Original Article

Serosal Cavities Contain Two Populations of Innate-like Integrin $\alpha_4^{\text{high}}CD4^+$ T Cells, Integrin $\alpha4\beta1^+\alpha6\beta1^+\alpha4\beta7^-$ and $\alpha4\beta1^+\alpha6\beta1^-\alpha4\beta7^+$ Cells

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ABSTRACT

We previously reported peritoneal innate-like integrin $\alpha_4$ (CD49d)$^{\text{high}}$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 $\alpha_4^{\text{high}}$CD4$^+$ T cells and investigated the functional heterogeneity of the subpopulations based on the integrin expression. Pleural cavity contained a lower ratio of integrin $\alpha_4^{\text{high}}$CD4$^+$ T cells to integrin $\alpha_4^{\text{low}}$CD4$^+$ T cells than peritoneal cavity, but the pleural integrin $\alpha_4^{\text{high}}$CD4$^+$ T cells have the same characteristics of the peritoneal integrin $\alpha_4^{\text{high}}$CD4$^+$ T cells. Most of integrin $\alpha_4^{\text{high}}$CD4$^+$ T cells were integrin $\beta1^{\text{high}}\beta7^-$, but a minor population of integrin $\alpha_4^{\text{high}}$CD4$^+$ T cells was integrin $\beta1^+\beta7^-$. Interestingly, the integrin $\alpha_4^{\text{high}}\beta1^{\text{high}}\beta7$ CD4$^+$ T cells expressed high levels of integrin $\alpha4\beta1$ and $\alpha6\beta1$, whereas integrin $\alpha_4^{\text{high}}\beta1^+\beta7$ CD4$^+$ T cells expressed high levels of integrin $\alpha_4\beta1$ and $\alpha4\beta7$, suggesting an alternative expression of integrin $\alpha6\beta1$ or $\alpha4\beta7$ in combination with $\alpha4\beta1$ in respective major and minor populations of integrin $\alpha_4^{\text{high}}$CD4$^+$ T cells. The minor population, integrin $\alpha_4^{\text{high}}\beta1^+\beta7$ CD4$^+$ T cells, were different from the integrin $\alpha_4^{\text{high}}\beta1^{\text{high}}\beta7$ 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 $\alpha_4^{\text{high}}\beta1^{\text{high}}\beta7$ CD4$^+$ T cells. In summary, the innate-like integrin $\alpha_4^{\text{high}}$CD4$^+$ T cells could be divided into 2 populations, integrin $\alpha_4\beta1^+\alpha6\beta1^+\alpha4\beta7^-$ and $\alpha_4\beta1^-\alpha6\beta1^-\alpha4\beta7^+$ cells. The functional significance of serosal integrin $\alpha4\beta7$ 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
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 α4 (CD49d) and rapidly secreted Th1 cytokines upon stimulation similarly to memory T cells (3). The innate-like integrin α4highCD4+ 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 α4highCD4+ 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 α4 is an integrin chain that can combine with either of 2 β chains, β1 (CD29) or β7, to form integrin α4β1 (VLA-4) or integrin α4β7 (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 α4β1 or α4β7 directs transendothelial migration into distinct anatomical sites; α4β1 through binding to vascular cell adhesion protein 1 (VCAM-1) for the entry into inflammatory sites and α4β7 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 α4β1 is reciprocally correlated to the expression of α4β7 (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 β1 can combine with at least 12 α integrin chains to form different kinds of integrin combinations that bind to extracellular matrix proteins, such as α1β1 (VLA-1), α2β1 (VLA-2), α3β1 (VLA-3), α4β1 (VLA-4), α5β1 (VLA-5), and α6β1 (VLA-6) (11). Since the adhesive activity of these β1 integrins is enhanced by T cell receptor (TCR)-mediated signaling, the expression of β1 integrins is important for the function of memory/effector T cells that express a higher level of integrin β1 than naïve T cells (12). What determines the kind of integrin α chain expressed in combination with integrin β1 is not well understood, but the expression of different β1 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 α4highCD4+ T cells. We observed that most of them preferentially expressed the α4β1 and α6β1 integrins, but a minor population of integrin α4highCD4+ T cells expressed a high level of α4β7 integrin. We further reanalyzed the functional differences of 2 kinds of serosal integrin α4highCD4+ T cells.

