of polymicrobial sepsis, mice were subjected to caecal ligation and puncture (CLP) according to the method described by Chung *et al* [22]. In brief, mice were anaesthetized with sodium pentobarbital (50 mg/kg body weight ip). A midline incision (1.5–2.0 cm) was made, and the caecum was ligated with a 3-0 silk suture and punctured through and through with a 22-gauge needle, and gently compressed to extrude a small amount of caecal contents through the puncture holes. The caecum was then returned to the abdomen and the incision was closed. For the sham-operated controls, the caecum was exposed but not ligated or punctured and then returned to the abdominal cavity. All mice were resuscitated with 1 ml of sterile saline administered subcutaneously during the post-operative period. Six mice were sacrificed at 6, 12, and 24 h after surgery.

Histological investigation

Mouse colon samples were taken, fixed in 10% neutral buffered formalin, and embedded in paraffin. Tissue sections were cut at 5 μm and stained with haematoxylin and eosin (H&E).

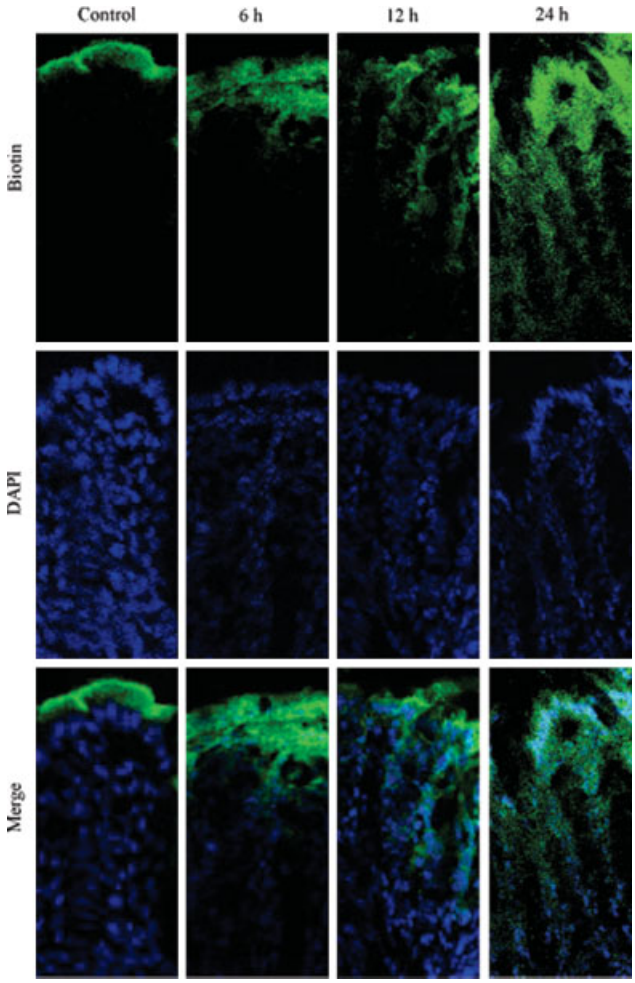

Figure 4. Barrier permeability of mouse colon determined with biotin as a molecular tracer. The tracer molecule is held to the luminal border of the colon in sham-operated mice (left column). Fluorescent detection reveals that biotin penetrated the epithelium into tissue in CLP mice. Nuclei were stained with DAPI (blue). Merged biotin and DAPI images are also presented

Transmission electron microscopy (TEM)

Mouse colons were washed and fixed with 4% glutaraldehyde for 2 h and then post-fixed with 1% osmium tetroxide (OsO_4). Tissues were embedded in Epon 812 and thin-sectioned. Sections were stained with uranyl acetate and lead citrate and then viewed and photographed on a Hitachi H-600 (Tokyo, Japan) transmission electron microscope operated at 75 kV.

Mucosal permeability assessment

In the present study, EZ-link Sulfo-NHS-Biotin (443 Da) was used as a small molecular tracer to measure the permeability of colons in CLP mice as reported by Guttman *et al* [23]. Biotin was diluted to 2 mg/ml in PBS containing 1 mM $CaCl_2$ just prior to use. After the animals were killed by cervical dislocation, biotin was slowly injected into the colon for 3.5 min via a syringe attached to a 22 G blunt-end needle. Following this, the region of colon just rostral to the area contacting the syringe was removed (1 cm) and placed in 3.7% paraformaldehyde in PBS, pH 7.3 for 3 h. The tissue was then washed extensively with PBS and sectioned by Wax-It Histology Services (Vancouver, BC, Canada). Tissue sections were incubated with a 1 : 500 dilution of streptavidin conjugated to Alexa 488 for 30 min and imaged by a Leica TCS SP2 confocal scanning microscope (Leica Microsystems, Heidelberg GmbH, Mannheim, Germany).

Immunofluorescence

Colon tissues were fixed in 3% paraformaldehyde for 3 h and then washed with PBS. The fixed colons were embedded in optimal cutting temperature embedding medium (Tissue-Tek OCT Compound, Sakura Finetek USA, Inc, Torrance, CA, USA) and frozen onto an aluminium stub. Sections of 5 μm were cut, attached to glass slides, and permeabilized with 0.2% Triton X-100 in PBS buffer for 20 min. Slides were washed with PBS extensively and blocked with 5% normal goat serum PBS containing 0.05% Tween-20 and 0.1% bovine serum albumin for 20 min at room temperature. Primary antibodies were added to the slides and incubated overnight at 4 °C in a humidity chamber. After washing, sections were incubated with Alexa Fluor 635-conjugated secondary antibodies for 60 min. The slides were again washed extensively and stained with DAPI (4′,6′-diamidino-2-phenylindole) for 5 min. Sections were visualized using a Leica TCS SP2 confocal scanning microscope (Leica Microsystems).

