RESEARCH ARTICLE

The role of $\alpha 9 \beta 1$ integrin and its ligands in the development of autoimmune diseases

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Abstract Adhesion of cells to extracellular matrix proteins through integrins expressed on the cell surface is important for cell adhesion/motility, survival, and differentiation. Recently, $\alpha 9 \beta 1$ integrin was reported to be important for the development of autoimmune diseases including rheumatoid arthritis, multiple sclerosis, and their murine models. In addition, ligands for $\alpha 9 \beta 1$ integrin, such as osteopontin and tenascin-C, are well established as key regulators of autoimmune diseases. Therefore, this review focused on the role of interactions between $\alpha 9 \beta 1$ integrin and its ligands in the development of autoimmune diseases.

Keywords $\alpha 9$ integrin · Autoimmune diseases · Multiple sclerosis · Osteopontin · Rheumatoid arthritis · Tenascin-C

Abbreviations

AEIDGIEL	Alanine-Glutamic acid-Isoleucine-Aspartic acid-Glycine-Isoleucine-Glutamic acid-Leucine; collagen antibody-induced arthritis
CIA	Collagen-induced arthritis
DCs	Dendritic cells
EAE	Experimental autoimmune encephalomyelitis
ECM	Extracellular matrix
EGF	Epidermal growth factor

FBG	Fibrinogen-like globe
FCA	Freund's complete adjuvant
LC-MS/MS	Liquid chromatography–tandem mass spectrometry
G-CSF	Granulocyte colony stimulating factor
LECs	Lymphatic endothelial cells
LYVE-1	Lymphatic vascular endothelial gene-1
MMP	Matrix metalloproteinase
MOG	Myelin oligodendrocyte glycoprotein
MS	Multiple sclerosis
OPN	Osteopontin
pp.-vWF	Propolypeptide of von Willebrand factor
RA	Rheumatoid arthritis
RGD	Arginine-Glycine-Aspartic acid
S1P	Sphingosine-1-phosphate
SLAYGLR	Serine-Leucine-Alanine-Tyrosine-Glycine-Leucine-Arginine
SVVYGLR	Serine-Valine-Valine-Tyrosine-Glycine-Leucine-Arginine
tTG	Tissue transglutaminase
Th	T helper
TLR	Toll-like receptor
TN-C	Tenascin-C
TNfn3	Third fibronectin type III domain of tenascin-C
VCAM-1	Vascular cell adhesion molecule-1
VEGF	Vascular endothelial growth factor

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Introduction

Integrins are major cell transmembrane receptors for cell adhesion to extracellular matrix (ECM) proteins. As heterodimer receptors composed of α -subunits and β -subunits, specific combinations of these subunits give rise to 24 different integrins (Hynes 1987). Interactions between integrins and

ECM play key roles in regulating multiple cellular functions, including cell migration, proliferation, and differentiation (Hynes 1992). Intensive research using integrin-deficient mice or neutralizing antibodies over the last few decades has revealed the involvement of a variety of integrins in disease development. Indeed, several have been identified to be critical molecules for disease pathogenesis.

$\alpha 9$ integrin, which consist of the same integrin subfamily with $\alpha 4$ integrin, is expressed on cell surfaces as a heterodimer with $\beta 1$ integrin (Palmer et al. 1993). $\alpha 9\beta 1$ integrin is widely distributed throughout normal tissues such as airway epithelium, skeletal muscle, smooth muscle, and hepatocytes (Palmer et al. 1993; Singh et al. 2004). Ligands of $\alpha 9\beta 1$ integrin include some types of ECM, such as osteopontin (OPN) (Smith et al. 1996; Yokosaki et al. 1999) and tenascin-C (TN-C) (Yokosaki et al. 1994), as well as growth and coagulation factors (Table 1). Arginine-glycine-aspartic acid (RGD)-recognizing integrins (such as $\alpha v\beta 3$, $\alpha 5\beta 1$, and $\alpha 8\beta 1$) bind to ECM containing an RGD motif including fibronectin and vitronectin. In contrast, $\alpha 9\beta 1$ integrin recognizes several non-RGD sequences. For example, the recognition sequence of $\alpha 9$ integrin is SVVYGLR in OPN (Yokosaki et al. 1999) and AEIDGIEL in TN-C (Yokosaki et al. 1998). $\alpha 9$ integrin-deficient mice normally survive during embryonic

development, but all mice die by 12 days after birth as the result of respiratory failure secondary to the accumulation of pleural fluid (Huang et al. 2000). In addition, $\alpha 9$ integrin is critically involved in lymphatic valve morphogenesis (Bazigou et al. 2009). Evaluation of peripheral and bone marrow cells from $\alpha 9$ integrin-deficient mice revealed severe impairment of granulocyte development by inhibition of G-CSF receptor signaling, indicating a requirement for $\alpha 9$ integrin in neutrophil development (Chen et al. 2006).

