

Original Article



Serosal Cavities Contain Two Populations of Innate-like Integrin $\alpha 4^{\text{high}}\text{CD4}^+$ T Cells, Integrin $\alpha 4\beta 1^+\alpha 6\beta 1^+\alpha 4\beta 7^-$ and $\alpha 4\beta 1^+\alpha 6\beta 1^-\alpha 4\beta 7^+$ Cells

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Received: Sep 28, 2017

Revised: Nov 3, 2017

Accepted: Nov 7, 2017

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Conflict of Interest

The authors declare no potential conflicts of interest.

Abbreviations

IFN, interferon; PEC, peritoneal cavity; PLC, pleural cavity; TNF, tumor necrosis factor

ABSTRACT

We previously reported peritoneal innate-like integrin $\alpha 4$ (CD49d)^{high}CD4⁺ T cells that provided help for B-1a cells. Here we analyzed the expression of various integrin chains on the peritoneal and pleural integrin $\alpha 4^{\text{high}}\text{CD4}^+$ T cells and investigated the functional heterogeneity of the subpopulations based on the integrin expression. Pleural cavity contained a lower ratio of integrin $\alpha 4^{\text{high}}\text{CD4}^+$ T cells to integrin $\alpha 4^{\text{low}}\text{CD4}^+$ T cells than peritoneal cavity, but the pleural integrin $\alpha 4^{\text{high}}\text{CD4}^+$ T cells have the same characteristics of the peritoneal integrin $\alpha 4^{\text{high}}\text{CD4}^+$ T cells. Most of integrin $\alpha 4^{\text{high}}\text{CD4}^+$ T cells were integrin $\beta 1^{\text{high}}\beta 7^-$, but a minor population of integrin $\alpha 4^{\text{high}}\text{CD4}^+$ T cells was integrin $\beta 1^+\beta 7^+$. Interestingly, the integrin $\alpha 4^{\text{high}}\beta 1^{\text{high}}\beta 7^-$ CD4⁺ T cells expressed high levels of integrin $\alpha 4\beta 1$ and $\alpha 6\beta 1$, whereas integrin $\alpha 4^{\text{high}}\beta 1^+\beta 7^+$ CD4⁺ T cells expressed high levels of integrin $\alpha 4\beta 1$ and $\alpha 4\beta 7$, suggesting an alternative expression of integrin $\alpha 6\beta 1$ or $\alpha 4\beta 7$ in combination with $\alpha 4\beta 1$ in respective major and minor populations of integrin $\alpha 4^{\text{high}}\text{CD4}^+$ T cells. The minor population, integrin $\alpha 4^{\text{high}}\beta 1^+\beta 7^+$ CD4⁺ T cells, were different from the integrin $\alpha 4^{\text{high}}\beta 1^{\text{high}}\beta 7^-$ CD4⁺ T cells in that they secreted a smaller amount of Th1 cytokines upon stimulation and expressed lower levels of Th1-related chemokine receptors CCR5 and CXCR3 than the integrin $\alpha 4^{\text{high}}\beta 1^{\text{high}}\beta 7^-$ CD4⁺ T cells. In summary, the innate-like integrin $\alpha 4^{\text{high}}\text{CD4}^+$ T cells could be divided into 2 populations, integrin $\alpha 4\beta 1^+\alpha 6\beta 1^+\alpha 4\beta 7^-$ and $\alpha 4\beta 1^+\alpha 6\beta 1^-\alpha 4\beta 7^+$ cells. The functional significance of serosal integrin $\alpha 4\beta 7^+$ CD4⁺ T cells needed to be investigated especially in view of mucosal immunity.

Keywords: Peritoneal cavity; Pleural cavity; CD4-positive T-lymphocytes; Integrin alpha4; CD49d; Th1 cells; CXCR3 receptor; CCR5 receptor

Author Contributions

Conceptualization: Kim TJ; Data curation: Yang JI, Park C, Lee S; Formal analysis: Yang JI, Park C, Koh I; Funding acquisition: Kim TJ, Suh KS; Investigation: Kim TJ, Park C, Lee S; Methodology: Park C, Yang JI, Koh I; Project administration: Kim TJ, Suh KS; Resources: Yang JI, Lee S; Software: Yang JI; Supervision: Kim TJ; Validation: Koh I, Lee S; Visualization: Yang JI, Park C; Writing - original draft: Yang JI, Kim TJ; Writing - review & editing: Kim TJ, Suh KS.

INTRODUCTION

Confronted with various kinds of pathogens, serosal cavities harbor distinctive types of lymphocytes including innate-like B-1a cells as well as innate immune cells, such as mast cells, macrophages, and natural killer (NK) cells (1,2). Recently, we reported the abundance of innate-like CD4⁺ T cells in the peritoneal cavity (PEC), which characteristically expressed a high level of integrin $\alpha 4$ (CD49d) and rapidly secreted Th1 cytokines upon stimulation similarly to memory T cells (3). The innate-like integrin $\alpha 4^{\text{high}} \text{CD4}^+$ T cells developed very early before the age of 3 days and provided help for B-1a cells. These peritoneal innate-like B-1a and integrin $\alpha 4^{\text{high}} \text{CD4}^+$ T cells are presumed to be responsible for the immediate response to pathogenic invasion into the PEC that results from uncontrolled gastrointestinal infection. The pleural cavity (PLC) similarly harbors innate-like lymphocytes including B-1a cells that had previously described as innate response activator B cells (4), but the characteristics of PLC T cells are not well investigated.

Integrin $\alpha 4$ is an integrin chain that can combine with either of 2 β chains, $\beta 1$ (CD29) or $\beta 7$, to form integrin $\alpha 4\beta 1$ (VLA-4) or integrin $\alpha 4\beta 7$ (LPAM-1) heterodimers, respectively (5). Both integrin heterodimers are not highly expressed on naive lymphocytes, but highly on specific types of memory lymphocytes (6). Integrin $\alpha 4\beta 1$ or $\alpha 4\beta 7$ directs transendothelial migration into distinct anatomical sites; $\alpha 4\beta 1$ through binding to vascular cell adhesion protein 1 (VCAM-1) for the entry into inflammatory sites and $\alpha 4\beta 7$ through binding to mucosal vascular addressin cell adhesion molecule-1 (MAdCAM-1) for recirculation to sites of intestinal inflammation and intestinal secondary lymphoid tissues (7). In human memory T cells, the expression of $\alpha 4\beta 1$ is reciprocally correlated to the expression of $\alpha 4\beta 7$ (8). Therefore, the fine regulation of the integrin β chain expression is important for the preferential recruitment of memory lymphocytes and intestinal pathology (9,10).