Isolation of TJ membrane microdomains and western blot analysis

Colonic tissue samples were homogenized in lysis buffer (50 mM Tris, 25 mM KCl, 5 mM $MgCl_2.6\ H_2O$, 2 mM EDTA, 40 mM NaF, 4mM Na_3VO_4, pH 7.4) containing 1% Triton X-100 and a protease inhibitor mixture solution. TJ membrane microdomains were isolated and TJ proteins were analysed by western blot according to our previously reported method [14].

Statistical analysis

The results presented are the mean ± SEM. Statistical analysis was performed using Student's *t*-test. A *p* value less than 0.05 was considered statistically significant.

Results

Gross morphological change of intestine in sepsis mice

In the present study, the mouse intestine was examined during polymicrobial sepsis. A different distribution of stool pellets was shown in the colon of

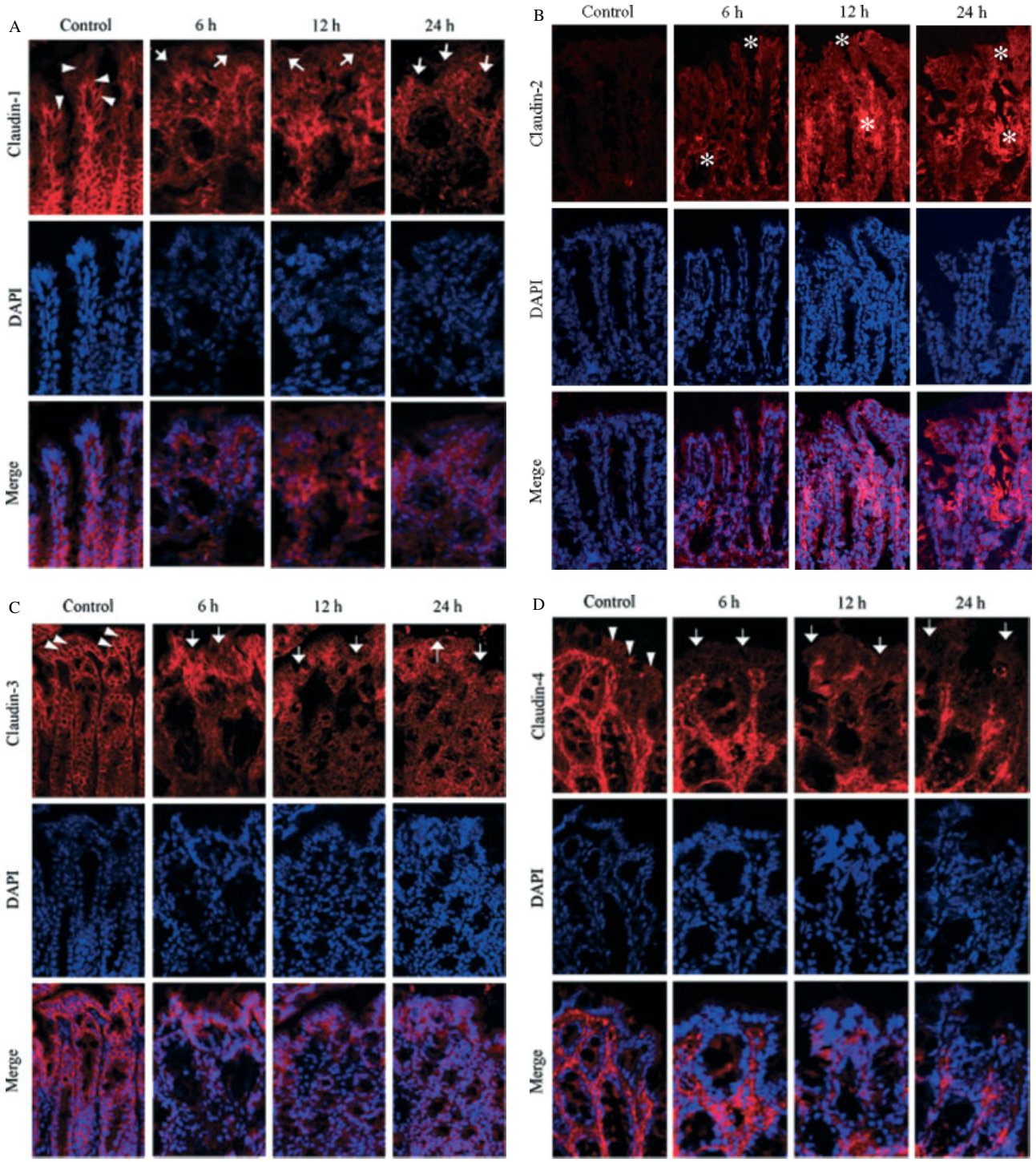

Figure 5. Claudin-1 (A), -2 (B), -3 (C), -4 (D), -5 (E), and -8 (F) and DAPI (DNA) localization on sections of colon from sepsis mice and controls. Claudin staining (red) and DAPI staining (blue), as well as merged claudin and DAPI images are presented. Arrowheads indicate areas of staining at the lateral boundaries of colonocytes. Arrows show the lack of focused staining in sepsis mice. Asterisks indicate elevated expression of claudin-2

sepsis mice (Figure 1). The intestine of sham control mice contained more pellets of stool in the colon. The amount of stool pellets in the colon from sepsis mice appeared smaller than that of control mice. Stool pellets were not seen in the colon for 24 h after surgery. The caecum was significantly engorged in CLP animals 6, 12, and 24 h after surgery.

