

Junctional adhesion molecule-C mediates leukocyte infiltration in response to ischemia reperfusion injury

SCHEIERMANN, Christoph, *et al.*

Abstract

OBJECTIVE: Junctional adhesion molecule-C (JAM-C) is an adhesion molecule that has multiple roles in inflammation and vascular biology, but many aspects of its functions under pathological conditions are unknown. Here we investigated the role of JAM-C in leukocyte migration in response to ischemia reperfusion (I/R) injury. **METHODS AND RESULTS:** Pretreatment of mice with soluble JAM-C (sJAM-C), used as a pharmacological blocker of JAM-C-mediated reactions, significantly suppressed leukocyte migration in models of kidney and cremaster muscle I/R injury (39 and 51% inhibition, respectively). Furthermore, in the cremaster muscle model (studied by intravital microscopy), both leukocyte adhesion and transmigration were suppressed in JAM-C-deficient mice (JAM-C^{-/-}) and enhanced in mice overexpressing JAM-C in their endothelial cells (ECs). Analysis of JAM-C subcellular expression by immunoelectron microscopy indicated that in I/R-injured tissues, EC JAM-C was redistributed from cytoplasmic vesicles and EC junctional sites to nonjunctional plasma membranes, a response that may account for the role of JAM-C in both leukocyte [...]

Reference

SCHEIERMANN, Christoph, *et al.* Junctional adhesion molecule-C mediates leukocyte infiltration in response to ischemia reperfusion injury. *Arteriosclerosis, thrombosis, and vascular biology*, 2009, vol. 29, no. 10, p. 1509-15

PMID : 19574560

DOI : 10.1161/ATVBAHA.109.187559

Available at:

<http://archive-ouverte.unige.ch/unige:5404>

Disclaimer: layout of this document may differ from the published version.



UNIVERSITÉ
DE GENÈVE

Junctional Adhesion Molecule-C Mediates Leukocyte Infiltration in Response to Ischemia Reperfusion Injury

Christoph Scheiermann, Bartomeu Colom, Paolo Meda, Nimesh S.A. Patel, Mathieu-Benoit Voisin, Alessandra Marrelli, Abigail Woodfin, Costantino Pitzalis, Christoph Thiemermann, Michel Aurrand-Lions, Beat A. Imhof, Sussan Nourshargh

Objective—Junctional adhesion molecule-C (JAM-C) is an adhesion molecule that has multiple roles in inflammation and vascular biology, but many aspects of its functions under pathological conditions are unknown. Here we investigated the role of JAM-C in leukocyte migration in response to ischemia reperfusion (I/R) injury.

Methods and Results—Pretreatment of mice with soluble JAM-C (sJAM-C), used as a pharmacological blocker of JAM-C-mediated reactions, significantly suppressed leukocyte migration in models of kidney and cremaster muscle I/R injury (39 and 51% inhibition, respectively). Furthermore, in the cremaster muscle model (studied by intravital microscopy), both leukocyte adhesion and transmigration were suppressed in JAM-C-deficient mice (JAM-C^{-/-}) and enhanced in mice overexpressing JAM-C in their endothelial cells (ECs). Analysis of JAM-C subcellular expression by immunoelectron microscopy indicated that in I/R-injured tissues, EC JAM-C was redistributed from cytoplasmic vesicles and EC junctional sites to nonjunctional plasma membranes, a response that may account for the role of JAM-C in both leukocyte adhesion and transmigration under conditions of I/R injury.

Conclusions—The findings demonstrate a role for EC JAM-C in mediating leukocyte adhesion and transmigration in response to I/R injury and indicate the existence of a novel regulatory mechanism for redistribution and hence function of EC JAM-C in vivo. (*Arterioscler Thromb Vasc Biol.* 2009;29:1509-1515.)

Key Words: JAM-C ■ ischemia reperfusion injury ■ leukocyte transmigration ■ inflammation ■ adhesion molecules

Leukocyte migration into inflamed tissues is a characteristic feature of inflammatory disorders, including numerous cardiovascular conditions such as atherosclerosis, myocardial infarction, and stroke. This response involves a cascade of cellular and molecular events that have culminated in the paradigm of the leukocyte adhesion cascade.¹ The final step in this process involves leukocyte migration through endothelial cells (ECs) which can occur via both para- or transcellular routes, and the subsequent breaching of the basement membrane underlying ECs and embedding pericytes.¹ Leukocyte transendothelial cell migration involves a number of adhesion molecules, the expression of which is highly concentrated at junctions between adjacent ECs. These molecules include PECAM-1, ICAM-2, CD99, ESAM, and members of the junctional adhesion molecule (JAM) family.¹⁻³ Our understanding of the roles, mechanisms of action, and potential interactions of these molecules has significantly enhanced in recent years, but many aspects of their functions in particular under in vivo pathological conditions remains unclear.

JAMs are members of an immunoglobulin subfamily, currently composed of JAM-A, -B, -C, JAM-4, ESAM (EC-selective adhesion molecule), and CAR (coxsackie virus and adenovirus receptor) that localize to cell-cell contacts and are specifically enriched at tight junctions with some being directly implicated in leukocyte transendothelial cell migration.³ Among these molecules, JAM-C is unique in terms of its broad expression and functional profile. Specifically, JAM-C expression has been reported on ECs, spermataids, intestinal epithelial cells, smooth muscle cells, fibroblasts, and has recently been detected on Schwann cells in the peripheral nervous system.⁴⁻¹¹ Furthermore, in humans, JAM-C is expressed on platelets and lymphocytes, whereas murine hematopoietic cells only express JAM-C during early development.^{4,12-16} Because of this wide expression pattern, JAM-C has been implicated in numerous events such as leukocyte trafficking, regulation of cell polarity, vascular permeability, and angiogenesis and appears to be critical in maintaining the integrity of the myelin sheath and the function of peripheral nerves.^{3,5,6,10,17-19} A number of ligands

Received March 5, 2009; revision accepted June 16, 2009.