OPN, an extracellular matrix (ECM) protein also known as Eta-1, is expressed in restricted adult cells and tissues under normal conditions and expression is augmented after insults such as inflammation and tissue injury. It exerts many functions in biological process such as wound healing, tissue remodeling, and immune responses (Morimoto et al. 2010; Patarca et al. 1989; Uede 2011). OPN contains a classical cell-binding motif, an RGD sequence, which is recognized by RGD-recognizing integrins on the cell surface such as $\alpha v\beta 3$, $\alpha 5\beta 1$, and $\alpha 8\beta 1$ (Barry et al. 2000b; Denhardt et al. 2001; Hu et al. 1995). OPN also contains SVVYGLR sequence, that can be recognized by non-RGD recognizing integrins such as $\alpha 4\beta 1$, $\alpha 4\beta 7$, and $\alpha 9\beta 1$ (Barry et al. 2000a; Green et al. 2001; Smith et al. 1996; Yokosaki et al. 1999) (Fig. 1). A thrombin-cleavage site $^{168}\text{RS}^{169}$ exists at the

Table 1 Ligands of $\alpha 9\beta 1$ integrin and their binding sequences. Several $\alpha 9\beta 1$ integrin ligands can also bind to $\alpha 4\beta 1$ integrin

Ligand	Amino acid sequence	$\alpha 9\beta 1$	$\alpha 4\beta 1$	Reference
OPN	SVVYGLR	+	+	(Barry et al. 2000a, Yokosaki et al. 1999)
Tenascin-C	AEIDGIEL	+	–	(Yokosaki et al. 1998)
VCAM-1	RTQIDSPNG	+	+	(Chan et al. 1992, Taooka et al. 1999)
Fibronectin-EIIIA	PEDGIHELFP	+	+	(Liao et al. 2002, Shinde et al. 2008)
FXIII	unknown	+	+	(Cui et al. 2009)
tTG	unknown	+	+	(Takahashi et al. 2000)
pp-vWF	DCQDHSFSIVIETVQ	+	+	(Cui et al. 2009)
Angiostatin	unknown	+	+	(Tarui et al. 2001)
Plasmin	kringle domain	+	–	(Majumdar et al. 2004)
Thrombospondin-1	unknown	+	+	(Staniszewska et al. 2007)
Emilin	globular C1q domain	+	+	(Danussi et al. 2011, Verdone et al. 2008)
ADAM-1, 2, 3, 9, 15	RX(6)DLPEF	+	?	(Eto et al. 2002)
ADAM-8	unknown	+	?	(Rao et al. 2006)
ADAM-12, 15	RX(6)DLPEF	+	?	(Eto et al. 2000)
ADAM-7, 28	unknown	+	+	(Bridges et al. 2005)
ADAM-33	unknown	+	–	(Bridges et al. 2005)
VEGF-A	unknown	+	?	(Vlahakis et al. 2007)
VEGF-C, D	unknown	+	?	(Vlahakis et al. 2005)
NGF, BDNF, NT-3	unknown	+	?	(Staniszewska et al. 2008)
XCL1	unknown	+	–	(Matsumoto et al. 2017)

