

Crucial role for CD69 in allergic inflammatory responses: CD69-Myl9 system in the pathogenesis of airway inflammation

Motoko Y. Kimura¹  | Koji Hayashizaki¹ | Koji Tokoyoda² | Shiki Takamura³ | Shinichiro Motohashi⁴ | Toshinori Nakayama¹

¹Department of Immunology, Graduate School of Medicine, Chiba University, Chiba, Japan

²Department of Osteoimmunology, German Rheumatism Research Centre (DRFZ) Berlin, Berlin, Germany

³Department of Immunology, Kindai University Faculty of Medicine, Osaka, Japan

⁴Department of Medical Immunology Graduate School of Medicine, Chiba University, Chiba, Japan

Correspondence

Motoko Y. Kimura and Toshinori Nakayama, Department of Immunology, Graduate School of Medicine, Chiba University, Chuo-Ku, Chiba, Japan.

Emails: kimuramo@chiba-u.jp; tnakayama@faculty.chiba-u.jp

Funding information

Ministry of Education, Culture, Sports, Science and Technology, Japan: Grants-in Aid for Scientific Research (S), Grant/Award Number: 26221305; JSPS Fellows, Grant/Award Number: 2502883; Japan Agency for Medical Research and Development (AMED)

Summary

CD69 has been known as an early activation marker of lymphocytes; whereas, recent studies demonstrate that CD69 also has critical functions in immune responses. Early studies using human samples revealed the involvement of CD69 in various inflammatory diseases including asthma. Moreover, murine disease models using *Cd69*^{-/-} mice and/or anti-CD69 antibody (Ab) treatment have revealed crucial roles for CD69 in inflammatory responses. However, it had not been clear how the CD69 molecule contributes to the pathogenesis of inflammatory diseases. We recently elucidated a novel mechanism, in which the interaction between CD69 and its ligands, myosin light chain 9, 12a and 12b (Myl9/12) play a critical role in the recruitment of activated T cells into the inflammatory lung. In this review, we first summarize CD69 function based on its structure and then introduce the evidence for the involvement of CD69 in human diseases and murine disease models. Then, we will describe how we discovered CD69 ligands, Myl9 and Myl12, and how the CD69-Myl9 system regulates airway inflammation. Finally, we will discuss possible therapeutic usages of the blocking Ab to the CD69-Myl9 system.

KEYWORDS

allergy, CD69, CD69-Myl9 system, inflammation, Myl9, platelets

1 | CD69 STRUCTURE AND FUNCTION

1.1 | Discovery of CD69

CD69 was first identified in the late 1980s as a molecule rapidly induced on T cells, NK cells and B cells upon activation.¹⁻⁴ Later, two groups independently cloned human CD69,^{5,6} and demonstrated that CD69 is a type-2 membrane glycoprotein and is expressed as a disulfide linked homo-dimer^{1,5-8} (Figure 1). CD69 is a highly glycosylated protein, which has two N-glycosylation sites in the human CD69 extracellular domain and three in the murine CD69 extracellular domain. Biochemical analysis showed that each CD69 molecule has different levels of glycosylation.¹⁻⁴ The CD69 gene is encoded in

the NK-gene complex that is located in human chromosome 12 and mouse chromosome 6, where a group of receptors that have C-type lectin-like domains are encoded.^{6,9} CD69 also has a C-type lectin-like domain; however, three-dimensional structure analysis revealed that CD69 lacks a Ca²⁺-dependent carbohydrate binding site. Instead, CD69 contains a hypothetical ligand-binding site constituted of a hydrophobic patch surrounded by acidic residues.¹⁰ These data suggest that CD69 may bind to its ligands by protein-protein interaction rather than via sugar chains.

CD69 has been known as an early activation marker of leucocytes including T cells, B cells, NK cells, granulocytes as well as macrophages and dendritic cells (DCs)^{8,11}: CD69 is not detectable on these cells at steady state but is only induced upon stimulation.^{8,11} Interestingly, some cells such as iNKT cells and tissue-resident memory T (T_{RM}) cells constitutively express a marginal level of CD69,^{8,12-21} although

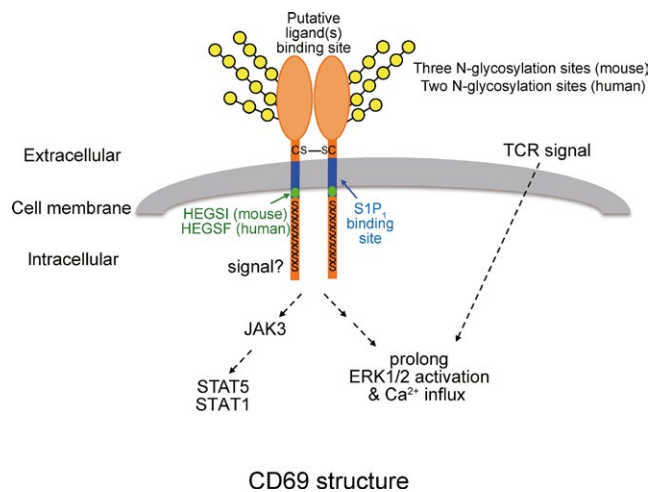


FIGURE 1 CD69 structure and its important domains. CD69 is a type-2 transmembrane glycoprotein and is expressed as disulfide-linked homo-dimer. The extracellular domain of CD69 has two N-glycosylation sites in human and three N-glycosylation sites in mouse, and each CD69 molecule appears to have different levels of glycosylation. CD69 has a C-type lectin-like domain; however, it lacks the features involved in Ca^{2+} dependent carbohydrate binding. Instead, CD69 contains the putative ligand-binding site constituted of a hydrophobic patch surrounded by acidic residues. The transmembrane domain of CD69 together with the membrane proximal region called HEGSI motif in mouse is important for S1P_1 binding. The cytosolic tail of CD69 is relatively short (only 40 amino acids), but both human and murine CD69 contain eight serine residues, some of which are phosphorylated in activated lymphocytes and thymocytes, suggesting its signaling potential. It is also reported that the CD69 cytosolic tail is important for prolongation of ERK activation and Ca^{2+} influx in the presence of phorbol ester stimulation, and for JAK3 mediated STAT5 and STAT1 activation

importance of CD69 expression for the functions of these cells are not fully understood. Platelets are also reported to express CD69²²; however, another report showed that CD69 expression is limited in platelets,¹ indicating that the expression level of CD69 on platelets is likely very low. CD69 has three distinct regions: an extracellular domain including C-type lectin-like domain, a transmembrane domain, and a cytosolic tail, and each domain has unique roles in CD69 function (Figure 1), as discussed in the following sections.

1.2 | Role of the transmembrane domain: CD69- S1P_1 system regulates lymphocyte egress from lymphoid organs

Sphingosine-1-phosphate (S1P) is known as a central mediator of lymphocyte egress from lymphoid organs such as thymus and lymph node (LN).²³ S1P is a ligand for at least five S1P receptors including S1P_1 , S1P_2 , S1P_3 , S1P_4 and S1P_5 , and each interaction plays essential roles in various biological reactions.^{23,24} Among five S1P receptors, S1P_1 plays important roles in lymphocyte egress by responding to S1P , depending on its concentration gradient. Since S1P concentration is very high in blood ($\sim 1 \mu\text{M}$) and lymph ($\sim 100 \text{ nM}$), but low in lymphoid organs such as the thymus and the LN,²⁵ S1P_1 -expressing lymphocytes

exit from lymphoid organs and return to blood circulation through lymphatic fluid at steady state.²³

One of the well-known functions of CD69 in the immune system is the regulation of cell surface expression of S1P_1 by a direct molecular interaction at cell surface membrane, which decreases lymphocyte egress from lymphoid organs such as the thymus and the LN.²³ In this review, we call this system the “CD69- S1P_1 system”. Biochemical studies using the domain-swapping technique between CD69 and the related type-2 transmembrane protein, NKRp1A, revealed that the CD69 transmembrane and membrane proximal regions (HEGSI motif) interact with the S1P_1 membrane helix 4²⁶ (Figure 1). This direct interaction induces internalization of S1P_1 , resulting in the inhibition of lymphocyte egress from lymphoid organs.^{23,26,27}

The biological importance of the CD69- S1P_1 system is demonstrated as cell egress from the thymus.^{28–30} During thymocyte development, CD69 expression is induced upon positive selection and the expression is maintained throughout positive selection.³¹ As CD69 surface expression is decreased, S1P_1 surface expression is induced, and cells leave the thymus.^{23,32} The experiments using CD69 full length transgenic (Tg) mice showed that mature thymocytes accumulate in the thymus, demonstrating that CD69 downregulation is required for egress of mature thymocytes.^{33,34} In addition, experiments using an S1P_1 agonist, FTY720, demonstrated that FTY720 treatment induced rapid internalization of S1P_1 ; therefore, in vivo injection of this drug resulted in the disappearance of lymphocytes from peripheral blood and the accumulation of cells in the thymus as well as in the LN.²⁸

Later studies showed that the CD69- S1P_1 system is also required for immune responses upon infection. At the beginning of an immune response (approximately within 24 hours), lymphocyte egress from the regional LN is transiently shut down^{27,35,36}; this phenomenon appears to be induced by the abundant presence of type I interferon ($\text{IFN}\alpha/\beta$) in the regional LN. $\text{IFN}\alpha/\beta$ rapidly induced CD69 expression and results in the inhibition of S1P_1 surface expression.²⁷ In this situation, antigen-specific T cells are efficiently stimulated by antigen-presenting cells (APCs) that have migrated from the inflammatory tissues, which induces further upregulation of cell surface expression of CD69 on T cells. CD69-expressing T cells are retained in the LN because they have little surface S1P_1 expression and are not in contact with the cortical sinus, sites of S1P_1 -dependent T cell egress from the LN,³⁶ until they become CD69⁺ effector T cells and are ready to migrate to inflamed local tissues.¹⁹ Thus, the CD69- S1P_1 system plays an important role in lymphocyte egress from lymphoid organs not only in steady states but also during immune responses.