From Barts and London School of Medicine and Dentistry (C.S., B.C., N.S.A.P., M.-B.V., A.M., A.W., C.P., C.T., S.N.), Queen Mary University of London, William Harvey Research Institute, London, UK; the Department of Cell Physiology and Metabolism (P.M.) and the Department of Pathology and Immunology (B.A.I.) University of Geneva, CMU, Switzerland; and INSERM (M.A.-L.), Centre de Recherche en Cancérologie de Marseille, Institut Paoli-Calmettes, France. Current affiliation for C.S.: the Division of Hematology/Oncology, Mount Sinai School of Medicine, New York. C.S. and B.C. contributed equally to this study.

Correspondence to Sussan Nourshargh, WHRI, Barts and The London School of Medicine and Dentistry, QMUL, Charterhouse Square, London, EC1M 6BQ, UK. E-mail s.nourshargh@qmul.ac.uk

© 2009 American Heart Association, Inc.

Arterioscler Thromb Vasc Biol is available at <http://atvb.ahajournals.org>

DOI: 10.1161/ATVBAHA.109.187559

have been reported for JAM-C, namely JAM-C, JAM-B, and Mac-1,^{3,17} although their contributions in the diverse functions of JAM-C remains unclear.

The functional role of JAM-C has largely been investigated using *in vitro* models of cell–cell interactions,^{8,11,13,20} but more recently a growing body of *in vivo* studies have demonstrated a significant role for this molecule in inflammatory and vascular events.^{7,12,18,21–23} Despite these findings, however, many aspects of the role(s) of JAM-C remain unknown, in particular its role in different stages of the leukocyte adhesion cascade and regulation of expression under *in vivo* pathological conditions. In the present study we have investigated the functional role of JAM-C in leukocyte migration in two murine models of I/R injury, namely I/R injury in the kidney and the cremaster muscle, the latter being investigated by intravital microscopy (IVM). The role of JAM-C was investigated in these models using both a pharmacological blocker of JAM-C (soluble JAM-C; sJAM-C) and genetically modified mice deficient in JAM-C or selectively overexpressing JAM-C in their ECs.^{6,12} Collectively, the findings demonstrate a role for JAM-C in leukocyte infiltration as elicited by I/R injury and indicate that JAM-C can support this response by mediating both leukocyte adhesion and transmigration, two distinct phases of the leukocyte adhesion cascade. Furthermore, analysis of venules by immunoelectron microscopy (IEM) detected for the first time the expression of JAM-C in EC intracellular vesicles *in vivo* and indicated that I/R injury can lead to redistribution of JAM-C within ECs, most notably from EC junctions and intracellular vesicles to EC nonjunctional membrane sites. The findings provide novel insights into the role and mechanism of action of JAM-C and highlight a potentially novel mechanism through which regulated expression of JAM-C may mediate different phases of leukocyte–vessel wall interactions under pathological inflammatory conditions.

Methods

Mouse strains used were C57BL/6 (WT), JAM-C^{-/-}, and mice overexpressing JAM-C in their ECs (EC JAM-C transgenics).^{6,12} Mice were purchased from Harlan-Olse, Bicester, UK or Charles River, Margate, UK. Analysis of JAM-C and PECAM-1 expression in murine tissues was performed by immunofluorescence staining and confocal microscopy. Mice pretreated with flag-tagged sJAM-C (3 mg/kg, *i.v.*) or a control molecule (flag-tag peptide or soluble fibronectin) were subjected to I/R injury. In the renal I/R injury model (30 minutes/24 hours), leukocyte infiltration into the kidneys was quantified by immunofluorescence and immunohistochemistry. Leukocyte adhesion and transmigration responses in mouse cremasteric venules as elicited by I/R injury (30 minutes/2 hours) was studied by IVM using WT mice (pretreated with a control molecule or sJAM-C), JAM-C^{-/-}, and EC JAM-C transgenic mice, as compared with relevant controls. The expression level of different adhesion molecules was investigated in blood cells from JAM-C^{-/-} and WT mice by flow cytometry. Cell transfer experiments were performed between WT and JAM-C^{-/-}, and the response of fluorescently-labeled leukocytes in recipient mice was analyzed by fluorescent IVM in the cremasteric vasculature. Subcellular localization and redistribution of JAM-C by I/R injury was investigated by IEM. (Please see supplemental materials, available online at <http://atvb.ahajournals.org>).

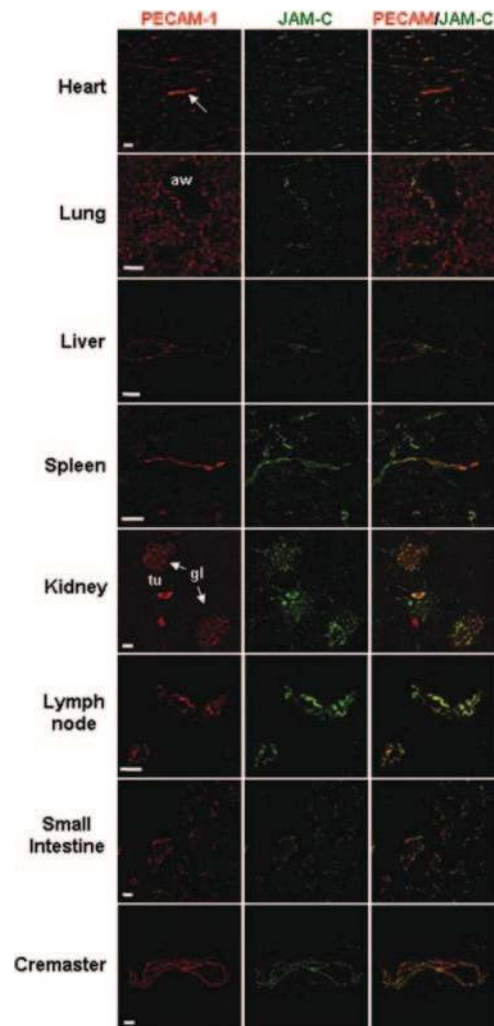


Figure 1. Investigation of the expression profile of JAM-C as compared to that of PECAM-1 in multiple murine organs by immunofluorescence staining and confocal microscopy. Scale bar: 20 μ m.