Histological analysis of tissue from sepsis mice 6, 12, and 24 h after surgery

Intact architecture and normal lymphoplasmacellular population were observed in sham-operated mice (Figure 2A). The appearance of mucosal injury and marked neutrophilic infiltration in the colon were found in CLP mice (Figures 2B–2D). The mice

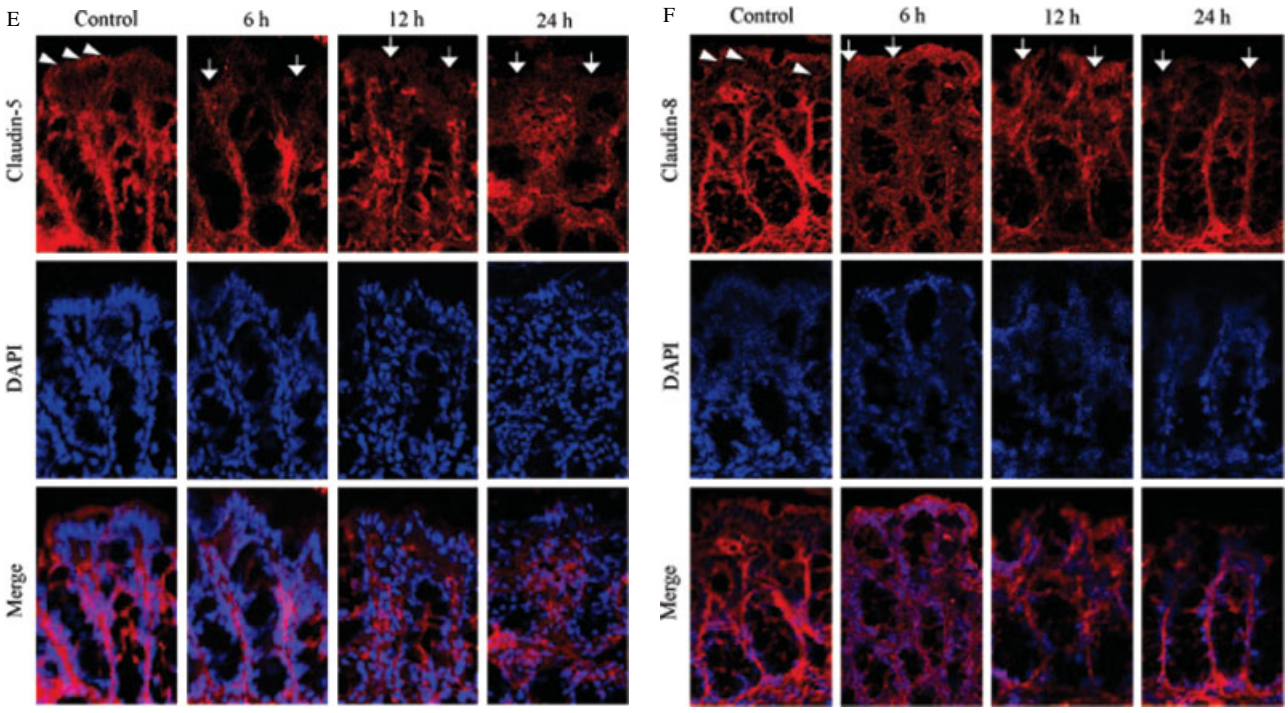

Figure 5. Continued

studied at 24 h post-CLP showed more pronounced disintegration of the mucosa compared with sham-operated mice. The degree of histological destruction increased when the CLP period was prolonged.

Alteration of TJ ultrastructure in sepsis mice

TJs are intercellular junctions crucial for barrier function. To assess the effects of polymicrobial sepsis on TJ ultrastructure, TEM was performed on the colon. Figure 3 shows that TEM of the colon revealed an intact TJ structure in the control animals (Figure 3A). The architecture of TJs was altered in CLP animals 6, 12, and 24 h after surgery (Figures 3B–3D). There were morphological differences in the epithelial TJ ultrastructure in the colons of CLP animals compared with that in sham-operated mice. In sepsis mice, the electron-dense materials present between the adjoining cells near the brush border decreased, indicating the disruption of normal TJ morphology.

TJs in the colon are functionally disrupted in CLP mice

The ultrastructure of the TJs in the colon was altered in sepsis; therefore, we determined whether TJ barrier function and permeability were impaired in CLP mice utilizing the tracer molecule biotin in the colon. Following the administration of biotin into the colon, fluorescence-labelled biotin was found to be restricted to the luminal border of the colon and did not penetrate the epithelium into the tissue in sham-operated mice (Figure 4). During polymicrobial sepsis, biotin staining permeated the epithelium into tissue and the fluorescent staining pattern was significantly different in the colons of mice 6, 12, and 24 h after CLP surgery (Figure 4). The biotin fluorescent staining penetrated deep into the epithelium and had penetrated throughout the tissue of the mice 24 h after CLP surgery (Figure 4), which indicated that TJs were disrupted and the permeability of the epithelial barrier was markedly increased.

Redistribution of claudins in the surface epithelium of the colon

It has been demonstrated that claudin-1, -2, -3, -4, and -5 are present in the colon [24,25]. Claudins contribute to barrier function and the redistribution of sealing claudin-8 leads to altered TJ structure and pronounced barrier dysfunction in active Crohn's disease [26]. Until now, there has been little known about the redistribution of claudins during polymicrobial sepsis. We therefore investigated the expression and distribution of claudin-1, -2, -3, -4, -5, and -8 in colon sections from sham-operated and sepsis mice.

We found that claudin-1 was immunolocalized at the cell apices and the lateral cell membrane in the tissue of controls (Figure 5A). Claudin-1 staining showed a different distribution pattern which was localized diffusely within the cells (Figure 5A) and the nucleus detected with DAPI was disarranged in the sepsis mice colon sections. Claudin-2 was scarcely detectable in controls, whereas it showed intense tight junctional staining both in the crypts and in the surface epithelium in sepsis mice (Figure 5B). Claudin-3 staining was predominantly junctional, as the areas of staining were at the lateral boundaries of colonocytes in controls (Figure 5C). Claudin-3 staining distribution in controls was similar to that