1.3 | Function of the CD69 cytosolic tail

Several studies have reported that the cytosolic tail of the CD69 molecule has some signaling capacity. In fact, there are eight serine and two threonine residues in the human CD69 cytosolic tail, and eight serine and one threonine residues in the murine CD69 cytosolic tail; six of them are conserved between human and mouse^{5,6} (Figure 1). Furthermore, it is reported that some serine residues of the cytosolic tail of CD69 are constitutively phosphorylated in activated

TABLE 1 CD69 expression in various human inflammatory diseases

Diseases	CD69 expression	References
Asthma	Bronchoalveolar lavage eosinophils sputum CD4 T and CD8 T cells	Hartnell et al. ⁴⁸ Julius et al. ⁴⁹ Lourenco et al. ⁵⁰
Eosinophilic Pneumonitis	Bronchoalveolar lavage eosinophils	Nishikawa et al. ⁵¹
COPD	Lung CD8 T cells	Freeman et al. ⁵²
Chronic bronchitis	Bronchoalveolar lavage and respiratory mucosal lymphocytes	Ekberg-Jansson et al. ⁵³
ECRS	Nasal polyp CD4 T cells	Shinoda et al. ⁵⁴ Hayashizaki et al. ⁴²
Arthritis	Synovial fluid and peripheral blood T cells Synovial membrane and fluid T cells	Laffon et al. ⁵⁵ Afeltra et al. ⁵⁶ Rollin et al. ⁵⁷
Sarcoidosis	Bronchoalveolar lavage and peripheral blood CD4 T and CD8 T cells	Wahlstrom et al. ⁵⁸
Atopic dermatitis	Peripheral blood eosinophils	Toma et al. ⁵⁹
Atherosclerosis	Atherectomy specimens	Hosono et al. ⁶⁰
Systemic sclerosis	Peripheral blood CD4 T and CD8 T cells	Radstake et al. ⁶¹ Radstake et al. ⁶²
Multiple sclerosis	Cerebrospinal fluid lymphocytes	Perrella et al. ⁶³
Systemic lupus erythematosus	Peripheral blood NK cells	Hervier et al. ⁶⁴
Weagner's granulomatosis	Peripheral blood CD4 T cells	Abdulhad et al. ⁶⁵
Neuromyelitis optica	Peripheral blood CD4 T cells	Matsuya et al. ⁶⁶
Autoimmune thyroiditis	Peripheral blood CD4T and CD8T cells Thyroid gland and peripheral blood T cells	Gessl et al. ⁶⁷ Marazuela et al. ⁶⁸

lymphocytes and thymocytes,^{8,22} indicating their possible roles for cell signaling. Early in vitro studies showed that the engagement of CD69 by the cross-linking of Ab in the presence of phorbol esters prolonged Ca^{2+} influx and extracellular signal-regulated kinase (ERK)1/2 kinase activation in human T cells^{8,37} (Figure 1). In addition, there is a report demonstrating that the cytosolic tail of CD69 can associate with the transcription factors STAT5 and STAT1 through Janus family kinase (JAK)3 activation³⁸; however, it is not clear if this association is direct or indirect since the CD69 cytosolic tail has neither the conserved box1 motif that is required for association with JAK, nor any tyrosine residues that are required for STAT binding to the receptors (Figure 1). The authors suggest that the JAK3-mediated signaling downstream of CD69 may negatively regulate T helper 17 (Th17) differentiation³⁸ while positively regulate the development of regulatory T cells (Tregs).³⁹

As we discussed in the previous section, CD69 Tg mice have an accumulation of mature thymocytes in the thymus.^{33,34} Interestingly, mice with a CD69 transgene that lacks the cytosolic tail but includes the S1P₁ binding site also showed accumulation of mature T cells in the thymus, suggesting that cytosolic tail is not crucial for the egress from the thymus via the CD69-S1P₁ system.^{26,34} However, the accumulation of mature thymocytes in the thymus of the tailless CD69 Tg mice was slightly lower compared to those in mice with the full length

CD69 transgene. This difference may be due to different surface expression levels of CD69 transgene,^{33,34} but also may be due to some roles for CD69 cytosolic tail in the egress of thymocytes, although the detailed mechanisms are still under investigation.

1.4 | Role of the extracellular domain: CD69 interacting molecules

Recently, various CD69 interacting molecules were reported.^{40–42} Human CD69 was reported to interact with galectin-1 expressed on DCs, which was identified by pull-down assay using human DCs and recombinant human CD69 extracellular domain protein. The authors showed that the CD69 and galectin-1 interaction negatively regulates Th17 cell differentiation, at least in vitro.⁴¹ Galectins are a family of glycan-binding proteins with carbohydrate recognition domain (CRD), have 15 family members, and are known to bind to many glycosylated receptors on variety of cells including T cells, regulating cell survival and cell fate.⁴³ Among them, galectin-1 is reported to also interact with CD45, CD43, and pre-B cell receptor (pre-BCR), in addition to CD69,^{44–46} suggesting a wide spectrum of binding of galectin-1 to various glycoproteins.

Another group reported that human CD69 interacts with S100A8/A9 (calprotectin), a heterodimer of S100A8 and S100A9, which was

identified by immuno-precipitation assay using PMA-stimulated human PBMC lysates and anti-CD69 Ab. The authors showed that the interaction between CD69 and S100A8/A9 supports Treg differentiation in the experiments using human PBMCs.⁴⁰ S100A8 and S100A9 are the calcium-binding protein, and expressed and secreted by granulocytes, monocytes and DCs. In addition, S100A8/A9 expression is increased under inflammatory conditions and during tumorigenesis, suggesting that S100A8/A9 may function in inflammation and within the tumor microenvironment.⁴⁷

Our group discovered that Myl9/12 are functional ligands for CD69 in inflammatory settings, which was identified by pull-down assay using bone marrow (BM) lysates and recombinant murine CD69 extracellular domain protein. We demonstrated that the CD69 and Myl9/12 interaction is crucial for induction and exacerbation of inflammatory responses in the airway.⁴² We will discuss this in a later section of this review.

It has not been directly addressed whether the interaction of CD69 with these molecules provides any signals through CD69 cytosolic tail; the molecular mechanisms for the biological relevance influenced by CD69 interaction with these molecules need to be investigated.

2 | CD69 AND INFLAMMATORY DISEASES

2.1 | CD69 expression in human diseases

Early studies reported that CD69 expression is often detected on samples from inflamed tissues and/or peripheral blood from the patients with several different diseases (Table 1), including asthma,^{48–50} eosinophilic pneumonia,⁵¹ chronic obstructive pulmonary disease (COPD),⁵² chronic bronchitis,⁵³ eosinophilic chronic rhinosinusitis (ECRS),^{42,54} arthritis,^{55–57} sarcoidosis,⁵⁸ atopic dermatitis,⁵⁹ atherosclerosis,⁶⁰ systemic sclerosis,^{61,62} multiple sclerosis,⁶³ systemic lupus erythematosus,⁶⁴ granulomatosis with polyangiitis (Wegener's granulomatosis),⁶⁵ neuromyelitis optica,⁶⁶ and autoimmune thyroiditis.^{67,68} These clinical findings indicate that CD69 expression is a possible activation marker for leukocytes, especially for T cells in inflamed tissues. Moreover, these findings suggest the possible contribution of CD69 to the pathogenesis of tissue inflammation.

2.2 | Roles of CD69 in murine models of inflammatory disease

A number of studies have found evidence that CD69 indeed plays crucial roles in the pathogenesis of various inflammatory diseases using murine disease models (Table 2). The first evidence indicating the requirement of CD69 for induction of inflammatory diseases was reported in a murine arthritis model, which was induced by injection of anti-type II collagen Ab together with lipopolysaccharide (LPS).⁶⁹ Expression of CD69 on the infiltrating neutrophils in arthritic joints of *Cd69^{+/+}* mice suggested a requirement for CD69 in migration of neutrophils into the local inflamed-tissue. Indeed, *Cd69^{-/-}* mice showed substantial attenuation of arthritic inflammation accompanied by reduced infiltration of neutrophils in the joint.⁶⁹

In 2009, we reported that *Cd69^{-/-}* mice showed ameliorated airway inflammation and reduced airway hyperresponsiveness (AHR) in a model of airway inflammation using ovalbumin (OVA).⁷⁰ Furthermore, anti-CD69 Ab treatment strongly ameliorated airway inflammation and AHR, and a significant therapeutic effect was detected.⁷⁰ Importantly, we found that the number of inflammatory cells in the lung was significantly reduced both in *Cd69^{-/-}* mice and in the mice with anti-CD69 Ab treatment. The adoptive transfer of ectopic CD69-expressing *Cd69^{-/-}* CD4 T cells into *Cd69^{-/-}* host mice developed airway inflammation upon OVA inhalation, indicating that CD69 expression on CD4 T cells is sufficient for the induction of airway inflammation, at least in our model.^{42,70} Recently this result was followed and supported by another group,⁷¹ in which the pretreatment with dexamethasone (DXM) together with anti-CD69 Ab treatment after OVA exposure completely inhibited airway inflammation; simple anti-CD69 Ab treatment inhibited established airway inflammation as effectively as DXM pretreatment.⁷¹ It is apparent that CD69 plays crucial roles in the infiltration and/or maintenance of inflammatory cells in inflamed tissues; whereas, there may also be a possibility that CD69 is involved in efficient immune responses, especially during the early priming phase, since CD4 T cells from *Cd69^{-/-}* mice showed less ability to differentiate into Th2 cells under in vitro culture conditions using APCs and also under in vivo inflammatory conditions.⁷⁰ Since the CD69 cytosolic tail may have some signaling potential, as we described in the previous section, the CD69 molecule itself may contribute to the activation of T cells and Th2 cell differentiation, which is known to be efficiently induced by strong TCR-mediated signaling.⁷²

The importance of CD69 expression on CD4 T cells in the pathogenesis of inflammatory diseases was also reported in a dextran sulfate sodium (DSS)-induced colitis model.⁷³ By carefully changing the concentration of DSS and the period of DSS-water administration, we have established both acute and chronic colitis models and examined the requirement for CD69 in the pathogenesis of colitis with *Cd69^{-/-}* mice. In both experimental settings, *Cd69^{-/-}* mice showed significant amelioration of disease severity, which was measured by body weight loss, stool consistency, fecal blood and survival rate.⁷³ Furthermore, anti-CD69 Ab treatment also ameliorated acute DSS-induced colitis. Importantly, lamina propria CD4 T cells from the mice administered DSS-water showed significantly higher CD69 expression, and the adoptive transfer of *Cd69^{+/+}* CD4 T cells into *Cd69^{-/-}* mice was sufficient to induce DSS-induced colitis.⁷³ Thus, these data demonstrate that CD69 expression on CD4 T cells plays important roles in the pathogenesis of DSS-induced colitis, although the molecular mechanisms remain unknown. It may be due to less inflammatory cells at the inflammatory sites, and/or due to less functional differentiation of effector T cells in the absence of CD69.