MATERIALS AND METHODS

Mice

C57BL/6 mice were purchased from Orient Bio (Sungnam, Korea). Cd1d−/− mice were kindly provided by L. Van Kaer (Vanderbilt University School of Medicine). This study was approved...
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), α3 (polyclonal), α7 (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 (SH4), CD127 (A7R34), α1 (HMa1), α2 (DX5), α5 (SH10-27, MFR5), α6 (GoH3), β1 (HM81.1), β7 (FIB504), α4β7 integrin (DATK32), interferon (IFN)-γ (XMG1.2), IL-10 (JESS16E3), TGF-β (TW7-20B9), tumor necrosis factor (TNF)-α (MP6-XT22) from BioLegend (San Diego, CA, USA); and CXCR4 (2B11), CXCR5 (SPRC5), CCR6 (Slrx6), CCR7 (4B12), ICOS (7E.IG9), α4 (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 µg/ml during the last 3 h of stimulation. Cells were stained with anti-CD4, β1, β7, and anti-α4 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 α4β6CD4+ T cells with a memory phenotype similar to that of peritoneal integrin α4β6CD4+ T cells
Since B-1a cells are abundant in PLC as well as PEC, we first investigated whether the integrin α4β6CD4+ T cells are present in the PLC. The integrin α4β6CD4+ 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 α4β6CD4+ T cells cavity also showed the memory and pro-inflammatory phenotypes found in the peritoneal integrin α4β6CD4+ 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 Cdd−/− mice.
The serosal integrin α4(high)CD4+ T cells are divided into 2 populations based on the expression of α6β1 or α4β7 integrins

We next investigated the expression of integrin β1 or β7 chains in the serosal integrin α4(high)CD4+ T cells since the integrin α4 is known to heterodimerize with integrin β1 or β7. When we checked the expression of integrin β1 or β7 chains in serosal CD4+ T cells, the memory phenotype CD4+ T cells were recognized as integrin β1'CD4+ T cells (Fig. 2A). The integrin β1 CD4+ T cells were not found in the gated integrin α4(high)CD4+ T cells, which indicates that the integrin α4β1 is a principal integrin complex in the serosal integrin α4(high)CD4+ T cells. Notably, a small population of integrin α4(high)β1'β7'CD4+ T cells was also observed. The level of the integrin β1 expression is higher in the integrin α4(high)β1'β7'CD4+ T cells than in α4(high)β1'β7'CD4+ T cells. Therefore, we divided the integrin α4(high)CD4+ T cells into α4(high)β1'highCD4+ and α4(high)β1'β7'CD4+ T cells. We presumed that some fraction of integrin
Serosal Integrin α4β1·α6β1·α4β7 and α4β1·α6β1·α4β7·CD4⁺ Cells

β1 in the integrin α4⁹⁶β1⁹⁶CD4⁺ T cells might associate with some other integrin α chain(s) to form other β1 integrin complexes.

In contrast to integrin α4 that associate with only 2 kinds of integrin β chains, the integrin β1 chain can associate with many integrin α chains (Fig. 2B). To find additional integrin β1 combination(s) in the integrin α4⁹⁶β1⁹⁶CD4⁺ T cells, we investigated the expression of individual integrin α chains in the 2 integrin α4⁹⁶CD4⁺ T cell populations as well as integrin α4⁹⁶CD4⁺ T cells (Fig. 2C). Notably, the high expression of integrin α6 is prominent only in the integrin α4⁹⁶β1⁹⁶CD4⁺ T cells, but not in the other populations. This result suggests that α6β1 (VLA-6) is another inflammatory integrin chain expressed on the serosal integrin α4⁹⁶β1⁹⁶CD4⁺ T cells.