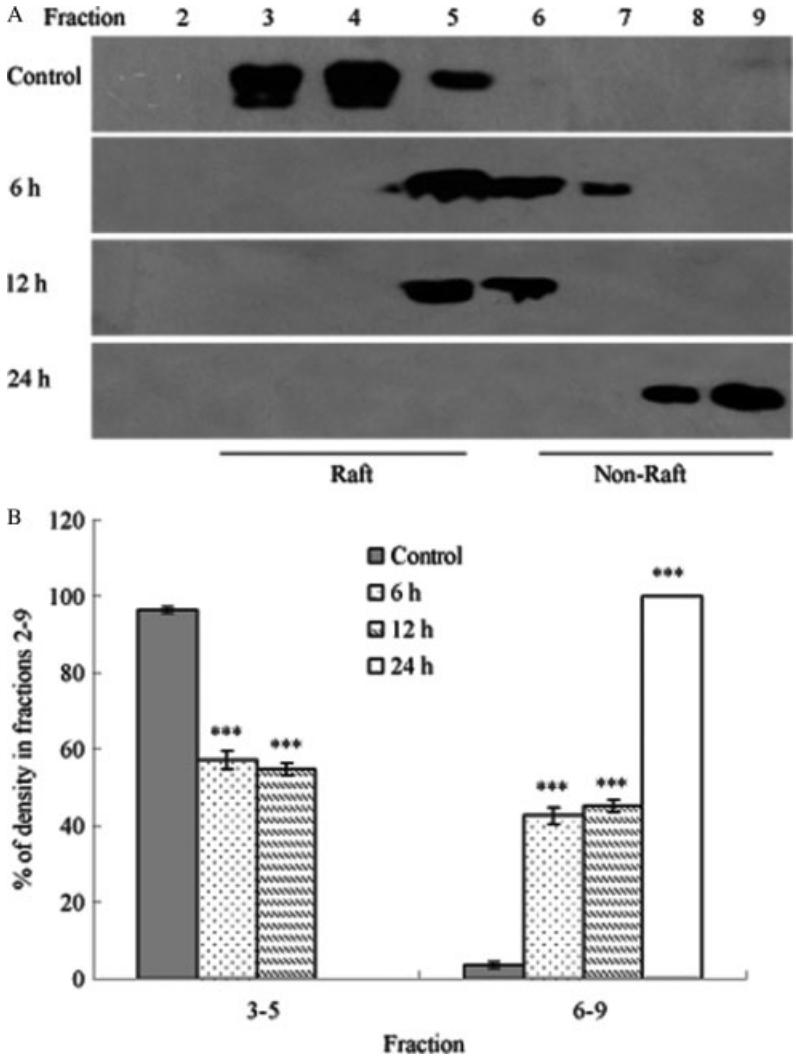

Figure 6. Effect of sepsis on the localization of caveolin-1 in raft fractions. Lipid rafts were isolated by Triton X-100 detergent extraction and sucrose gradient centrifugation as described in the Materials and methods section. Caveolin-1 was used as a marker for the localization of lipid rafts in the sucrose gradients, which was detected mainly in lipid raft fractions (fractions 3–5) in controls. Data are shown as means ± SEM (***$p < 0.001$)

reported by Guttman *et al* [23]. In contrast, claudin-3 was redistributed from the TJ and showed cytoplasmic staining in sepsis mice (Figure 5C). Claudin-4 and -5 were predominantly in the microvillus surface of epithelial cells and along the lateral membranes of the cells (Figures 5D and 5E). The distribution of claudin-4 and -5 was different between control and sepsis mice, and localization of claudin-4 and -5 in colonocytes was completely disrupted in colon sections of sepsis mice (Figures 5D and 5E). Claudin-8 was detected in the surface of epithelial cells and the crypt epithelium of controls (Figure 5F), and weak cytoplasmic staining was shown in sepsis murine colon sections.

Redistribution of TJ proteins in membrane microdomains of TJs induced by sepsis

We recently found a major pool of TJ proteins in membrane microdomains of TJs, and the distribution of occluding and claudin isoforms was markedly influenced in membrane microdomains of TJs in UC [14]. In the present study, we examined the distribution of TJ proteins including occludin and claudin-1, -3, and -5 in sucrose density gradient fractions from sepsis mice. Membrane microdomains of TJs are considered as specialized lipid raft-like membrane microdomains [19]. To fix the localization of lipid rafts within the sucrose gradients, we used caveolin-1 as a marker. The majority of caveolin-1 (96.4%) was found in TX-100 insoluble raft fractions (fractions 3–5) of controls (Figures 6A and 6B). We also found that caveolin-1 was removed from raft fractions to non-raft fractions (fractions 6–9) in sepsis mice (Figures 6A and 6B).

The distribution of occludin and claudin isoforms (claudin-1, -3, and -5) from different fractions was analysed by western blot (Figures 7–10). Densitometric analysis was presented as a percentage of the total protein detected on the same immunoblot, as shown in Figures 7–10. Western blot analysis demonstrated that it amounted to 22.5 ± 1.1% for occludin in membrane microdomains of TJs in controls, 38.8 ± 1.9% for claudin-1, 19.4 ± 1.3% for claudin-3, and 24.7 ± 0.8% for claudin-5, respectively. The distribution of