Other disease models such as cigarette smoke-induced pulmonary inflammation⁷⁴ and bleomycin-induced lung injury fibrosis⁷⁵ were also ameliorated in *Cd69^{-/-}* mice. In both models, the authors demonstrated that infiltration of macrophages, neutrophils and lymphocytes (lymphocytes are only in bleomycin-induced fibrosis) to the inflamed

TABLE 2 Roles of CD69 in various mouse disease models

Diseases	Experimental design	Methods	Effects	References
Asthma	OVA-induced allergic airway inflammation	Cd69 ^{-/-} (a) mouse	Inhibition	Miki-Hosokawa et al. ⁷⁰
		Anti-CD69 (H1.2F3)Ab	Inhibition	
	OVA-induced allergic airway inflammation	Anti-CD69 (H1.2F3)Ab	Inhibition	Wang et al. ⁷¹
	OVA-induced allergic airway inflammation HDM-induced allergic airway inflammation	Cd69 ^{-/-} (a) mouse	Inhibition	Hayashizaki et al. ⁴²
		Anti-Myl9/12 Ab	Inhibition	
	OVA-induced allergic airway inflammation	Anti-CD69 (H1.2F3)Ab	Inhibition	Martin et al. ⁷⁷
		Cd69 ^{-/-} (b) mouse	Enhancement	
CHS (Contact Hyper Sensitivity)	Oxazolone-induced contact hyper sensitivity	Anti-CD69 (2.2)Ab	Enhancement	Martin et al. ⁷⁷
		Cd69 ^{-/-} (b) mouse	Enhancement	
COPD	Cigarette smoke-induced pulmonary inflammation	Cd69 ^{-/-} (a) mouse	Inhibition	Tsuyusaki et al. ⁷⁴
Arthritis	Anti-type II collagen antibody-induced arthritis	Cd69 ^{-/-} (a) mouse	Inhibition	Murata et al. ⁶⁹
	Anti-type II collagen antibody-induced arthritis	Cd69 ^{-/-} (b) mouse	None	Lamana et al. ⁷⁸
		Anti-CD69 (2.2)Ab	None	
	Collagen-induced arthritis (CIA)	Cd69 ^{-/-} (b) mouse	Enhancement	Sancho et al. ¹⁴²
	Collagen-induced arthritis (CIA)	Anti-CD69 (2.2)Ab	Enhancement	Sancho et al. ⁸¹
		Anti-CD69 (2.3)Ab	Inhibition	
Fibrosis	Bleomycin-induced lung injury	Cd69 ^{-/-} (a) mouse	Inhibition	Yamauchi et al. ⁷⁵
IBD	DSS-induced colitis	Cd69 ^{-/-} (a) mouse	Inhibition	Hasegawa et al. ⁷³
		Anti-CD69 (H1.2F3)Ab	Inhibition	
	Antigen-specific transfer colitis	Cd69 ^{-/-} (a) mouse	Enhancement	Radulovic et al. ⁸⁶
	CD45RB ^{high} CD4 T cell transfer colitis	Cd69 ^{-/-} (a) mouse	Enhancement	Radulovic et al. ⁸⁵
Tumor	RM-1 prostatic carcinoma	Cd69 ^{-/-} (b) mouse	Enhanced anti-tumor activity	Esplugues et al. ⁸²
	RMA-S and YAC-1 lymphoma	Anti-CD69 (2.2)Ab	Enhanced anti-tumor activity	
	RM-1 prostatic carcinoma	Cd69 ^{-/-} (b) mouse	Enhanced anti-tumor activity	Esplugues et al. ⁸⁷
	RMA-S and YAC-1 lymphoma	Anti-CD69 (2.2)Ab	Enhanced anti-tumor activity	
Pneumonitis	Listeria infection	Cd69 ^{-/-} (b) mouse	Enhancement	Vega-Ramos et al. ¹⁴³

lung was significantly impaired in *Cd69*^{-/-} mice. They also showed that infiltrated macrophages in the inflamed lung expressed CD69, suggesting the importance of CD69 expression on macrophages infiltrating into the lung in cigarette smoke-induced pulmonary inflammation and bleomycin-induced lung injury fibrosis.

Taken together, these results demonstrate that CD69 plays critical roles in the pathogenesis of inflammatory diseases in various tissues regardless of the types of inflammation, such as type 1 or type 2 inflammation. Furthermore, the fact that anti-CD69 Ab treatment ameliorated inflammatory responses in OVA-induced allergic airway inflammation and DSS-induced colitis indicates the possibility that the pathogenesis of the disease depends on the interaction between CD69 and CD69 ligand(s) expressed in inflamed tissues, although such ligands had not been identified for a long time and were recently discovered in 2016.⁴²

2.3 | Two lines of *Cd69*^{-/-} mice with some variations in implication of the role for CD69

There are two independently established lines of germline *Cd69*^{-/-} mice; one is the line with a deleted exon 1 including the first ATG, which was made by our group (*Cd69*^{-/-} (a) mice in Table 2)⁶⁹; and the other is the line established by Sanchez-Madrid's group with deleted exon 2 but still including exon 1, according to their construct structure (*Cd69*^{-/-} (b) mice in Table 2).⁷⁶ Both lines of *Cd69*^{-/-} mice do not express any surface expression of CD69. There are some discrepancies in the implication of the role for CD69 in the pathogenesis of inflammation using these two lines of *Cd69*^{-/-} mice. As we discussed in the earlier sections, our *Cd69*^{-/-} (a) mice showed limited inflammatory responses in several mouse models such as OVA-induced allergic airway inflammation,⁷⁰ DSS-induced colitis,⁷³ anti-type II collagen

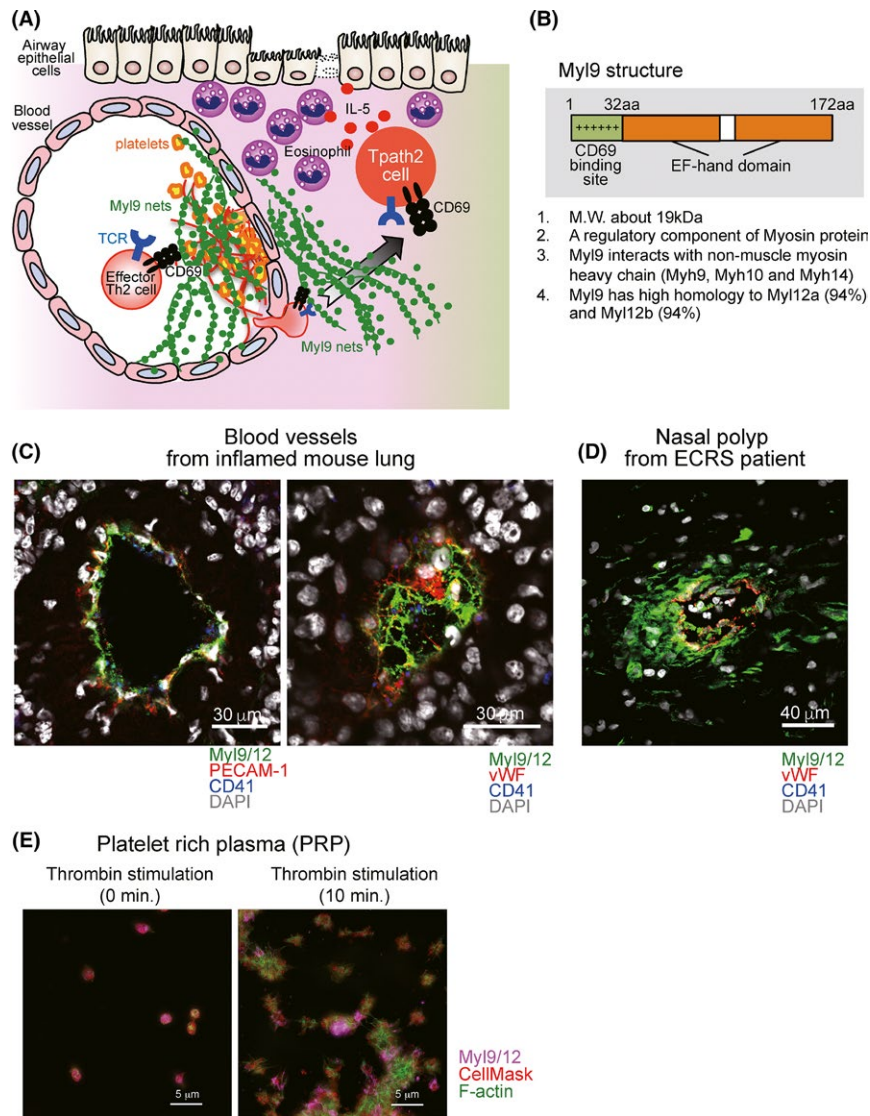


FIGURE 2 The CD69-Myl9 system and distribution of Myl9/12 in inflamed tissues. A, The CD69-Myl9 system is a novel mechanism that regulates the infiltration of inflammatory cells into inflamed tissues through blood vessels. In inflamed lungs, platelet-derived Myl9 is detected on the luminal surface of blood vessels and forms net-like structures together with platelets that we named “Myl9 nets”. Effector T cells from regional LN are re-activated and re-express CD69 at the vasculature in the inflamed lung, and then interact with Myl9 nets to migrate into tissues, leading to efficient induction of airway inflammation. Myl9 nets may act as a “platform” for effector T cells to migrate into inflamed tissues. In chronic inflammation such as the polyps of ECRS patients, Myl9 nets were detected not only inside the vessels but also in perivascular regions, which may facilitate inflammatory cell maintenance within inflammatory sites for long periods, leading to exacerbation and persistence of tissue inflammation. B, Myl9 structure and important domains. CD69 binds to Myl9 through its N-terminal region that contains positively charged amino acids. The C-terminal region of Myl9 contains two EF-hand domains including Ca²⁺ binding sites. C, Confocal microscopy analysis of lung sections after OVA inhalation showed that Myl9/12 (green) is expressed on the luminal surface of blood vessels (left) and some vessels contain Myl9 nets together with vWF (red) and platelets (CD41, blue) (right). D, Confocal microscopy analysis of polyps from ECRS patients showed Myl9/12 expression (green) on luminal surface of blood vessels and at perivascular regions together with vWF (red) and platelets (CD41, blue). E, Structured Illumination Microscopy (SIM) using platelet rich plasma prepared from murine peripheral blood stained with Myl9 (pink), CellMask (red) and F-actin (green) revealed that Myl9 was highly expressed in platelets and that Myl9 protein moved to the edge of platelet aggregates after 10 minutes stimulation with thrombin

Ab induced arthritis,⁶⁹ cigarette smoke-induced pulmonary inflammation⁷⁴ and bleomycin-induced lung injury fibrosis.⁷⁵ In contrast, the other line of *Cd69*^{-/-} (b) mice showed enhanced inflammation in OVA-induced allergic airway inflammation⁷⁷ and no difference in inflammatory responses in anti-type II collagen Ab induced arthritis.⁷⁸ The reason for these contradictory results obtained by different research groups has not yet been determined, particularly in the airway

inflammation models.^{70,77} One possible explanation is that different strategies of construction for *Cd69*^{-/-} mice may result in the differences in the results obtained. *Cd69*^{-/-} (b) mice contain the residual exon 1 gene fragment encoded 21 amino acids including five serine residues of cytosolic tail of CD69 molecule, which may have artificial effects on the function of CD69-mediated signaling. Another possibility is the different experimental protocols and conditions used by the

two different groups. The third possibility is that different microbiota at the mucosa in the mice maintained in the different animal facilities might create such a discrepancy, as several reports indicate that the microbiota strongly influences the immune system.^{79,80}

Importantly, there have also been three CD69 Abs used in in vivo mouse studies: two murine CD69 Abs (CD69.2.2 and CD69.2.3) and one hamster CD69 Ab (H1.2F3).^{42,70,73,77,81} Treatment with either H1.2F3 or CD69.2.3 has inhibitory effects on inflammatory diseases.^{42,70,73,81} CD69.2.3 is reported to have some ability to induce antibody-dependent cellular cytotoxicity (ADCC).^{81,82} In contrast, CD69.2.2 treatment appears to exacerbate inflammation^{77,81} (Table 2). Since CD69.2.2 was reported to induce rapid internalization of CD69,^{81,82} which may indicate that this Ab might work as an agonistic Ab so that the treatment with CD69.2.2 results in exacerbating inflammation. H1.2F3 appears to have a small effect on internalization of CD69 and we think it acts as a blocking Ab.^{42,70,73,83} As blocking antibodies and agonistic antibodies generally have different effects on target responses, careful consideration of the function of CD69 Ab treatment in inflammation is required.