Results

JAM-C Is Expressed in the Vasculature of Multiple Organs in Mice

As JAM-C protein expression has not been investigated in a systematic manner in murine tissues, initial studies aimed to address this point by immunofluorescence staining and confocal microscopy. In all tissues studied (heart, lung, liver, spleen, kidney, lymph nodes, small intestine, and cremaster muscle), JAM-C expression was closely associated with the EC marker PECAM-1, although the extent of colocalization varied between different organs (Figure 1). Please see the supplemental materials for more details. As strong vascular expression of JAM-C was noted in kidneys and the cremaster muscle, these organs were analyzed for the functional role of JAM-C under conditions of I/R injury.

Soluble JAM-C Inhibits Leukocyte Infiltration in Models of Kidney and Cremaster Muscle I/R Injury

The role of JAM-C in leukocyte migration in two models of I/R injury, kidney and cremaster muscle, was investigated

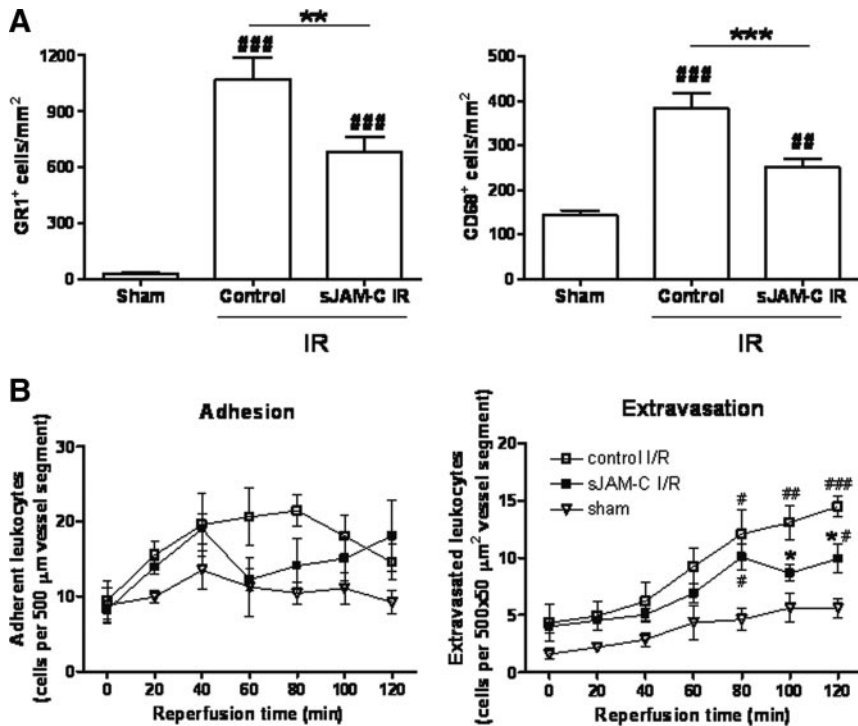


Figure 2. sJAM-C decreases leukocyte migration in kidney and cremaster muscle models of I/R injury. A, GR1⁺ (infiltrated inflammatory leukocytes) and CD68⁺ (monocytes/macrophages) cells quantified in kidney sections. B, The number of adherent and extravasated leukocytes was quantified in cremasteric venules by IVM. (Please see the supplemental materials).

using sJAM-C as a pharmacological blocker of JAM-C-mediated responses.^{12,23} In the kidney model, a significant leukocyte infiltration was noted in mice subjected to I/R injury as compared to sham-operated mice. In this model, the infiltrating leukocytes consisted of both neutrophils and monocytes/macrophages, though the latter appeared to form a minority population ($\approx 1:4$ ratio of CD68⁺ cells [monocyte/macrophage] to GR1⁺ cells [neutrophils and GR1⁺ inflammatory monocytes]); Figure 2A and supplemental Figure I). Pretreatment of mice with sJAM-C (3 mg/kg, i.v.), but not a flag control peptide, significantly suppressed the infiltration of both GR1⁺ and CD68⁺ cells into inflamed kidneys (37% and 55% inhibition, respectively), suggesting a role for JAM-C in both neutrophil and monocyte migration in this model. To investigate the stage in the leukocyte adhesion cascade mediated by JAM-C under conditions of I/R injury, the role of JAM-C in leukocyte migration was also investigated in real time by IVM²⁴ in the mouse cremaster muscle.

In the I/R injury of the cremaster muscle, after a 30-minute ischemia period, leukocyte–vessel wall interactions were observed by IVM during a 2-hour reperfusion period. In this model, leukocyte adhesion to and extravasation through venular walls increased in a time-dependent manner over the 2-hour reperfusion period, as compared to sham-operated animals. In mice pretreated with sJAM-C a marked reduction in leukocyte adhesion was noted (Figure 2B, left), which was associated with a significant suppression of leukocyte transmigration, as compared to mice receiving a flag-control peptide (51% inhibition at 120 minutes reperfusion time; Figure 2B, right). Fibronectin, used as a control soluble protein, had no significant effect on leukocyte adhesion or transmigration as compared to responses obtained in flag-control peptide-treated mice (not shown). These results demonstrate that sJAM-C suppresses leukocyte migration in the

kidney and cremaster muscle models of I/R injury, indicating a role for JAM-C in this inflammatory scenario.

