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REVIEW

Function of Junctional Adhesion Molecules (JAMs) in Leukocyte Migration and Homeostasis

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Abstract Homeostasis is a word widely used in the scientific community to refer to the property of a system to maintain its uniformity and functionality. In living organisms, the word refers to the concept enunciated 150 years ago by C. Bernard by which external variations must be compensated for in order to maintain internal conditions compatible with life. This is especially true in the case of highly dynamic system such as the hematopoietic system that requires the coordinated control of cell proliferation and death within specialized microenvironments that are anatomically distinct. As a consequence, hematopoietic cell adhesion and migration must be tightly controlled in order for hematopoietic cells to reach and to be maintained in appropriate microenvironments. The junctional adhesion molecules (JAMs) are adhesion molecules that belong to the immunoglobulin superfamily (IgSf) and that have been initially identified as important players controlling vascular permeability and leukocyte transendothelial migration. This involves the regulated localization of the JAMs at lateral endothelial cell/cell borders and their interaction with leukocyte integrins. More recently, some of the JAM family members have also been found to be expressed by stromal cells and to regulate chemokine secretion within lymphoid organs, acting not only on leukocyte transendothelial migration, but also on hematopoietic cell retention within specialized microenvironments. This review summarizes recent progress in understanding the role of the JAMs in leukocyte adhesion and migration to tentatively draw an integrated view of the homeostatic function of the JAMs within the hematopoietic system.

Keywords Transendothelial migration · Lymph node · Bone marrow · Chemokine secretion

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The JAM Family

The proteins of the junctional adhesion molecules (JAM) family belong to the immunoglobulin superfamily (IgSf) and present two extracellular immunoglobulin domains of V and C2 type. The C2 domains encountered in JAM family members share the characteristic features of C2 domains found in the larger cortical thymocyte marker for Xenopus (CTX) protein family, which has been proposed to represent the ancestral precursor sequences of the B cell and T cell receptors as a result of ancient chromosomal duplications (Du Pasquier et al. 2004). The JAM family is composed of three classical members, JAM-A, JAM-C, JAM-B sharing up to 35 % of amino-acid identity (Aurrand-Lions et al. 2000), and four non-classical members ESAM-1, JAML, CAR and JAM-4 diverging from JAM-A, -B and -C by the nature of their cytoplasmic tails (Bazzoni 2003; Ebnet et al. 2004). These latter parts of JAM-A, -B and -C are relatively conserved in length and composition with membrane proximal and C-terminal sequences that are almost identical between these three proteins (Bradfield et al. 2007a). While the function of the membrane proximal domain has not been addressed so far, the C-terminal

sequence is a type II PDZ binding motif able to interact with several proteins containing PDZ domains known to act as intracellular scaffolds linking cell surface molecules to the intracellular machinery involved in cell migration, polarity, or signaling (Ebnet et al. 2003 Mandell and Parkos 2005). Another structural property shared by JAM-A and JAM-C is their ability to be released from inflammatory endothelial cells by the metalloproteinases, ADAM-10 and ADAM-17 (Koenen et al. 2009; Rabquer et al. 2010; Salifu et al. 2007).Whether this property extends to JAM-B and beyond endothelial cells must still be addressed, but the finding by Rabquer et al. showing that soluble JAM-C is pro-angiogenic argues for a general molecular mechanism that regulates JAMs-mediated events, instead of a simple side effect of inflammatory reaction.

In addition to these common structural features, JAM-A, -B and -C share in common their vascular expression and have all been involved in inter-endothelial and leuko-endothelial interactions through their differential expression pattern at endothelial cell junctions (Weber et al. 2007). However, their expression pattern is not restricted to endothelial cells (Table 1). Among JAM-A, -B and -C, the first complete sequence to be identified was JAM-A in mouse endothelioma cells as the surface antigen recognized by the BV11 monoclonal antibody. In this seminal study, the authors have shown that JAM-A is an adhesion molecule selectively enriched at the tight junction of endothelial and epithelial cells (Martin-Padura et al. 1998). This protein sequence turned to be identical to the previously reported partial sequence encoding the platelet receptor for the antibody F11 (F11R) known to induce platelet activation and aggregation (Naik et al. 1995; Sobocka et al. 2000). Soon after JAM-A, two other members of the family were cloned in the context of new endothelial adhesion molecules: JAM-B also named VE-JAM or JAM2 and JAM-C also named JAM2 or JAM3 depending on the species (Arrate et al. 2001; Aurrand-Lions et al. 2001; Cunningham et al. 2000; Palmeri et al. 2000). For clarity, the nomenclature JAM-A, JAM-B and JAM-C proposed by Muller (2003) will be used throughout the remaining text of this review.