2.4 | CD69 suppresses immune responses in some experimental settings

As we discussed in the earlier section, CD69 is crucial for the induction of inflammatory diseases and for immune responses upon infection. However, CD69 is also expressed on a subset of Tregs,⁸⁴ and may therefore negatively regulate some immune responses. For instance, *Cd69*^{-/-} mice showed exacerbated colitis, induced by CD25⁺CD45RB^{high} T cell injection, due to less function of Tregs (*Cd69*^{-/-} (a) mice in Table 2).^{85,86} In this experimental setting, Tregs play crucial roles in the prevention of colitis, indicating a requirement for CD69 in efficient generation and/or function of Tregs. It is also reported that *Cd69*^{-/-} mice showed augmented anti-tumor immunity due to enhanced NK cell activity.^{82,87} These data may indicate that CD69 is important for immuno-suppressive activity in some experimental settings. We think that contradictory results in the different colitis models^{73,85,86} may be due to their different levels of involvement of CD69-expressing effector T cells and Tregs.

3 | THE CD69-MYL9 SYSTEM IN AIRWAY INFLAMMATION

Our group recently identified a system regulating the pathogenesis of airway inflammation, and named it as the "CD69-MyI9 system"⁴² (Figure 2A). We found that the CD69 ligand, MyI9/12, is expressed on the luminal surface of blood vessels in inflamed lungs, and works as a "platform" through which activated CD69-expressing T cells migrate into the inflamed lung. The CD69-MyI9 system may promote efficient recruitment of activated T cells into the inflamed airway. We will discuss the details of this scenario in the following sections.

3.1 | Identification of MyI9/12 as CD69 ligands

Our group recently succeeded in identifying the functional ligands for CD69 in inflammatory settings.⁴² By using recombinant murine CD69 extracellular protein as bait, pull-down with lysates from BM, which was previously suggested to express CD69 ligands,⁸³ was performed, and subsequent LS-MS/MS analysis revealed that the CD69-interacting protein is MyI9 (Figure 2B). Physical association between CD69 and MyI9 molecules was confirmed by two methods: immunoprecipitation-western blotting assay and ELISA assay. Myosin light chain family has 10 family members and MyI9 has high homology to MyI12a (94%) and MyI12b (94%). We confirmed that CD69 binds to not only MyI9 but also MyI12a and MyI12b, at least in vitro. Further studies using MyI9 mutants revealed that CD69 binds to the N-terminal region of MyI9 and that the interaction is dependent on positive charges of lysine residues of MyI9.⁴² By using the N-terminal region of MyI9 protein as an immunogen, we succeeded to make functional monoclonal (mouse anti-MyI9/12 monoclonal Ab (114-2G9), KAN Research Institute Inc; rabbit anti-MyI9/12 monoclonal Ab (F-6), Abwiz Bio Inc) and polyclonal Abs that inhibit the binding of CD69 to MyI9, MyI12a and MyI12b; and, therefore, we named these Abs as MyI9/12 Ab.⁴²

3.2 | MyI9/12 Ab treatment ameliorates airway inflammation in two mouse models

We next examined the impacts of anti-MyI9/12 Ab treatment on OVA-induced and house dust mite (HDM)-induced allergic airway inflammation.⁴² Anti-MyI9/12 Ab treatment significantly ameliorated lung inflammation; mononuclear cell numbers within bronchoalveolar lavage (BAL) fluid were significantly decreased, mucus secretion was reduced and methacholine-induced AHR was significantly reduced. Importantly, the inhibition of airway inflammation by anti-MyI9/12 Ab treatment was strong, and almost identical to the inhibition by treatment with anti-CD69 Ab. These results are consistent with the notion that anti-MyI9/12 Ab and anti-CD69 Ab inhibited the interaction between CD69 and MyI9 molecules.⁴²

3.3 | Increased MyI9/12 expression in inflamed local tissues

MyI9 is a regulatory light chain that forms non-muscle myosin II by associating with myosin heavy chain such as Myh9, Myh10 and Myh14,⁸⁸ suggesting that MyI9 is a cytosolic protein. If so, how is it possible that MyI9 works as a ligand for CD69? We first examined when and where MyI9/12 is expressed during inflammation.⁴² MyI9 protein expression was limited in non-inflamed lungs from normal mice; whereas MyI9 protein expression was significantly increased upon inflammation. Confocal microscopy analysis revealed that MyI9 expression was detected on the luminal surface of blood vessels upon inflammation (Figure 2C left). Furthermore, we found that MyI9 forms net-like structures together with platelets (CD41) and von Willebrand factor (vWF) inside of the vessels in the inflamed lung (Figure 2C

right), demonstrating that Myl9 clearly exists as extracellular protein. We named the net-like structure detected in the blood vessels in inflamed lungs as "Myl9 nets".⁴²

We next examined the expression of Myl9 in inflamed human tissue. ECRS is an intractable airway inflammatory disease and often develops nasal polyps accompanied by massive infiltration of eosinophils, lymphocytes and plasma cells.⁸⁹ ECRS patients usually undergo surgical resection of nasal polyps to improve the clinical symptoms; however, the recurrence rate is high and is often associated with intractable asthma so that new therapeutic strategies are always desired.^{90–93} Interestingly, Myl9 nets were frequently detected in nasal polyps from the patients with ECRS, not only inside the blood vessels but also around the perivascular regions (Figure 2D). Furthermore, CD69-expressing T cells were identified within the polyps,⁵⁴ and they appeared to be trapped in the perivascular regions by Myl9 nets.⁴² Thus, the CD69-Myl9 system could be a new therapeutic target for the treatment of chronic inflammatory diseases such as ECRS.

3.4 | Source of Myl9/12 molecules in the inflamed lung

It has been reported that Myh9 forms a complex with Myl9 and is highly expressed in platelets,^{94,95} and that Myl9 can be detected in granules released from platelets upon activation.⁹⁶ In fact, we confirmed that Myl9 protein expression was extremely high in megakaryocytes and platelets.⁴² Of note, Myl9 mRNA expression was extremely high but Myl12a and Myl12b mRNA expression was limited in platelets; therefore, we think that the major protein that we detected by Myl9/12 Ab is Myl9. Our experiment using Structured Illumination Microscopy (SIM) showed that Myl9 was highly expressed in platelets (Figure 2E) and that Myl9 relocated into the edge of platelet aggregates within 10 minutes after thrombin stimulation (Figure 2E) and was released outside of platelets within 2 hours after thrombin stimulation.⁴² However, it is not yet clear whether Myl9 is secreted from platelets or is released due to platelet rupture. The depletion of platelets by GP1b α Ab treatment 1 day before and during OVA challenge significantly decreased the amounts of Myl9 detected on the luminal surface of blood vessels in the lung, demonstrating that the source of Myl9 in inflamed lungs is most likely platelets.⁴²

Platelets are a major player in the coagulation cascade, which is an ordered-process and favors clot formation. Tissue injury readily activates platelets in the intravascular space, and platelets attach to the inflamed endothelial cells to form plugs (platelet plug). Almost simultaneously, endothelial cells are activated, and release tissue factors that induce the activation of coagulation factor X (a serine endopeptidase). This activation cascade results in thrombin activation and the generation of fibrin strands, leading to a stable platelet-fibrin clot.^{97,98} We propose that Myl9 is released from platelets and then incorporated into the clot, creating Myl9 nets that form a platform for inflammatory cells to be recruited into the tissue parenchyma.

Importantly, recent reports suggest that abnormalities in the coagulation cascade may play an etiologic role in many diseases, such as asthma and other inflammatory conditions.⁹⁸ For instance, it is

reported that platelets from patients with allergy show abnormalities^{99,100} and that platelets play a crucial role in the recruitment of inflammatory cells upon antigen exposure in allergic diseases including asthma, allergic rhinitis and eczema.¹⁰¹ We think that involvement of platelets in the induction or exacerbation processes of airway inflammation may be dependent on CD69-Myl9 system, at least in part.

3.5 | Role of the CD69-Myl9 system in immune responses

The first step of immune responses is the activation and recruitment of innate immune cells to inflammatory tissues through inflammatory vessels, APCs then capture antigens and migrate into regional LNs where they present these antigens to T cells. Antigen-specific naïve T cells that recognize antigens are activated, differentiate into effector T cells and eventually move to inflamed sites via the blood stream.^{102–105} During the activation of T cells in LNs, they express substantial amounts of CD69 on their surface; however, these T cells need to down-regulate their CD69 expression for egress from the LN and migration into inflamed tissues via the blood stream.²³ This process seems to not fit with our CD69-Myl9 system in which CD69-expressing effector T cells must exist in the lung vasculature. To address this discrepancy, we performed intravascular staining, which allows staining of the cells that are only in the vasculature.¹⁰⁶ We found that CD69-expressing T cells were not detected in the systemic blood stream, but do exist in the vasculature in inflamed lung.⁴²

How do antigen-specific T cells re-express CD69 in the inflamed vasculature? One possibility is that the inflammatory cytokines are rich in small vessels in the inflamed lung, and the exposure to these cytokines may induce cell surface expression of CD69 on T cells in the small vessels. In fact, inflammatory cytokines such as IFN α/β , TGF β , TNF α and IL-33 can rapidly induce CD69 on T cells without antigen stimulation,^{107–112} suggesting the possible involvement of these inflammatory cytokines in the surface expression of CD69 on T cells in the inflamed lung vasculature. It is also reported that CD69 protein is present in the cytoplasm even in the resting T cells from human PBMCs.¹¹³ As some of the antigen-specific T cells in the inflamed lung vasculature are pre-activated by antigen stimulation in the regional LN, they may contain CD69 protein in the cytoplasm. Thus, antigen-specific T cells may upregulate cell surface expression of CD69 readily upon inflammatory stimulation; whereas naïve T cells have little CD69 protein in the cytoplasm so that they may induce very limited levels of CD69 surface expression in the inflammatory vessels. We think that Myl9 nets may transiently trap various kinds of cells; however, only pre-activated antigen-specific T cells can readily upregulate CD69 upon inflammatory stimulations so that they are able to tightly attach to Myl9 nets, and efficiently migrate into the inflamed tissues. Since naïve T cells do not express enough CD69, they may just pass through the Myl9 nets and be returned to circulation. A similar scenario has been reported in a study demonstrating that circulating CCR5⁺ resting memory CD8 T cells rapidly induce surface CCR5 expression upon inflammatory stimulation, and the CCR5 expression is crucial for the recruitment of these cells into lung tissues.¹¹⁴ An alternative

possibility is that there may exist MHC class II (MHC-II)-expressing cells in the inflamed vasculature that can induce T cell activation in the vessels. In fact, endothelial cells in human allografts appeared to express both MHC class I (MHC-I) and MHC-II, which may induce T cell activation.^{115–118} Although endothelial cells do not express CD80 or CD86,¹¹⁵ these costimulatory molecules are known to be unnecessary to induce cell surface expression of CD69. Furthermore, in addition to DCs and monocytes, eosinophils, basophils, neutrophils, mast cells and innate lymphocytes are known to express MHC-II,¹¹⁸ although their contribution to the induction of CD69 on T cells remains unknown and further investigation is required.