JAM-C^{-/-} Mice Exhibit Reduced Leukocyte Adhesion and Transmigration in Cremasteric Venules as Induced by I/R Injury

To further investigate the role of JAM-C in regulating leukocyte–vessel wall interaction, the response of JAM-C^{-/-} mice to I/R injury was studied by IVM in the cremaster muscle. Initially, some characterization of these mice was performed. Figure 3A shows the EC junctional colocalization of JAM-C with the EC marker PECAM-1 in WT cremasteric venules and its lack of expression in JAM-C^{-/-} mice. The expression of PECAM-1 and other EC adhesion molecules such as VE-Cadherin and ICAM-1 appeared normal in JAM-C^{-/-} mice (Figure 3A and not shown). In addition, flow cytometry analysis revealed that the expression of key leukocyte adhesion molecules (PECAM-1, ICAM-2, Mac-1, L-selectin, and α_4 integrins) on blood neutrophils, monocytes (inflammatory and noninflammatory), and lymphocytes was normal in JAM-C^{-/-} mice (data not shown). Finally, as this is the first reported IVM study on JAM-C^{-/-} mice, some hematologic parameters were also quantified (supplemental Table I), which showed elevated circulating leukocyte number in JAM-C^{-/-} mice in line with previous reports,⁷ whereas no differences in mean arterial blood pressure was noted between WT, JAM-C^{-/-}, JAM-C^{+/-}, and EC JAM-C transgenic mice.

In the cremaster muscle I/R injury model, JAM-C^{-/-} mice exhibited a significantly reduced leukocyte adhesion (88% inhibition at 40 minutes reperfusion) and extravasation (66% inhibition at 120 minutes reperfusion) response as compared to their littermate JAM-C^{+/-} control mice (Figure 3B), findings that are in line with the results obtained under conditions of pharmacological blockade of JAM-C with

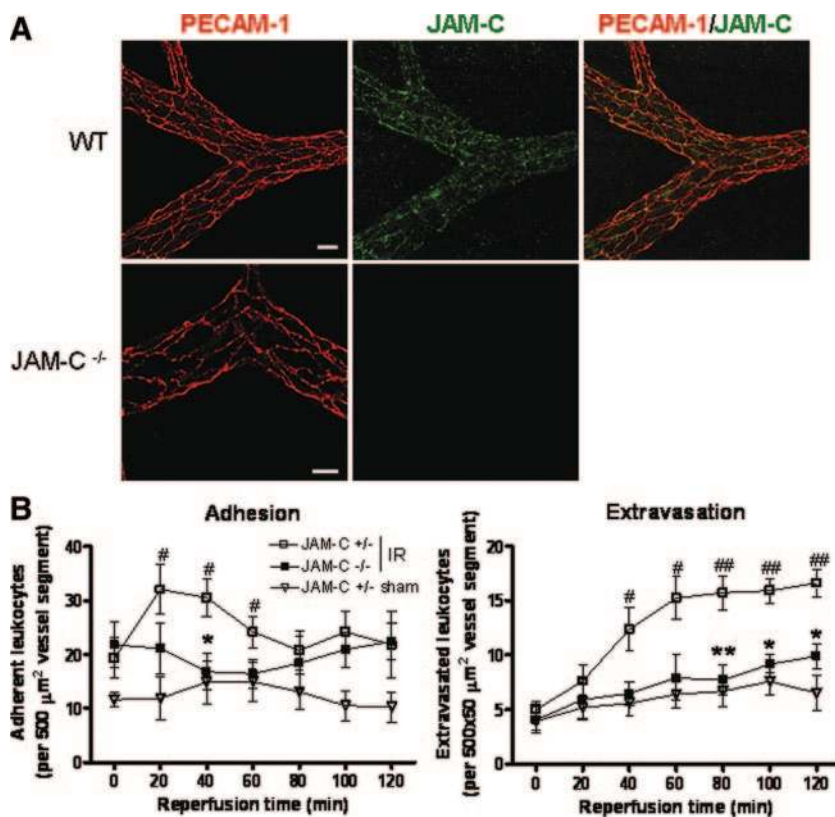


Figure 3. JAM-C^{-/-} exhibit reduced leukocyte adhesion and transmigration in cremasteric venules in response to I/R injury. A, Confocal images of cremasteric venules. Scale bar=20 μm . B, The number of adherent and extravasated leukocytes was quantified in cremasteric venules by IVM. (Please see the supplemental materials).

sJAM-C (Figure 2B). Of relevance, no differences in leukocyte responses were noted between JAM-C^{+/-} mice and WT animals (not shown). Because the inhibitory effect noted in the JAM-C^{-/-} may have been caused by defects in either leukocyte or EC functions, preliminary cell transfer experiments were performed to address this issue. Briefly, calcein-labeled JAM-C^{-/-} leukocytes injected into WT recipients responded in the same manner as WT leukocytes, whereas fluorescently-labeled control leukocytes (from WT or JAM-C^{+/-} mice) injected into JAM-C^{-/-} recipients exhibited a reduced transmigration response (81% inhibition at 120 minutes reperfusion; not shown). Collectively these data provide the first indication that EC JAM-C plays a key role in mediating leukocyte adhesion and extravasation in vivo. To further address this point, I/R injury was assessed in mice overexpressing JAM-C in their ECs.