Role of JAM-A in Trans-Endothelial Migration

As mentioned in the previous paragraph, the initial identification of JAM-A as an endothelial adhesion molecule concentrated at inter-endothelial junctions has prompted the authors to test if JAM-A may play a role in leukocyte transmigration. They found that monocyte trans-endothelial migration and recruitment of leukocytes in the mouse air-pounch model were specifically inhibited by the BV11 mAb, but not by a second mAb against JAM-A suggesting

Table 1 Summary of JAM family member expression patterns, putative functions and ligands in mouse

Molecule	Expression pattern	Function	Ligands	References
JAM-A	Epithelial cells, endothelial cells, neutrophils, monocytes, DC, lymphocytes, platelets, hematopoietic progenitors	Leukocyte transmigration, tight junction maintenance, polarization, regulation of DC migration into LNs	JAM-A LFA-1 $\alpha_V\beta_3$	(Ebnet et al. 2001; Corada et al. 2005; Sugano et al. 2008)
JAM-B	Endothelial cells, fibroblasts, MSC	Leukocytes transmigration, tight junction maintenance, regulation of stem cell homeostasis	JAM-C, $\alpha_4\beta_1$	(Ludwig et al. 2009; Arcangeli et al. 2011; Stelzer et al. 2012)
JAM-C	Endothelial cells, fibroblasts, epithelial cells, hematopoietic progenitors	Leukocytes transmigration, tight junction maintenance, regulation of naïve T cell homeostasis	JAM-C, JAM-B, CAR, $\alpha_M\beta_2$, $\alpha_X\beta_2$	(Santoso et al. 2002; Mirza et al. 2006; Morris et al. 2006)
ESAM-1	Endothelial cells, hematopoietic progenitors	Leukocytes transmigration	ESAM-1	(Nasdala et al. 2002; Wegmann et al. 2006; Ooi et al. 2009)
CAR	Epithelial cells, cardiomyocytes	Transepithelial migration of neutrophils, costimulation	JAML JAM-C	(Zen et al. 2005; Mirza et al. 2006)
JAML	Neutrophils TCR$\gamma\delta$ T cells	Transepithelial migration of neutrophils, costimulation	CAR	(Witherden et al. 2011; Luissint et al. 2008; Verdino et al. 2011)
JAM-4	Epithelial cells, hematopoietic cells	Unknown	Unknown	(Hirabayashi et al. 2003; Nagamatsu et al. 2006)