In summary, the CD69-Myl9 system is a novel system that regulates the pathogenesis of allergic airway inflammation by efficiently recruiting and maintaining inflammatory cells in the inflamed lung. Since Myl9 expression is observed in not only acute mouse allergic airway inflammation models but also in the polyps from patients with ECRS, a human chronic inflammatory disease, we think that the CD69-Myl9 system plays important roles in both acute and chronic inflammatory diseases.

3.6 | Pathogenic Th2 cells migrate into the inflammatory sites via the CD69-Myl9 system

Recently we have identified a subpopulation of memory Th2 cells that produces high amounts of IL-5 and appears to be responsible for the pathology of various Th2-type chronic inflammatory diseases, including asthma^{72,119}; the cells we call pathogenic Th2 (Tpath2) cells. Tpath2 cells are generated from memory Th2 cells by exposure to IL-33 produced by epithelial cells, endothelial cells and airway smooth muscle cells under inflammatory conditions, such as the lung in asthma patients.^{120,121} While these Tpath2 cells stay within structures called inducible bronchus-associated lymphoid tissue (iBALT) in the inflamed lung,⁵⁴ some Tpath2 cells seem to circulate and migrate into inflamed tissues upon antigen exposure. For instance, it is reported that CD161^{hi} CRTH2^{hi} IL-5-producing Tpath2 cells are detected in PBMCs of patients with eosinophilic gastrointestinal disease.¹²² We think that migration of Tpath2 cells into inflamed tissue is highly dependent on the CD69-Myl9 system. Furthermore, IL-5-producing Tpath2 cells

were readily detected in polyps from patients with ECRS.¹²⁰ As we described in the earlier section, Myl9 nets were detected inside of the blood vessels as well as at the perivascular area of blood vessels in the polyps from ECRS patients. We think that the Myl9 nets also play a role in maintenance of Tpath2 cells within inflamed tissues, leading to exacerbation and persistence of tissue inflammation.

4 | ROLES OF CD69 EXPRESSION IN T_{RM} CELLS AND BM MEMORY T CELLS

T_{RM} cells spend their life within specific tissues without circulation, and contribute to tissue homeostasis and barrier immunity by surveillance for secondary pathogen invasion.^{15–21} Since CD69 is expressed on T_{RM} cells, CD69 has been considered as a marker for T_{RM} cells^{15–21}; whereas a recent study has shown that a substantial proportion of T_{RM} cells in the pancreas, salivary glands and female reproductive tract of mice do not express CD69.^{18,123} These results suggest that CD69 is a good marker for T_{RM} cells in general, but not a perfect marker in some tissues.

How is CD69 expression induced and maintained on T_{RM} cells? Antigen-induced TCR stimulation is important for CD69 up-regulation on CD8 T cells.¹²⁴ Several studies using virus infection models such as influenza or vaccinia viruses indicated that, even after virus clearance, viral residual antigens exist in the local infection sites, which contributes to the expression of CD69 on CD8⁺ T_{RM} cells and promotes residency of both CD4⁺ and CD8⁺ T_{RM} cells.^{125–128} Furthermore, as we discussed in the earlier sections, several studies suggested that inflammatory cytokines such as IFN α/β , TGF β , TNF α and IL-33 can also induce CD69 on T cells.^{107–112} However, it remains unclear why CD69 expression is continuously expressed and if any signals are required for the long-term maintenance of CD69 expression on T_{RM} cells.

The importance of CD69 expression on CD8⁺ T_{RM} cells has been examined by using *Cd69*^{-/-} mice^{112,124,129,130} (Table 3). *Cd69*^{-/-} CD8 T cells were less well maintained in skin epidermis¹³⁰ or lungs,¹²⁴ demonstrating that CD69 expression is crucial for CD8⁺ T_{RM} cells residing in local tissues. Furthermore, S1P₁-overexpressing CD8 T cells

TABLE 3 Roles of CD69 on CD8⁺ T_{RM} cells and BM memory CD4 T cells

Experimental design	Methods	Tissue	Cells	Results	References
Influenza virus infection	<i>Cd69</i> ^{-/-} (a) mouse	Lung	CD8T	CD69 is required	Lee et al. ¹²⁴ Takamura et al. ¹²⁹
Herpes simplex virus (HSV) infection	<i>Cd69</i> ^{-/-} (b) mouse	Skin	CD8T	CD69 is required	Mackay et al. ¹³⁰
LCMV infection DNFB treatment	S1P ₁ introduction to CD8 T cells	Non-lymphoid organs (kidney, salivary gland, lamina propria, intestine intraepithelium, skin)	CD8T	CD69 is required	Skon et al. ¹¹²
OVA plus LPS immunization NP-KLH with alum immunization NP-CGG plus IFA immunization	<i>Cd69</i> ^{-/-} (a) mouse anti-CD69 (H1.2F3)Ab	Bone marrow	CD4T	CD69 is required	Shinoda et al. ⁸³

could not be retained in non-lymphoid tissues.¹¹² Since CD69 inhibits S1P₁ surface expression, as we discussed in the earlier section, these results suggest that the CD69-S1P₁ system is not only required for lymphocyte egress from lymphoid organs such as thymus and LN, but also required for the retention of CD8⁺ T_{RM} cells in non-lymphoid tissues. Interestingly, a recent study has indicated that the CD69-S1P₁ system is only required for the establishment of CD8⁺ T_{RM} cells in non-lymphoid tissues, but no longer required for long-term maintenance of CD8⁺ T_{RM} cells in the tissues at least in an infection model, indicating a limited requirement of the CD69-S1P₁ system for established T_{RM} cells in the tissues.¹²⁹ As we discussed in the earlier sections, Myl9 nets were abundantly detected inside of the blood vessels as well as perivascular regions in the polyps from ECRS patients. Together with the fact that T_{RM} cells express CD69, we think that, under inflammatory conditions such as asthma, the CD69-Myl9 system may contribute to the retention of pathogenic T_{RM} cells at inflamed sites, exacerbating inflammatory diseases. Thus, CD69 appears to be required for both the establishment and retention of CD8⁺ T_{RM} cells in non-lymphoid tissues; however, the precise molecular mechanisms by which CD69 is involved in each process of the establishment and retention of CD8⁺ T_{RM} cells in different models (ie, infection vs. inflammation) remain unknown. Moreover, little is known about the role for CD69 in CD4⁺ T_{RM} cells and further investigation is required.

In addition to CD8⁺ T_{RM} cells, resting memory CD4⁺ T cells in the BM require CD69 expression for their recruitment and maintenance in BM.^{83,131} (Table 3). It has been reported that, in an immune response, memory T cells preferentially reside and rest on stromal niches of the BM,^{131–134} suggesting that the BM works as a “reservoir” for long-lived memory T cells.^{131,135–137} Because we identified Myl9 in BM lysates as a CD69 ligand by pull-down assay using the murine CD69 extracellular domain,⁴² it is likely that the CD69-Myl9 system may also work in the recruitment and maintenance of memory CD4 T cells in the BM, although Myl9-expressing cells to recruit CD69⁺ cells into the BM should be identified. One possibility is megakaryocytes, which contain high amounts of Myl9,⁴² and are intimately associated with sinusoidal endothelial cells, the site of cellular exchange between the circulation and the BM.¹³³ We think that activated platelets detach from vessels within inflamed tissues as site-specific inflammation subsides, and the Myl9 nets resolve; some of the CD69-expressing antigen-specific T cells that do not enter or stay in the inflamed tissues may migrate into the BM to generate long-term pathogenic and resting memory. The systemic dynamics of CD69-expressing T cells in pathogenic memory should be further investigated.

5 | CD69 AS A POTENTIAL TARGET FOR THERAPEUTICS

Several humanized Abs are now undergoing clinical trials for intractable asthma patients.^{138–141} Although these trials are showing some encouraging results, some limitations have also been reported. As we discussed above, the interaction between CD69 and Myl9 appears to be a critical process in various inflammatory diseases. Therefore, blocking this interaction by either anti-CD69 or anti-Myl9/12 Abs could be

an effective therapeutic target for the intractable inflammatory diseases such as ECRS or steroid-resistant asthma. As we discussed earlier in this review, Myl9 protein expression becomes detectable in the lung upon inflammation but less detected under healthy conditions; therefore, anti-Myl9/12 Ab may work only within the inflamed tissues. Thus, treatment with anti-Myl9/12 Ab may have advantages in selective effects and smaller side effects as compared to other drugs, such as steroids. Myl9 expression is extremely high in platelets, which raises the question whether anti-Myl9/12 Ab can alter platelet function. However, we think this is unlikely, because Myl9 is only released from platelets upon activation, suggesting that anti-Myl9/12 Ab should not have any influence on platelets at steady state.

Anti-CD69 Ab could also be useful for the treatment of intractable asthma. All activated inflammatory cells in chronic inflammatory lesions are thought to express CD69 on their cell surface; therefore, anti-CD69 Ab can target all inflammatory cells and could result in strong and broad reductions in inflammatory responses. In fact, the majority of infiltrated T cells in the polyps of ECRS express CD69.⁵⁴ Because CD69 is also expressed on T_{RM} cells, anti-CD69 Ab may also impact on these cells. However, *Cd69*^{−/−} mice showed no detectable increases in spontaneous inflammation or infection when they are maintained under SPF conditions, and thus it is unlikely that severe defects in immune responses or autoimmune diseases occur after anti-CD69 Ab treatment, although further careful studies are still required. Finally, we want to point out that blocking the interaction between CD69 and Myl9 can be used not only for intractable asthma but also other intractable inflammatory diseases, including colitis, arthritis and atopic dermatitis.