Transgenic Mice Overexpressing JAM-C in ECs Exhibit Enhanced Leukocyte Adhesion and Transmigration in Response to I/R Injury

To extend the findings above, the cremaster muscle model was used to study I/R injury in mice overexpressing JAM-C in their ECs (EC JAM-C transgenic).¹² Confocal microscopy studies revealed that these mice exhibited an overall enhanced expression of JAM-C in ECs (not shown) as previously reported.¹² Interestingly, in a small number of tissue samples ($\approx <1\%$), some ECs (in both venules and arterioles) exhibited a “patchy” expression profile of JAM-C where the distribution of the molecule was very different to that seen in WT mice (compare Figures 3A and 4A). Specifically, whereas in WT tissues JAM-C expression appeared to be

largely localized at EC junctions, in some vessels of the transgenic mice JAM-C was detected both at EC junctions and also showing a strong apical/cytoplasmic expression. Of relevance the expression of other molecules under the control of this promoter has been reported to be unevenly distributed or patchy.²⁵

The EC JAM-C transgenic mice exhibited a consistently higher level of leukocyte adhesion and extravasation in cremasteric venules (eg, 62% increase at 120 minutes reperfusion; Figure 4B) as compared to WT littermate controls in response to I/R injury. These results together with the findings of studies using sJAM-C and JAM-C^{-/-} mice demonstrate that in this inflammatory scenario, EC JAM-C can support both leukocyte adhesion and transmigration. To gain a better understanding of the expression and regulation of expression of EC JAM-C under conditions of I/R injury, JAM-C subcellular localization was studied in cremaster muscle tissues by IEM.

I/R Injury Promotes JAM-C Redistribution in ECs In Vivo

Our immunofluorescent and confocal microscopy studies in WT animals indicated the EC JAM-C expression to be predominantly junctional (Figures 1 and 3A), in line with previous reports.⁵ More recent studies have also reported on the intracellular expression of JAM-C in cultured microvascular (HDMECs) and macrovascular (HUVECs) ECs.^{19,26} To investigate the expression profile of EC JAM-C in vivo and more importantly to assess how this may be regulated under conditions of I/R injury, JAM-C expression was studied by IEM,^{10,27} an approach that allows in situ localization of

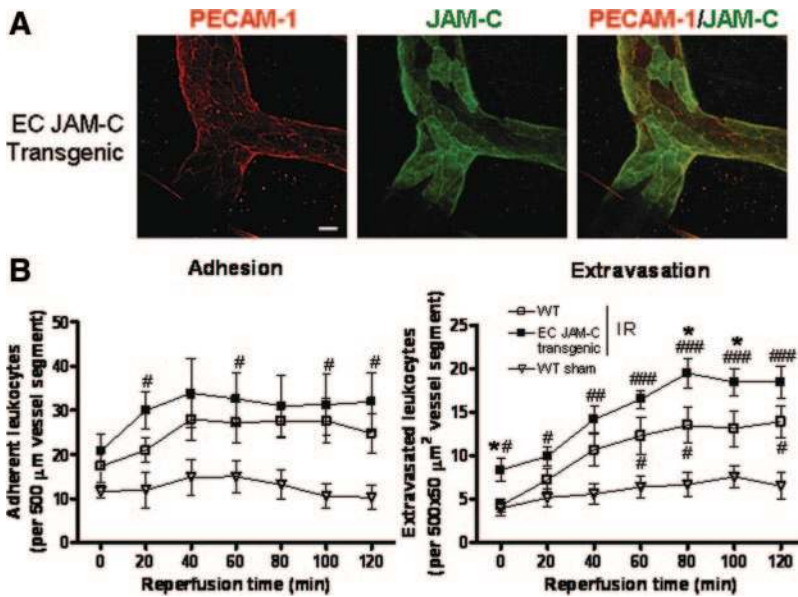


Figure 4. Transgenic mice overexpressing JAM-C in their ECs exhibit enhanced leukocyte adhesion and transmigration in cremasteric venules in response to I/R injury. A, Cremasteric venules from JAM-C transgenic mice. Bar=20 μm. B, Adherent and extravasated leukocytes were quantified in cremasteric venules by IVM. (Please see the supplemental materials).

JAM-C at the subcellular level. Using this technique, in control sham operated mice, JAM-C expression was noted both in ECs and in the myelin sheath of nearby nerves (not shown) as previously reported.¹⁰ In ECs, the expression of JAM-C was mostly at junctions between adjacent cells (3.6±0.4 particles per junction, n=28 ECs; Figure 5Ai) with a significantly lower level of immunolabeled JAM-C being found along the nonjunctional plasma membrane of ECs (1.4±0.1 particles/field, n=49 ECs; Figure 5Aii). Interestingly, JAM-C was also localized in small single membrane-bound cytoplasmic vesicles (56±1.8 nm diameter vesicles expressing 2.2±0.2 particles/vesicle, n=41; Figure 5Aiii). These vesicles represented <1% of all EC vesicles and were found throughout the cytoplasm.

In cremaster muscles subjected to I/R injury, JAM-C was detected in the same cellular compartments but there was a clear shift in the distribution of JAM-C ($P<0.0001$ by the χ^2 test). Specifically, the number of particles per field of nonjunctional membrane was higher and the number of

cytosolic vesicles and of junctional membrane domains immunolabeled for JAM-C was lower as compared to sham-operated tissue samples (Figure 5B). Hence, under conditions of I/R injury JAM-C expression in cytoplasmic vesicles was reduced by ≈7-fold while increasing in the nonjunctional membrane by ≈2.5-fold. This in vivo redistribution of JAM-C was associated with an unusually frequent presence of leukocytes in the lumen of the vessels (not shown). Collectively, the present results provide the first in vivo indication for redistribution of JAM-C from EC junctional sites and intracellular compartments toward the plasma membrane under inflammatory conditions, demonstrating the existence of a novel regulatory mechanism for relocalization and hence function of EC JAM-C in vivo.