that the BV11 epitope was specifically involved in the control of leukocyte transmigration (Martin-Padura et al. 1998). One year later, the remarkable finding that JAM-A redistributes away from inter-endothelial junctions upon combined treatment with TNF-α and IFN-γ has suggested that two different pools of JAM-A may exist: a first one corresponding to the junctional pool and a second one representing the luminal pool (Ozaki et al. 1999). Whether this differential localization corresponds to different dimerization states of JAM-A remains to be addressed, but the specific recognition of JAM-A monomers and/or dimers by BV11 and BV12 mAbs, and results showing that JAM-A signaling in epithelial cells depends on dimerization demonstrate that JAM-A exists in two different conformational states on the cell surface (Bazzoni et al. 2000; Mandell et al. 2004; Nava et al. 2011; Severson et al. 2008). This is in agreement with the crystal structure of JAM-A showing that the protein must first *cis*-dimerize before forming homotypic trans-interaction in a zipper-like fashion (Kostrewa et al. 2001). At that stage, the question remained if this structural duality of JAM-A reflected dual functions for the junctional versus luminal pools of the protein. The answer came from a landmark study by Ostermann et al. (2002) who have shown that JAM-A controls LFA-1-induced trans-endothelial migration of T cells and neutrophils. In this work, the authors have shown that endothelial JAM-A participates to the LFA-1 dependent arrest of T cells and monocytes on endothelium after PMA or CXCL12 activation of endothelial cells. They have further demonstrated a direct interaction between LFA-1 and the C2 membrane proximal domain of JAM-A while the membrane distal V domain was rather involved in *cis*-dimerization and homotypic trans-interaction. This is consistent with the fact that during leukocyte transmigration, JAM-A and ICAM-1 are redistributed at the endothelial cell surface and colocalize with LFA-1 expressed on adherent neutrophils (Shaw et al. 2004). More recently LFA-1/JAM-A interaction has been shown to destabilize JAM-A homodimers (Wojcikiewicz et al. 2009), suggesting that the engagement of LFA-1 with JAM-A induces the intercellular junction remodeling during leukocyte transmigration (Fig. 1). In addition to trans-interaction with LFA-1, JAM-A has also been reported to form protein complexes with the integrin $\alpha_v\beta_3$ at cell–cell borders of quiescent endothelial cells (Naik et al. 2003). This protein complex is disrupted upon angiogenic stimulation with bFGF that induces a redistribution of JAM-A to the cell surface. Whether this contributes to the specific modulation of leukocyte trans-endothelial migration in angiogenic endothelial cells remains to be addressed (Griffioen et al. 1996, 1998). Another insight into JAM-A function in leukocyte trans-endothelial migration came from the study of *jam-a* deficient mice (Cera et al. 2004).

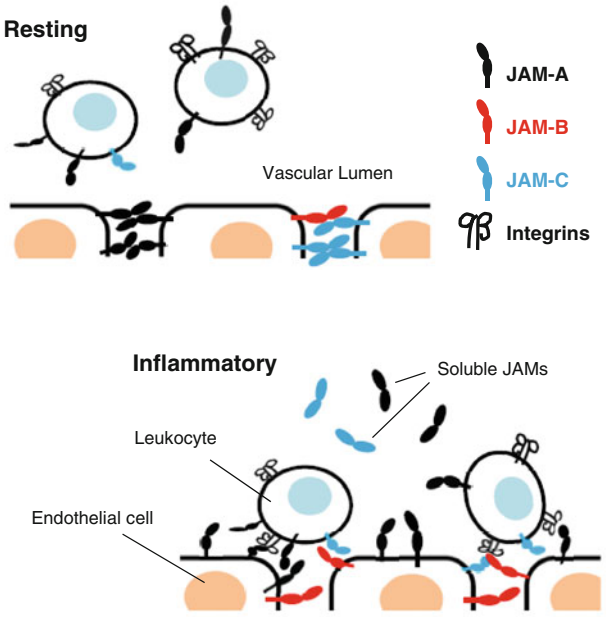

Fig. 1 JAMs remodeling during leukocyte-transendothelial migration. In the absence of inflammation (*upper panel*), the junctional adhesion molecules are concentrated at lateral cell borders between endothelial cells. Leukocyte subsets express integrins, JAM-A and JAM-C depending on the species. When inflammation occurs (*lower panel*), JAM-A is relocalized at the apical surface of endothelial cells and engages with the leukocyte integrin $\alpha_L\beta_2$. In the meantime, JAM-A and JAM-C are shedded from endothelial cells in soluble forms leading to destabilization of inter-endothelial junctions. The integrins $\alpha_4\beta_1$ and $\alpha_M\beta_2$ are able to interact, respectively, with JAM-B and JAM-C expressed on endothelial cells, while JAM-B expressed by endothelial cells may counter-interact with JAM-C expressed on certain leukocyte subsets

Indeed, an unexpected increased trafficking of Dendritic Cells (DC) to lymph nodes has been observed in these mice due to a cell autonomous promigratory phenotype of DC lacking JAM-A expression. In contrast, polymorphonuclear leukocytes exhibited a reduced diapedesis in *Jam-a*$^{-/-}$ mice. This is most likely due to a cell autonomous function of JAM-A in neutrophils in which the protein controls internalization and recycling of β_1 integrin after chemotactic stimulation, suggesting that JAM-A positively contributes to directional integrin-dependent migration while it prevents spontaneous motility (Bazzoni et al. 2005; Cera et al. 2009; Corada et al. 2005). This would be in agreement with the recent finding that DC migration occurs in an integrin independent manner within three-dimensional environments (Lammermann et al. 2008).