On the other hand, integrin $\beta 1$ can combine with at least 12 α integrin chains to form different kinds of integrin combinations that bind to extracellular matrix proteins, such as $\alpha 1\beta 1$ (VLA-1), $\alpha 2\beta 1$ (VLA-2), $\alpha 3\beta 1$ (VLA-3), $\alpha 4\beta 1$ (VLA-4), $\alpha 5\beta 1$ (VLA-5), and $\alpha 6\beta 1$ (VLA-6) (11). Since the adhesive activity of these $\beta 1$ integrins is enhanced by T cell receptor (TCR)-mediated signaling, the expression of $\beta 1$ integrins is important for the function of memory/effector T cells that express a higher level of integrin $\beta 1$ than naïve T cells (12). What determines the kind of integrin α chain expressed in combination with integrin $\beta 1$ is not well understood, but the expression of different $\beta 1$ integrin complexes regulates their adhesive and migratory behavior of memory T cells (13-15).

In this study, we systemically analyzed the expression of various kinds of integrin chains in the serosal integrin $\alpha 4^{\text{high}} \text{CD4}^+$ T cells. We observed that most of them preferentially expressed the $\alpha 4\beta 1$ and $\alpha 6\beta 1$ integrins, but a minor population of integrin $\alpha 4^{\text{high}} \text{CD4}^+$ T cells expressed a high level of $\alpha 4\beta 7$ integrin. We further reanalyzed the functional differences of 2 kinds of serosal integrin $\alpha 4^{\text{high}} \text{CD4}^+$ T cells.

MATERIALS AND METHODS**Mice**

C57BL/6 mice were purchased from Orient Bio (Sunnam, Korea). *Cd1d*^{-/-} mice were kindly provided by L. Van Kaer (Vanderbilt University School of Medicine). This study was approved

by the Institutional Animal Care and Use Committee of Sungkyunkwan University School of Medicine. All procedures were performed in a pathogen-free facility, according to institutional guidelines.

Cell preparation and flow cytometric analysis

Peritoneal and pleural cells were isolated by flushing the serosal cavities with PBS. Cells were stained on ice for 30 min with the appropriate combinations of fluorochrome-conjugated Abs in FACS buffer (5% bovine calf serum [BCS] and 0.05% sodium azide in PBS). Following fluorochrome-labeled monoclonal antibodies were used: CXCR6 (221002), $\alpha 3$ (polyclonal), $\alpha 7$ (334908) from BD Biosciences (San Jose, CA, USA); CD4 (GK1.5), CD62L (MEL-14), CD44 (IM7), CXCR3 (CXCR3-173), CCR4 (2G12), CCR5 (HM-CCR5), PD-1 (29F.1A12), SLAM (TC15-12F12.2), CD122 (5H4), CD127 (A7R34), $\alpha 1$ (HMa1), $\alpha 2$ (DX5), $\alpha 5$ (5H10-27, MFR5), $\alpha 6$ (GoH3), $\beta 1$ (HM81-1), $\beta 7$ (FIB504), $\alpha 4\beta 7$ integrin (DATK32), interferon (IFN)- γ (XMG1.2), IL-10 (JES5-16E3), TGF- β (TW7-20B9), tumor necrosis factor (TNF)- α (MP6-XT22) from BioLegend (San Diego, CA, USA); and CXCR4 (2B11), CXCR5 (SPRCL5), CCR6 (Slrx6), CCR7 (4B12), ICOS (7E.17G9), $\alpha 4$ (R1-2), IL-4 (11B11) from eBioscience (San Diego, CA, USA). After washing with FACS buffer, the stained cells were analyzed on a FACSCanto II system (BD Biosciences). Data were analyzed using FlowJo software (Tree Star, San Carlos, CA, USA).

Intracellular staining for cytokines

Cells were suspended in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), 25 mM sodium bicarbonate, 2 mM glutamine, 50 U/ml penicillin, 50 mg/ml streptomycin, and 10 mM HEPES (all from Invitrogen Life Technologies, Carlsbad, CA, USA) and then stimulated with 50 ng/ml PMA (Sigma-Aldrich, St. Louis, MO, USA) and 1.5 mM ionomycin (Sigma-Aldrich) for 4 h. Brefeldin A (Sigma-Aldrich) was added to 10 μ g/ml during the last 3 h of stimulation. Cells were stained with anti-CD4, $\beta 1$, $\beta 7$, and anti- $\alpha 4$ Abs, fixed with 2% paraformaldehyde in PBS, permeabilized with 0.1% BSA/0.05% Triton X-100 in PBS, and stained with Abs against IFN- γ , IL-2, IL-4, IL-5, IL-10, IL-13, TGF- β , or TNF- α .

Statistical analysis

Student's t-test (unpaired) and 1-way or 2-way ANOVA tests were used to assess the statistical significance of differences between groups. The p-values <0.05 were considered to be statistically significant for all tests. Histograms were plotted using GraphPad Prism 4.0 (GraphPad Software, San Diego, CA, USA).

RESULTS

PLC contains integrin $\alpha 4^{\text{high}}$ CD4⁺ T cells with a memory phenotype similar to that of peritoneal integrin $\alpha 4^{\text{high}}$ CD4⁺ T cells

Since B-1a cells are abundant in PLC as well as PEC, we first investigated whether the integrin $\alpha 4^{\text{high}}$ CD4⁺ T cells are present in the PLC. The integrin $\alpha 4^{\text{high}}$ CD4⁺ T cells were also abundant in the PLC although their proportion among CD4⁺ T cells was smaller in the PLC than in the PEC (**Fig. 1**). As expected, the pleural integrin $\alpha 4^{\text{high}}$ CD4⁺ T cells cavity also showed the memory and pro-inflammatory phenotypes found in the peritoneal integrin $\alpha 4^{\text{high}}$ CD4⁺ T cells, which include a high expression of CD44, CXCR3, PD-1, ICOS, SLAM, CD122, and CD127, a low expression of CD62L, and a rapid secretion of IFN- γ upon stimulation (data not shown). These cells were not natural killer T (NKT) cells as they were present in *Cd1d*^{-/-} mice.