**The serosal integrin α4⁹⁶β1⁹⁶CD4⁺ and α4⁹⁶β1⁹⁶β7⁹⁶CD4⁺ T cells are distinct memory phenotype T cells**

We addressed whether the integrin α4⁹⁶β1⁹⁶CD4⁺ and α4⁹⁶β1⁹⁶β7⁹⁶CD4⁺ 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
α<sup>4</sup>β<sup>1</sup> high β<sup>7</sup> CD4<sup>+</sup> 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 β<sup>1</sup>β<sup>7</sup>CD4<sup>+</sup> T cells than in β<sup>1</sup>highCD4<sup>+</sup> T cells. Accordingly, integrin α<sup>4</sup>β<sup>1</sup>β<sup>7</sup>CD4<sup>+</sup> T cells secreted smaller amounts of Th1 cytokines such as IFN-γ and TNF-α than integrin α<sup>4</sup>lowβ<sup>1</sup>β<sup>7</sup>CD4<sup>+</sup> T cells (Fig. 4). Furthermore, the integrin α<sup>4</sup>highβ<sup>1</sup>β<sup>7</sup>CD4<sup>+</sup> T cells secreted small but significant amounts of IL-10. These results suggest that the serosal integrin α<sup>4</sup>highCD4<sup>+</sup> T cells are heterogeneous population with different migratory properties and cytokine secretion.

**DISCUSSION**

Integrin α<sub>4</sub>β<sub>1</sub> (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 α<sub>4</sub>β<sub>1</sub> 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<sup>+</sup> T cells expressed a high level of integrin α<sub>4</sub>highβ<sub>1</sub> as we designated these cells as integrin α<sup>4</sup>highCD4<sup>+</sup> 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 α<sup>4</sup>highCD4<sup>+</sup> T cells expressed other important integrin complexes to gain insights into their functional characteristics and checked whether the PLC also contains...
this type of T cells. We found that the integrin α4\(\beta^4\)CD4\(^+\) T cells were divided into the major integrin α4\(\beta^4\)α6\(\beta^1\)β7CD4\(^+\) T cells and the minor integrin α4\(\beta^4\)β1\(\beta^7\)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 α4 and β1 are preferentially expressed on memory T cells rather than naïve T cells. In addition to the pro-inflammatory integrin α4β1, each α4 and β1 integrin chain form alternative combinations that provide additional functional characteristics. Integrin α4 chain is able to combine with integrin β1 or β7, but the probabilities of the combination of α4 chain with 2 β chains are not equal as the integrin β7 is advantageous over the β1 chain in the binding to α4 chain (23). Therefore, the level of the β1 chain expression would determine the expression level of the integrin α4β1 in the presence of a given level of the α4 chain expression. Given the finding that the integrin α4 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 β1 and β7 chains; β1\(\beta^4\), β1\(\beta^7\), and β1β7 cells. As β1β7 CD4\(^+\) T cells were integrin α4\(\beta^4\) cells, integrin α4\(\beta^4\)CD4\(^+\) T cells could be divided into α4\(\beta^4\)β1\(\beta^4\)CD4\(^+\) and α4\(\beta^4\)β1\(\beta^7\)CD4\(^+\) T cells. Integrin α4\(\beta^4\)β1\(\beta^7\)CD4\(^+\) T cells, the smaller population, are thus thought to principally express