Role of JAM-B and JAM-C in Trans-Endothelial Nigration

Similar to JAM-A, JAM-B and JAM-C have been cloned and characterized in the context of inter-endothelial and

leuko-endothelial interactions (Fig. 1). Indeed, JAM-B has been identified from a cDNA library prepared from High Endothelial Venules (Palmeri et al. 2000). Six months later, the same sequence was identified by Cunningham et al. (2000) as a sequence encoding an adhesion molecule able to bind to immortalized human T lymphocytes. Soon after, we have reported the characterization of JAM-C as a differentially regulated transcript in mouse endothelioma cells cocultured with tumor cells (Aurrand-Lions et al. 2000, 2001). The generation of monoclonal antibodies against mouse JAM-C confirmed the high level of JAM-C expression by endothelial and sinus lining cells of the lymph node, while expression on mature mouse lymphocytes was not found. This expression pattern in mice was in sharp contrast with the expression of JAM-C on human T, NK, DC and activated T cells. However, both in mouse and human species, JAM-C was identified as the main counter receptor for JAM-B expressed by endothelial cells (Lamagna et al. 2005; Arrate et al. 2001; Liang et al. 2002). In the meantime, JAM-C was also identified as a human platelet adhesion molecule able to interact with the leukocyte integrins $\alpha_M\beta_2$ and $\alpha_x\beta_2$ (Santoso et al. 2002), while human JAM-B was shown to interact with the integrin $\alpha_4\beta_1$ when JAM-C is coexpressed with the integrin (Cunningham et al. 2002). This suggested that JAM-B and JAM-C were participating to an intricated network of interactions between hematopoietic and endothelial cells that may contribute to the final transendothelial transmigration step of the leuko-endothelial adhesion cascade (Nourshargh et al. 2010).

Along this line, JAM-B and JAM-C have been involved in leukocyte adhesion, rolling and transendothelial migration (Johnson-Leger et al. 2002 Ludwig et al. 2005). In most of the studies, JAM-C requirement in leukocyte transmigration was studied using soluble recombinant proteins and anti-JAM-C blocking antibody. Indeed, when HUVEC were treated with anti-JAM-C blocking antibody or recombinant soluble JAM-C protein, lymphocyte transmigration across treated HUVEC was reduced. Inversely, when HUVEC overexpressed JAM-C, lymphocyte transmigration was increased (Johnson-Leger et al. 2002). Similarly, the in vitro treatment of human epithelial cells with anti-JAM-C blocking antibody has been shown to reduce reverse neutrophil trans-epithelial migration most likely due to the engagement of JAM-C with $\alpha_M\beta_2$ expressed on neutrophils (Chavakis et al. 2003, 2004; Zen et al. 2004). However, these results must be interpreted with caution since transmigration assays were conducted in static conditions and some available anti human JAM-C antibodies cross-react with phosphorylated Keratin 8 (Betanzos et al. 2009). In contrast to conclusions drawn from static transmigration assays, experiments conducted under flow have demonstrated that JAM-C has minimal, if any, role in neutrophil or monocyte trans-endothelial migration (TEM) in vitro (Sircar et al. 2007). However, pharmacological blockade of JAM-C has been shown to inhibit leukocyte recruitment in various inflammatory models in mice and to increase the frequency of reverse transmigrated monocytes (Bradfield et al. 2007b; Palmer et al. 2006; Scheiermann et al. 2009; Vonlaufen et al. 2006). This has prompted Woodfin et al. (2011) to revisit the function of JAM-C in neutrophil transmigration in vivo using single beam laser confocal microscopy and three dimensional imaging in real time. In this study, the authors show that several modes of neutrophils paracellular TEM exist in vivo and could be classified as normal TEM in a luminal-to-abluminal direction, hesitant multidirectional TEM, and reverse TEM in which leukocytes migrate through endothelial junctions in an abluminal-to-luminal direction. These three modes of TEM co-exist but their relative contributions to the overall neutrophil recruitment are dependent on the inflammatory stimulus, with a predominance of hesitant and reverse TEM during ischemia reperfusion injury. In addition, the proportion of neutrophils undergoing hesitant and reverse TEM is correlated with the disappearance of JAM-C from inter-endothelial contacts and is increased in mice in which JAM-C expression at inter-endothelial contacts is disrupted, suggesting that JAM-C plays a role in maintaining the polarized luminal-to-abluminal leukocyte TEM. Whether this depends on the stabilization of JAM-C by JAM-B at inter-endothelial contacts remains to be addressed, but this study shows for the first time that measuring leukocyte transmigration in endpoint experiments may be biased by reverse transmigration events that are dependent on the nature of the stimulus. It would therefore be interesting to revisit some negative results in the light of this newly described mode of leukocyte transmigration.