6 | CONCLUDING REMARKS

In this review, we have summarized current knowledge of CD69 biology, especially how the CD69 molecules regulates inflammatory diseases such as airway inflammation. CD69 has been known to be crucial for the pathogenesis of various inflammatory diseases; however, the molecular mechanisms regulating how CD69 works was not investigated. Our recent discovery of Myl9/12 as a functional ligand for CD69 and the CD69-Myl9 system defines how CD69 regulates the pathogenesis of airway inflammation. As we discussed in this review, Myl9 is produced by platelets upon inflammation and creates Myl9 nets inside of blood vessels. We think that CD69-expressing inflammatory cells use Myl9 nets as a platform to migrate into inflamed tissues; the system we named the CD69-Myl9 system. Since Myl9 nets were also detected in the perivascular region in the nasal polyps of ECRS patients, we think that the CD69-Myl9 system works also in human chronic inflammation as a machinery to maintain inflammatory CD69-expressing cells inside the inflammatory lesions. As there are many reports showing the correlation between coagulation system function and the pathogenesis of inflammation, we think that the CD69-Myl9 system may play a role in a variety of inflammatory diseases. We have already made both anti-CD69 and anti-Myl9/12 humanized monoclonal Abs, and we think that both Abs could be new therapeutic tools not only for airway inflammatory disorders but also other inflammatory diseases.

ACKNOWLEDGEMENTS

We thank Drs. D. Tumes, K. Hirahara and A. Singer for helpful discussions and crucial reviewing this manuscript. The authors acknowledge grants from the Ministry of Education, Culture, Sports, Science and Technology, Japan: Grants-in Aid for Scientific Research (S) #26221305 (to T. N.), JSPS Fellows #2502883 (to K. H.), and Practical Research Project for Allergic Diseases and Immunology from Japan Agency for Medical Research and Development (AMED).

CONFLICT OF INTEREST

The authors have no conflicts of interest to disclose.

REFERENCES

- Hara T, Jung LK, Bjorn Dahl JM, Fu SM. Human T cell activation. III. Rapid induction of a phosphorylated 28 kD/32 kD disulfide-linked early activation antigen (EA 1) by 12-o-tetradecanoyl phorbol-13-acetate, mitogens, and antigens. *J Exp Med*. 1986;164:1988-2005.
- Lanier LL, Buck DW, Rhodes L, et al. Interleukin 2 activation of natural killer cells rapidly induces the expression and phosphorylation of the Leu-23 activation antigen. *J Exp Med*. 1988;167:1572-1585.
- Cosulich ME, Rubartelli A, Risso A, Cozzolino F, Bargellesi A. Functional characterization of an antigen involved in an early step of T-cell activation. *Proc Natl Acad Sci USA*. 1987;84:4205-4209.
- Cebrian M, Yague E, Rincon M, Lopez-Botet M, de Landazuri MO, Sanchez-Madrid F. Triggering of T cell proliferation through AIM, an activation inducer molecule expressed on activated human lymphocytes. *J Exp Med*. 1988;168:1621-1637.
- Ziegler SF, Ramsdell F, Hjerrild KA, et al. Molecular characterization of the early activation antigen CD69: a type II membrane glycoprotein related to a family of natural killer cell activation antigens. *Eur J Immunol*. 1993;23:1643-1648.
- Lopez-Cabrera M, Santis AG, Fernandez-Ruiz E, et al. Molecular cloning, expression, and chromosomal localization of the human earliest lymphocyte activation antigen AIM/CD69, a new member of the C-type animal lectin superfamily of signal-transmitting receptors. *J Exp Med*. 1993;178:537-547.
- Yokoyama WM, Koning F, Kehn PJ, et al. Characterization of a cell surface-expressed disulfide-linked dimer involved in murine T cell activation. *J Immunol*. 1988;141:369-376.
- Testi R, D'Ambrosio D, De Maria R, Santoni A. The CD69 receptor: A multipurpose cell-surface trigger for hematopoietic cells. *Immunol Today*. 1994;15:479-483.
- Ziegler SF, Levin SD, Johnson L, et al. The mouse CD69 gene. Structure, expression, and mapping to the NK gene complex. *J Immunol*. 1994;152:1228-1236.
- Llera AS, Viedma F, Sanchez-Madrid F, Tormo J. Crystal structure of the C-type lectin-like domain from the human hematopoietic cell receptor CD69. *J Biol Chem*. 2001;276:7312-7319.
- Sancho D, Gomez M, Sanchez-Madrid F. CD69 is an immunoregulatory molecule induced following activation. *Trends Immunol*. 2005;26:136-140.
- Wilson SB, Byrne MC. Gene expression in NKT cells: Defining a functionally distinct CD1d-restricted T cell subset. *Curr Opin Immunol*. 2001;13:555-561.
- Marzio R, Mauel J, Betz-Corradin S. CD69 and regulation of the immune function. *Immunopharmacol Immunotoxicol*. 1999;21:565-582.
- Montufar-Solis D, Garza T, Klein JR. T-cell activation in the intestinal mucosa. *Immunol Rev*. 2007;215:189-201.
- Mueller SN, Gebhardt T, Carbone FR, Heath WR. Memory T cell subsets, migration patterns, and tissue residence. *Annu Rev Immunol*. 2013;31:137-161.
- Shin H, Iwasaki A. Tissue-resident memory T cells. *Immunol Rev*. 2013;255:165-181.
- Fan X, Rudensky AY. Hallmarks of tissue-resident lymphocytes. *Cell*. 2016;164:1198-1211.
- Mueller SN, Mackay LK. Tissue-resident memory T cells: Local specialists in immune defence. *Nat Rev Immunol*. 2016;16:79-89.
- Schenkel JM, Masopust D. Tissue-resident memory T cells. *Immunity*. 2014;41:886-897.
- Mackay LK, Kallies A. Transcriptional regulation of tissue-resident lymphocytes. *Trends Immunol*. 2017;38:94-103.
- Thome JJ, Farber DL. Emerging concepts in tissue-resident T cells: Lessons from humans. *Trends Immunol*. 2015;36:428-435.
- Testi R, Pulcinelli F, Frati L, Gazzaniga PP, Santoni A. CD69 is expressed on platelets and mediates platelet activation and aggregation. *J Exp Med*. 1990;172:701-707.
- Cyster JG, Schwab SR. Sphingosine-1-phosphate and lymphocyte egress from lymphoid organs. *Annu Rev Immunol*. 2012;30:69-94.
- Rosen H, Stevens RC, Hanson M, Roberts E, Oldstone MB. Sphingosine-1-phosphate and its receptors: Structure, signaling, and influence. *Annu Rev Biochem*. 2013;82:637-662.
- Spiegel S, Milstien S. Sphingosine-1-phosphate: An enigmatic signalling lipid. *Nat Rev Mol Cell Biol*. 2003;4:397-407.
- Bankovich AJ, Shiow LR, Cyster JG. CD69 suppresses sphingosine 1-phosphate receptor-1 (S1P1) function through interaction with membrane helix 4. *J Biol Chem*. 2010;285:22328-22337.
- Shiow LR, Rosen DB, Brdickova N, et al. CD69 acts downstream of interferon-alpha/beta to inhibit S1P1 and lymphocyte egress from lymphoid organs. *Nature*. 2006;440:540-544.
- Matloubian M, Lo CG, Cinamon G, et al. Lymphocyte egress from thymus and peripheral lymphoid organs is dependent on S1P receptor 1. *Nature*. 2004;427:355-360.
- Allende ML, Dreier JL, Mandala S, Proia RL. Expression of the sphingosine 1-phosphate receptor, S1P1, on T-cells controls thymic emigration. *J Biol Chem*. 2004;279:15396-15401.
- Pappu R, Schwab SR, Cornelissen I, et al. Promotion of lymphocyte egress into blood and lymph by distinct sources of sphingosine-1-phosphate. *Science*. 2007;316:295-298.
- Yamashita I, Nagata T, Tada T, Nakayama T. CD69 cell surface expression identifies developing thymocytes which audition for T cell antigen receptor-mediated positive selection. *Int Immunol*. 1993;5:1139-1150.
- Schwab SR, Cyster JG. Finding a way out: Lymphocyte egress from lymphoid organs. *Nat Immunol*. 2007;8:1295-1301.
- Feng C, Woodside KJ, Vance BA, et al. A potential role for CD69 in thymocyte emigration. *Int Immunol*. 2002;14:535-544.
- Nakayama T, Kasprlowicz DJ, Yamashita M, et al. The generation of mature, single-positive thymocytes in vivo is dysregulated by CD69 blockade or overexpression. *J Immunol*. 2002;168:87-94.
- Hall JG, Morris B. The immediate effect of antigens on the cell output of a lymph node. *Br J Exp Pathol*. 1965;46:450-454.
- Grigoroza IL, Panteleev M, Cyster JG. Lymph node cortical sinus organization and relationship to lymphocyte egress dynamics and antigen exposure. *Proc Natl Acad Sci USA*. 2010;107:20447-20452.
- Gonzalez-Amaro R, Cortes JR, Sanchez-Madrid F, Martin P. Is CD69 an effective brake to control inflammatory diseases? *Trends Mol Med*. 2013;19:625-632.
- Martin P, Gomez M, Lamana A, et al. CD69 association with Jak3/Stat5 proteins regulates Th17 cell differentiation. *Mol Cell Biol*. 2010;30:4877-4889.
- Sanchez-Diaz R, Blanco-Dominguez R, Lasarte S, et al. Thymus-derived Treg cell development is regulated by C-type-lectin-mediated BIC/miRNA155 expression. *Mol Cell Biol*. 2017;37:e00341-16.