Fig. 1 Structure and receptors of osteopontin (OPN). OPN contains RGD and SVVYGLR motifs for the binding of RGD-recognizing integrins such as $\alpha v \beta 3$ and $\alpha 4 \beta 1 / \alpha 4 \beta 7 / \alpha 9 \beta 1$ integrins, respectively. Positions of thrombin and metalloproteinase-3/7 cleavage sites are indicated. OPN cleaved by thrombin generates N-half (OPN-N) and C-half fragments (OPN-C)

center of the OPN molecule. $\alpha 9 \beta 1$ integrin recognized SVVYGLR sequence only when OPN is cleaved by thrombin, while the SVVYGLR sequence in intact OPN or thrombin-cleaved OPN can be recognized by both $\alpha 4 \beta 1$ and $\alpha 4 \beta 7$. OPN is further cleaved by matrix metalloproteinases (MMP)-3 and -7, at the position between G¹⁶⁶ and L¹⁶⁷ (Agnihotri et al. 2001). Recently, MMP-cleaved OPN was reported to also be recognized by $\alpha 9 \beta 1$ integrin (Kon et al. 2014). Moreover, polymerized OPN catalyzed by transglutaminase-2 is bound to $\alpha 9 \beta 1$ (Nishimichi et al. 2011; Nishimichi et al. 2009). Mice

lacking OPN are viable, fertile, and developmentally normal, but display altered osteoclast formation (Rittling et al. 1998).

TN-C, an ECM protein associated with tissue injury and repair (Brellier et al. 2009; Imanaka-Yoshida et al. 2004; Trebaul et al. 2007), is not normally expressed in most adult tissues, but is up-regulated during wound healing, tumorigenesis, and inflammation (Chiquet-Ehrismann and Chiquet 2003). TN-C is composed of epidermal growth factor (EGF)-like repeats, fibronectin type III repeats, and a C-terminal fibrinogen globe (FBG) (Fig. 2). Fibronectin type

Fig. 2 The modular structure and receptor of tenascin-C (TN-C). TN-C forms hexamers. The TN-C monomer is composed of four domains: an assembly domain, epidermal growth factor-like repeats, fibronectin type III repeats, and a C-terminal fibrinogen globe. Domains of integrin binding are indicated

III repeats within TN-C contain two distinct cell-binding motifs: an RGD motif for RGD-recognizing integrins (Joshi et al. 1993; Prieto et al. 1993; Sriramarao et al. 1993) and an AEIDGIEL motif for $\alpha 9 \beta 1$ integrin (Yokosaki et al. 1998). Notably, TN-C knockout mice are fertile, without gross deficit, and phenotypically normal (Saga et al. 1992).

OPN and TN-C, two common ligands of $\alpha 9 \beta 1$ integrin, have similar structures, expression patterns, and functions, i.e. they contain an RGD cell-binding motif and an $\alpha 9 \beta 1$ integrin-binding sequence, are highly induced upon inflammation, and exhibit persistent expression associated with chronic inflammation. In addition, they are involved in toll-like receptor (TLR) signaling (Midwood et al. 2009; Shinohara et al. 2006).

As $\alpha 9 \beta 1$ integrin and its ligands are widely expressed in a variety of cells, aberrant interactions have been speculated to exacerbate pathological conditions. Moreover, as $\alpha 9$ integrin-deficient mice have an extremely short lifespan, alternative neutralization approaches are needed to examine roles of $\alpha 9 \beta 1$ integrin in vivo. Recently, the development of a neutralizing antibody against murine $\alpha 9 \beta 1$ integrin facilitated clarification of its role in several diseases. Therefore, this review focuses on the interaction between $\alpha 9 \beta 1$ integrin and its ligands, especially OPN and TN-C. Moreover, we introduce the physiological significance of these interactions in the development of autoimmune diseases, especially rheumatoid arthritis (RA) and multiple sclerosis (MS).

Role of $\alpha 9$ integrin and its ligands in rheumatoid arthritis (RA)

RA is one of the major chronic inflammatory diseases. Accelerated proliferation of synovial cells in joints leads to cartilage destruction. In the joint synovium, various ECMs, such as OPN and TN-C, are up-regulated in RA patients or murine inflammatory arthritis models (Hasegawa et al. 2007; Ohshima et al. 2002a, b; Page et al. 2012), suggesting interactions between ECMs and integrins on synovial cells are important for the development of RA.