Non-Classical JAM Members and Transendothelial Migration

Although initially identified as endothelial-cell specific adhesion molecule, ESAM has also been reported to be expressed by platelets (Hirata et al. 2001; Nasdala et al. 2002). It has later been shown to play a role in leukocyte TEM by tightening inter-endothelial junctions. Analysis of neutrophil transmigration by intravital microcopy in *esam* deficient mice has revealed that neutrophil transmigration was reduced up to 50 % while adhesion and rolling of leukocytes were not affected (Wegmann et al. 2006). This was correlated to the function of ESAM in maintaining tightness of inter-endothelial junctions since VEGF-induced vascular permeability was reduced in *esam* deficient mice. In addition to ESAM, JAML and CAR also

play a role in leukocyte transmigration. JAML is encoded by a transcript induced by retinoic acid in acute promyelocytic leukemia cells (Moog-Lutz et al. 2003). It is prominently expressed by granulocytes and monocytes and is a ligand for CAR expressed by epithelial and endothelial cells. The couple JAML/CAR has been involved in neutrophil transmigration across epithelial cells after inflammatory stimuli (Zen et al. 2005). More recently, this couple has also been involved in leukocyte adhesion to endothelial cells and TEM (Guo et al. 2009; Luissint et al. 2008). Indeed, Luissint et al. have reported that JAML expression is restricted to leukocytes and that it interacts with CAR only upon dimerization. When leukocytes expressing JAML do not express the integrin $\alpha_4\beta_1$, JAML is dimeric and its interaction with CAR is independent of integrin activation. However, when JAML and $\alpha_4\beta_1$ are coexpressed, JAML forms a complex with $\alpha_4\beta_1$ and accumulates in monomer. After integrin activation through chemokine stimulation, JAML and $\alpha_4\beta_1$ dissociate, allowing JAML homodimerization and interaction with CAR. This indicates that promiscuous interactions between cytokine signaling, integrin activation and JAMs may exist, and suggests that JAM members may regulate hematopoietic homeostasis beyond the control of leukocyte migration.

Role of JAM-C in Lymph Node Homeostasis

Lymph nodes are encapsulated organs localized at the interface between blood and lymphatic systems in which primary antigenic activation of naive lymphocytes occurs (von Andrian and Mempel 2003).They are subdivided in three regions: the subcapsular sinus just beneath the capsule, the cortex, and the medulla (Roozendaal et al. 2008).These three regions are composed of different stromal cell types. The subcapsular sinus is composed of lymphatic cells and lined by a thin layer of sinus lining cells (SLC) and macrophages that capture antigens or microorganisms from the lymph (Junt et al. 2007). The cortex is mainly composed of two areas: the T cell zone or paracortex and B cell follicles. In the T cell zone, there are specialized vascular structures named high endothelial venules (HEV) by which lymphocytes access lymph nodes from the bloodstream. Once in the tissue, T lymphocytes accumulate in the T cell zone whereas B cells migrate into follicles suggesting that lymphocyte intra nodal migration is controlled by distinct and specific cues provided by fibroblastic reticular cells (FRC) and follicular dendritic cells (FDC) (Worbs and Forster 2009; Mueller and Germain 2009). In absence of antigenic activation, lymphocytes return to the blood circulation via efferent lymphatic vessels within hours. This highly orchestrated process is called lymphocyte recirculation and contributes to the maintenance of lymphocyte homeostasis since cells entering the lymph nodes must compensate for cells exiting the organ. It implies that entry and retention time of naive lymphocytes within lymph nodes must be adjusted at each instant, a process in which HEVs play an active role through their ability to form a waiting area for lymphocytes requiring access to lymph nodes (Mionnet et al. 2011). However, even if several studies have shown that members of the JAM family such as JAM-A, -B, -C and ESAM are differentially expressed by HEVs, blood vessels, lymphatic vessels and subcapsular sinuses in lymph nodes, little is known about the adhesive function of the JAM family members at these entry and exit sites of lymph nodes (Pfeiffer et al. 2008; Ueki et al. 2008). More recently, the unexpected finding that JAM-C is expressed by FRCs, although at lower levels than on HEVs, has prompted us to reconsider the function of JAM-C in the maintenance of lymph node homeostasis. Indeed, FRCs express and secrete the homeostatic chemokines CXCL12, CCL21 and CCL19 making them attractive to manipulate T cell homeostasis (Malhotra et al. 2012; Forster et al. 2008; Link et al. 2007). We found that inhibition of JAM-C function by pharmacological blockade, silencing or knock-in results in inhibition of chemokine production in lymph nodes fibroblastic reticular cells (Frontera et al. 2011). Moreover, anti-JAM-C blocking antibody increased retention and accumulation of naive T cells within the lymph nodes suggesting that reducing intranodal chemokine content may delay lymphocyte exit from lymph nodes. In addition, one could not exclude that JAM-C expressed by FRCs serve as a guidance cue for T lymphocytes within lymph nodes.