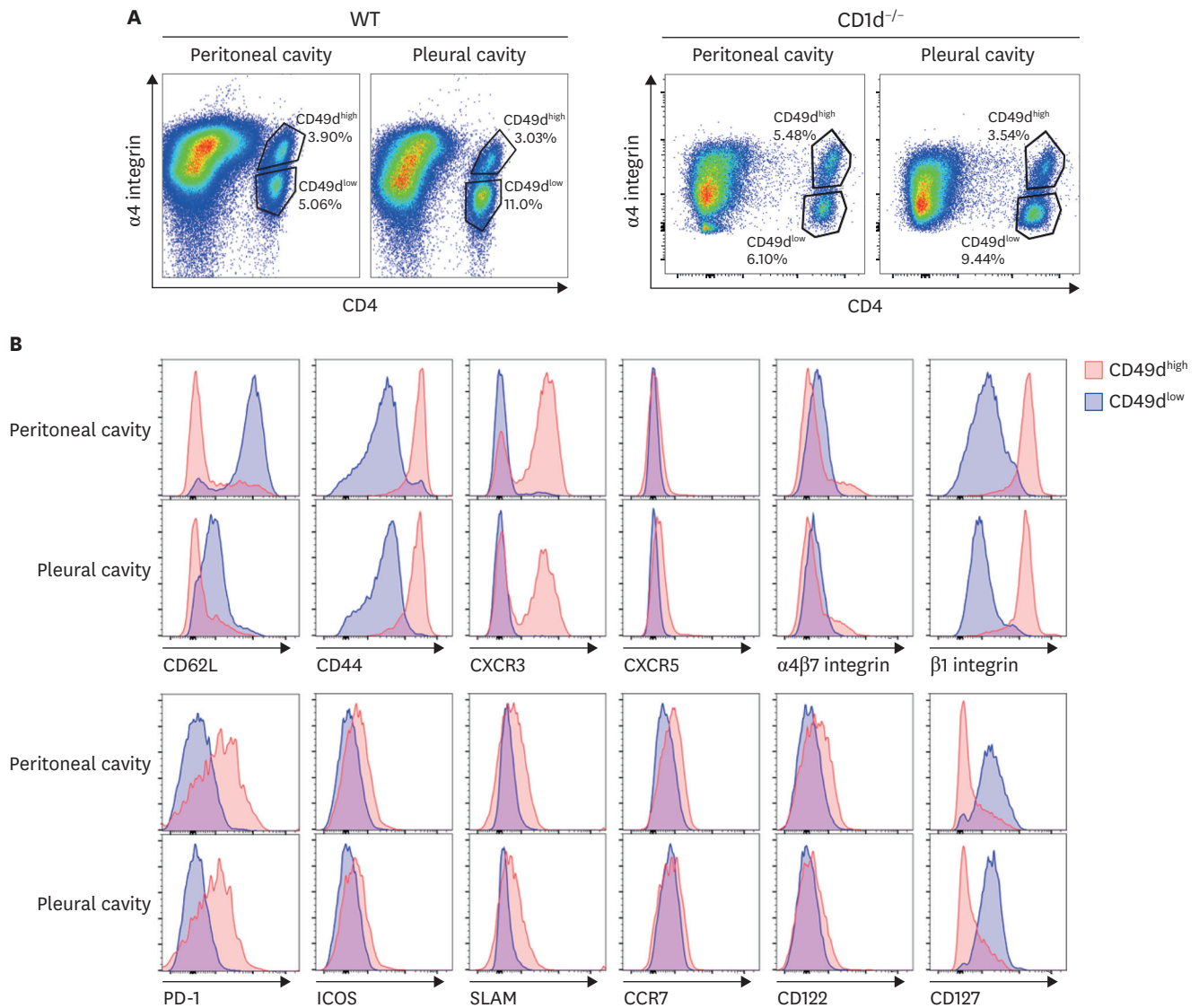


Figure 1. Expression of activation-related cell surface molecules in peritoneal and pleural integrin $\alpha 4^{\text{high}}$ CD4⁺ T cells. (A) PEC and PLC cells obtained from 8-week-old C57BL/6 WT or *CD1d*^{-/-} mice were examined for the expression of CD4 and integrin $\alpha 4$ (CD49d). (B) Expression of given cell surface proteins in gated peritoneal and pleural integrin $\alpha 4^{\text{high}}$ CD4⁺ T cells (red line, CD49d^{high}) or CD49d^{low} T cells (blue line, CD49d^{low}). Data are representative of 12 separate experiments. WT, wild type.

The serosal integrin $\alpha 4^{\text{high}}$ CD4⁺ T cells are divided into 2 populations based on the expression of $\alpha 6\beta 1$ or $\alpha 4\beta 7$ integrins

We next investigated the expression of integrin $\beta 1$ or $\beta 7$ chains in the serosal integrin $\alpha 4^{\text{high}}$ CD4⁺ T cells since the integrin $\alpha 4$ is known to heterodimerize with integrin $\beta 1$ or $\beta 7$. When we checked the expression of integrin $\beta 1$ or $\beta 7$ chains in serosal CD4⁺ T cells, the memory phenotype CD4⁺ T cells were recognized as integrin $\beta 1^+$ CD4⁺ T cells (Fig. 2A). The integrin $\beta 1$ CD4⁺ T cells were not found in the gated integrin $\alpha 4^{\text{high}}$ CD4⁺ T cells, which indicates that the integrin $\alpha 4\beta 1$ is a principal integrin complex in the serosal integrin $\alpha 4^{\text{high}}$ CD4⁺ T cells. Notably, a small population of integrin $\alpha 4^{\text{high}}\beta 1^+\beta 7^+$ CD4⁺ T cells was also observed. The level of the integrin $\beta 1$ expression is higher in the integrin $\alpha 4^{\text{high}}\beta 1^+\beta 7^+$ CD4⁺ T cells than in $\alpha 4^{\text{high}}\beta 1^+\beta 7^-$ CD4⁺ T cells. Therefore, we divided the integrin $\alpha 4^{\text{high}}$ CD4⁺ T cells into $\alpha 4^{\text{high}}\beta 1^{\text{high}}$ CD4⁺ and $\alpha 4^{\text{high}}\beta 1^+\beta 7^+$ CD4⁺ T cells. We presumed that some fraction of integrin