To analyze the role of OPN in RA, a collagen antibody-induced arthritis (CAIA) model was used. This mouse model of inflammatory arthritis was induced by administration of an antibody cocktail against distinct epitopes of type II collagen, followed by injection of lipopolysaccharide (LPS). OPN-deficient mice were resistant against arthritis development in the CAIA model (Yumoto et al. 2002), indicating the involvement of OPN in inflammatory arthritis. To identify the OPN epitope underlying development of CAIA in this model, an antibody against the murine $\alpha 4 \beta 1$ and $\alpha 9 \beta 1$ integrin binding epitope SLAYGLR within mouse OPN was generated (M5 Ab) (Yamamoto et al. 2003). Prophylactic treatment of CAIA mice with M5 Ab significantly inhibited joint inflammation, reduced inflammatory cytokine expression (such as

TNF- α and IL-6), and prevented osteoclast-mediated bone resorption and osteoclast formation, indicating that SLAYGLR (corresponding to SVVYGLR in human) is involved in the development of CAIA. However further studies were needed to clarify the receptor that bind to SLAYGLR; obviously two possible candidates are $\alpha 4 \beta 1$ and $\alpha 9 \beta 1$ integrin. A neutralizing antibody, which interferes the binding of murine $\alpha 9 \beta 1$ integrin and its ligands was successfully developed (Kanayama et al. 2009). Surprisingly, therapeutic administration of the anti- $\alpha 9$ integrin antibody, which was injected after disease onset, induced significant amelioration of CAIA. The authors also found that $\alpha 9 \beta 1$ integrin was expressed on synovial fibroblasts and macrophages. Upon stimulation with $\alpha 9 \beta 1$ integrin ligand, these cells expressed pro-inflammatory cytokines TNF- α , IL-6, and IL-1 β , as well as chemokines CCL2, CCL3, CCL4, CXCL2, and CXCL5, which contributes to the development of joint inflammations and destruction.

Furthermore, novel post-translational processing of OPN, specifically polymerization catalyzed by transglutaminase-2, facilitated binding to $\alpha 9 \beta 1$ integrin (Nishimichi et al. 2009). Expression of transglutaminase-2 and its transglutaminase activity were associated with cartilage degradation in joints of collagen-induced arthritis (CIA) mice (Lauzier et al. 2012). These reports indicated that conformational modifications of OPN by enzymes yield at least 3 different forms of OPN, which acquire the binding activity for $\alpha 9 \beta 1$ integrin not observed for intact OPN, and are involved in murine inflammatory models.

The contribution of TN-C to inflammatory joint disease was elucidated by intra-articular injection with methylated bovine serum albumin, a model that induces pathological changes similar to RA. In this model, TN-C-deficient mice were protected from joint destruction (Ruhmann et al. 2012). Domain analysis of TN-C, clarified that the FBG domain causes TLR4 activation and subsequent induction of joint inflammation, suggesting that TN-C acts as an endogenous TLR4 activator. Furthermore, the role of TN-C in DC function was analyzed, as activated DCs express high levels of TN-C (Goh et al. 2010). CD4⁺ T cells co-cultured with DCs harvested from TN-C-deficient mice were defective in IL-17 synthesis. However, these reports did not address the issue if $\alpha 9 \beta 1$ integrin is involved in TN-C-mediated joint inflammation. The relationship between TN-C and $\alpha 9 \beta 1$ integrin in inflammatory joint disease was clarified by following manuscript. Conventional dendritic cells (cDCs) and macrophages expressing $\alpha 9 \beta 1$ integrin were increased in lymph node tissue after the first collagen II-immunization in a CIA model (Kanayama et al. 2011). $\alpha 9 \beta 1$ integrin-expressing cDCs and macrophages produced OPN and TN-C, and these ECM proteins induced the production of Th17-related cytokines, such as IL-23, by cDCs and macrophages via TLR2 and TLR4 signaling. To analyze the relationship between TN-C and

$\alpha 9 \beta 1$ integrin in inflammatory cytokine production, the TN-C mutant TNfn3RAA was used as an $\alpha 9 \beta 1$ integrin-specific ligand. Stimulation of cDCs by TNfn3RAA promoted the production of inflammatory cytokines including IL-6, TNF- α , and IL-12. Notably, production of these cytokines was not altered by the deletion mutant RNfn3RAA, which lacks the $\alpha 9 \beta 1$ integrin-binding motif AEIDGIEL. Furthermore, increased cytokine expression induced by TNfn3RAA was inhibited by an $\alpha 9 \beta 1$ integrin-neutralizing antibody. Thus, $\alpha 9 \beta 1$ is critically involved in TN-C-mediated joint inflammation.