JAM-B/JAM-C Regulation of CXCR4/CXCL12 Axis in Inflammation and Hematopoiesis

It remains to be formerly explored if JAM-C plays a similar function in more complex homeostatic feedback loops occurring after inflammatory challenge or immune activation, although two reports using different strains of *jam-c* deficient mice indicate that it could be the case. In a first study, we have shown that inflammation does not resolve normally after inflammatory challenge, and that regulation of CXCR4 expression on granulocytes is altered in the few *jam-c* deficient mice that survived after weaning (Imhof et al. 2007). Since JAM-C is not expressed on mouse granulocytes, this suggests that JAM-C may control CXCR4 expression in a paracrine manner through the regulation of CXCL12 production in stromal cells of peripheral tissues. In a second study, it has been reported that leukocyte homeostasis is altered in the bone marrow of

jam-c$^{-/-}$ surviving mice with an increase in the number of myeloid committed progenitors that differentiate from hematopoietic stem cells (HSC) (Praetor et al. 2009). Since this step is under the control of cytokines and growth factors, this has prompted the authors to hypothesize that JAM-C expressed by HSC mediates cues from the microenvironment influencing the balance between self-renewal and differentiation of HSCs. This is in agreement with our recent finding that JAM-B, one counter-receptor for JAM-C, is expressed by bone marrow endothelial cells, a fraction of mesenchymal stem cells (MSC) defined by the phenotype CD45$^-$ CD31$^-$ Sca-1$^+$ integrin α_v^+ and a fraction of fibroblastic like stromal cells defined by the phenotype CD45$^-$ CD31$^-$ Sca-1$^-$ integrin α_v^+. These stromal subsets are the source of CXCL12 which plays an essential role in homeostasis and in the retention of HSCs within bone marrow microenvironment (Mercier et al. 2011; Nagasawa et al. 2011) as illustrated by the fact that CXCR4/CXCL12 inhibition induces HSC and hematopoietic progenitor mobilization. We have reported that CXCL12 secretion is increased in the bone marrow of *jam-b* deficient mice and that CXCR4 cell surface expression is downregulated on HSCs, suggesting that JAM-B signaling in bone marrow stromal cells may inhibit CXCL12 secretion (Arcangeli et al. 2011). Together with the observation that inhibition of JAM-C expression in lymph node stromal cells increases CXCL12 secretion (Frontera et al. 2011), this allows us to propose a model in which JAM-C would positively control homeostatic chemokine secretion while JAM-B would negatively regulate chemokine production (Fig. 2). In this model, inhibiting JAM-B or JAM-C expression in stromal cells coexpressing both proteins will result in opposite effect on chemokine secretion based on signaling occurring at cell–cell contacts between neighboring cells. This would be consistent with a recent study showing that the gap junction proteins Connexins 43 and 45 control BM homeostasis by regulating CXCL12 expression and secretion through signaling that is dependent on cell–cell contacts between stromal cells (Schajnovitz et al. 2011). Whether the JAMs are involved in similar signaling mechanisms remains to be tested, but these studies have highlighted an unexpected function for cell–cell contacts protein in the regulation of chemokine secretion within lymphoid microenvironments.