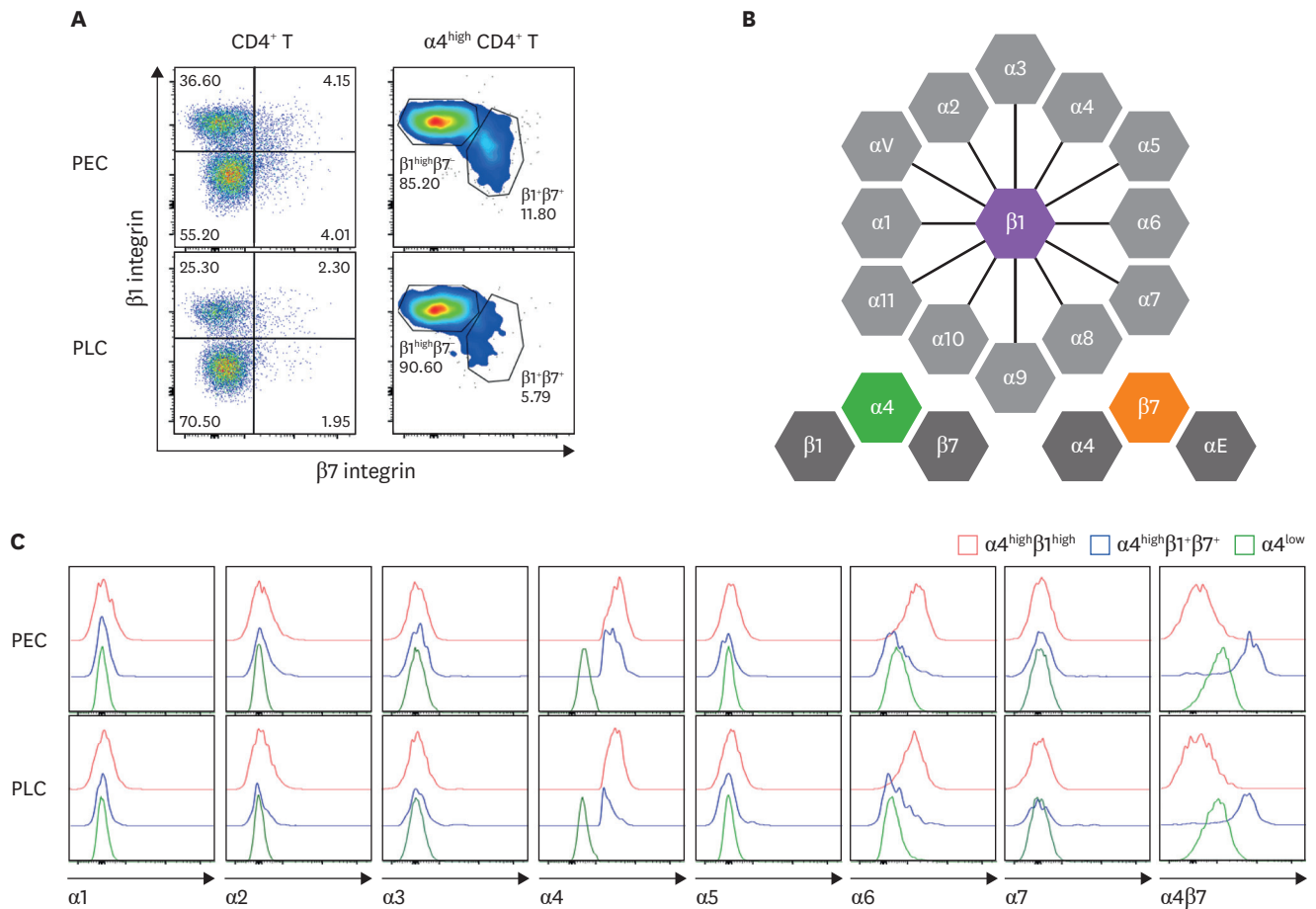


Figure 2. Serosal integrin $\alpha 4^{\text{high}}$ CD4⁺ T cells are divided into integrin $\alpha 4^{\text{high}}\beta 1^{\text{high}}$ CD4⁺ T and $\alpha 4^{\text{high}}\beta 1^+\beta 7^+$ CD4⁺ T cells. (A) PEC and PLC CD4⁺ T cells or gated integrin $\alpha 4^{\text{high}}$ CD4⁺ T cells obtained from 8-week-old C57BL/6 wild type mice were shown for the expression of integrin $\beta 1$ and $\beta 7$. (B) Illustration for integrin combinations of integrin $\alpha 4$, $\beta 1$, and $\beta 7$ chains. (C) Cell surface expression of various integrin α chains in integrin $\alpha 4^{\text{high}}\beta 1^{\text{high}}$ CD4⁺ T cells (red line, $\alpha 4^{\text{high}}\beta 1^{\text{high}}$), integrin $\alpha 4^{\text{high}}\beta 1^+\beta 7^+$ CD4⁺ T cells (blue line, $\alpha 4^{\text{high}}\beta 1^+\beta 7^+$), and integrin $\alpha 4^{\text{low}}$ CD4⁺ T cells (green line, $\alpha 4^{\text{low}}$). Data are representative of at least 3 separate experiments.

$\beta 1$ in the integrin $\alpha 4^{\text{high}}\beta 1^{\text{high}}$ CD4⁺ T cells might associate with some other integrin α chain(s) to form other $\beta 1$ integrin complexes.

In contrast to integrin $\alpha 4$ that associate with only 2 kinds of integrin β chains, the integrin $\beta 1$ chain can associate with many integrin α chains (**Fig. 2B**). To find additional integrin $\beta 1$ combination(s) in the integrin $\alpha 4^{\text{high}}\beta 1^{\text{high}}$ CD4⁺ T cells, we investigated the expression of individual integrin α chains in the 2 integrin $\alpha 4^{\text{high}}$ CD4⁺ T cell populations as well as integrin $\alpha 4^{\text{low}}$ CD4⁺ T cells (**Fig. 2C**). Notably, the high expression of integrin $\alpha 6$ is prominent only in the integrin $\alpha 4^{\text{high}}\beta 1^{\text{high}}$ CD4⁺ T cells, but not in the other populations. This result suggests that $\alpha 6\beta 1$ (VLA-6) is another inflammatory integrin chain expressed on the serosal integrin $\alpha 4^{\text{high}}\beta 1^{\text{high}}$ CD4⁺ T cells.