40. Lin CR, Wei TY, Tsai HY, Wu YT, Wu PY, Chen ST. Glycosylation-dependent interaction between CD69 and S100A8/S100A9 complex is required for regulatory T-cell differentiation. *FASEB J*. 2015;29:5006-5017.
41. de la Fuente H, Cruz-Adalia A, Martinez Del Hoyo G, et al. The leukocyte activation receptor CD69 controls T cell differentiation through its interaction with galectin-1. *Mol Cell Biol*. 2014;34:2479-2487.
42. Hayashizaki K, Kimura MY, Tokoyoda K, et al. Myosin light chains 9 and 12 are functional ligands for CD69 that regulate airway inflammation. *Sci Immunol*. 2016;1:eaaf9154.
43. Mendez-Huergo SP, Blidner AG, Rabinovich GA. Galectins: Emerging regulatory checkpoints linking tumor immunity and angiogenesis. *Curr Opin Immunol*. 2017;45:8-15.
44. Stillman BN, Hsu DK, Pang M, et al. Galectin-3 and galectin-1 bind distinct cell surface glycoprotein receptors to induce T cell death. *J Immunol*. 2006;176:778-789.
45. Starossom SC, Mascanfroni ID, Imitola J, et al. Galectin-1 deactivates classically activated microglia and protects from inflammation-induced neurodegeneration. *Immunity*. 2012;37:249-263.
46. Bonzi J, Bornet O, Betzi S, et al. Pre-B cell receptor binding to galectin-1 modifies galectin-1/carbohydrate affinity to modulate specific galectin-1/glycan lattice interactions. *Nat Commun*. 2015;6:6194.
47. Gebhardt C, Nemeth J, Angel P, Hess J. S100A8 and S100A9 in inflammation and cancer. *Biochem Pharmacol*. 2006;72:1622-1631.
48. Hartnell A, Robinson DS, Kay AB, Wardlaw AJ. CD69 is expressed by human eosinophils activated in vivo in asthma and in vitro by cytokines. *Immunology*. 1993;80:281-286.
49. Julius P, Luttmann W, Knoechel B, Kroegel C, Matthys H, Virchow JC Jr. CD69 surface expression on human lung eosinophils after segmental allergen provocation. *Eur Respir J*. 1999;13:1253-1259.
50. Lourenco O, Mafalda Fonseca A, Taborda-Barata L. T cells in sputum of asthmatic patients are activated independently of disease severity or control. *Allergol Immunopathol (Madr)*. 2009;37:285-292.
51. Nishikawa K, Morii T, Ako H, Hamada K, Saito S, Narita N. In vivo expression of CD69 on lung eosinophils in eosinophilic pneumonia: CD69 as a possible activation marker for eosinophils. *J Allergy Clin Immunol*. 1992;90:169-174.
52. Freeman CM, Han MK, Martinez FJ, et al. Cytotoxic potential of lung CD8(+) T cells increases with chronic obstructive pulmonary disease severity and with in vitro stimulation by IL-18 or IL-15. *J Immunol*. 2010;184:6504-6513.
53. Ekberg-Jansson A, Andersson B, Avra E, Nilsson O, Lofdahl CG. The expression of lymphocyte surface antigens in bronchial biopsies, bronchoalveolar lavage cells and blood cells in healthy smoking and never-smoking men, 60 years old. *Respir Med*. 2000;94:264-272.
54. Shinoda K, Hirahara K, Iinuma T, et al. Thy1⁺IL-7⁺ lymphatic endothelial cells in iBALT provide a survival niche for memory T-helper cells in allergic airway inflammation. *Proc Natl Acad Sci USA*. 2016;113:E2842-E2851.
55. Laffon A, Garcia-Vicuna R, Humbria A, et al. Upregulated expression and function of VLA-4 fibronectin receptors on human activated T cells in rheumatoid arthritis. *J Clin Invest*. 1991;88:546-552.
56. Afeltra A, Galeazzi M, Ferri GM, et al. Expression of CD69 antigen on synovial fluid T cells in patients with rheumatoid arthritis and other chronic synovitis. *Ann Rheum Dis*. 1993;52:457-460.
57. Rollin R, Marco F, Jover JA, et al. Early lymphocyte activation in the synovial microenvironment in patients with osteoarthritis: Comparison with rheumatoid arthritis patients and healthy controls. *Rheumatol Int*. 2008;28:757-764.
58. Wahlstrom J, Berlin M, Skold CM, Wigzell H, Eklund A, Grunewald J. Phenotypic analysis of lymphocytes and monocytes/macrophages in peripheral blood and bronchoalveolar lavage fluid from patients with pulmonary sarcoidosis. *Thorax*. 1999;54:339-346.
59. Toma T, Mizuno K, Okamoto H, et al. Expansion of activated eosinophils in infants with severe atopic dermatitis. *Pediatr Int*. 2005;47:32-38.
60. Hosono M, de Boer OJ, van der Wal AC, et al. Increased expression of T cell activation markers (CD25, CD26, CD40L and CD69) in atherectomy specimens of patients with unstable angina and acute myocardial infarction. *Atherosclerosis*. 2003;168:73-80.
61. Radstake TR, van Bon L, Broen J, et al. The pronounced Th17 profile in systemic sclerosis (SSc) together with intracellular expression of TGFbeta and IFNgamma distinguishes SSc phenotypes. *PLoS ONE*. 2009;4:e5903.
62. Radstake TR, van Bon L, Broen J, et al. Increased frequency and compromised function of T regulatory cells in systemic sclerosis (SSc) is related to a diminished CD69 and TGFbeta expression. *PLoS ONE*. 2009;4:e5981.
63. Perrella O, Carrieri PB, De Mercato R, Buscaino GA. Markers of activated T lymphocytes and T cell receptor gamma/delta⁺ in patients with multiple sclerosis. *Eur Neurol*. 1993;33:152-155.
64. Hervier B, Beziat V, Haroche J, et al. Phenotype and function of natural killer cells in systemic lupus erythematosus: Excess interferon-gamma production in patients with active disease. *Arthritis Rheum*. 2011;63:1698-1706.
65. Abdulahad WH, Stegeman CA, Limburg PC, Kallenberg CG. Skewed distribution of Th17 lymphocytes in patients with Wegener's granulomatosis in remission. *Arthritis Rheum*. 2008;58:2196-2205.
66. Matsuya N, Komori M, Nomura K, et al. Increased T-cell immunity against aquaporin-4 and proteolipid protein in neuromyelitis optica. *Int Immunol*. 2011;23:565-573.
67. Gessi A, Waldhauf W. Elevated CD69 expression on naive peripheral blood T-cells in hyperthyroid Graves' disease and autoimmune thyroiditis: Discordant effect of methimazole on HLA-DR and CD69. *Clin Immunol Immunopathol*. 1998;87:168-175.
68. Marazuela M, Postigo AA, Acevedo A, Diaz-Gonzalez F, Sanchez-Madrid F, de Landazuri MO. Adhesion molecules from the LFA-1/ICAM-1,3 and VLA-4/VCAM-1 pathways on T lymphocytes and vascular endothelium in Graves' and Hashimoto's thyroid glands. *Eur J Immunol*. 1994;24:2483-2490.
69. Murata K, Inami M, Hasegawa A, et al. CD69-null mice protected from arthritis induced with anti-type II collagen antibodies. *Int Immunol*. 2003;15:987-992.
70. Miki-Hosokawa T, Hasegawa A, Iwamura C, et al. CD69 controls the pathogenesis of allergic airway inflammation. *J Immunol*. 2009;183:8203-8215.
71. Wang HY, Dai Y, Wang JL, Yang XY, Jiang XG. Anti-CD69 monoclonal antibody treatment inhibits airway inflammation in a mouse model of asthma. *J Zhejiang Univ Sci B*. 2015;16:622-631.
72. Nakayama T, Hirahara K, Onodera A, et al. Th2 cells in health and disease. *Annu Rev Immunol*. 2017;35:53-84.
73. Hasegawa A, Iwamura C, Kitajima M, et al. Crucial role for CD69 in the pathogenesis of dextran sulphate sodium-induced colitis. *PLoS ONE*. 2013;8:e65494.
74. Tsuyusaki J, Kuroda F, Kasuya Y, et al. Cigarette smoke-induced pulmonary inflammation is attenuated in CD69-deficient mice. *J Recept Signal Transduct Res*. 2011;31:434-439.
75. Yamauchi K, Kasuya Y, Kuroda F, et al. Attenuation of lung inflammation and fibrosis in CD69-deficient mice after intratracheal bleomycin. *Respir Res*. 2011;12:131.
76. Lauzurica P, Sancho D, Torres M, et al. Phenotypic and functional characteristics of hematopoietic cell lineages in CD69-deficient mice. *Blood*. 2000;95:2312-2320.
77. Martin P, Gomez M, Lamana A, et al. The leukocyte activation antigen CD69 limits allergic asthma and skin contact hypersensitivity. *J Allergy Clin Immunol*. 2010;126:355-365, 365 e351-353.
78. Lamana A, Sancho D, Cruz-Adalia A, et al. The role of CD69 in acute neutrophil-mediated inflammation. *Eur J Immunol*. 2006;36:2632-2638.