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**Figure 4.** Cytokine production by integrin α4\(\beta^4\)β1\(\beta^7\)CD4\(^+\) T cells, integrin α4\(\beta^4\)β1\(\beta^7\)CD4\(^+\) T cells, and integrin α4\(\beta^4\)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 α4\(\beta^4\)β1\(\beta^7\)CD4\(^+\) T cells (green, α4\(\beta^4\)β1\(\beta^7\)), α4\(\beta^4\)β1\(\beta^7\) CD4\(^+\) T cells (purple, α4\(\beta^4\)β1\(\beta^7\)), and α4\(\beta^4\)CD4\(^+\) T cells (yellowish green, α4\(\beta^4\)). 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
integrin α4β1 and α4β7. As integrin α4β7 (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 β1 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 β1 integrin is involved in the entrance of memory T cells into bone marrow (24). Although the integrin αβ1 is likely to be responsible for this migratory behavior, other β1 integrins may be responsible for their unique migration patterns (25). Especially, the very high expression of integrin β1 in the serosal α4α6β1α4β7− and α4β1 CD4+ T cells suggested that these cells contained another β1 integrin complex besides α4β1. Integrins α1β1, α5β1, and α6β1 were thought to be good candidates for another β1 integrin expressed on the serosal α4α6β1α4β7− 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 β1 integrin in the α4α6β1β1highCD4+ T cells resulted in the clear identification of the integrin α6β1 in this population. As the integrin α6β1 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 summary, we could identify the distinctive major and minor populations of serosal integrin α4β1 CD4+ T cells that are different based on the migratory behavior and cytokine secretion. The mutually exclusive expression of integrin α4β7 or α6β1 appears to be an important functional feature of memory or innate T cells determining their preferential pattern of migration.

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REFERENCES

1. Haas KM. B-1 lymphocytes in mice and nonhuman primates. *Ann NY Acad Sci* 2015;1362:98-109.

https://doi.org/10.4110/in.2017.17.6.392
2. Ganshina IV. Serous cavities of coelomic origin as possible organs of the immune system. Part 1. *Biol Bull Rev* 2016;6:497-504.

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.

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.

5. Rose DM, Han J, Ginsberg MH. Alpha4 integrins and the immune response. *Immunol Rev* 2002;186:118-124.

6. Woodland DL, Kohlmeier JE. Migration, maintenance and recall of memory T cells in peripheral tissues. *Nat Rev Immunol* 2009;9:153-161.

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.

8. 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.

9. Kurmaeva E, Lord JD, Zhang S, Bao JR, Kevil CG, Grisham MB, Ostanin DV. T cell-associated alpha4beta1 but not alpha4beta1 integrin is required for the induction and perpetuation of chronic colitis. *Mucosal Immunol* 2014;7:1354-1365.

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.

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.

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.

13. Bauer M, Brakebusch C, Coisne C, Sixt M, Wekerle H, Engelhardt B, Fassler R. Beta1 integrins differentially control extravasation of inflammatory cell subsets into the CNS during autoimmune. *Proc Natl Acad Sci U S A* 2009;106:1920-1925.

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.

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.

16. Loetscher P, Ugucioni M, Bordoli L, Baggioioli M, Moser B, Chizzolini C, Dayer JM. CCR5 is characteristic of Th1 lymphocytes. *Nature* 1998;391:344-345.

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. *Immun Netw* 2016;16:126-133.

18. Soricini D, Bruscoli S, Frammartino T, Cimino M, Mazzon E, Galuppo M, Bramanti P, Al-Banchaabouchi M, Farley D, Ermakova O, et al. Wnt/beta-catenin signaling induces integrin alpha4beta1 in T cells and promotes a progressive neuroinflammatory disease in mice. *J Immunol* 2017;199:3031-3041.
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 α5β1 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, Pagan AJ, Mitchell JS, Shimizu Y. Control of α4β7 integrin expression and CD4 T cell homing by the β1 integrin subunit. *J Immunol* 2010;184:2458-2467.
PUBMED | CROSSREF

24. DeNucci CC, Shimizu Y. β1 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, Lämmertmann 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, Mojcik 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 alpha6beta1 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-β1 in coordinated expression of mucosal integrins by T cells. *Mucosal Immunol* 2011;4:66-82.
PUBMED | CROSSREF