The serosal integrin $\alpha 4^{\text{high}}\beta 1^{\text{high}}$ CD4⁺ and $\alpha 4^{\text{high}}\beta 1^+\beta 7^+$ CD4⁺ T cells are distinct memory phenotype T cells

We addressed whether the integrin $\alpha 4^{\text{high}}\beta 1^{\text{high}}$ CD4⁺ and $\alpha 4^{\text{high}}\beta 1^+\beta 7^+$ CD4⁺ T cells were functionally distinct or not. We compared the expression of activation-related molecules in the 2 populations. Both populations showed the memory phenotype as shown by a high expression of CD44 and a low expression of CD62L and CCR7 (**Fig. 3**). However, the

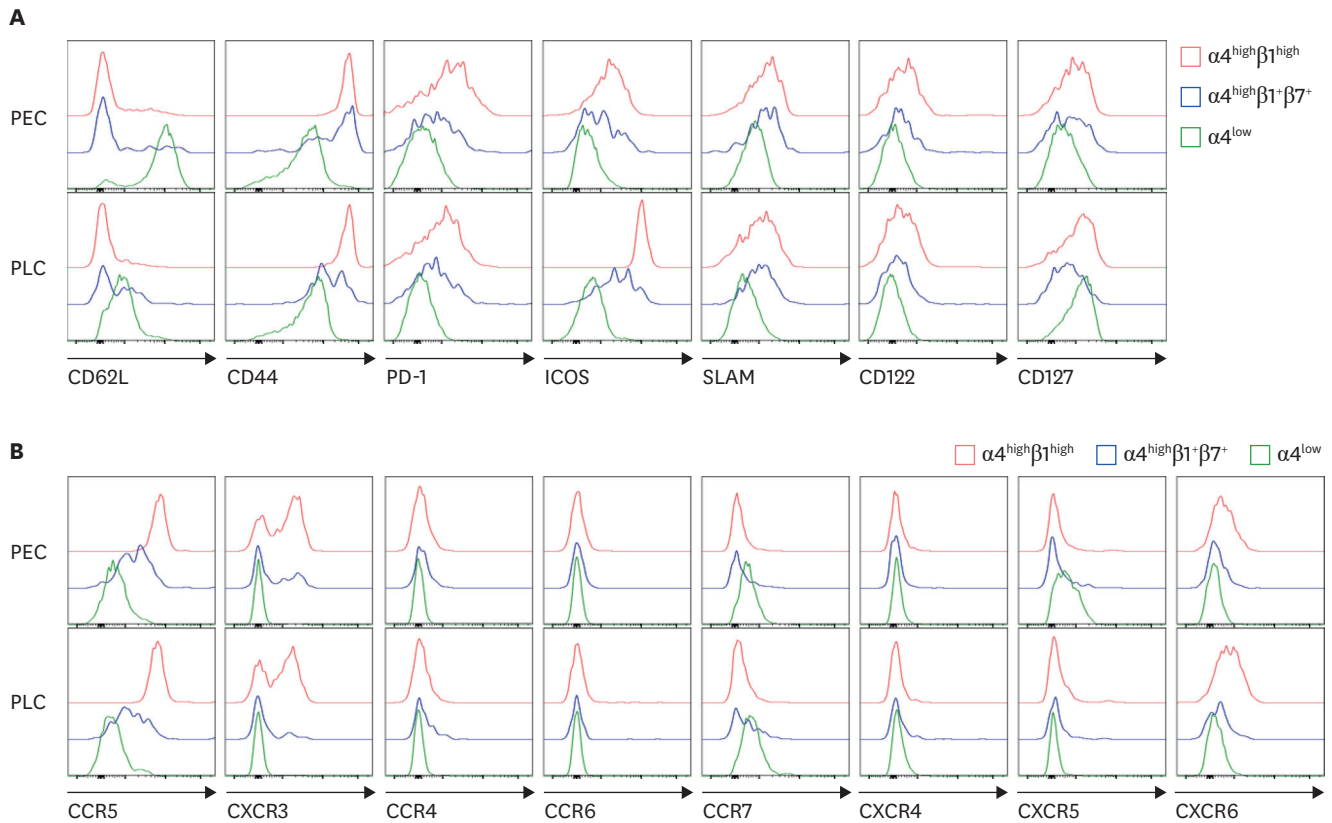


Figure 3. Comparison of various activation-related molecules (A) and chemokine receptors (B) among integrin $\alpha 4^{\text{high}}\beta 1^{\text{high}}\text{CD4}^+$ T cells (red line), integrin $\alpha 4^{\text{high}}\beta 1^+\beta 7^+\text{CD4}^+$ T cells (blue line), and integrin $\alpha 4^{\text{low}}\text{CD4}^+$ T cells (green line). PEC and PLC cells obtained from 8-week-old C57BL/6 mice were examined for the expression of indicated cell surface proteins. Data are representative of 12 separate experiments.

$\alpha 4^{\text{high}}\beta 1^+\beta 7^+\text{CD4}^+$ T cells expressed lower levels of CCR5 and CXCR3, 2 representative Th1 cell chemokine receptors (16,17). The expression of PD-1 and ICOS was also lower in the integrin $\beta 1^+\beta 7^+\text{CD4}^+$ T cells than in $\beta 1^{\text{high}}\text{CD4}^+$ T cells. Accordingly, integrin $\alpha 4^{\text{high}}\beta 1^+\beta 7^+\text{CD4}^+$ T cells secreted smaller amounts of Th1 cytokines such as IFN- γ and TNF- α than integrin $\alpha 4^{\text{high}}\beta 1^{\text{high}}\text{CD4}^+$ T cells (Fig. 4). Furthermore, the integrin $\alpha 4^{\text{high}}\beta 1^+\beta 7^+\text{CD4}^+$ T cells secreted small but significant amounts of IL-10. These results suggest that the serosal integrin $\alpha 4^{\text{high}}\text{CD4}^+$ T cells are heterogeneous population with different migratory properties and cytokine secretion.

DISCUSSION

Integrin $\alpha 4\beta 1$ (VLA-4) is a principal integrin complex that is essential for T cells to enter the peripheral inflammatory sites such as brain, lung, and pancreatic islets during autoimmune or infectious pathogenetic processes (7,18,19). Integrin $\alpha 4\beta 1$ is not normally expressed on both naïve and memory T cells in the resting condition, implicating a careful regulation of this integrin to prevent excessive infiltration of T cells into peripheral sites. Notably, we previously observed that almost half of peritoneal CD4⁺ T cells expressed a high level of integrin $\alpha 4\beta 1$ as we designated these cells as integrin $\alpha 4^{\text{high}}\text{CD4}^+$ T cells, which suggests that the PEC is a reservoir of pro-inflammatory T cells (3). In this manuscript, we addressed whether the peritoneal integrin $\alpha 4^{\text{high}}\text{CD4}^+$ T cells expressed other important integrin complexes to gain insights into their functional characteristics and checked whether the PLC also contains