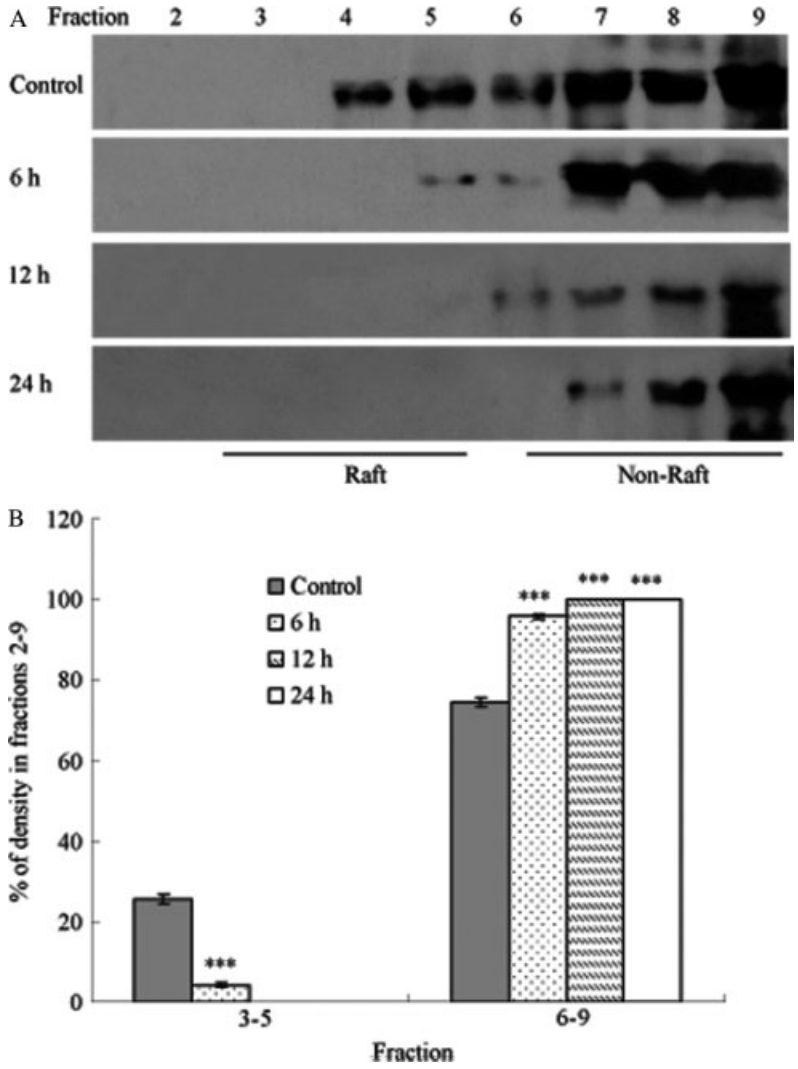

Figure 7. Sepsis induced redistribution of the TJ protein occludin. Homogenates of murine colon tissues were subjected to sucrose density gradient centrifugation and analysed by immunoblotting. Blots were probed with an antibody against occludin. Blots were analysed and quantified by densitometry. The blots shown are representative of three experiments ($^{***}p < 0.001$)

occludin and claudin isoforms was markedly altered in membrane microdomains of TJs in sepsis mice. The amount of occludin in raft fractions decreased to 4.2%, 0%, and 0%, respectively, for sepsis mice of 6, 12, and 24 h after surgery (Figures 7A and 7B). Claudin-1 and -3 were completely displaced from TJ membrane microdomains in the sepsis mice of 12 and 24 h after surgery (Figures 8 and 9), and claudin-5 was also completely removed for 24 h after surgery (Figure 10).

Discussion

Gut dysfunction during sepsis is a common problem, resulting in loss of intestinal mucosal barrier selectivity and multiple organ dysfunction [2]. As a result, the gut is viewed by many as an 'engine' that drives sepsis. Despite extensive studies on TJ disruption *in vitro,* there are no studies that have determined alterations of TJs and barrier dysfunction, or the cellular and molecular basis on the changes in TJs during polymicrobial sepsis. Our present findings demonstrated for the first time that the architecture of TJs is altered and functional tracer experiments showed that a molecular tracer, which does not normally penetrate epithelia, passed across in sepsis animals. TJ disruption was characterized by the redistribution of TJ proteins in sections of colon taken during the progression of polymicrobial sepsis. Subcellular fractionation using discontinuous sucrose density gradients demonstrated changes in TJ protein expression in membrane microdomains of TJs in response to sepsis. Our observations provide new data on the role of TJs in the pathogenesis of polymicrobial sepsis.

Because disruption of TJ barrier function may be a major part of the pathology through a mechanism of altered TJ permeability in sepsis, we examined the intestine barrier permeability after polymicrobial sepsis. Permeability studies were performed using a low-molecular-weight tracer of fluorescence signal. In various phases of sepsis, there were significant changes in the permeability for the low-molecular-weight tracer

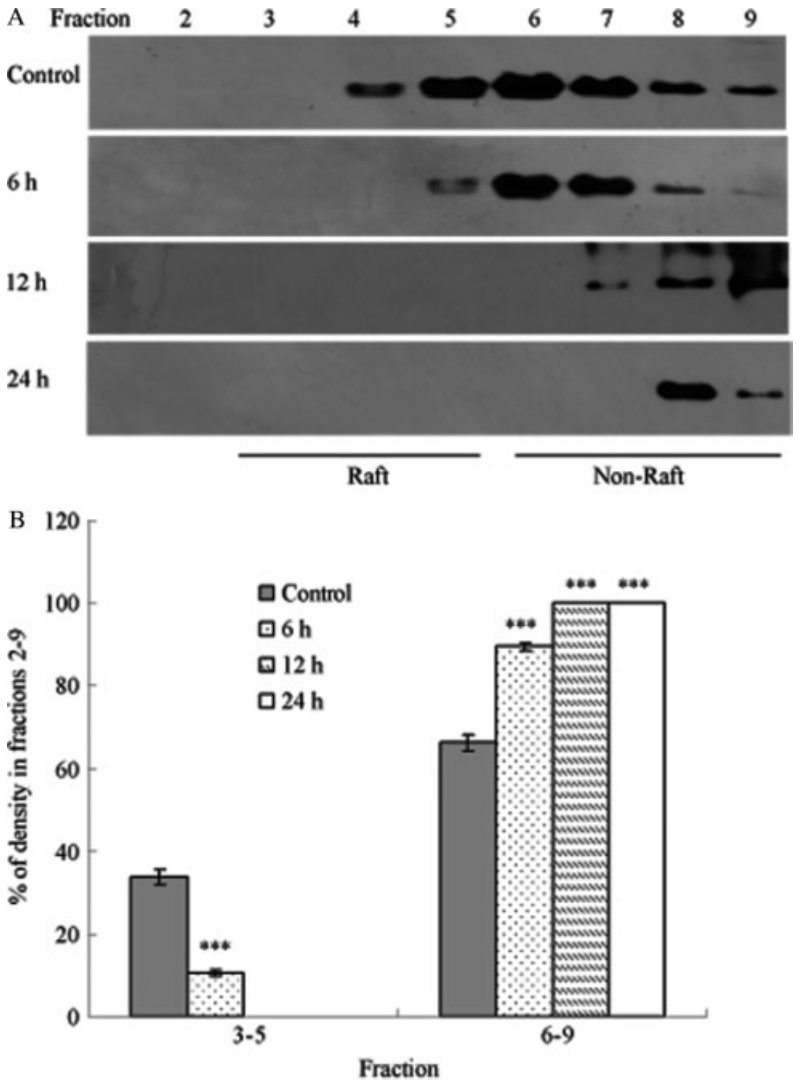

Figure 8. Distribution of claudin-1 in membrane microdomains of TJs. Homogenates were subjected to sucrose density gradient centrifugation. Equal amounts of protein from each fraction were analysed by immunoblotting for claudin-1 ($^{***}p < 0.001$)