In RA, osteoclastic bone resorption was increased. Integrins such as $\alpha \nu \beta 3$ have been identified as important players for osteoclast formation and activation (Zou and Teitelbaum 2010). In 2006, $\alpha 9 \beta 1$ integrin was reported to be expressed on osteoclasts and was involved in osteoclast differentiation and function (Rao et al. 2006). Expression of $\alpha 9$ integrin mRNA was increased during osteoclast differentiation of murine bone marrow cells, and this process could be blocked by an anti- $\alpha 9 \beta 1$ integrin antibody. Osteoclasts from $\alpha 9$ integrin-deficient mice exhibited impaired bone resorption ability. As a ligand of $\alpha 9 \beta 1$ integrin, this manuscript also suggested that ADAM8 is involved in osteoclast function. In other reports, roles of OPN and TN-C in osteoclast differentiation and function have been demonstrated (Kiyeko et al. 2016; Yamamoto et al. 2003). Fig. 3 shows a schematic diagram of possible roles for the interaction of $\alpha 9 \beta 1$ integrin with OPN or TN-C in the development of RA.

Importantly, $\alpha 9$ integrin is also expressed in human osteoclasts. Synovial fibroblasts and macrophages were obtained from human RA patients (Asano et al. 2014). Similar to the

mouse RA models, $\alpha 9$ integrin is expressed on human synovial fibroblasts and macrophages. However, there were significant differences between murine and human arthritic joint microenvironments. Although OPN production is much higher than TN-C production in murine synovial tissues during inflammatory arthritis, TN-C production in RA patients was greater than OPN. In addition, OPN was mainly produced by synovial fibroblasts in mice, whereas in RA patients, synovial fibroblasts produced a greater amount of TN-C than synovial macrophages. Importantly, synovial cells stimulated by TN-C induced the expression of MMPs and IL-6 in synovial fibroblasts, as well as TNF- α and IL-1 β in synovial macrophages. Unlike murine arthritis, the interaction between $\alpha 9 \beta 1$ integrin and TN-C, but not OPN, might be a useful target for RA. Indeed, in a clinical trial, it was recently reported that OPN blockade by an antibody was unlikely to benefit human RA patients. Moreover, TN-C levels in synovial cells and serum are elevated in RA patients, with serum TN-C levels correlating to joint erosion (Hasegawa et al. 2007; Page et al. 2012).

Role of $\alpha 9$ integrin and its ligands in MS and its murine model, experimental autoimmune encephalomyelitis (EAE)

MS is a demyelinating disease characterized by inflammation within the central nervous system, possibly due to autoimmunity. In active lesions, evidence indicates that autoreactive T cells play a main role in MS induction (Brocke et al. 1994). EAE is a widely used experimental model of MS. Among Th

Fig. 3 The interaction between $\alpha 9 \beta 1$ integrin and osteopontin (OPN) or tenascin-C (TN-C) regulates the pathogenesis of rheumatoid arthritis (RA). $\alpha 9 \beta 1$ integrin is expressed on synovial fibroblasts, macrophages, conventional dendritic cells, and osteoclasts, all of which express OPN and TN-C. OPN undergoes post-translational modifications by thrombin or metalloproteinase. Enzyme-modified OPN or TN-C stimulate $\alpha 9 \beta 1$ integrin-expressing cells to promote the production of various inflammatory cytokines and differentiation of RA-related cells, causing an elevated inflammatory response and bone destruction

cells, Th1 and Th17 subgroups are the major encephalitogenic populations in EAE (Petermann and Korn 2011). Microarray analysis revealed overexpression of OPN transcripts in both spinal cords of rats with EAE and brain tissues of MS patients (Chabas et al. 2001). OPN has well-described pleiotropic functions during bacterial infections, including generation of Th1 response, enhanced production of IFN- γ and IL-12, and diminished IL-10 production (Ashkar et al. 2000). It must be noted that later studies also demonstrated the involvement of OPN in Th17 cell polarization (Chen et al. 2010; Shinohara et al. 2008). These data suggested OPN might play an important role in the pathogenesis of EAE and MS.