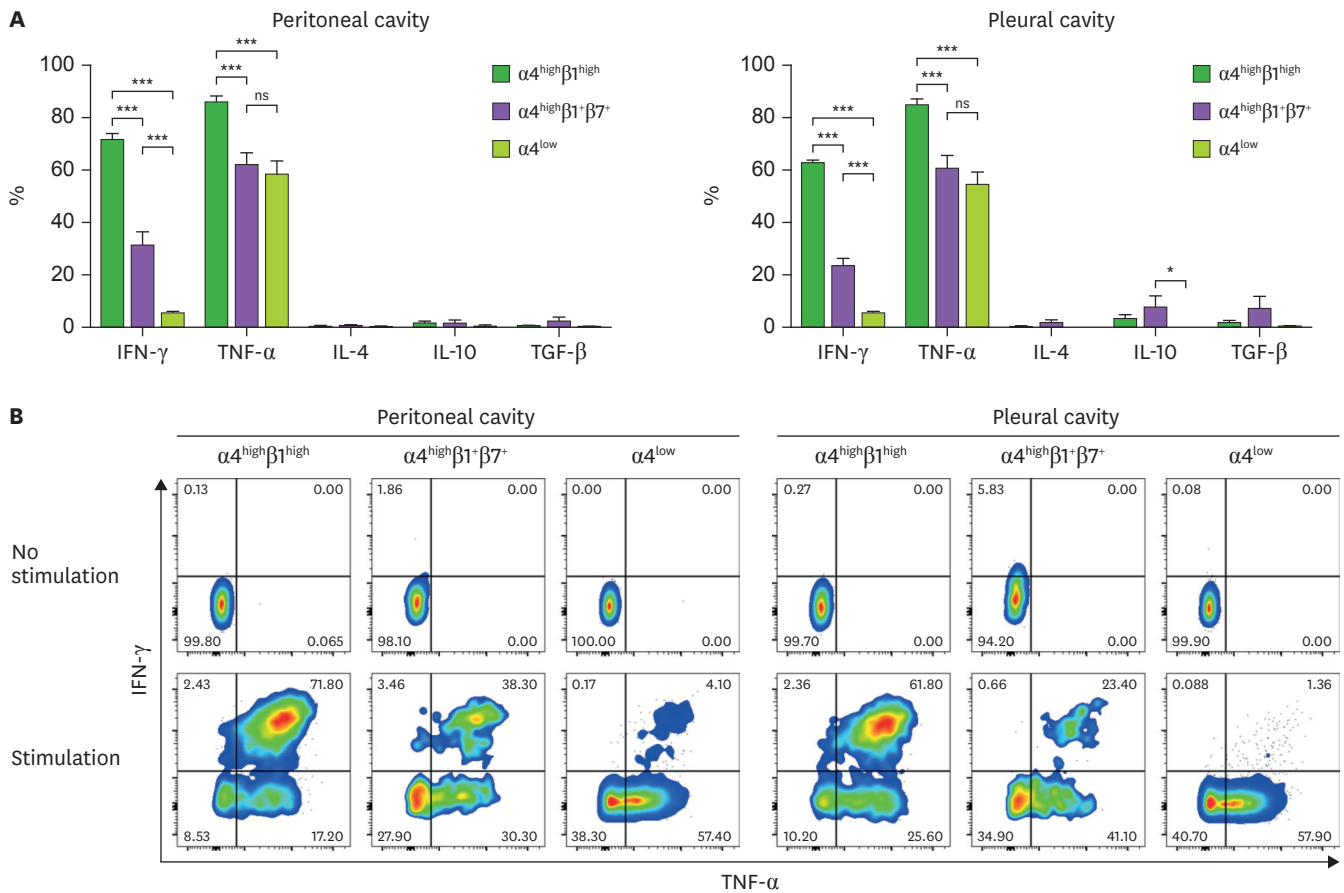


Figure 4. Cytokine production by integrin $\alpha 4^{\text{high}}\beta 1^{\text{high}}$ CD4⁺ T cells, integrin $\alpha 4^{\text{high}}\beta 1^+\beta 7^+$ CD4⁺ T cells, and integrin $\alpha 4^{\text{low}}$ CD4⁺ T cells. (A, B) Peritoneal and pleural cavity cells harvested from 8-week-old C57BL/6 mice were stimulated *in vitro* with 50 ng/ml PMA and 1.5 mM ionomycin for 4 h. (A) Individual bars represent the percentages of given cytokine-producing cells among $\alpha 4^{\text{high}}\beta 1^{\text{high}}$ CD4⁺ T cells (green, $\alpha 4^{\text{high}}\beta 1^{\text{high}}$), $\alpha 4^{\text{high}}\beta 1^+\beta 7^+$ CD4⁺ T cells (purple, $\alpha 4^{\text{high}}\beta 1^+\beta 7^+$), and $\alpha 4^{\text{low}}$ CD4⁺ T cells (yellowish green, $\alpha 4^{\text{low}}$), detected by intracytoplasmic staining of given cytokines. (B) Representative flow cytometric data for given cytokines are shown with or without stimulation. Data are representative of 12 separate experiments.

ns, not significant.
*P<0.05; ***P<0.001

this type of T cells. We found that the integrin $\alpha 4^{\text{high}}$ CD4⁺ T cells were divided into the major integrin $\alpha 4^{\text{high}}\alpha 6^+\beta 1^{\text{high}}$ CD4⁺ T cells and the minor integrin $\alpha 4^{\text{high}}\beta 1^+\beta 7^+$ CD4⁺ T cells.

The combinations of integrin α and β chains are diverse to form different kinds of adhesion molecules for other cells or extracellular matrix, complement receptor, or receptor for bacterial protein (20-22). Integrin $\alpha 4$ and $\beta 1$ are preferentially expressed on memory T cells rather than naïve T cells. In addition to the pro-inflammatory integrin $\alpha 4\beta 1$, each $\alpha 4$ and $\beta 1$ integrin chain form alternative combinations that provide additional functional characteristics. Integrin $\alpha 4$ chain is able to combine with integrin $\beta 1$ or $\beta 7$, but the probabilities of the combination of $\alpha 4$ chain with 2 β chains are not equal as the integrin $\beta 7$ is advantageous over the $\beta 1$ chain in the binding to $\alpha 4$ chain (23). Therefore, the level of the $\beta 1$ chain expression would determine the expression level of the integrin $\alpha 4\beta 1$ in the presence of a given level of the $\alpha 4$ chain expression. Given the finding that the integrin $\alpha 4$ is highly expressed on the memory phenotype CD4⁺ T cells, we divided the serosal CD4⁺ T cells into 3 populations based on the expression of integrin $\beta 1$ and $\beta 7$ chains; $\beta 1^{\text{high}}$, $\beta 1^+\beta 7^+$, and $\beta 1^-\beta 7^-$ cells. As $\beta 1^-\beta 7^-$ CD4⁺ T cells were integrin $\alpha 4^{\text{low}}$ cells, integrin $\alpha 4^{\text{high}}$ CD4⁺ T cells could be divided into $\alpha 4^{\text{high}}\beta 1^{\text{high}}$ CD4⁺ and $\alpha 4^{\text{high}}\beta 1^+\beta 7^+$ CD4⁺ T cells. Integrin $\alpha 4^{\text{high}}\beta 1^+\beta 7^+$ CD4⁺ T cells, the smaller population, are thus thought to principally express

integrin $\alpha 4\beta 1$ and $\alpha 4\beta 7$. As integrin $\alpha 4\beta 7$ (LPAM-1) is required for the entry into intestine and the pathogenesis of chronic colitis (9), this population appears to have the ability to enter the inflammatory intestinal sites. The role of this peritoneal CD4⁺ T cell population in gastrointestinal inflammation needs to be addressed in the future.