(Figure 4). An increase of the intestinal epithelium permeability for biotin was detected, which indicated that the integrity of the TJs in the colon was functionally disrupted in sepsis. Our finding that intestinal permeability was significantly increased during the progression of polymicrobial sepsis indicated that the increased permeability occurred most likely via the disruption of TJ ultrastructure.

The integrity of TJs is maintained by TJ proteins, and paracellular permeability is associated with proper expression and localization of TJ proteins. To investigate the possible role of TJ proteins in TJ barrier dysfunction induced by sepsis, we performed western blot analysis of TJ proteins in membrane microdomains of TJs. A significant change in the expression of TJ proteins including occludin and claudins in membrane microdomains of TJs was found (Figures 7–10), which is responsible for intestinal epithelial permeability secondary to sepsis. Sepsis-induced changes in TJ barrier function are associated with the redistribution of TJ proteins in membrane microdomains of TJs. This finding is consistent with our previous results showing that the distribution of TJ proteins in membrane microdomains of TJs is altered in response to UC [14]. The TJ membrane microdomain is an integral part of the TJ structure [19,20]. Lipid rafts are specialized membrane microdomains enriched in cholesterol and sphingolipid, and preferentially partitioned in the apical membrane of polarized epithelia. Our results indicate that the loss of TJ barrier function is correlated with translocation of lipid raft-associated TJ proteins, suggesting that membrane microdomains of TJs may play an important role in the *in vivo* epithelial barrier dysfunction.

Previous studies have demonstrated that claudin-1, -3, -4, -5, and -8 are present in the colon [24–26]. To confirm further the underlying cellular mechanisms with special attention to tight junctions in sepsis, we used confocal laser scanning microscopy to investigate changes in the expression and distribution of claudins associated with altered TJ structure and TJ barrier dysfunction in sepsis. Our results demonstrated that immunolocalization of claudin-1, -3, -4, -5, and -8 on tissue sections of sepsis mice labelled TJs and

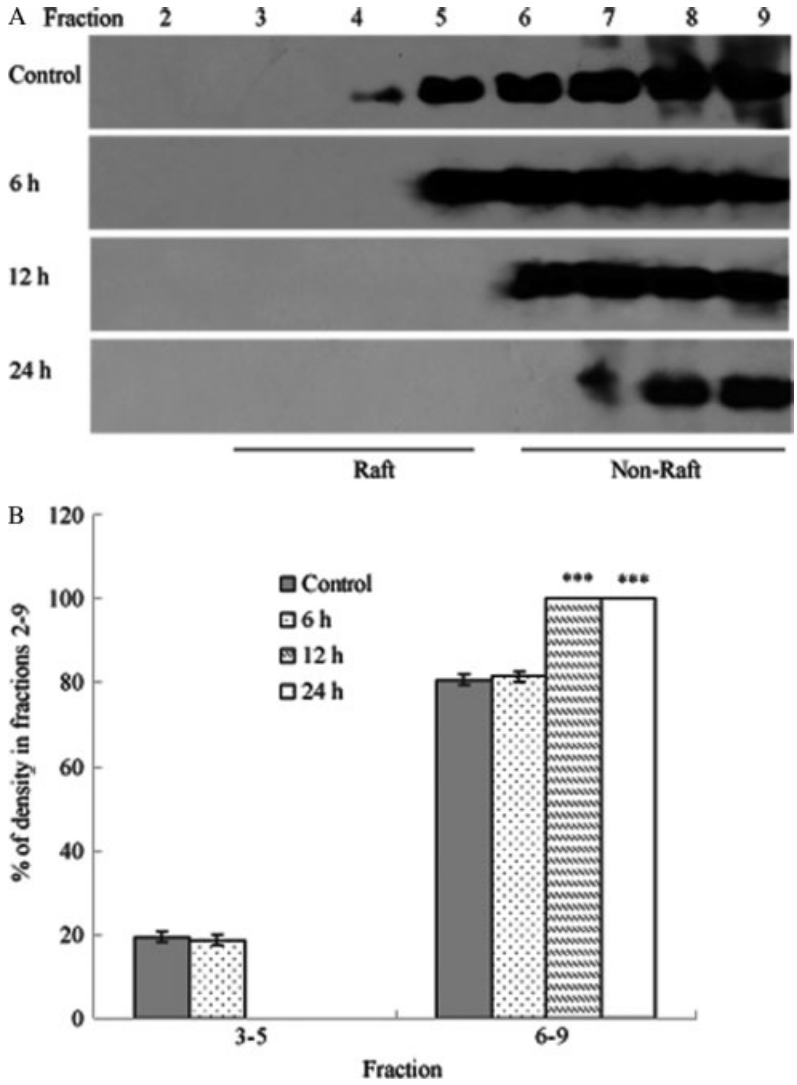

Figure 9. Distribution of claudin-3 in membrane microdomains of TJs. Homogenates were subjected to sucrose density gradient centrifugation. Equal amounts of protein from each fraction were analysed by immunoblotting for claudin-3 ($^{***}p < 0.001$)

the lateral boundaries of colonocytes, and appeared markedly altered compared with sham-operated controls. The results were consistent with previous results showing that claudins are redistributed in response to different pathological conditions such as CD and bacteria invasion [18,23]. However, claudin-2 was dramatically up-regulated in sepsis mice. Our results are similar to the findings in Crohn's disease [18]. Therefore, the redistribution of sealing claudins together with the increased expression of the pore-forming TJ protein claudin-2 may provide the molecular basis for the disruption of tight junctions during polymicrobial sepsis *in vivo*.