The severity of EAE in OPN-deficient mice was significantly reduced as compared with wild-type counterparts (Chabas et al. 2001; Jansson et al. 2002). Moreover, OPN-deficient mice were 100% protected from EAE-related death. Myelin-reactive T cells from OPN-deficient mice exhibited attenuated T cell activation, as evidenced by inhibition of cell growth, enhanced IL-10 production, and reduced IFN- γ production. Daily administration of recombinant OPN protein to OPN-deficient mice with EAE during the spontaneous recovery counteracted ongoing disease remission and exacerbated clinical paralysis (Hur et al. 2007). Moreover, all mice treated with recombinant OPN protein died as a result of EAE. These authors also found that OPN inhibits cell death by inhibiting the transcription factor Foxo3a (a downstream substrate of ALT involved in apoptosis) and NF- κ B (an important molecule regulating mature T cell survival). After cleavage by thrombin, OPN generates N-half (OPN-N) and C-half fragments (OPN-C) (Fig. 1). To evaluate the effect of thrombin-cleavage on OPN activity, recombinant full-length OPN, OPN-N, OPN-C, and a mixture of OPN-N and OPN-C were administered during the remission phase occurring after the first peak (Boggio et al. 2016). As a result, each OPN form and the mixture of OPN-N and OPN-C induced a similarly strong relapse. It is possible that intrinsic thrombin in inflammatory foci leads to the cleavage of full-length OPN. In addition, thrombin-resistant murine OPN, which is generated by a mutation within the thrombin-cleavage site (from R¹⁵³ and S¹⁵⁴ to S¹⁵³ and F¹⁵⁴), exhibited no effect on relapse, suggesting that thrombin-cleaved form of OPN is involved in pathogenesis of EAE. It should be reminded that thrombin-cleaved form of OPN exposed cryptic binding sites for both α 4 β 1 and α 9 β 1 integrins (Barry et al. 2000a; Green et al. 2001; Smith et al. 1996; Yokosaki et al. 1999). Anti- α 4 integrin antibody, natalizumab has already shown beneficial therapeutic effects for relapsing-remitting MS and Crohn's disease. However, the role of α 9 β 1 integrin in EAE was yet to be elucidated.

The detailed analysis how interaction of α 9 β 1 integrin and its ligands in vivo leads to the development of EAE was carried out by Ito et al. (Ito et al. 2014). α 9 β 1 integrin and TN-C were expressed and co-localized in the lymphatic vascular endothelial gene-1⁺ (LYVE-1⁺) medullary and cortical sinuses

of lymph nodes. Blockade of α 9 β 1 integrin by neutralizing antibody in Freund's complete adjuvant (FCA)-immunized mice resulted in almost hollowed out medullary and cortical sinuses in lymph nodes, as this tissue was devoid of lymphocytes (the author referred to these sinuses as "vacant"). Authors further analyzed how T cell recruitment from regional lymph node to pathological foci was regulated in EAE mice. Naive T cells migrate from peripheral blood into draining lymph nodes. Egress of naive T cells from lymph nodes via lymphatic vessels is regulated by the sphingosine-1-phosphate receptor (S1PR). The lymphocyte egress inhibitor FTY720, a S1PR agonist, induced vacant sinuses within lymph nodes, suggesting α 9 β 1 integrin might be a functional molecule for lymphocyte egress. Indeed, α 9 β 1 integrin-blockade by neutralizing antibody enhanced lymphocyte accumulation within lymph nodes after immunization with FCA. In addition, stimulation of α 9 β 1 integrin on LECs by TNfn3RAA, an α 9 β 1 integrin-specific ligand, induced S1P secretion (Fig. 4). A single injection of neutralizing antibody against α 9 β 1 integrin prophylactically ameliorated EAE-related symptoms and significantly reduced infiltrating cells in the spinal cord. Thus, the association of α 9 β 1 to TN-C might be a target of EAE via regulation of lymphocyte egress from lymph nodes. Recently, as the first evidence, direct function of TN-C in the induction and progression of EAE were reported using TN-C-deficient mice (Momcilovic et al. 2017). TN-C deficiency impaired the induction of EAE by down-regulating IFN- γ - and IL-17-producing T cells.