The $\beta 1$ integrin is reported to be highly expressed on memory T cells and critical in the maintenance of T cell memory in bone marrow, suggesting that $\beta 1$ integrin is involved in the entrance of memory T cells into bone marrow (24). Although the integrin $\alpha 4\beta 1$ is likely to be responsible for this migratory behavior, other $\beta 1$ integrins may be responsible for their unique migration patterns (25). Especially, the very high expression of integrin $\beta 1$ in the serosal $\alpha 4^{\text{high}}\beta 1^{\text{high}}\text{CD4}^+$ T cells suggested that these cells contained another $\beta 1$ integrin complex besides $\alpha 4\beta 1$. Integrins $\alpha 1\beta 1$, $\alpha 5\beta 1$, and $\alpha 6\beta 1$ were thought to be good candidates for another $\beta 1$ integrin expressed on the serosal $\alpha 4^{\text{high}}\beta 1^{\text{high}}\text{CD4}^+$ T cells as these integrins were reported to be expressed on some T cells (14,26,27). Our screening to find the second α integrin partner with the $\beta 1$ integrin in the $\alpha 4^{\text{high}}\beta 1^{\text{high}}\text{CD4}^+$ T cells resulted in the clear identification of the integrin $\alpha 6\beta 1$ in this population. As the integrin $\alpha 6\beta 1$ is mainly expressed on macrophages and its activity is upregulated by inside-out signaling upon stimulation with PMA (28,29), its expression on these innate-like CD4⁺ T cells is a very interesting feature that may reveal their characteristic migratory pattern such as interstitial migration after transendothelial migration (30,31).

In the serosal integrin $\alpha 4^{\text{high}}\text{CD4}^+$ T cells, the expression of integrin $\alpha 4\beta 7$ or $\alpha 6\beta 1$ appeared to be mutually exclusive, arguing that the 2 integrin $\alpha 4^{\text{high}}\text{CD4}^+$ T cell populations are functionally distinctive. As we investigated the activation-related cell surface molecules and cytokine production profiles, we could confirm that the $\alpha 4^{\text{high}}\beta 1^{\text{high}}\text{CD4}^+$ T cells were composed of integrin $\alpha 4\beta 1^+ \alpha 6\beta 1^+ \alpha 4\beta 7^-$ and $\alpha 4\beta 1^+ \alpha 6\beta 1^- \alpha 4\beta 7^+$ cells. It is interesting to address whether these 2 population originate from the common ancestor population or not. Most importantly, the functional significances of these 2 serosal T cell subsets in diseases such as infection, autoimmunity, cancer, or transplantation need to be investigated in the future. We think that integrin $\alpha 4$ is not a stable marker of these populations as the expression of integrin $\alpha 4$ is altered upon adoptive transfer to Rag-1^{-/-} mice (3) and upregulated by retinoic acid signal (32). The elucidation of developmental markers such as transcription factors is required to follow these cells and human counterpart populations.

In summary, we could identify the distinctive major and minor populations of serosal integrin $\alpha 4^{\text{high}}\text{CD4}^+$ T cells that are different based on the migratory behavior and cytokine secretion. The mutually exclusive expression of integrin $\alpha 4\beta 7$ or $\alpha 6\beta 1$ appear to be an important functional feature of memory or innate T cells determining their preferential pattern of migration.

ACKNOWLEDGEMENTS

This study was supported by a grant from Ministry of Health and Welfare, Republic of Korea (HI15C2939).