Sepsis is associated with a systemic inflammatory response syndrome that is characterized by copious amounts of pro-inflammatory cytokines, suggesting that regulation of the inflammatory response has been compromised [26]. The pathogenesis of sepsis has implicated pro-inflammatory cytokines (eg TNF-α and IFN-γ) as harmful mediators [27]. The important findings of the deleterious effects of pro-inflammatory cytokines on intestine barrier function and TJ structure have been previously documented *in vitro* and *in vivo* [14,17,28,29]. Intestinal permeability is significantly increased and TJ proteins are redistributed from the TJ membrane microdomains to the cytosolic compartment, and then TJ disruption is induced in sepsis.

Here, we have presented evidence that TJ barrier dysfunction is correlated with alterations in TJ architecture and TJ protein redistribution in TJ membrane microdomains during polymicrobial sepsis. We have also demonstrated that claudin distribution in epithelial cells is altered in the murine colon of sepsis. These observations support the important role of TJ changes to barrier impairment in sepsis.

Acknowledgements

We acknowledge the following financial support: National Basic Research Programme (973 Programme) in China (Nos 2007CB513005 and 2009CB522405); the Key Project of the National Natural Science Foundation in China (30830098); the National Natural Science Foundation in China (30672061); the Key Project of Nanjing Military Command (06Z40); and the Military Scientific Research Fund (0603AM117);

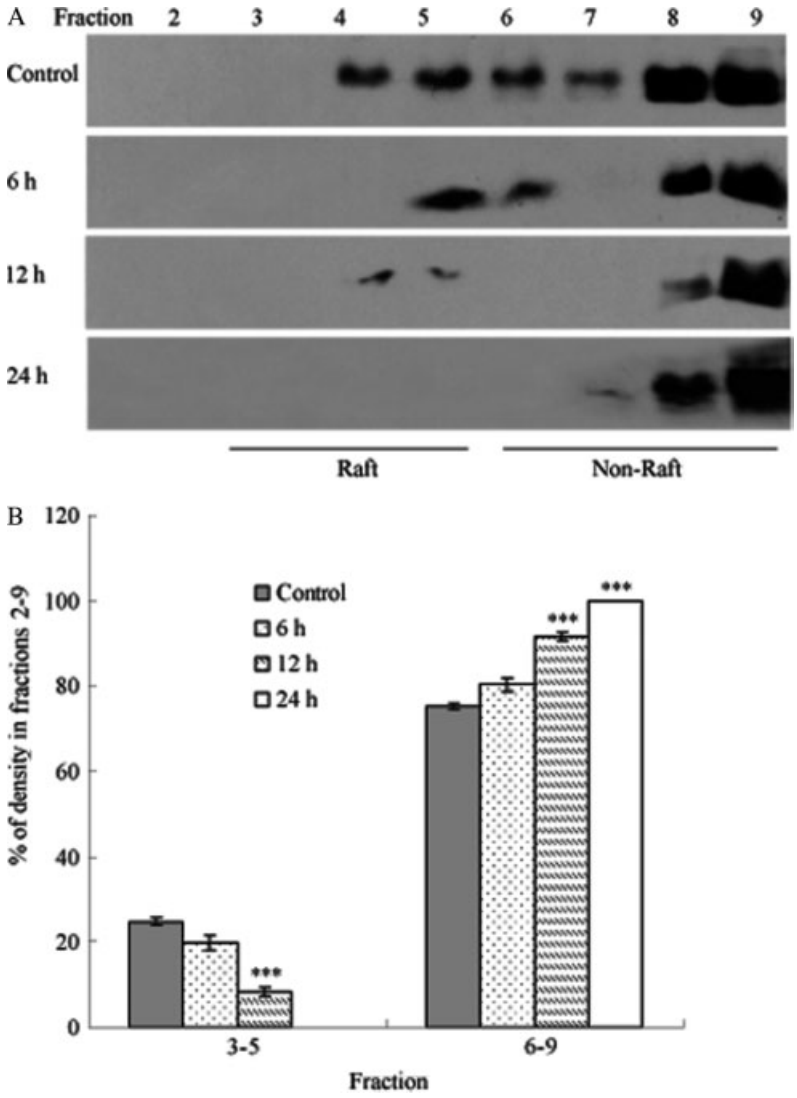

Figure 10. Distribution of claudin-5 in membrane microdomains of TJs. Homogenates were subjected to sucrose density gradient centrifugation. Equal amounts of protein from each fraction were analysed by immunoblotting for claudin-5 ($^{***}p < 0.001$)

and we thank the Deutscher Akademischer Austauschdienst Researcher Fellowship (Bioscience Special Program, Germany) for supporting Dr Qiurong Li.