A novel α 9 β 1 integrin ligand involved in autoimmune diseases

During the course of α 9 β 1 integrin analysis in various disease conditions, we found the existence of spliced form of α 9 (SF α 9) (Kon et al. 2011). SF α 9 exhibited no binding ability to known α 9 β 1 integrin ligands, despite SF α 9 being expressed on the cell surface. Yet SF α 9 is involved in α 9 β 1 integrin-mediated cell adhesion as a promoter. Subsequently, the C-class chemokine XCL1 was identified to be a SF α 9-binding protein from a screen using LC-MS/MS analysis of purified SF α 9 protein from the cell surface-enriched fraction (Matsumoto et al. 2017). XCL1 bound not only to SF α 9, but also α 9 β 1 integrin, and enhanced α 9 β 1 integrin-dependent cell migration. Recombinant XCL1 protein exacerbated inflammatory joint disease in CAIA, which could be reduced by administration of an anti- α 9 β 1 integrin antibody. In addition, a neutralizing antibody to XCL1 inhibited the development of both EAE and inflammatory arthritis CAIA. Therefore, XCL1 is an important ligand of α 9 β 1 integrin in the development of autoimmune diseases.

Fig. 4 The interaction between $\alpha 9 \beta 1$ integrin and tenascin-c (TN-C) inhibits the egress of lymphocytes from lymph nodes. Naive T cells enter lymph nodes via afferent lymphatics during circulation between blood and lymphatic tissues. After T cell proliferation and differentiation in lymph nodes, activated T cells up-regulate sphingosine-1-phosphate (S1P) receptor and migrate to the lymphatic sinus in response to a S1P

concentration gradient. Interactions between $\alpha 9 \beta 1$ integrin on lymphatic endothelial cells and TN-C promote S1P secretion from lymphatic endothelial cells, thereby augmenting T cell egress. Augmentation of T cell egress affects inflammatory processes including experimental autoimmune encephalomyelitis

Conclusions and future prospective

In this review, we summarized the pathological and physiological significance of interactions between $\alpha 9 \beta 1$ integrin and OPN or TN-C during the development of RA and MS. As a result of their structural and functional similarity, $\alpha 9$ and $\alpha 4$ integrins are in the same subfamily within integrin family (Palmer et al. 1993). Furthermore, both integrins share several common ligands, as shown in Table 1, and their signaling cascades are also considered to be similar (Liu et al. 2001, 1999; Young et al. 2001). Even though the anti- $\alpha 4$ integrin antibody drug Natalizumab has been used to treat autoimmune diseases, dual blockade of $\alpha 4$ and $\alpha 9$ integrins might yield better therapeutic effects in some conditions.

There are two isoforms of OPN: a secreted form (sOPN) and an intracellular form (iOPN). iOPN mRNA transcripts are commenced from a downstream alternative start site, such that iOPN lacks the signal sequence and remains inside of cells. It is important to know the role of iOPN in immune reactions, and most of our understanding of related outcomes comes from OPN-deficient mice. sOPN and iOPN have distinct functions as a result of their localization. First, IFN- α production by virus infection leads to the induction of antiviral responses. Plasmacytoid DC cells (pDCs) sense viruses through TLR-7 and TLR-9, whose ligation induces iOPN. In pDCs, the iOPN associated with MyD88, an essential adaptor protein in TLR signaling, translocates IRF-7 to the nucleus, leading to production

of IFN- α and proinflammatory cytokines. Second, iOPN expressed in cDCs enhanced Th17 cell responses by suppressing IL-27 expression, which is known to inhibit Th17 cell development. Thus, iOPN in cDCs is a critical regulator of Th17 responses. In this review, we focused on sOPN only, as iOPN is an intracellular protein and, therefore, not a ligand of $\alpha 9 \beta 1$ integrin. Several excellent review articles addressing roles for iOPN in immune reactions have been published (Cantor and Shinohara 2009; Inoue and Shinohara 2011).

Numerous ligands of $\alpha 9 \beta 1$ integrin have been identified (Table 1), however few interactions underlying pathological roles observed during the development of autoimmune diseases have been revealed, with the exception of OPN and TN-C. As such, one important means to resolve this problem is the generation of an antibody against known $\alpha 9 \beta 1$ integrin-recognizing sequences.

Further insights into signaling pathways activated by the binding of various $\alpha 9 \beta 1$ integrin ligands in multiple tissues are urgently needed to develop more refined $\alpha 9 \beta 1$ integrin-based therapeutics.

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