REFERENCES

1. Haas KM. B-1 lymphocytes in mice and nonhuman primates. *Ann N Y Acad Sci* 2015;1362:98-109.
PUBMED | CROSSREF

2. Ganshina IV. Serous cavities of coelomic origin as possible organs of the immune system. Part 1. *Biol Bull Rev* 2016;6:497-504.
[CROSSREF](#)
3. Moon H, Park C, Lee JG, Shin SH, Lee JH, Kho I, Kang K, Cha HS, Kim TJ. Early development in the peritoneal cavity of CD49dhigh Th1 memory phenotype CD4⁺ T cells with enhanced B cell helper activity. *J Immunol* 2015;195:564-575.
[PUBMED](#) | [CROSSREF](#)
4. Weber GF, Chousterman BG, Hilgendorf I, Robbins CS, Theurl I, Gerhardt LM, Iwamoto Y, Quach TD, Ali M, Chen JW, et al. Pleural innate response activator B cells protect against pneumonia via a GM-CSF-IgM axis. *J Exp Med* 2014;211:1243-1256.
[PUBMED](#) | [CROSSREF](#)
5. Rose DM, Han J, Ginsberg MH. Alpha4 integrins and the immune response. *Immunol Rev* 2002;186:118-124.
[PUBMED](#) | [CROSSREF](#)
6. Woodland DL, Kohlmeier JE. Migration, maintenance and recall of memory T cells in peripheral tissues. *Nat Rev Immunol* 2009;9:153-161.
[PUBMED](#) | [CROSSREF](#)
7. Kadioglu A, De Filippo K, Bangert M, Fernandes VE, Richards L, Jones K, Andrew PW, Hogg N. The integrins Mac-1 and alpha4beta1 perform crucial roles in neutrophil and T cell recruitment to lungs during *Streptococcus pneumoniae* infection. *J Immunol* 2011;186:5907-5915.
[PUBMED](#) | [CROSSREF](#)
8. Rott LS, Briskin MJ, Andrew DP, Berg EL, Butcher EC. A fundamental subdivision of circulating lymphocytes defined by adhesion to mucosal addressin cell adhesion molecule-1. Comparison with vascular cell adhesion molecule-1 and correlation with beta 7 integrins and memory differentiation. *J Immunol* 1996;156:3727-3736.
[PUBMED](#)
9. Kurmaeva E, Lord JD, Zhang S, Bao JR, Kevil CG, Grisham MB, Ostanin DV. T cell-associated $\alpha 4\beta 7$ but not $\alpha 4\beta 1$ integrin is required for the induction and perpetuation of chronic colitis. *Mucosal Immunol* 2014;7:1354-1365.
[PUBMED](#) | [CROSSREF](#)
10. Millward JM, Løbner M, Wheeler RD, Owens T. Inflammation in the central nervous system and Th17 responses are inhibited by IFN-gamma-Induced IL-18 binding protein. *J Immunol* 2010;185:2458-2466.
[PUBMED](#) | [CROSSREF](#)
11. Brakebusch C, Hirsch E, Potocnik A, Fässler R. Genetic analysis of beta1 integrin function: confirmed, new and revised roles for a crucial family of cell adhesion molecules. *J Cell Sci* 1997;110:2895-2904.
[PUBMED](#)
12. Epler JA, Liu R, Chung H, Ottoson NC, Shimizu Y. Regulation of beta 1 integrin-mediated adhesion by T cell receptor signaling involves ZAP-70 but differs from signaling events that regulate transcriptional activity. *J Immunol* 2000;165:4941-4949.
[PUBMED](#) | [CROSSREF](#)
13. Bauer M, Brakebusch C, Coisne C, Sixt M, Wekerle H, Engelhardt B, Fässler R. Beta1 integrins differentially control extravasation of inflammatory cell subsets into the CNS during autoimmunity. *Proc Natl Acad Sci U S A* 2009;106:1920-1925.
[PUBMED](#) | [CROSSREF](#)
14. Hauzenberger D, Klominek J, Sundqvist KG. Functional specialization of fibronectin-binding beta 1-integrins in T lymphocyte migration. *J Immunol* 1994;153:960-971.
[PUBMED](#)
15. Baaten BJ, Cooper AM, Swain SL, Bradley LM. Location, location, location: the impact of migratory heterogeneity on T cell function. *Front Immunol* 2013;4:311.
[PUBMED](#) | [CROSSREF](#)
16. Loetscher P, Uguccioni M, Bordoli L, Baggiolini M, Moser B, Chizzolini C, Dayer JM. CCR5 is characteristic of Th1 lymphocytes. *Nature* 1998;391:344-345.
[PUBMED](#) | [CROSSREF](#)
17. Park HJ, Lee A, Lee JJ, Park SH, Ha SJ, Jung KC. Effect of IL-4 on the development and function of memory-like CD8 T cells in the peripheral lymphoid tissues. *Immune Netw* 2016;16:126-133.
[PUBMED](#) | [CROSSREF](#)
18. Sorcini D, Bruscoli S, Frammartino T, Cimino M, Mazzon E, Galuppo M, Bramanti P, Al-Banchaabouchi M, Farley D, Ermakova O, et al. Wnt/ β -catenin signaling induces integrin $\alpha 4\beta 1$ in T cells and promotes a progressive neuroinflammatory disease in mice. *J Immunol* 2017;199:3031-3041.
[PUBMED](#) | [CROSSREF](#)

19. Yang XD, Michie SA, Tisch R, Karin N, Steinman L, McDevitt HO. A predominant role of integrin alpha 4 in the spontaneous development of autoimmune diabetes in nonobese diabetic mice. *Proc Natl Acad Sci U S A* 1994;91:12604-12608.
[PUBMED](#) | [CROSSREF](#)
20. Jun HK, Lee SH, Lee HR, Choi BK. Integrin $\alpha 5\beta 1$ activates the NLRP3 inflammasome by direct interaction with a bacterial surface protein. *Immunity* 2012;36:755-768.
[PUBMED](#) | [CROSSREF](#)
21. Hynes RO. Integrins: a family of cell surface receptors. *Cell* 1987;48:549-554.
[PUBMED](#) | [CROSSREF](#)
22. Plow EF, Haas TA, Zhang L, Loftus J, Smith JW. Ligand binding to integrins. *J Biol Chem* 2000;275:21785-21788.
[PUBMED](#) | [CROSSREF](#)
23. DeNucci CC, Pagán AJ, Mitchell JS, Shimizu Y. Control of $\alpha 4\beta 7$ integrin expression and CD4 T cell homing by the $\beta 1$ integrin subunit. *J Immunol* 2010;184:2458-2467.
[PUBMED](#) | [CROSSREF](#)
24. DeNucci CC, Shimizu Y. $\beta 1$ integrin is critical for the maintenance of antigen-specific CD4 T cells in the bone marrow but not long-term immunological memory. *J Immunol* 2011;186:4019-4026.
[PUBMED](#) | [CROSSREF](#)
25. Sixt M, Bauer M, Lämmermann T, Fässler R. Beta1 integrins: zip codes and signaling relay for blood cells. *Curr Opin Cell Biol* 2006;18:482-490.
[PUBMED](#) | [CROSSREF](#)
26. Gebhardt T, Wakim LM, Eidsmo L, Reading PC, Heath WR, Carbone FR. Memory T cells in nonlymphoid tissue that provide enhanced local immunity during infection with herpes simplex virus. *Nat Immunol* 2009;10:524-530.
[PUBMED](#) | [CROSSREF](#)
27. Chang AC, Salomon DR, Wadsworth S, Hong MJ, Mojcić CF, Otto S, Shevach EM, Coligan JE. Alpha 3 beta 1 and alpha 6 beta 1 integrins mediate laminin/merosin binding and function as costimulatory molecules for human thymocyte proliferation. *J Immunol* 1995;154:500-510.
[PUBMED](#)
28. Shaw LM, Mercurio AM. Regulation of alpha 6 beta 1 integrin laminin receptor function by the cytoplasmic domain of the alpha 6 subunit. *J Cell Biol* 1993;123:1017-1025.
[PUBMED](#) | [CROSSREF](#)
29. Wei J, Shaw LM, Mercurio AM. Integrin signaling in leukocytes: lessons from the $\alpha 6\beta 1$ integrin. *J Leukoc Biol* 1997;61:397-407.
[PUBMED](#)
30. Krummel MF, Bartumeus F, Gérard A. T cell migration, search strategies and mechanisms. *Nat Rev Immunol* 2016;16:193-201.
[PUBMED](#) | [CROSSREF](#)
31. Friedl P, Weigelin B. Interstitial leukocyte migration and immune function. *Nat Immunol* 2008;9:960-969.
[PUBMED](#) | [CROSSREF](#)
32. Kang SG, Park J, Cho JY, Ulrich B, Kim CH. Complementary roles of retinoic acid and TGF- $\beta 1$ in coordinated expression of mucosal integrins by T cells. *Mucosal Immunol* 2011;4:66-82.
[PUBMED](#) | [CROSSREF](#)