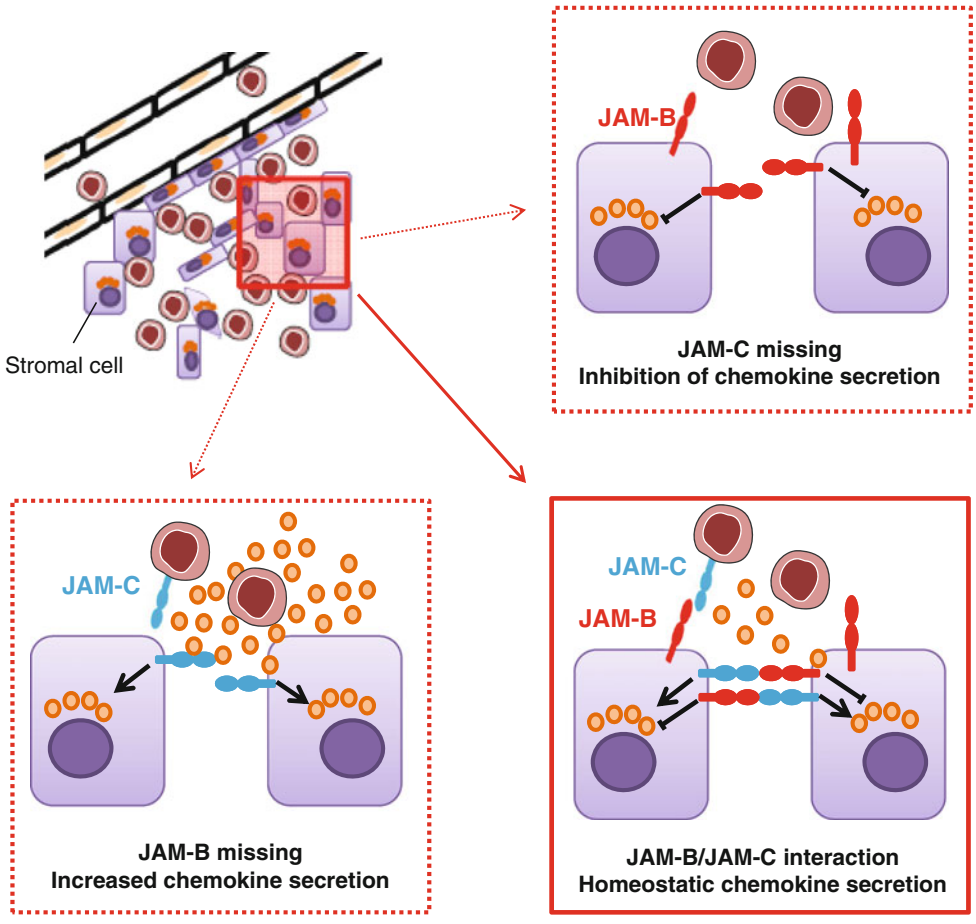

Fig. 2 Model of JAM-B/JAM-C control of chemokine secretion in lymph nodes and bone marrow. At the steady state, JAM-B and JAM-C expressed on stromal cells of lymphoid organs are preferentially engaged in heterotypic interactions resulting in constitutive homeostatic chemokine secretion (*lower right panel*). When JAM-C expression is artificially reduced, chemokine secretion is inhibited in lymph node stromal cells (*upper right panel*). Conversely, the loss of JAM-B expression in knock-out mice results in increased CXCL12 secretion in the bone marrow (*lower left panel*), suggesting opposite effects of JAM-B and JAM-C on constitutive chemokine secretion

Concluding Remarks

JAM proteins are tight junction associated proteins which have been first considered as simple adhesion molecules, then as guardians of the para-vascular space, before the recent discovery that they exist in a soluble form and that they influence chemokine secretion. Whether there is a link between soluble JAM production and chemokine secretion remains to be addressed, but growing interests in therapeutic targeting of chemokines and adhesion molecules in inflammatory and cancer diseases ensure that they will remain at the forefront of research in immunology for the upcoming years.

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