79. Hooper LV, Littman DR, Macpherson AJ. Interactions between the microbiota and the immune system. *Science*. 2012;336:1268-1273.
80. Honda K, Littman DR. The microbiota in adaptive immune homeostasis and disease. *Nature*. 2016;535:75-84.
81. Sancho D, Gomez M, Martinez Del Hoyo G, et al. CD69 targeting differentially affects the course of collagen-induced arthritis. *J Leukoc Biol*. 2006;80:1233-1241.
82. Esplugues E, Vega-Ramos J, Cartoixa D, et al. Induction of tumor NK-cell immunity by anti-CD69 antibody therapy. *Blood*. 2005;105:4399-4406.
83. Shinoda K, Tokoyoda K, Hanazawa A, et al. Type II membrane protein CD69 regulates the formation of resting T-helper memory. *Proc Natl Acad Sci USA*. 2012;109:7409-7414.
84. Cortes JR, Sanchez-Diaz R, Bovolenta ER, et al. Maintenance of immune tolerance by Foxp3⁺ regulatory T cells requires CD69 expression. *J Autoimmun*. 2014;55:51-62.
85. Radulovic K, Manta C, Rossini V, et al. CD69 regulates type I IFN-induced tolerogenic signals to mucosal CD4 T cells that attenuate their colitogenic potential. *J Immunol*. 2012;188:2001-2013.
86. Radulovic K, Rossini V, Manta C, Holzmann K, Kestler HA, Niess JH. The early activation marker CD69 regulates the expression of chemokines and CD4 T cell accumulation in intestine. *PLoS ONE*. 2013;8:e65413.
87. Esplugues E, Sancho D, Vega-Ramos J, et al. Enhanced antitumor immunity in mice deficient in CD69. *J Exp Med*. 2003;197:1093-1106.
88. Vicente-Manzanares M, Ma X, Adelstein RS, Horwitz AR. Non-muscle myosin II takes centre stage in cell adhesion and migration. *Nat Rev Mol Cell Biol*. 2009;10:778-790.
89. Nakayama T, Yoshikawa M, Asaka D, et al. Mucosal eosinophilia and recurrence of nasal polyps—New classification of chronic rhinosinusitis. *Rhinology*. 2011;49:392-396.
90. Hamilos DL. Chronic rhinosinusitis: Epidemiology and medical management. *J Allergy Clin Immunol*. 2011;128:693-707; quiz 708-699.
91. Van Bruaene N, Perez-Novo CA, Basinski TM, et al. T-cell regulation in chronic paranasal sinus disease. *J Allergy Clin Immunol*. 2008;121:1435-1441, 1441 e1431-1433.
92. Stevens WW, Lee RJ, Schleimer RP, Cohen NA. Chronic rhinosinusitis pathogenesis. *J Allergy Clin Immunol*. 2015;136:1442-1453.
93. Hulse KE. Immune mechanisms of chronic rhinosinusitis. *Curr Allergy Asthma Rep*. 2016;16:1.
94. Gilles L, Bluteau D, Boukour S, et al. MAL/SRF complex is involved in platelet formation and megakaryocyte migration by regulating MYL9 (MLC2) and MMP9. *Blood*. 2009;114:4221-4232.
95. Seri M, Cusano R, Gangarossa S, et al. Mutations in MYH9 result in the May-Hegglin anomaly, and Fechtner and Sebastian syndromes. The May-Hegglin/Fechtner Syndrome Consortium. *Nat Genet*. 2000;26:103-105.
96. Garcia BA, Smalley DM, Cho H, Shabanowitz J, Ley K, Hunt DF. The platelet microparticle proteome. *J Proteome Res*. 2005;4:1516-1521.
97. Zelaya H, Alvarez S, Kitazawa H, Villena J. Respiratory antiviral immunity and immunobiotics: Beneficial effects on inflammation-coagulation interaction during influenza virus infection. *Front Immunol*. 2016;7:633.
98. Kim DY, Cho SH, Takabayashi T, Schleimer RP. Chronic rhinosinusitis and the coagulation system. *Allergy Asthma Immunol Res*. 2015;7:421-430.
99. Caspary EA, Comaish JS. Release of serotonin from human platelets in hypersensitivity states. *Nature*. 1967;214:286-287.
100. Pitchford SC, Page CP. Platelet activation in asthma: Integral to the inflammatory response. *Clin Exp Allergy*. 2006;36:399-401.
101. Page C, Pitchford S. Platelets and allergic inflammation. *Clin Exp Allergy*. 2014;44:901-913.
102. von Andrian UH, Mempel TR. Homing and cellular traffic in lymph nodes. *Nat Rev Immunol*. 2003;3:867-878.
103. Griffith JW, Sokol CL, Luster AD. Chemokines and chemokine receptors: Positioning cells for host defense and immunity. *Annu Rev Immunol*. 2014;32:659-702.
104. Krummel MF, Bartumeus F, Gerard A. T cell migration, search strategies and mechanisms. *Nat Rev Immunol*. 2016;16:193-201.
105. Hasegawa A, Hayashi K, Kishimoto H, et al. Color-coded real-time cellular imaging of lung T-lymphocyte accumulation and focus formation in a mouse asthma model. *J Allergy Clin Immunol*. 2010;125:461-468 e466.
106. Anderson KG, Mayer-Barber K, Sung H, et al. Intravascular staining for discrimination of vascular and tissue leukocytes. *Nat Protoc*. 2014;9:209-222.
107. Ledgerwood LG, Lal G, Zhang N, et al. The sphingosine 1-phosphate receptor 1 causes tissue retention by inhibiting the entry of peripheral tissue T lymphocytes into afferent lymphatics. *Nat Immunol*. 2008;9:42-53.
108. Casey KA, Fraser KA, Schenkel JM, et al. Antigen-independent differentiation and maintenance of effector-like resident memory T cells in tissues. *J Immunol*. 2012;188:4866-4875.
109. Takamura S, Roberts AD, Jelley-Gibbs DM, Wittmer ST, Kohlmeier JE, Woodland DL. The route of priming influences the ability of respiratory virus-specific memory CD8⁺ T cells to be activated by residual antigen. *J Exp Med*. 2010;207:1153-1160.
110. Kohlmeier JE, Miller SC, Woodland DL. Cutting edge: Antigen is not required for the activation and maintenance of virus-specific memory CD8⁺ T cells in the lung airways. *J Immunol*. 2007;178:4721-4725.
111. Lopez-Cabrera M, Munoz E, Blazquez MV, Ursa MA, Santis AG, Sanchez-Madrid F. Transcriptional regulation of the gene encoding the human C-type lectin leukocyte receptor AIM/CD69 and functional characterization of its tumor necrosis factor- α -responsive elements. *J Biol Chem*. 1995;270:21545-21551.
112. Skon CN, Lee JY, Anderson KG, Masopust D, Hogquist KA, Jameson SC. Transcriptional downregulation of S1pr1 is required for the establishment of resident memory CD8⁺ T cells. *Nat Immunol*. 2013;14:1285-1293.
113. Risso A, Smilovich D, Capra MC, et al. CD69 in resting and activated T lymphocytes. Its association with a GTP binding protein and biochemical requirements for its expression. *J Immunol*. 1991;146:4105-4114.
114. Kohlmeier JE, Miller SC, Smith J, et al. The chemokine receptor CCR5 plays a key role in the early memory CD8⁺ T cell response to respiratory virus infections. *Immunity*. 2008;29:101-113.
115. Hughes CC, Savage CO, Pober JS. Endothelial cells augment T cell interleukin 2 production by a contact-dependent mechanism involving CD2/LFA-3 interaction. *J Exp Med*. 1990;171:1453-1467.
116. Shiao SL, McNiff JM, Pober JS. Memory T cells and their costimulators in human allograft injury. *J Immunol*. 2005;175:4886-4896.
117. Shiao SL, Kirkiles-Smith NC, Shepherd BR, McNiff JM, Carr EJ, Pober JS. Human effector memory CD4⁺ T cells directly recognize allogeneic endothelial cells in vitro and in vivo. *J Immunol*. 2007;179:4397-4404.
118. Kambayashi T, Laufer TM. Atypical MHC class II-expressing antigen-presenting cells: Can anything replace a dendritic cell? *Nat Rev Immunol*. 2014;14:719-730.
119. Endo Y, Iwamura C, Kuwahara M, et al. Eomesodermin controls interleukin-5 production in memory T helper 2 cells through inhibition of activity of the transcription factor GATA3. *Immunity*. 2011;35:733-745.
120. Endo Y, Hirahara K, Iinuma T, et al. The interleukin-33-p38 kinase axis confers memory T helper 2 cell pathogenicity in the airway. *Immunity*. 2015;42:294-308.
121. Molofsky AB, Savage AK, Locksley RM. Interleukin-33 in tissue homeostasis, injury, and inflammation. *Immunity*. 2015;42:1005-1019.

122. Mitson-Salazar A, Yin Y, Wansley DL, et al. Hematopoietic prostaglandin D synthase defines a proeosinophilic pathogenic effector human T(H)2 cell subpopulation with enhanced function. *J Allergy Clin Immunol*. 2016;137:907-918 e909.
123. Steinert EM, Schenkel JM, Fraser KA, et al. Quantifying memory CD8 T cells reveals regionalization of immunosurveillance. *Cell*. 2015;161:737-749.
124. Lee YT, Suarez-Ramirez JE, Wu T, et al. Environmental and antigen receptor-derived signals support sustained surveillance of the lungs by pathogen-specific cytotoxic T lymphocytes. *J Virol*. 2011;85:4085-4094.
125. Zammit DJ, Turner DL, Klonowski KD, Lefrancois L, Cauley LS. Residual antigen presentation after influenza virus infection affects CD8 T cell activation and migration. *Immunity*. 2006;24:439-449.
126. Kim TS, Hufford MM, Sun J, Fu YX, Braciale TJ. Antigen persistence and the control of local T cell memory by migrant respiratory dendritic cells after acute virus infection. *J Exp Med*. 2010;207:1161-1172.
127. Khan TN, Mooster JL, Kilgore AM, Osborn JF, Nolz JC. Local antigen in nonlymphoid tissue promotes resident memory CD8⁺ T cell formation during viral infection. *J Exp Med*. 2016;213:951-966.
128. Jelley-Gibbs DM, Brown DM, Dibble JP, Haynes L, Eaton SM, Swain SL. Unexpected prolonged presentation of influenza antigens promotes CD4 T cell memory generation. *J Exp Med*. 2005;202:697-706.
129. Takamura S, Yagi H, Hakata Y, et al. Specific niches for lung-resident memory CD8⁺ T cells at the site of tissue regeneration enable CD69-independent maintenance. *J Exp Med*. 2016;213:3057-3073.
130. Mackay LK, Rahimpour A, Ma JZ, et al. The developmental pathway for CD103(+)CD8⁺ tissue-resident memory T cells of skin. *Nat Immunol*. 2013;14:1294-1301.
131. Di Rosa F, Gebhardt T. Bone marrow T cells and the integrated functions of recirculating and tissue-resident memory T cells. *Front Immunol*. 2016;7:51.
132. Tokoyoda K, Zehentmeier S, Hegazy AN, et al. Professional memory CD4⁺ T lymphocytes preferentially reside and rest in the bone marrow. *Immunity*. 2009;30:721-730.
133. Tokoyoda K, Hauser AE, Nakayama T, Radbruch A. Organization of immunological memory by bone marrow stroma. *Nat Rev Immunol*. 2010;10:193-200.
134. Sercan Alp O, Durlanik S, Schulz D, et al. Memory CD8(+) T cells colocalize with IL-7(+) stromal cells in bone marrow and rest in terms of proliferation and transcription. *Eur J Immunol*. 2015;45:975-987.
135. Mazo IB, Honczarenko M, Leung H, et al. Bone marrow is a major reservoir and site of recruitment for central memory CD8⁺ T cells. *Immunity*. 2005;22:259-270.
136. Zhang X, Dong H, Lin W, et al. Human bone marrow: A reservoir for "enhanced effector memory" CD8⁺ T cells with potent recall function. *J Immunol*. 2006;177:6730-6737.
137. Akatsuka Y, Torikai H, Inamoto Y, et al. Bone marrow may be a reservoir of long-lived memory T cells specific for minor histocompatibility antigen. *Br J Haematol*. 2006;135:413-414.
138. Pelaia G, Vatrella A, Maselli R. The potential of biologics for the treatment of asthma. *Nat Rev Drug Discov*. 2012;11:958-972.
139. Rothenberg ME. Humanized anti-IL-5 antibody therapy. *Cell*. 2016;165:509.
140. Bel EH, Wenzel SE, Thompson PJ, et al. Oral glucocorticoid-sparing effect of mepolizumab in eosinophilic asthma. *N Engl J Med*. 2014;371:1189-1197.
141. Castro M, Zangrilli J, Wechsler ME, et al. Reslizumab for inadequately controlled asthma with elevated blood eosinophil counts: Results from two multicentre, parallel, double-blind, randomised, placebo-controlled, phase 3 trials. *Lancet Respir Med*. 2015;3:355-366.
142. Sancho D, Gomez M, Viedma F, et al. CD69 downregulates autoimmune reactivity through active transforming growth factor-beta production in collagen-induced arthritis. *J Clin Invest*. 2003;112:872-882.
143. Vega-Ramos J, Alari-Pahissa E, Valle JD, et al. CD69 limits early inflammatory diseases associated with immune response to *Listeria monocytogenes* infection. *Immunol Cell Biol*. 2010;88:707-715.

How to cite this article: Kimura MY, Hayashizaki K, Tokoyoda K, Takamura S, Motohashi S, Nakayama T. Crucial role for CD69 in allergic inflammatory responses: CD69-Myl9 system in the pathogenesis of airway inflammation. *Immunol Rev*. 2017;278:87-100. <https://doi.org/10.1111/imr.12559>