References

1. Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J, Pinsky MR. Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. *Crit Care Med* 2001;**29:**1303–1310.
2. Nieuwenhuijzen GA, Deitch EA, Goris RJ. The relationship between gut-derived bacteria and the development of the multiple organ dysfunction syndrome. *J Anat* 1996;**189:**537–548.
3. Mitic LL, Anderson JM. Molecular architecture of tight junctions. *Annu Rev Physiol* 1998;**60:**121–142.
4. Mitic LL, Van Itallie CM, Anderson JM, Schneeberger EE, Fanning AS. Molecular physiology and pathophysiology of tight junctions I. Tight junction structure and function: lessons from mutant animals and proteins. *Am J Physiol Gastrointest Liver Physiol* 2000;**279:**G250–G254.
5. Anderson JM, Van Itallie CM. Tight junctions and the molecular basis for regulation of paracellular permeability. *Am J Physiol Gastrointest Liver Physiol* 1995;**269:**G467–G475.
6. Ma TY. Intestinal epithelial barrier dysfunction in Crohn's disease. *Proc Soc Exp Biol Med* 1997;**214:**318–327.
7. Söderholm JD, Perdue MH. Stress and the gastrointestinal tract II. Stress and intestinal barrier function. *Am J Physiol Gastrointest Liver Physiol* 2001;**280:**G7–G13.
8. Furuse M, Fujimoto K, Sato N, Hirase T, Tsukita S. Overexpression of occludin, a tight junction-associated integral membrane protein, induces the formation of intracellular multilamellar bodies bearing tight junction-like structures. *J Cell Sci* 1996;**109:**429–435.
9. Furuse M, Hirase T, Itoh M, Nagafuchi A, Yonemura S, Tsukita S. Occludin: a novel integral membrane protein localizing at tight junctions. *J Cell Sci* 1993;**123:**1777–1788.
10. Noda A, Kubo A, Tsukita S. Claudin-based tight junctions are crucial for the mammalian epidermal barrier: a lesson from claudin-1-deficient mice. *J Cell Sci* 2002;**156:**1099–1111.
11. Stevenson BR, Anderson JM, Goodenough DA, Mooseker MS. Tight junction structure and ZO-1 content are identical in two strains of Madin–Darby canine kidney cells which differ in transepithelial electrical resistance. *J Cell Biol* 1988;**107:**2401–2408.
12. Kompan L, Kompan D. Importance of increased intestinal permeability after multiple injuries. *Eur J Surg* 2001;**167:**570–574.
13. Schmitz H, Barmeyer C, Fromm M, Runkel N, Foss HD, Bentzel CJ, *et al*. Altered tight junction structure contributes to the impaired epithelial barrier function in ulcerative colitis. *Gastroenterology* 1999;**116:**301–309.

14. Li QR, Zhang Q, Zhang M, Wang CY, Zhu ZX, Li N, *et al*. Effect of *n*-3 polyunsaturated fatty acids on membrane microdomain localization of tight junction proteins in experimental colitis. *FEBS J* 2008;**275:**411–420.
15. Sartor RB. Current concepts of the etiology and pathogenesis of ulcerative colitis and Crohn's disease. *Gastroenterol Clin North Am* 1995;**24:**475–507.
16. Muza-Moons MM, Schneeberger EE, Hecht GA. Enteropathogenic *Escherichia coli* infection leads to appearance of aberrant tight junction strands in the lateral membrane of intestinal epithelial cells. *Cell Microbiol* 2004;**6:**783–793.
17. Li QR, Zhang Q, Wang M, Zhao SM, Ma J, Luo N, *et al*. Interferon-γ and tumor necrosis factor-α disrupt epithelial barrier function by altering lipid composition in membrane microdomains of tight junction. *Clin Immunol* 2008;**126:**67–80.
18. Zeissig S, Bürgel N, Günzel D, Richter J, Mankertz J, Wahnschaffe U, *et al*. Changes in expression and distribution of claudin-2, -5 and -8 lead to discontinuous tight junctions and barrier dysfunction in active Crohn's disease. *Gut* 2007;**56:**61–72.
19. Nusrat A, Parkos CA, Verkade P, Foley CS, Liang TW, Innis-Whitehouse W, *et al*. Tight junctions are membrane microdomains. *J Cell Sci* 2000;**113:**1771–1781.
20. Nusrat A, von Eichel-Streiber C, Turner JR, Verkade P, Madara JL, Parkos CA. *Clostridium difficile* toxins disrupt epithelial barrier function by altering membrane microdomain localization of tight junction proteins. *Infect Immun* 2001;**69:**1329–1336.
21. Wichterman KA, Baue AE, Chaudry IH. Sepsis and septic shock — a review of laboratory models and a proposal. *J Surg Res* 1980;**29:**189–201.
22. Chung C-S, Wang WY, Chaudry IH, Ayala A. Increased apoptosis in lamina propria B cells during polymicrobial sepsis is FasL but not endotoxin mediated. *Am J Physiol Gastrointest Liver Physiol* 2001;**280:**G812–G818.
23. Guttman JA, Samji FN, Li YL, Wayne A, Finlay BB. Evidence that tight junctions are disrupted due to intimate bacterial contact and not inflammation during attaching and effacing pathogen infection *in vivo*. *Infect Immun* 2006;**74:**6075–6084.
24. Rahner C, Mitic LL, Anderson JM. Heterogeneity in expression and subcellular localization of claudins 2, 3, 4, and 5 in the rat liver, pancreas, and gut. *Gastroenterology* 2001;**120:**411–422.
25. Zeissig S, Bojarski C, Buergel N, Mankertz J, Zeitz M, Fromm M, *et al*. Downregulation of epithelial apoptosis and barrier repair in active Crohn's disease by tumour necrosis factor alpha antibody treatment. *Gut* 2004;**53:**1295–1302.
26. Chinnaiyan AM, Huber-Lang M, Kumar-Sinha C, Barrette TR, Shankar-Sinha S, Sarma VJ, *et al*. Molecular signatures of sepsis multi-organ gene expression profiles of systemic inflammation. *Am J Pathol* 2001;**159:**1199–1209.
27. Weaver JGR, Rouse MS, Steckelberg JM, Badley AD. Improved survival in experimental sepsis with an orally administered inhibitor of apoptosis. *FASEB J* 2004;**18:**1185–1191.
28. Li QR, Zhang Q, Wang M, Zhao SM, Xu GW, Li JS. *n*-3 polyunsaturated fatty acids prevent disruption of epithelial barrier function induced by proinflammatory cytokines. *Mol Immunol* 2008;**45:**1356–1365.
29. Bruewer M, Luegering A, Kucharzik T, Parkos CA, Madara JL, Hopkins AM, *et al*. Proinflammatory cytokines disrupt epithelial barrier function by apoptosis-independent mechanisms. *J Immunol* 2003;**171:**6164–6172.