Discussion

JAM-C is a relatively new addition to the growing number of EC junctional adhesion molecules implicated in leukocyte trafficking,^{3,12,17,20,23} and as such many aspects of its mechanism of action, in particular within in vivo pathological scenarios, remain unknown. Here we provide evidence for the involvement of JAM-C in leukocyte infiltration as elicited by I/R injury and demonstrate that in mediating this response, JAM-C can support both leukocyte adhesion and transmigration, two distinct phases of the leukocyte adhesion cascade. Furthermore, the study reports on the in vivo existence of EC vesicular stores of JAM-C that are redistributed to the plasma membrane in response to I/R, providing a novel regulatory mechanism for EC JAM-C function in vivo.

JAM-C is expressed by multiple cell types such as ECs, leukocytes, Schwann cells, spermatids, and intestinal epithelial cells^{4-7,9-11} and is expressed in the vasculature of most organs investigated at mRNA level²⁸ and at protein level, as demonstrated in the present study. Specifically, in murine tissues, JAM-C was detected at a high expression level in HEVs of lumbar lymph nodes, in line with previous reports,^{5,28} and was also detected in the vasculature of the heart, lungs, liver, spleen, kidneys, the small intestine, and cremas-

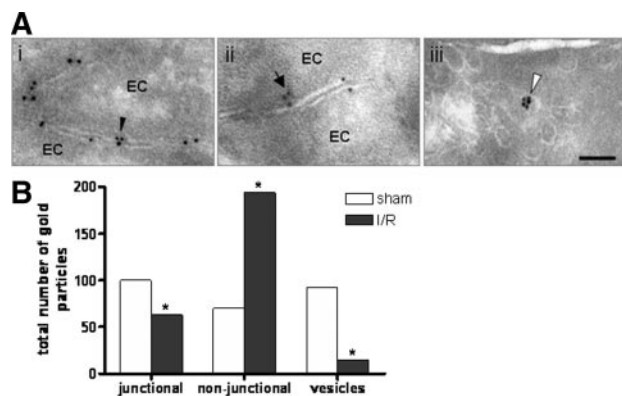


Figure 5. JAM-C localizes in different compartments within ECs and redistributes after I/R injury. Localization (A) and quantification (B) of JAM-C at EC junctional sites (i), at nonjunctional domains of the EC membrane (ii), and within cytoplasmic vesicles (iii). Scale bar=100 nm. (Please see the supplemental materials).

ter muscle in close association with that of PECAM-1. The broad cellular and tissue distribution of JAM-C suggests a role for this molecule in multiple essential biological functions, as illustrated by the diverse and severe defects (eg, immune-deficiency, growth retardation, and neurological and reproductive defects) exhibited by JAM-C-deficient mice.^{6,7,10} JAM-C has also been implicated in the pathogenesis of numerous inflammatory and cardiovascular conditions such as arthritis, acute pancreatitis, peritonitis, pulmonary inflammation, and atherosclerosis as largely investigated using murine disease models.^{7,8,21,23,29} The aim of the present studies was to further investigate the role of JAM-C under pathological cardiovascular conditions by investigating its involvement in leukocyte infiltration as induced by I/R injury. For this purpose, two murine models were used, namely I/R injury in the kidney and in the cremaster muscle, the latter being studied in real-time by IVM. The functional role of JAM-C was investigated here using both a pharmacological blocker of JAM-C, sJAM-C, and genetically modified mice lacking JAM-C (JAM-C^{-/-}) or overexpressing JAM-C in their ECs (EC JAM-C transgenic).^{5,6,12,27} Treatment of mice with i.v. sJAM-C resulted in a significant suppression of leukocyte infiltration (both neutrophils and monocytes) into kidneys subjected to I/R injury. Analysis of leukocyte-vessel wall interactions in the cremaster muscle model by IVM showed that sJAM-C could suppress leukocyte adhesion and extravasation in response to I/R injury. These results were in line with findings in JAM-C^{-/-} mice where a significant suppression of leukocyte adhesion and extravasation was observed under conditions of I/R injury. Overall, the findings suggest that the noted suppression of leukocyte extravasation under conditions of pharmacological or genetic deletion of JAM-C may be at least partly attributable to reduced leukocyte adhesion. As murine circulating leukocytes are reported not to express JAM-C,^{12,16} the results are likely to be caused by loss of function of endothelial JAM-C. In agreement with this hypothesis, studies performed using a cell transfer technique demonstrated that vascular (but not leukocyte) JAM-C deficiency lead to suppression of leukocyte-vessel wall interactions. In addition, EC JAM-C transgenic mice exhibit consistently higher levels of leukocyte adhesion and a significantly enhanced leukocyte extravasation response as induced by I/R injury.

Because JAM-C is largely expressed at EC junctions and has to date been heavily implicated in leukocyte transendothelial cell migration, identifying a role for JAM-C in leukocyte adhesion in vivo was of interest. We hypothesized that JAM-C may mediate leukocyte adhesion, an earlier step in the leukocyte adhesion cascade that is prerequisite to leukocyte transendothelial cell migration, under conditions where JAM-C is enhanced on the luminal surface of the endothelium. In this context, a number of in vitro studies have demonstrated enhanced expression of EC junctional molecules on nonjunctional regions. Specifically, nonjunctional expression of PECAM-1 and JAM-A on cultured ECs has been shown to be enhanced by certain cytokine combinations.^{30,31} More recently, Keiper et al found that cultured ECs stimulated with oxidized LDL can support enhanced monocyte adhesion in a manner that is partly JAM-C-dependent

and appears to be associated with enhanced expression of JAM-C on ECs at nonjunctional sites.⁸ A similar phenomenon was also observed under conditions of blocking JAM-B-JAM-C interactions.²⁷ To explore the potential mechanisms that may account for JAM-C-mediated leukocyte adhesion, the subcellular expression and localization of EC JAM-C was studied in cremaster muscles by IEM. The findings identified JAM-C in three distinct EC regions, junctional membrane, nonjunctional membrane, and an intracellular vesicular store of JAM-C. Although representing a small proportion of EC vesicles, the latter novel finding may provide mechanistic insights to regulation of expression and function of JAM-C under different inflammatory conditions. Indeed, comparison of tissues from control and I/R injured mice showed a clear redistribution of JAM-C from junctional and vesicular domains to nonjunctional regions, a finding that could well account for JAM-C-mediated leukocyte adhesion under conditions of I/R injury. Such a redistribution may provide a means of enhancing leukocyte-vessel wall interaction within the vascular lumen, possibly via JAM-C-Mac-1 interactions.²⁷ In addition, increased luminal expression of JAM-C may promote migration of leukocytes to EC junctions through increased intravascular crawling, a response that appears to support efficient leukocyte transmigration and is reportedly Mac-1-dependent,³² as well as by creating an adhesive haptotactic gradient that guides luminal leukocytes to EC junctions. Of relevance, in humans, vascular expression of JAM-C appears to be enhanced under certain inflammatory disease conditions such as atherosclerosis and rheumatoid arthritis,^{8,29} though the associated mechanisms are unknown, highlighting the need for a better understanding of the molecular events that regulate JAM-C expression under different disease conditions.

Collectively, the present results show a role for JAM-C in leukocyte adhesion and transmigration in response to I/R injury and demonstrate the redistribution of EC JAM-C during this vascular insult in a manner that could potentially determine the functional role of JAM-C.

Acknowledgments

The authors thank P. Hammel and D. Caille for excellent technical assistance.

Sources of Funding

This work was supported by funds from The Wellcome Trust, UK and The British Heart Foundation (to S.N.; grants 081172/Z/06/Z and PG/03/123/16102, respectively). Other authors were funded as follows: N.S.A.P. by the WHR foundation and Kidney Research UK (PDF4/2009); P.M. by the Swiss National Science Foundation (310000-122430), Juvenile Diabetes Research Foundation International (1-2007-158), the EU (FP-7 BETAIMAGE 222980) and Novo Nordisk; M.A.-L. by the Swiss National Foundation (310000-112551) and Avenir Program, Inserm; B.A.I. by Oncosuisse (OCS-01812-12-2005) and the Swiss National Science Foundation (310000-120184).

Disclosures

None.

References

- Ley K, Laudanna C, Cybulsky MI, Nourshargh S. Getting to the site of inflammation: the leukocyte adhesion cascade updated. *Nat Rev Immunol*. 2007;7:678–689.
- Muller WA. Leukocyte-endothelial-cell interactions in leukocyte transmigration and the inflammatory response. *Trends Immunol*. 2003;24:327–334.
- Bradfield PF, Nourshargh S, Aurrand-Lions M, Imhof BA. JAM family and related proteins in leukocyte migration. *Arterioscler Thromb Vasc Biol*. 2007;27:2104–2112.
- Arrate MP, Rodriguez JM, Tran TM, Brock TA, Cunningham SA. Cloning of human junctional adhesion molecule 3 (JAM3) and its identification as the JAM2 counter-receptor. *J Biol Chem*. 2001;276:45826–45832.
- Aurrand-Lions M, Duncan L, Ballestrem C, Imhof BA. JAM-2, a novel immunoglobulin superfamily molecule, expressed by endothelial and lymphatic cells. *J Biol Chem*. 2001;276:2733–2741.
- Gliki G, Ebnet K, Aurrand-Lions M, Imhof BA, Adams RH. Spermatid differentiation requires the assembly of a cell polarity complex downstream of junctional adhesion molecule-C. *Nature*. 2004;431:320–324.
- Imhof BA, Zimmerli C, Gliki G, Ducrest-Gay D, Juillard P, Hammel P, Adams R, Aurrand-Lions M. Pulmonary dysfunction and impaired granulocyte homeostasis result in poor survival of Jam-C-deficient mice. *J Pathol*. 2007;212:198–208.
- Keiper T, Al-Fakhri N, Chavakis E, Athanasopoulos AN, Isermann B, Herzog S, Saffrich R, Hersemeyer K, Bohle RM, Haendeler J, Preissner KT, Santoso S, Chavakis T. The role of junctional adhesion molecule-C (JAM-C) in oxidized LDL-mediated leukocyte recruitment. *FASEB J*. 2005;19:2078–2080.
- Morris AP, Tawil A, Berkova Z, Wible L, Smith CW, Cunningham SA. Junctional adhesion molecules (JAMs) are differentially expressed in fibroblasts and co-localize with ZO-1 to adherens-like junctions. *Cell Commun Adhes*. 2006;13:233–247.
- Scheiermann C, Meda P, Aurrand-Lions M, Madani R, Yiangou Y, Coffey P, Salt TE, Ducrest-Gay D, Caille D, Howell O, Reynolds R, Lobrinus A, Adams RH, Yu AS, Anand P, Imhof BA, Nourshargh S. Expression and function of junctional adhesion molecule-C in myelinated peripheral nerves. *Science*. 2007;318:1472–1475.
- Zen K, Babbin BA, Liu Y, Whelan JB, Nusrat A, Parkos CA. JAM-C is a component of desmosomes and a ligand for CD11b/CD18-mediated neutrophil transepithelial migration. *Mol Biol Cell*. 2004;15:3926–3937.
- Aurrand-Lions M, Lamagna C, Dangerfield JP, Wang S, Herrera P, Nourshargh S, Imhof BA. Junctional adhesion molecule-C regulates the early influx of leukocytes into tissues during inflammation. *J Immunol*. 2005;174:6406–6415.
- Santoso S, Sachs UJ, Kroll H, Linder M, Ruf A, Preissner KT, Chavakis T. The junctional adhesion molecule 3 (JAM-3) on human platelets is a counterreceptor for the leukocyte integrin Mac-1. *J Exp Med*. 2002;196:679–691.
- Ody C, Jungblut-Ruault S, Cossali D, Barnet M, Aurrand-Lions M, Imhof BA, Matthes T. Junctional adhesion molecule C (JAM-C) distinguishes CD27+ germinal center B lymphocytes from non-germinal center cells and constitutes a new diagnostic tool for B-cell malignancies. *Leukemia*. 2007;21:1285–1293.
- Cunningham SA, Rodriguez JM, Arrate MP, Tran TM, Brock TA. JAM2 interacts with alpha4beta1. Facilitation by JAM3. *J Biol Chem*. 2002;277:27589–27592.
- Praetor A, McBride JM, Chiu H, Rangell L, Cabote L, Lee WP, Cupp J, Danilenko DM, Fong S. Genetic deletion of JAM-C reveals a role in myeloid progenitor generation. *Blood*. 2009;113:1919–1928.
- Weber C, Fraemohs L, Dejana E. The role of junctional adhesion molecules in vascular inflammation. *Nat Rev Immunol*. 2007;7:467–477.
- Lamagna C, Hodivala-Dilke KM, Imhof BA, Aurrand-Lions M. Antibody against junctional adhesion molecule-C inhibits angiogenesis and tumor growth. *Cancer Res*. 2005;65:5703–5710.
- Orlova VV, Economopoulou M, Lupu F, Santoso S, Chavakis T. Junctional adhesion molecule-C regulates vascular endothelial permeability by modulating VE-cadherin-mediated cell-cell contacts. *J Exp Med*. 2006;203:2703–2714.
- Johnson-Leger CA, Aurrand-Lions M, Beltraminelli N, Fasel N, Imhof BA. Junctional adhesion molecule-2 (JAM-2) promotes lymphocyte transendothelial migration. *Blood*. 2002;100:2479–2486.
- Vonlaufen A, Aurrand-Lions M, Pastor CM, Lamagna C, Hadengue A, Imhof BA, Frossard JL. The role of junctional adhesion molecule C (JAM-C) in acute pancreatitis. *J Pathol*. 2006;209:540–548.
- Ludwig RJ, Zollner TM, Santoso S, Hardt K, Gille J, Baatz H, Johann PS, Pfeffer J, Radeke HH, Schon MP, Kaufmann R, Boehncke WH, Podda M. Junctional adhesion molecules (JAM)-B and -C contribute to leukocyte extravasation to the skin and mediate cutaneous inflammation. *J Invest Dermatol*. 2005;125:969–976.
- Chavakis T, Keiper T, Matz-Westphal R, Hersemeyer K, Sachs UJ, Nawroth PP, Preissner KT, Santoso S. The junctional adhesion molecule-C promotes neutrophil transendothelial migration in vitro and in vivo. *J Biol Chem*. 2004;279:55602–55608.
- Woodfin A, Reichel CA, Khandoga A, Corada M, Voisin MB, Scheiermann C, Haskard DO, Dejana E, Krombach F, Nourshargh S. JAM-A mediates neutrophil transmigration in a stimulus-specific manner in vivo: evidence for sequential roles for JAM-A and PECAM-1 in neutrophil transmigration. *Blood*. 2007;110:1848–1856.
- Anghelina M, Moldovan L, Moldovan NI. Preferential activity of Tie2 promoter in arteriolar endothelium. *J Cell Mol Med*. 2005;9:113–121.
- Sircar M, Bradfield PF, Aurrand-Lions M, Fish RJ, Alcaide P, Yang L, Newton G, Lamont D, Sehrawat S, Mayadas T, Liang TW, Parkos CA, Imhof BA, Luscinskas FW. Neutrophil transmigration under shear flow conditions in vitro is junctional adhesion molecule-C independent. *J Immunol*. 2007;178:5879–5887.
- Lamagna C, Meda P, Mandicourt G, Brown J, Gilbert RJ, Jones EY, Kiefer F, Ruga P, Imhof BA, Aurrand-Lions M. Dual interaction of JAM-C with JAM-B and alpha(M)beta2 integrin: function in junctional complexes and leukocyte adhesion. *Mol Biol Cell*. 2005;16:4992–5003.
- Aurrand-Lions M, Johnson-Leger C, Wong C, Du Pasquier L, Imhof BA. Heterogeneity of endothelial junctions is reflected by differential expression and specific subcellular localization of the three JAM family members. *Blood*. 2001;98:3699–3707.
- Palmer G, Busso N, Aurrand-Lions M, Talabot-Ayer D, Chobaz-Peclat V, Zimmerli C, Hammel P, Imhof BA, Gabay C. Expression and function of junctional adhesion molecule-C in human and experimental arthritis. *Arthritis Res Ther*. 2007;9:R65.
- Ozaki H, Ishii K, Horiuchi H, Arai H, Kawamoto T, Okawa K, Iwamatsu A, Kita T. Cutting edge: combined treatment of TNF-alpha and IFN-gamma causes redistribution of junctional adhesion molecule in human endothelial cells. *J Immunol*. 1999;163:553–557.
- Romer LH, McLean NV, Yan HC, Daise M, Sun J, DeLisser HM. IFN-gamma and TNF-alpha induce redistribution of PECAM-1 (CD31) on human endothelial cells. *J Immunol*. 1995;154:6582–6592.
- Phillipson M, Heit B, Colarusso P, Liu L, Ballantyne CM, Kubus P. Intraluminal crawling of neutrophils to emigration sites: a molecularly distinct process from adhesion in the recruitment cascade. *J Exp Med*. 2006;203:2569–2575.